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IJST contact Information:
P.O. Box 2793 Amman 11953 Jordan
Tel. +96265602285
E-mails: info@ijst-jo.com / info.icast@yahoo.com
URL: www.ijst-jo.com

EDITORIAL BOARD

FORWARD

It is my pleasure to welcome you back and present you this issue, Volume 5, No. 3 (2010), of ***International Journal for Sciences and Technology (IJST)***. The members of Editorial Board, the ICAST and TSTC teamwork and I hope you will find this collection of research articles useful and informative.

In this issue, I am pleased to welcome you all in our journal's new website with new vision to serve your requests in both English and Arabic languages. Also I would like to inform you that our journal had been added as a ***Refereed Journal in Ulrich's International Journals Directory*** which had added another value and supportive position to IJST.

The journal is one of the scientific contributions offered by ***the International Centre for Advancement of Sciences and Technology*** to the science and technology community (Arab region with specific focus on Iraq and International).

Finally, on behalf of *the International centre*, I would like to express my gratitude and appreciation to the efforts of the Editorial Board, Advisory group with their valuable efforts in evaluating papers, Researchers and the Editorial Secretary for managing the scientific, design, technical and administrative aspects of the Journal and for preparing this volume for final printing and publishing.

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IJST

Abdul Jabbar Al- Shammari

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ENGLISH SECTION

Building "E- Manara"
Towards Demonstrating Personal Knowledge Unit

Akram M. Othman (1), Samer F. Lolo (2)

Amman Arab University / Amman (1)
Jordan

Emails: (1) akram.othman@aau.edu.jo (2) esamerlolo@gmail.com

ABSTRACT

This work aimed to demonstrate the procedure for implementing Personal Knowledge Unit (PKU) principle. The basic proposal is to modelize the knowledge by units that highly depend on the style and behavior of the personal integration process.
Modeling knowledge as units can improve the usage of information and minimize the effects of information overload to enhance the quality and enable persons to manage and assess their knowledge in efficient and effective ways.
The proposed methodology is using existent Content Management (CM) tools to assist persons in building and integrating their knowledge into units. The applied PKU is called "eManara".

Keywords: *E-Manara, PKU*

الملخص باللغة العربية

هدف هذا العمل إلى عرض سياق تنفيذ مفهوم وحدات المعرفة الشخصية (Personal Knowledge Units PKU)، بواسطة بناء نموذج يسمح بربط مجموعة من وحدات المعلومات المختلفة لتكوين وحدة معرفة شخصية كبيرة متكاملة تعتمد على نمط و طريقة استخدام كل فرد لمتطلباته من المعلومات .
تم في البحث اختبار فرض أن تكامل المعلومات وأسلوب تمثيلها كوحدات معرفة يحسن من طريقة الاستخدام، ويقلل من تأثير ظاهرة الحمل المعلوماتي الزائد(Information Overload)، إضافة إلى تحسين نوعية المعلومات، وتمكين الأشخاص من إدارة وفحص وحدات معارفهم الشخصية بكفاءة وفعالية أكبر.
تستخدم الطريقة المقترحة أدوات نظم إدارة المحتوى (Content Management Systems CMS) وسيلة رئيسية في فحص وتطوير وتنفيذ وحدة المعرفة الشخصية (eManara) كنموذج ريادي تطبيقي للبحث.

INTRODUCTION

This paper is a demonstration of building a practical Personal Knowledge Unit (PKU) called "eManara". Personal Knowledge Unit (PKU) is the fusion of personal information forms (contents) and the personal tools needed to manage it. Tools are smoothed from enterprise knowledge and content management systems, to be as portable units, this Personal Portable Units (PPU) used to carry out all the processes of personal knowledge/content management. PPU must be independent of platforms and the background culture of the person, and so be easy and simple to extend, enhance, and use within innovations and needs.
The paper is consisting of the following sections: section 2 is discussing the basic principle of PKU, then in section 3, eManara infrastructure and basic model were presented. Section 4 shows the evaluation procedure to assess the PPU used in eManara. Finally, we conclude the paper in section 5.

Preview of PKU as PUW

The term of “Personal Unit Ware” (PUW) refers to the conceptual framework for implementing the concepts PPU and PKU.
PKU Production Net (PPN) is the PKU production process, accomplished through a grid of tasks that linked together to reflect the production, management, and union processes.
Personal Unit Ware is being architected by using Open Source and Unit based Virtual Architecture (UVA) principles, which were designated using web spiral methodology and implemented by web technologies and tools (4).
Blogging and Wiki are common applications used in individual domain to log and share knowledge, beyond the limitations and boundaries of technology and background culture.

Portable Personal Unit (PPU)

It is a standard personal form of functional, moveable, and manageable, software tool, can be integrated with other units to build a larger unit.
The main object of this unit is to serve the personal need of managing information-knowledge/content effectively and efficiently. The personal dimension is the personal perspective of the unit; as the needs are vary from user to other.
PPUs must be easy to use, simple to install and manage, independent of platforms, be plug and play i.e., can be transferred to personal wares[1] without effecting on its function and role, free or not expensive so any one can get it, open sourced so be transparent and extendable to fits the need of the user.
The basic functions of PPU actually are the merge (union) of Personal Knowledge Management (PKM) and Content Management (CM) tools and features, i.e., a collection of processes that an individual needs to carry out in order to gather, classify, store, search and retrieve knowledge/content in his/her daily activities (2) (3).

[1] Personal wares are any of personal assets of hardware or software.

Activities are not confining to business/work-related tasks but also include personal interests, hobbies, home, family, and leisure activities.

The following is the summarized points of PPU:

1. Standard in scope and domain
2. Can work (be operated) individually.
3. Open interfaced, sourced, accessed in its scope, domain
4. Pluggable and joinable, in order to form a larger unit. The super unit must -at least- have all characteristics of its subordinates.
5. Variable size (volume) due to its features and embedded objects.
6. Dynamically changed over its operating time
7. Examining these characteristics, PPU is suitable for web environment and open systems that security is not very important issues. (Fig.1)

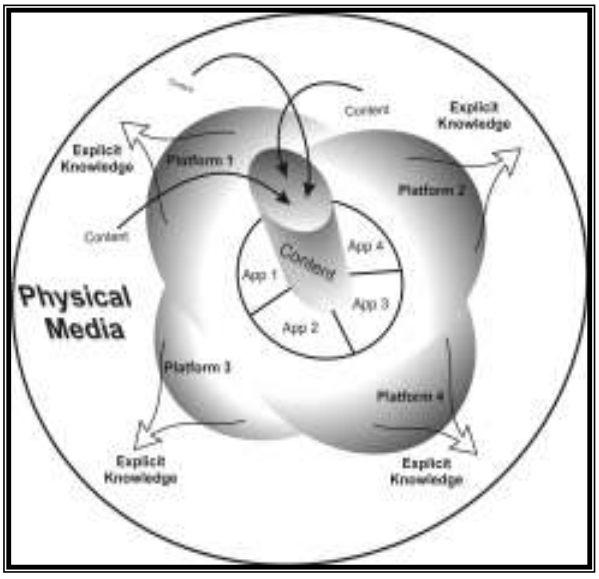


Figure (1) Content/Knowledge Based Open Systems

In content/knowledge based open system, contents are shared among applications that make use of these contents in its way without changing or affecting each other. All these applications can dynamically work in platforms that process the task of the application as a basis to trigger an action. Each task will produce its own knowledge over its specific domain. The whole process is act as qualification of contents to produce explicit knowledge, which feed to the system as contents, hence enhancing and increasing the whole knowledge in spiral rapid fashion.

Unit based Virtual Architecture (UVA) is modeled using the principles of virtual units and abstracted tasks. The layers of UVA can be demonstrated as follows:

Multithreading/Multiprogramming technologies enable multiple piece of codes to run on one processor, hence each unit of code is virtually has its own processor. Virtual Machine technology enables programs to run independently from physical operating system and platform (for example Sun JVM - Java Virtual Machine- and Microsoft .NET framework). Internet and WEB applications are make use of Client/Server and Peer-2-Peer technologies to be accessed and operated independently from anywhere, on whatever wares and via virtual ports that established to serve a specific task, using various protocols and technologies. Extensible Markup Language (XML) and technologies of RSS (Rich Site Summery or Really Simple Syndication), Resource Description Framework (RDF),

Ontology Web Language (OWL) and extensible Markup Language-Remote Procedure Call (XML-RPC) are being developed to permit complex data structures to be transmitted, processed, and returned between different operating systems running in different environments. Contents are charging to serve a virtual task. Figure (2) is illustrates UVA layers.

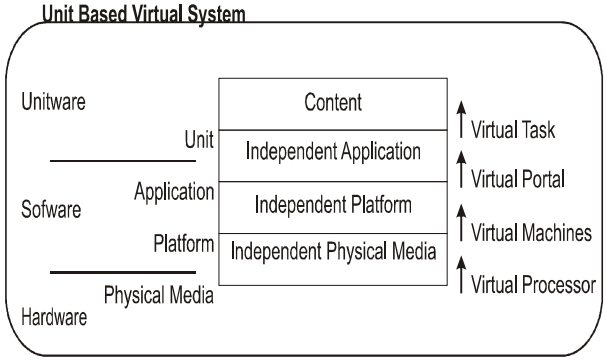


Figure (2) Unit based Virtual Architecture

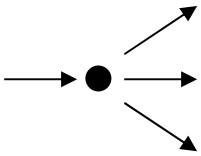
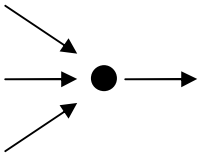
Personal Knowledge Unit (PKU)

Knowledge is highly depends to the personal perspectives and skills over the personal scope of information. Community knowledge is the accumulated personal knowledge.

Standardizing personal knowledge is very important to make the accumulation process simple and free form errors. Following specific rules may waste and confuse user's attention. By using PPU, this problem can be minimized as the tools are highly leveled to the user and -without loosing flexibility- are closed to low level wares of various applications, operating systems, machines and distributed systems. Hence, PKU is the fusion of personal content with personal experience over this content, basically, by using Personal Portable Units.

The fusion acts as weaving the content with personal image of content's role/value/properties. The common image is the answer of how, what, when, where, why, who, whom, whose, and yes/no questions. This image can be extended to include the personal memory and view of the specific content. Blogging and Wiki are the essential technologies used to implement the principles of eManara. Table (1) summarizes the PKU view of Blogging and Wiki.

Table (1)

	Blogging	Wiki
1		
2	Physically (one) Author (writer) Virtually many readers.	Physically many Authors (writers) Virtually one reader.
3	Experience Based (i.e. Subjective)	Content Based (i.e. Objective)
4	The major tasks are EPN.	The major tasks are CPN.
5	Expands Organizational memory to specific Personal memory.	Extends Personal memory to Organizational Memory.
6	Unique content, multiple experiences.	Unique experience, multiple contents.

PKU Production Net (PPN)

PPN is a production process illustration. PPN demonstrates the building of PKM from experience (the subject) and content (the object) of a desired matter. PPN consists of four nodes: Matter, Experience (subject), Content (object), and PKU, and of nine tasks (directed links) distributed into three nets: experience production net (EPN), Content Production NET (CPN) and Management Net (MN). MN includes Experience Management Net (EMN) tasks: code-mark-clip, Content Management Net (CMN) tasks: post-construct-qualify, and unite task to produce PKUs.

The whole process begins from the desired matter, which represents implicit aim of the user work. The PKU produced is represents the modeling of the knowledge extracted from the matter. Hence the produced PKU is the explicit aim of the user work. Figure (3) shows the PPN illustration.

Thinking and needs are basic source of experience which exploited by invention and search. The explicit experience subjects will be managed by coding its forms, marking its indices, and prepare it to clip.

Classification and verification are the basic contention of the object. Unification and organization tasks are used to structure contents. The objectified content is to be managed through posting and constructing its contexts, hence can be qualified.

The clipped subjects are being united with the qualified objects i.e. joining the explicit experience with the structured content. The units are personally built to produce PKUs.

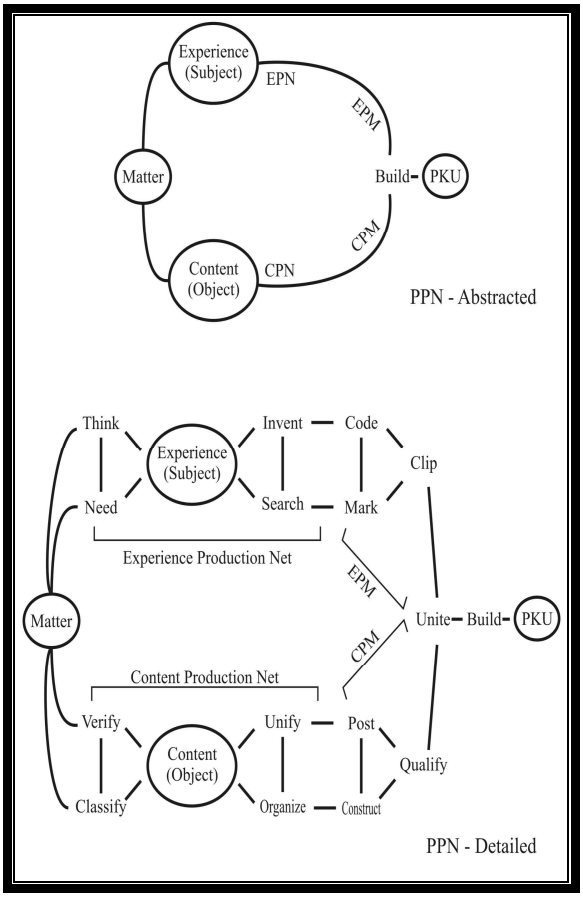


Figure (3) PPN illustration

E-MANARA EXPLORATION

This section presents the software tools used to develop eManara and the developed PPUs, which integrated in the user interface "eMishkah".

1- EManara Infrastructure

Web application and development principles, technologies, tools, environment and access are the base kernel that used to implement the PKU concepts in eManara. Blogging and Wiki are the basic application. Open source and spiral-incremental are the basic

development principles. Local-host server and user-agent (HTML browser) are basic accessing processors. All tools used in developing, processing and accessing are free and fits personal's need. Tools are:

1. Apache 2 – local host server.
2. PHP 5.3 - programming language of the server side script
3. MYSQL 4.1 - Data Base
4. IE6 - User Agent
5. BLOG: CMS - Nucleus CMS 3.6- Blogging
6. DokuWiki - Wiki.
7. Coppermine Photo Gallery 1.3.3 - images repository and meta-image indexer
8. Google Desktop search – local search tool

2- Basic PPUs Used To build eManara

Four PPUs are used. eMBlog, eMWiki, eMPhoto, and Google Desktop search tool. Each is adapted from a universal open sourced web application. The four PPUs are ported in eMishkah - the user interface. It is play the role of gate way or port that all PPUs and the external/internal contents tools access.(Fig.4)

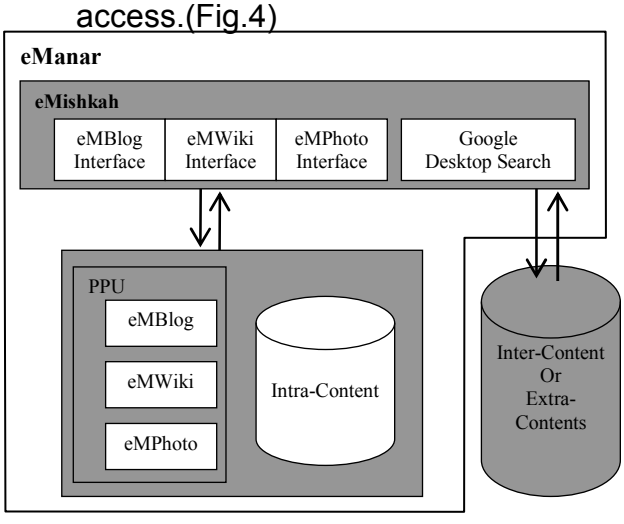


Figure (4) Basic structure of eManara

Inter-Content is all kinds of contents that can be accessed in the local system (e.g. PC and LAN). Extra-Content is all somewhat content that can be accessed via Internet or mobile devices. Intra-Content is the content that processed by the PPUs that is defined in PKU (eManara). Figure[2] (a1) shows a snap shot of eMishkah.

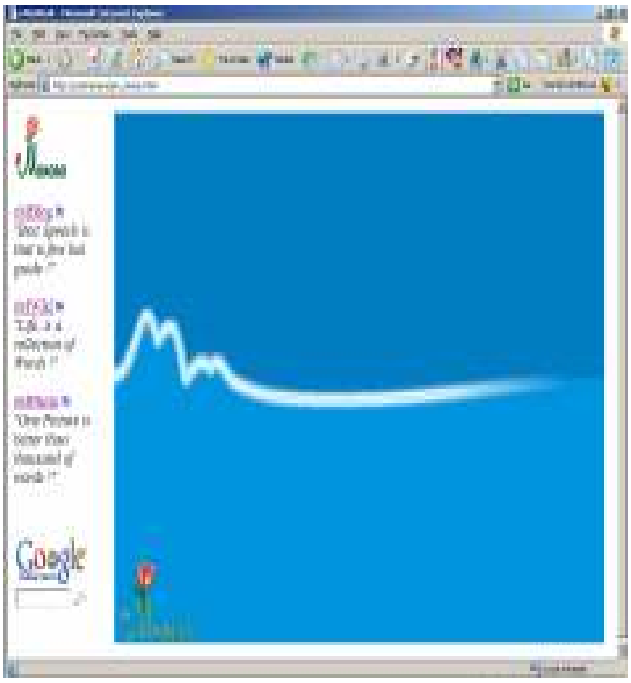


Figure (a1) a snap shot of EMishkah

EMishkah consists of both side panel and main frame. The PPUs are organized in the side panel and modified by editing the em_side.html file. Each PPU when clicked will show its home page in the main frame as will shown in figures (a2, a3, a4, and a5). There is small icon beside each link to use PPU in a separated window. Except for Google Desktop Search, where it can be able to click on its logo to open the search home

[2] The figures of a# are listed in the last of paper.

page. Finally, by clicking the logo of eManara will get the logo page of eMishkah in the main frame.

2-1 EMBlog

EMBlog is based on Nucleus-BLOG: CMS. BLOG: CMS is a CMS: a powerful set of PHP scripts that allow user to maintain one or more weblogs or online journals. A short summary of the most important features are given below[3]:

- One or more weblogs, even on the same page
- Plug-in-interface to add extra functionality
- Comments
- Archives
- Categories
- Search
- Multiple authors
- Future items
- Drafts
- Extensive administration area
- Media library and file upload
- Fully customizable skins and templates
- Skin import/export
- Easy installation
- Easy backups
- XML-RPC interface (implementing the Blogger API and Meta Weblog API).
- XHTML-ready

Figure (a2) shows a snap shot of eMBlog.

2-2 eMwiki

EMBlog is based on DokuWiki. DokuWiki is a standard compliant, simple to use Wiki, mainly aimed at creating documentation of any kind.

Figure (a2) A snap shot of eMBlog.

It has a simple but powerful syntax, which makes sure the data files remain readable outside the Wiki and eases the creation of structured texts. All data is stored in plain text files – no database is required. A short summary of the most important features is given below[4]:

- Works on plain text files.
- Simple syntax and easy editing with quick buttons and access keys
- Section Editing allows user to edit small parts of a page.
- Automatic generation of content tables
- Unlimited page revisions
- Colored side by side diff support.
- Support for read only pages.
- Interwiki Links.

[3] The detailed features can be get from URL: www.blogcms.com/admin/documentation/default.htm

[4] The detailed features can be get from URL: http://www.wiki.splitbrain.org/wiki:features

- Uploading and embedding images
- Image caching and resizing
- Multilanguage Support
- custom text replacements
- to avoid edit conflicts

Figure (a3) shows a snap shot of eMWiki.

Figure (a3) A snap shot of eMWiki.

2-3 eMPhoto

EMPhoto is based on Coppermine Photo Gallery. Coppermine Photo Gallery is a picture gallery script. Users can upload pictures with a web browser (thumbnails are created on the fly), rate pictures, add comments and send e-cards. The admins can manage the galleries and batch adds pictures that have been uploaded on the server by FTP. Support for multimedia files has been added recently.

Images are stored in albums and albums can be grouped by categories. The script also supports multiple languages and has a theme system[5].

Figure (a4) shows a snap shot of eMPhoto.

Figure (a4) A snap shot of eMPhoto.

2-4 Google Desktop Search

Google Desktop Search is Google technology on user desktop. It can be used to search for files and communications on personal hard drive, as well as chats, web pages and networked files user have seen recently. Like Google, Desktop Search processes documents to create a searchable index. Unlike Google, Desktop Search's technology and index live on user's own computer, so results can be seen only from there. Google Desktop Search can be used to locate:

[5] The detailed features can be get from FAQs in URL: http://coppermine.sourceforge.net

- Web pages that previously seen using Internet Explorer, Netscape, Mozilla, or Firefox browser.
- Email that sent or received using Outlook, Outlook Express, or Netscape, Thunderbird, or Mozilla's email products.
- IM chats using AOL and Instant Messenger (AIM).
- Files in Microsoft Word, Excel, PowerPoint, PDF, and plain text formats.
- Image, audio, and video files (by searching for information about these files, such as names, titles, captions, and artist info).

Figure (a5) shows a snap shot of Google Desktop search tool which integrated in eMishkah user interface.

2-5 Evaluating eManara using Quality Metrics

This section discusses eMBlog, eMWiki and Google Desktop Search using comparisons of the core tools used with universal tools and the information quality metrics (1). As follows:

i. EMBlog core is based on Nucleus CMS 3.15. Table (2) shows the comparison of Nucleus CMS and other four CMSs.
ii. EMWiki core is based on DokuWiki. The following section aims at comparing DokuWiki against another specific Wiki Engines to verify its superiority over them. The engines includes: MediaWiki, Twiki, WikkaWiki (5).

⇒ DokuWiki vs. MediaWiki

When compared to MediaWiki, DokuWiki's following features:

- Raw Text Files.
- Window Shares.
- Syntax Highlight. (There're two GeSHi plug-ins available, though)
- Table of Contents. With TOC Level setting.
- Quoting and Footnotes Text Syntax.
- Has a more intuitive text syntax, overall.
- CSS compliant - through W3C validator.
- Great RSS features: RSS Feeder + XML Exporting of Namespace documents.

Table (2) Comparing Various CMS s[8]

Product	Nucleus CMS 3.15	PHP Nuke 6	Baseline CMS 1.95	Complete Site Manager 1.1.0	Movable Type 3.0
Last Updated	12/1/2004	9/25/2002	4/27/2005	4/24/2005	6/5/2004
System Requirements					
Application Server	Apache	mod_php	IIS	PHP 4 or 5, with XML support.	PHP4 or more
Approximate Cost		Free	$2,500	$150.00	Free version available
Database	MySQL	MySQL, Postgres, mSQL, Interbase, Sybase	Microsoft SQL Server 2000	MySQL	MySQL, mSQL, PostgreSQL, BerkeleyDB, Flat File, sqlite
License	GNU GPL	GNU GPL	Commercial per installation	Commercial, per Web site	Commercial, includes full source code
Operating System	Any	Any	Win 2000, 2003	OS Independent	Unix, Linux, FreeBSD & All BSD's, HP-UX, Windows 2000, 2003, XP, Solaris, AIX
Programming Language	PHP	PHP	ASP	PHP, Java Script, DHTML	Perl 5
Web Server	Apache	Apache, IIS	MS-IIS	Any php enabled server	Apache, Jetty, Tomcat, IIS
Ease of Use					
Drag-N-Drop Content	No		No	Yes	No
Friendly URLs	Yes	No	No	Yes	Yes
Server Page Language	Yes	Yes	No	No	Yes
WYSIWYG Editor	Limited	No	Yes	Yes	Free Add On
Management					
Asset Management	No	No	Yes	No	No
Clipboard	No	No	No	No	No
Content Scheduling	Yes[6]	No	Yes	No	Yes
Online Administration	Yes	Yes	Yes	Yes	Yes
Sub-sites / Roots	No	No	Yes	No	Yes
Themes / Skins	Yes[7]	Yes	Yes	No	Yes
Web Statistics	No	Yes	Yes	No	No
Web-based Style/Template Management	Yes	Limited	No	Yes	Yes

[6] This item is modified due to observation.
[7] This item is modified due to observation.
[8] These comparison items are adapted from results of 'cms matrix' (http://www.cmsmatrix.org/matrix).

Concluding table (2), Nucleus CMS has useful features and some lack in management. The benefits are: Nucleus CMS is based on Open Source and GPL license. Ease of use features is good if compared to its competitor PHP Nuke. The lack of management can overcome in future by further development.
Hence nucleus cms seems to be the best choice over these five CMSs.

But MediaWiki also has strong features, missing in DokuWiki:

- Mail Notification, for users watching pages (articles). (Actually, MediaWiki doesn't currently have this feature. It's available in alpha releases of MediaWiki (1), (5)
- Users have profiles, to store personal settings. It also allows for automatic signature (~~~).
- Discussion pages, working more or less like comments to page content.
- Detects editing conflict and resolves (merges) non conflicting changes.
- Statistics, including Most Popular and Least Popular pages, among others.
- Wiki Site Admin can lock pages and ban hosts. DokuWiki can lock pages and namespaces, based on users or groups.
- Wiki Site Admin can delete or rename pages. DokuWiki has a simple while elegant trigger to remove pages, and without control any user can trigger a page to be removed.
- Allows Math formulas to be edited.
- Allows paragraphs to be indented and understands definition lists.

⇒ DokuWiki vs. Twiki

TWiki actually seems to have a lot more support and a lot more users. Moreover there isn't anything that any other wiki can do that TWiki can't. It is large corporate user base allows for quite a large number of extensions and plugins.

Although some of the missing features are usually not so important, such as strikethrough, superscript/subscript, footnotes, among others there are still some that I consider most valuable:

* Installation - TWiki [http://twiki.org/cgi-bin/view/Codev/RCS requires Revision Control System (RCS)] which is a real headache to install on windows servers, and not much better on *nix. DokuWiki Install is easier.

* Syntax Highlight - not through a plug-ins or extension, but maintained by the developers in the core code. This is a must for code documentation purposes (a common application of wikis)

* Windows Shares - a very nice-to-have option, on intranets, where many companies still use Windows on the local area network. Although it may open some security breaches, these are minimal on an intranet and the feature is well worth it.

* Localization - any open-source web application should meet UTF-8 requirements and be localized in as many languages as the user community is able to.

- GUI Editor - this is not WYSIWYG, but a more simple and practical editor that assists the user in inserting Wiki Markup.
- XHTML/CSS - last but not least, one of the most important features is the compliance (certification) against the web standards. It's incredible how so many well-known web applications make no efforts to meet this requirement, or when it meets is just by "accident" and is not considered a worthy-feature.

⇒ DokuWiki vs. WikkaWiki

When compared to WikkaWiki, DokuWiki's following features:

- Raw Text Files; doesn't require a database engine.
- Window Shares.
- Table of Contents. With TOC Level setting.
- Footnotes Text Syntax.
- Has very intuitive text syntax, overall.
- Camel Case linking is optional.
- Namespaces.

But WikkaWiki also has strong features, missing in DokuWiki:-

- Advanced support for inline Free Mind maps (or mind maps), embedded Flash objects, and embedded RSS.
- Footer comments as well as inline comments (quote).
- Orphan/Wanted pages, among other nice-to-have information about Wiki Pages.
- Page Cloning, which is tied together with Category Templates.
- Full revision history, displaying differences between any two revisions of the Wiki Page.
- Wiki Ping.
- Basic Statistics.
- Users have profiles, to store personal settings.
- Password Management.
- Wiki Site Admin has Access control list (ACL) User Interface to define access level permissions and settings.
- Advanced Referrer Management.
- Install Wizard, through any Web Browser.
- Calendar and other Actions.
- Easier to customize by editing header.php and footer.php.
- Smart Title function for optimized Search Engine Results.

They both have a clean and nice user interface:

- Great RSS features: RSS Feeder + XML Exporting of Namespace documents.
- XHTML and CSS compliant - through W3C validator.
- GUI Editor.

iii. Google Desktop Search are compared to six other desktop search tools. Table (3) shows this comparison. Noting that the reason to use Google Desktop Search tool in eManara is its familiarity and ease of use. Other tools can be used as needed.

Table (3) Comparing Various desktop Search tools

Vendor	Operating Systems	System Requirements	Searchable File Types
Google Desktop Google Enterprise Desktop	Windows XP and Windows 2000 Service Pack 3 and above	128MB of RAM and a 400MHz Pentium processor, 500MB of space available on your hard disk. must have administrator privileges	Microsoft Word, Excel, Power point, Web Pages, Text, AOL IM, PDF files, Multimedia files, including audio (MP3, WMA, WAV and more), images (JPG, GIF, PNG, BMP and more), and videos (AVI, MPG, WMV, and more), by meta-tag. Third Party Plug ins add more file types ,Lotus Notes
Ask Jeeves	Windows2000 Windows XP	P3 400MHz and 128 MB RAM minimum 1 GHz and 256 MB RAM recommended	Office 2000+ for Office doc formats, Outlook for email Images – jpg, gif and png Music Files – mp3, wma and wav Video Files – mpeg and wmv Plus: bookmarks, web page history and more
Blinkx	Windows 98,2000,ME,XP, 2003, MAC OS	Hard Disk 100MB Memory (64 MB) and processor (200 Mhz CPU	Internet Explorer 4- 6, Outlook Express, Outlook 2000, Outlook 2003, Outlook XP, Firefox, Eudora, Mozilla Firebird, Mozilla, AOL Instant Messenger, html, pdf, txt, MS Excel, MS Word, MS PowerPoint, mp3, jpg, AAC (iTunes),WMV, MPEG, Real, AVI, QuickTime MOV,TIFF,GIF,JPEG, Zip files,BMP, Lotus Notes, Windows Media, Attachments for Outlook, Outlook Express, and Eudora
Lycos Hotbot	Windows 98, ME, 2000 or XP	N/A	Search files, email, internet, browser history, and RSS news feeds
MSN Toolbar Suite • MSN Deskbar • MSN Toolbar for Internet Explorer and Windows Explorer • MSN Toolbar for Outlook	Windows 2000 Service Pack 4 or later, Windows XP	Pentium 233MHz processor or better (500MHz recommended). Minimum 128MB of RAM (256MB recommended) MSN Desktop Search requires Microsoft Outlook 2000+ or Microsoft Outlook Express 6.0+ to index and search your email	Outlook Email, Contacts, Calendar, Tasks, Notes, Email Word, Excel, PowerPoint , OneNote Plain Text Files AOL IM .MSN IM PDFs, HTML Pages MP3, WMA, AVI GIF, JPG, BMP Outlook Express Email Hotmail via OE or Outlook
Yahoo Desktop	Windows 98SE/ME/2000/XP	• 128 MB RAM required • 256 MB RAM recommended • 50 MB free hard disk space	Over 200 file types including: Email Desktop and Yahoo Word, Excel, PowerPoint PDF, Music, Internet Explorer, Text ,Images

CONCLUSION

EManara is presented and investigated. Starting from its basic concepts and foundation frame work which lay basically in web oriented platform, through the PPUs used which consider CMS technologies, ending by evaluating the whole unit by examining the tasks needed and accomplished via its tools and the quality indices from practical comparisons.

The investigation shows that each PPU has its way to process contents. The way is effects on the behavior of the resultant units. But by integrating the features of the PPUs, the usage of eManara will improved. The tools used, which selected from various types of tools show that there are many tools supportthe PUW principle, and implementing and developing such system is simple and not needs much tools or components.
The evaluation shows that selected tools are verified to be best choice to match the requirements of PKU. Both Nucleus CMS and DokuWiki have many superior features than other compared systems.

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Detection of some bacterial infection in urinary tract and their antibiotic sensitivity

Janet A. Dishvarian

Institute of Medical Technology Mansour/ Baghdad -Republic of Iraq

Email: janet_1966@yahoo.com

ABSTRACT

A Survey study had been done for urinary tract bacterial infection. 572 urine samples were collected from patients who are suffering from urinary tract infections. 289 cases out of the total samples indicated positive results and the rest were negative .The highest incidence of bacterial infection was for *E. coli* 39.7% followed by *Proteus* 24.2% and *Klebsiella pneumoniae* 20.8%, while the lowest incidence was for *Morganella morganie* 6.6% , *Pseudomonas aeruginosa* 4.9% , *Citrobacter* spp. 3.5% and for *Staphylococcus aureus* 0.3%.

The infection incidences expressed higher level in summer. It reached the peak from July to September with a percentage of 60% ; while it reached 40% for the rest of the year .The percentage of bacterial incidence in females was higher than males in most of the examined samples ,except in *Klebsiella* which was higher in males .The cases studied were in ages ranged between (1 - 65) years old and the most positive cases were in elder patients than in children .The antibiotic sensitivity ; for ciprofloxacin gentamycin ,tetracycline ,keflex, chlaforam , nitrofurantin , ceftriaxone , fucidin ,cefalexin ,amikacin ,trimethoprim + sulfamethaxazole , tobramycin and amoxicillin ; was examined. Detected species of bacteria were more sensitive to ciprofloxacin and chlaforan, while the resistances to the rest were in different ranges.

الملخص باللغة العربية

تم إجراء مسح إحصائي للإصابات البكتيرية التي تسبب خمج المسالك البولية .حيث زرعت 572 عينة إدرار من أشخاص مصابين بإعراض التهاب المجاري البولية على مدى أكثر من سنة للفترة من نيسان/2007 إلى كانون الأول /2008 . أعطت (289) حالة نتيجة موجبة و (283) حالة أعطت نتيجة سالبة . تركزت الإصابات بالاشرشيا القولونية بنسبة 39.7% يتبعها الإصابة بالبروتيس والكلبسييلا بنسبة 24.2% و20.8% على التوالي , ثم النسب الأقل كانت بواقع موركانيلا موركاني ، السيدوموناس ،سيتيروبكتر والمكورات العنقودية بنسب 6.6% ،4.9% ،3.5% و 0.3% على التوالي . كما تركزت الإصابات في أشهر الصيف بشكل عام حيث وصلت الذروة في شهري تموز ولحد أيلول بنسبة 60% بينما توزعت نسبة 40% على باقي أشهر السنة . كما كانت النسبة في الإناث لكل الأنواع البكتيرية أكثر مما في الذكور عدا الإصابة بالكليبسييلا كانت في الذكور أعلى . تم دراسة الحالات بأعمار مختلفة من (1– 65) سنة وكانت نسبة الإصابة عند الكبار أكثر من الصغار بالعمر

فحصت الحساسية للمضادات الحياتية ؛ للسبروفلوكساسلين ، جنتامايسين ، تيتراسايكلين ، كفلكس، كلافورام ، نايتروفيورانتين ، فيوسيدين ،سفترياكسون ، سيفالكسين ،اميكاسين ،ترايميثيبريم + سلفاميثاكسازول ، توبرامايسين واموكسيسيلين ؛ واتضحت بان اكثر البكتريا كانت حساسة للسبروفلوكساسلين والكلافورام بينما المقاومة للباقي كانت بدرجات متفاوتة .

INTRODUCTION

Urinary tract infection UTI is a condition where one or more structures of urinary tract become infected after bacteria overcome the structures` strong natural defenses. UTIs are the most common of all infections and can occur at any time in the life of an individual (1). From a microbiologic perspective, urinary tract infection exists when pathogenic microorganisms are detected in the urine. Every female has a 20% lifetime risk of coming down with a UTI (2). In children, approximately 5% of girls and 1% of boys have a UTI by 11 years of age (3). It is also the most common cause of nosocomial infections in adults.

Most pathogens causing UTIs are the Coliforms ; including *E.coli* , *Klebsiella pneumoniae , Proteus mirabilis* and *Citrobacter spp.* these strains produce substance such as P- fimbriae that tend to make these bacteria more infectious; *Staphylococcus* aureus which is responsible for 5% -15% of primary infection (4).

An African study reported that *Escherichia coli* (32%) and *Proteus* spp. (22%) form more than 50% of the total isolates (5). Most strains are resistant to several antibiotics such as cefotaxime because of the presence of R-factor or plasmids (6). Antibiotics which have been recommended to treat UTIs include Ampicillin, Trimethoprim-Sulfamethoxazole, Flouroquinolones and Nitrofurantoin (7).

However due to increase abuse of these antibiotics, extensive resistance of micro-organisms to these antibiotics has developed.

This study is aimed at establishing the main organisms causing of UTIs in our environment and ascertaining the extent of resistance of the causal organisms to commonly prescribed antibiotics. Clinical laboratory records of cases of urinary tract infection were studied for the spectrum of bacterial isolates and their antibiotic susceptibility results were analyzed for recommending suitable therapy.

MATERIALS & METHODS

572 urine samples were collected from patients in ages between (1 – 65 years old) in both gender, with UTI symptoms referred to Al-Yarmok teaching hospital at Baghdad city ;were studied during the period from April \ 2007 till December \ 2008 . These urine samples were undergoing urinalysis and cultured; whenever pus cell or bacteria found; at 37 ˚C for 24 hr. on the following media:-

- Blood agar with 5% human blood (oxoid).
- MacConkey agar (oxoid).
- Mannitol salt agar

All positive cultures were repeated again to confirm result and to be accepted as UTI in association with abnormal urinalysis and UTI symptoms.

The strains isolated were identified and diagnosed by Gram's stain and biochemical reactions which were done by using Api 20 E and Api Staph system (bio Merieux ,France) in addition to the characteristics of bacterial colonies on culture media .

Antibiotic sensitivity was performed for all isolates by disc diffusion method on Nutrient agar (oxoid) (8). Different antibiotic disc were used (Table 1) .The inhibition zones of antibiotic discs were measured according to the method of Baron *et al*., 1990 (9)

Table(1) The antibiotic discs which are used to study the sensitivity of the isolates

Antibiotic	symbol	Concentration g\discµ	Reference
Gentamycin	GM	10	Mast diagnostic UK
Ciprofloxacin	CIP	100	=
Tetracycline	TE	10	=
Keflex	KF	30	=
Chlaforam	C	30	=
Nitrofurantin	F	300	=
Ceftriaxon	CRO	30	=
Fucidin	FU	30	=
Cefalexin	CE	30	SDI
Amikacin	AK	30	Mast diagnostic UK
Trimethoprim+ Sulfamethaxazole	S+T	25	Hi Media,India
Tobramycin	TOB	10	Mast diagnostic UK
Amoxicillin	AM	30	=

RESULTS & DISCUSSION

Bacteria species that had been seen in UTIs were of fecal origin. These organisms are a subset of the organisms found in the feces, more than 90% of acute UTIs in patients with normal anatomic structure and function are caused by certain strains of *E. coli.*(10) , 10 -20 % are caused by coagulase-negative *Staphylococcus saprophyticus* and 5% or less are caused by other enterobacteriaceae organisms or enterococci, while in complicated cases of UTI, such as UTI's resulting from anatomic obstructions, or from catheterization the most common causes of UTI are *E. coli, Klebsiella pneumoniae, Proteus mirabilis, Enterococcus* sp., *Pseudomonas aeruginosa* 53% ,12% ,6% ,12% , 0-4% (11) .

Out of 572 urine samples, 289 samples showed a positive culture growth while 283 samples have no growth. Seven different isolates obtained with highest incidence for *E. coli* 115 isolate (39.7%) followed by *Proteus* 70 isolate (24.2%)and *Klebsiella pneumoniae* 60 (20.8%) , while the lowest incidence was for *Morganella morganie ,Pseudomonas aeruginosa , Citrobacter spp.* and *Staphylococcus aureus* with 19 (6.6%) , 14 (4.9 %) , 10 (3.5%) , and 1 (0.3%) respectively. (Table 2)

Regarding sex the study showed that the percentage of UTI in female was higher than in male for most isolates obtained except in *Klebsiella pneumoniae* was higher in male than in female (Table 3) .

Table (2) Types of isolates obtained and their percentages

Isolates	No. of isolates	Percentage %
E.coli	115	39.7
Proteus mirabilis	70	24.2
Klebsiella pneumoniae	60	20.8
Moganella morganii	19	6.6
Pseudomonas aerogenosa	14	4.9
Citrobacter spp.	10	3.5
Staphylococcus aureus	1	0.3
Total	289	100

Table (3) The sex prevalence in the positive isolates

Isolates	No. of isolates	Male		Female	
		No.	%	No.	%
E.coli	115	40	34.8	75	65.2
Proteus mirbilis	70	25	35.7	45	64.3
Klebsiella pneumoniae	60	37	61.66	23	38.33
Moganella morganii	19	8	42.1	11	57.9
Pseudomonas aerogenosa	14	4	28.6	10	71.4
Citrobacter spp.	10	5	50	5	50
Staphylococcus aureus	1	0	0	1	100
Total	289	119		170	

Women are at great risk for UTI primarily because of the significantly shorter urethra and closer proximity to the rectum. The female genitalia may become colonized with pathogenic bacteria that can more easily enter the urethra. In addition, woman lack the bacteriostatic protection that prostatic secretions offer the male (2) American women are 30 time more likely to have UTI than men (12) .

All patients taken were between 1-65 years old ,Table (4) showed the age prevalence of UTI and revealed highest incidence in elder patients than in younger ones (17.99 , 28.72 , 22.5) % were for ages (40-49 , 50-59 , 60-65) respectively. 20 -25 % of elder women have UTI because of number of biological factors including estrogen loss, after menopause the wall of urinary tract thin out weakening the mucous membrane and reducing the ability to resist bacteria, the bladder may lose elasticity and fail to empty completely, besides poor over all health.

Table (4): Age prevalence of UTI

Age (years)	No. of positive cases	%
1 - 9	46	15.9
10 – 19	22	7.62
20 – 29	12	4.15
30 – 39	9	3.12
40 – 49	52	17.99
50 – 59	83	28.72
60 – 65	65	22.5
Total	289	100

5 -15 % of men older than 50 -65 will have asymptomatic UTI more likely because of prostate problems (13).

Regarding month distribution of the UTI obtained in this study, (Table 5) the highest incidence was in summer season. It reaches the peak during July, August and September about 60% (58.06%) while 41.94 % distributed on the rest of the months of the year .infections caused by these bacteria has seasonal variation with higher incidence in summer and full in winter and spring may be due to hot weather and bad hygiene management during summer (14).

Table (5) monthly prevalence of UTI

Month	Positive case	Negative case	Total	% of + ve
April / 2007	9	8	17	3.12
May	11	8	28	3.81
June	12	8	20	4.15
July	22	12	34	7.61
August	35	25	60	12.11
September	29	20	49	10.04
October	11	21	32	3.81
November	4	16	20	1.38
December	3	12	15	1.04
January / 2008	2	8	10	0.69
February	3	12	15	1.04
March	3	14	17	1.04
April	9	10	19	3.12
May	12	11	23	4.15
June	12	10	22	4.15
July	25	12	37	8.65
August	33	19	52	11.4
September	25	5	30	8.65
October	15	20	35	5.19
November	9	15	24	3.12
December	5	8	13	1.73
Total	289	283	572	100

The increasing rates of resistance to uropathogenic *E. coli* isolates reported worldwide , antibiotic susceptibility pattern of these isolates revealed that for outpatients, first generation cephalosporins, nitrofurantoin, norfloxacin/ciprofloxacin were effective for treatment of urinary tract infection but for inpatients, parenteral therapy with newer aminoglycosides and third generation cephalosporins need to be advocated as the organisms for nosocomial UTI exhibit a high degree of drug resistance.

Trimethoprim and sulphamethoxazole combination was not found to be effective for the treatment of urinary tract infections as all the uropathogens from inpatients and outpatients showed high degree of resistance to co-trimoxazole. Culture and sensitivity of the isolates from urine samples should be done as a routine before advocating the therapy (15).

In order to determine current levels of resistance to antibiotics commonly used locally for empirical treatment, we reviewed susceptibility to (ciprofloxacin gentamycin,tetracycline ,keflex, chlaforam , nitrofurantin , ceftriaxone , fucidin ,cefalexin ,amikacin ,trimetheprim +sulfamethaxazole ,tobramycin and amoxicillin) amongst all urinary isolates obtained in our study over a 1 year period since April\2007 till December\2008 reveal increases in resistance to tetracycline, chlaforam , amoxicillin, , gentamicin and trimetheprim +sulfamethaxazole and increases in sensitivity to ciprofloxacin and chlaforam.

The inhibition zones of the antibiotic discs were measured on nutrient agar for all isolates .The sensitivity of bacterial isolates to the antibiotics were tested ,the results in figures 1,2,3,4,5,6,7 showed that most isolates were resistant to antibiotic in different ranges as follows:

1/ *E.coli* sensitive to (CIP ,C) 100% ,Gm 78.3% , S+T 74.8% (CE , F) 66.9% ,(CRO , AK) 61.7% , Am 50.4% , Kf 36.5% .
Resistant to (Tob , Fu , TE)100% .
2-*Proteus mirabilis* sensitive to (CIP ,C , CRO) 100% ,Gm 67.1% ,(AK ,Tob) 67.1% , F 60% ,Am 45.7% , Kf 41.4% .
Resistant to (Tob , FCE , S+T , TE)100% .
3- *Klebsiella pneumoniae* sensitive to CIP 100% , AK 70% , CE 66.6% , S+T 60% , (Kf , CRO) 50% ,(C , Tob Am) 40% ,Gm 13.3% .

Resistant to TE 100% , Gm 86.7%, C 60% .

4- *Morganella morgani* sensitive to CIP 100% , Kf 68.4% ,(S+T , Tob)63.2% , (CE , AK) 57.9% CRO 52.6% , Am 47.4% ,C 42.1% , Gm 31.6% .

Resistant to TE 100% , Gm 68.4%, C 57.9% .

5-*Pseudomonas aeurogenosa* sensitive to(CIP , Kf ,C , CRO , CE , Am) 100% , Gm 92.8% , AK 71.4% .

Resistant to(TE, S+T , Tob) 100% .

6-*Citrobacter spp.* sensitive to(CIP, Am)100% , CRO 70% ,(Gm , Kf , CE , S+T) 60% (AK , Tob)40% , C 30% .

Resistant to TE 100% , C 70% AK 60% .

7- *Staphylococcus aureus* sensitive to (Gm , CIP , Kf , C , F , CRO , Fu , CE , AK , Tob , Am)100% .

Resistant to (TE , S+T) 100%

Fig . 1

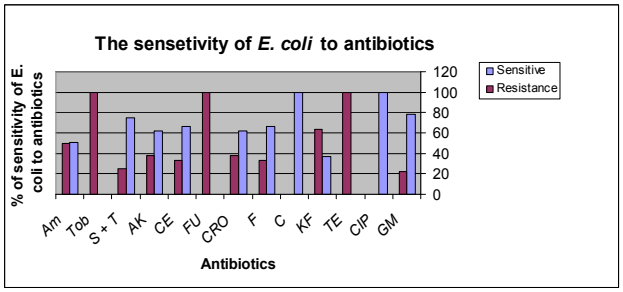

Figure (1) The sensitivity of *E. coli* to antibiotics

Fig .2

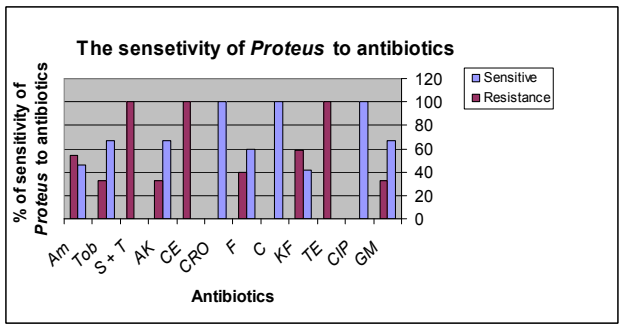

Figure (2) The sensitivity of *Proteus mirabilis* to antibiotic

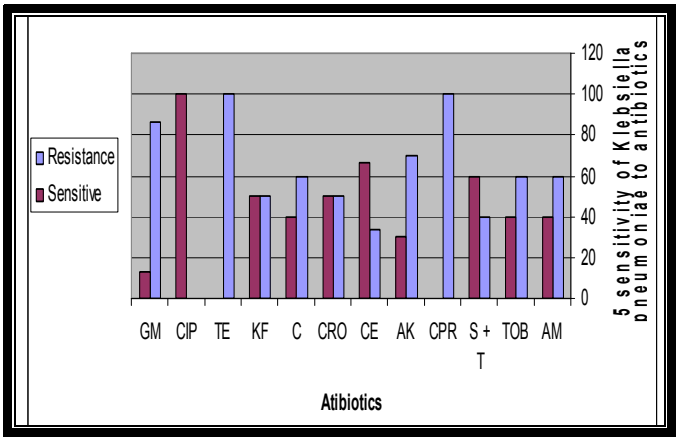

Figure (3) the sensitivity of *Klebsiella* to antibiotics

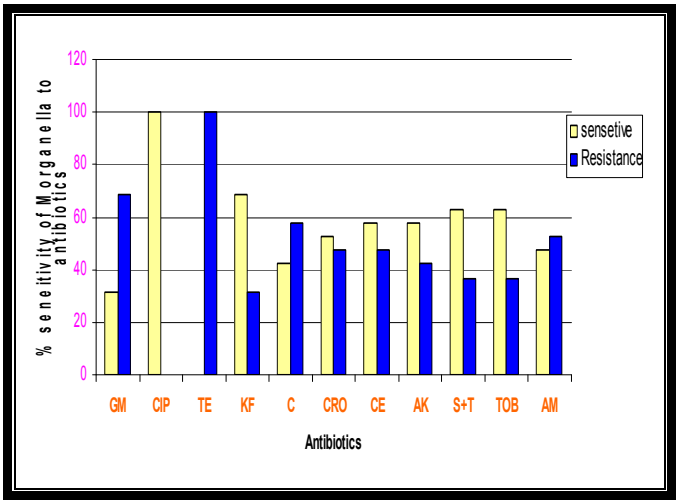

Figure (4) The sensitivity of *Morganella morganii* to antibiotics

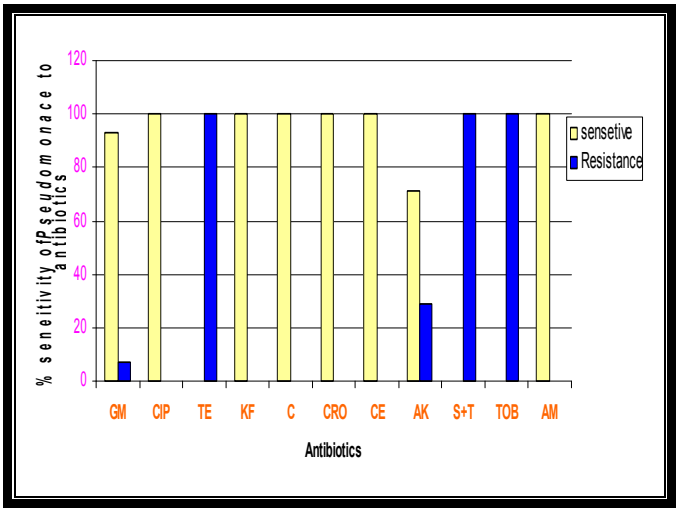

Figure (5) The sensitivity of *Pseudomonas aerogenosa* to antibiotics

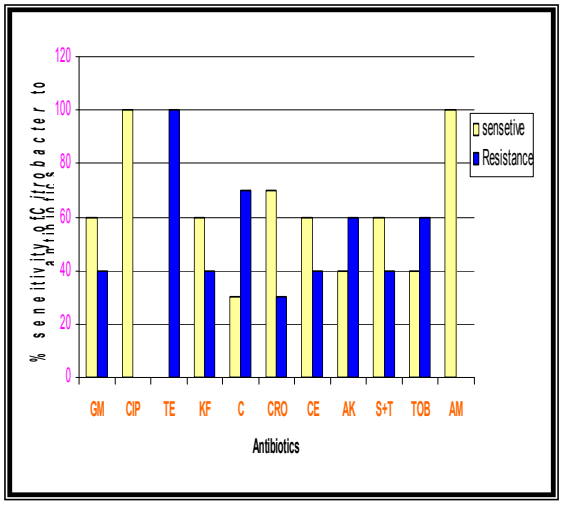

Figure (6) The sensitivity of *Citrobacter spp.* To antibiotic

Further studies employing maximum and minimum inhibitory concentration for each isolate against the above antibiotic discs will be needed to obtain more accurate results.

In previous studies nitrofurantoin was the most active agent (94% susceptible), followed by gentamicin and cefpodoxime. High rates of resistance to ampicillin (55%) and trimethoprim (40%), often in combination were observed in both sets of isolates. Although isolates exhibiting resistance to multiple drug classes were rare, resistance to cefpodoxime, indicative of extended spectrum β-lactamase production, was observed in 5.7% of community and 21.6% of nosocomial isolates(16). The Gram-positive organisms were very sensitive to Augmentin and the Fluoroquinolones. E. coli showed the highest sensitivity to Nitrofurantoin (76%) while it was also very susceptible to the Fluoroquinolones (74%).

The above study clearly shows that Nitrofurantoin is a very effective first line drug for UTIs(17).

Antibiotic Resistant to(Amoxicillin, Augmentin, Ciprofloxacin, Gentamicin, Nalidixic Acid, Nitrofurantoin, Cotrimoxazole, Tetracycline) were(65.7 , 42.1, 19.7, 47.5, 65.8, 24.8, 86.4, 76.7) respective , besides antimicrobial prophylaxis is not associated with decreased risk of recurrent UTI, but more likely associated with increased risk of resistant infections(18).

CONCLUSION

As a conclusion, resistance to agents commonly used as empirical oral treatments for UTI was extremely high. Levels of resistance to tetracycline and tobramycin render them unsuitable for empirical use. Continued surveillance and investigation of other oral agents for treatment of UTI in the community is required.

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Isolation and Characterization of Sera Adenosine Deaminase, AMP- Deaminase and 5´-Nucleotidase Forms from Uterine Tumor Patients

Hathama R. Hasan & Shatha A. Al- Shammari

Chemistry Dept. , College of Science, Baghdad University / Republic of Iraq

E-mail: shath_a@yahoo.com

ABSTRACT

Isolation and characterization of sera Adenosine deaminase (ADA), AMP- deaminase (AMPDA), and 5´-Nucleotidase (5´-NT) from control healthy women, and patients with benign and malignant uterine tumors were carried out using Con A- Sepharose (6B) affinity chromatography.

ADA and 5´-NT seem to be present in three forms (with molecular weights M.wt range (250-350 KD), and iso- electric point (pI) values of 5.9), and two forms(with M.wt 200 ,and 250 KD and pI values of 5.0, and 5.9 respectively in the sera of malignant uterine tumor patients, while two forms of ADA(with M.wt of 250, and 350 KD, and pI values of 5.1, and 5.6) and three forms of 5´-NT(with M.wt 160, 200 ,and 250 KD and pI values of 5.0, and 5.9) were found in sera of control and benign uterine tumor groups. As far as AMPDA is concerned, only one form was detected (with M.wt 60 KD and pI value of 5.4) in the sera of control women, benign and malignant uterine tumors patients, as judged by polyacrylamide gel electrophoresis and isoelectric focusing electrophoresis. Concentrated filtrate had the highest significant cytotoxic effect on growth of both tumor cell lines when compared with S-layer proteins. We concluded that S-layer proteins and concentrated filterate of *L.acidophilus* and *L.casei* had cytotoxic effect on growth of both tumor cell lines RD and L20B depending on concentrations. The concentrated filtrate gave highest cytotoxic effect than S-layer protein.

Abbreviations

ADA**:** Adenosine deaminase.
5´-NT**:** 5´-Nucleotidase,
AMPDA: AMP- deaminase,
pI: iso-electric point**,**
ADCP: adenosine deaminase complexing protein**,**
AMP: adenosine monophosphate,
IMP: inosine monophosphate,
PAGE: polyacrylamide gel electrophoresis**,**
Rm: relative mobility,
p.p: partially purified,
M.wt: molecular weight

الملخص باللغة العربية

تم فصل الأنزيمات 5′-NT,AMPDA, ADAمن مصول النساء الأصحاء ومصول المصابات بأورام الرحم الحميدة والخبيثة باستخدام تقنية كروماتوغرافيا الألفه (عمود سفاروز Con-6B).
تم تحليل الأنزيمات المنقاة جزئيا بواسطة عملية الرحلان الكهربائي على متعدد الأكريل أمايد وبالاعتماد على صبغة الفعالية الأنزيمية لكل أنزيم فقد ظهر شكلين لأنزيم 5′-NT وثلاث أشكال لأنزيم ADAفي مصول المصابات بسرطان الرحم بينما ظهرت ثلاث أشكال 5′-NTوشكلين لأنزيم ADAفي مصول النساء الأصحاء والمصابات بأورام الرحم الحميدة.
أما أنزيم AMPDAالمنقى جزئيا فقد ظهر بشكل واحد في جميع النماذج .تم دراسة بعض الصفات الفيزوكيميائيه لهذه الأنزيمات المنقاة جزئيا والتي تتضمن أوزانها الجزيئية ونقطة التعادل الكهربائي وثباتها خلال الخزن.

INTRODUCTION

It was suggested that changes in protein properties or in their rate of biosynthesis might be a fundamental property of the malignant cell. Moreover changes in the physicochemical (size and charge), characteristics of some enzymes associated with tumor development were reported [1]. The presence of different enzyme forms in patients may be of usefulness in clinical evaluation of the course of the disease, as well as in the development of an effective chemotherapeutic regimen [2].

ADA is widely distributed in mammalian tissues and is known to occur in several isoenzymes and molecular forms [3,4]. These forms are ADA-L and ADA-S, the last form is non- covalently bound to a complexing protein ADCP (adenosine deaminase complexing protein) to form ADA-L. ADCP; an essential factor: firstly for the association of ADA to plasma membrane of mammalian cells to produce an efficient protection against the toxic effect of different nucleotides that could enter from extracellular space, and secondly for participation of the ecto-enzyme complex in the cell-cell adhesion to modulate an efficient immune competent system [5]. These different enzyme forms can be resolved on the basis of their electrophoretic migration or their molecular weights [2].

Specific changes in ADA are associated with tumorgenesis, and a new form of tumor ADA was reported. Changes observed in the tumor ADA was suggested to be due to alteration, or absence of the conversion factor, or changes in the regulation of enzyme biosynthesis [2].

5´-NT is a very well known enzyme, which catalyzes the dephosphorylation of purine and pyrimidine nucleoside monophosphates to their corresponding nucleosides. Two forms of 5´-NT are present in human lymphocytes: The first form: ecto5´-NT, which is a dimer of two identical 70 KD subunits bound by a glycosyl phosphotidylinositol linkage to the external face of the plasma membrane of various cells, with the preferred substrate being AMP. The second form which called C-N-II, prefers IMP, as substrate, and controls the intracellular levels of nucleoside 5´-monophosphates [6].

Concerning some purine metabolizing enzyme, the activity of 5´-NT is always regarded to be linked to cell maturation and differentiation. In addition to that,

also it has an imbalance link to transformation and / or progression of cancer cells [7], as was reported. 5´-NT from human lymphocytes was reported as a dimer of two identical 70 KD subunits and three isoenzymes of 5´-NT were observed in lymphocytes of normal and patients with B-cell chronic lymocytic leukemia but with less activity from that of normal group [6].On the other hand, multiple isoenzymes of AMPDA were reported to present in mammalian tissues and that they could be distinguished from each other on the basis of kinetic, physical, and immunological properties [8].

The differences in the activities of sera ADA, AMPDA, and 5´-NT of uterus cancers patients, which was reported in our previous work [9], lead us to carry out more investigation on these enzymes, in order to follow up the changes in these forms, and to have a more detailed look at these forms and their physicochemical properties which are result by cell transformation. Such study enables us to determine the possibility of using the most remarkable of the present study in clinical evaluation of the course of the disease and, may be in the development of an effective chemotherapy

MATERIALS AND METHODS

Chemicals:

All chemicals and reagents used throughout this work were of Analar grade.

Samples:

Sera of control subjects, benign and malignant uterine tumors patients were collected, as mentioned in our previous work [9], and used separately for the purification and characterization of ADA, AMPDA, and 5´-NT enzymes. The sera were diluted with washing buffer {acetate buffer (0.1 M; pH 6.0) containing 0.1% Triton x-100, NaCl (0.1 M), $CaCl_2$ (0.001 M), $MgCl_2$ (0.001 M), $MnCl_2$ (0.001 M), and NaN_3 (0.0005 M)} to give a final protein concentration (20 mg/ml).

Purification of sera ADA, AMPDA, and 5´-NT by affinity chromatography:

Affinity chromatography on Con A-Sepharose in a column of (1x5 ml) in dimension with bed volume of (1ml), was used to separate and partially purifying ADA, AMPDA, and 5´-NT enzymes from sera of the mentioned samples.

The elution buffer:

α-Methyl mannoside (0.2 M) was prepared by dissolving (0.9707 g) in (10) ml of the washing buffer, and then the solution was made up to (25) ml with the same buffer.

Procedure:

Appropriate amount of Con A-Sepharose was placed in a (5) ml column of (1x5 ml syringe) to get a (1) ml bed volume and washed with the washing buffer at flow rate of (4) ml/h. 1 ml of (20) mg protein was applied on the column and allowed to penetrate through the gel, then left for (30) min at (4) ºC to allow binding to the lectin.

Elution of the enzymes from the column was carried out using (1) ml portions of the elution buffer at the same flow rate (4) ml/h and fractions of (1) ml were collected until no protein found in the last fraction by measuring the

absorbance at (280) nm. The fractions were kept at (-20) ºC until being used.

Protein concentrations [10] and activities of ADA [11], AMPDA [11], and 5´-NT [12] enzymes were measured in all collected fractions from (washing and elution steps). Fractions with the highest enzymatic activities were collected and frozen at (-20) Cº for further analysis .The fractions, which have the highest enzyme activity, were concentrated using Sephadex G-25 method [13].

Characterization of crude and partially purified enzymes:

1-Analysis by conventional polyacrylamide gel electrophoresis (PAGE):

Depending on enzymes activities stains, PAGE technique was used to detect the different forms of each enzyme, and the extent of its purity. The samples used were the fractions with the highest specific activities, which were concentrated using Sephadex G-25 [13] method. Polyacrylamide gel (7.5 %); was prepared and applied on the LKB 2117 multiphor electrophoresis system. The optimum conditions used were according to the procedure in the application note 306 of LKB Company (current 40mA for three hours). After the bromophenol blue reached near the gel margin, the electrophoresis was stopped and the gel was divided into four parts where: Part one was used for proteins staining (Coomassie brilliant blue stain CBB R-250) [14].

Part two was used for 5´-NT activity staining. Part three was used for ADA activity staining.

Part four was used for AMPDA activity staining, and as described below.

Gel staining:

I- For protein:

Coomassie brilliant blue (CBB R-250) stain [14] was used to stain proteins on the gel according to the application note 306 of LKB Company.

II- For ADA and AMPDA activities:

Staining of the gel for ADA and AMPDA enzyme activities was carried out using Brady and O'Connell method [15] with some modifications as described in the discussion section.

III- For 5´-NT activity:

The 5´-NT enzyme activity staining was performed following Dvorak and Heppel method [16] with some modifications, as described in the discussion.

IV- For glycoprotein:

The gel was stained for carbohydrates according to Leach *et.al* [17] method.

2. Molecular weight determination:

The approximate M.wt of the partially purified (p.p.) enzymes were estimated by conventional PAGE where activity staining for each p.p. enzyme was used to reveal its band position on the gel, and the approximate M.wt was determined using the M.wt calibration curve. The calibration curve was constructed by plotting the Log of M.wt of each of the standard proteins: Ferritine (440KD), Catalase (232 KD), Aldolase (158 KD), Albumin (67 KD), Ova albumin (43 KD) against its relative mobility (Rm) on the gel Fig (1).

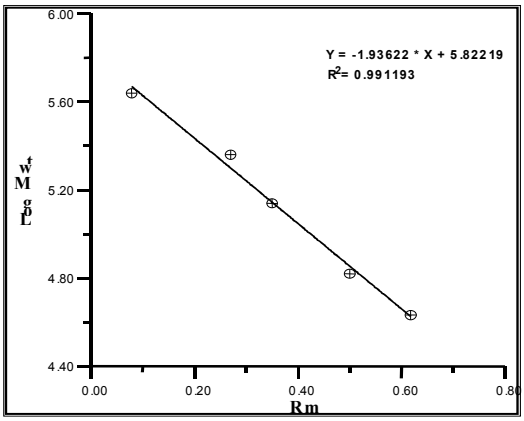

Figure (1) Standard curve for M.wt. determination

Calculations:
The relative mobility (Rm.) of each standard protein and sample was measured as follows:

$$Rm = \frac{\text{The distance of protein migration}}{\text{The distance of bromophenol blue migration}}$$

3. Determination of the isoelectric point (pI):
The pI of each enzyme form was determined by using (5%) polyacrylamide gel containing (40%) Ampholine with pH range (3.5 –10.5) [14]. The electrophoresis was carried out according to LKB instructions by applying (1500) volts and a current of (50) mA for (2) hour. After that the gel was divided into five parts leaving one track for pH measurement, which was sliced into (0.5) cm segments and each slice was put in a tube containing (2) ml of boiled cooled de-ionized water and left overnight at (4) °C. The pH was measured for each tube and a calibration curve was plotted between the recorded pH and the number of the gels segment. The other four parts were stained for protein and enzyme activity, as mentioned above. The pI of each band can be determined from the calibration curve Fig. (2) from measuring the distance of each band from the cathode in centimeters.

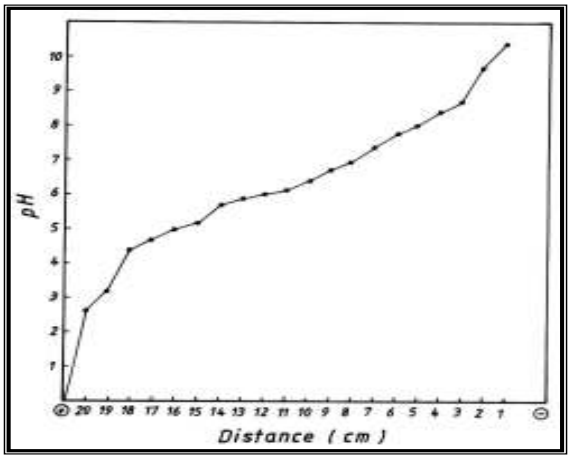

Figure (2) Iso- electric focusing profile of p.p. enzymes on Ampholine PAG with pH gradient (3.5-10.5).

RESULTS

1- Purification of ADA, AMPDA, and 5´-NT enzymes:
Different methods were used for purification of ADA, AMPDA, and 5´-NT enzymes. Most of them depend on there separation according to either enzyme affinity for its substrate or the affinity of their glycan moiety to bind to different lectin. [18,19,20]. Throughout this study, affinity chromatography on Con A-Sepharose column was used for this purpose.

5´-NT and the multiple forms of ADA are glycoproteins [21,22] as confirmed from the effect of different lectins on their activities [19,20]. Figs. (3), (4), and (5) show the elution profile of each enzyme isolated from the sera of the three sample groups. It was clear that AMPD A was eluted in the washing step, while ADA and 5´-NT were eluted upon using (0.2) M of α -methyl mannoside in the acetate buffer.

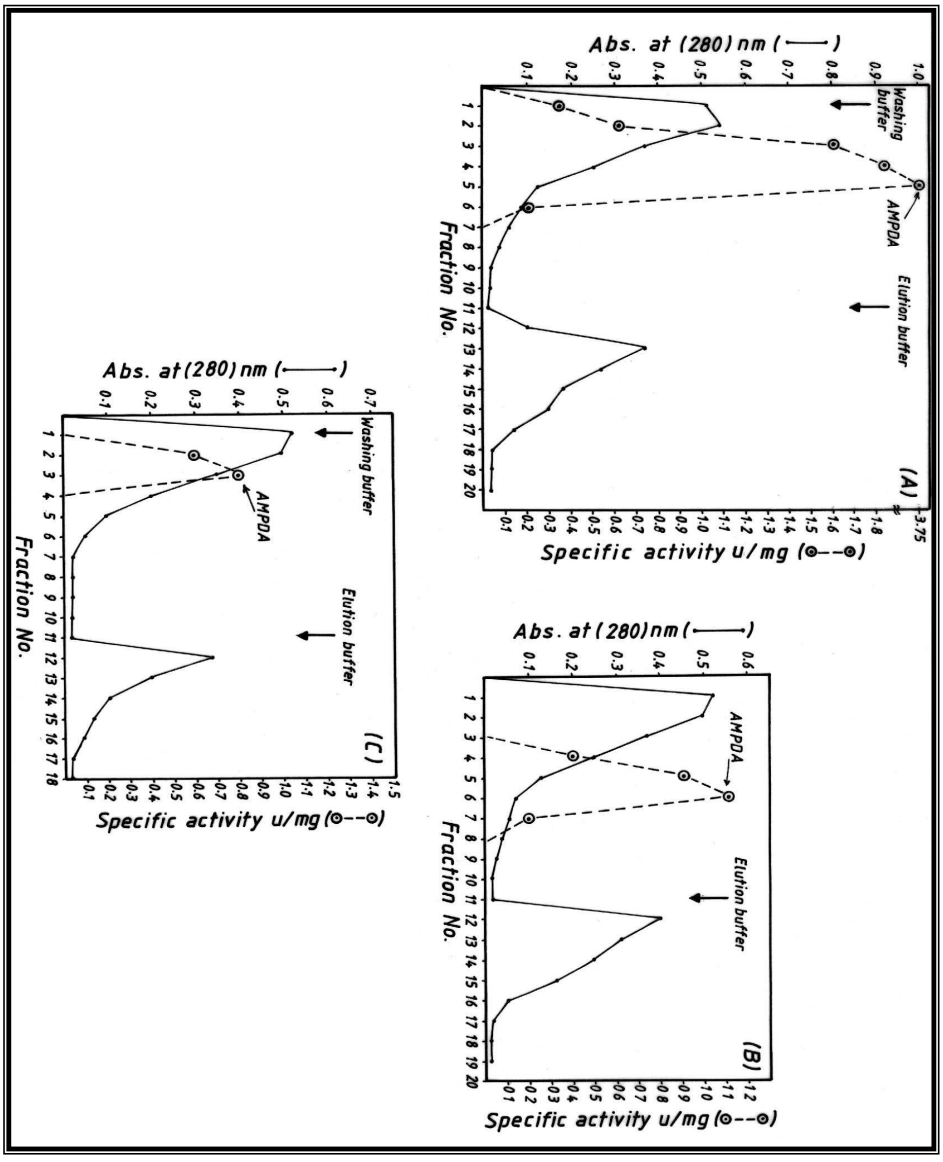

Figure (3) Affinity chromatography of sera AMPDA on Con A-Sepharose 6B (1x5 ml) column with flow rate of (4) ml/h, bed volume (1ml), and fraction vol. (1ml)
(A-control, B- Benign , C- Uterus cancer).

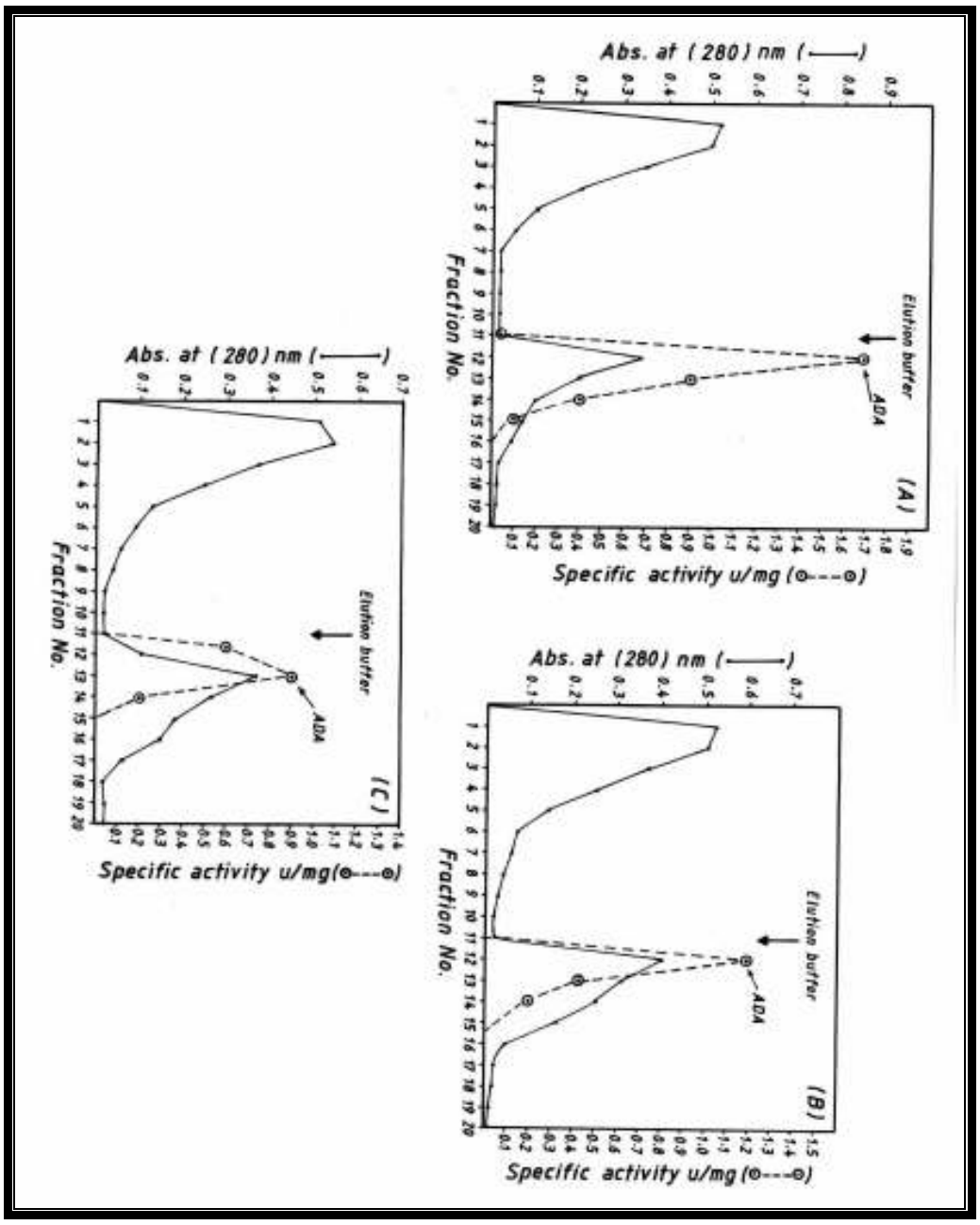

Figure (4) Affinity chromatography of sera ADA on Con A-Sepharose 6B column (1x5 ml) with flow rate of (4) ml/h, bed volume (1ml), and fraction vol. (1ml)
(A- Control, B- Benign, C- Uterus cancer).

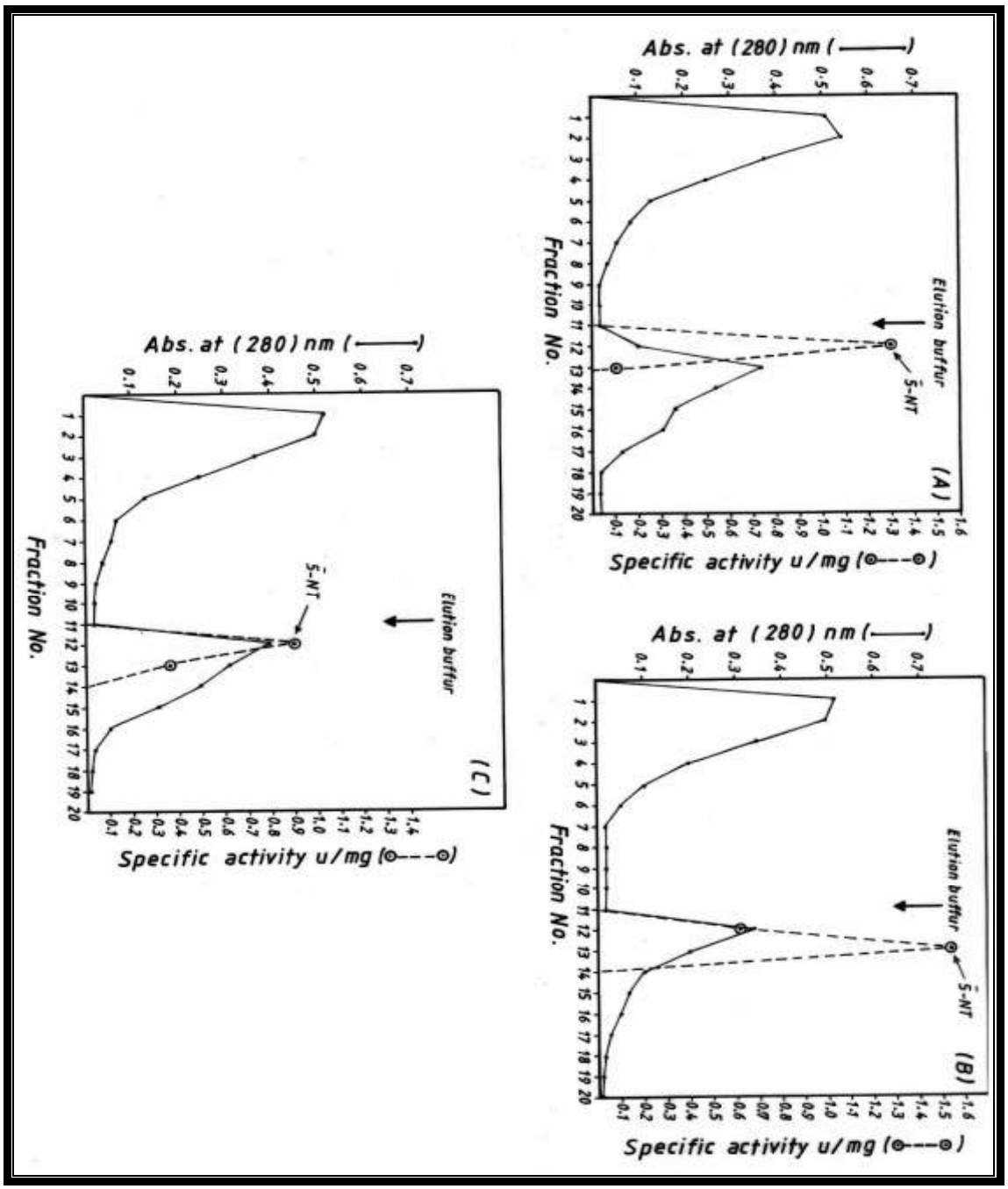

Figure (5) Affinity chromatography of sera 5′-NT on Con A-Sepharose 6B column(1x5 ml) with flow rate of (4) ml/h, bed volume (1ml), and fraction vol. (1ml)
(A- control, B- Benign , C- Uterus cancer)

The partially purified AMPDA from sera of control, benign and malignant uterine tumors patients has a purification fold and yield value of (32.32), (93.64%), (12.79) (86.84%), and (17.29), (82.03%) respectively as illustrated in Table (1).

Table (1): Purification of sera AMPDA from control and patients with benign and malignant uterine tumors.

AMPDA	Protein mg	Total activity U/L	Specific activity U/mg	Yield %	Fold of purification
Control					
Crude	39.05	4.56	0.116	100	1
P. purified	1.138	4.27	3.75	93.64	32.32
Benign uterine tumors					
Crude	55.23	4.75	0.086	100	1
P. purified	3.75	4.125	1.1	86.84	12.79
Uterus cancer					
Crude	55.47	2.56	0.046	100	1
P. purified	2.625	2.10	0.8	82.03	17.29

ADA enzyme of sera of the three samples group, showed (18.41), (13.79), and (16.36) folds of purification with a yield value of (81.81%), (81.85%), and (88.94%) respectively, as shown in Table (2).

Table (2): Purification of Sera ADA from control, and patients with benign and malignant uterine tumors

ADA	Protein mg	Total activity U/L	Specific Activity U/mg	Yield %	Fold of Purification
Control					
Crude	40.50	3.74	0.092	100	1
P. purified	1.80	3.06	1.7	81.81	18.41
Benign uterine tumors					
Crude	40.92	3.56	0.087	100	1
P. purified	2.4	2.89	1.2	81.18	13.79
Uterus cancer					
Crude	39.56	2.17	0.055	100	1
P. purified	2.14	1.93	0.9	88.94	16.36

While the obtained purification fold of 5´-NT enzymes was (16.88), (28.19), and (14.59) folds of purification with a yield value of (96.41%), (85.85%), and (45.94%) respectively as shown in Table (3)

Table (3): Purification of sera 5′ -NT from control, benign and malignant uterine tumors patients

5′-NT	Protein Mg	Total activity U/L	Specific activity U/mg	Yield %	Fold of purification
Control					
Crude	39.51	3.07	0.077	100	1
P. purified	2.27	2.96	1.3	96.41	16.88
Benign uterine tumors					
Crude	40.38	2.22	0.055	100	1
P. purified	1.23	1.906	1.55	85.85	28.19
Uterus cancer					
Crude	42.25	5.5	0.130	100	1
P. purified	1.33	2.52	1.9	45.94	14.59

2- Analysis of the p.p. enzymes By PAGE:

The purity of ADA, AMPDA, and 5´-NT were analyzed and confirmed by PAGE using CBB-R 250 stain, glycoprotein stain, while enzymes activity stains was used for enzymes detection Fig (6). The approximate M.wt of the enzymes were estimated using PAGE, depending the specific enzyme activity staining, and by comparison with a mixture of standard polypeptides of known M.wt.. Isoelectric points were determined

using PAG-Ampholine with a pH gradient from (3.5 to 10.5).

Phenol violet stain was used to detect ADA and AMPDA activities on PAGE based on Brady and O'Connell method [15]. This method was used previously to detect ADA activity after starch gel electrophoresis. The detection depends on color changes of the phenol violet stain at the site of the enzymes activity, which is pH dependant, where a dark brown band of ADA activity appears.

Throughout this work, the same stain was used with the following modifications: The stain was tried with PAG instead of starch gel used in the original method. Also in the original method, the staining solution contained agar, while here the staining carried out in the absence of the agar. The other modification was that here the incubation of the gel with the substrate was for (24) hour, where a bright orange-colored bands appeared, that were more detectable than those obtained without an incubation step as reported in the original method.

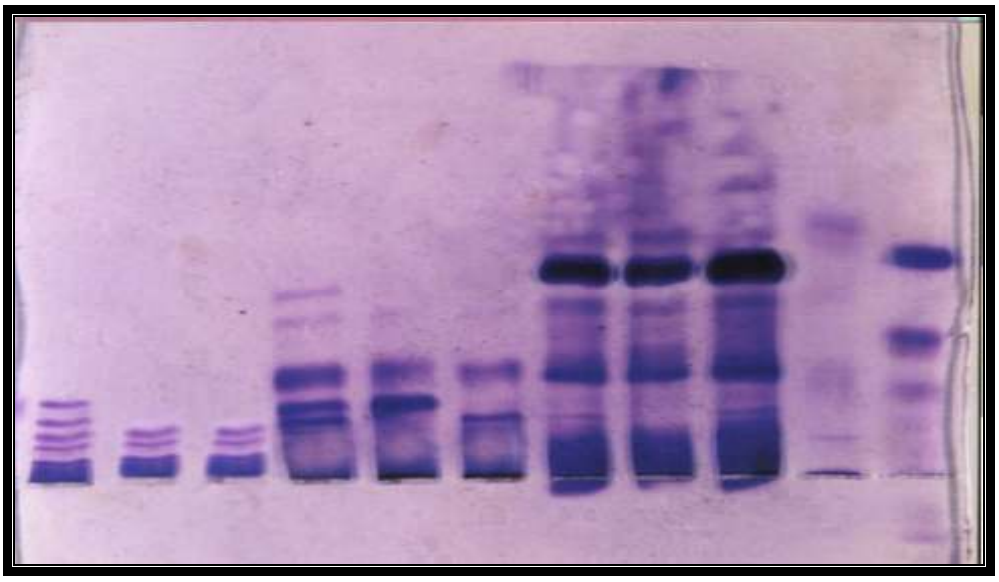

Figure (6): Conventional-PAGE 7.5% profile of crude serum and P.P. enzymes. The gel was stained for proteins with CBB R-250 (from right to left):

1, 2- Standard M.wt proteins **3- Crude serum (control)**

4- Crude serum (benign) **5- Crude serum (malignant)**

6- P.P. AMPDA (control) **7- P.P. AMPDA (benign)**

8- P.P. AMPDA (malignant) **9-P.P.ADA and 5′-NT (control)**

10- P.P.ADA and 5′-NT (benign) **11-P.P.ADA and 5′-NT (malignant)**

Fig.(7) shows that two bands with ADA activity were present in sera of control and of benign groups with approximate M.wt of (250) and (300) KD. These two forms (isoenzymes) have pI values of (5.1) and (5.6) as calculated from the isoelectric focusing profile Fig. (8).

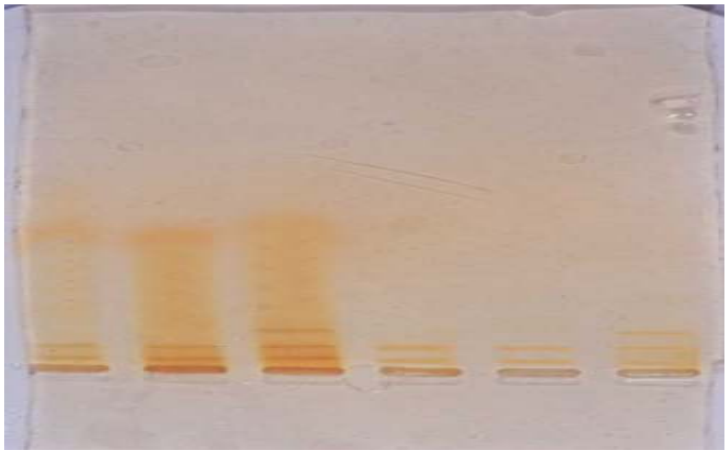

Figure (7): Conventional-PAGE 7.5% profile of sera ADA. The gel was stained for ADA activity (from left to right):

1- Crude serum (control) 2- Crude serum (benign)

3- Crude serum (malignant) 4- P.P. ADA (control)

5- P.P. ADA (benign) 6- P.P. ADA (malignant)

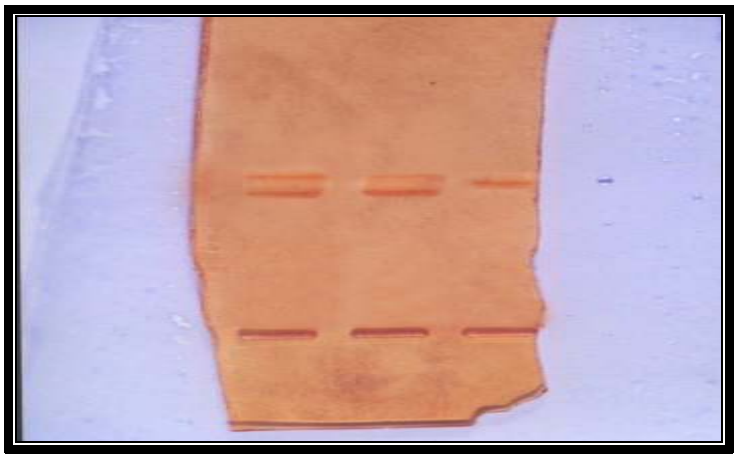

Fig. (8): Isoelectricfocusing gel electrophoresis 5% profile. The gel was stained for ADA activity (from left to right):

2- P.P. ADA (benign) 1- P.P. ADA (control)

3- P.P. ADA (malignant)

While three bands of ADA activity were detected in sera samples of malignant uterine tumors group with M.wt range between 250-350 KD.

When the same sera sample was separated on isoelectrofocusing gel, only one band appeared to stain for ADA activity, which separated in a region, correspond to a pI of (5.9). AMPDA from sera of the above three groups were detected on poly acryl amide gel using the same phenol violet staining procedure mentioned above. Fig. (9) and Fig. (10) show that one band of AMPDA was detected in sera of the three samples groups with approximate M.wt of (60) KD and pI value of (5.4).

In order to detect the presence of 5´-NT activity in the sera of the different groups used throughout this study, electrophoresis was carried out using polyacrylamide gel as a medium for the enzyme activity staining procedure which was modified as follows:

The original method [16] for the staining step based on 5´-NT action on AMP, which liberate phosphate (Pi) according to the following equation:

$$\text{5′-AMP} \xrightarrow{5'-\text{NT}} \text{Adenosine + Pi}$$

The produced phosphate was precipitated as white lead phosphate bands upon the addition of lead nitrate; this white band is converted to a dark color upon the addition of sodium sulfite solution. Our modification of this method involved the replacement of the sodium sulfite solution by solutions that are usually used in the test tube assay for measurement of this enzyme activity {namely: stannous chloride (0.008M), and ammonium molybdate solutions (0.008M)}.By such modification a faint blue background of the gel with a dark blue bands was obtained, instead of getting a gel with a dark background, which make the detection of the rapidly developed bands very difficult to be detected.

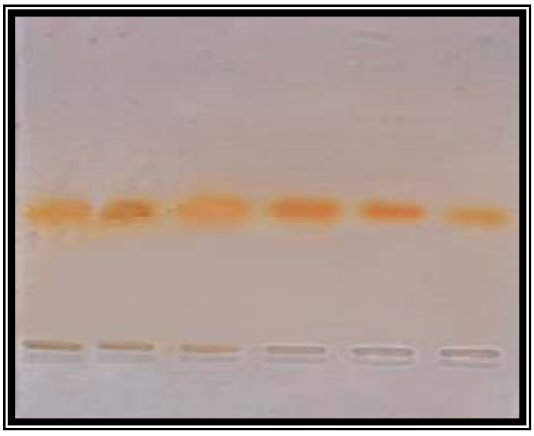

Figure (9) conventional- PAGE 7.5% of profile AMPDA activity

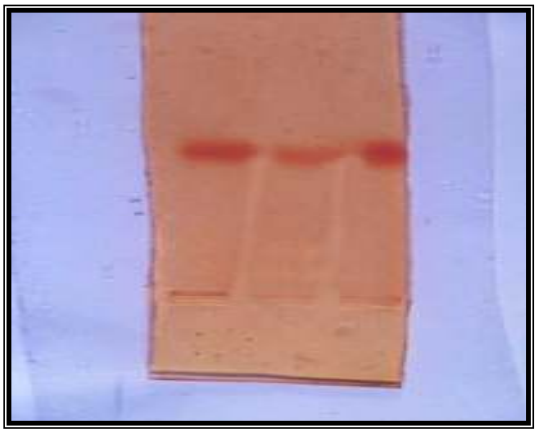

Figure (10) Isoelectricfocusing gel electrophoresis 5% profile.

The result in Fig (11) shows the presence of three bands of 5´-NT activity with approximate M.wt of (160), (200), and (250) KD in the control and benign uterine tumors groups. While only two bands were detected in these groups, when isoelectrofocusing of the samples were carried out with pI values of (5.0) and (5.9), as shown in Fig (12).

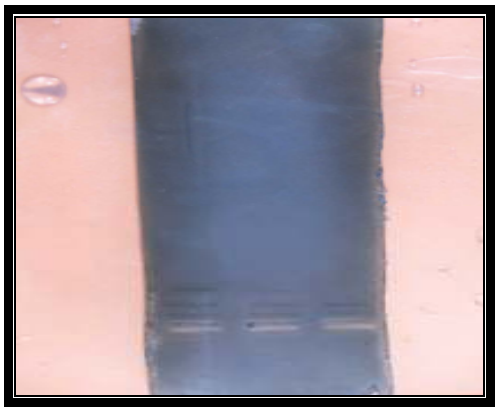

Figure (11): Conventional-PAGE 7.5% profile of sera P.P. 5′-NT (from left to right):

1- P.P. 5′-NT (control)

2- P.P. 5′-NT (benign)

3- P.P. 5′-NT (malignant)

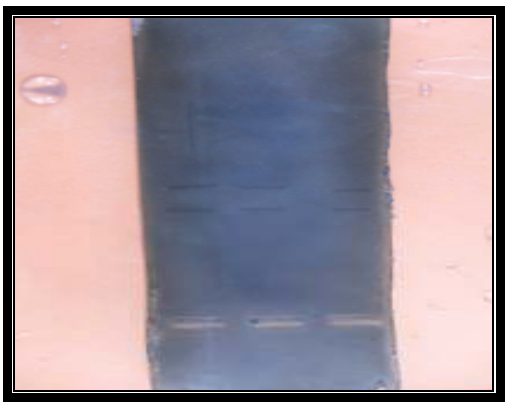

Figure (12): Isoelectricfocusing gel electrophoresis 5% profile. The gel was stained for 5′-NT activity (from left to right):

1- P.P. 5′-NT (control)

2- P.P. 5′-NT (benign)

3- P.P. 5′-NT (malignant)

For sera of malignant uterine tumor group ,when samples of isolated 5´-NT forms electophorized on polyacrylamide gel ,two bands were stained for the activity with M.wt of (200) and (250) KD and with pI values of (5) and (5.9).

Glycoproteins staining Fig. (13) of the ADA and 5´-N confirmed that they are glycoproteins, as revealed by their binding to Con A-Sepharose column. Also, the same stain revealed that even though AMPDA did not bind to Con A-Sepharose column, it is a glycoprotein since it gives a positive pink color bands with glycoprotein staining.

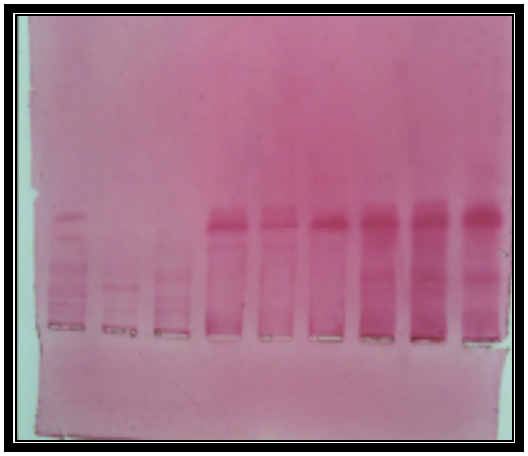

Figure (13): Conventional-PAGE 7.5% profile of crude serum and P.P. enzymes. The gel was stained for glycoproteins (from right to left):

1- Crude serum (control)
2- Crude serum (benign)
3- Crude serum (malignant)
4- P.P. AMPDA (control)
5- P.P. AMPDA (benign)
6- P.P. AMPDA (malignant)
7-P.P.ADA and 5′-NT (control)
8- P.P.ADA and 5′-NT (benign)
9-P.P.ADA and 5′-NT (malignant)

DISCUSSION

Con A-Sepharose chromatography was used for the purification of 5´-NT and ADA. This based on the fact that this lectin was reported to be an inhibitor to these enzymes activities, which indicate that their glyco moiety are of a high mannose type and this inhibition may be interpreted in terms of the specific binding of lectins to the carbohydrate residues near their active sites [18,20].

The results of PAGE of isolated different enzyme's forms of ADA, AMPDA, and 5´-NT in control, benign and malignant uterine tumors groups revealed the appearance of a new ADA form, and disappearance of one of 5´-NT forms from the sera of uterus cancer's group, while only one form of AMPDA was detected in the three sera samples groups. These results were in agreement with the fact that the enzyme complement of a tumor cell differ in many ways from that of its normal counterpart reflected its altered metabolism [23].

ADA exhibits substantial electrophoretic heterogeneity. Several forms of ADA have been distinguished previously in erythrocytes by their electrophoretic mobility on starch gel electrophoresis [1,5].

Tissues other than erythrocytes have been reported to exhibit additional forms termed “tissue-specific” isoenzymes, which vary in their electrophoretic mobility in a manner specific for that particular tissue [20,21].

The functional role of the highest M.wt ADA in living organism has been the subject of several studies that associated its variation with tumoral transformation of the cell in the tissue [1], also it was reported that the absence or modification of conversion factor may be a property of the malignant cell [2,5].

On the other hand, it was reported using preparative isoelectricfocusing in a study used chemically induced rat colon tumor, demonstrated ADA was to be present in two electrophoretic variants, These variants which were undetectable in normal rat colon, were called tumor ADA (I) and (II) exhibited close pI values of approximately (4.85) and (4.74)

respectively [2].

Many possible mechanisms may explain the appearance of the new form of tumor ADA that was detected in sera of uterus cancer patient throughout the present study with M.wt and pI value differ from that of other groups. These mechanisms may include either of the following suggestion: de-repression or activation of a distinct gene, modification of a pre-existing low M.wt. protein, (which promotes dimerization), or interaction of the low M.wt. enzyme with a modified form of conversion factor [2].ADA activity was found to be modulated by the membrane bound adenosine deaminase complexing protein[23]. It has been suggested that a change in the thickness of bilayer below and above the main phase transition may modify the orientation of CP in the membrane, thereby affecting substrate accessibility of ADA[24]. Alterations in enzyme levels in cancer are well known and ADA, an enzyme of purine salvage pathway, has been found to be altered in various malignancies [9,25,26,27].As it is known that the changes that occurs in cell membrane upon transformation of the cell into a cancer one may explain such alterations[28].

The disappearance of one of 5´-NT forms, with a M.wt. of (160KD), that was observed in sera of uterus cancer patients may be due, at gnomic basis, to difference in expression and co-operation between the genes, or may be due to an alteration of the 5´-NT gene [6], or to the presence of a serine protease, that was observed to be highly active in cancer cells [29,30].

AMPDA, the functional unit in the purine “nucleotide cycle”, is important for the control of cell levels of AMP and thus monitoring the energy state of the cell [31]. AMPDA was found as one form in sera of the three groups throughout this work, although it was reported that multiple forms were found in different human tissue, yet the source of serum AMPDA is still unknown [32]. There is no published data about the nature of AMPDA, whether it is a glycoprotein or simple protein. The results of the analysis of the enzyme from the three sample groups under study, showed that AMPDA is a glycoprotein, but its glyco moiety is not of the mannose type, so it did not bind to Con –A, further investigations are required to detect the type of the glycan moiety that present in AMPDA.

Stability of crude and purified ADA, AMPDA and 5´-NT enzymes was carried out on sera of control, benign, malignant uterine tumors. The enzymes stability were checked during storage at (20) Cº. These enzymes were found stable for more than half a year. These results were in agreement with other studies carried on these enzymes stability where the purified human erythrocyte ADA retained its activity for up to 3 weeks at (4) Cº [33].ADA isoenzymes activity in human colorectal adenocarcinomas were found unaltered during the storage for more than (7) months [34], and the purified 5´-NT, which isolated from a rat glioblastoma cell line, found to be stable for at least 2 weeks at (4) Cº and for months at ((20) Cº [18]. Purified 5´-NT from

human liver showed no loss of activity during (3) months [35], and it was reported that human erythrocyte AMPDA retained its full activity after two weeks of storage at (4) Cº [36].

CONCLUSION

As a conclusion based on the results of this study: An appearance of a new ADA form (with pI of 5.9) in the patients with malignant tumors while one of the 5´-NT enzyme's forms (with M.wt. of 160 KD) was disappeared. These findings may be used as an adjuvant tool to diagnose the presence of uterus cancer.

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Removal of Chromium and Cobalt Ions by *Anabaena Sp. Alga* from Aqueous Solutions

Ithar K. Al- Mayali (1) Altaf .A.Issa & Abbas .M.Ismail (2)

College of sciences / Baghdad University - Republic of Iraq (1) College of science for women/ Baghdad University- Republic of Iraq (2)

E-mail: ithar_abbas@yahoo.com

ABSTRACT

Anabaena Sp. was exposed for different concentrations 0.5 , 1 , 2 , 3 and 4 ppm for both chromium and cobalt ions.

The study explained the high ability removing both elements with high percentages which reached to 99.9 , 99.9 , 99.7 , 99.75 and 99.75 % for concentrations 0.5 , 1, 2, 3 and 4 ppm of chromium in last day of the experiment . Also the percentages reached to 97.20, 97.20, 99.20, 98.50 and 98.75% for the concentrations 0.5, 1, 2, 3 and 4 ppm of cobalt in the last day of the experiment.

The study concluded that this alga could highly tolerate for both chromium and cobalt because of highly removal percentages of these heavy metals until the last days of the experiment.

According to the concluded results, they explain that this alga can be used to remove chromium and cobalt ions from polluted water with high efficiency.

الملخص باللغة العربية:

تم تعريض طحلب *Anabaena Sp.* لتراكيز مختلفة 0.5 و 1 و 2 و3 و4 جزء بالمليون من عنصري الكروم و الكوبلت ، وأوضحت الدراسة إن لهذا الطحلب القابلية العالية على إزالة العنصرين بنسبة عالية جدا وصلت إلى 99.9 و 99.9 و 99.7 و 99.75 و 99.75 % للتراكيز 0.5 و 1و 2و 3و4 جزء بالمليون في اليوم الأخير من التجربة. وكذلك وصلت إلى 97.20 و 97.20 و 99.20 و 98.50 و 98. 75 % للتراكيز 0.5 و 1 و 2و 3 و4 جزء بالمليون من عنصر الكوبلت في اليوم الأخير من التجربة.

وعليه يمكن الاستنتاج إن هذا الطحلب له القابلية العالية على تحمل عنصري الكروم و الكوبلت وذلك لقابليته على الإزالة العالية و التي تكاد تكون تامة للعنصرين حتى الأيام الأخيرة من التجربة . وتبعا" للنتائج التي تم الحصول عليها يتضح بأنه يمكن استخدام الطحلب في إزالة ايونات عنصري الكروم و الكوبلت من المياه الملوثة بها و بكفاءة عالية .

INTRODUCTION

Heavy metals are considered of the most important water pollutants. In recent years, many low cost sorbents such as algae, fungi, bacteria and plants have been investigated for their biosorption capacity toward heavy metals (1).
Heavy metals make a significant contribution to environment because of human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, intensive agriculture, sludge dumpling and melting operation. Some heavy metals such as Mn ,Fe, Cu, Zn, Mo, and Ni are essential as micronutrients for microorganisms, plants and animals (1) while others have no known biological functions. All heavy metals at high concentrations have strong toxic effects and regarded as environmental pollutants (2,3).
Aquatic plants and \ or algae are known to accumulate metals and other toxic elements from contaminated water (4,5) .The bio removal process using aquatic plants often exhibit a two-stage uptake process: an initial fast , reversible , metal –binding process (biosorption),followed by slow, irreversible ,ion -sequestration step (bioaccumulation) .
The initial metal biosorption by different parts of cells can occur via complexation, coordination, chelating of metals, ion exchange, adsorption and micro precipitation. The bioaccumulation process is an active mode of metal accumulation by living cells.
This process depends on the metabolic activities of the cell, which in turn can be affected by the presence of metallic ions (6).
The bio removal application merits consideration when comparing with other methods (7).
Anabaena Sp., one of blue green algae, usually found in water as solitary or in free cluster. Its filamentous contain vegetative cells with cylindrical or barrel –shaped. There are heterocyst and aconite between vegetative cells. The heterocyst may be terminal or intercalary heterocyst (7).

Classification of *Anabaena Sp.:*

Kingdom : Bacteria
Division : Cyanophyta
Class: Cyanophyceae
Order: Nostocales
Family : Nostocaceae
Genus : *Anabaena sp.*

Scope of study

this study examines the removal of chromium and cobalt ions from aqueous solutions by *Anabaena Sp.* in order to use in bioremediation programs of water pollution treatment.

MATERIALS AND METHODS

Chu-No-10 medium which described by Chu, 1942 (8) and modified by Kassim, 1998 (9) was used to cultivate the alga Anabaena *sp.* The medium prepared as stock solutions and kept stored in 4° C.
2.5 ml had been taken from each stock solution and added to one liter of deionizer water, to prepare the medium.
PH was about 6.8-7 .sterilized by autoclave with 121°C and pressure

1.5 for 15 minutes (8). Kept in 4 C° until use.

In order to get axenic culture, study followed Paterson method (10), which is represented by: the algal culture will be kept in the dark for 24 hours, then take about 10 ml of this culture and transfer into sterilized and new medium and kept it in the dark again for 3 hours, then do sedimentation for the algal cells by centrifugation at 3000 cycle\ min .for 5 minutes (for 15 times) and wash the algal sediment with distilled water and later cultivate the algal strain in a new medium in order to activate the isolated algal species

To ensure there is no microbial contamination, streaking a swap of the culture on the nutrient agar medium and incubated in 37 C° for 48-72 hours (10).

The stock solutions of chromium and cobalt concentrations were prepared with initial concentration of 1000 ppm by dissolved 0.0496 mg\l aqueous dichromate potassium K_2CrO_4 and 0.0476 gm\l aqueous cobalt sulfate $COSO_4.7H_2O$ solution in deionizer water and then the following concentrations were prepared: 0.5, 1, 2,3 and 4 ppm according to the following equation:

$$C_1V_1 = C_2 V_2$$

Where:

$C_{1=}$ first concentration,

V_1= first volume (of standard solution),

C_2= second concentration (wanted to get it),

$V_{2=}$ second volume.

It was add about 50 ml of axenic culture to 1000 ml of the medium containing chromium ions , also another 50 ml of axenic culture to 1000 ml of medium containing cobalt ions.

Three replicates were used for each concentration, and then kept in 25 C° and light intensity 380 micro anchtain $.m^{-2}.sec^{-1}$ (with light period about 16 hours and 8 hours of dark) .

The alga exposed to these concentrations to point out the ability of alga to remove and tolerate chromium and cobalt ions by comparing the initial concentration of these two elements which exposed to alga and the concentrations of it in filtrate (usually, the alga separate from the solution of culture and chromium or cobalt by filtration) and then measure the concentration of chromium and cobalt in filtrate by using atomic absorption device in central laboratory of college of Science\ Baghdad university.

Statistical Analysis

SPSS Program (Version 7.0) had been used to analysis the data.

RESULTS AND DISCUSSION

Biosorption is a process owned some unique characteristics. It can effectively sequester dissolve metals from very dilute complex solutions with high efficiency. This makes biosorption an ideal method for the treatment of high volume low concentration complex wastewaters.

The results reveled that the studied alga has high affinity to remove chromium (Table 1).
The statistical analysis LSD indicated that there were significant differences among the removal percentages with each other in first, second and third days but not in the sixth , eighth and tenth days.

In addition, these analytical results indicated there were significant differences among the days of experiment for each concentration in probable P>0.05.
There is more than one type of functional group contributory to biosorption process, each of which has a different affinity for sorbing heavy metals (11& 12).

Table 1: Removal percentage of chromium by Anabaena Sp. and The Statically Analysis (LSD).

Days	Concentrations (ppm)					LSD
	0.5	1	2	3	4	
1	Nil	Nil	Nil	Nil	Nil	9.35*
2	Nil	Nil	Nil	90.9	90.9	12.58*
4	90.9	99.0	99.0	99.7	99.7	6.14 *
6	99.4	99.6	99.6	99.6	99.6	4.75 ns
8	99.5	99.6	99.6	99.75	99.75	4.79 ns
10	99.9	99.9	99.9	99.75	99.75	3.97 ns
LSD	12.67*	12.63*	12.72*	12.65*	5.37 *	

Differences = significant differences, ns= non-significant *(P<0.05).

Table (2) explains the highly efficiency of *Anabaena Sp.* to remove cobalt in all the concentrations (0.5, 1, 2, 3 and 4) ppm which exposured to them during ten days.

The statistical analysis showed there were no significant differences among the concentrations with each other or among the days of the experiment for each concentration.

Table 2: Removal percentage of Cobalt by Anabaena Sp. and the Statically Analysis (LSD).

Days	Concentrations (ppm)					LSD
	0.5	1	2	3	4	
1	96	97	98	98	98.4	4.27 ns
2	95.6	97.5	98.5	98	98.36	4.08 ns
4	98.40	98.60	98.25	98.24	98.60	2.67 ns
6	96.30	96.90	98.20	98.54	98.60	3.93 ns
8	96.00	98.80	98.40	98.40	98.30	3.87 ns
10	97.20	97.20	99.20	98.50	98.75	3.68 ns
LSD	4.84ns	4.07ns	3.79ns	2.17ns	2.04ns	___

Differences = significant differences, ns= non significant (*P<0.05)

Numerous chemical groups had been proposed to contribute to metal biosorption, such as carboxyl, carbonyl, sulfonate, sulfhdryl, phosphonate and hydroxyl groups (13, 14).

Some previous researches indicated that the carboxyl group was found to be the major binding sites for positively – charged heavy –metals ions (14).

According to these results, this alga has highly ability to tolerant for both chromium and cobalt ions from polluted water because of its ability to remove these two metals. Therefore, it is hopeful for this alga to use as removing agent for these heavy metals from polluted water, as a type of bioremediation.

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The competitiveness of Jordan Phosphate Mines Company (JPMC)

Rami Alrawashdeh (1) & Ahmad Al-Sukkar (2)

Department of Engineering / Al-Hussein Bin Talal University – Jordan / /Ma'an 20(1), Management and Financial College for Graduate Studies / Amman Arab University for Graduate Studies- Jordan (2)

E-mail: r_rawash@yahoo.com.au

ABSTRACT

One purpose of this paper is to study the main distribution channels of Jordan Phosphate Mines Company (JPMC) and highlight the markets lost and gained during the last three decade.

Another purpose of this paper is to examine the factors that have affected the competitiveness of the phosphate industry and to compare Jordan Phosphate Mines Company with its peers in the global market by considering factors such as the size of the phosphate reserves, the status of mineral policies, distance to major markets, human capital stock levels, and relevant social factors.

It has been observed that there has been a tremendous shift in market structure of Jordan phosphate exports in the last three decades. Though JPMC lost most of the markets in East Europe countries and Western Europe this has been compensated by gaining markets in south Asia. In comparing JPMC with other producers, results showed that Jordan outperformed and underperformed some major phosphate producers regarding policy environment and government effectiveness, distance to major markets, size of the reserve and human capital. For example Policy environment and government effectiveness in Jordan have outperformed China, Senegal, Morocco, Egypt, Syria and underperformed Tunisia, South Africa, Israel, US and Canada.

Key words*: Competitiveness, Marketing strategy; Competitive advantage;Resource management; Diversification; Market share*

الملخص باللغة العربية:

تهدف هذه الورقة البحثية لدراسة أهم القنوات التسويقية لشركة مناجم الفوسفات الأردنية وتلقي هذه الدراسة الضوء على الأسواق التي حصلت عليها الشركة والأسواق التي خسرتها خلال الثلاثة عقود الماضية. كما أن هذه الدراسة تهدف لدراسة أهم العوامل التي أثرت على تنافسية شركة مناجم الفوسفات الأردنية مع الأخذ بعين

الاعتبار مقارنة أداء الشركة مع منافسيها من المنتجين في السوق العالمي من خلال اخذ بعض العوامل الرئيسية مثل حجم احتياطيات الفوسفات، وضع السياسات القانونية المعدنية، المسافة عن الأسواق الرئيسية، مستوى رأس المال البشري و بعض العوامل الاجتماعية

ظهر من خلال تحليل بيانات الشركة ان هنالك تغيير كبير في التركيبة السوقية لشركة مناجم الفوسفات الأردنية خلال العقود الثلاثة السابقة. فعلى الرغم أن الشركة قد خسرت معظم الأسواق في أوروبا الشرقية وأوروبا الغربية إلا إنها قد ربحت معظم الأسواق في جنوب آسيا. ومع مقارنة شركة الفوسفات مع المنتجين الآخرين، فإن النتائج تظهر بأن الشركة قد تفوق أداؤها مقارنة مع بعض المنتجين وتدنى أداؤها مقارنة مع بعض المنتجين الآخرين فيما يخص البيئة القانونية والفعالية الحكومية ، المسافة عن الأسواق الرئيسية ، حجم احتياطيات الفوسفات وحجم رأس المال البشري. فعلى سبيل المثال، فيما يخص البيئة القانونية والفعالية الحكومية فإن الشركة قد تفوقت في أدائها على المنتجين في الصين، السنغال، المغرب، مصر وسوريا، بينما تدنت في أدائها على المنتجين في تونس، جنوب أفريقيا، إسرائيل، الولايات المتحدة وكندا.

INTRODUCTION

Jordan's open economy can only rely on limited natural resources. Only six percent of the country is arable land and water resources are among the scarcest in the world. However, there are sizeable mining resources of potash and phosphates in Jordan.

Mining currently represents around three per cent of Gross Domestic Product (1). As the major producer of phosphates in the world, Jordan is a significant exporter into world markets. Jordan recently ranked as the sixth largest producer and the second largest exporter of phosphate and it exports to more than thirty countries.

The term *international competitiveness* is now widely used in business and economics literature. Writers in the field have suggested several measures but perhaps the most common view is that a country is internationally competitive when it sustains and even increases its share in international trade over an extended period. As can be seen from Figure 1 Jordan has increased its share of world phosphate rock exports in the past two decades from ten per cent in 1985 to 13 per cent in 2007.

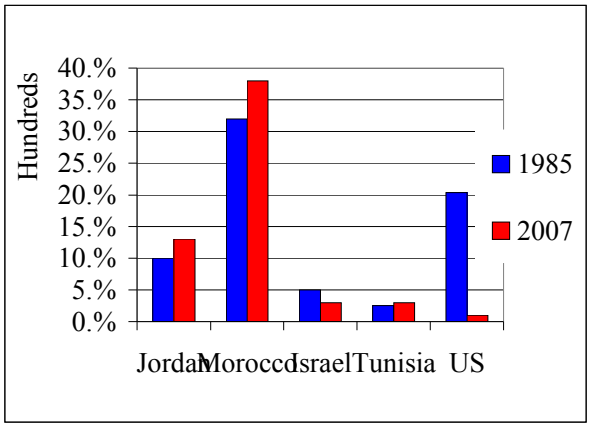

Figure (1) World export market share of phosphate rock from 1985 to 2007 (2)

One of the interesting trends in Figure 1 is the falling share of exports from the United States and the rise of Morocco. This raises issues about whether there has been a loss of competitiveness through exhaustion of mineral endowment or for some other

reasons, or whether it signals greater value adding and perhaps a growth in broader market competitiveness. The US industry has greatly reduced its exports of phosphate rock to nearly zero preferring to concentrate on downstream processing. More than 95% of the U.S phosphate rock ore mined is mined currently to be used in manufacturing of wet-process phosphoric acid and super phosphoric acid, which are used as intermediate feedstocks in the manufacture of granular and liquid ammonium phosphate fertilizers and animal feed supplements.
Jordan exports its product to most regions in the world.

Jordan exports to Eastern Europe, Western Europe, the Middle East (Turkey, Lebanon and Iran) South Asia (India, Pakistan, Bangladesh and Sri Lanka), East Asia (China, Japan, Taiwan, South Korea and North Korea), South East Asia (Indonesia, Malaysia, Thailand and the Philippines) and Oceania.

Table 1 shows regional exports of Jordanian phosphate rock between 2001 and 2007. In this period nearly sixty per cent of Jordanian exports went to South Asia, almost ten per cent to other Middle East nations and just six per cent to South-East Asia. European customers had reported less than twenty per cent.

Table 1: Average annual regional exports of Jordanian phosphate rock (thousands tons) from 2001 to 2007 (2)

Region	*Countries*	*Exports*
Eastern Europe	**Bulgaria, Poland, Czech Republic, Romania, Yugoslavia, Lithuania, Albania, Russia**	**431**
Western Europe	**Italy, Greece, France, Norway, Cyprus, West Germany, Austria, Sweden, Holland, Britain, Belgium, Denmark, Finland,**	**263**
South Asia	**India, Pakistan, Bangladesh, , Sri Lanka.**	**2329**
East Asia	**Taiwan, Japan, Vietnam, China, North Korea, South Korea, ,**	**244**
South East Asia	**Indonesia, Malaysia, Philippines, Thailand**	**237**
Middle East	**Iran, Turkey**	**374**
Oceania	**Australia, New Zealand**	**0**

As Figure 2 shows, some regions that had increased imports of Jordanian phosphate in the 1970s and 1980s, suffered reductions in the early 1990s (East Europe and East Asia) and the mid-1990s (Western Europe). They started to rise again slightly from the beginning of the new millennium.

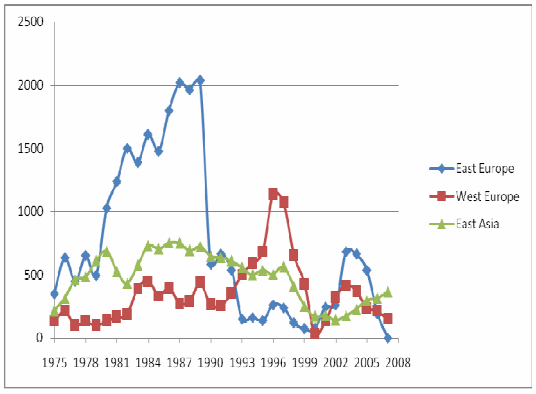

Figure 2: Regional exports of Jordanian phosphate rock (thousands tonnes) from 1975 to 2007 (2)

Southern East of Asia imports had been increased in the 1980s, stagnated in the 1990s, and have fallen since 2000. South Asia, which increased its imports of Jordanian phosphate in the 1970s and 1980s, declined until late 1990s and rose again until 2007 - see Figures 2 and 3. Jordan's exports to South Asia increased from 142,000 tones in 1975 to 2.3 million tones in 2007.

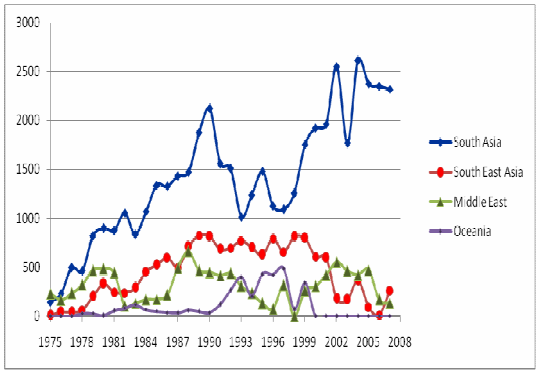

Figure 3: Regional exports of Jordanian phosphate rock (thousands tones) from 1975 to 2007 (2)

One can argue that to increase its competitiveness, Jordan would need to regain the regional markets that it previously serviced. Its exports to Western Europe market fell from 1.2 million tones in 1996 to 148,000 tones in 2007, and those to Eastern Europe from two million tones in 1989 to zero in 2007. It had also to regain Oceania market, which disappeared completely after the late 1990s.

In recent years, the Jordan Phosphate Mines Company has concentrated its marketing activity on the growing and profitable Indian market. Between 2001 and 2007, India accounted for 56 per cent of Jordan's phosphate exports. The next largest export destinations were Netherlands, Turkey, Indonesia, Poland and Iran which formed 7.2, 6.3, 6.1, 5.8 and 5.7 per cent respectively.

A good export strategy is to diversify and spread exports as much as possible because higher concentration and lower spread of the exports makes the exporter more vulnerable to market disturbances . Whereas, a lower concentration and higher spread makes the exporter less vulnerable to market disturbances and so the aim of this paper is to study the direction of Jordan Phosphate Mines company and to compare JPMC operational strategies with other producers in the market.

Direction of Jordan's phosphate exports

Table 2 shows the percentage shares of key importers of Jordanian phosphate exports between 1975 and 2007. Continuing strong customers have

included Portugal, Poland, India, Pakistan, Bangladesh, Turkey, Taiwan, Philippines, Indonesia, Japan and Malaysia. The key market that stands out is India, whose share increased consistently from 15.9 per cent in 1975-1979 to 58 per cent in 2000-2007.

After 1989, Jordan relinquished markets in Cyprus, Sweden, and Lebanon, and after 1995 the Czech Republic, Albania, France, Norway and Britain all fell away. Then after 1999, Jordan lost Romanian, Yugoslavian, West German, Austrian, Belgian, Danish, South Korean, Australian and New Zealand markets. Losing the Western European markets and the Oceania markets was due in part to environmental restrictions. Fertilizer plant closures in Western Europe assist in explaining the decline in phosphate exports to this region, while a new phosphate mine in Queensland serving the Australian and New Zealand markets, tended to crowd out the Jordanian product.

Some of the lost markets previously accounted for a large market share. Romania had average annual share of 18.3 per cent from 1980 to 1984. Yet the transition to capitalism and new political environment meant that its average annual share declined to 0.1 per cent between 1995 and 1999 and to zero between 2000 and 2007.

Table 2: Percentage shares of key importers of Jordan's phosphate rock exports between 1975 and 2007 (2)

Country	*1975-1979*	*1980-1984*	*1985-1989*	*1990-1994*	*1995-1999*	*2000-2007*
Eastern Europe						
Poland	5.8	8.0	11.1	1.3	0.3	5.6
Czech Republic	3.8	2.1	1.9	0.4	-	-
Romania	12.4	18.3	10.6	1.3	0.1	-
Yugoslavia	2.6	5.7	9.0	4.1	0.9	-
Lithuania	-	-	-	-	-	1.3
Albania	-	-	0.3	1.5	-	-
Western Europe						
Portugal	4.1	1.5	0.8	1.1	2.8	-
Italy	5.2	2.1	1.0	0.1	-	0.8
Greece	0.1	0.8	1.5	1.9	2.3	0.5
France	2.7	2.9	3.0	0.6	-	-
West Germany	-	-	0.3	0.1	3.1	-
Austria	-	0.4	0.1	0.1	0.3	-
Sweden	-	0.6	0.4	-	-	-
Netherlands	-	-	0.0	6.9	10.6	7.0
Belgium	-	-	-	0.1	1.0	-
Middle East						
Iran	0.2	-	-	-	1.5	4.9
Turkey	12.2	6.7	7.0	8.7	3.6	5.6
Lebanon	2.6	0.5	-	-	-	-
South Asia						

India	15.9	17.3	20.4	29.2	31.6	58
Pakistan	3.0	5.4	4.4	4.3	2.1	1.1
Bangladesh	1.7	2.2	2.1	1.8	0.5	0.1
Sri Lanka	0.9	0.0	-	--	-	-
South East Asia						
Thailand	-	-	-	-	2.5	1.2
Philippines	0.1	0.2	0.2	0.7	0.2	0.5
Indonesia	1.4	6.3	8.9	14.6	14.6	5.6
Malaysia	1.5	1.6	2.2	2.5	1.3	0.6
Vietnam	-	-	-	-	-	0.6
East Asia						
South Korea	-	0.9	3.0	2.7	3.4	2.3
Taiwan	10.5	5.4	4.7	6.5	4.1	1.5
Japan	9.7	6.8	5.2	4.5	3.8	3.2
China	1.7	2.3	0.4	0.5	-	-
Oceania						
Australia	0.2	0.7	0.5	3.4	7.1	-
New Zealand	-	0.5	0.4	0.8	1.3	-

The transition at the beginning of 1990, from a centralized economy to a free market economy, resulted in significant economic and social difficulties. During this transition to capitalism, Romania has displayed symptoms of considerable economic recession.

Thus, devaluation of the national currency, an increase in the unemployment rate, a decrease of internal production, the accumulation of external debt and the inconsistency of the policies for economic recovery are all issues that Romania has been forced to face in its economic restructuring by means of implementing the principles of a free market economy. Taiwan, Turkey and Japan contributed to around 10.5, 12.2 and 9.7 per cent respectively of Jordan exports from 1975 to 1979. These amounts fell to 1.5, 5.6 and 3.2 per cent respectively between 2000 and 2007 because of aggressive marketing strategies by competing producers. New markets for Jordan's phosphate exports after 1995 were Iran and Thailand and since the new millennium Lithuania and Vietnam have become new customers.

The port of Aqaba is the key Jordanian exit point for JPMC exports. Exports to Asia are transported pass through the Red Sea, while those to Europe must go through the Suez Canal . One of the handicaps of JPMC exports are the high fees which are charged by the Egyptian government in order to pass phosphate through the Suez Canal – see Figures 4 and 5.

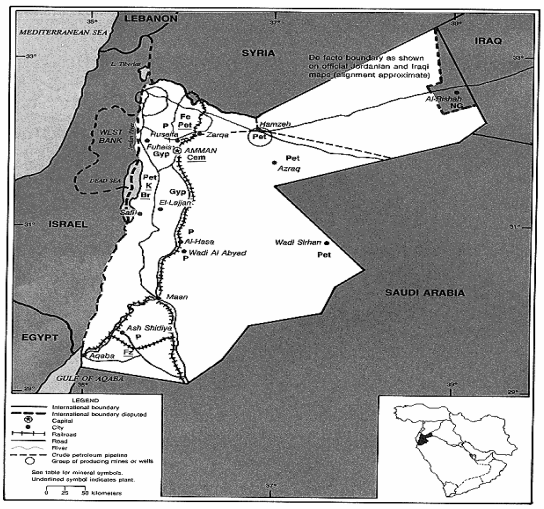

Figure 4: Jordan phosphate mines and the port of Aqaba

Figure 5: Suez Canal Jordan phosphate Channel to Europe

In 2001, Jordanian officials negotiated with the Egyptian to obtain preferential prices on exports of Jordanian phosphate, but they failed. If this had taken place Jordan may have improved its competitiveness in European markets and competed more effectively with Morocco.

When c.i.f. prices (i.e. cost, insurance and freight price) are reduced, Fob prices are affected. For example, in 1998 Jordan shipped phosphate fertilizers on the basis of Fob prices and its customers had to pay for freight costs and insurance but it was more cheaper for them to buy phosphate from other phosphate producing countries on the basis of c.i.f. price, and so Jordan had to reduce its Fob fertilizers prices in order to market its phosphate fertilizers to its customers.

The competitiveness of the Jordanian phosphate industry

Tilton (3, 4, 5) suggests two schools of thought concerning national and company mineral competitiveness. These are “the traditional view” and the “alternative view”. The traditional view is that competitiveness and wealth creation in mining is largely a transitory gift of nature. Companies and countries with the best deposits are the most competitive and generate the most wealth. Once their deposits are exhausted, however, competitiveness will shift to those companies and countries with the next best set of deposits. In this view, resource endowment is the overriding determinant of competitiveness in mining,

Tilton’s “alternative view” sees a key role for technology and innovation in reversing mining’s otherwise declining fortunes by maintaining and enhancing the competitiveness of the industry. Here government plays a role in

providing an economic climate that encourages innovative activities. In this view of the world, the role of government shifts from ensuring that society gets its fair share of wealth created by mining and that it is used in a manner that achieves intergenerational equity, to creating an economic climate conductive to the innovative activities of firms and individuals. In short, public policy focuses more on how to increase the benefits flowing from mining and less on how best to divide them.

In considering the contribution of copper to the economic development of Chile, Maxwell (6) suggested a broad relationship of the form:

Mining competitiveness	=f (Policy environment	Size of mineral endowment,	Human capital stock,
	Cultural homogeneity and political harmony,	Distance from major markets)	

This framework provides a useful broader view of the factors that will influence country‘s mineral sector competitiveness.

The policy environment variable reflects the combination of factors such as the quality of civil service, political decision makers, policies formulated and lack of corruption. In spite of the difficulties surrounding the Jordanian economy, the government has made several structural reforms aimed at transforming Jordan into a dynamic market economy, including privatization of some state-owned enterprises, and liberalization of the trading regime. In order to maximize the contribution of the mining sector to the national product, the Jordanian Government adopted policies that boost local value-adding capacity and improve efficiency by privatizing the Jordan Phosphate Mines Company in 2006.

One way to measure the policy factor is by using the government effectiveness index. This index combines responses on the quality of public service provision, the quality of the bureaucracy, the competence of civil servants, the independence of the civil service from political pressures, and the credibility of the government's commitment to policies. It ranges from -2.5 to +2.5, the -2.5 is the worst and +2.5 is the best.

As described in the World Bank governance indicators (7), Jordan’s government effectiveness in 2007 was + 0.19 which is better than other phosphate producers such as China (-0.08), Senegal (-0.21), Morocco (-0.05), Egypt (-0.51), Syria (-1.01) and Togo (-1.59) and lower than Tunisia (0.50), South Africa (+0.75), Israel (+1.26), US (+1.67), and Canada (+2.09).

A significant mineral endowment is also a necessary condition for resource sector competitiveness. When a country has few phosphates, it is clear that to maintain its competitiveness, strong exploration activity will be important. According to the US Geological Survey (8), Jordan’s phosphate reserves amounted to 0.9 billion tones. Compared to other producers, it is higher than Israel (0.2 billion tones), Tunisia (0.1 billion tones), Syria (0.1 million tones), Senegal (50 million tones), Togo (30 million tones), and lower than China (6.6 billion tones), Morocco (5.7 billion tones), South

Africa (1.5 billion tones) and the United States (1.2 billion tones).

The emergence of the mining industry in the 1970s and 1980s provided a foundation for building human capital stock, which is essential for the appropriate application of new technology and innovation.

Despite the other challenges it has faced because of its geographical location, Jordan has performed relatively well in terms of measures of its human development. On its change in Human Development Index from 1975 to 2007, it has outperformed Togo, USA, South Africa and underperformed Morocco, Tunisia, Togo and China - see Table 3.

Table 3: Human development trends and change for phosphate producing countries from 1975 to 2007 (9)

Country	*Change in Human Development Index 1975- 2007*
Jordan	0.13
Morocco	0.21
Tunisia	0.25
Senegal	0.16
Togo	0.09
South Africa	0.02
Israel	0.13
China	0.24
US	0.08

According to the cultural homogeneity and political harmony factor, Jordan is a stable country. There has been strong co-operation between the two main national groups - Palestinians and Jordanians - who have worked work together harmoniously to improve the main sectors of the economy including mining.

Distance from markets is a direct determinant of transport costs. Because phosphate rock is a low value commodity and its transport costs are relatively high, a producer's geographical location is an important factor affecting international competitiveness.

The Jordan Phosphate Mines Company (JPMC) has a strong competitive position because of its relatively close geographic location to the large densely populated Asian nations such as India, Indonesia and Pakistan. These countries have had growing needs for food and fertilizers.

The first two columns of Table 4 show Jordan's major markets and their percentage of total JPMC phosphate rock exports in the 2001 to 2007 period. The remaining columns show the distances from Jordan to major customers, as well as that of other major producers such as Morocco, Togo, South Africa, China, Egypt, Palestine and the United States. Jordan's geographic location appears to have provided a competitive edge particularly in the Indian, Iranian and Pakistani markets, though religious and cultural links must also have been important influences in the latter three nations.

Table 4: The percentage shares of major importers of Jordanian phosphate rock from 2001 to 2007, and the distances (in kilometers) from Jordan and other major phosphate exporters to the key ports in the importing nations (10,11)

		Distance in kilometers from key exporting to importing ports							
Major phosphate importers	*Share of Jordan's market*	*Jordan*	*Morocco*	*Togo*	*China*	*South Africa*	*Tunisia*	*Palestine*	*Syria*
India	56.0 %	4638	8624	11481.6	4852	5838	6694	4640	5313
Indonesia	8.1 %	8203	11993	12414	2862	7060	10259	8204	8879
Netherlands	7.5 %	5899	2366	6558	15632	11155	3878	5430	5435
Turkey	6.4 %	1184	3635	7916	10916	7724	1960	1185	206
Poland	5.7 %	7161	3628	7820	16894	12417	5140	6692	6697
Iran	5.0 %	4312	8102	11651	7745	6134	6368	4313	4987
Thailand	3.0 %	7352	11142	12964	4086	7534	9408	7353	8027
Japan	2.6 %	11622	15406	16697	1720	11395	13672	11624	12291
Pakistan	1.9 %	4339	8129	11596	6955	6080	6395	4340	5014
Malaysia	1.0 %	8350	12134	13000	2344	7699	10400	8352	9019

Though they are neighbors, Palestine has a geographical competitive edge over Jordan European markets such as the Netherlands, Turkey and Poland and its share in these markets was larger than Jordan's share. China competes with Jordan in the East Asian and South-East Asian markets such as Indonesia, Japan, South and North Korea, Indonesia, Thailand, and Malaysia. Its closer location and new phosphate developments have recently ensured a greater market share in these countries than Jordan.

Morocco has a comparative location advantage over Jordan in Western European nations such as Spain (14.2 per cent of its market) and France (seven per cent). This also applies in the North American and Latin America markets, especially the United States (24 per cent), Mexico (ten per cent) and Brazil (3.2 per cent) - see Table 5.

Table 5: The major importers of phosphate rock from Morocco in 2006 and the distance of key ports in these markets from the key ports in Jordan and Morocco (in kilometers) (12)

Country	*Morocco's percentage market share (2006)*	*Distance from key port in Jordan*	*Morocco*
Spain	**14.2**	**3883**	**579**
France	**7.0**	**3035**	**1601**
United States	**24.0**	**10460**	**6571**
Brazil	**3.2**	**8681**	**4580**
Mexico	**10.0**	**11448**	**7558**

The port of Aqaba in Jordan is 3883 kilometers from Barcelona, the closest port from Jordan to Spain, compared to the port of Safi in Morocco, which is 579 kilometers from Seville, the closest Spanish port. The closest port in France to both Jordan and Morocco is Marseilles. It is 3035 km from Aqaba and 1601 km from Safi in Morocco.

Some other aspects in the competitiveness of JPMC

Another aspect of competition in the phosphate industry arises because Jordan's customers in the raw phosphate rock market may become its competitors in the phosphate fertilizer market. Jordan has the advantage of an indigenous raw material, but its Gulf States neighbors such as Saudi Arabia have previously purchased Jordanian phosphate rock for the manufacturing of fertilizers. The "Jubail" plant in Saudi Arabia had lower cost energy inputs such as oil and this gave Saudi fertilizers a competitive advantage over Jordanian fertilizers in global fertilizer markets.

The arrival of new producers in markets close to Jordan's competitive location may also affect the future market share of the JPMC. The large Saudi Arabian Al-Jalamid phosphate project, located close to its border with both Jordan and Iraq, has a new capital investment of $US 2 billion. When it commences operations in 2010, this may have a significant impact on the downstream market for Di-ammonium phosphate (DAP) and it seems likely to position Saudi Arabia as the third or fourth largest phosphate producing nation. It is a potential threat to Jordan's future mining capacity expansions to feed integrated downstream capacity targeted at the export market.

Saudi Arabia is one of the main importers of Jordanian phosphoric acid in which it does also its own fertilizing plants. However, Al-Jalamid phosphate new deposits located in the northern side of Saudi Arabia will yield enough phosphate rock to produce three million tones of Di-ammonium phosphate a year.

Over the past half century, the United States has been the largest

phosphate rock producing country. This will continues over the short to medium term. However, it is destined to change in the end. A major problem facing US phosphate rock producers is the depletion of the high-grade phosphate rock in Florida. This will provide an opening for countries such as Jordan.

Some phosphate producers have also worked on reducing impurities and improving their phosphate content to increase their competitiveness. In the past, for example, potential buyers of Syrian rock for phosphoric acid production were discouraged by its relatively high chloride content. Recently Syria has improved its beneficiation technology to reduce the Chlorine content. As a result, its phosphate rock has become a strong competitor to Jordan phosphate.

Enhancing linkages between phosphate and potash companies is another potential key to exploit Jordan's comparative advantage. This is the case particularly if it is possible to produce high value added compounds composed of both potash and phosphate. Unfortunately there has been little such co-operation in Jordan between the JPMC and the Arab Potash Company. By contrast, in Israel the phosphate producing Rotem Amfert Negev Company and the potash producing Dead Sea Works Company have co-operated closely in manufacturing advanced fertilizers.

Government practices have played an important role in JPMC management. In the mid-1980s phosphate and fertilizer sales were often conducted on a barter basis. India, for example, provided textiles or capital items such as industrial equipment in exchange for phosphate supply. India used to provide equipment for the Aqaba railway and even an engine repair workshop. However, many of the bartered goods were not needed and accepted only in the interest of export promotion.

Counter trading at that time was often conducted on a package basis, where either the whole deal was agreed to or no trade occurred. To obtain items that were required, unwanted goods had to be accepted, and Jordanian exporters often urged the authorities to accept such goods. Otherwise, they would have lost their contracts.

The Jordanian government often arranged re-exports of bartered goods that were surplus to Jordanian requirements. This occurred at considerable discounts compared to the prices that the Indians considered the goods were worth. Trade on a cash basis would have been preferable to these arrangements, but countries in this position may not have purchased Jordanian phosphate as their first choice.

To encourage investments in the industry, the Jordanian cabinet decided in 1997 to exempt JPMC from mining fees placed on phosphate sales from joint venture projects during the first five years of operation. This was subject to an additional time extension based on the projects' profits**.** Furthermore, it exempted the fertilizer unit in Aqaba from custom duties for nine years .

One area in which Jordan has lacked experience is in negotiating joint ventures with foreign

companies. While such arrangements may guarantee a market for Jordan phosphate products, market changes should also be taken into considerations when contracts are signed on fixed prices. For example, Jordan signed a joint venture with the Japanese to sell phosphoric acid at JD 207 per tonne. By 2004, its production costs were JD 214 per tonnes and the JPMC made a loss of JD 7 per tonne. If Jordan had sold its output to joint ventures on international markets, it would have made a large profit. At that time Jordan was selling phosphoric acid to international customers by around JD 215.6 per tonne.

The demand and supply balance situation may also affect the Jordan phosphate market profitability. This typically occurs as an industry becomes more competitive. For example, in 1998, there were several new entrants to the phosphate fertilizers markets. They included Ukraine and Lithuania, while governments in Poland and Romania privatized their fertiliser plants. This meant that several European countries suddenly became either new competitors or stronger competitors in the phosphate fertiliser industry.

New producers may flood markets or reduce their prices to establish a customer base. Established producers in the fertilizers market such as Jordan had to reduce its prices too in order to sell its products.

CONCLUSION

The aim of the paper was to highlight the challenges that surrounded JPMC competitiveness. From the above discussion, it has been observed that there has been a tremendous shift in market structure of Jordan phosphate exports in the last three decades. Though Jordan lost most of the markets in East Europe countries and Western Europe this has been compensated by gaining markets in south Asia.

In comparing Jordan with other producers, results showed that Jordan outperformed and underperformed some major phosphate producers regarding policy environment and government effectiveness, distance to major markets, size of the reserve and human capital. For example Policy environment and government effectiveness in Jordan have outperformed China, Senegal, Morocco, Egypt, Syria and underperformed Tunisia, South Africa, Israel, US and Canada.

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The Role of ERP in Supply Chain Performance

Mohammad J. Adaileh (1) & Khaled M. Abu-alganam (2)

Dept. of Information Systems & Management/ Al- Qassim - KSA (1)
Amman Arab University/ Amman – Jordan (2)

E-mails: madaileh@aabfs.org (1) khaledganam72@yahoo.com (2)

ABSTRACT

Many firms are deploying ERP systems in supply chain applications. Recently Jordan has embarked upon an ambitious plan to make full use of the IT capabilities. In Jordan, the application of the ERP systems is relatively immature. In addition, it is evolving, and the number of organizations involved is growing. Raising awareness and knowledge is essential for adopting ERP systems in supply chain in Jordan, at both the organizational and inter-organizational levels. Firms need to identify and understand the critical factors that affect the using of ERP systems successfully in firm performance, and address them effectively to ensure that the promised benefits can be realized and failures can be avoided.

الملخص باللغة العربية

تبنت العديد من المنظمات نظم تخطيط موارد المنظمة (ERP), في تطبيقها لإدارة سلسلة التوريد, وقد اتخذ الأردن حديثا خطط طموحه نحو الاستخدام الكامل لتطبيقات تكنولوجيا المعلومات. تعتبر تطبيقات نظم تخطيط موارد المنظمة (ERP) غير ناضجة بشكل كافي, وتمر في فترة تحول مع تزايد عدد المنظمات التي تطبقها, لذا فأن الإرشاد والمعرفة ضروريان لتبني نظم تخطيط موارد المنظمة (ERP) في سلسلة التوريد داخل المنظمة, وبين المنظمات الأخرى. إن المنظمات بحاجه لتحديد وفهم عناصر النجاح الحرجة التي تؤثر على أداء المنظمة, من خلال استخدام نظم تخطيط موارد المنظمة بنجاح, وهي بحاجه أيضا إلى أن تبلور بفعالية الفوائد الواعدة والسلبيات المتوقعة .

INTRODUCTION

According to Stephen (1), ERP is a system that effectively integrates all information required by the operating process functions including finance, accounting, human resources, production, material management, quality management, allocation and distribution, and sales by organization or process reengineering and information technology. ERP is an integrated information system that integrates enterprise internal function working processes, standardizes internal data processing procedures, and combines the operational data generated by different functions (1) ;(2). Future ERP will integrate supply chain management (SCM) to provide enterprise management more accurate information (3) ;(4) ;(5).

Enterprise Resource Planning and Supply Chain Management

Organizations have felt the need for going beyond mere transaction processing and automation of business processes. What is required to operate in complex business environment is a tool which can help in identifying and planning resources based on certain organizational constraints that are dynamic in nature (6). SCM (Supply Cain Management) is concept which look at a business as a chain of will inter connected entities and thus providing a see through perspective of the entire business. The supply chain can be modeled to reduce inventory, lead times and cost at each link under the given constraints. Supply chain management has been used by a few organizations and they have obtained immense benefit from the supply chain integration with suppliers and customers (7). The high growth of ERP investment and significance of SCM in a global economy prompt researchers and practitioners to seriously think about the design and implementation of ERP in SCM (8). This may be resorted so as to capitalize on the strengths of the two systems (ERP and SCM) (9). Sophisticated middleware interface software, which enable sharing of data and processes are used. These software help in linking the ERP and SCM systems at the points where they have overlapping features (10). The relationship between ERP and SCM have been studied by Akkermans , (11) who produced a research in 23 separate firms about the results and future expectations of ERP systems implementations in a SCM perspective. The authors inferred that many firms deploying ERPs considered extending system scope mainly to integrate their suppliers, customers or both to the system, to provide additional e-commerce or e-business operations and to increase supply chain functionalities.

ERP systems success (synonymous with ERP success) refers to the use of such systems to enhance organizational effectiveness (12); (13), which is different from the technical implementation success of such systems wherein measurement indicators such as cost overruns, project management metrics, and

time estimates are the main concerns (14). In the work of DeLone and McLean, (15) concluded "By studying the interactions along these components of the model [dimensions of IS success], as well as the components themselves, a clearer picture emerges as to what constitutes information systems success." Moreover, other researchers (e.g. Akkermans, (16) have studied the interrelations among critical success factors in the early stages of ERP implementations; this study complements such efforts. Importantly, insights from this research may benefit both ERP practitioners and IS success evaluations researchers. Over the past three decades, evaluating the value and success of IT systems for organizations has been a recurring issue (15); (12), and various assessment approaches have surfaced, (6). In response, DeLone and McLean, (15) developed an integrated, multidimensional, and inter-related IS success model that has become the most dominant framework for assessing IT systems success at the micro level (6). Drawing from the work of DeLone and McLean, (15), Gable and colleagues (12); (17), developed an additive ERP systems success measurement model that redefines the dimensions in the original D&M IS success model. It is important to point out that ERP systems are different from other IT systems (8); (18), because ERP implementation includes technological, operational, managerial, strategic, and organizational related components (18). As a consequence, success measurement models used for other typical IT systems' evaluation may not be adequate for ERP systems (12); (6). Thus, it is illuminating when attention is paid to ERP systems particularly, rather than just lumping them together with other IT systems.

Indeed, DeLone and McLean, (15) stress that researchers should take into account the specific characteristics of the IT system under investigation when evaluating its success. Given that ERP systems are a different class of IT systems, it is therefore vitally important for a specialized success measurement framework or model to be used when evaluating or measuring the success of such systems. Gable et al, (12) eliminated (through multi-stage data collection and statistical analysis) the Use (UE) and User satisfaction (US) dimensions in the D&M model. Arguments against dropping them are also available in the literature (13) ;(6). The retained ERP success dimensions in Gable and colleagues' model are System Quality (SQ), Information Quality (IQ), Individual Impact (II), and Organizational Impact (OI). Through literature reviews and case studies, Ifinedo (13) ;(6), proposed an extended ERP system success measurement model to include Workgroup Impact (WI) not included in the Gable et al. model. The author argues that any ERP success measurement model should include a dimension related to (WI) because ERP systems are often adopted to enhance efficient cross-functional operations (19). Here, "workgroup" refers to the sub-units and/or functional departments of an organization. A

version of the ERP success measurement model proposed by Ifinedo, (13) is illustrated in Figure (1)

Dimensions	Definition	References
System Quality	Measure of the information system itself, and concerned with the performance characteristics of the ERP systems.	(DeLone and McLean, 1992; Rai et al, 2002; Ifinedo, P. 2006, 2007; Ifinedo, P. & Nahar, N. 2006; Hong and Kim, 2001)
Information Quality	Measure of the information system output, and concerned with timeliness, relevance, and usefulness of information generated by an information system, and focuses on the quality of the information system output	DeLone and McLean, 1992; McKinney, Yoon and Zahedi, 2002) Seddon and Kiew, 1996; Rai et al, 2002; DeLone and McLean, 2003; Ifinedo, P. 2006, 2007; Ifinedo, P. & Nahar, N. 2006; Bailey and Pearson (1983; Saaksjavi and Talvinen (1993; Rainer and Watson (1995)
Individual Impact	Measure of the effect of information on the behavior of the recipient, and concerned with the effect of information on the behavior of the user	DeLone and McLean, 1992; Umble and Umble 2002; Kim and Lee, 1986; Ein-Dor, Segev, Steinfeld, 1981; Dickson, Senn, Chervany, (1977)
Workgroup Impact	This dimension refers to the impact of ERP acquisitions on the workgroups, subunits and/or departments within organizations. And encompasses issues relating to the use of ERP to improve inter-departmental coordination, communication, and productivity	Davenport 2000, Abdinnour-Helm et al. 2003; Ifinedo, P. 2006, 2007; Ifinedo, P. & Nahar, N. 2006
Organizational Impact	Measure the effect of information on organizational performance, This refers to the value or benefits accruing to the organization for adopting a particular ERP system	Ifinedo 2006a; Ifinedo, P. 2006, 2007; Ifinedo, P. & Nahar, N. 2006; Umble and Umble 2002; DeLone and McLean, 1992; McKinney, Yoon and Zahedi, 2002; Saaksjavi and Talvinen (1993)

Figure (1): ERP Systems Success Measurement Models adopted by Ifinedo (13)

Supply Chain Performance

A number of experts and practitioners from Supply Chain Strategy (a monthly newsletter from the MIT Center for Transportation and Logistics) recommend four metrics for executives' attention. Those metrics incorporate all the dimensions of supply chain performance and respond to the factors that external stakeholders, analysts and venture capital firms take into consideration when evaluating a firm. (20) ;(21). In literature much attention has been devoted to three main aspects of performance: financial, organizational and strategic performance.

After comparing different measures of performance, they suggest that multiple dimensions of performance should be considered where possible, including both financial and non-financial measures.
Accounting-based indicators, with efficiency, sales growth rate and profitability (e.g. return on sales or on investments) are the financial indicators most commonly used. In addition, operational (non-financial) performance measures, such as product quality, customer satisfaction and market shares are often examined. In this study, the operational (non-financial) performance measure was used, see table (1), however, the major key performance indicators that were adapted form many authors and used in this research as follow:

Table (1) Classification literature in Non-Financial Performance

Dimensions	Definition	References
Reduce cycle time	end-to-end delay in the process**,** lead time is the time required to convert the raw materials into final products plus the time needed for the products to reach the customer	(Whyte, 2000; Harwick, 1997; Ferdows et al, 2004)
Reduce inventory	Inventory includes raw materials, work-in-process, finished goods. When compared with optimal levels, inventory is an indicator of efficiency.	Ferdows et al, 2004; Whyte, 2000)
Improve resource utilization	Resource utilization efficiency can be measured by comparing value added with the value of the resources and assets used.	Womack et al, 1990; Ferdows et al, 2004
Improve quality	Better identification of functions to avoid errors and improve the quality of performance	Frohlich & Westbrook, 2001; Vickery et al., 2003; Gimenez & Ventura, 2005
Improve service.	full rate, backorder level, which is the number of orders waiting to be filled. On-time delivery, which is the fraction of customer orders that are fulfilled on time, i.e., within the agreed-upon due date	Van Donk & Van der Vaart, 2004,2005; Van der Vaart & Van Donk, 2006; Lohman et al. 2004)
Flexibility	ability to adapt to changing environment	(Muhlemann *et al.* 2000
Delivery	Processes that provide finished goods and services, including order management, transportation management, and warehouse	(Beamon 1999)

	management, for meeting planned or actual demand	
Responsiveness	velocity at which a supply chain provides products to the customer, and it includes the ablity to respond to the environmental changes	(Beamon 1999; Li 2002; Luning *et al*. 2002; Aramyan *et al*. 2006; Nicoll*, 1994*

RESEARCH METHODOLOGY

There is a need for provide Jordanians supply chains members with better understanding, and a clear picture of the relationship between ERP systems and performance and its success requirements, especially for manufacturing firms which are at the heart of the supply chain that are insufficiently informed about ERP systems. Although many studies covered the role and the impact of IT in SC performance, there are a few contributions about the CSFs that would support practitioners in their efforts to successfully implement ERP systems in supply chain. There is no clear definition of constructs and conceptual frameworks on CSFs and outcomes of ERP systems in SC performance in the current literature. The findings of previous studies can be described as fragmented, and have not been holistic. And there are limited studies that discuss the relationship between CSFs of ERP systems and their impact on the performance of the supply chain.

Research Design

The three common design of research used in social sciences research are exploratory, explanatory, and descriptive studies. Exploratory research is often employed to develop a preliminary understanding of some phenomena. Explanatory is carried out to discover and report relationships among different aspects of the phenomena. Descriptive studies are conducted to describe the precise measurements and reporting of the characteristics of the phenomena under investigation (22). Explanatory research approach can be used when it is necessary to show that one variable causes or determine the value of the other variables. Therefore, the nature of this research is both exploratory and explanatory.

This research aims to cover a wide variety of manufacturing firms from different industries in Jordan that use ERP systems to integrate with supply chain members. However, no comprehensive sampling frames of firms that use ERP systems were available. There were no specialized databases to identify the ERP systems user companies in Jordan. This has influenced the sampling method, the size of selected sample, and the gross response rate. Therefore, the sampling method used was judgment sampling. We took a survey of Jordanian manufacturing firms from various industries. Based on the selection criteria; nine firms were selected to conduct this research. However, this was the best list available after strenuous efforts for the present research, which relied on multiple manufacturing firms in Jordan use

ERP systems. These companies are: Arab Potash Company, Jordan Cement, Jordan, Phosphate Company, Arab Center for Pharmaceuticals and Chemicals Co (ACPC), Petra Aluminum Co, Pharma International, Hammoudeh Dairy Co, Jordan Ceramics, and Al-Razi Pharmaceutical Co. For the purpose of the present research, the target respondents group involved managers and employees in the fields of purchasing, distribution, sales and marketing, transportation, IT, inventory and warehousing, research and development, and financial and accounting.

The instrument used in this research was questionnaire to measure the research's different variables. The final version of the questionnaire consists of (51) statements with close ended questions. Individuals were asked to indicate the extent of importance with the questionnaire items on a five-point Likert-type scale ranging from 1 to 5. Cronbach's alpha was employed as the criterion to evaluate reliability of the constructs by examining their internal consistency. Estimate greater than 0.70 are generally considered to meet the criteria for reliability. Validity concerns with weather the researcher is actually measuring what he claims, this study uses the four different types of validity as follows: *Face validity*, *Content validity*, *Criterion validity, Construct validity that* testifies to how well the results obtained from the use of the measures fit the theories around which the test is designed (23). We used the two subcategories of construct validity: Convergent Validity and Discriminant validity. Exploratory Factor analysis was conducted to analyze the scale items of the research constructs, and to check the construct validity of the measurement scale. (24)& (25).

Model Operationalisation using EFA And Data Analysis

Factor analysis was conducted to analyze the scale items of the 13 research constructs, and to check the construct validity of the measurement scale. For this research the result of reliability test are shown in table (3) in which the (α) value are grater than 0.6 for all variables. All of these percentages represent a significance amount of data explanation. **Eigenvalues** Also called characteristic roots was utilized to measure the amount of variation in the total sample accounted for by each factor (24). Note that the eigenvalue is not the percent of variance explained but rather a measure of amount of variance in relation to total variance (since variables are standardized to have means of 0 and variances of 1, total variance is equal to the number of variables (26). For this research, the values of the 13 variables are grater than 1 which leads to keeping all the presented factor. The **KMO Measure** (Kaiser-Meyer-Olkin) was used to assess which variables to drop from the model because they are too multicollinear. KMO varies from 0 to 1.0 and KMO overall should be .60 or higher to proceed with factor analysis. (Some researchers use a more lenient .50 cut-off. To assess the suitability of data analysis, Bartlett's Test of Sphericity suggests that the intercorrelation matrix contains sufficient common

variance to make factor analysis worthwhile. Referring to table (3), all KMO's values are grater than 0.6 or the 0.5 cut-of, which indicate that the data of the research support the use of factor analysis, and suggest the data my be grouped into a smaller set of underlying factor. And the Bartlett value (sig) is Zero for all that means all values are significant for all variables which specify the relationships between the variables. Total of Variance Explained (TVE %) was utilized considering these criteria: Some researchers simply use the rule of keeping enough factors to account for 90% (sometimes 80%) of the variation. Where the researcher's goal emphasizes parsimony (explaining variance with as few factors as possible), the criterion could be as low as 50%.

Table (2): Major Indicators of the Factor Analysis

Construct	No. of Items	KMO, BTS (Sig)	Loading	TVE (%)	α value
System quality (SQ)	9	0.87, 1208.35 (0.0)	0.68-0.88	60.94	0.92
Information quality (IQ)	8	0.86, 1299.93 (0.0)	0.77-0.90	67.52	0.93
Individual impact (II)	4	0.77, 349.430 (0.0)	0.75-0.88	68.95	0.85
Workgroup impact (WI)	5	0.84, 667.184 (0.0)	0.78-0.91	73.71	0.91
Organizational impact (OI)	7	0.80, 945.997 (0.0)	0.79-0.87	68.07	0.92
Reduce cycle time (RC)	5	0.82, 798.835 (0.0)	0.85-0.92	77.43	0.93
Reduce inventory (RI)	3	0.77, 457.396 (0.0)	0.93-0.94	87.61	0.93
Improve resource utilization (RU)	3	0.70, 268.931 (0.0)	0.86-0.92	77.64	0.85
Improve quality (QU)	4	0.79, 485.855 (0.0)	0.80-0.90	75.52	0.89
Improve service (SER)	4	0.83, 573.206 (0.0)	0.87-0.94	80.30	0.92
Flexibility (FLEX)	3	0.71, 304.605 (0.0)	0.85-0.92	79.66	0.87
Delivery (DEL)	3	0.75, 445.859 (0.0)	0.92-0.95	86.85	0.92
Responsiveness (RES)	6	0.83, 922.989 (0.0)	0.76-0.90	71.57	0.92

Hair (27) suggests that for any factor to be meaningful, at least 5% of the total variance explained should be attributable to that factor. Keep as many factors as are required to explain 60%, 70%, 80-85%, or 95%.
There is no general consensus and one should check what is common in the field. It seems reasonable that any decent model should have at least 50% of the variance in the variables explained by the common factors (24). Summated scales are a collection of related questions that measure underlying constructs, the result of summated scale analysis can be shown in table (2). It shows that Items loading on all factors for each construct were higher than the 0.05.

The Model

By using the exploratory Factor Analysis (EFA), there are two dimension of the study, the first five variables describe the ERP success dimension, and then the eight variables describe the supply chain performance.

Multiple Regression Analysis
According to Maxwell, (25), multiple regression attempts to find a relationship between a dependant variable and greater than one independent variables. Multiple regression analysis is used in more complex data analysis with more than one factor changing the dependant variable (28). There are some values to report when using multiple regressions which are: Adjusted R square value and F-Value and its significance, Significance, Beta coefficient

The Path Variables
This model consists of five regression paths as shown below, and the relations are designed as the functions below:

$$Y' = \alpha + \beta\, 1X1 + \beta\, 2X2 + E$$

Where

Y' = A predicted value of Y (which is dependant variable).

α = the value of Y when X is equal to zero. This is also called the "Y Intercept".

β = the change in Y for each 1 increment change in X.

(X1 X2)= an X score on independent variable for which we are trying to predict a value of Y.

E= standard Error.

Regression Path: The relationships between and ERP and Non-Financial Performance (ERP → Non-Financial Performance).
ERP construct with five independent sub-variables. The relationships are designed to test the hypothesis H1 through H8.

1- $H1: RC = \alpha + \beta 1 (SQ) + \beta 2 (IQ) + \beta 3 (II) + \beta 4 (WI) + \beta 5 (OI) + E$
2- $H2:RI = \alpha + \beta 1 (SQ) + \beta 2 (IQ) + \beta 3 (II) + \beta 4 (WI) + \beta 5 (OI) + E$
3- $H3:RU = \alpha + \beta 1 (SQ) + \beta 2 (IQ) + \beta 3 (II) + \beta 4 (WI) + \beta 5 (OI) + E$

4- *H4:QU* = α + ***β1*** *(SQ) + β2 (IQ)+ β3 (II)+ β4 (WI)+ β5 (OI)+ E*
5- *H5:SER*= α + ***β1*** *(SQ) + β2 (IQ)+ β3 (II)+ β4 (WI)+ β5 (OI)+ E*
6- *H6:FLEX* = α + ***β1*** *(SQ) + β2 (IQ)+ β3 (II)+ β4 (WI)+ β5 (OI)+ E*
7- *H7:DEL* = α + ***β1*** *(SQ) + β2 (IQ)+ β3 (II)+ β4 (WI)+ β5 (OI)+ E*
8- *H8:RES* = α + ***β1*** *(SQ) + β2 (IQ)+ β3 (II)+ β4 (WI)+ β5 (OI)+ E*

Hypotheses Testing

In order to assess the hypotheses of model, number of means of evaluation was used. First, we have to determine adjusted R squared and analysis of variance to approve model fit, adjusted R squared provide a measure of fit for each variable and represent the changes in R^2. Analysis of variance F tests to determine how well the model fits the data and in effect tests for the joint significance of the explanatory variables. Second, the standardized estimation coefficient of (Beta), this beta can closely approximate the magnitude of the effect. A beta close to Zero has little, if any, substantive effect, while an increase in value corresponds to increase importance in the casual relationships. Third, co linearity statistics which contain Tolerance (Regression) to determine how much the independent variable are linearly related to one another (multicollinear). A variable with very low tolerance contributes little information to a model, and can cause computational problems. It is calculated as 1 minus R squared for an independent variable when it is predicted by the other independent variables already included in the analysis. Variance Inflation Factor (VIF) which is the reciprocal of the tolerance. As the variance inflation factor increases, so does the variance of the regression coefficient, making it an unstable estimate. Large VIF values are an indicator of multicollinearity. VIF should be less than 10. A stepwise method selection was used, Stepwise variable entry and removal examines the variables at each step for entry or removal. *Observed Significance Level* Often called the P value was used, if the observed significance level is small enough, usually less than 0.05 or 0.01, the null hypothesis is rejected. Table (4) shows the results of conducting the evaluation test on the research data, the tables content is the result of testing the regression analysis for the models which will discussed below.

Analysis of Regression Path

The Regression Path concern with the existence of significant relationship between ERP variables and Non-Financial Performance. Looking at table (3) eight hypotheses was created to determine these relations. For example, Reduce cycle time was hypothesized to be positively associated with ERP variables, stepwise method was used with settings at 0.05 α levels, based on

the significance (Probability) of the F value and the F value itself which equal 88.19 ($p<0.001$), adjusted R Squared (represent the changes in R^2) was investigated, it equal (0.476) that means the model is fit for each variable. A stepwise regression analysis fits a variety of models to the data, adding and deleting variables as their significance in the presence of the other variables is either significant or non-significant, respectively. Using the stepwise method, two variables of ERP was entered, because two variables of ERP have significant effect, namely (SQ) and (IQ). Beta was found to equal (0.425) for (SQ), and (0.290) for (IQ) which implies the existence of a positive significant relationships between ERP and (RC) , and the t-value of the hypothesized model was significant with a value of (3.90) for (SQ), and (2.66) for (IQ). Other three variables namely (II, WI, and OI) was excluded because standardized estimation coefficient of Beta were close to Zero that mean it has little, if any, substantive effect. That means these relationship are not significant, and it was founded that the t-value of regression paths between the variables have no significant and less than (1.96 and 2.54) on the significance level (0.05 or 0.01). Co linearity statistics has been determined. The tolerance is less than (1) for two independent variables. This means that independent variables are linearly related to one another (multicollinear). The (VIF) was also and less than ten, that mean there are not multicollinearity in the independent variables. Durban-Watson test Values (1.264) less than 2 that indicate positive autocorrelation between variables. For other hypotheses see table (3) which shows the relations related to these hypotheses.

Discussion and Recommendation for future researches

While the current research made significant contributions from both a theoretical and practical point of view, the findings of our research should be evaluated in the light of the following limitations: we adopted the single-informant approach from the manufacturer's perspective to identify the success dimensions of ERP systems. Given that a single response reflected each supply chain member, our findings may well be vulnerable to the threat of single-source bias. Second, Since the data collection was restricted to Jordan; our findings may have a sampling bias and may not be wholly applicable to firms in different cultures. Third This research did not consider the impact of sample characteristics, including characteristics of the respondents (Job Title, Job Function, Years working), characteristics of surveyed organizations (Major Industry, number of employees), and sample characteristics of the technology applications (the numbers of years of using ERP systems, the members that company integrate with using the ERP systems, on determining the success dimensions of ERP systems and their impact on firm performance.

This research aims to identify and improve understanding of the Critical Success Factors and dimension of Enterprise Resource Planning (ERP) implementation from the manufacturing members' perspectives in Jordan. Furthermore, this research is to investigate the impact on firm performance. The results of the statistical analysis are interpreted to arrive at practical suggestion that manufacturing companies in Jordan can benefit from when deciding to adapt the ERP systems; each hypothesis is examined, and explained. All variables were metric satisfying the conditions for multiple regression analysis. The stepwise method was used with settings at 0.05 α levels. T-tests were conducted on each independent variable. As no multi co linearity was detected among the included variables, all variables were included in the analysis. Further examination of tolerance and variance inflation factor (VIF) statistics did not reveal any multi-co linearity concerns. All variables in the model demonstrated normal distribution following a test for unvaried normality by applying the Kolmogorov-Smirnov test and low skewers and kurtosis statistics. The significant results of the regression analysis show that Eight performance metrics were hypothesized to be positively associated with ERP. The variables of ERP that entered to the model have significant effect,. Beta was measured, and the t-value of the hypothesized model was significant, which implies the existence of positive significant relationships between ERP and firm performance metrics. As a result, new constructs, and new multi-item measurement scales for measuring these constructs associated with the ERP systems dimensions and supply chain performance. The framework of the study provides a foundation for future research. In the future, new constructs may be added to provide in-depth understanding of ERP-SC theory. Then provides the inferences made from an instrument that is valid and reliable for the current research's context. All the scales have been tested through rigorous statistical methodologies including, reliability analysis, factor analysis, content validity, and construct validity analysis. All the scales are shown to meet the requirements for reliability and validity and thus, can be used in future research. The development of these scales will greatly stimulate and facilitate theory development in this field.

Future research should conduct factorial invariance. Using the instruments developed in this research, one may test for factorial invariance tests by bringing some contextual variables into the model, that allow the comparison across industries, different organization size, the numbers of years of using ERP systems, the members that company integrate with using the ERP-SCM applications and so on. For the technology (IT) related variables or factors, we believe additional factors can be identified and used in future studies. The inclusion of additional issues or factors will improve the variance explained in casual models involving such factors.

When operationalizing the technology (IT-related) issues in future studies, it is also important to use multiple indicators that are validated in the literature. For example "employee IT skills" and "satisfaction with legacy IT systems" could have benefited from such.

Table (3): Regression Analysis (The Relationships):

Regression Path	Variables Entered	Model Fit		Test Statistics			Collinearity Statistics		Durban-Watson Test	Variables removed
		Adjusted R squared	F value (P)	Standardized Beta	t-value	Sig.	Tolerance	VIF		
	SQ		88.19	0.425	3.901	0	0.23	4.352		
ERP → RC	*IQ*	0.476	0	0.29	2.661	0.008	0.23	4.352	1.264	II,WI,OI
	WI		79.14	0.379	4.426	0	0.391	2.558		
ERP → RI	*II*	0.449	0	0.335	3.91	0	0.391	2.558	1.548	SQ,IQ,OI
	WI		86.03	0.416	5.04	0	0.405	2.471		
ERP → RU	*SQ*	0.47	0	0.315	3.815	0	0.405	2.471	1.672	IQ,II,OI
	WI		68.79	0.363	4.242	0	0.405	2.471		
ERP → QU	*SQ*	0.414	0	0.32	3.685	0	0.405	2.471	1.827	IQ,II,OI
	IQ		88.39	0.539	6.709	0	0.422	2.369		
ERP → SER	*WI*	0.477	0	0.189	2.357	0.019	0.422	2.369	1.876	SQ,II,OI
	II		68.54	0.346	3.829	0	0.374	2.676		
ERP → FLEX	*IQ*	0.413	0	0.338	3.732	0	0.374	2.676	1.31	SQ,WI,OI
	IQ		70.76	0.457	5.4	0	0.422	2.369		
ERP → DEL	*WI*	0.421	0	0.235	2.781	0.006	0.422	2.369	1.671	SQ,II,OI
			172.23							
ERP → RES	*IQ*	0.471	0	0.689	13.124	0	1	1	1.423	SQ,II,WI,OI

More studies are needed to determine the nature of relationship between "satisfaction with legacy IT systems" and ERP success. The literature suggests that ERP success did not have adverse effect on the satisfaction with legacy IT systems for firms.

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The Systemic Resistance induction of Barely That Infected With *Fusarim oxysporum* Using *Serratia marcescens*

Ali Q. Khazaal

Biotechnology Dept./ College of Sciences / Baghdad University- Republic of Iraq

E-mail: aliqasim14@yahoo.com

ABSTRACT

The inhibition activity of seven strains of *Serratia marcescens* against *Fusarium oxysporum* was studied. All the strains were able to inhibit the *F.oxysporum* growth *in vitro* and the strain SM5 was the most efficient strain with 60.8%, this strain was able to induce the systemic resistance of barely which infected previously with *F.oxysporum* after test the sensitive of barely seeds toward the fungus. The fresh weight as well as the specific activity of peroxidase and polyphenol oxidase was increased in plants that treated with SM5 comparing to other plant that treated with distilled water or fungus only.

الملخص باللغة العربية

اختبرت القابلية التثبيطية لسبعة عزلات بكتيرية عائدة لجنس *Serratia marcescens* ضد الفطر *Fusarium oxysporum* وقد وجد ان لهذه العزلات قابلية تثبيطية ضد هذا الفطر وكانت العزلة SM5 الأكفأ في التثبيط وبنسبة بلغت .60.8% . وجد ان لهذه العزلة قابلية على حث المقاومة الجهازية لنبات الشعير المصاب بالفطر*F.oxysporum* بعد ان وجد ان بذور الشعير المستخدمة كانت حساسة لهذا النوع الفطري. تمكنت العزلة SM5 من زيادة الوزن الطري وكذلك الفعالية النوعية لانزيمي peroxidase وpolyphenol oxidase في النباتات المعاملة بهذه البكتريا مقارنة بتلك النباتات المعاملة بالماء المقطر او بالفطر الممرض لوحده.

Key words: induced systemic resistance, *Serratia marcescens, Fusarium oxysporum,* polyphenol oxidase and peroxidase

INTRODUCTION

Fusarium oxysporum is a highly destructive pathogen of both greenhouse and field grown plants in warm vegetable production areas. The disease caused by this fungus is characterized by wilted plants, yellowed leaves and minimal or absent crop yield, there may be a 30 to 40% yield. (1)

Various strategies for controlling *F. oxysporum have* been introduced over the years e.g., soil cultural practices, fungicide treatments etc., but serious losses still occur, largely because the effectiveness of these approaches is variable and often short lived in addition to the phytotoxicity and fungicide residues which are leading to major problems such as environmental pollution, human health hazards and development of pathogen resistance. (2, 3)

Alternative treatments for control of plant diseases are needed. The use of microorganism to control plant pathogen, known as biological control is now in practice. It is accepted as a suitable and environmentally friendly alternative or a supplemental way of reducing the use of chemicals in agriculture against plant disease management. (4)

Plants possess various inducible defense mechanisms to protect themselves against pathogen attacks. The first one of this is the Systemic Acquired Resistance (SAR), which is induced by the exposure of root or foliar tissues to biotic or abiotic elicitors, is dependent of the phytohormone salicylate (salicylic acid). While the second is Induced Systemic Resistance(ISR),which is induced by the exposure of roots to specific strains of Plant Growth Promoting Rhizobacteria(PGPR), is dependent of the phytohormones ethylene and jasmonate (jasmonic acid).(5)

Many plant enzymes are involved in defense reactions against plant pathogens. These include oxidative enzymes such as peroxidase (PO) and polyphenol oxidase (PPO), which catalyse the formation of lignin and other oxidative phenols that contribute to the formation of defense barriers for reinforcing the cell structure. Other enzymes such as tyrosine ammonia-lyase (TAL) and phenylalanine ammonia-lyase (PAL) are involved in phytoalexin or phenolic compound biosynthesis.(6)

Plant Growth Promoting Rhizobacteria can suppress diseases through antagonism between bacteria and soil-borne pathogen through competition for nutrients and production of antimicrobial or lytic enzymes for fungal cell wall, as well as by inducing systemic resistance in the plant against both root and foliar pathogen. (7, 8)

Some root colonizing nonpathogenic rhizobacteria may also trigger disease resistance in the host plant, by induced systemic resistance (ISR). It is effective against different types of plant pathogens. The triggering of disease resistance by nonpathogenic bacteria depends on plant species to a different extent. Hence, it is interesting to find strains that can stimulate a wide array of plants in order to select them for potential commercial uses. (9)

Recently, the term "induced systemic resistance (ISR) was introduced to designate the resistance induced in leaves of plants by inoculation of roots with non-pathogenic rhizobacteria and increased expression of natural defense mechanisms of plants against various type of pathogens. Induced resistance exploiting natural defense machinery of plants could be proposed as an alternative, non-conventional and ecologically-friendly approach for plant protection. Its introduction into agricultural practice could minimize the scope of chemical control, thus contributing to the development of sustainable agriculture. (10, 11)

The aim of this research was to test the ability of *Serratia marcescens* to inhibit the *F. oxysporum in vitro* and study the ability of these bacteria to induce the systemic resistance of barely that infected with fungus by increasing the specific activity of peroxidase and polyphenol oxidase which represent the two types of defense enzyme in plants.

MATERIALS & METHODS

Bacterial and fungal strains

The 7 bacterial strains of *S. marcescens* were obtained from Biotechnology Department- College of Science- Baghdad University. While the fungal strain of *F. oxysporum* was obtained from Institution of Biotechnology and Genetic Engineering - Baghdad University

Spore Suspension preparation

F.oxysporum culture was inoculated on potato dextrose agar slants, incubated for one week, micro conidia were harvested by adding 5 ml of sterilized water in each tube and surface of medium was scraped with the help of sterilized spatula. Spore suspension from each tube was passed through two layers of gauze to remove mycelial fresh weight. Spores were counted with haemocytometer and suspension was adjusted to 10^4 spores/ml by adding sterilized distilled water. (2)

The Pathogenicity Test

25 Barely seeds were soaked in *F.oxysporum* spore suspension with concentration 1×10^4 spore/ml for 18 hours. The control treatment was soaked in distilled water. Then seeds were put on filter paper and watered with sterilized distilled water and leave in room temperature. The germination percent was calculated after 3 days. (12)

Bacterial antagonism test

Antagonism between the bacterial strains and fungi was determined as described by (13). A 5mm disc of *F.oxysporum* was placed on one side of potato dextrose agar and incubated at 25 c. After two days of incubation, a loopfull of overnight culture of *S.marcescens* strains with concentration 1×10^7 was streaked on the opposite side of the fungus growth and incubated. The diameter (mm) of the inhibition zone between the bacteria and the fungus was used as an indication of the extent of antagonism. Tree plates were used as replicates for

each strain. Percentage Inhibition was calculated as:

Inhibition percentage = Colony growth diameter in control plate **-** Colony growth diameter in each treatment **/** Colony growth diameter in control plate

Preparation of talc based formulation

A loop full of SM5 was inoculated in to the nutrient broth medium and incubated for 48 hours at 28 c at 125 rpm. After incubation, the broth containing 1×10^7 cfu/ml was used for the preparation of talc based formulation. To 400 ml bacterial suspension 1 kg of talc powder, calcium carbonate 15 g (to adjust the pH to neutral and carboxy methyl cellulose 10 g (as additive) were mixed under sterile condition. The product was shade dried to reduce the moisture content to 20% and then packed in polypropylene bags and sealed. (1)

Pot Experiment

25 Barely seeds (Ipa'a 99) from State Board for Seeds Testing and Certification/ Ministry of Agriculture were sterilized with 1% sodium hypochlorite and rinsed tree times with distilled water are sowed in plastic pots with 20 cm diameters and 14 cm height which sterilized with 70% ethanol and filled with 3 Kg of sterilized soil by autoclave for 20 minute. The soil prosperities were the following: sand 62%, silt 16% and clay 22%.

Four treatments were performed including the following:

Control: the seeds were soaked for 18 hours in distilled water and sowed. This treatment was symbol as A.

Bacteria: the seeds were soaked for 18 hours in distilled water and sowed. After 2 weeks 5g of *S.marcescens* SM5 talc based formation was added to each plant. This treatment was symbol as B.

Fungi: the seeds were soaked for 18 hours in spore suspension and sowed. This treatment was symbol as C.

Fungi + Bacteria: the seeds were soaked for 18 hours in spore suspension and sowed. After 2 weeks 5g of S. *marcescens* SM5 talc based formation was added to each plant. (14), this treatment was symbol as D.

These tests were performed at the plant field of Baghdad University/ college of science. The plant was watered twice in week with same amount of tap water. After 15 days the germination percent was calculated. The germination plants were reduced to 7 in every pot.

After 2, 3 and 4 weeks of plating, the random plant from each pot was carefully uprooted and wash under tap water to calculate the fresh weight. Then 1 gram of leaf tissue was homogenized with 1 ml of ice sodium phosphate buffer (pH 7.0). Homogenized samples were centrifuged at 10.000 rpm for 10 minute. The supernatant solution was used as sources for analyzing peroxidase and polyphenol oxidase. (15)

Assay of peroxidase

The reaction mixture consisted of 1.5 ml 0.05 M pyrogallol, 0.5 ml of the enzyme extract and 0.5 ml of 1% H2o2. The changes in absorbance at 420 nm were recorded at 30 second intervals for 3 minute.

Assay of polyphenol oxidase

The reaction mixture consisted of 200 µl of the enzyme extract and 1.5 ml of 0.1 M sodium phosphate buffer (pH 6.5). To start the reaction, 200 µl of 0.01 M catechol. The changes in absorbance at 495 nm were recorded at 30 second intervals for 3 minute. (16)

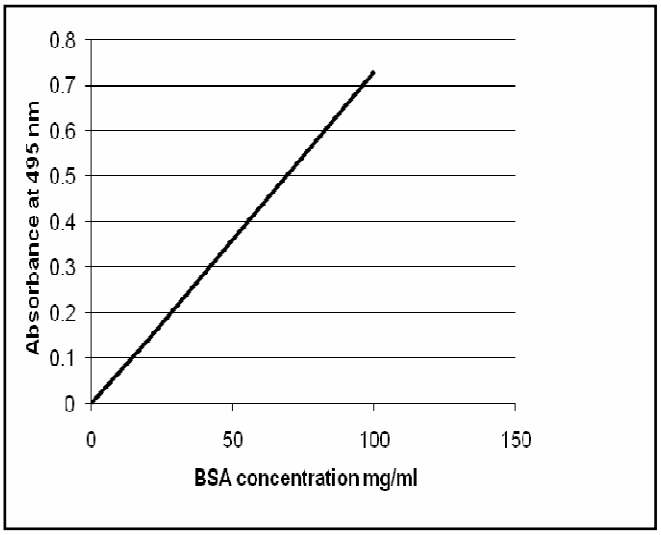


Figure (1): the standard curve of bovine serum albumin (BSA)

Statistical analysis

All experiments were performed according to the Complete Random Design (C.R.D) and the average means was compared according to the (L.S.D). The degree for significant variation used in statistical analysis was $P<0.05$.(7)

RESULTS

Pathoginicity test

The results showed that the germination percentage in control treatment was 76.6%.While was 34.2% when seeds soaked in the *F.oxysporum* spore suspension.

Antagonism test

As shown in table (1) the *S. marcescens* strains have ability to antagonism the *F.oxysporum* with different range. The SM5 strain have highest ability with 60.8%.This strain was chosen to complete other experiment of this study.

Pot Experiment

The results showed that the germination percentage was 17.6% when seeds soaked in distilled water while this percentage decreased to 13.1% when seeds soaked in spore suspension as shown in table (2).The fresh weight and specific activity of peroxidase and polyphenol oxidase was higher in the treatment B and D when talc based formulation of SM5 was added to the root after infect with fungi, while this activity was decreased in the plants which are infect with *F.oxysporum* and did not receive any antagonism bacteria (treatment A and C).

DISCUSSION

Pathoginicity test

The results showed that the *F.oxysporum* have ability to infect the barely seeds. The *F.oxysporum* produces many types of toxins like Phytotoxic, Naphthoquinones, Isomarticin, Javanicin, Fusarubin, and Dihydrofusarubin that cause serious diseases and may cause the death of plant cell (17, 18, 19)

Antagonism test

S. marcescens SM5 was able to inhibit the *F.oxysporum In vitro* and this may be because the different materials that produced by this type of bacteria. Many previous studies showed the ability of *S.*

marcescens to produce substances like red pigment (Prodigiosin) and chitinase that able to inhibit many types of fungi like *Alternaria alternate, Aspergillus niger, Fusarium Oxsysporum , Botrytis cinerea, Cochliobolus miyabeanus, Pythium spinosum and P.ultimum* .(13,20,21)

Pot Experiment

After 2 weeks of plating, the germination percentage, fresh weight and the specific activity of both peroxidase and polyphenol oxidase in the treatments A and B as well as C and D was to approach one another because the bacterial talc based formulation was not added yet as shown in table(2) and figure(2,3). But when bacterial formulation added, the fresh weight was increased in the treatment B and D as shown in table (2) and figure (2, 3). This increased fresh weight and the specific activity of both peroxidase and polyphenol oxidase may be because the effect of *S. marcescens* SM5 which contribute in the improvement the plant growth and induce the systemic resistance of plant against pathogen. Ramomoorty *et.al.* found that application of talc-based formulation of *Pseudomonas fluorescens* isolate Pf1 increases plant growth in the field and reduced disease incidence. The increase in plant growth might be associated with secretion of auxins ,gibberellins and cytokinins and suppression of deleterious microorganisms in the rhizosphere(3). The use of antagonists as seed treatment or soil application will help in managing the disease spread through induction the systemic resistance which helps in reducing the secondary spread of the disease by zoospores. If the infection is minimized by induced systemic resistance (ISR), the management of the disease will become cost effective and efficient. (22)

It has been found that ISR induction was correlated to the up-regulation of different pathogenesis related (PR) and defense related proteins (chitinases, glucanase, peroxidases and specific phytoalexins) and enzyme activities, especially phenyl alanine ammonia lyase and synthesis of other phenols and related proteins (20). The increased activities of peroxidase and polyphenol oxidase can limit disease development through the formation of polymerized phenolic barriers around the sites of infection. In addition, peroxidase and polyphenoloxidase can contribute to synthesis of anti-nutritive, antibiotic, and cytotoxic compounds leading to enhanced resistance against pathogens (23). The induction of ISR relies on specific plant PGPR strain interactions. In *Arabidosis thaliana*, the PGPR strain *P. fluorescens* WCS417r was capable of eliciting an ISR response on most ecotypes, but not on ecotypes RLD and Wassilewskija. Subsequent genetic studies led to the identification of the *ISR1* locus that not only controls the ability to respond to *P.fluorescens* WCS417r, but also basal resistance to *Pseudomonas syringae* pv .tomato. After further research, the *ISR1* locus was found to play a role in the ethylene signaling path way of *A.thaliana*,

therefore ecotypes RLD and Wassilewskija carried a recessive trait that affected ISR by disrupting ethylene signaling while leaving SAR intact. This demonstrates that among *A.thaliana* ecotypes, allelic variability exists in regulatory genes that influence ISR pathways. (5)

These results agree with many previous studies which able to induction of systemic resistance of different plants such as tomato and hot pepper by using many types of microorganisms like *Bacillus*, *Pseudomonas*, and *Trichoderma* (3, 22, 24, 25)

The present work suggests to using the microorganisms in plant diseases protection as alternative way to use the chemicals and pesticide which harmful to environment and human.

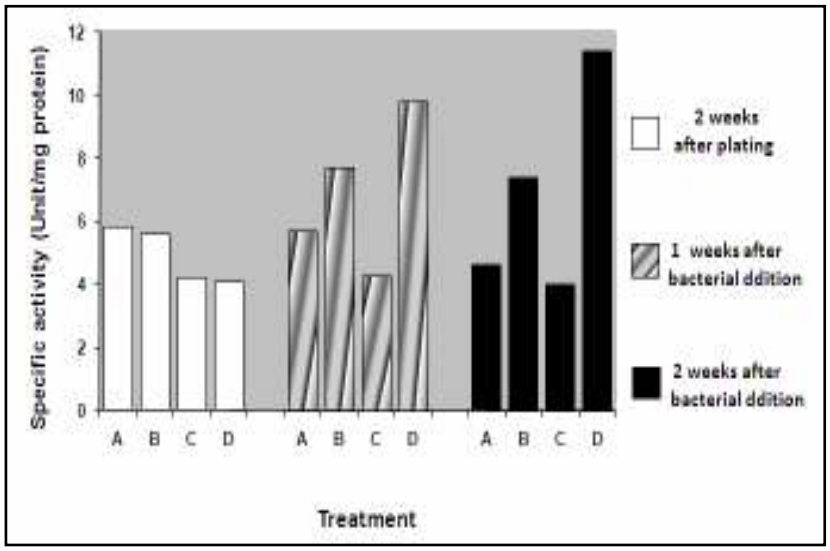
Table (1) The antagonism activity of *S.marcescens* strains against *F.oxysporum*

Bacterial Strains	Inhibition (%)
SM1	48.3
SM2	55.4
SM5	60.8
SM6	35.2
SM7	39.1
SM8	30.8
SM10	43.7

Table (2): The effect of different treatment on barely germination and fresh weight

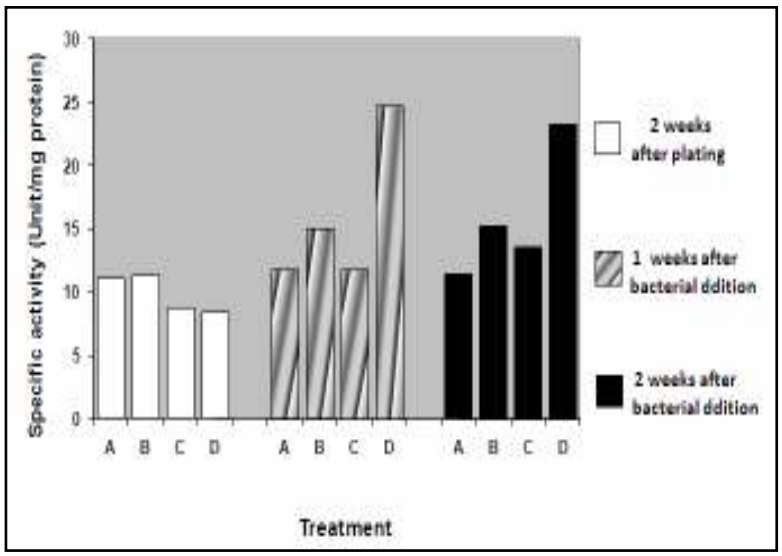
Treatment	2 weeks after plating		1 week after bacterial addition	2 week after bacterial addition
	Germination percent %	Fresh weight (g)	Fresh weight (g)	Fresh weight (g)
A	17.3 a	1.03 a	1.36 b	2.23 b
B	17.1 a	9.96 a	1.54 a	2.43 a
C	11.4 b	0.72 b	0.82 c	1.37 c
D	11.3 b	0.71 b	0.95 d	1.58 d
L.S.D.	1.0871	0.2977	0.3261	0.4175

A= seeds were soaked for 18 hours in distilled water only.
B= seeds were soaked for 18 hours in distilled water. After 2 weeks 5g of SM5 talc based formation was added to the root.
C= seeds were soaked in spore suspension only.
D= seeds were soaked in spore suspension. After 2 weeks 5g of SM5 talc based formation was added to the root.



Figure(2): the specific activity of peroxidase

A= seeds were soaked for 18 hours in distilled water only. **B=** seeds were soaked for 18 hours in distilled water. After 2 weeks 5g of SM5 talc based formation was added to the root. **C=** seeds were soaked in spore suspension only **D=** seeds were soaked in spore suspension. After 2 weeks 5g of SM5 talc based formation was added to the root.



Figure(3): the specific activity of polyphenol oxidase

A= seeds were soaked for 18 hours in distilled water only

B= seeds were soaked for 18 hours in distilled water. After 2 weeks 5g of SM5 talc based formation was added to the root.

C= seeds were soaked in spore suspension only.

D= seeds were soaked in spore suspension. After 2 weeks 5g of SM5 talc based formation was added to the root.

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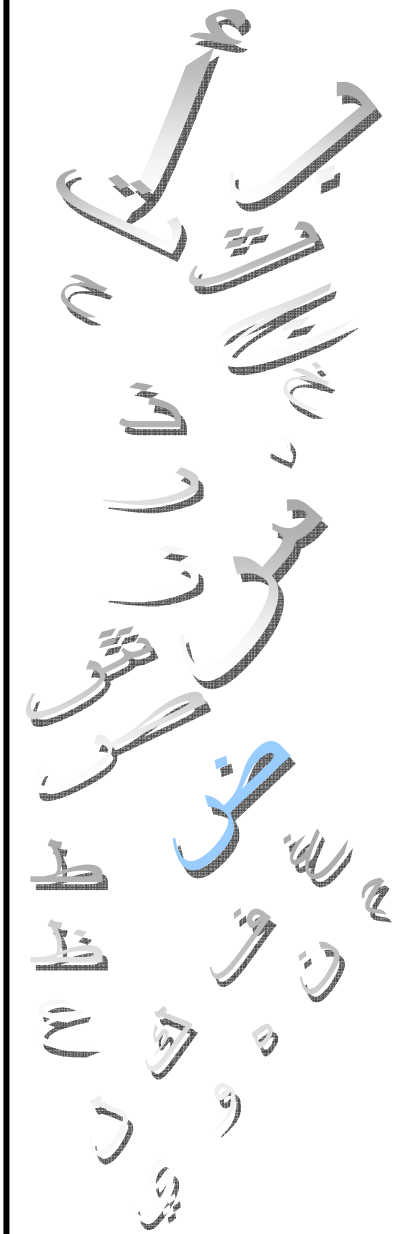
قسم الدراسات العربية

ARABIC SECTION



التغيرات النسيجية في رئات الفئران المتسببة من الإصابة بجرثومة *Listeria Monocytogenes*

تغريد خضر محمد

المعهد الطبي التقني المنصور / بغداد – جمهورية العراق

البريد الإليكتروني: taghreidkheder@yahoo.com

ABSTRACT

Listeria monocytogenes were diagnosed by using API LISTEIA Kit , selective and differential culture media. The colonies of ***Listeria monocytogenes*** appeared on blood agar base with transparent halo of Beta haemolysis . Tryptic soy agar was used for the secondary culture, whereas Oxford agar; Modified Fraser broth ; blood agar base and CHROM agar medium were used to isolate and diagnose ***Listeria monocytogenes*** after 24 hr of incubation period at 37 c^0 .
Histopathological studies of infected lungs in male and female mice with ***Listeria monocytogenes*** and crud or purified Listeriolysin O toxin had shown the filtration of neutrophils and macrophages in the branches of alveoli. The effect of LLO in female of mice severe than in male was more . It caused necrosis and pulmonary hemorrhages. Pregnant female mice represented the high risk group of ***Listeria monocytogenes*** infection. The infectious tissues of pregnant mice with ***L. monocytogenes*** represented the accumulation of neutrophils and macrophages in alveolar spaces and many pulmonary blood vessels. In some areas, alveoli had been obliterated. Abortion took place in many female mice as a result of infection with ***L. monocytogenes*** .

الملخص باللغة العربية

تـــم تـــشخيص جرثومـــة ***Listeria monocytogenes*** بواسـطة عـدة API LISTERIA Kit , والأوسـاط الزرعيـة الانتقائية والتفريقية، حيث ظهرت مستعمرات ***L. monocytogenes*** على وسط blood agar الأساس محاطة بهالة شفافة نتيجة لتحلل الدم نـوع بيتـا. و استخدم وسط treptic soy agarلأغراض الـزرع الثـانوي. كمـا اسـتخدم وسط Listeria selective agar base , Oxford وتـــشخيص جرثومـــة ***L. monocytogenes*** بعد مرور (24) ساعة من الحضن بدرجة حرارة (37) م^0 .
وقد أظهرت الدراسة النسيجية إن عالق جرثومة ***L. monocytogenes*** وذيفـان (LLO) Listeriolysin O الخـام والمنقـى قـد سـبب ارتـشاحا للخلايـا المتعادلـة وخلايـا الـبلاعم الكبيرة في تفرعات القصبات الهوائية في إنـاث الفئران المحقونة . وكان تأثير الـذيفان أشد علـى إناث الفئران المحقونة مقارنة مع ذكور الفئـران المحقونـة بالـذيفان ,إذ سـبب تخثـرا ونزفـا فـي الرئة , كما أظهرت الدراسة النسيجية ان النـزف كان اشد فـي رئتـي الفئـران الحوامـل فضلا عن حدوث إجهاض في بعض إناث الفئران الحوامل

المقدمة

تصيب جرثومة ***Listeria monocytogenes*** العاملين في المزارع [1] , والمسالخ [2] ، والنساء الحوامل ، والأجنة والأطفال حديثي الولادة, ومرضى السرطان ,والسكري , ومرضى العوز المناعي المكتسب (Acquired Immunodeficiency Syndrome - AIDS) (3، 4) وكذلك تصيب العديد من الحيوانات مثل الدجاج والأبقار والأغنام والفئران وخنازير غينيا.

أكدت العديد من الدراسات انه بالإمكان تشخيص جرثومة ***Listeria monocytogenes*** في المرأة الحامل بأخذ مسحات المهبل (Vaginal Swabs) والدم لغرض زرعها على الأوساط الزرعية الانتقائية وغير الانتقائية لتشخيص جرثومة الليستريا ,أو بأخذ المصل لإجراء الفحوصات المصلية , كما يمكن تشخيص جرثومة ***L. monocytogenes*** في عينات المشيمة(Placenta) وذلك بزرعها على الأوساط الانتقائية, وأصبح بالإمكان ملاحظة التغيرات النسيجية التي تطرأ على أنسجة المشيمة خلال مدة الإصابة بجرثومة ***L. monocytogenes*** باستخدام المجهر الالكتروني والمجهر الضوئي [5و6]. وقد يلجأ بعض الأطباء إلى اخذ عينات من كبد وطحال ودماغ جثث الأجنة الميتة أو الطفل الميت بعد الولادة والتي تسمى بمواد تشريح الجثث (Necropsy materials) لغرض عزل وتشخيص جرثومة ***Listeria monocytogenes*** في تلك المقاطع النسيجية [7 و 8].

تسبب جرثومة ***Listeria monocytogenes*** تنخرا (Necrosis) ونزفا (Haemorrhage) في رحم ومشيمة الأم الحامل بسبب إنتاج (Tumor necrosis factor-TNF) من الخلايا المناعية(الخلايا البيض Leukocytes) مما يؤدي إلى فقدان الجنين [9] ,كما أشار الباحثان غوليريا وبولاريد [1] ، إلا إن كلا من (Macrophage) (Growth Factor,MGF) و (Colony stimulating factor- (CSFتنتجان بكميات كبيرة من الخلايا الظهارية(Epithelial cells) المبطنة للرحم خلال مدة الإصابة بجرثومة الليستريا، حيث يقوم هذان العاملان بتحفيز (Trophblasts) الموجودة في المشيمة لتكوين عوامل جذب كيميائية للخلايا المتعادلة، فتجذبها إلى مكان تواجد الجرثومة ***monocytogenes*** في المشيمة والخلايا البيض(Leukocytes) في أنسجة الجنين كالرئتين والكبد [10] .

يعد الذيفان Listeriolysin O من أهم العوامل ذات التأثير القوي والمتسبب بإصابات عديدة في الفئران البالغة، حيث تغزو جرثومة ***L. monocytogene*** (Macrophages) بمساعدة ذيفان Listeriolysin O الذي يسبب تحلل الجسيمات الحالة(Phagolysosomes) وبذلك يمكن التمييز بين السلالة المسببة للمرض والسلالة الغير ممرضة في الإنسان والفئران .[11]

أثبتت بعض الدراسات إن جرثومة ***L. monocytogenes*** بإمكانها التكاثر بسرعة في مشيمة الإنسان والفئران وخنازير غينيا بعد حقنها في الوريد وبجرعة (10^3 - 10^5) خلية حية /مل بعد مرور 24 ساعة من الحقن وان هذه الجرثومة لها القدرة على الانتقال من المشيمة إلى أعضاء الجسم الأخرى مثل الرئتين والكبد والطحال وسائل النخاع ألشوكي عن طريق الدم .[12]

في هذه الدراسة الحالية سيتم الكشف عن تأثير غزو جرثومة ***Listeria monocytogenes*** وذيفان (Listeriolysin O (LLO في رئتي إناث وذكور الفئران المحقونة داخل الصفاق(Intraperitonial) واثبات إن ذيفان LLO له دور فعال في حدوث الامراضية وتعتبر هذه اول دراسة في العراق يتم فيها دراسة تاثير الذيفان في رئتي الفئران.

المواد وطرق العمل

1- **عزلة** ***Lisreria monocytogenes*** :

تم الحصول على عزلة ***Lisreria monocytogenes*** والتي يرمز لها بالرمز Lis.T2 بواسطة الباحثة تغريد خضر محمد حيث قامت بعزلها من امراة عراقية كانت تعاني من الاجهاض المتكرر في بغداد وتم ذلك في مختبرات الصحة المركزي الخاص لعزل

وتشخيص المايكروبات ,وكانت العزلة مخزونة في وسط Maknur)(Tryptic soy broth- (TSB) المضاف له 10% كليسرول بدرجة (70-) م^0 , وقد كان مصدر هذه العزلة من مسحة مهبل المرأة التي كانت تعاني من الإسقاط المتكرر (Repeated abortion)

2- تشخيص جرثومة Lisreria monocytogenes :

تم تشخيص جرثومة ***Lisreria monocytogenes*** باستخدام الأوساط الزرعية والعدد التالية:

أ. وسط اغارتريتون الصويا Bacto)(Tryptic soy Agar –TSA)
تم تحضير الوسط وفقا لتعليمات الشركة المجهزة للوسط بإذابة (41) غراما من الوسط في (1000) مليلتر من الماء المقطر ثم ضبط pH إلى (7.3) . تم تعقيم الوسط ألزرعي بالموصدة بدرجة حرارة (121) م^0 لمدة (15) دقيقة , ثم برد إلى(45) م^0 , وأضيف له عينة دم بشرية حديث السحب بنسبة (5%) .
استخدم هذا الوسط لعزل وتشخيص وحفظ جرثومة ***Lisreria monocytogenes*** بدرجة حرارة (4) م^0 ولمدة تتراوح بين (4-5 أسابيع).

ب. مرق تربتون الصويا Maknur)(Tryptic soy broth-TSB)
حضر الوسط مختبريا وفق تعليمات شركة Maknur، استخدم هذا الوسط لعزل جرثومة ***Lisreria monocytogenes*** واستخلاص ذيفان Listeriolysin O مع إضافة 0.5% من الكلوكوز و0.5% من مسحوق خلاصة الخميرة Yeast extract .

ج. أغار لستيريا الانتقالي الأساس Listeria (selective agar base) :
يسمى الوسط باسم – Oxoid)(Oxford agar (OXA) , حضر الوسط مختبريا وفق تعليمات شركة اكسويد (Oxoid) , يمكن استخدام هذا الوسط لتشخيص جرثومة ***Lisreria monocytogenes***.

د. CHROM agar Listeria medium
حضر الوسط مختبريا وفق تعليمات شركة (Chromagar ,Franch) .

ه. وسط مرق فريسر المحور Modified (Fraser Broth-MTB) :
حضر الوسط وفق تعليمات منظمة الغذاء والدواء الأمريكية [13] .

و. وسط الدم الأساس (Difco) (Blood agar base-BAB)) .
حضر الوسط وفق تعليمات منظمة الغذاء والدواء الأمريكية [13] مضافا له دم إنسان بنسبة (5%)

ز. (Bio Mereiux)(API LISTERIA Kit)
حضنت جميع الأطباق المزروعة والعدة الخاصة بتشخيص اللستيريا بدرجة حرارة (37) م^0 لمدة (24) ساعة.

3- استخلاص وتنقية ذيفان O: Listeriolysin
تم استخلاص ذيفان Listeriolysin O-LLO الخام على وفق كريفت وجماعته[14] , كما تم تنقية ذيفان LLO من طافي عزلة Lis.T2 على وفق طريقة جينكز وجماعته ولوبيتال وجماعته[15 and 16] . استخدمت طريقة فولن –لوري [17] لتقدير البروتين خلال مراحل الاستخلاص والتنقية .

4- تركيز نموذج ذيفان Listeriolysin O :
ركز ذيفان Listeriolysin O الذي تم الحصول عليه بعد الترشيح الهلامي بهلام - Pharmacia) Sephadex G-200 (Sweden. باستخدام هلام Sepadex G-25 , إذ وزن (0.042) غرام من هلام Sepadex G-25, ثم وضع في أنبوبة ابندروف نظيفة ومثقوبة من الأسفل بواسطة الابرة المستخدمة لحقن الأنسولين,وضعت الأنبوبة المثقوبة في أنبوبة بلاستيكية صغيرة مقعرة لتثبيت أنبوبة الابندروف ,و جمع بروتين (LLO) بعد عملية الطرد المركزي , بعدها أضيف (150) مايكروليتر من النموذج المراد تركيزه في أنبوبة ابندروف , وضع النموذج مع الهلام بدرجة (4) م^0 لمدة (15) دقيقة, وعرض النموذج للطرد المركزي بسرعة (3000) دورة/دقيقة لمدة(3) دقائق ثم جمع النموذج.

5- دراسة التغيرات النسيجية الناتجة عن ذيفان Listeriolysin O :

تم حقن مجموعتين من الفئران البيض بعمر يتراوح بين(18-22) يوما بوزن (22-25) غراما , ومجموعة ثالثة بجرثومة ***Lisreria monocytogenes*** بذيفان Listeriolysin O ,وللسيطرة حقنت مجموعة رابعة من الفئران بمحلول (0.5) مليلتر من دارئ الفوسفات ذي القم الهيدروجيني (7.6) pH , كما في جدول (1) .

جدول (1) : مجاميع الفئران البيض الذكور والإناث المحقونة بجرعات محددة من ذيفان Listeriolysin O وجرثومة *Lisreria monocytogenes* .

الحيوان	مادة الحقن	التركيز	الجرعة(مليلتر)	منطقة الحقن
(5) فئران ذكور (5) فئران إناث	ذيفان LLO الخام	625 مايكرو غرام/مليلتر بروتين	0.5	داخل الصفاق
(5) فئران ذكور (5) فئران إناث	ذيفان LLO المنقى	200 مايكرو غرام/مليلتر بروتين	0.25	داخل الصفاق
(5) فئران إناث حوامل	***Lisreria monocytogenes***	$10x2^8$ خلية/مليلتر	0.5	داخل الصفاق
(5) فئران إناث	دارئ الفوسفات PH(7.6)	----------	0.5	داخل الصفاق

قتلت الفئران المحقونة التي لم تمت بعد(72) ساعة وشرحت مباشرة وأخذت الرئتين من الفئران المحقونة بذيفان Listeriolysin O الخام أو المنقى, ومن إناث المحقونة بجرثومة ***Lisreria monocytogenes*** استخدمت طريقة هوماسون [18] لتحضير المقاطع النسيجية.

النتائج

تم الكشف عن وجود جرثومة ***Lisreria monocytogenes*** من خلال الفحص ألمجهري للشرائح الملونة بملون Gram stain باستخدام المجهر الضوئي الاعتيادي, وكانت ذات شكل عصوي كروي (Coccobacilli) ايجابية لملون Gram stain ,وقد اتخذت بعض الخلايا الشكل المنحني وهذا عائد إلى ظاهرة تعدد الإشكال (Pleomorphism) التي تتميز بها هذه الجرثومة .بينت الاختبارات الكيموحيوية إن عزلة ***Lisreria monocytogenes*** لها القابلية على تخمير سكر الارابيتول α-methyl-D-Glucoside على أنها لم تبد قابلية في تخمير سكر الراميزوز ,كما إن العزلة ***Lisreria monocytogenes*** كانت ايجابية لاختبار الاسكيولين, إذ تكون اللون الأسود في الحفر الخاصة باستهلاك الاسكيولين (ESC) في عدة API LISTERIA Kit.

ظهرت مستعمرات جرثومة ***Lisreria monocytogenes*** على وسط (Oxford) agar-OXA بلون اسود محاط بهالة سوداء ، وبلغ قطر المستعمرة(1) مليمتر ، محدبة وذات ارتفاع قليل على سطح الاغار بعد مرور (24) ساعة من فترة الحضن .وكانت صفات المستعمرات العائدة لجرثومة ***Lisreria monocytogenes*** مماثلة إلى ماجاء في دراسات عالمية عند استخدام وسط (OXA) . [19 ,20 and 21]

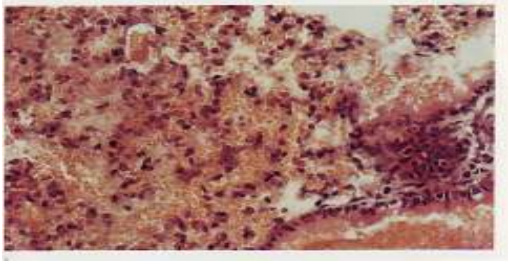
تكون لون اسود داكن في وسط Modified Fraser Broth-MFB ، نتيجة لاستهلاك الاسكيولين من قبل جرثومة ***Lisreria monocytogenes*** وذلك بعد مرور (24) ساعة من فترة الحضن بدرجة(37) ^{0} م . كما ظهرت مستعمرات جرثومية ***Lisreria monocytogenes***على وسط أغار الدم الأساس (Blood agar base-BAB) محاطة بهالة شفافة نتيجة لإنتاج هذه المستعمرات ذيفان listeriolysin O عند حضن الأطباق بدرجة (37)^{0}م ولمدة (24) ساعة .

يعد وسط (CHROMagar *Listeria* medium) من الأوساط الزرعية التفريقية

(Differential) والانتقائية الحديثة المستخدمة حاليا في معظم الدول الغربية المتقدمة لعزل جرثومة ***L. monocytogenes*** وتميزها عن L. ivanovii . أما في دراستنا الحالية ، فقد استخدم وسط (CHROMagar *Listeria* medium) لأول مرة في القطر العراقي ، إذ ظهرت مستعمرات ***L. monocytogenes*** زرقاء مخضرة ومنتظمة ومحاطة بهالة بيضاء (white) halo بعد مرور (24) ساعة من فترة الحضن بدرجة (37)^{0}م.

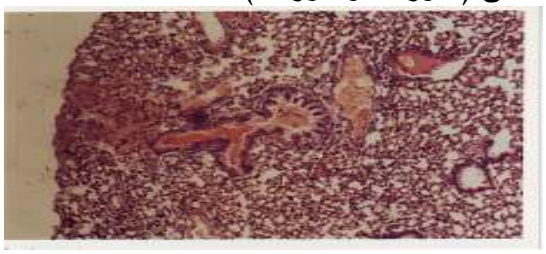
أظهر فحص المقاطع النسيجية بالعين المجردة حدوث تغييرات واضحة في أعضاء الحيوانات المحقونة بعالق جرثومة ***Lisreria monocytogenes*** بتركيز ($10x2^{8}$) خلية/مليلتر، وكذلك التغييرات في أعضاء الحيوانات المختبرية المحقونة بمستخلص الهيمولايسين بيتا الخام (ذيفان listeriolysin O) المنتج من جرثومة الليستريا، إذ لوحظ حدوث زيادة في حجم الكبد مع تكون بقع لونية بيضاء مصفرة على سطح الكبد المصاب لكلا الجنسين من الفئران،في حين أنها لم تظهر في كبد مجموعة السيطرة في الحيوانات غير المحقونة، كما لوحظ حدوث انتفاخ في أمعاء الفئران لكلا الجنسين .

كان تأثير العالق الجرثومي لجرثومة ***Lisreria monocytogenes*** ومستخلص ذيفان Listeriolysin O الخام والمنقى في المقاطع النسيجية الخاصة برئتي الإناث اكبر في احداث التغييرات النسيجية التي ظهرت في المقاطع النسيجية الخاصة برئتي ذكور الفئران ، إذ تضمنت التغيرات حدوث ارتشاح الخلايا المتعادلة (Neutrophils) وخلايا البلاعم الكبيرة (Macrophages) في تفرعات القصبات الهوائية ونزف دموي شديد(Haemorrage) داخل القصبات الهوائية (صورة 1) وتحلل جدران الحويصلات.

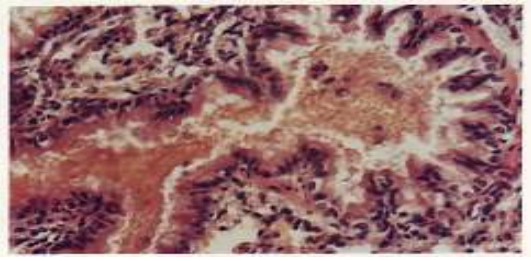


صورة(1) : مقطع في رئة لأنثى فارة حامل محقونة بجرثومة *Listeria monocytogenes* بتركيز ($10x2^{8}$) خلية/مليليتر، تميز بارتشاح الخلايا المتعادلة والبلاعم الكبيرة وحدوث نزف (x100).

كما ظهر تجمع الخلايا المتعادلة وخلايا البلاعم الكبيرة ونزف داخل الوريد الرئوي وتجمع الخلايا الالتهابية (Inflammatory cells) في القصبات وحدوث تليف (Fibrosis) في رئتي إناث الفئران الغير حوامل المحقونة بذيفان LLO المنقى (صورة 2 وصورة 3).

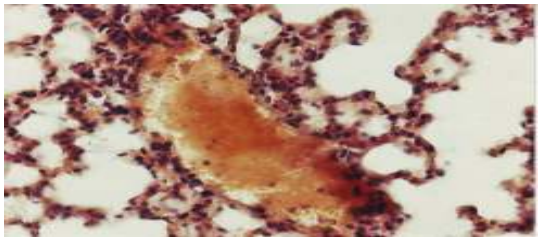


صورة(2) : مقطع في رئة لأنثى فارة غير حامل محقونة بذيفان LLO المنقى بتركيز (200 مايكرو/مليلتر)، تميز بتجمع الخلايا المتعادلة وخلايا البلاعم الكبيرة داخل الوريد الرئوي (X100) .



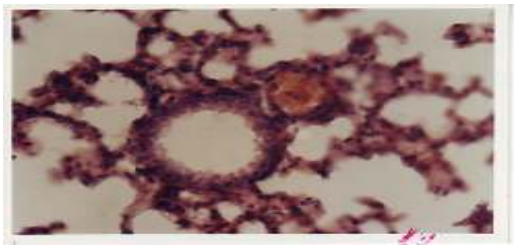
صورة(3) : مقطع في رئة لأنثى فارة غير حامل محقونة بذيفان LLO المنقى ، تميز بحدوث نزف دموي في القصبات الهوائية (X400) .

كان تأثير LLO الخام بتركيز (625مايكروغرام/مليلتر) اقل تأثيرا مما هو عليه في رئتي إناث الفئران المحقونة بذيفان LLO المنقى، إذ تميزت المقاطع النسيجية بوجود نزف دموي في الرئة وارتشاح عدد قليل من الخلايا المتعادلة داخل نسيج الرئة والقصبات الهوائية (صورة 4).

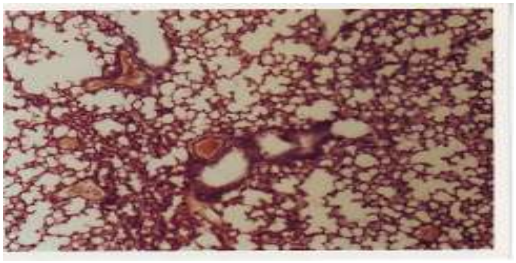


صورة(4) : مقطع في رئة لأنثى فأرة غير حامل محقونة بذيفان LLO الخام، تميز بحدوث نزف دموي وارتشاح عدد فليل من الخلايا المتعادلة (X400) .

لوحظ في بعض المقاطع النسيجية لرئتي الفئران الإناث المحقونة بذيفان LLO الخام والمنقى عدم وجود جدران للحويصلات الهوائية وتنخر (Necrosis) (صورة 5 و6)

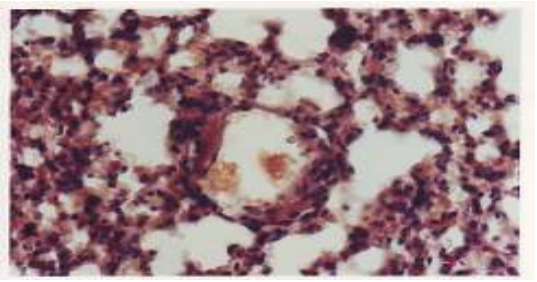


صورة(5) : مقطع في رئة لأنثى فأرة غير حامل محقونة بذيفان LLO الخام ، تميز بعدم وجود جدران للحويصلات الهوائية وتنخر(X400) .

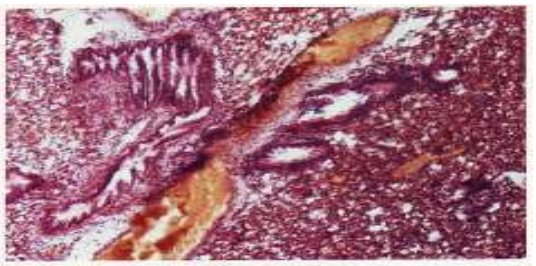


صورة(6) : مقطع في رئة لأنثى فأرة غير حامل محقونة بذيفان LLO المنقى، تميز بعدم وجود جدران للحويصلات الهوائية (X100) .

أما تأثير ذيفان LLO الخام والمنقى في رئتي ذكور الفئران المحقونة فقد تمثل بحدوث نزف دموي وتجمع للخلايا الالتهابية في القصبات اقل مما هو عليه في رئتي إناث الفئران المحقونة بالذيفان (صورة 7و8).



صورة(7) : مقطع في رئة ذكر الفأر محقون بذيفان LLO الخام، تميز بحدوث نزف دموي وتجمع الخلايا الالتهابية . (X400)



صورة(8) : مقطع في رئة ذكر الفأر محقون بذيفان LLO المنقى، تميز بحدوث نزف وتجمع الخلايا الالتهابية . (X100)

المناقشة

وجد من دراستنا الحالية انه عند حقن الفئران بذيفان Listeriolysin O (LLO) الخام أو المنقى فان تاثيره يكون اشد مما هو عليه عند حقنها بعالق جرثومة ***Lisreria monocytogenes*** ، وذلك بسبب إن تأثير ذيفان LLO يكون مباشر على الخلية المستهدفة (Target cell) ، بينما عند حقن الفئران بجرثومة ***Lisreria monocytogenes*** فأنة يحتاج إلى وقت معين للارتباط مع الخلية المستهدفة اعتمادا على جنس وعمر ومناعة المضيف ، بعدها تبدأ بالتضاعف داخل خلايا المضيف، ثم تنتج الجرثومة ذيفان LLO الذي يسبب تغيرات نسيجية في الرئتين وباقي أعضاء جسم المضيف .

بينت بعض الدراسات انه بعد حقن خنازير غينيا بعالق جرثومة ***Listeria monocytogenes*** ($10x1^8$) خلية/مليلتر داخل الوريد أو ($10x2.5^8$) خلية/مليلتر داخل الصفاق ، وبعد مرور (24) ساعة من الحقن ثم قتل الحيوانات المحقونة وفحص رئتيها ، فوجود (edema) و(congestion) في الرئة المصابة وزيادة سمك جدران الحويصلات نتيجة لغزو جرثومة الليستريا لخلايا وحيدة النواة (Mononuclear cells) ولخلايا متعددة النواة (Polymorphnuclear cells) . [22 and 23]

تمتلك خلايا البلاعم الكبيرة الموجودة في رئتي الفئران (Alveolar macrophages) على سطحها مستقبلات Fc-receptor للجلوبيولين المناعي IgG الذي يقوم بدور هام في المقاومة المبكرة (Early resistance) ضد جرثومة الليستريا، كما إن لخلايا البلاعم الكبيرة والخلايا المتعادلة (Neutrophils) دور مهم في مقاومة

البكتريا الداخل خلوية (Intracellular bacteria) بوجود O_2 والإنزيمات الحالة للجسيمات (Lysosomes) فيزداد إنتاج جذر(Superoxide-O_2) فتقتل جرثومة الليستريا بعملية الأكسدة (Oxidative mechanisms) ، لذا يلاحظ وجود خلايا (Alveolar macrophages) في القصيبات الهوائية وما بين القصبات (Alveolar spaces) [24]. أظهرت بعض الدراسات قابلية جرثومة ***Listeria monocytogenes*** على غزو خلايا البلاعم الكبيرة في الحويصلات الرئوية في إناث وذكور الفئران المحقونة ،ونادرا ما تغزو جرثومة الليستريا الخلايا الطلائية لرئتي الفئران [25] . كما تمكن بعض الباحثين من تشخيص وعزل جرثومة الليستريا في بعض الحيوانات الأخرى مثل الفرس، إذ لوحظ إن جرثومة ***L. monocytogenes*** وذيفان listeriolysin O لها القابلية على إحداث آفات (Lesions) ونزف حاد في الرئتين والتهاب السحايا [27 and 26] .

تسبب جرثومة ***Listeria monocytogenes*** الإجهاض المتكرر في النساء والفئران والقردة وذلك لقدرة هذه الجرثومة على عبور الأمعاء الدقيقة وحواجز الدم –المشيمة (Blood-Placenta barriers) نتيجة لامتلاك جرثومة الليستريا عوامل فوعة عديدة أهمها ذيفان Listeriolysin O، الذي ينتج فقط من السلالات الممرضة [28].

أكدت الدراسات أنة بالإمكان فحص مشيمة الفأرة المجهضة أما باستخدام المجهر الضوئي الاعتيادي أو بالعين المجردة، فعند دراسة المشيمة بالعين المجردة يلاحظ وجود خراجات كبيرة (Macroabscesses) بيضاء اللون تميل إلى اللون الرصاصي الفاتح، وتعد هذه من الصفات المهمة التي تساعد على تشخيص مرض (Listeriosis) عند فحص المشيمة [29] ، كما وجد إن الخراجات تتكون من تجمع الخلايا المتعادلة داخل الزغابيات (Intravillous) وبين الزغابيات (Intervillous) [30]، كما تسبب جرثومة ***Listeria monocytogenes*** خراجات صغيرة (Micro abscesses) في نسيج المشيمة وترشيح الخلايا البيض (Leukocytes) في أنسجة الجنين [32 and 31]، وتصيب جرثومة ***Listeria monocytogenes*** الخلايا البطانية (Endothelial cells) المبطنة لوريد الحبل السري محفزة تلك الخلايا على إنتاج (IL-6, Interleukine-6) و(IL-8, Interleukine-8) خلال (6) ساعات من الإصابة [33]، وقد أشارت البحوث إلى إن الخلايا المستهدفة (Target cells) المصابة بجرثومة ***Listeria monocytogenes*** المنتجة لذيفان Listeriolysin O تعاني من التحلل، أما السلالات غير المنتجة لذيفان Listeriolysin O فإنها تستطيع غزو الخلايا المستهدفة ولكن ليس لها القابلية على إحداث تحلل في الخلايا المستهدفة [34]، وقد وجد إن السلالات العائدة إلى جرثومة ***Listeria monocytogenes*** المنتجة لذيفان Listeriolysin O وغير المنتجة للذيفان تظهر قابلية على غزو الخلايا الكبدية (Hepatocytes)، الا ان السلالات المنتجة للذيفان تستطيع التضاعف داخل الخلايا الكبدية[35,36 and 37] .

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استخدام محلول شودانس مع مسحوق صبغة الأمارانث لتلوين الفطريـــات فـــي المسحة الرطبة وحفظها

شهرزاد عبد الستار الجنابي (1) ، لمياء يعقوب العاني (2) وشيماء إسماعيل (3)

المعهد الطبي التقني المنصور/ بغداد – جمهورية العراق (1)، (2)، كلية البنات للعلوم/ جامعة بغداد– جمهورية العراق

البريد الإليكتروني: shahrzadaljanabi@yahoo.com

ABSTRAC

In this study an attempt was done to use $CuSo_4$ Schuadinn's solution and Amaranth (the food color powder) in preservation of fungi and in preparation of staining solution for fungi in the wet mount smear. The results showed that the food color powder was dissolved in $CuSo_4$ Schuadinn's solution very easily, the most appropriate concentration of staining solution was 4% which was showing an efficiency in staining the fungi in the wet mount smear with stable coloration during two years , $CuSo_4$ Schuadinn's solution has proved to be efficient to preserved the four species of fungi for a period not less than two years.

الملخص باللغة العربية

تتضمن هذه الدراسة محاولة للاستفادة من محلول حفظ الطفيليات شودنس($CuSo_4$ Schaudinn's solution) ومسحوق صبغة الأمارانث الحمراء في حفظ الفطريـــات, وتلـــوين الفطريـــات فـــي المسحات الرطبة. أظهرت النتائج ما يلي: الذوبان السريع لمسحوق صبغة الأمارانث الحمراء بدرجة حـــرارة الغرفـــة فـــي محلـــول $CuSO_4$ Schaudinn's. كما أظهـــر محلـــول $CuSO_4$ Schaudinn's قدرته على حفظ الفطريات لمـــدة سنتين بدرجة حرارة الغرفـــة. أعطـــى محلـــول التلوين بالتركيز 4% أفضل تلوين للفطريات فـــي المسحة الرطبة, ويحتفظ محلول التلوين بقدرتـــه على تلوين الفطريات في المسحة الرطبـــة لمـــدة سنتين.

الكلمات المفتاحيـــة: محلـــول الحفـــظ شـــودنس $CuSO_4$ Schaudinns, مـــــسحوق صـــــبغة الأمارانث الحمراء.

المقدمة

اكتسبت الفطريات(Fungi) أهميه كبيره لما لبعضها من أهميه في مجال الصناعة كإنتاج الأنزيمات الخارجية و الأحماض العضوية مثل حامض الستريك (Citric acid) وحامض الكلوكونيك (Gluconic acid) اللذان يستعملان في مجال الصناعات الغذائية، وكذلك أنتاج المضادات الحياتية مثل البنسلين Penicillin و الكرسيوفولفين (Griseofulvin) اللذان يفرزان من قبل أنواع من جنس (***Penicillium***) , هذا بالإضافة إلى الدور الذي تلعبه الخمائر في أنتاج الكحولات المستعملة في مجال الصناعة لاسيما الكحول الأثيلي (Ethanol alcohol) , وهي أيضا مصدرا لإنتاج الفيتامينات الغذائية الخام (Crude dietary vitamins) , وأنها مصدر لإنتاج الأحماض الأمينيه(Amino acid) (1, 2, 3, 4, 5, 6) . كما إن الفطريات قد اكتسبت أهميتها من خلال الأمراض المختلفة التي تسببها للإنسان والحيوان والنبات, وكذلك التلف الذي تسببه لعدد من المواد المختلفة مثل المواد الغذائية, المواد القطنية, المواد الخشبية والمواد الورقية (7 , 8, 9, 10 , 11). استخدمت طرق كثيرة ومتعددة لدراسة وتشخيص الفطريات و أن الفحص المجهري باستخدام المسحة الرطبة من أهم تلك الطرق وأظهرت الدراسات بأن صبغة اللاكتوفينول كوتن بلو Lactophenol cotton blue) (LPCB) من أكثر الصابغات المعتمدة (12, 13, 14, 15) وفي تقارير لعدد من البحوث التي تتضمن بعض الدراسات الخاصة اعتبرت النتائج التي أعطتها صبغة (LPCB) أكثر دقه من النتائج التي أعطتها صبغة كرام وأنها تتميز بسهولة استخدامها وسرعتها في أنجاز عملية التلوين (16, 17).

يستخدم محلول شودنس(Schaudinns) لتثبيت وحفظ عينات البراز وكذلك حفظ الطفيليات المعوية (intestinal parasites) التي تشمل الأوالي(Protozoa) وبيوض الديدان المعوية (eggs of intestinal worms) (18, 19, 20, 21, 22, 23, 24, 25, 26). يستخدم كلوريد الزئبق(mercuric chloride) $HgCl_2$في تحضير هذا المحلول و هو من المواد السامة ولهذا السبب فأن استخدام هذا المحلول فيه خطورة, أجريت بعض الدراسات لاستبدال($HgCl_2$) بمادة كبريتات النحاس($CuSO_4$), أظهرت تلك الدراسات بأن كفاءة محلول Schaudinn's $CuSo_4$ في حفظ عينات البراز والطفيليات المعوية لا تقل عن كفاءة محلول Schaudinns $HgCl_2$ (27, 28). تتوفر في جميع الأسواق داخل القطر وخارجه مسحوق صبغة الأمرانث الحمراء Amaranth والتي رقم منسبها اللوني هو (C.I. NO.16185) إن هذا الرقم يحمل مفتاح تركيب الصبغة وهو ثابت في جميع مصادر الإنتاج في حين قد يتغير أسم الصبغة التجاري حسب مصادر الإنتاج, هذه الصبغة من الصبغات الصناعية synthetic dye ( ) وتستخدم في الصناعات الغذائية وتُصنع من القطران (29, 30, 31, 32). الهدف من هذه الدراسة هو محاولة الاستفادة من محلول الحفظ Schaudinns $CuSO_4$ ومسحوق صبغة الأمارنث الحمراء في تحضير صبغه لتلوين الفطريات في المسحة الرطبة و كذلك محاولة الاستفادة من محلول Schaudinns $CuSO_4$ في حفظ الفطريات .

المواد وطرائق العمل

استمر العمل بهذه الدراسة من 5/1 /2008 إلى 5/1 / 2010

تحضير الوسط الزرعي وجمع العزلات الفطرية:

حضر وسط البطاطا دكستروز أغار (Potato Dextrose Agar) (PDA) حسب تعليمات الشركة المجهزة أوكسويد (Oxoid)وعقم بجهاز الموصدة (Autoclave) بدرجة حرارة 121 ْم و ضغط 15 بار/ أنج^2 لمدة 15 دقيقه وبعد إنهاء عملية التعقيم تم تبريده إلى 45 ْم ثم أضيف المضاد الحيوي كلوريمفينيكول (Chloramphenicol) لمنع نمو البكتريا (35), ثم وزع الوسط بأطباق بتري (Petri dishes) وفي أنابيب محكمة الغلق لتتصلب بشكل مائل, تم الحصول على العزلات الفطرية من مصادر مختلفة مثل التربة, المواد التالفة, والحبوب ثم زرعت على الوسط الزرعي (PDA) , وحضنت الأطباق تحت درجة حرارة (25 ± 1) ْم لمدة خمسة عشر يوما , وبعد مدة الحضانة تم عزل الفطريات المختلفة من الأطباق ثم شخصت وبعدها أخذت مسحه من الأبواغ لتنميتها في أنابيب اختبار حاويه على الوسط الزرعي (PDA)المائل وحضنت تحت درجة الحرارة والوقت السابقين نفسهما , ثم حفظت في الثلاجة بدرجة حرارة 4ْم للاستعمال.

الفحص المجهري للعزلات: بعد التشخيص العياني تم تحضير مسحات على الشرائح المجهريه لغرض تصنيفها وذلك بوضع قطره من محلول صبغة (LPCB) على شريحة زجاجيه ونقل جزء من المستعمرة الفطرية المراد تشخيصها ومزجها بهدوء جدا مع القطرة ثم وضع غطاء الشريحة (cover slip) وفحصت الشريحة تحت المجهر الضوئي وشخصت الأجناس المختلفة حسب المفتاح التصنيفي (36) وكما مبين في الجدول رقم (1).

الجدول (1): تصنيف الأجناس الأربعة من الفطريات التي تم عزلها واستخدامها في هذه الدراسة

مرتبة التصنيف	أجناس الفطريات			
	Aspergillus	***Penicillium***	***Fusarium***	***Candida***
الشعبة	***Ascomycota***	***Ascomycota***	***Ascomycota***	***Ascomycota***
الصنف	***Plectomycetes***	***Plectomycetes***	***Monileicioushyphomycetes***	***Archiascomyctes***
الرتبة	***Eurotiales***	***Eurotiales***	//////	***Saccharomycetales***
العائلة	***Trichocomaceae***	***Trichocomaceae***	//////	***Saccharomycetceae***
الجنس	***Aspergillus***	***Penicillium***	***Fusarium***	***Candida***

تحضير المحاليل

- تم تحضير محلول صبغة (LPCB) حسب المصدر (34).
- تم تحضير 500مل من محلول ($CuSO_4$ Schaudinns) حسب المصدر (33) وحفظ لمدة سنتين بدرجة حرارة الغرفة لاستعماله عند الحاجة إليه خلال مدة الدراسة .
- تم شراء مسحوق صبغة الأمارانث الحمراء (Amaranth) (C.I.NO.16185) من السوق المحلية.

تحضير تراكيز محلول التلوين:تم تحضير خمس تراكيز (1%, 2%, 4%, 6%, 8%,) وذلك من إذابة مسحوق صبغة الأمارانث الحمراء في محلول $CuSO_4$ Schaudinns ثم نقل 20 مل من كل تركيز إلى قنينة محكمة الغلق (كل تركيز في قنينة) وحفظت الخمس تراكيز لمدة سنتين بدرجة حرارة الغرفة.

تحضير عالق الفطريات: أعدت أربع قناني حجم 25 مل ومحكمة الغلق, ووزع 20مل من محلول $CuSO_4$ Schaudinns ثم تم نقل إلى كل قنينة عدد من المستعمرات النقية لجنس واحد فقط من الفطريات الأربعة التي تم عزلها وتشخيصها لهذه الدراسة (***spegillus, Penicillum,*** **Candida*** **(*Fusarium,*** النامية على الوسط الزرعي ثم مزجت مع المحلول الموجود في القنينة , حفظت هذه القناني بدرجة حرارة الغرفة لمدة سنتين.

طرق تحضير المسحات الرطبة

الطريقة الأولى: تحضير المسحات الرطبة من الوسط الزرعي مباشره.

■ حضرت خمس مسحات لكل فطر من أجناس الفطريات الأربعة المشمولة بالدراسة ***Aspergillus, Penicillum, Fusarium, Candida*** كل مسحه أستخدم في تحضيرها احد هذه التراكيز(1%, 2%, 4%, 6%, 8%) من محلول التلوين الذي ذكر تحضيره أعلاه.

■ وضعت قطره من واحد من التراكيز أعلاه على شريحة زجاجية ومن ثم تم نقل جزئ صغير من المستعمرة الفطرية الفتية النامية مباشرة من الوسط الزرعي لجنس واحد فقط ومزج مع القطرة بهدوء وبعد تغطيتها بغطاء الشريحة طليت حافات غطاء الشريحة الزجاجية بطلاء الأظافر.

الطريقة الثانية: تحضير المسحات الرطبة من العالق الفطري.

■حضرت خمس مسحات رطبه لكل فطر من أجناس الفطريات الأربعة المشمولة بالدراسة ***Aspergillus, Penicillum, Fusarium, Candida*** كل مسحه أستخدم في تحضيرها احد هذه التراكيز(1%, 2%, 4%, 6%, 8%) المحضرة من إذابة مسحوق صبغة الأمارانث في محلول$CuSO_4$ Schaudinns.

■ وضعت قطره من واحد من التراكيز التي ذكر تحضيرها أعلاه على شريحة زجاجية ومن ثم أضيفت قطره واحده من المحلول المعلق لجنس واحد من الفطريات ومزجت القطرتان بهدوء وبعد تغطيتها بغطاء الشريحة طليت حافات غطاء الشريحة الزجاجية بطلاء الأظافر.

■ مجموع المسحات التي تم تحضيرها حسب الطريقتين أعلاه 40 مسحه.

■ بعد الانتهاء من تحضير أي مسحة رطبة من التي تم تحضيرها أعلاه فحصت بالمجهر الضوئي ثلاث مرات حسب الجدول الزمني التالي:

1- بعد تحضير المسحة مباشره.
2- بعد مرور ساعة واحده على تحضير المسحة.
3- بعد مرور 24 ساعة على تحضير المسحة.

تحضير مسحات السيطرة: وضعت قطره واحده من صبغة (LPCB) على شريحة زجاجيه ثم نُقل جزئ صغير من المستعمرة الفطرية لجنس واحد فقط مباشرة من الوسط الزرعي ومزجت مع قطره من صبغة (LPCB) بهدوء وكانت تغطى بغطاء الشريحة ثم طليت حافات غطاء الشريحة بطلاء الأظافر, بأتباع هذه الطريقة حُضرت أربع مسحات سيطرة للأجناس ***Aspergillus, Penicillum, Fusarium, Candida*** مسحه لكل جنس, بعد الانتهاء من عمل كل مسحه كانت تفحص بالمجهر الضوئي مباشرةً.

كان عمل جميع المسحات والفحوصات المجهريه المذكورة أعلاه يتكرر مرة واحدة في كل شهر ولمدة سنتين وذلك لمتابعه عمل محلول الحفظ ومحلول التلوين بالتراكيز الخمسة لمعرفة صلاحية استخدامهما لمدة سنتين. ولهذا تم التأكيد على استخدام نفس المحاليل المعلقة ومحاليل الصبغة التي تم تحضيرها عند بدأ العمل.

النتائج

صفات مسحوق الصبغة

كان ذوبان مسحوق صبغة الأمارانث الحمراء في محلول $CuSO_4$ Shaudinns عند درجة حرارة الغرفة سريع وتام ولم يتكون أي راسب في أي تركيز من التراكيز الخمسة (1%, 2%, 4%, 6%, 8%) التي تم تحضيرها. وكذلك لم يظهر أي راسب في أي مسحه من المسحات الرطبة المحضرة من أي تركيز عند فحصها بالمجهر.

نتائج الفحوصات المجهريه للمسحات الرطبة: من خلال الفحوصات المجهريه للمسحات الرطبة على مدى سنتين ظهر تطابق بأشكال الفطريات وبعددها النسبي للفطريات من الجنس الواحد في المسحات الرطبة المحضرة بالطريقة الأولى مع المسحات الرطبة المحضرة بالطريقة الثانية ومسحات السيطرة, إن هذه النتيجة قد تدل على كفاءة محلول الحفظ $CuSO_4$ Shaudinns في حفظ الفطريات بأجناسها الأربعة لمدة سنتين بدرجة حرارة الغرفة. وأن مسحوق صبغة الأمارانث الحمراء ليس له تأثير سلبي على أشكال الفطريات بالأجناس الأربعة. وكانت نتائج تلوين تراكيب الفطر (mycelia, foot cell, conidiophore , phialids, conidiospore وغيرها من التراكيب حسب كل جنس من أجناس

الفطريات الأربعة المستخدمة في هذه الدراسة) في جميع المسحات الرطبة المحضرة حسب الطريقة الأولى والطريقة الثانية الوارد ذكرهما في فقرة المواد و طرائق العمل ولجميع التراكيز(1%, 2%, 4%, 6%, 8%) كما يلي :

■ التركيزين 1% و 2% : ظهرت ألوان الفطريات في المسحات المحضرة باستخدام هذين التركيزين بدون لون أو باللون الوردي الفاتح مع خلفيه عديمة اللون. درجة وضوح شكل الفطريات كانت ضعيفة الجدول (2) .

■ التركيز4% : ظهرت ألوان الفطريات في المسحات المحضرة باستخدام هذا التركيز باللون الأحمر مع خلفيه عديمة اللون أو باللون الوردي الفاتح . كانت درجة وضوح شكل الفطريات قويه الجدول (2) والصور (1 أ, 2أ, 3 أ, 4 أ) .

■ التركيزين6 % و 8% : ظهرت ألوان الفطريات في المسحات المحضرة باستخدام هذين التركيزين باللون الأحمر الغامق مع خلفيه حمراء اللون أو أحمر غامق. كانت درجة وضوح شكل الفطريات ضعيفة الجدول (2) .

الجدول (2): جدول يوضح نتائج تلوين المسحات الرطبة لأجناس الفطريات الأربعة التي أستخدم في تلوينها خمس تراكيز من محلول التلوين المحضر من إذابة مسحوق صبغة الأمارانث الحمراء في محلول الحفظ $CuSo_4$ Schaudinns في الفحوصات المجهريه في المتابعة الشهرية على مدى سنتين وللفترات الزمنية الثلاث.

تراكيز محلول التلوين	أجناس الفطريات							
	Aspergillus		*Penicillum*		*Fusarium*		*Candida*	
	لون الفطريات	لون الخلفية	لون الفطريات	لون الخلفية	لون الفطريات	لون الخلفية	لون الفطريات	لون الخلفية
%1	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**	**عديمة اللون**
%2	**وردي فاتح**	**عديمة اللون**	**وردي فاتح**	**عديمة اللون**	**وردي فاتح**	**عديمة اللون**	**وردي فاتح**	**عديمة اللون**
%4	أحمر	**عديمة اللون**	أحمر	**عديمة اللون**	أحمر	**عديمة اللون**	أحمر	**عديمة اللون**
%6	أحمر	أحمر	أحمر	أحمر	أحمر	أحمر	أحمر	أحمر
%8	**أحمر غامق**	أحمر	**أحمر غامق**	أحمر	**أحمر غامق**	أحمر	**أحمر غامق**	أحمر

* التركيز4% : ظهرت ألوان الفطريات في المسحات المحضرة باستخدام هذا التركيز باللون الأحمر مع خلفيه عديمة اللون أو باللون الوردي الفاتح . كانت درجة وضوح شكل الفطريات قويه الجدول (2) والصور (1 أ, 2أ, 3 أ, 4 أ) .

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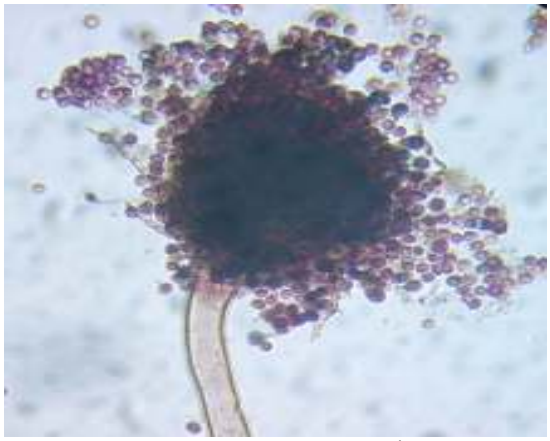
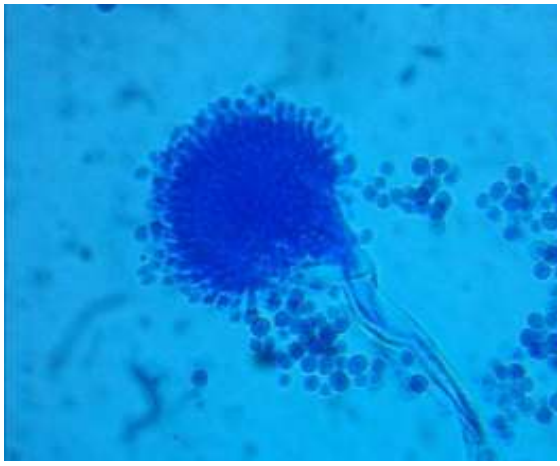
■ أعطى التركيز4% أفضل وضوح لشكل الفطريات في كل المسحات المحضرة ولم يظهر فرق يذكر بين تلوين المسحات التي تم تحضيرها بأتباع أي من الطريقتين الأولى أو الثانية, ولم يوجد فرق يذكر بالتلوين بين جنس وآخر من أجناس الفطريات الأربعة التي استخدمت في هذه الدراسة, وكذلك لم يظهر فرق يذكر عند استخدام

هذا التركيز بين ألوان المسحات سواء فحصت مباشره بعد تحضير كل مسحة . وفحصت بعد نصف ساعة أو فحصت بعد مرور24 ساعة من تحضير كل مسحه الجدول (2) والصور (1 أ, 2أ, 3 أ, 4 أ) .

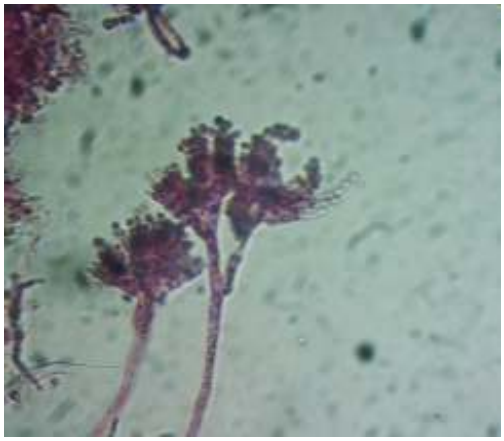
■ من خلال المتابعة لمدة سنتين أظهرت نتائج الفحوصات المجهريه للمسحات الرطبة المحضرة حسب الطريقة الأولى والطريقة الثانية الوارد ذكرهما في فقرة المواد و طرائق العمل وباستخدام محلول التلوين بالتراكيز الخمسة بعدم ظهور تغير بألوان الفطريات التي تشمل أجناس الفطريات الأربعة (***Fusarium, Aspergillus, Penicillum, Candida***) أو لون خلفيات المسحات الرطبة.



صورة 1(أ): صورة لفطرمن جنس *Aspergillus* كان قد ظهر في الفحص المجهري للمسحه الرطبه التي أستخدم في تلوينها محلول التلوين بالتركيز 4%.

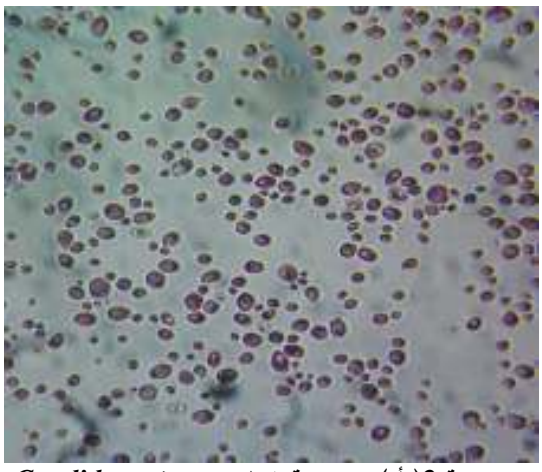


صورة 1(ب): صورة لفطرمن جنس *Aspergillus* كان قد ظهر في الفحص المجهري لمسحة السيطره التي تم تلوينها بصبغة (LPCB) قوة التكبير 40مرة .



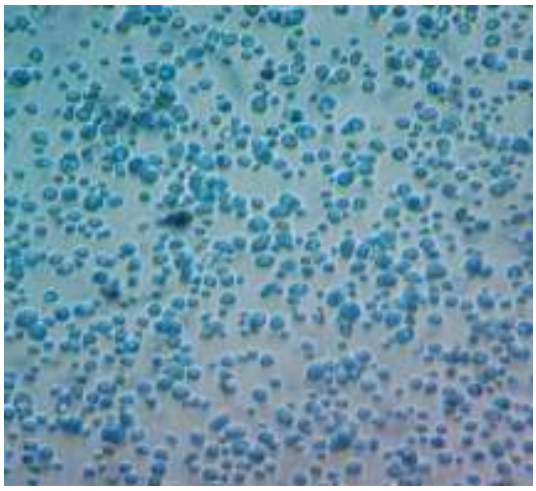
صورة 2(أ): صورة لفطر من جنس *Penicillium* كان قد ظهر في الفحص المجهري للمسحة الرطبه التي أستخدم في تلوينها محلول التلوين بالتركيز 4%

صورة 2(ب): صورة لفطرمن جنس *Penicillium* كان قد ظهر في الفحص المجهري لمسحة السيطره التي تم تلوينها بصبغة (LPCB) قوة تكبير 40 مره.

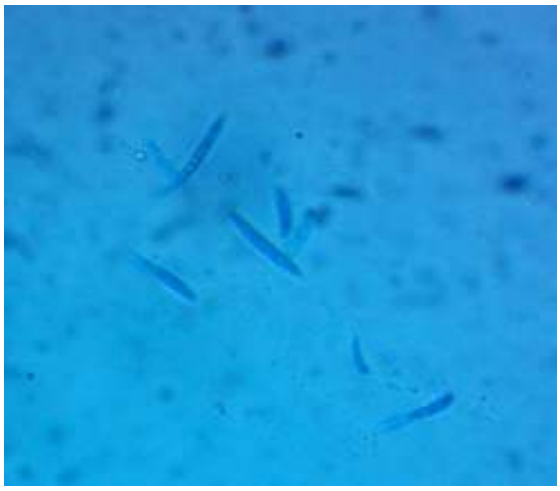


صورة 3(أ): صورة خمائر من جنس *Candida* كانت قد ظهرت في الفحص المجهري للمسحه الرطبه التي أستخدم في تلوينها محلول التلوين بالتركيز 4%.

صورة 4(أ): صورة لفطرمن جنس *Fusarium* كان قد ظهر في الفحص المجهري للمسحه الرطبه التي أستخدم في تلوينها محلول التلوين بالتركيز 4%.



صورة 3(ب): صورة خمائرمن جنس *Candida* كان قد ظهر في الفحص المجهري لمسحة السيطره التي تم تلوينها بصبغة (LPCB) قوة التكبير 40مرة.



صورة 4(ب): صورة لفطرمن جنس *Fusarium* كان قد ظهر في الفحص المجهري لمسحة السيطره التي تم تلوينها بصبغة (LPCB) قوة التكبير 40مرة.

المناقشة

عند تحضير الخمس تراكيز لمحلول التلوين كان مسحوق صبغة الأمارانث الحمراء سريع الذوبان في محلول $CuSo_4$Schaudin , إن هذه الصفة لمسحوق صبغة الأمارانث تتفق مع ما ورد في الدراسة (36) التي اختصت بصفات هذا المسحوق والتي تذكر بأن جميع مساحيق الصبغات الغذائية يدخل في تركيبها رمز السلفونيت وهي أملاح لحامض السلفونيك ويعتبر هذا الحامض قويا نسبيا ويمنحها قابليه سريعة على الذوبان سواء في الماء أو الكحول, إن هذه الخاصية أسهمت في تسريع وتسهيل عملية تحضير محلول التلوين بحيث كانت عملية الإذابة لا تحتاج إلى تسخين المحلول أو إلى عملية ترشيح للتخلص من الرواسب هذا من جهة ومن جهة أخرى إن خلو المسحة الرطبة من الرواسب يساعد في رفع درجة وضوح الفطريات المتلونة عند فحصها بالمجهر. إن ثبات ألوان الفطر في المسحات الرطبة لمدة 24 ساعه وثبات ألوان التراكيز الخمسة لمحلول التلوين في تلوين المسحات الرطبة لمدة سنتين نجد تفسيره في الدراسة (36) التي ورد فيها على أنه لا يظهر تلاشي بلون الصبغة عند وجودها في وسط أسه الهيدروجيني 3 (P^H 3), أوفي وسط أسه الهيدروجيني 7 (P^H 7) , أو في وسط أسه الهيدروجيني 8 (P^H8). تعتمد آلية عمل مسحوق صبغة الأمارانث الحمراء في التلوين على نظرية الارتباط بين جزيئات المادة ذات الشحنات السالبة مع جزيئات المادة ذات الشحنات الموجبة, إن جميع الصبغات الغذائية يدخل في تركيبها رمز السلفونيت ويعتبر هذا الحامض قويا نسبيا مما يجعل وسطها حامضي وأسها الهيدروجيني منخفضا ولهذا فأن جزيئات مسحوق الصبغة ستحمل الشحنات السالبة , وتحمل جزيئات البروتين الشحنات الموجبة, وبالتالي سيحصل ارتباط الكتروني بين جزيئات كلا هاتين المادتين (36). لو تمعنا قليلا في المواد التي تدخل في تركيب جدار الخلية وحسب ما تذكر الدراسة (39) أن عديد السكريد (Polysaccharide) يشكل 80% من مكوناته وأن البروتين يشكل 20% ولذلك قد يكون من المستبعد أن يلوّن المحلول جدار الخلية ونتوقع أن تلوّن هذه الصبغة التراكيب البروتينيه في داخل الخلية, قد يكون هذا ممكنا حسب ما ورد في الدراسة (39) والتي تنص على أن جدار الخلية يقوم بدور الوسيط بين مكونات الخلية الداخلية مع محيط الخلية الخارجي ويعزز هذا الرأي ما ورد في الدراسة (29) التي تذكر بأن صبغة (LPCB) تميل إلى تلوين السيتوبلازم أكثر من تلوين جدار الخلية. إن قدرة محلول الحفظ $CuSo_4$Schaudinns في حفظ الفطريات له جوانب إيجابيه مهمة حيث انه يعتبر سجل دائمي يمكن الرجوع إليه في الاستشارات الطبية وكذلك عند الاستفادة من الفطريات المحفوظة في تدريس الطلبة بالمؤسسات التعليمية (33).لا يحتوي محلول التلوين بالتركيز 4% وكذلك محلول الحفظ $CuSo_4$ Schaudinns على مواد سامه أو مسرطنة فكلا المحلولين لا توجد فيهما ماده خطره سامه أو مسرطنة ما عدا حامض الخليك الثلجي فهو يعتبر ماده مخدشه مع ذلك فأن هذا الحامض موجود بنسبة قليله تبلغ 1/ 20 وهذا يقلل كثيراً من تأثيره(33). أما مسحوق صبغه الأمارانث الحمراء فهذه المادة غير خطره وأن جميع الصبغات الغذائية لا تحتوي على مواد سامه أو مسرطنه (37). أما من الناحية الاقتصادية فأن جميع الصبغات الغذائية متوفرة في جميع الأسواق داخل القطر وخارجه و بثمن زهيد ويمكن حفظها لفترة زمنية غير محدودة وذلك عندما تحفظ في حاويات بلاستيكيه محكمة الغلق وبعيده عن ضوء الشمس, الرطوبة والماء (36). قد يمكننا أن نعتبر محلول التلوين بالتركيز 4% هو محلول له قدره على تلوين الفطريات في المسحات الرطبة ويحتفظ بقدرته على التلوين لمدة سنتين وقد يتميز بعديد من الصفات الإيجابية مثل خلوه من المواد السامة أو المسرطنه , وكذلك ثبات ألوانه , وسهوله وسرعه تحضيره , و السهولة والسرعة عند استخدامه في تلوين المسحة الرطبة .كما أن محلول الحفظ $CuSo_4$ Schaudinns له قدره جيده في حفظ الفطريات لمدة لا تقل عن سنتين.

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إيجاد الطرق المثلى لتنقية اللايكوبين ودراسة فعاليته المضادة للأكسدة في داخل وخارج جسم الكائن الحي

سندس حميد أحمد وآخرون

وزارة العلوم والتكنولوجيا- جمهورية العراق

البريد الإلكتروني: hams_63@yahoo.com

ABSTRACT

Lycopene is the antioxidant of the very effective and important to reduce the incidence of cancers and other chronic diseases. In our study was to find a way to refine the optimal lycopene from tomato residues (peel) and the outcome is high, as when conducting a comparative study of the concentration of lycopene in the peel, tomato juice and the mixture of the whole tomatoes we found that the total peel gave the highest concentration of pigment lycopene than other parts. At the time of purification using silica gel and the emergence of a single package of lycopene compared with standard lycopene, and gave (RF) value of 0.52 in addition to the (RT) 11.3 min identical to the time of holding the top of lycopene standard, when a measurement of the light spectrum between (300-600 nm) gave the pure lycopene three peaks at wavelengths 440, 460, 503 nm and the coefficient of detention is 0.98, 1, 0.96.

When we study the feasibility of removing free radicals generated by using the DPPH found that partially purified lycopene gave the highest efficiency compared to purified lycopene and tomato juice. In a study of the effectiveness of the enzymes (SOD) and (GPX) in the CCl4-treatment substance found that the effectiveness of the enzymes increased when we use different kinds of lycopene and evidence of the susceptibility effect of lycopene in the removal of free radicals resulting from the use of CCl4.

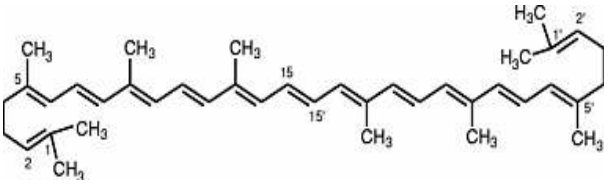
الملخص باللغة العربية

يعد اللايكوبين من مضادات الأكسدة الفعالة جداً والمهمة لخفض نسبة الإصابة بالسرطانات والأمراض المزمنة الأخرى. في دراستنا تم التوصل إلى إيجاد طريقة مثلى لتنقية اللايكوبين من متبقيات الطماطم (القشور) وبحصيلة عالية، إذ عند إجراء دراسة مقارنة لتركيز اللايكوبين في القشور، العصير وخليط الطماطم الكلي وجد ان القشور أعطت أعلى تركيز لصبغة اللايكوبين مقارنة بالأجزاء الأخرى. وعند إجراء التنقية باستخدام هلام السليكا وظهور حزمة واحدة للايكوبين مقارنة باللايكوبين القياسي وأعطت معامل ترحيل (RF) بقيمة 0.52 بالإضافة إلى ظهور قمة عند زمن احتجاز (RT) 11.3 دقيقة مطابقة لزمن احتجاز قمة اللايكوبين القياسي، وعند إجراء قياس الطيف الضوئي بين

(300-600 نانوميتر) أعطى اللايكوبين النقي ثلاث قمم عند الأطوال الموجية 440، 460، 503 نانوميتر وان معامل الاحتجاز (COD) هي 0.98، 1، 0.96 . وعند دراسة قابلية إزالة الجذور الحرة المتولدة باستخدام مادة إل DPPH وجد أن اللايكوبين المنقى جزئياً أعطى أعلى فعالية مقارنة باللايكوبين النقي وعصير الطماطم. وعند إجراء دراسة فعالية الانزيمات (SOD) و (GPX) عند المعاملة بمادة الـ CCl_4 وجد ان فعالية الانزيمين قد ارتفعت عند استخدام اللايكوبين بأنواعه وذلك دليل على قابلية اللايكوبين في إزالة تأثير الجذور الحرة الناتجة عن استخدام مادة CCl_4 .

المقدمة

تعد منتوجات الطماطم كمصدر رئيس لفيتامينات A,C ولمعادن البوتاسيوم K والمغنيسيوم mg وبالاضافة الى الالبان، وتعد الطماطم ذات سعرات حرارية واطئة اذ يحتوي الـ 100غم منها على 32 سعرة وغنية بالكاروتينويدات والبيتا كاروتين واللايكوبين (1). اللايكوبين(Lycopene)هو مادة مضادة للاكسدة فعالة جداً وهي المسؤولة عن اعطاء اللون الاحمر الداكن للطماطم. شكل رقم (1) يبين فيه تركيب اللايكوبين (3). وان تناول ثمرة واحدة من الطماطم في اليوم يعمل على زيادة تركيز اللايكوبين في مجرى الدم ولكن هناك بعض المحاذير عند تناولها اذ ان استهلاك كميات كبيرة من الطماطم مفيد من حيث زيادة تركيز نسبة اللايكوبين ولكن هناك زيادة في نسبة الاملاح التي تسبب تكون الحصى لذا تم توجه العالم الى انتاج مادة اللايكوبين ذات الاهمية الطبية لتفادي زيادة نسبة الاملاح (4).



شكل رقم (1) يبين التركيب الكيماوي اللايكوبين

ان تناول اللايكوبين يعمل على خفض نسبة الاصابة بسرطانات القولون، الرئة، البروستات، البنكرياس والثدي (5). ان فعالية اللايكوبين المضادة للسرطان لها علاقة بفعاليته المضادة للاكسدة ويكون اكثر فعالية بوجود البيتا كاروتين ويعمل اللايكوبين على حماية المادة النووية DNA، البروتينات واللبيدات وتعمل على خفض نسبة ضغط الجهد التاكسدي المسؤول عن احداث الامراض المزمنة (القلب، ارتفاع ضغط الدم، ارتفاع نسبة الكولسترول) والامراض السرطانية (7,6).

اشارت منظمتا الـ FAO/WHO ان باحثي اللجنة الخاصة بالاضافات الغذائية (JECFA) قاموا بتقسيم اللايكوبين (الطبيعي والصناعي) لاستعماله كملون غذائي منذ عام (1965، 1975، 1978) لكن لم يتمكنوا انذاك من تحديد الجرعة المسموح بها يومياً (ADI) وذلك لقلة المعلومات انذاك، ولكن في المؤتمر السابع والستين للـ (JECFA) حصلت الموافقة على استعمال اللايكوبين كملون غذائي وتم تحديد الجرعة اليومية للفرد 0.5 ملغم/كغم من قبل منظمتي الـ (FAO/WHO).

الهدف من البحث

ايجاد الطريقة المثلى لاستخلاص اللايكوبين وباعلى حصيلة ونقاوة عالية ودراسة الفعالية المضادة للاكسدة.

المواد وطرائق البحث

استخلاص اللايكوبين:

ثبتت طريقة الاستخلاص من قبل الفريق البحثي للحصول على مستخلص اللايكوبين النقي جزئياً من الطماطم، اذ تم تقسيم مقدار 300غم من الطماطم الى ثلاث اجزاء وكل جزء بوزن 100غم، الجزء الاول هرست الطماطم بالكامل، الجزء الثاني جمع العصير فقط والجزء الاخير اخذت القشور فقط. وتم معاملة الاجزاء بخليط المذيبين العضويين اثيل اسيتيت:ايثانول بنسبة 1:2 وتم خلطهما جيداً باستعمال خلاط (Vortex) مع اضافة المذيبات العضوية على دفعات وفي درجة حرارة المختبر الى ان اصبحت القشور عديمة اللون واضيف 5% من محلول

كبريتات الصوديوم اللامائية، وخلط المستخلص جيداً لحين انفصاله الى طبقتين، فصلت طبقة الايثانول عن طبقة الاثيل اسيتيت واعيد غسل طبقة الاثيل اسيتيت ثم تم تركيزه الى حجم 30 مل بدرجة حرارة 35م. تم تقدير تركيز اللايكوبين باستعمال جهاز الامتصاص الضوئي على طول موجي 503 نانوميتر.

اجراء عملية الصوبنة:

اتبعت طريقة (8) مع بعض التحوير لاجراء الصوبنة لازالة الليبيدات، اذ يضاف 100 مل من محلول هيدروكسيد البوتاسيوم بتركيز 10% الى اللايكوبين ويترك لمدة 18 ساعة بدرجة حرارة الغرفة وبعدها يهمل الجزء المائي ويتم تجفيف اللايكوبين بوجود كبريتات الصوديوم اللامائية بدرجة حرارة 30م ويعلق اللايكوبين مرة اخرى بـ 1مل من الاثيل اسيتيت والميثانول (50:50) حجم:حجم.

تنقية اللايكوبين:

تم تثبيت طريقة تنقية اللايكوبين من قبل الفريق البحثي، اذ تم تعبئة هلام السليكا بعمود بابعاد (1.5×10 سم) وتم امرار اللايكوبين الخام واجريت عملية الازالة باستخدام (9:1) بنزين : ايزوبروبانول وبسرعة جريان امل/دقيقة

تقنية الـ HPLC:

تم تشخيص اللايكوبين باستخدام تقنية الـ HPLC في مختبرات ابحاث ابن سينا وبالتعاون مع كلية الصيدلة جامعة بغداد.
استخدمت تقنية الـ HPLC لتحديد نقاوة اللايكوبين مقارنة باللايكوبين القياسي، استخدم عمود الفصل C18 vydac2181954 بابعاد (4.6×250 ملم) يحتوي على جزيئات بقطر (5مايكرومتر) هو الطور الثابت وتتم الازالة باستخدام 100% ميثانول وبسرعة جريان امل/دقيقة بدرجة حرارة 15م وبطول موجي 450 نانوميتر، وتم تشخيص الكاروتينويدات بزمن ظهور (Retention time) بالمقارنة مع المكونات القياسية (λ max). بالاضافة الى شكل القمم عند استخدام الطيف الضوئي (UV) بين (600–300 نانوميتر).
اجراء الكشف عن اللايكوبين بطريقة كروماتوغرافيا الطبقة الرقيقة:
تم تحديد ظروف التجربة من قبلنا لاجراء فحص كروماتوغرافيا الطبقة الرقيقة (TLC) باستخدام هلام السليكا (Silica gel)، يؤخذ كمية صغيرة من اللايكوبين الخام ويذاب في البنزين : ايزوبروبانول حجم:حجم (9:1) ويتم اجراء الترحيل باستخدام المذيبات العضوية المذكورة.
الطريقة السريعة للكشف عن قابلية اللايكوبين في ازالة الجذور الحرة:
اتبعت طريقة (18) مع بعض التحوير.

معاملة الحيوانات المختبرية

قسمت الحيوانات المختبرية ارانب الى ستة مجاميع:
المجموعة الاولى: (السيطرة) تم تجريعها جرعة ماء بتركيز (1مل/كغم) فموياً.
المجموعة الثانية: (السيطرة) جرعت (1:1) البارافين السائل : رابع كلوريد الكاربون.
المجموعة الثالثة: جرعت (200 ملغم/كغم) من عصير الطماطم.
المجموعة الرابعة: جرعت (200 ملغم/كغم) اللايكوبين المنقى جزئياً.
المجموعة الخامسة: جرعت (200 ملغم/كغم) اللايكوبين النقي.
تم تجريع المجاميع (5،4،3) بمادة (1:1) البارافين السائل : رابع كلوريد الكاربون بتركيز (2 مل/كغم) وبعد مرور 30 دقيقة يتم سحب الدم عن طريق طعنة القلب ويوضع في انابيب حاوية على الـ (EDTA) وتحفظ في الثلاجة لحين اجراء الفحوصات البيوكيمياوية.

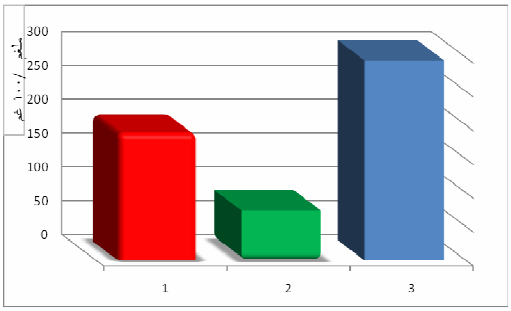
قياس فعالية الانزيمات المضادة للاكسدة في داخل جسم الكائن الحي:

تم قياس فعالية انزيم الكلوكاثايون بيروكسيديز (GPX) وفعالية انزيم السوبر اوكساید ديسميوتيز (SOD) اتبعت طريقة (10)، استخدمت العدة التشخيصية (Randox Laboratories, Ireland).

النتائج والمناقشة

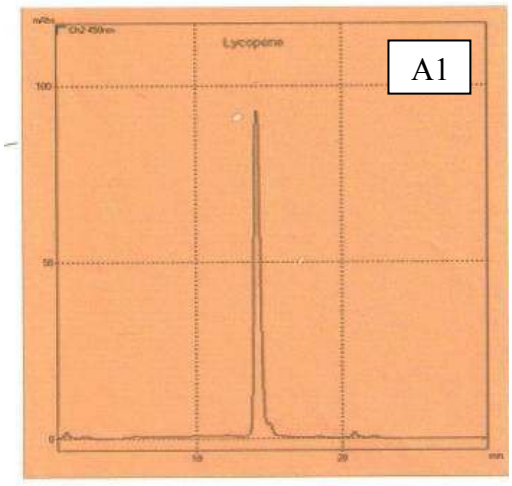
يبين شكل رقم (2) تركيز صبغة اللايكوبين المنقاة جزئياً من القشور والعصير والطماطم الكلية وكان تركيز اللايكوبين 275 ملغم، 70 ملغم، 171 ملغم/100غم. وجد ان القشور تحتوي على اعلى تركيز من اللايكوبين وهذا يتفق

مع ما توصل اليه (11)، اذ وجـدوا ان القـشور واللب تحتوي على اعلى تركيز من اللايكوبين من العصير والطماطم الكاملة.



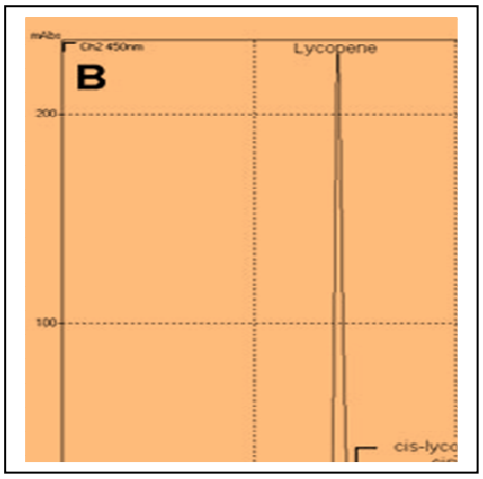
1–الطماطة كاملة 2– عصير الطماطا 3– قشور الطماطا

شكل (2) تركيز اللايكوبين في الطماطا والقشور والعصير



شكل رقم (3) آ تشخيص اللايكوبين المنقى مقارنة باللايكوبين القياسي

شكل رقم (3) آ يبين فيه تـشخيص اللايكـوبين المنقى مقارنة باللايكوبين القياسـي، اذ وجـد ان وقت الاحتجاز (RT) للايكوبين القياسي هو 11 دقيقة واللايكوبين المنقى هو 11.3 دقيقة شـكل (3) ب.

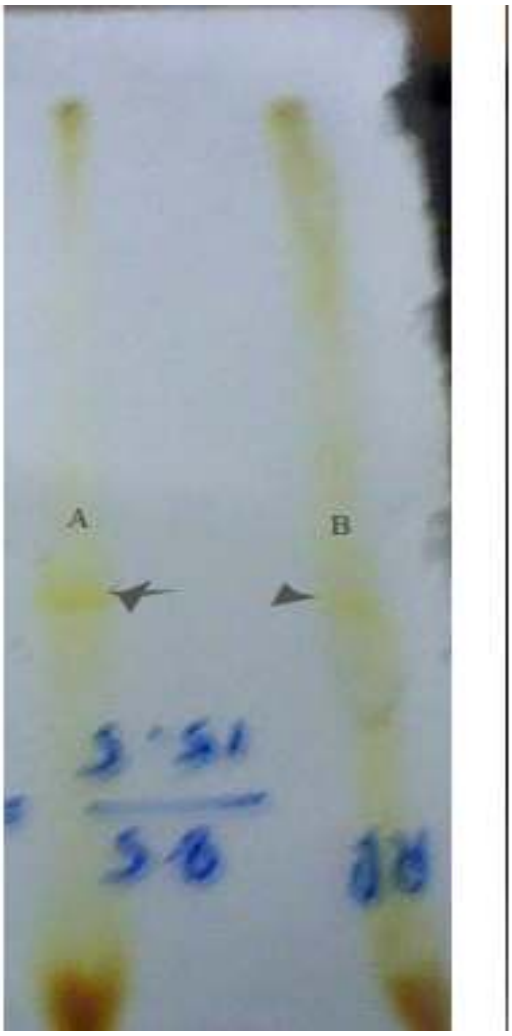


شكل (3) ب: تركيز اللايكوبين المنقى

وعند قراءة الطيـف للاشـعة فـوق البنفـسجية (300–600 نانوميتر) شكل (4) ظهرت ثـلاث قمم القمة الاولى عنـد الطـول المـوجي 440 نانوميتر والقمة الثانية عند الطول المـوجي 460 نانوميتر والقمة الثالثة عنـد 503 نـانوميتر وان معامل التحديد Coefficient determination هو 0.9 و 1.00 و 0.96 وهي مماثلة لقراءات اللايكوبين القياسي شكل رقم (4) يظهر فيه نتائج قراءات الطيف للاشعة الفوق البنفسجية وشـكل (5) يبين فيهااللايكوبين المنقى جزئيا والنقي.

وهذا يتفق مع ما توصل اليه (3،12) اذ اعطـت نتـائج فحـص قـراءات الطيـف (300–600 نانوميتر) للاشعة الفـوق البنفـسجية للايكـوبين المنقى من الكريب فروت ذو اللون الوردي ومن الطماطم نفس القمم التي قد تم الحصول عليها في دراسـتنا، واعطـت نتـائج الكـشف باسـتخدام كروماتوغرافيا الطبقة الرقيقـة حزمـة واحـدة للايكوبين المنقى مقارنة بـاللايكوبين القياسـي، وعند احتساب معامل الترحيل (Rf) وجـد انـه 0.52 مطابق لحزمة اللايكوبين القياسـي وهـذا يتفق مع ما توصل اليه (14) اذ وجد عند ترحيل اللايكوبين المنقى مـن البطـيخ الاحمـر ومـن الطماطم اعطى نفس قيمة (Rf) المذكورة.

ان اللايكوبين يوجد في الطماطم، البطيخ الاحمـر والكريب فروت الوردي وله دور فعال في خفض نسبة الامراض المتولـدة عـن زيـادة الـضغط التأكسدي في الجسم نتيجة لارتفاع نسبة الجـذور الحرة في جسم الانسان وله فوائد لصحة الجهاز الهـضمي وامـراض الـسرطان المختلفـة (15،16،17).



شكل رقم(4) يبين فيه قراءة الطيف للاشعة الفوق البنفسجية (300-600) نانوميتير عمود الهلام المستخدم C18 Vydac 218TP54 column , 250 x 4.6 mm الطور االثابت 100% ميثانول وبسرعة جريان 1 مل لكل دقيقة

أ- اللايكوبين القياسي

ب- اللايكوبين المنقى

شكل رقم(5) أ- اللايكوبين المنقى جزئيا

شكل (5) ب- اللايكوبين النقي

يبين جدول رقم (1) الكشف السريع عن ازالة الجذور الحرة اذ وجد ان اللايكوبين المنقى جزئيا ذو فعالية عالية جدا في ازاة الجذور الحرة يليه اللايكوبين النقي واخيرا عصير الطماطم كما يبين جدول (2) قابلية اللايكوبين في ازالة الجذور الحرة المتولدة بوجود الـ DPPH بواسطة اللايكوبين المنقى جزئياً والنقي وعصير الطماطم. اذ اظهر اللايكوبين المنقى جزئيا فعالية عالية مضادة للاكسدة يليه اللايكوبين النقي واخيرا عصير الطماطم وذلك لامتلاك اللايكوبين عدد كبير من الاواصر المزدوجة والتي لها القدرة على اقتناص جذور الاوكسجين المتولدة اذ يعمل اللايكوبين على تثبيط تأكسد الدهون الغير مشبعة (18)، اذ وجد ان اللايكوبين المنقى جزئياً اعطى اعلى فعالية مقارنة باللايكوبين النقي.

جدول رقم (1) الكشف السريع عن ازالة الجذور الحرة

النموذج	سرعة ظهور البقعة	كثافة البقعة
اللايكوبين المنقى جزئي	اكثر سرعة	+++
اللايكوبين النقي	سريع	++
عصير الطماطم	بطيء	+

+ بطيء بعد مرور 20 دقيقة
+++ ظهور البقعة لحظة الكشف
++ ظهور البقعة بعد مرور 15 دقيقة من اجراء الكشف

جدول (2) فعالية اللايكوبين في ازالة الجذور الحرة المتكونة بواسطة ال DPPH

النماذج	تركيز ميكروغرام / مل	% لازالة الجذور الحرة لل Dpph	التركيز الذي يحدث 50% تثبيط للجذور الحرة لل Dpph
للايكوبين المتقى جزئيا	1000	98.3±0.12a	0.6a
	500	85±0.21b	
	250	70.33± 0.22c	
	125	65.7± 0.03d	
اللايكوبين النقي	1000	80.65 ± 0.37a	1.3b
	500	73.33 ± 0.01b	
	250	62 ± 0.74 c	
	125	51.60± 0.33d	
عصير الطماطم	1000	61.70 ± 0.32a	7.1c
	500	44.16 ± 0. 33b	
	250	30.54 ± 0.11c	
	125	20.50 ± 0.32d	

جدول (3) تاثير اللايكوبين على فعالية الانزيمات Glutathione peroxidase (GPX) and superoxide dismutase (SOD

المجـــــــاميع	المعاملة	SOD (U/ml)	GPX (U/L)
1.	السيطرة	41±15.56a	375.42±1.69a
2.	المعاملة + CCL	18±16.97b	368.95±1.89b
3.	اللايكوبين النقي	88±16.97c	420.60±1.89c
4.	اللايكوبين النقي CCL4	62±0.01d	378.51±1.84d
		64±16.97e	588.84±4.59e

	اللايكوبين المنقى جزئيا	53±32.55f	386.95±1.89f
5	اللايكوبين المنقى جزئيا CCL4	44.36 ± 1.02g	376.56± 1.32g
6.	عصير الطماطم	44.53 ± 0.32h	366.01± 1.43h
7	عصير الطماطم و CCL4		

اجريت التجربة بثلاث مكررات واظهرت النتائج فروق معنوية P< 0.05

جدول رقم (3) يبين فيه دراسة فعالية الانزيمات (GPX) و (SOD) في داخل جسم الحيوانات المختبرية, وجد عند اجراء المعاملة بـ CCl_4 انخفضت فعالية الانزيمين GPX و SOD نظراً لتولد الجذور الحرة التي قد احدثتها هذه المادة والتي قد ادت الى حصول ضرر في نسيج الكبد نتيجة تولد الجذور الحرة واكسدة الدهون الغير مشبعة ولكن عند المعاملة باللايكوبين المنقى جزئياً وكلياً وعصير الطماطم وجد ان هناك زيادة في فعالية الانزيمين دليل على فعاليتها في ازالة السمية المتولدة نتيجة لتأثيرات مادة الـ CCl_4 واعادة اصلاح ما تضرر من الكبد من بروتينات، مادة نووية، دهون غير مشبعة وبالاضافة الى ذلك وجد ان اللايكوبين المنقى جزئياً اعطى اعلى فعالية يليه اللايكوبين النقي واخيراً عصير الطماطم وان سبب كون اللايكوبين المنقى جزئياً اكثر فعالية وذلك لاحتوائه على الكاروتينويدات الاخرى واهمها البيتا كاروتين (19). وان هذينالانزيمين مهمين لحماية الكبد ودراسة الفعالية المضادة للاكسدة للادوية والمستخلصات النباتية(1).

نستنتج من هذه الدراسة بأنه قد تم التوصل الى طريقة ذات جدوى اقتصادية لكونه يمكن الاستفادة من متبقيات الطماطم في انتاج اللايكوبين النقي والمنقى جزئياً بحصيلة عالية مقارنة لما قد ثبت في الدراسات السابقة ولاهميته الطبية تم استكمال دراستنا في دراسة فعالية اللايكوبين كمادة مضادة للاكسدة ووجد انها فعالة جداً في ازالة السمية الناتجة عن الجذور الحرة وذلك يعود لاحتواء تركيبه الكيميائي على عدد كبير جداً من الاواصر المزدوجة وبذا سوف يكون هذا البحث ذا جدوى في اعادة تدوير متبقيات الطماطم لانتاج مادة فعالة مهمة للوقاية من السرطانات والامراض المزمنة الاخرى بالاضافة الى خفض الكولسترول وحماية الكبد والقلب من الضغط التأكسدي الذي يتولد عن وجود الجذور الحرة في جسم الانسان.

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دراسة مقارنة لكفاءة عملية غسل الكلية للدم (الـديلزة) للمرضـى المـصابين بالفشل الكلوي المزمن باستخدام محلول غسل كليـة محـضر محليـا ومحلـول مستورد

بشرى خالد حسن

المعهد الطبي التقني المنصور / بغداد- جمهورية العراق

البريد الإلكتروني: osamah_1991@yahoo.com

الملخص باللغة العربية

أجريت عملية الغسل الكلوي للـدم فـي إحـدى مستشفيات بغداد عام 2006 علـى 38 مـريض مصابين بالعجز الكلوي المزمن وذلك باسـتخدام محاليل غسل كلوي جاهزة ومـستوردة مطابقـة للمواصفات القياسية وقورنت مع محاليـل غـسل كلوي محضرة محليا بالمختبر. وفي كلتا الحالتين اسـتخدم نفـس جهـاز الكليـة الـصناعية ، المرشح،والماء المعقم الخالي من الايون والجراثيم . وكانت محاليل الغسل في كلتا الحالتين خالية من البيكربونات نوع (acetate) وقيست نسبة تركيز العناصـــــــر الحيويـــــــة اليوريـــــــا ،الكرياتنين،الـــصوديوم،البوتاسيوم ،الكالـــسيوم، والفوسفات قبل وبعد الديلزة في الحالتين لمعرفـة مقدار الانخفاض الحاصل في تراكيزها بعد الديلزة وتبين ان المحلول الجاهز والمستورد خفض معدل تركيز اليوريا بنسبة 42.2% والكرياتنين بمعـدل 40.2% اما المحلول المحضر مختبريا فقد خفض اليوريا بمعدل 32.7% والكرياتنين بنسبة 39.6% ويستنتج من ذلك ان المحلول المحلي كفوء نـسبيا ويمكن استخدامه في حالة عدم توفر محاليل الغسل الكلوي القياسية الجاهزة.

ABSTRACT

The hemodialysis was done in one of Baghdad hospital in 2006 by 38 chronic kidney failure patient. Two kind of dialysate solution were used one of them was standard imported solution and the other was manual prepared in chemical lab two cases were used the same hemodialysis mashaine , filter .sterilized and deionized water and these two dialysate were without bicarbionate (acetate). The important biochemical substances such as urea , creatinine, sodium , potassium , calcium ,phosphate were measured before and after dialysis in two cases in order to know the amount of reducing value after dialysis

The standard imported solution reduced urea value by 42.2 % and creatinine by 40.2% greater than urea 32.7% and creatinine 39.6 % that be taken by manual prepared in chemical lab this value improved that the manual prepared solution had relative efficiency and can be used when the standard imported dialysate solution not available.

المقدمة

ان وظيفه الكليه هو طرح الفضلات الناجمه عن الفعاليات الايضيه في الجسم وتحافظ على توازن الماء وتعتمد في اداء وظيفتها على التكامل الحاصل بين خلايا الكبيبه والانبيبات الكلويه وعلى التجهيز الاعتيادي للدم الى الكليه وعلى الاداء التنظيمي الاعتيادي للهرمونات التي التي تسيطر على عمل الكليه['3 '2 '1]كما تقوم بانتاج بعض الهرمونات مثل Erythropoiten و Renin.[' 4] تصفي الكلية في اليوم الواحد 200 لتر من البلازما وهذه التصفية تعتمد على الضغط الهيدروستاتيكي للاوعية الدموية الشعرية والذي يكون اعلى منه في Tubular lumen ['6 '5]. يحتوي الراشح على الالكتروليتات مثل الصوديوم ،البوتاسيوم،الكالسيوم، الكلوكوز،الفوسفات ['9 '8 '7]وتطرح مع الادرار الفضلات النيتروجينية غير البروتينية مثل اليوريا الكرياتنين، حامض البوليك اضافة الى الكتروليتات الزائدة والماء الزائد عن حاجة الجسم['12 '11 '10] ويؤدي انحباس الماء وزيادة الكتروليتات في الجسم الى الوفاة في ساعات قليلة .لذا من الضروري طرح المواد الزائدة عن حاجة الجسم اما المواد المهمة والمفيدة للجسم مثل البروتينات والبروتينات المرتبطه فتترشح بكميات قليلة جدا ويعاد امتصاص المواد المهمة التي يحتاجها الجسم ['14 '13]

في حالة قصور وعجز الكلية المزمن والمتسبب بعدم قدرة الكلية على ترشيح الدم وطرح الفضلات السامة الناجمة عن العمليات الايضية في الجسم مع زيادة تركيز الاملاح اللاعضوية في الغذاء الى ما يفقدة الجسم بواسطة التعرق والغائط ['15].لذا يجب تخفيض نسبة البروتينات الماخوذة لمنع حموضة الدم ['16] ومراقبة مقدار تركيز يوريا الدم فاذا ارتفعت الى اكثر من (150) ملغم/100 مل وانخفض سرعة الترشيح الكبيبي الى 25مل / دقيقة فان السيطرة الغذائية لن تكون فعالة وكافية ويتطلب الامر الى عملية غسل كلوي بواسطة كليه صناعية او زرع كليه لادامة الحياة.['17]

يستند مبدا الغسل الكلوي للدم بواسطة جهاز الكلية الصناعية الى مبدا النفوذية حيث يدخل دم المريض الى الكلية الصناعية ويفصل دم المريض عن محلول الغسل غشاء نصف ناضح يسمح الغشاء للاملاح اللاعضوية الزائدة والفضلات الايضية ان تنتشر من خلاله الى محلول الغسل الكلوي ولكنه يمنع مرور خلايا الدم وبروتينات البلازما الى محلول الغسل .لذا يتخلص الجسم من الفضلات الايضيه والاملاح اللاعضوية والماء الزائد عن حاجة الجسم .['20 '19 '18]

يهدف البحث الى قياس كفاءة محلول الغسل الكلوي المحضر محليا من حيث مكوناتة الكيمياوية،تركيزها ،نقاوتها ومقارنتها مع محلول غسل كلوي جاهز ومستورد وذلك من خلال تجريبهما على مرضى العجز الكلوي المزمن ومقارنة النتائج المستحصلة بعد عملية الغسل الكلوي في خفض نسبة تركيز العناصر الحيوية في الدم كاليوريا ، الكرياتنين ، الصوديوم ، المرشح والماء الخالي من الايون والجراثيم وتقارن كلتا النتائج مع نتائج مستلة من مصادر علمية في هذا المضمار.

<u>المواد وطريقة العمل</u>: اجري البحث في مستشفى اليرموك في بغداد عام 2006 على 38 مريض من الذين يعانون عجز كلوي مزمن ومن الذكور فقط وتتراوح اعمارهم بين (19–64) سنه واجريت لهم عملية غسل كلوي للدم بصورة دوريه بمعدل مرتين في الاسبوع . تستغرق كل عملية غسل كلوي للدم ثلاث ساعات .

غسل دم المرضى بنوعين من محاليل الغسل الكلوي الخاليه من البيكربونات نوع (acetate) احدهما محلي التحضير والاخر محلول غسل جاهز مطابق للمواصفات القياسيه مستورد . سحبت نماذج من دم المرضى قبل الديلزه وحسبت نسبة تركيز اليوريا ، كرياتنين ، الصوديوم ، البوتاسيوم ، الكالسيوم الفوسفات فيها ثم اجريت عملية الديلزه باستخدام محلول الغسل المحضر محليا بالمختبر الكمياوي واستمرت عملية الغسل ثلاث ساعات ثم قيست نسبة تركيز الالكتروليتات الانفة الذكر واليوريا والكرياتنين بعد عملية الديلزة بساعتين لمعرفة مقدار التخفيض في نسبهما بعد الغسل الكلوي وطبق هذا الغسل الكلوي على (20) مريض

سحبت نماذج دم اخرى من مرضى اخرين قبل الديلزه وقيست تركيز الالكتروليتات الصوديوم البوتاسيوم الكالسيوم الفوسفات واليوريا والكرياتنين فيها ثم اجريت عملية الديلزه باستخدام محلول غسل جاهز مستورد قياسي (سعودي المنشا) واستمرت عملية الغسل ثلاث ساعات . ثم قيست نسب تركيز الالكتروليتات السابقه الذكر واليوريا والكرياتنين فيها بعد الغسل الكلوي لمعرفة مقدار التخفيض الحاصل في نسبهما بعد

الديلزه وطبق هذا الغسل الكلوي على 18 مريض وقد استخدم في كلتا الحالتين نفس المرشح النصف الناضح والمصنوع من مادة السليلوز المركب Cuproammonium cellulose والمجهز من شركة (Nipro) كما استخدم نفس جهاز الكليه الصناعيه في الغسل في الحالتين نوع B- Brown واستخدمت نفس مواصفات الماء المزال منه الايونات والخالي من الجراثيم في الحالتين .

حضر المحلول المحلي في المختبر الكيمياوي باذابة كلوريد الصوديوم ، كلوريد البوتاسيوم ، كلوريدالكالسيوم ، كلوريد المغنيسيوم وخلات الصوديوم بنسب وزنية معينة وحسب الجدول رقم(4) في 34 لتر ماء مزال منه الايونات والجراثيم . وكانت الاملاح المستخدمة نقية خالية من الشوائب نوع Analar ومخصصة لاغراض طبية وصيدلانية ومستوردة من مناشئ مختلفة وخالي من البيكاربونات .

اما المحلول المستورد الجاهز الخالي من البيكاربونات ايضا فهو مجهز بعبوة بلاستيكية سعة (5) لتر سعودي المنشا ويحتوى على نفس الاملاح السابقة اضافة الى الدكستروز . ويخفف بالماء الخالي من الايون الى 34 لتر وحسب نسب المكونات الوزنية المعطاة في الجدول رقم (4).

النتائج والمناقشة

من الامور المهمة التي تؤثر على عملية الديلزة هو عمر المريض ،نوعية محلول الغسل ،تركيزمكوناته،نوع الماء المستخدم في عملية الديلزة ، اضافة الى نوع المرشح المستخدم في جهاز الديلزة من حيث ملائمتة للمريض['21] اضافة الى كفاءة ونوعية جهاز الكليه الصناعية المستخدم من حيث تزويدة بكل ما يحتاجه من معدات ومواد ضرورية خاصة بعملية الغسل والتي تتناسب مع كل مريض['22] كما ان سلامة المريض من الامراض المصاحبة للعجز الكلوي مثل داء السكر وامراض القلب ،الضغط والاستخدام المفرط لبعض الادوية والمسكنات وبجرعات عالية تؤثر على كفاءة الديلزة اضافة الى عامل مهم اخر هو المرحلة التي بدأ فيها الغسل الكلوي للدم هل كانت مرحله مبكره او متأخره من حالة عجز الكليه المزمن '25 '23] 24]

يبين الجدول رقم (1) التغير الحاصل على معدل تركيز العناصر الحيويه التي تتأثر في حالة العجز الكلوي المزمن قبل وبعد عملية الديلزه والمستل من المصادر العلميه التي تستخدم جهاز مناسب للديلزه ومحلول غسل قياسي مطابق للمواصفات القياسه ومرشح ملائم للمريض وحالته الصحيه ['26] .

جدول رقم (1) يبين التغير الذي يطرأ على معدل تركيز المواد الحيويه في الدم بعد عملية الديلزه والمستل من المصادر العلميه

العناصر الحيويه	تركيز ها قبل عملية الديلزه	تركيز ها بعد عملية الديلزه	مقدار التغير بالتركيز بعد عملية الديلزه	النسبه المئويه لمقدار الانخفاض بالتركيز بعد عملية الديلزه
اليوريا (ملغم/100مل)	132	63	69	52.2
كرياتنين (ملغم/100مل)	10.3	5.6	4.7	45.6
البوتاسيوم (ملي مكافئ /لتر)	5.4	4	1.4	25
الكالسيوم (ملغم/100مل)	9.8	11.2	1.4	14.3
الصوديوم (ملي مول /لتر)	137	138	1	14
الفوسفات (ملغم/100 مل)	4.9	3.6	1.3	26.5

اما الجدول رقم (2) فيظهر التغير الحاصل على معدل تركيز العناصر الحيويه التي تتاثر بالعجز الكلوي قبل وبعد عملية الديلزه وباستخدام جهاز غسل كلوي نوع B- Brown ومحلول غسل كلوي جاهز مستورد مطابق للمواصفات القياسيه سعودي المنشأ

والجدول رقم (3) يبين التغير الذي يطرأ على معدل تركيز العناصر الحيويه قبل وبعد عملية الديلزه وباستخدام محلول غسل كلوي محلي التحضير حضر في المختبر من مواد كمياويه نقيه مخصصه لاغراض طبيه وصيدلانيه من مناشئ مختلفه.

جدول رقم (2) يبين التغير الذي يطرأ على معد تركيز العناصر الحيويه في الدم بعد عملية الديلزه وباستخدام محلول غسل الكليه جاهز ومستورد ومطابق للمواصفات القياسيه ومطبق على 18 مريض

M= mean ---------- SD= _+ standard deviation

العناصر الحيويه	معدل تركيزها قبل عملية الديلزه	معدل تركيزها بعد عملية الديلزه	مقدار الانخفاض بمعدل التركيز بعد الديلزه	النسبه المئويه لمعدل الانخفاض بالتركيز بعد عملية الديلزه
اليوريا (ملغم/100مل)	M = 181.5 SD = 13.2	M=104.9 SD=11.7	76.6	42.2
الكرياتنين (ملغم/100مل)	M = 10.7 SD = 0.79	M=6.5 SD=0.4	4.3	40.2
البوتاسيوم(ملي مكافئ /لتر)	M = 5.1 SD = 2.21	M=3.6 SD=2.13	1.3	29.4
الكالسيوم (ملغم/100مل)	M = 8.1 SD = 4.3	M=1.05 SD=4.5	2.4	29.6
الصوديوم(ملي مول /لتر)	M = 140.5 SD = 11.8	M=140.9 SD=11.1	0.4	0.3
الفوسفات ملغم/100مل	M = 7.4 SD =2.1	M=5.1 SD=1.9	2.3	31.1

جدول رقم (3) يبين التغير الذي يطرأ على معدل تركيز العناصر الحيويه في الجسم بعد عملية الديلزه باستخدام محلول غسل محضر محليا ومطبق على عشرين مريض

العناصر الحيويه	تركيزها قيل الديلزه	تركيزها بعد الديلزه	مقدار الانخفاض بالتركيز بعد الديلزه	النسبه المئويه لمعدل تخفيض التركيز بعد الديلزه
اليوريا ملغم/100مل	M=214 SD=13.2	M=144.3 SD=11.8	70	32.7
الكرياتنين ملغم/100مل	M=9.1 SD=1.1	M=5.5 SD=0.5	3.6	39.6
البوتاسيوم ملي مكافئ /لتر	M=4.84 SD=1.9	M=4.4 SD=1.7	0.44	8.3
الكالسيوم ملغم/100مل	M=6.8 SD=5.1	M=7.3 SD=4.8	0.5	7.4
الصوديوم ملي مول /لتر	m=134.6 SD=10.2	M=141.6 SD=9.8	7	5.2
الفوسفات ملغم/100مل	M=7.4 SD=3.2	M=4.7 SD=3.8	2.7	36.5

ويبن الجدول رقم (4) مكونات محلول الغسل الكلوي القياسي المطابق للمواصفات العالمية ومتكون من محلولين Hypertonic solution و Hypotonic solution يعطى للمريض حسب حالته االصحيه ومضاف له مواد كمياويه اخرى تعدل من قيمة الدم (PH) ويمكن ان يستخدمها الاثنان معا اذا احتاج الامر['27]

جدول رقم (4) يبين تركيز مكونات العناصر الداخله في المحلول الغسل الكلوي المحلي التحضير والمحلول الجاهز المستورد

اسم الماده	تركيز محلول الغسل المحلي التحضير مقاس ملي مول /لتر	تركيز محلول الغسل الجاهز المستورد مقاس ملي مول /لتر
الصوديوم	**139**	**135**
البوتاسيوم	**2**	**1**
الكالسيوم	**1.75**	**1.75**
المغنيسيوم	**0.37**	**0.5**
الكلورايد	**103**	**93**
الخلات	**30**	**32**
الدكستروز	–	**1 غرام /لتر**
البيكاربونات	–	–

بينما الجدول رقم (5) يظهر مكونات الوزنيه للمحلول الغسل الكلوي للدم المحلي مقارنه مع مكونات الوزنيه لمحلول الغسل الكلوي الجاهز المستورد ويمتاز المحلول الجاهز باختلافات طفيفه في التركيز مع المحلول المحضر مختبريا عدا ان المحلول الجاهز حاوي على الدكستروز والاثنان خاليان من البيكربونات ويطلق عليهما محلول الغسل الكلوي نوع Acetateحيث تقوم الخلات بتنظيم PH الدم ['28]

اما بالنسبه للمرشحات المستخدمه في عملية الديلزه فهناك انواع مختلفه من المرشحات والتي تتلائم مع عمر المريض وحالته الصحيه ولكن المتوفر حاليا هو نوعين فقط وهذا بدوره يوثر على كفاءة الديلزه باعتباره عامل مهم في هذه العمليه [29]

جدول رقم (5) يبين تركيز عناصر محلول الغسل الكلوي في حالة Hypotonicsolution و Hypertonicsolution والمستل من المصادر العلميه

اسم العنصر	تركيز محلول الغسل الكلوي Hypotonicsolutionمقاس في الملي مول /لتر	تركيز محلول الغسل الكلوي Hypertonicsolutionمقاس في الملي مول /لتر
الصوديوم	**27.69**	**238**
البوتاسيوم	**1.52**	**10**
الكالسيوم	**0.96**	**7.3**
المغنيسوم	**6.52**	**2**
الخلات	**10.4**	**41.8**
البيكربونات	**12.5**	**47.9**
الكلورايد	**9.28**	**220**
لاكنات	–	**1.67**
النترات	–	**0.3**
الدكستوز	**60**	**60**

يلاحظ من مقارنة الجداول (1.2.3) ان عملية الغسل الكلوي للمرضى المصابين بعجز الكليه المزمن بدأت بمرحلة متقدمة من المرض اي بعد ان ارتفعت نسبة اليوريا كثيرا عن القيمة الطبيعية لها (14 – 40) ملغم /100 والكرياتنين عند 10.7 ملغم/100 مل بينما تشير المصادر العلمية ان عملية الغسل في الدول المتقدمة تتم عندما تكون نسبة اليوريا في الدم اقل من 150ملغم/100مل والكرياتنين اقل من10 ملغم /100مل

يظهر من مقارنة النتائج المدونة في الجداول رقم (1.2.3) ان معدل تركيز اليوريا في الدم قد انخفض بعد الديلزة بنسبة 46.6% هذا ماوضحة الجدول رقم (1) المستل من المصادر العلمية والتي تم فيها عملية الديلزة بكفاءة وتقنية عالية ولكن هذا الانخفاض لم يصل الى القيمة الطبيعية.

يبين الجدول رقم 2 النسبة المئوية لمعدل انخفاض تركيز اليوريا في الدم كانت 42.2%والنسبه المئويه لمعدل انخفاض الكرياتنين هو 40.2% ولكنهما بعد الديلزة لم تنخفض قيمتهما الى القيمة الطبيعية وان مقدار الانخفاض المبين في الجدول رقم 1 هو اكبر من مقدار الانخفاض المبين في الجدول رقم 2 وربما يعزى ذلك الى كفاءة التقنية العالية المستخدمة في عملية الديلزة في البلدان المتقدمة. .

اما يالنسبة لمعدل انخفاض تركيز اليوريا والكرياتنين في الجدول رقم (3) فبلغ 32.7 % بالنسبة لليوريا و39.6% بالنسبة للكرياتنين ربما يعزى ذلك الى عدم توفر المرشحات المناسبة للمريض كما ان عملية الغسل الكلوي قد بدات في مرحله متقدمه من مراحل العجز الكلوي المزمن مقارنة مع معدل تركيز اليوريا في الجدولين(1)(2) ومن ملاحظة التغير الحاصل في تراكيز الالكتروليتات في الجداول (1 2 3) ومقارنتها مع بعضها يظهر الجدول رقم(2)ان نسبه الانخفاض للبوتاسيوم هو 29.4 % افضل مما هو في الجدول رقم 1 والجدول رقم(3) وان تركيز البوتاسيوم فيه بعد الديلزه مساوي للقيمة الطبيعيه له .اما بالنسبه للجدول رقم (3) فكانت النسبه المئويه للانخفاض معدل تركيز البوتاسيوم في الدم هو 8.3 % وهو اقل من الحالتين السابقتين وربما يعود الى اختلاف تركيزة في محلول الغسل المحلي عن المحاليل القياسية. لذا يوصى بتخفيض مستوى تركيز البوتاسيوم في المحلول المحضرمحليا بنسبة قليلة مع مراعاة المحافظة على تركيز كل من الالكتروليتات (K,Ca,Mg) ضمن الحدود الطبيعية لان التغير في تركيزها يوثر على ثبات كهربائية الجسم ويؤثر على وظيفة القلب.

يظهر الجدول رقم (1) ان نسبة الكالسيوم في الدم بعد الديلزة مساوية للقيمة الطبيعية ومثله الجدول رقم (2) مما يدل على كفاءة محاليل الغسل الكلوي المستخدمة.اما الجدول رقم (3) فيشير الى ان تركيز الكالسيوم بعد الغسل الكلوي اقل من القيمة الطبيعية فيجب مراعاة ذلك عند تحضير محلول الغسل الكلوي .

اما بالنسبة لمعدل تركيز الصوديوم والفوسفات فأ ن نسبتهم بعد الديلزة في الجدول رقم (2) وفي هذا الاخير افضل مما في الجدول رقم (3) وربما يعزى ذلك الى وجود شوائب في محلول الغسل الكلوي مثل (So4.Zn.NO3.Cl.Cu.Al) مع مراعاة ان يكون محلول الغسل محافظا على ضغط الدم الاعتيادي وpH للدم ويمنع تحطيم مكونات الدم وبروتينات البلازما .

التوصيات

1 –يجب ان تكون المواد الداخلة في تحضير محاليل الغسل الكلوي للدم تامة النقاوة خالية من الشوائب والعناصر الثقيلة .

2–ان يكون الماء المستخدم في غملية تحضير محلول الغسل الكلوي من النوع الخالي من الايون .خالي من الجراثيم لا يحتوي على شوائب كيمياوية (النترات .الكلوريدات الكبريتات. الالمنيوم .الخارصين).

3– توفير مرشحات مناسبة تلائم الحالة الصحية لكل مريض .

4– يجب ان يجرى فحص دم بعد الديلزة لمعرفة كفاءة عملية الديلزة وذلك من معرفة نسبة التخفيض الحاصل في نسب اليوريا والكرياتنين والالكتروليتات الاخرى

5–يعتبر المحلول المحضر مختبريا كفوء نسبيا في عملية الديلزة ويمكن استخدامة قي حالة شحة محاليل الغسل الكلوي الجاهزة ويفضل ان يكون قريبا اكثر من المواصفات القياسية.

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