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EDITORIAL BOARD - 2019

FORWARD

Dear Colleagues,

IJST was a fruitful effort issued by the International Centre for Advancement of Sciences and Technology – ICAST, which tries to take part in both globalization and revolution in information and communication technologies, because S&T development becoming not only the key elements of economic growth and industrial competitiveness, but also essential for improving the social development, the quality of life and global environment. ICAST took then a decision to establish a scientific alliance with TSTC (Tharwa for scientific Training & Consultations) and this alliance comes to support the efforts towards publishing IJST.

Today, we announce a new issue of our journal, that is the second issue from the fourteen volume of IJST, June, 2019.

Finally, I hope that all significant figures of sciences whom joined the editorial board, the researchers, and the readers of our journal will keep IJST between their eyes and contribute in continuing its journey, with their remarks, valuable recommendations and their researching outcomes.

Thanks a lot for all who support IJST.

Editor-in-Chief

IJST

Abdul Jabbar Al- Shammari

The Referees for this Issue

** The referees and advisory group below are listed according to alphabetical order, with deep appreciation for all.*

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A comparative study on fracture healing in dogs' femur with induced diabetes mellitus treated by Glyburide

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ABSTRACT

The aim of this study was to evaluate the effect of diabetes mellitus and glyburide on bone healing of experimentally induced fractured of the fumeral bones in dogs. Twelve adult male dogs of age of 1–3 years and weights 14–20 kg . (Mean ±SE, weight 16 ± 0.32 kg) were used. The sample was divided randomly into three equal groups, control group, diabetic group and diabetic with glyburide treated group. Diabetes mellitus was induced in both diabetic and diabetic with glyburide treated groups by an intravenous injection of alloxan monohydrate at a dose (100 mg /kg) . The animals were anesthetized by giving pre-medication with atropine sulphate 0.04 mg/kg B.W. intramuscularly, after 10 minute, intramuscular injection of a mixture from xylazine 5 mg/kg and ketamine at dose 15mg/kg B.W**.** A mid shaft transverse femur fracture was a achieved and fixed by Intramedullary pinning. Control group was left for healing naturally. In diabetic group inducing of diabetes mellitus and fracture of femur bone similar as control group, while in diabetic with glyburide treated group same as in diabetic group in addition to oral glyburide drug at dose (20 mg/animal) daily.
Clinical examination at 1- 35 days show dysfunction of the fore limbs movement, swelling the site of operation , heat and response to digital palpation, which were severe in diabetic group than in diabetic with glyburide treated group but less than that in control group. Also in diabetic group and diabetic with glyburide treated group there was increase blood glucose concentration, depression, polyuria and polydipsia .
Radiological examination at 7 ,14, 21, 28 and 35 days postoperative were done. Control non diabetic group showed at 7 days visible fracture line, no periosteal reaction around fracture site, while at 14 days showed visible fracture, slight periosteal reaction around fracture site. At 21days the same group showed increase of callus formation, but still visible fracture line and at 28 days showed more dense callus formation but still visible fracture line. At 35 days showed un visible fracture line with alignment of cortices of fracture ends, slight periosteal reaction around fracture site, visible fracture line while at 21days. In diabetic with glyburide treated group at 14 days, show slight periosteal reaction with visible fracture line. At 21 days, show present callus formation and still visible fracture line. At 35 days show increase callus formation in fracture line. , good alignment as cortices and complete healing.
Histopathological findings at 35 post-operative days in control group showed a new formation of osteoid materials are present with normal osteocytes in the bone also trabiculi area of stem cells of bone marrow and large area of ossification with active osteoblast indication presence of good mineralization. In diabetic group showed a large area of not ossified bone with bone marrow without stem cells. In diabetic with glyburide treated group show large number of stem cells in order to formation of new bone(healing) , presence of osteocytes and active osteoblasts with area of trabeculi formation , present of osteoblast in the border and presence of osteocyte (large number) and stem cells of bone marrow with new trabeculi formation. In conclusion of the study showed that diabetes mellitus causes delay in bone healing after fracture, while glyburide enhances healing in diabetic animals.

Keywords: diabetes mellitus, glyburide, bone healing, fracture, alloxan monohydrate

INTRODUCTION

The goal of the treatment of a fracture is the restoration of bone structure, composition, and function. The vast majority of fractures (90- 95%) are treated successfully (1). The highly complex process of fracture repair is still not fully understood; however, research of recent years has identified various associations between factors that affect the repair process and healing outcome the most dominant mechanical factors is the fracture geometry, described by fracture type and gap size. In comminuted fractures and fractures with large butterfly fragments, angulation and displacement of the fragments may result in a delayed healing). Diabetes mellitus is one of the chronic diseases that occur either due to decrease in insulin secretion or lack of insulin peripheral activity (2).

Alloxan induces diabetes by destroying the insulin producing beta cells of the pancreas. In vitro studies have shown that alloxan is selectively toxic to pancreatic beta cells, leading to induction of cell necrosis, leading to induction of cell necrosis. This action is mediated by reactive oxygen species with a simultaneous massive increase in calcium concentration leading to a rapid destruction of beta cells. The use of lower dose alloxan produces partial destruction of pancreatic beta cells even though the animals became permanently diabetic (3).

Diabetes mellitus is one of the chronic diseases that occur either due to decrease in insulin secretion or lack of insulin peripheral activity (4) Fracture healing Long bone fractures are very common in dogs and are presented in a variety of forms. There are four main principles of fracture repair set forth by the Arbeitsgeme in Schaft Osteosynthese Fragen. The relevant literature of repair of fractured bone has been reviewed under the sub-heads mentioned in reference (5).

METERIALS AND METHODS

Twelve (12) male dogs were aged between 1 and 3 years. Their weights ranged from 14 to 20 kg (16.± 0.32). These dogs were brought from scattered areas of the province of Basra and placed in individual cages in the animal house at the veterinary college. These animals were examined clinically and radiophysiologically to ensure that they were healthy.

The dogs were divided into three groups. Group one, the fracture occurred in femur were left to heal naturally. Group two, the femur was fractured with the induced of diabetes by Alloxan 100 mg/ kg. Group three, the fracture of the femur with the induction of diabetes and treated by the glyburide (6).

Animals were not allowed to eat for 24 hrs and then stopped from intravenous dioxin from 100 mg per kg with distilled water by 0.9% and then measuring the blood sugar level were done before and after injection after taking diabetes to 160 days after birth.

Animals were not allowed to eat for 24 hrs. and then stopped from intravenous dioxin from 100 mg per kg with distilled water by 0.9% and then measuring the blood sugar level were done before and after injection after taking diabetes to 160 days after birth.

Normal blood glucose concentration of dogs > 80 to 100 mg/dl (If it was above 200mg/dl and polydipsia and polyurea for 3-7 days were considered to be signs of diabetes mellitus and selected for the experiment (7). In some dogs whose plasma glucose levels were below 200 mg/dl after injection of alloxan, a second injection of alloxan was given. The dogs were maintained on dextrose saline for a further 8-9 hours in order to prevent any hypoglycemic episodes. The dogs were returned to their cages and fed. The blood glucose level was determined by using an apparatus (Accu-chek) (8). After 3 days of alloxan injection blood glucose levels were measured by taken a few drops of blood from each dog's ear.

Surgical Procedure:

The animals were anesthetized by giving pre-medicated with atropine sulphate 0.04 mg/kg B.W. intramuscularly. After 10 minutes, intramuscular injection a mixture of xylazine at dose 5 mg/kg and ketamine hydrochloride at dose 15mg/kg B.W were administered (9).

The operations were performed with the animals under standard aseptic techniques. The animal recumbent on its left side, an incision (10cm) was made at the middle lateral surface of fumeral region, incision the skin. When the fumeral bone was exposed curved artery forceps introduce around the bone achieve a complete transverse fracture of mid shaft by hand saw (Figure 1).

The fracture was fixed by intramedullary pins (Steinmann pin), suitable diameter of medullary bone. The proximal end of the bone was clung by using bone chuck and inserted steinmann pin in fracture site using pin-chuck by retrograde method up to penetrating the head of bone (figure 2), then the alignment of the fracture ends and then inserted the pin into distal fracture bone. The pin was cut using pin cutter. Muscles were reconstructed and sutured by using 3/0 chromic catgut 3-0, the skin closed by blanket suturing pattern using silk**.** These operations were done in animal of group Control group, while These operations were performed in diabetic group and diabetic with glyburide treated group after inducing of diabetes mellitus.

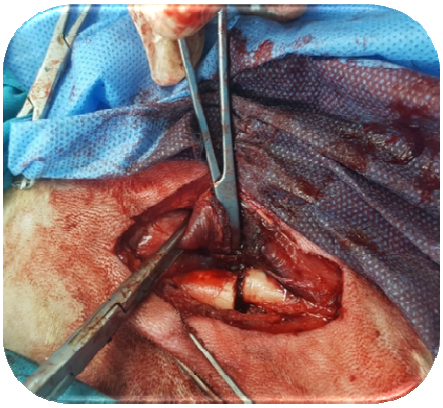

Figure (1): Inducing of fracture in the mid- shaft of femur

Figure (2): Insertion of Steinmann pin through proximal part of femur fracture part

Follow up radiographic bone healing was conducted by taking x-ray of the lateral inspect of the leg before the animal was euthanized at 7, 14, 21, 28, 35days. The radiographic examination included assessment of the callus formation and the presence or absence of fracture line, x-ray apparatus (GNATUS,70VP.15 mA at0>25) and X-ray films.

Histopathological evaluation:

Femur bone was cut by 2 cm for each limb side of the fracture line and then washed with water after washing with sodium citrate. For 12 days it had become soft after washing with water daily. Three days later, bone was removed. Bone samples were washed with ethyl alcohol (10). Specimens were collected from all the animals from the site of the fracture after a period of 35 days postoperatively for the three groups were taken an immediately fixed in 10% buffered formalin, routinely processed, sectioned and stained with Hematoxylin and Eosin (H&E).

Postoperative care:

The animals were given antibiotics represented by penicillin streptomycin at a dose of 1 ml per 25 kg of body weight for three days.
The topical oxytetracycline sprays (oxytetracyclineHcl 4.2 gm., Gention 420 mg) were used in the clinical case as the following increased the consumption of water quantities as well as the increase in the number of urination and also the severe case of inactivity and deflation that were observed every two hours for three days (3).

RESULTS

Blood glucose evaluation:

In control and the diabetes groups, diabetic animals were observed a rise in day 14, although not significant, noting a decrease in the level of diabetes 28 days significant decrease compared to the previous two groups. It was observed in the three groups with diabetes.
Therefore, there was increase in sugar but was observed in the three groups on 14 days Therefore, there was a decrease in diabetes. However, in the three groups in the 28 days period, significant increase was observed in total non-treated diabetes compared with total diabetes treatment and controlled outcome. There was therefore a significant reduction in treated diabetes when compared with total diabetes (Table 1).

Table (1): Mean values of blood glucose levels of the three experimental groups

Group	0 day	14 days	28 days
Control	61.16 c ±15.43 a	63.33 c ±10.80 a	75.83 c ±16.55 a
Diabetic without treatment	175.00 A ±59.16 a	190.00 A ±34.05 a	166.66 A ±21.60 a
Diabetic with treated glyburide	110.00 B ±25.29 a	134.16 B ±31.68 a	94.16 B ±22.00 b

Clinical findings:

Radiological findings: The radiographic images have been taken as following at 7, 14, 21, 28, 35 days post-operative.

Control group: At 7 days showed visible fracture line, no periosteal reaction around fracture site. At 14 days showed visible fracture, slight periosteal reaction around fracture site).At 21 days showed increase of callus formation, but still visible fracture line. At 28 days showed denser callus formation but still visible fracture line. At 35 days showed invisible fracture line with alignment of cortices of fracture ends (Figure 3).

Diabetic group: At 7 days, showed visible fracture line, no periosteal reaction around fracture site. At 14 days, slight periosteal reaction around fracture site, visible fracture line. At 21 days, showed periosteal reaction around fracture site, visible fracture line. At 28 days showed periosteal reaction around fracture site, visible fracture line. At 35 days showed without periosteal reaction still visible fracture line (Figure 4).

Diabetic with glyburide treated group: In 7 days, no reaction was caused around the periosteal around the fracture line In 14 days a thick sesame reaction occurs with the survival of the fracture line. At 21, the calcification of the core is marked with the fracture line remaining. In 28 days it increased in calcification while the fracture line remained.
In 35 days, there was a sharp increase in the calcification of the un visible line of fracture (Figure 5).

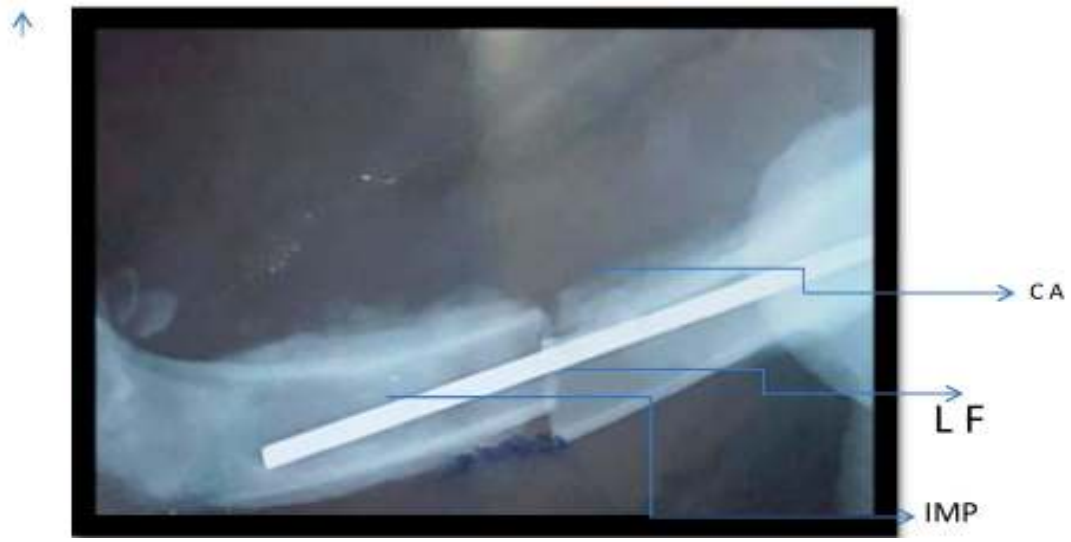

Figure (3): A phtographaic picture for control group (lateral view) on 35 days showed invisible fracture line with alignment of cortices of fracture ends.(line fracture LF, intra midullary pin IMP callus area CA).

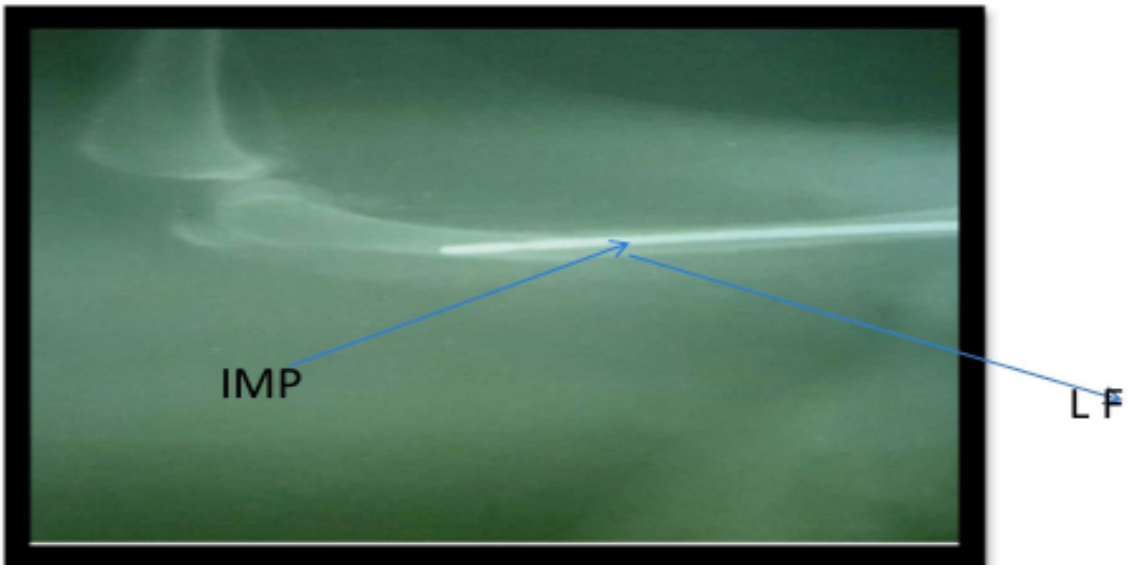

Figure (4): A phtographaic picture for diabetic group (lateral view) on 35 days showed loss periosteal reaction around fracture site, visible fracture line (line fracture LF, intra midullary pin IMP).

Figure (5): Diabetic with glyburide treated group (lateral view) on 35 days showed increase callus formation in fracture line, but still visible (line fracture LF, intra midullary pin IMPcallus area CA).

Histopathological Examination:

Control group : Remodeling phase involves the formation and mineralization of the callus and replacement of the mineralized callus with mineralized bone and sculpting of the bone back to its original shape, size, and biomechanical competency via modeling and remodeling days a new formation of ossifcation are present with normal osteocytes in the bone also trabecular area of stem cells of bone marrow and large area of ossification with active osteoblast). Presence of good area of mineralization Good area of trabeculae and endosteal cells, osteoblast and osteocyte (Figure 6).

Diabetic group: As fibroplasia phase begins, necrotic bone resorption is carried out by osteoclasts that are derived from the circulating monocytes in the blood and by monoblastic precursor cells originating from the local bone marrow. The fibroplasia phase is characterized by the formation of callus and begins with continued vascular in growth. In 35 days, there is a large area of non-fossilized bones with bone marrow without stem cells, as well as bone cells that make up the tumor cells. Active bone necrosis is on the border of bone trabeculi, with stem cell loss in the bones and bilateral hallo significant for the late maximization Active bone cells are bone present with a small mineralization of small trabeculi formation and bone formation (Figure 7).

Diabetic with glyburide treated group: In 35 days a cartilage osteoarthritis occurred in the region in a large number of stem cells in order to form a new healing bone, and indicated the presence of endosteal cells of bone and bone marrow cells.
The region of trabeculea formation and present bone cell osteocyte cellFigur Bone presence (large number) and stem cells for bone marrow new trabeculae formation (Figure 8).

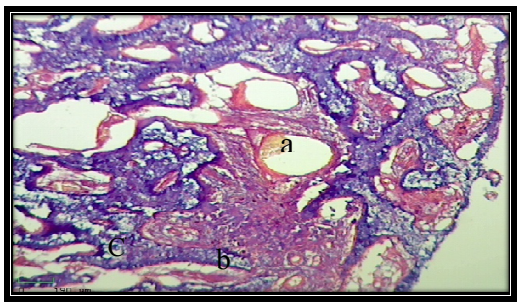

Figure (6): The cross-section of the control group after 35 days: (a) a good area of trabeculae (compressed bone); (b) osteocytes bone cells; (c) active osteoblast. (H&E, 40x).

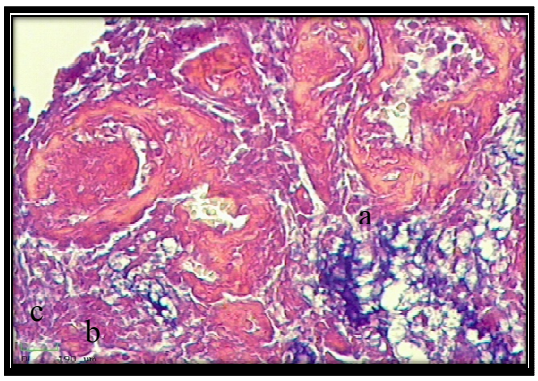

Figure (7): The cross-section of the diabetes group after 35 days: (a) formation of trabeculae; (b) osteocyte. (c) bone marrow. (H&E,40x).

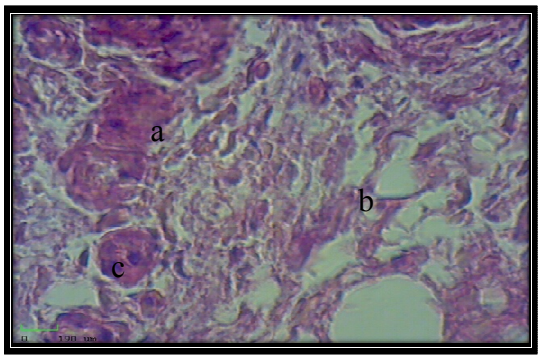

Figure (8): The cross-section of diabetics with the group treated with glibrid in 35 days: (a) the formation of bone marrow. (b) Osteocyte. (large number) (C) Stem cells for bone marrow. (H&E,40x).

DISCUSSION

Induction of diabetes mellitus in diabetic and diabetic with glyburide treated groups: The dose used in this trial (100) mg / kg was clinically effective and showed an increase in the blood sugar level during the first seven days. This is in consistent with (11). The results indicated that there were differences of statistical significance in the glucose levels between total cyclic, and those who agree with who had similar results for this dose. In this study there were signs of the bed was characterized by severe depression after the first two weeks in the total diabetes and treated diabetes. Alloxan selectively harms the beta of the pancreas and causes hypoglycemia in the blood with disorders that causes high sugar.

Radiological findings in control group: In the first week there was a fracture line where it was visible and there was no reaction on the perineum around the fracture position. Within 14 days, the line of fracture was observed. There was a small amygdala interaction around the fracture line. In the 21 day period, the fracture was visible, while in 35 days it was noticed that the fracture line had disappeared and the bone was almost normal and the calcification was very double (12).

Diabetic group: In the first week there was a fracture line where it was visible and there was no reaction on the perineum around the fracture position. Within 14 days, the line of fracture was observed. There was a small amygdala interaction around the fracture line. In the 21 days period, the fracture was visible, while in 35 days it was noticed that the fracture line had appeared and the bone calcification was slow. The calcification process depends on the delay may be due to diabetes, which causes disorders in the metabolism and atrophy of the blood vessels, which in turn works as a barrier between the formation of bone and cartilage, and this study was in consistent with (13), as the insulin signals cause the extraction of carboxylic bone.

Diabetic with glyburide treated group : In the first week there was a fracture line where it was visible and there was no reaction on the perineum around the fracture position. Within 14 days, the line of fracture was observed. There was a small amygdala interaction around the fracture line. In the 21 days period, the fracture was visible, while in 35 days it was noticed that the fracture line had disappeared and the bone was almost in normal calcification. The calcification process depends on the condition of the healthy body as well as the nutrition and activity of the bone cells.

The formation of good alignments for bone and crustaceans and bones and the repair itself and that the cause of healing is that the substance produced by insulin, which promotes the process of bone formation and calcification through its work in the metabolic processes and this is consistent with (14), As the family of Spaniel Urea act on the activation of insulin by the effect of cells Beta (15). This in the non-diabetic group was faster than the group treated with diabetes and the treatment group, as well as the treatment group is faster than the non-treated group.

Histopathological Changes:

Control group: The results of the anatomical pathology of this group showed the presence of new formulations of materials and materials and the presence of bone density of bones of bone and cells of Gaia and cells of osteoblast, and also a cell culture of the cells of active bone corresponds exactly with the images taken to this group in our study and this corresponds.

Diabetic group: The results of the pathological anatomy of this group showed a wide area of bone non-fossil bone marrow and the number of a number of stem cells with the loss of magnification with the number of a number of sub-cells with great loss of stem cells and delay the maximization and provide active bone oocytes with a few mineralization and also a little trabeculae and this corresponds to the results showed that all the minerals in type 1 diabetes have decreased.

Diabetic with glyburide treated group: The results showed in this group a large number of stem cells and active bone cells in order to form an area osteocyte and trabeculai of the same cells from the bone marrow and this corresponds to the radiographic images in the study which showed the disappearance of the fracture line and that antipsychotics may occur activity of bone (16), which supports the hypothesis of control of the proportion of sugar, which showed the effect of the burden of healing, the results showed satisfactory in the study that bone healing in the group did not have diabetes was compared with the group of diabetes and diabetes treatment, while the results of osteoporosis in diabetes treatment was faster than diabetes in this study supports the previous precedents related to the role of diabetes and high altitude healing fractures the sulphonyl urease so the reduce serum glucose level. Possibly paying for its hypoglycemia effect (17).

Glyburide is used along with diet and exercise, and sometimes with other medications, to treat type 2 diabetes (condition in which the body does not use insulin normally and, therefore, cannot control the amount of sugar in the blood). Glyburide is in a class of medications called sulfonylureas. Glyburide lowers blood sugar by causing the pancreas to produce insulin (a natural substance that is needed to break down sugar in the body) and helping the body use insulin efficiently. This medication will only help lower blood sugar in people whose bodies produce insulin naturally. Glyburide is not used to treat type 1 diabetes (condition in which the body does not produce insulin and, therefore, cannot control the amount of sugar in the blood) or diabetic ketoacidosis (a serious condition that may occur if high blood sugar is not treated) (18).

REFERENCES

1. Wentzensen N.; Ridder R.; Klaes R.; Vinokurova S.; Schaefer U. and vonKnebel Doeberitz M. (2002). Characterization of viral-cellular fusion transcripts in a large series of HPV16 and 18 positive anogenital lesions. *Oncogene*. 21(3):419.

2. Mutalik S.; Chetana M.; Sulochana B.; Devi PU. and Udupa N. (2005). Effect of Dianex, a herbal formulation on experimentally induced diabetes mellitus. *Phytother. Res.* 19(5): 409-415.

3. Russel J.; Tan DX.; Mayo JC.; Sainz RM.; Leon J. and Czarnocki Z. (2003). Melatonin as an antioxidant: biochemical mechanisms and pathophysiological implications in humans. *ACTA. BIOCHIMICA. POLONICA-ENGLISH EDITION.* 50(4):1129-1146.

4. Kumar A.; Kumar D.; Mohindroo J. and Saini NS. (2015). Feasibility Novel three-component domino reactions of ketones, isatin and amino acids: Synthesis and discovery of antimycobacterial activity of highly functionalized novel dispiropyrrolidines. *Europ. J. Med. Chem.* 45(1):411-422.

5. Vaananen HK.; Zhao H.; Mulari M. and Halleen JM. (2000). The cell biology of osteoclast function. *J. Cell. Sci*. 113(3): 377-381.

6. Fulzele K.; Fuller PM.; Saper CB.; Pedersen NP.; Lu J. and Scammell TE. (2010). Sleep state switching. *Neuron*. 68(6):1023-1042.

7. Ogbu JU. (2013). A cultural ecology of competence among inner-city blacks. *Beginnings:* The social and affective development of Black children, 22,45-66. Open approach. *Vet. Med*. 48: 133-140.

8. Kim JS.; Lee YY. and Kim TH. (2016). A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. *Bioresource. Technol.* 199: 42-48.

9. Bader J. and Eckart Z. (2011). HypE: An algorithm for fast hypervolume- based many-objective optimization. *Evolution. Comput.* 19.1: 45-76.

10. Zhen Lei E. and David Z. (2007). The economics of pesticides and pest control. *Int. Rev. Environ. Resource. Econom.* 1.3:271-326.

11. Devaki K. and Gopalakrishnan VK. (2015). Evaluation of antidiabetic potential of aqueous extract of Passiflora edulis sims on Alloxan induced diabetes mellitus in Wistar albino rats. *Int. J. Pharm. Sci.* 34(1): 171-177.

12. Strube M. and Filippova K. (2008). Sentence fusion via dependency graph compression. Pp.:177-185.

13. Hong Woo H. and Lee L. (2005). Molecular design, synthesis, and hypoglycemic and hypolipidemic activities of novel pyrimidine derivatives having thiazolidinedione. *Europ. J. Med. Chem.* 40(9):862-874.

14. Maria D.; Alma M.; Lara T.; Annalina L.; Maria CB.; Vincenzo C. and Simona R. (2015). Intake of sugar-sweetened beverages and weight gain: a systematic review. *Amer. J. Clin. Nutr.* 84(2):274-288.

15. Santosh NM.; Akash DP.; Pritam ND.; Nikhil SS. and Pankaj BM. (2016). Design, synthesis and *in vivo* screening of some novel quinazoline analogs as anti-hyperlipidemic scavenging properties of Zea

mays female inflorescences (maize silks) from Mexican maize landraces. J. Food. 16(1):96-104.
16. Huibin Z.; Ya-an Z.; Guan-zhong W.; Jin-pei Z.; Wen-long H. and Xiao-wen H. (2009). Hypolipidemic effects of flavonoid rich extract from Eugenia jambolana seeds on streptozotocin induced diabetic rats. Food and hypolipidemic activities of novel pyrimidine derivatives having thiazolidinedione. *Eur. J. Lett*. 19: 1740-1744.
17. Danieal S.; Luca R.; Glan PC. and Pamela M. (2015). Sulphonyl urease and their uses dogs using external fixators with closed reduction and limited drought tolerance in chickpea (Cicer arietinum L.). *Theor. Appl. Gen.* 127(2):445-462.
18. NADAC. (2019). Centers for Medicare and Medicaid Services.

Burns statistical and retrosepective study regarding age and sex

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ABSTRACT

The aim of the study was to obtain the socio-demographic characteristics of burn patients in burn unit in Al-Yarmok hospital in Baghdad, Iraq. Data were obtained from medical records of patients with burns admitted to the burn unit, the referral etiology of burns, the total body surface area length of stay and mortality, adults, especially, between 16-60 years of age, run the highest risk of burn injuries, scalds own home are the major cause of admission.

Keywords: burns, patient, prevalence.

INTRODUCTION

A burn is a type of injury to skin, or other tissue, caused by heat, cold, electricity, chemicals, frictions, or radiation. In 2015, fire and heat had resulted in 67 million injuries. This resulted in about 209 million hospitalization and 176.000 deaths. Most deaths due to burns occur in the developing world, particularly in South-East Asia. Treatment developed since 1960 had improved outcomes, in the United States. Approximately 96% of children and young adults admitted to burn centers to serve their injuries (1). Burns are considered global public health problems, accounting for an estimated 180.000 death annually. The majority of these occur in low-and middle income countries and almost two thirds occur in African and South East regions. The rate of children deaths from burns is currently seven times higher in low-and middle- income countries than in high –income countries (2). In India, over 1000.000 people suffer moderately or severely from burn every year. Nearly 173.000 Bangladeshi children suffer moderately or severely burn every year. In Bangladesh, Colombia, Egypt and Pakistan, 17% of children with burn have temporary disability and 18% have a permanent disability. Burns are the second most common injury in rural Nepal accounting for 5% of disabilities. In 2008, over 410.000 burn injuries occurred in the USA with approximately 40.000 requiring hospitalization (3). Fifty years ago, the first international congress on research in burn was held at the national navel USA, a compilation of 64 papers was edited by Artz and published as the proceeding, the last 50 years have been tremendous advances in the management of the a cut burns and in the last decade these improvement have been documented in a series of annual reviews entitled "What's new in burns and metabolism" published by the journal of the American college of surgeons (4). According to World Health Organization (WHO), burns were ranked 9th in the overall mortality rank for people aged 5-14 years with an estimated 41.575 deaths, 15% for people aged 15-29 years with an estimated 62, 655 deaths (5). Studies worldwide have demonstrated that the incidence of burn injuries highest among children below 4 years of age (6). Studies from South African men are a particular risk group for burns related to interpersonal violence and women for self-inflicted burns. About 75% of the majority associated with burns injuries is related to sepsis especially in developing countries (7). The Lund and Browder chart is a tool useful in the management of burns for estimating the total body surface area affected (8). In this study we had analyzed patients requiring treatment for injuries, the purpose of the study is to evaluate the age groups most commonly affected, as well as the most common causes of burn injuries.

MATERIALS AND METHODS

The study is described as a retrospective, based on burn case reports (n=80), recorded in burn unit at Al-Yarmouk Hospital in Baghdad, Iraq, from December to May, 2018 to obtain demographic characteristics of the study population, including occupational burns profile, origin of the referral, etiology of burns, total body surface, length of stay and mortality. The average time between burns injury and initial evaluation (at hospital) was 1+4, 5 hrs. Patients with burns were resuscitated using park land formula for the initial dehydration. Incidence ratios were used to compare males and females. Rates in each age group of patients were up to 24 years (25 years -80 years). The hospital is opening 24 hrs daily and receive about 5-7 cases of burn in each week, patients could seek directly at the emergency centre of this hospital. Burn causes were mostly from hot liquid, fire, electrical and chemical. The degree of burns, length of stay at hospital was divided into three groups: 0-5 hrs. , 5-24 hrs. and more than 24 hrs. Patients' disposition were divided into treated and discharge. Statistical analyses were performed using SPSS version 14.0.

RESULTS

A total of 80 patients suffered from burns were admitted to the burns clinic at Al- Yarmouk hospital. The most affected gender was female with 50 cases (62.5%), compared to 30 cases (37.5%) male patients (table 1). The most affected age group with burn related injuries was the 16-60 years (43.75%), followed by 2-5 years (8.75%), as shown in table (2).

Table (1): Distribution of patients' sample according to gender

Gender	N	%
Males	30	37.5
Females	50	62.5
Total	80	100%

Table (2): Distribution of patients according to age group

Age group (year)	N	%
2-5	7	8.75
6-10	10	12.5
11-15	20	25
16-60	35	43.75
60-80	8	10
Total	80	100%

Regarding the cases of burn injuries, 50 patients (62.5%) cases were suffering from scalds, 2 cases (2.5%) from electrical and chemical burns, 28 cases (35%) from burns caused by fire (table 3). The main group of the burn degree was the third degree 60 (75%), followed by second degree which was noticed at low rate 20(25%) (table 4).

According to the occupational factors, 45 patients (56.25%) were housewives and 20 patients (25%) were students, and 15 patients (18.75%) were working with cooks, (table 5).

Table (3): Distribution of patients according to Etiology of burn

Cause	N	%
Scalds	50	62.5
Electrical and chemical	2	2.5
Fire	28	35
Total	80	100%

Table (4): Degree of burn in admitted patients

Degree	Total	%
Grade 2	20	25
Grade 3	60	75
Total	80	100%

Table (5): Distribution of patients' admission according to occupational status

Occupation	Total	%
Housewife	45	56.25
Students	20	25
Cooks	15	18.75
Total	80	100%

DISCUSSION

Burns are considered one of the most common and devastating forms of trauma. Patients with serious burn injuries shall be admitted immediately in specialized care settings in order to reduce morbidity and mortality. Although survival rates for patients have been improved substantially in the past few decades due to advances in modern medical care in specialized centres, burn wound is considered one of the major health problems in the world; burn injury is an important cause of hospital admission (9).

The results of the current study revealed that burn in females was 30 (37.5%). This result was disagreed with findings reported Mitchell (10), when they found that burn wound in males was more than females. This may be justified as females are exposed more to burns and near loose fitting clothes. In this study, results found that the distribution of burn wound were occurred mostly in age group (16-60) years 35(43.75%), which was in disagreement with the findings reported by Al-Kayleh, 1999 (11), who showed that the age group less than 10 years had the highest distribution burn wounds. In the current study, it was found that 60 (75%) had third degree burn and 20 (25%) had second degree burn, which were similarly with results obtained by Al- Kayleh, 1999, who showed that the highest distribution of burn wound were found in burn patients suffering from third degree burn. The current study showed that the housewives 45(56.25%) were the most susceptible group to burn wound followed by students 20(25%), and these results reflect agreement with findings reported by Hunt 2000 (12), who showed the housewife were the highest susceptible group to burn wound. The relation between patients job and burn wound showed statistical significant. In the current study, the scalds were the most common types of burns 50(62.5%), and this was in agreement with findings reported by Golshan *et al,* 2013 (13), who showed same results, where electrical and chemical burns were relatively minor causes.

CONCLUSION

Although the incidence of burns treated in emergency care was at its highest levels in the adults' ages, gender difference was observed among adults, females suffered more acute burn and the larger differences were among those aged 16-60 years. These differences were even greater in scalds burns.

REFERENCES

1. Forijuoh SN. (2006). Burns in low and middle income countries. A review of available literature on descriptive epidemiology, risk factors, treatment and prevention. Vol.:32. pp: 529-537.
2. Mock C.; Peck M. and Lerug E. (2008). World Health Organization plan for burn prevention and care. WHO, Geneva.
3. Sen GO. and George R. (2007). Unfair ineffective and inefficient gender in equity in health: why it exists and how we can change it. Final report to the WHO commission or social determinant of health women and gender equity knowledge network.
4. Allgomer A. (2005). Centennial perspective on burn treatment. *J. Coll. Surg.* 200: 463-475.
5. Fermandes F.and Torquato M. (2012). Burn injuries in children and adolescents: clinical and epidemiological characterization. *Rev. Gauch. Enferm.* pp: 133-141.
6. Gevaart A. and Durkin D. (2014). A study of energy-related injuries from hospital admission among children and adults in south Africa. *Burns.* 40(6):1209-1218.
7. Macedo JL. and Santos JB. (2005). Bacterial and fungal colonization of burn wounds. Memlust. Waldo. Craz. Pp.:100-539.
8. Diver A. (2008). The evolution of burn fluid resuscitation. *Int. J. Surg.* 6(4): 347-349.
9. Hettiaratchy S. and Papini R. (2004). Initial management of a major burn, assessment and resuscitation. BMJ. 329(7457): 101-103.
10. Mitchell K. (2014). New management strategy for fluid resuscitation, quantifying volume in the first 28 hours after burn injury. *J. Burn. Care. Res.* 34(1): 196.
11.Al- Kayleh AT. (1999). Invasive burn wound infection. *Annal. Burns. Fire Disas.* 7:1-3.
12. Hunt JL. (2000). The presidential address. Back to the future: the ABA and burn prevention. *J. Burn. Care.* 21: 474-783.
13. Golshan A.; Patel C. and Hyder AA. (2013). A systematic review of the epidemiology of international burn injuries, South Asia. *J. Public. Health.* 3(35): 384-396.

Effect of caffeine therapeutic dose on rat organs: A biochemical and histological study

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ABSTRACT

This study was designed to investigate the effect of caffeine on gonads function related with biochemical assess in male rats. Caffeine is a chemical compound that may interfere with many organs' functions in the body, it may cause endocrine dysfunction in diagnosis of many clinical cases. Twenty-four adult male rats were randomly allotted to constituently control and treated groups having 6 rats in each one, all rats gavage daily for 40 days as follows: control group (GI) received 0.5 ml of distilled water. The treated groups were distributed as follow GII received (25mg caffeine/kg), GIII (50 mg caffeine/kg) and GIV (100mg caffeine/kg). Blood samples were collected from inferior vena cava of the heart of sacrificed animals and divided into two tubes; one contains EDTA for hematological analysis and the second tube was centrifuged at 3000rpm for 15min., the serum collected in an Eppendorf tube and stored at -20C^{O} for further laboratory investigation. Rats organs collected for histopathological study. Results reveal that the caffeine significantly increase serum testosterone, a sperm abnormality, RBCs, Hb, PLT. While, caffeine significantly decrease in sperm count, sperm motility and serum ALT, urea, WBCs, in all treated groups when compared to non-treated rats. Histopathological study showed different degree of suppression of spermatogenesis in testes, atrophy of glomeruli, vacuolation of hepatocytes and myocardial muscle cells in a high dose manner.

Keywords: Caffeine, Testosterone, Sperm, CBC.

INTRODUCTION

Coffee and other caffeine-containing beverages are widely consumed on a daily basis (1). Caffeine is now being added to food products such as potato chips, chocolates and bottled water which confirms it is growing popularity (2). It is a naturally occurring substance found in coffee beans, tea leaves, kola nuts and cocoa beans. Recently there has been an increase in energy drink consumption leading to caffeine abuse, with aggressive marketing and poor awareness on the consequences of high caffeine use (3).

It is therefore important to define the possible risks and benefits associated with caffeine intake, in order to be able to better inform both health professionals and the public. In fact, coffee is a complex chemical mixture reported to contain more than a thousand different chemicals including carbohydrates, lipids, nitrogenous compounds, vitamins, minerals, alkaloids and phenolic compounds (4).

Caffeine (1, 3, 7-trimethylxanthine) is a purine alkaloid that occurs naturally in coffee beans (4). Caffeine appears to exert most of its biological effects through the antagonism of the A1 and A2 subtypes of the adenosine receptors (5,6). Caffeine is rapidly and almost completely absorbed in the stomach and small intestines and distributed to all tissues, including the brain. Caffeine metabolism occurs primarily in the liver, which are ultimately excreted in the urine. The structure of caffeine is similar to adenosine in the body.

The caffeine binds to adenosine cell membrane receptors found in the heart, brain, smooth muscles, adiposities, and skeletal muscles. Caffeine can simultaneously affect a wide number of tissues in the body (7); it stimulates the CNS and increases the release of epinephrine. Caffeine has been shown to increase heart rate, metabolic rate, respiratory center outputs, fat oxidation and diuresis. It also decreases perception of pain and fatigue (8).

Caffeine usage may cause anxiety, heart palpitations, trembling, nervousness and facial flushing. These adverse effects are usually dose related. More side effects were reported when subjects consumed greater than 6 to 9 mg/kg body weight (9). Lethal half dose of caffeine is 150 to 200 mg/kg body weight (roughly 100 cups of coffee). Acute caffeine toxicity can cause hematemesis, hyperventilation, hyperglycemia, hypokalemia, metabolic acidosis and cardiac arrhythmia (10). This study aimed to determine the effect of therapeutic doses of caffeine on biochemical parameters and study the histopathological changes on some organs of rats.

MATERIALS AND METHODS

Experimental animals:

Twenty four adult male rats (232 ± 2.5 gm body weight) were housed (6 rats/cage) under optimum identical conditions (12/12 light, dark cycle, 22 ± 2 C°) where in these are allowed free access to pelleted rat chow and tap water, 24 adult male rats were randomly allotted to constituently control and treated groups having 6 rats in each group, according to guide for laboratory animals (11). All rats were gavage daily for 40 days as follows: control group (GI) received 0.5 ml of distilled water. Also, treated groups were distributed as follow GII received (25mg caffeine/kg), GIII (50 mg caffeine/kg) and GIV (100mg caffeine/kg). At the end study, blood samples were collected from inferior vena cava of the heart of sacrificed animals and divided into two tubes; one contains EDTA for hematological analysis and the second tube was centrifuged at 3000 rpm for 15min. The serum was collected in an Eppendorf tube and stored at -20C^{O} for further laboratory investigation. Rats' organs were collected for histopathological study.

Studied parameters:

The animal in this study were weighted after the adaptation period (at 0 time) and at the end of the experiment (at 40 days time). The blood samples were collected from inferior vena cava of the heart of sacrificed animals in ETDE tube for hematological parameters were measured by hematology analyzer (Genex Inc., Florida USA) included: total Erythrocytes count, Hemoglobin concentration, Total Leukocytes count and platelets. The second tube of blood was centrifuged at 3000rpm for 15min. and serum collected in Eppendorf tube for biochemical and hormonal analysis as follows, Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity were measured by U.V assay according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) without pyridoxal phosphate activation (12). The urea level was measured by ultra violet assay and the creatinine level was measured by UV assay (Modified Jaffe' method) through the reagents used in automatic analyzer (Mindray) ACCENT-200 and ACCENT-200 II GEN (13). Serum testosterone was measured using AFIAS-6 (automated fluorescent immunoassay system) (14). The testes weight were measured immediately after collection from sacrificed animals. Measurement of sperm count, sperm motility and sperm normality was done as described by Evans and Maxwell. Caffeine sample used in this study was procured from Iraqi Sama Alfayhaa Pharmaceutical Industries, Himedia company, Iraq. Doses of caffeine prepared and used for this study were 25mg\kg, 50 mg\kg and 100mg\kg body weight of rats.

Histopathological study:

At the end of experiment, all animals were sacrificed and testes, kidney, liver and heart organs were collected, then preserved in 10% buffered formalin for histopathological examination. Slides

were stained with hematoxylin and eosin stain for examination according to Drury (15).

Statistical analysis:

Data were expressed as mean ± standard deviation (SD). In addition to used ANOVA analysis in experiment, least significant difference (LSD) was used to test the differences among means for ANOVA indicated a significant (P<0.05), using computerized SPSS version 11 (13).

RESULTS

Effect of caffeine on body weight of rats:

Table (1) showed no significant differences in body weight of rats before (0 time) and after treatment with caffeine among groups in comparison with control.

Table (1): The effect of caffeine on body weight of rats. N=6 (M ± SD)

Group	0 Time /gm	End time /gm
G1 Control	230.50 ± 22.53 a	294.75 ± 53.77 a
G2 25mg/kg	234.25 ± 18.76 a	299.75 ± 41.73 a
G3 50mg/kg	232.50 ± 16.58 a	297.50 ± 8.10 a
G4 100mg/kg	234.25 ± 20.66 a	298.50 ± 40.44 a
LSD	24.7	50.0

Values expressed in the small letters mean significant differences (P<0.05) level

Effect of caffeine on blood parameters:

Table (2) showed a significant (P<0.05) increase in RBCs count and PLT count in caffeine treated group 50 mg/kg and 100 mg/kg in comparison to the control group and 25 mg/kg caffeine treated group. On the other hand, there is a significant (P<0.05) increase in Hb concentration in high dose group when compared to other study groups, while the WBCs count are significantly (P<0.05) decreased in all caffeine treated group in comparison with control group.

Table (2): The effect of caffeine on blood parameters. N=6 (M ± SD)

Group	RBC $*10^6$cell/mm^3	HB g/dl	WBC $*10^3$cell/mm^3	PLT
G1 Control	5.87 ± 0.51 b	12.60 ± 0.59 bc	10.53 ± 0.75 a	431.50 ± 48.55 b
G2 25mg/kg	6.27 ± 0.14 b	13.11 ± 0.45 b	7.51 ± 0.53 b	438.71 ± 28.71 b
G3 50mg/kg	6.58 ± 0.24 a	13.66 ± 0.38 b	6.96 ± 0.71 b	561.83 ± 49.55 a
G4 100mg/kg	6.96 ± 0.52 a	14.68 ± 0.90 a	5.71 ± 0.49 bc	552.66 ± 56.71 a
LSD	0.68	1.01	1.25	114.5

Values expressed in the small letters mean significant differences (P<0.05) level

Effect of caffeine on serum AST and ALT level:

Table (3) showed no significant differences in serum AST level between caffeine treated groups when compared to control group while there is a significant (P<0.05) decrease in serum ALT level in caffeine treated groups in comparison to control group.

Effect of caffeine on serum urea and creatinine level:

Table (4) showed a significant (P<0.05) decrease in serum urea levels in all caffeine treated groups in comparison to control group. On the other hands, there is a significant (P<0.05) increase in serum creatinine level in G4 and G3 group when compared to G2 and control group.

Table (3): The effect of caffeine on serum AST and ALT level. N=6 (M ± SD)

Group	AST U/L	ALT U/L
G1 Control	106.5 ± 2.51 a	15.0 ± 2.58 a
G2 25mg/kg	95.75 ± 6.80 a	7.25 ± 1.50 c
G3 50mg/kg	103.2 ± 9.03 a	8.50 ± 1.29 c
G4 100mg/kg	106.0 ± 6.37 a	11.5 ± 3.00 b
LSD	8.32	2.77

Values expressed in the small letters mean significant differences (P<0.05) level

Table (4): The effect of caffeine on serum urea and creatinine level. N=6 (M ± SD)

Group	Urea mg/dL	Creatinine mg/dL
G1 Control	31.7 ± 3.53 a	0.45 ± 0.07 c
G2 25mg/kg	19.8 ± 6.27 b	0.44 ± 0.05 c
G3 50mg/kg	21.0 ± 3.74 b	0.49 ± 0.04 b
G4 100mg/kg	26.5 ± 5.80 b	0.61 ± 0.09 a
LSD	6.27	0.08

Values expressed in the small letters mean significant differences (P<0.05) level

Effect of caffeine on serum testosterone and testes weight:

Table (5) showed a significant (P<0.05) increase in serum testosterone level in all caffeine treated groups in comparison to control group while there is no significant differences in the weight of testes between caffeine treated groups and control group.

Effect of caffeine on sperm characteristics:

Table (6) showed a significant (P<0.05) decrease in sperm count, sperm motility and sperm normality in 100mg/kg and 50mg/kg caffeine group in comparison to control and 25mg/kg caffeine treated group. On the other hands, the table also showed a significant (P<0.05) increase in sperm abnormality in 100mg/kg and 50mg/kg caffeine group in comparison to control and 25mg/kg caffeine treated group.

Table (5): The effect of caffeine on testes weight and serum testosterone level. N=6 (M ± SD)

Group	Testes weight/ gm	Testosterone/ ng/ml
G1 Control	1.22 ± 0.20 a	0.66 ± 0.04 d
G2 25mg/kg	1.22 ± 0.22 a	1.06 ± 0.00 c
G3 50mg/kg	1.25 ± 0.26 a	3.49 ± 0.20 b
G4 100mg/kg	1.32 ± 0.09 a	5.43 ± 0.17 a
LSD	0.24	0.16

Values expressed in the small letters mean significant differences (P<0.05) level

Table (6): The effect of caffeine on sperm properties. N=6 (M ± SD)

Group	Sperm count $*10^6/\mu L$	Sperm motility %	Sperm normal %	Sperm abnormal %
G1 Control	55.49 ± 2.64 a	95.0 ± 0.81 a	90.0 ± 0.00 a	10.0 ± 0.00 c
G2 25mg/kg	54.46 ± 1.80 a	93.75 ± 1.25 a	90.0 ± 0.00 a	10.0 ± 0.00 c
G3 50mg/kg	48.44 ± 2.20 b	62.5 ± 2.88 b	87.5 ± 2.88 b	12.5 ± 2.88 b
G4 100mg/kg	47.83 ± 1.87 b	30.0 ± 0.00 c	50.0 ± 0.00 c	50.0 ± 0.00 a
LSD	2.70	2.04	1.81	1.81

Values expressed in the small letters mean significant differences (P<0.05) level

Histopathological study

Testes: Testes of control rats showed normal seminiferous tubules and normal spermatogenesis, the treated groups showed different degree of suppression of spermatogenesis which increase with high dose of caffeine administered as shown in figure (1).

Kidney: Kidney histopathological study of control group showed normal glomeruli and normal cortical tubules and the caffeine groups showed a little effect on kidney tissue presented as vacuolation of glomeruli as shown in figure (2).

Liver: There is no pathological changes in healthy control liver tissue of control groups showed a normal lobular hepatic architecture with central vein, while the treated groups showed mild changes in liver presenting as bile duct proliferation, central vein dilatation and vacuolation of hepatocytes that observed in high dose caffeine group as shown in figure (3).

Heart: Histopathological study of control group showed normal myocardial muscle cells. Caffeine treated groups showed vacuolation of myocytes, congestion of blood vessels and degeneration of cardiac muscle cells that noted in high dose caffeine group as shown in figure (4).

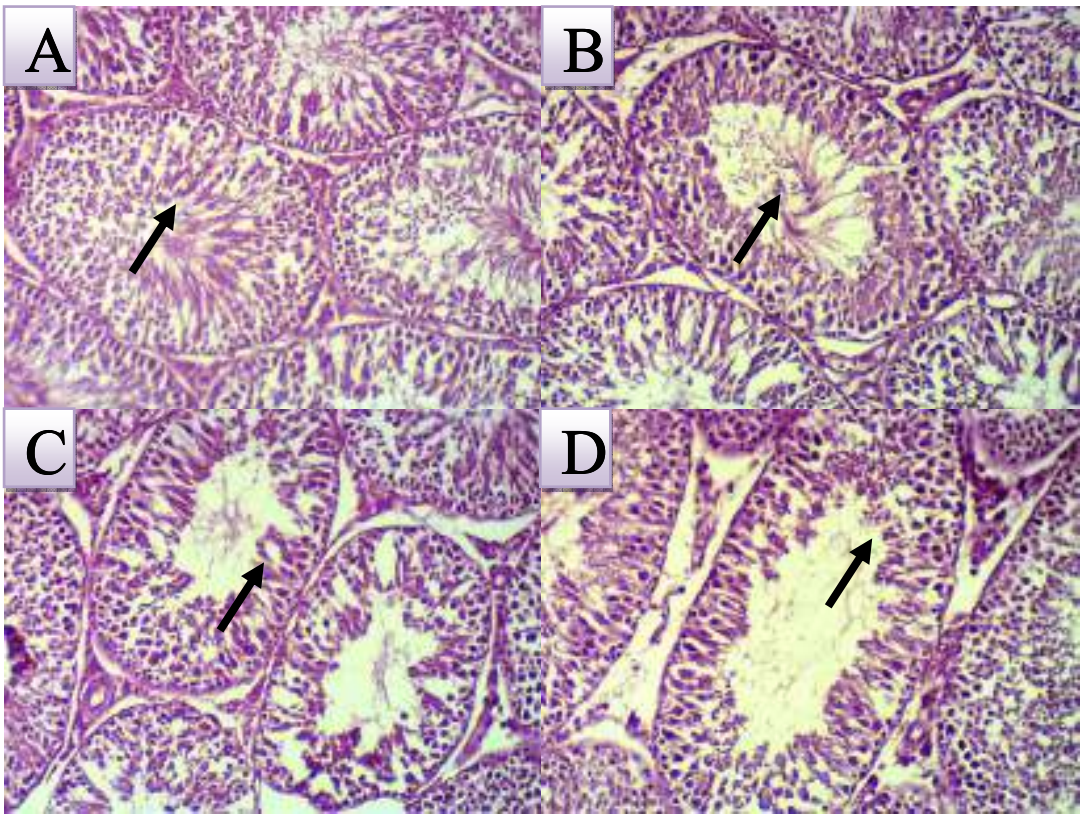

Figure (1): Testes of rat (A) control group showed normal spermatogenesis in seminiferous tubules. (B) 25mg/kg caffeine showed little effect on spermatogenesis. (C) 50mg/kg caffeine showed moderate suppression of spermatogenesis and vacuolation of primary spermatocytes. (D) 100mg/kg caffeine showed marked suppression of spermatogenesis, vacuolation of spermatogonia and primary spermatocytes. H&E stain 200X

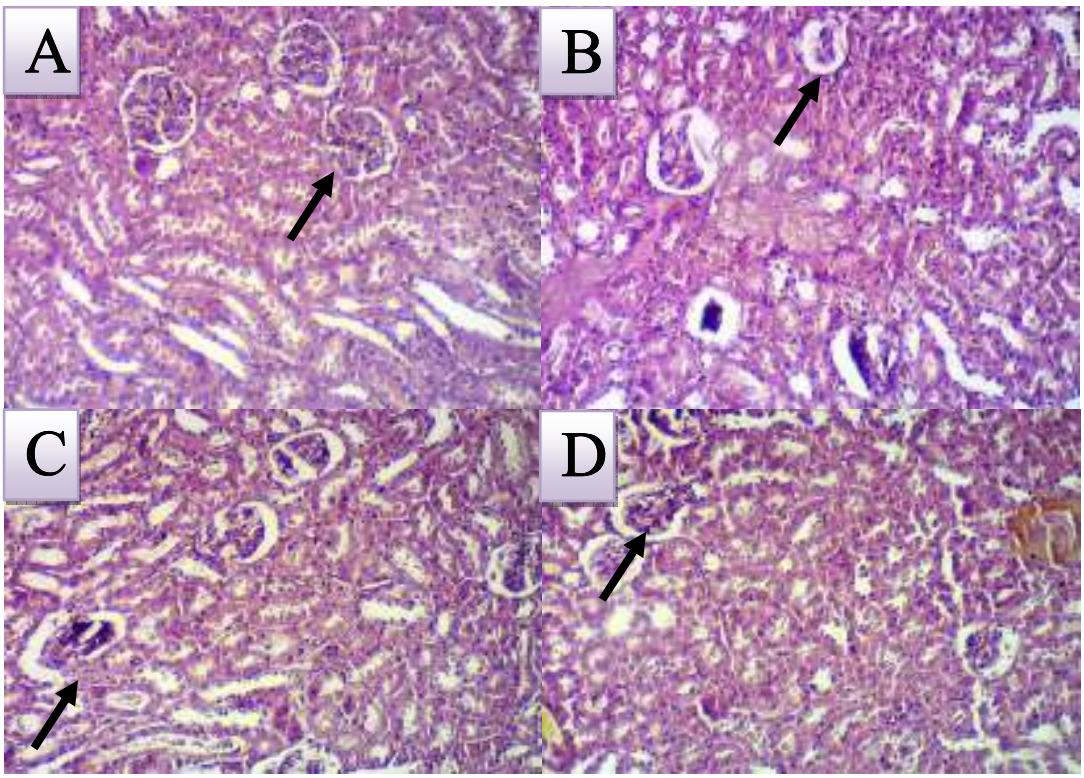

Figure (2): Kidney of rat (A) control group showed normal glomeruli and cortical tubules. (B) 25mg/kg caffeine showed atrophy of glomeruli in some field. (C) 50mg/kg caffeine showed a slight vacuolation of glomeruli. (D) 100mg/kg caffeine showed vacuolation of glomeruli. H&E stain 200X.

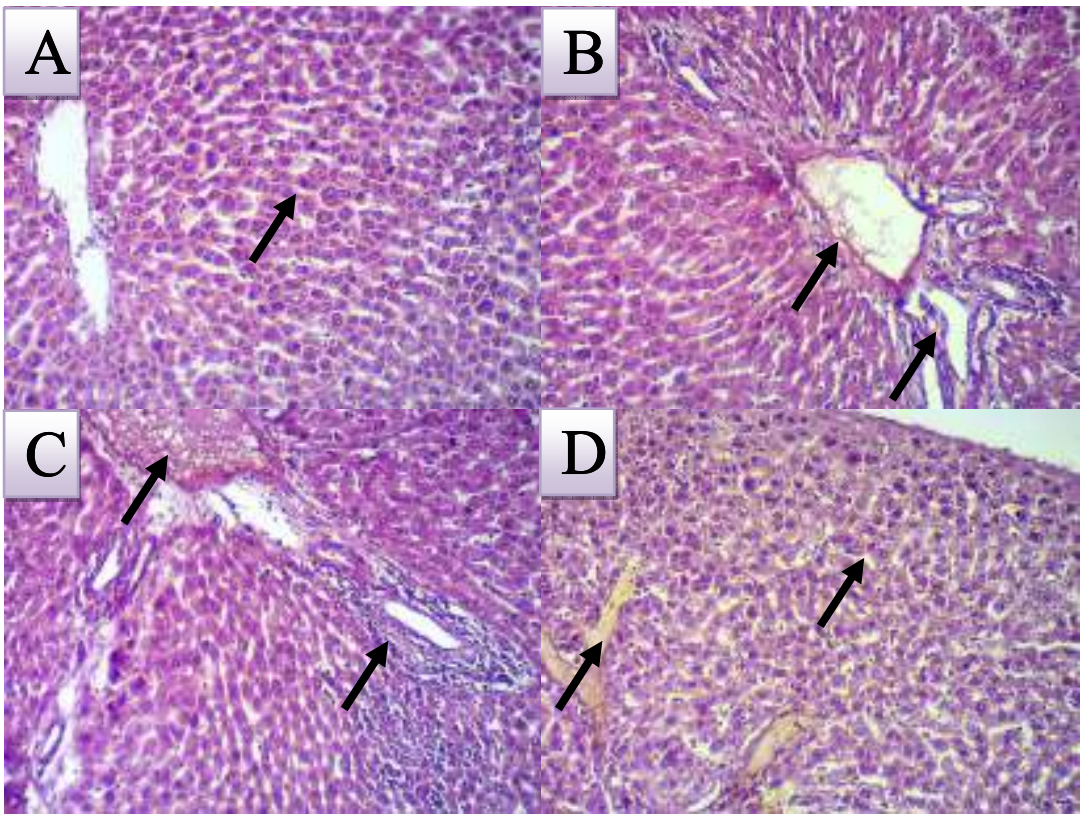

Figure (3): Liver of rat (A) control group showed normal hepatocytes architecture. (B) 25mg/kg caffeine showed dilation of central vein and bile duct proliferation. (C) 50mg/kg caffeine showed slight vacuolation of hepatocytes, bile duct proliferation and aggregation of inflammatory cells. (D) 100mg/kg caffeine showed marked vacuolation of hepatocytes and congestion of portal vein. H&E stain 200X.

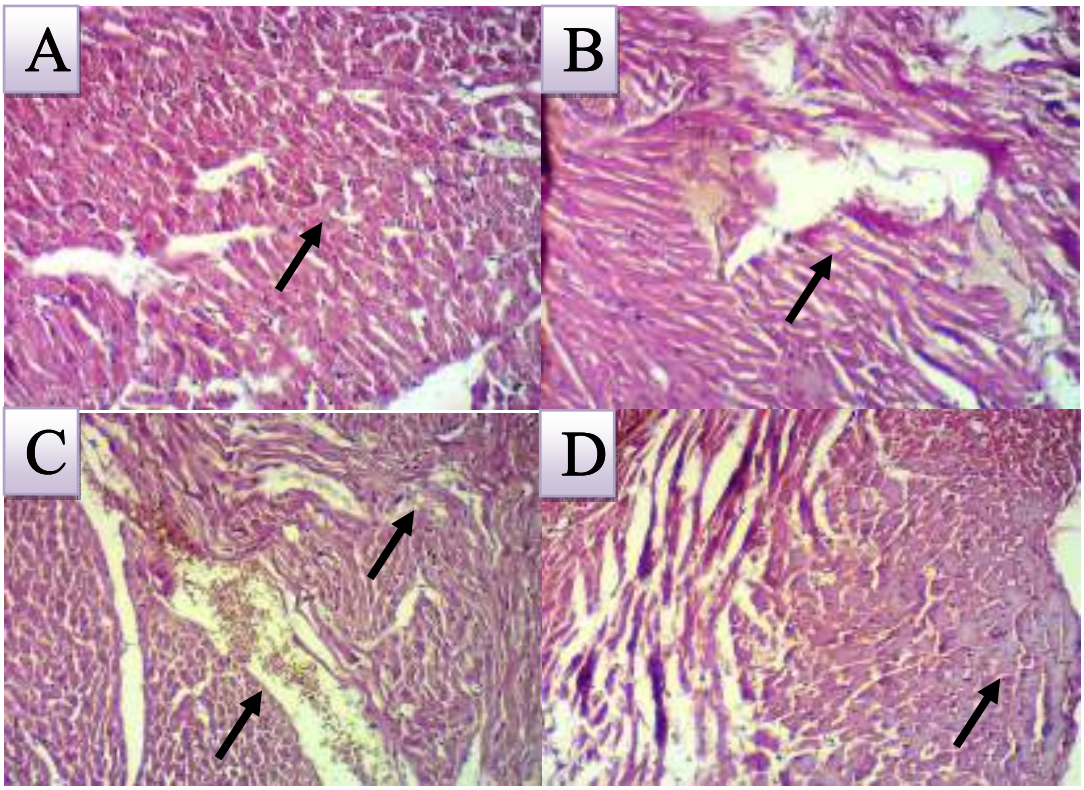

Figure (4): Heart of rat (A) control group showed normal myocardial muscle cells. (B) 25mg/kg caffeine showed vacuolation of some myocardial muscle cells. (C) 50mg/kg caffeine showed vacuolation of myocardial muscle cells and congestion of blood vessels. (D) 100mg/kg caffeine showed degeneration of myocardial muscle cells somewhat grayish in color. H&E stain 200X.

DISCUSSION

This study revealed the effect of caffeine therapeutic doses on male rat testes, kidneys, livers and hearts. Results showed no significant differences in body weight between treated and non-treated rats, similar findings were reported previously by others (16-18), while Shirali *et.al*. reported an increase in body weight of rat treated with caffeine (19). This may be related to the synergism effect of caffeine and carnitine.

The current study showed that the blood parameters monitored an increase in RBCs, Hb and PLT level, on the other hand, a decrease in WBCs level. These results are in consistent with (20), giving the reasons that may be related to the effect of raised testosterone which increases the body mass which associated with increase oxygen demands that lead to increase level of RBC that are provided by erythropoietin hormone, while another group of researchers had another findings (16, 21,22). The differences may be related to higher therapeutic dose of caffeine used by others.

Caffeine is metabolized in liver cells. Results showed no changes in AST and ALT levels in caffeine groups. This may be linked to use of therapeutic doses of caffeine that are overcome by hepatocyte with no severe changes. this finding was confirmed by histopathological examination of liver tissue, which included bile duct proliferation, a slight vacuolation of hepatocytes (figure 3), since the effect of therapeutic dose was the goal of this study. These findings were in agreement with (23,24) and disagreed with other findings (20,25,26). The differences may be related to higher dose administration of caffeine and its long duration.

Results revealed a decrease in urea level and increase in creatinine level, which may be due to diuretic effect produced by caffeine on kidney cells, while increase activity of muscle cells stimulated by caffeine lead to increase creatinine concentration and not due to kidney damage, although higher doses administration may result in kidney damage. These findings were confirmed by histopathological evaluation, which showed only a slight atrophy of some glomeruli of kidney (figure 2). This result wasn't in line with (16) who reported that caffeine administration caused increase urea and creatinine levels due to higher doses of caffeine used.

Several clinical and animal studies had mentioned that caffeine use caused serum testosterone elevation in adult human and adult animals (27,28). This result was in line with the results obtained by the current study and is also in agreement with Park *et.al.* and Joshua *et.al.* (29,30). This elevation of

testosterone may be related to increase activity of leydig cells by caffeine administration. However, this result was not in line with (31) due to immature rat used in the study. On the other hands, no effect of caffeine on rat testes weight were observed in this study as reported also by others (29,32-34). Results also indicated a decrease in sperm count, motility and sperm normality may be related to the effect of caffeine on spermatogenesis and sperm germinal cells this result is in line with (30), this result is supported by histopathology of testes (figure 1), that revealed a different degree of suppression of spermatogenesis in rat treated by caffeine depending on dose manner, this result is in agreement with (35).

Caffeine causes stimulation of nervous system that leads to increase activity of body tissues (8). In the current study heart tissue showed a vacuolation of cardiac muscle cells (figure 4), but with high doses cardiac muscles degenerated as reported by Happonen *et.al.* (36). This result may be linked to the effect of caffeine by rising level of cortisol and epinephrine which affect heart tissue cells (37,38).

CONCLUSION

The current study determined that high doses caffeine may have a deleterious effect on some organs and biochemical parameters and further studies also are required to determine the relationship between caffeine and endocrine organs.

REFERENCES

1. Nawrot P.; Jordan S.; Eastwood J.; Rotstein J.; Hugenholtz A. and Feeley M. (2003). Effects of caffeine on human health. *Food Add. Contam.* 20(1):1-30.
2. Temple JL.(2009). Caffeine use in children: what we know, what we have to learn and why we should worry. *Neurosci. Biobehav. Rev*. 33:793-806.
3. Bagwath PL. (2011). Energy drinks and the neurophysiological impact of caffeine. *Frontier. Neuroasci.* 5(116):1-8.
4. Spiller MA. (1998). The Chemical Components of Coffee. *Caffeine*. pp.: 97-161.
5. Evans SM. and Griffiths RR.(1999). Caffeine withdrawal: A parametric analysis of caffeine dosing conditions. *J. Pharm. Exp. Ther*. 289:285-294.
6. Battram DS.; Shearer J.; Robinson D. *et al*. (2004). Caffeine ingestion does not imp the re-synthesis of proglicogen and macroglicogen after prolonged exercise and carbohydrate supplementation in humans. *J. Appl. Physiol*. 96:943-950.
7. Neuwinger HD. (2000). African Traditional Medicine, a Dictionary of Plants Use and Application, Med pharm GmbH Publishers, Stuttgart, Germany.
8. Hursel R. and Westerterp-Planteng MS. (2013). Catechin-and caffeine-rich teas for control of body weight in humans. *Amer. J. Clini. Nutr.* 98(6):1682S-1693S.
9. Kendall KL.; Moon JR.; Fairman CM.; Spradley BD.; Tai CY.; Falcone PH. *et al*.(2014). Ingesting a pre-workout supplement containing caffeine, creatine, β-alanine, amino acids, and B vitamins for 28 days is both safe and efficacious in recreationally active men. *Nutr. Res.* 34(5):442-449.
10. Guide for the care and use of laboratory animals. (2011). 8th ed. Washington: National Academic Press.
11. Keisler BD. and Armsey TD. (2006). Caffeine as an ergogenic aid. *Curr. Sports. Med. Rep.* 5(4):215-219.
12. Khadhair AA. and Majeed SK. (2017). Study the toxicity of digoxin on liver and their transaminase enzymes level in rat. *Kufa. J. Vet. Med. Sci.* 8(2):73-80.
13. Khadhair AA. and Majeed SK. (2018). Toxopathological and biochemical study of multiple doses digoxin in sprague dawley rats. *Bas. J. Vet. Res*. 17(1):136-146.
14. Wilson JD.; George FW. and Griffin JE. (1981). The hormonal control of sexual development. *Science.* 211:1278-1284.
15. Drury RA. (1967). Carlton's Histological Technique. 4th ed. Oxford University Press., Toronto. P:140.
16. Emmanuel A.; Majesty D.; Benjamin A.; Peter A. and Princess U. (2017). Effect of Caffeine on Some Selected Biochemical Parameters Using Rat Model. *Adv. Bio.* Pp.:1-8.
17. Inoue H.; Kobayashi-Hattori K.; Horiuchi Y.; Oishi Y.; Arai S. and Takita T. (2006). Regulation of the body fat percentage in developmental-stage rats by methylxanthine derivatives in a high-fat diet. *Biosci. Bio. Biochem.* 70(5):1134-1139.
18. Zheng G.; Sayam K.; Okubo T.; Juneja LR. and Oguni I. (2004). Anti-obesity effects of three major components of green tea, catechins, caffeine and theanine in mice. *In Vivo*. 18(1): 55–62.
19. Shirali S.; Hosseini SA.; Ashtary-Larky D.; Daneghian M. and Mirlohi MS. (2016). Effect of caffeine co-ingested with carnitine on weight, body-fat percent, serum leptin and lipid profile changes in male teen soccer players: A randomized clinical trial. *Int. J. Pediatr.* 4(10):3685-3698.
20. Camerone A.; Sweet E.; Bottino A.; Bittar C.; Veiga C. and Cameron LC. (2007). Effect of caffeine supplementation on haematological and biochemical variables in elite soccer players under physical stress conditions. *Brit. J. Sports. Med.* 41(8):523-530.
21. Duru MK.; Agomuo EN. and Amadi B. A.(2012). Biochemical studies on 'Udu' an antimalarial concoction used in Umunchi village, Isiala Mbano L.G.A of Imo State, Nigeria. *Con. J. Pharm. Toxicol. Res.* 5(2): 28-34.
22. Adebayo AH.; Abolaji AO.; Opata TK. and Adegbenro IK. (2010). Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on

biochemical and haematological parameters of albino Wister rats. *Afr. J. Biol.* 9(14):2145-2150.
23. Tucker KL. (2003). Dietary Intake and Bone Status with Aging. *Current. Pharm. Des.* 9(31):1-18.
24. Kynast-Gales SA. and Massey LK. (1994). Effect of caffeine on circadian excretion of urinary calcium and magnesium. *J. Amer. Coll. Nutri.* 13(5):467-472.
25. Mathew A.(2001). Liver enzymes (elevated) in Principles of Health Choice. Media Inc. available at: http://www.liverenzyme.com/
26. Manne V. and Saab S. (2015). Coffee as modulator of liver injury: fact and fiction. *Clin. Liver Dis.* 6(6): 139-141.
27. Sarobo C.; Lacorte LM.; Martins M. *et al*. (2012). Chronic caffeine intake increases androgenic stimuli, epithelial cell proliferation and hyperplasia in rat ventral prostate. *Int. J. Exper. Pathol.* 93(6):429-437.
28. Ramlau-Hansen CH.; Thulstrup AM.; Bonde JP.; Olsen J. and Bech BH.(2008). Semen quality according to prenatal coffee and present caffeine exposure: two decades of follow-up of a pregnancy cohort. *Human Reprod.* 23(12):2799-2805.
29. Park M.; Choi Y.; Choi H.; Yim JY. and Roh J. (2015). High doses of caffeine during the peripubertal period in the rat impair the growth and function of the testes. *Int. J. Endocrinol.* 1-9.
30. Joshua O.; Sunday O.; John O. and Titilope O. (2017). Excessive Caffeine Intake Disrupts Testicular Architecture, Spermatogenesis and Hormonal Levels in Experimental Wistar Rats. *IOSR. J. Enviro. Sci. Toxicol. Food Technol.* 11(10):8-18.
31. Bae J.; CHoi H.; CHoi Y. and Roh J. (2017). Dose-and time-related effects of caffeine on the testis in immature male rats. *Exper. Animals*. 66(1):29-39.
32. Kwak Y.; Choi H. and Roh J. (2017). The effects of caffeine on the long bones and testes in immature and young adult rats. *Toxicol. Res.* 33(2):157-164.
33. Whitby KE.; Collins TF.; Welsh JJ.; Black TN.; O'Donnell M.; Gray GC. *et al*. (1986). Reproduction study of caffeine administration to male Osborne-Mendel rats. *Food Chem. Toxicol.* 24: 277–282.
34. Tinwell H.; Colombel S.; Blanck O. and Bars R. (2013). The screening of everyday life chemicals in validated assays targeting the pituitary-gonadal axis. *Regul. Toxicol. Pharmacol.* 66: 184-196.
35. Friedman L.; Weinberger MA.; Farber TM. *et al*. (1979). Testiscular atrophy and impaired spermatogenesis in rats fed high levels of themethyl xanthines caffeine, theobromine, or theophylline. *J. Enviro. Pathol. Toxicol.* 2(3):687-706.
36. Happonen P.; Voutilainen S. and Salonen JT. (2004). Coffee drinking is dose-dependently related to the risk of acute coronary events in middle-aged men. *J. Nutri.* 134(9):2381-2386.
37. Lochen ML. and Rasmussen K. (1996). Palpitations and lifestyle: impact of depression and self-rated health. The Nordland Health Study. *Scand. J. Social. Med.* 24(2):140-144.
38. Rosmarin PC. (1989). Coffee and coronary heart disease: a review. *Prog. Cardio. Dis.* 32(3):239-245.

Effects of electromagnetic fields (50 Hz and 27.5 Gauss) and heavy metal ions on rats' spermatogenesis

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ABSTRACT

Industrial growth and new technologies have led to a higher presence of heavy metal ions in our environments such as mercury (Hg^{2+}), cadmium (Cd^{2+}) and lead (Pb^{2+}), and therefore increasing the environmental pollution in air and food. The widely using of electromagnetic devices such as mobile and hei-tiki may induce a real threat to human health. The aim of the current study was to investigate the effects of heavy metal ions and exposed to EM field on male rats' spermatozoa properties. To approach the goal of the study, two groups of rats were used; Group A & B involved 3 subgroups depend upon the heavy metal used (HgCl2, CdCl2 and PbCl2) and on exposure to EM or not. Semen analysis was done by microscope and computer-assisted semen analysis. Results show significant changes in spermatozoa properties as compared with the control groups not administered heavy metals nor exposed to EM. This study concluded that synergism exposure of rats to heavy metal and EM have an important implication on the reproductive system.

Keywords: Electromagnetic field, heavy metal, rat's spermatozoa

INTRODUCTION

The European Parliament proposed the study of the possible effects of the concurrence of chemical agents with electromagnetic fields in biomedicine, because of its presence in food and in the environment respectively (1).

Extremely low frequency magnetic fields (ELF-MF) of 50 Hz involve the high-tension power lines and electro-domestic devices. Actually, they are submitted to scientific and social polemic because some opinions designed them to the possibility to be implicated to the potential injury on individual and Public Health (2,3). Some studies provoke in quietude principally for children and others unauthorized the precedents because the ELF-MFs are ubiquitous and also present at the proximities of the population densities (ICNIRP. Guidelines 1Hz to 100KHz, 2010) (4).

Epidemiological studies signalised a certain relationship between the exposition to ELF-MF of 50 Hz and the tumour induction in the brain and also leukaemia in children (5-8). The aim of this experiment is to study the effect of the ELF-MF of 50 Hz on the rat's spermatogenesis in order to furnish new data to complete the information on the possible effects to male infertility, and to hypothesize on the possible physiological and biochemical mechanism to induce pathology.

MATERIALS AND METHODS

Animals:

For each metal ion (Hg^{2+}; Pb^{2+}+Cd^{2+}) two groups of 20 g weighing young male BALB/c mice were used, each one with 8 animals, one of them was used as control group, and the other two groups submitted to ELF-MF. Table (1) describes the experimental design and groups. All groups were fed with standard diet and water "ad libitum", and maintained in metabolic cages, with the same conditions as 12 h light/12 h obscurity, humidity and temperature, one cage one meter near the other. After the calibration solenoid chamber, the magnetic fields were established and confined inside the solenoid area.

Table (1): The experimental design

Animal groups	Group description	Number of rats	Exposition to electromagnetic fields
G1	control without HgCl2	8	Not exposed
G1	control without HgCl2	8	Exposed
G1	control with HgCl2	8	Exposed
G2	Control without CdCl2+PbCl2	8	Not exposed
G2	Control without CdCl2+PbCl2	8	Exposed
G2	Control with CdCl2+PbCl2	8	Exposed

$HgCl_2$ solution: Mercury solution ($HgCl_2$) is administered by intraperitoneal route at the concentration of 0,01 mg Hg/kg/day/7 days in saline solution NaCl 0,8%, after a mother solution of 1 mgHg^{2+} /mL; with Insulin syringe with divisions of 0,01 ml.

$CdCl_2$ and $PbCl_2$ solutions: Solutions of both metal ions will be injected intraperitoneally in each case at the concentrations of 0,1 mg Me^{2+}/Kg/day/7days in saline solution of NaCl 0,8%, both Me^{2+} will be administered equally as mercury.

Exposition to the electromagnetic field of 50 Hz:

Each metabolic cage with 6 animals will be placed in the geometrical centre of the solenoid chamber, inside the confined electromagnetic fields, meanwhile the other cage with the control animals situated to 1 meter distance, with the same experimental conditions during 7 days.

Calibration of the chamber to be used:

The metabolic cages with 6 animals were submitted to an electromagnetic field with the intensity of 27.5 Gauss, 50 Hz (275 volts) generated by the solenoid conveniently evaluated through two graphics, one representing the calibration of the chamber and the other showing the calibration of the magnetic field intensity by one of us. The value of the field to which were submitted the animals during 7 days are 27.5 G (275 V) and the frequency of 50 Hz.

Collection of sperms:

After 7 days of exposure to the electromagnetic field, the rats were sacrificed by cervical dislocation under light Ether anaesthesia. The scarified male rats were dissected and both vas deferentia which closet to epididymis was extracted. The cut pieces were placed in physiological saline (0.9% NaCl) for 10 min. at 37 C (9).

Seminal analysis:

The sperm motility was analyzed as described by Adamkovicova *et al*. (10), briefly computer assisted CASA analyses (Mini Tub, Tiefenbach, Germany) with the aid of a light microscope (Olympus, Japan) was used. Collected sperms were diluted with 10 µl saline (0.9% NaCl) and pipetted into Neubauer counting chamber. Motility assessed for five fields (10). To evaluate the sperm morphology, collected sperms were fixed with Hancock's solution and slides were stained with Giemsa stain for 1 hour and examined by a light microscope at 400X magnification (9).

Statistical analysis:

One-way analysis of variance (ANOVA) was used to analysis the differences for statistical significance. Data were expressed as means± standard deviation (SD). The P value regards significant if it's ($P<0.05$).

RESULTS AND DISCUSSION

Tables (2 and 3) show the results of the sperm motility of the rats, exposed to synergism effects of heavy metal and electromagnetic fields (50 Hz and 27.5 Gauss).

Observable and significant changes were found in all parameters used in this study. Rats group that was exposed to HgCl2 showed significant decrease in all parameters in comparison with control rats that did not administrate the heavy metals, while the huge changes in all parameters when the rats exposed to synergism effects by heavy metal and electromagnetic field, similar results were reported when two heavy metals (CdCl2 and PbCl2) were used (table 3). Table (4) confirmed the results by changes in spermatozoa morphology, a significant changes percentage of spermatozoa as compared between treated and non-treated rats with normal morphology in all experimental. The effects of cadmium (Cd) and zinc have been studied before, Adamkovicova *et al.* (10) found that low doses of Cd and DZN impair sperm quality, and can reduce male fertility potential. Amara *et.al*, (11) found that Cd induced oxidative stress in rat testis and affect the sperm motility. On the other hand, Wang *et.al*. evaluated the urinary metal levels with human semen (12). Energy drinks have been found to affect the rat sperm concentration after long-term consumption (13). The significant findings in the current study were the effects of the electromagnetic field in the spermatozoa. In the previous study (published in the current volume) the authors found that exposure of rats to synergism effects of heavy metals and electromagnetic field affect the blood and tissue chemical and physical properties.

Table (2): Sperm motility analysis of rats administrated HgCl2 and exposed to ELF-MF of 50 Hz

Parameters	A* Mean±SD	B** Mean±SD	C*** Mean±SD
Motility (%)	17.12 ±19.8 8	40.12 ±21.11	55.05±8.58
Prog (%)	6.97±15.23**	20.00±14.54	27.69±7.34
DPA(µm)	32.32±2.57	24.55±3.57	21.95±3.29
ALH (µm)	9.74±0.69	6.76±1.06	4.52±1.26
DCL (µm)	55.31±4.70	37.54±5.41	33.91±4.98
velocity average path	134.74±11.30	95.01±14.47	81.10±13.88
Linearity (%)	0.45±0.02	0.53±0.04	0.53±0.04

*Group A administration HgCl2 (0,01 mg/Kg/day Hg^2) and exposed to ELF-MF of 50 Hz
**Group B administration HgCl2 (0,01 mg/Kg/day Hg^2) and not exposed to ELF-MF of 50 Hz
** Group C control (not administration HgCl2, non-exposed to ELF-MF of 50 Hz
Prog=progressive motility, DPA= distance average path. ALH= amplitude of lateral head displacement. ALH amplitude of lateral head displacement. DCL= distance curve line

Table (3): Sperm motility analysis of rats administrated $CdCl_2$ and $PbCl_2$ solutions and exposed to ELF-MF of 50 Hz

Parameters	A* Mean±SD	B** Mean±SD	C*** Mean±SD
Motility (%)	16.22 ±17.89	39.10 ±22.3 1	50.05±8.58
Prog (%)	5.97±15.23**	21.00±14.54	28.61±7.34
DPA(µm)	33.33±2.57	25.55±3.57	23.95±3.29
ALH (µm)	8.98 ±0.69	6.85 ±1.06	3.88±1.26
DCL (µm)	56.41±4.70	39.54±5.41	32.91±4.98
velocity average path	135.66 ±11.30	94.9 1±14.47	82.09 ±13.88
LIN linearity(%)	0.45±0.02	0.53±0.04	0.53±0.04

*Group A administration $CdCl_2$ and $PbCl_2$ solutions and exposed to ELF-MF of 50 Hz
**Group B administration $CdCl_2$ and $PbCl_2$ solutions and not exposed to ELF-MF of 50 Hz
** Group C control not administration $CdCl_2$ and $PbCl_2$ solutions, non-exposed to ELF-MF of 50 Hz
Prog=progressive motility, DPA= distance average path. ALH= amplitude of lateral head displacement. ALH amplitude of lateral head displacement. DCL= distance curve line

Table (4): Sperm morphology evaluation

Group Parameters	A Mean±SD	B Mean±SD	C Mean±SD
Normal morphology	64.10 ±1.12	90.40 ±2.91	96.56±1.12
Abnormal morphology	35.90 ±0.69	9.60. ±2.49	3.44 ±2.34

*Group A administration $CdCl_2$ and $PbCl_2$ solutions and exposed to ELF-MF of 50 Hz
**Group B administration $CdCl_2$ and $PbCl_2$ solutions and not exposed to ELF-MF of 50 Hz
** Group C control not administration $CdCl_2$ and $PbCl_2$ solutions, non-exposed to ELF-MF of 50 Hz

Nowadays heavy metal ions are found in our environment and induce polluted air and food (14), therefore the exposure of human to electromagnetics field through mobiles will induce huge pathological changes (1,2). The human male infertility regards as global problems, and the rate increases every year to reach 15-20% (15). Industrial growth and new technologies have led to a higher presence of heavy metal ions in our environments such as mercury (Hg^{2+}), cadmium (Cd^{2+}) and lead (Pb^{2+}), and therefore increasing the environmental pollution in air and food.

Heavy metals have high biological half-lives that inhibit and block protein and immunological mechanisms. They are accumulative and create several health implications (16). Therefore, the effects of spermatozoa by two factors (heavy metal and EFL) may increase the rate of human infertility.

These two factors and the possible biological synergism between heavy metals and electromagnetic fields (EMF) make metals a real threat to human health. Recent research works showed the interest among the scientific community to determine the effects in known ecosystems and human health. In addition, on-going research has also shown the necessity of developing bio-markers for pollution from heavy metals (17,18).

The outcomes of this study are very important, firstly the focus on subject not explored before; the effects of combination of heavy metal and electromagnetic field on reproductivity of rats. Secondly, it had given attention about using electromagnetic devices and their implications on human health.

REFERENCES

1. Scientific Comment of COST-281 (2002). On Epidemiologic Studies on the Health Impact of Mobile Communication Base-Stations. - Action 281: Potential Health Implications from Mobile Communication Systems.

2. Watchdog Report (2002). European Cooperation Report in the Field of Scientific and Technological Research. - Potential Health Implications from Mobile Telecommunications Systems. - COST-281.

3. Peyman A.; Gabriel C.; Grant EH.; Vermeeren G. and Martens L.(2009). Variation of the dielectric properties of tissues with age: the effect on the values of SAR in children when exposed to a walkie–talkie devices. *Phys. Med. Biol.* 54:227–241.

4. ICNIRP (2010). Guidelines 1 Hz-100KHz.

5. Ahlbom A. (1997). Residential epidemiological studies. In: Matthes R., Bernhardt J.H., and Repacholi M.H., eds. Biological effects of static and ELF electric magnetic fields. Oberschleissheim, Germany: ICNIRP publication 4/97, pp.:185-190.

6. Saffer JD. and Thurston SJ. (1995).Cancer risk and electromagnetic fields. *Nature.* 375:22-23.

7. Savitz DA.; Wachtel H.; Barnes FA.; John EM. and Tvrdyk JG. (1988). Case control study of childhood cancer and exposure to 60 Hz magnetic fields. *Am. J. Epidemiol.* 128:21-38.

8. Sancho M. and López E.(1995). Campos electromagnéticosy salud. *Rev. Esp. Fisiol.* 9:21.

9. Kourounma A.; Duan P.; Keita H.; Osamuyimen A.; Qi S.; Quan C.; Yu T. and Yang K. (2015). *In vitro* assessment of ROS on motility of epididymal sperm of male rat exposed to intraperitoneal administration of nonylphenol. *Asian. Pac. J. Rep.* 4(3):169-178.

10. Adamkovicova M.; Toman R.; Martiniakova M.; Omelka R.; Babosova R.; KrajcovicovaV.; Grosskopf B. and Massanyi P. (2016). Sperm motility and morphology changes in rats exposed to cadmium and diazinon. *Reprod. Biol. Endocrinol.* 14:1-7.

11. Amara S.; Abdelmelek H.; Garrel C.;Guiraud P.; Douki T.; Ravanat J.; Favier A.; Sakly M. and Ben RK.(2008). Preventive effect of zinc against cadmium-induced oxidative stress in the rat testis. *J. Reprod. Dev.* 54:129-134.

12. Wang YX.; Sun Y.; Feng .; Wang P.; Yang P.; Li J.; Huang Z.; Chen YJ.; Liu C.; Sun L.; Yue J.; Gu LJ.; Zeng Q. and Lu WQ. (2016). Association of urinary metal levels with human semen quality: A cross-sectional study in China. *Environ Int.* 91:51–59.

13. Dias TR.; Alves MG.; Bernardino RL.; Martins AD.; Moreira AC.; Silva J.; Barros A.; Sousa M.; Silva BM. and Oliveira PF. (2015). Dose-dependent effects of caffeine in human Sertoli cells metabolism and oxidative profile: relevance for male fertility. *Toxicol.* 328:12–20.

14. Ahlbom A.; Cardis E.; Green A.; Linet M.; Savitz D. and Swerdlow A.(2001). Review of the epidemiologic literature on EMF and health. Environ. *Health. Perspect.* 109 (Suppl 6): 911–933.

15. Zegers-Houchschild F.; Adamson GD.; de Mouson J.; Ishiara O.; Masour R.; Nygren K *et al.* (2009). International committee for monitoring

assisted reproductive technology (ICMART) and the world health organization (WHO) revised glossary of ART terminology. *Fertil Steril.* 92(5):1520-1524.
16. de Souza Predes F.; Diamante MA. and Dolder H.(2010). Testis response to low doses of cadmium in Wister rats. *Int. J. Exp. Pathol*. l91:125-131.
17. WHO. (1990). Environmental Health Criteria 118: Inorganic Mercury. World Health Organization. Geneva. P.:168.
18. World Health Organization (1998). Informative Bulletins, Nr 183; 184; 193; 205, of 1998.

Physical and chemical parameters in blood and tissues of rats and in cell culture after exposure to electromagnetic fields and heavy metal ions

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ABSTRACT

The European Parliament proposed the study of the possible effects of the concurrence of chemical agents with electromagnetic fields in biomedicine, because of its presence in food and in the environment respectively.
In this work, we had measured the dielectric properties of tissues (conductivity and permittivity) treated with heavy metal ions and exposed to EM fields, in addition, characterization of PC 12 cell cultures (Human Pheochromocytoma cells) were achieved because of their similarity to nerve cells in the brain. Precise cell exposure data in oxidative stress mechanisms as enzymatic defence activities are essential for evaluating perspectives of possible cellular effects on human health. Data will be furnished on cell viability and enzymatic activities of lactate-dehydrogenase, superoxide-dismutase, catalase, glutathione-peroxidase, glutathione-reductase, glutathione and lipid peroxidation, in order to observe molecular effects on oxidative stress as defensive reactions in mammals, induced by the influence of electromagnetic fields and heavy metal ions, either independently or by combined action. The present work completes our previous experiments on the chemical and physical effects of environmental toxic metals within biological tissues, and we show that the technique proposed is an excellent tool to evaluate the influence of the two mentioned external toxic agents in tissues in projection to human health. In addition, our preliminary results already showed the interest of this experimental project to human nutrition to avoid harmful effects on human health. Nowadays fish are contaminated with mercury (Hg^{2+}), toys and air with cadmium (Cd^{2+}) and water with lead (Pb^{2+}) and in general, all of these heavy metal ions are present in food. Therefore, this project will be useful and applicable to any area of interest where contamination is present and in particular to people of these affected areas.
To determine the complex dielectric properties (conductivity and permittivity) of biological tissues of rats exposed to the industrial frequency of 2.45 GHz we designed and built a simple experimental set-up. For this purpose, the scattering parameters of biological samples, which are placed in a sample holder inside a waveguide, are measured and compared with those, obtained from numerical analysis of the sample using the finite element (FE) technique with an adaptive mesh. Systematic errors are minimized by a precise calibration of the experimental system. The simplicity of the experimental set-up makes this technique a very practical tool for detecting and quantifying changes in the complex dielectric properties of organs poisoned with pollutants (heavy metal ions).

Keywords: conductivity, permittivity, finite element (FE) technique, dielectric properties, EM fields.

INTRODUCTION

Dielectric properties of biological materials are of high relevance in dosimetrist studies and biophysics sciences for the assessment of exposure to EMF in Public Health. In particular for people exposed to electromagnetic fields of antennas and cellular phones and other electric devices. It is for these reasons that we will center our interest in electromagnetic fields at cellular and microwave radiation frequencies applying rat blood cultures and tissues (1,2). Our preliminary analysis showed differences in the conductivity and permittivity of various body tissues in rats when treated with different heavy metals. In particular mercury (Hg^{2+}), cadmium (Cd^{2+}), lead (Pb^{2+}) and iron (Fe^{2+}) (ferromagnetic element) are the four contaminating ions elements in food which signalized higher differences in conductivity and permittivity of tissues (1-3). In the current study, authors continued the initial results and made a comparison between different tissues and different elements.

Heavy metal ions inhibit and blockage proteins and mechanisms of defence of the organism. In humans, heavy metals have a high biological half-life; they are accumulative and created a high concern in Public Health due to their pernicious effects in human health. These two factors, and the possible biological synergism between heavy metals and electromagnetic fields (EMF), make these metals a real threat to human health. Recent research works have shown the interest within the scientific community to determine the effects in known ecosystems and human health. In addition, on-going research has also shown the necessity of developing bio-markers for pollution from heavy metals (1-4).

Mammals, rats and nervous cells in culture (Pheochromocitoma cells), are the best biological models to do an experiment, because they are also exposed to electromagnetic fields and are the basic stages to know the effects to human beings and mankind. Brain and cerebral structures are also the focus to show effects on the proposed brain tumors and leukemia, induced by such low frequency radiations. To provide relevant dielectric data for children models, several studies have been carried out on dielectric properties of animal tissues from a range of ages (4-6). The data published in the scientific literature showed discussions on the extent to which the variation of dielectric data as a function of age would affect the results of dosimetry studies, and consequently, the possible implications for the exposure of children (7-9).

In the same context, recent dosimetrist studies are important to clarify the changes of dielectric properties in different tissues in single and under chronic exposure at the intensity doses, time in frequency like humans could suffer or they are exposed.

MATERIALS AND METHODS

Biological tissues:

Several groups of male Wister rats with six animals for each group were used for each metal, and we will apply an ANOVA statistical analysis to the results. Blood will be extracted with a syringe before the animal death and afterwards dissected in order to separate the organs and tissues. Biological samples were submitted to measurement: blood, and slices of tissues of a thickness up to 2 mm of brain, lung, liver, pancreas, kidney, muscle (10,11). The following parameters were measured: conductivity, permittivity and 20 blood parameters such as metal ions, enzymatic activity and normal biochemical blood parameters. In order to ensure no loss of moisture content, the samples were kept in Petri plates containing humidified cotton (NaCl 0.8%), closed hermetically with parafilm and kept at 0º C until the microwave measurements of the dielectric permittivity are made, usually less than thirty minutes after the sample tissue be available.

Enzymatic analysis:

In order to observe molecular effects on oxidative stress, due to the influence of electromagnetic fields, the cell viability and the enzymatic activities were measured (lactate-dehydrogenase, superoxide-dismutase, catalase, glutathione-peroxidase (with and without Se^{2+}), glutathione-reductase, glutathione and lipid peroxidation), according to methods described by Ribas-Ozonas *et.al.* (12).

Microwave system set-up:

For the determination of the dielectric properties of rat tissues at microwave frequencies, an experimental set-up was built using waveguides at the operating frequency of 2.45 GHz (25). To determine the dielectric properties of the tissue the reflection (S_{11}) and transmission (S_{21}) complex coefficients of a sample of tissue were measured by using a vector network analyzer (VNA). For this purpose, a sample holder formed by a methacrylate block with a rectangular cavity, 44 mm long, 11 mm high and 2 mm thick has been designed. The complex permittivity of the block was measured experimentally at 2.45 GHz and found to be $\varepsilon_r = 2.6$ and $\sigma = 4.96.10^{-4}$ S/m. The contribution of the dielectric loss of the methacrylate with no sample was taken into account in the calibration of the measuring system. The block was divided into two pieces to facilitate the positioning of the sample as it is shown in figure (1a). The methacrylate block completely fills a section of a WR430 waveguide suitable to work within the range 1.7 to 2.6 GHz (Figure 1b). At the selected operating frequency, the length of the sample (11 mm) was very small compared to the wavelength in the guide (148 mm) and therefore it may be assumed that a uniform

power is applied to the whole sample placed at the center of the waveguide. A coax-to-waveguide transition was placed at each end of the WR430 waveguide section and semi-rigid coaxial cables were used to connect each transition to the VNA, as shown in figures (1c and 1d). Microwave measurements were made at controlled room temperature (27º) and thermal effects during the measurements were negligible as the RF power applied to the sample was very low (-3 dBm).

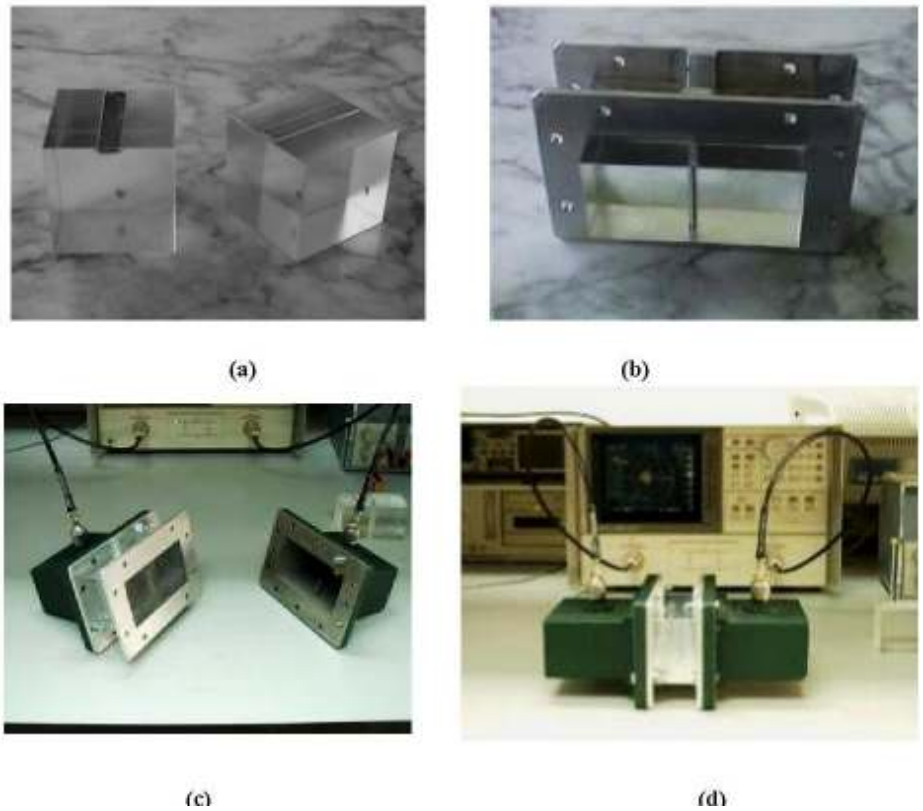

Figure (1): a) Sampler holder; b) waveguide WR430 section with methacrylate block; c) coax-to-waveguide transitions; and d) complete setup with VNA

Determination:

The main sources of random error are the accuracy of the value of the tissue sample volume (968 mm^3), the variation of the content of water or moisture and the intrinsic biological differences that exist among tissue samples. The first one is minimized by the design of the methacrylate sample holder that tightly encloses the sample. The use of translucent methacrylate makes it possible to thoroughly observe the sample inside the block, and, the effects of moisture absorption in this plastic are negligible during the very short time spent in the measurements. With respect to the second source of error, microwave measurements were repeated after 24 hrs, following the same procedure for storing and handling tissues (13,14).

The results could show the treated and exposed animal's data and from cell culture, compared to the results from freshly available samples. An attention will be payed to any variation in the permittivity of the samples due to the loss of water or moisture of the samples in order to be or not disregarded.

The calibration of the measuring system will be performed by using the WR430 waveguide section and three precision waveguide standard loads (short, open and wide band) and the error correction routines integrated into the internal processor of the VNA. Once the experimental system will be calibrated at the measuring frequency of 2.45 GHz, and the absolute error measured for the permittivity and for the conductivity.

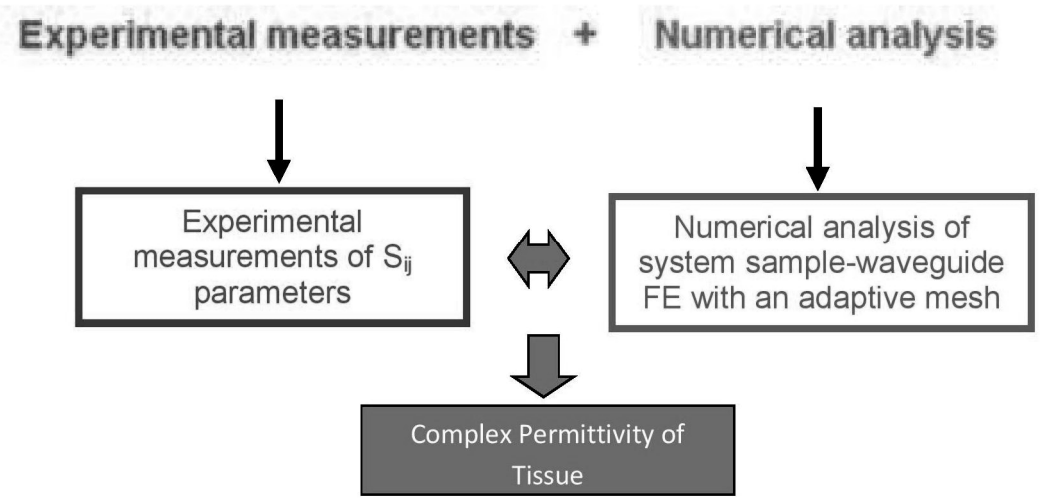

Figure (2): Schematic representation of the comparison of S_{ij} scattering parameters experimentally and numerically calculated to determine the complex permittivity of the tissue

To determine the complex permittivity of the sample, we performed a 3-D analysis based on the FE technique, of the electromagnetic-field distribution inside the WR430 waveguide section containing the sample of the biological tissue. The experimental analysis will provide the value of the complex dielectric permittivity of a tissue that would produce the values for S_{11} and S_{21}. For each analyzed biological tissue, four parameters, two moduli and two phases were analytically obtained. The correct values will be those that match with the experimental ones at the same reference planes. An adaptive mesh will be used for the different parts of the structure, so that 24000 and 12000 basic tetrahedral was applied for the tissue and the sample holder respectively. Figure (2) shows the combination of experimental and analytical comparison of scattering parameters that we will follow to determine the permittivity and conductivity of the biological tissues.

Cell culture to measure oxidative stress:

PC12 pheochromocytoma cells will be exposed to plane wave EM fields to the frequency 2.45 GHz generated within a GTEM chamber for 10, 30 y 60 minutes. The radiofrequency power will vary within the range 0.5 to 1 W. We decided to fix the exposure time at 30 minutes with and intensity of 0.75 W, and f = 2.45 GHz. We measured cell viability, enzymatic activities and lipid peroxidation, all parameters before mentioned, in order to observe molecular effects on oxidative stress, due to the influence of electromagnetic fields with and without the presence of heavy metal ions (15,16).

Methodology:

In our past experiments the four metal ions injected to rats, with higher effects in physical properties of tissues were Hg, Pb, Cd and Fe. Male Wister rats will be injected by the intraperitoneal way with 0,1 mg/Kg/day, except HgCl 0,01 mg/Hg/Kg/day, during 12 days, from a solution of 0,2 mL of 10 mg/L. After 12 days of blood extracted, rat organs dissected, cuts in 2 mm thickness and measured the dielectric conductivity and permittivity. Cuts will be placed in the cavity of methacrylate block with a WR430 waveguide operating at 2.45 GHz with a vector analyzer. As control between treated and not exposed organs and cells, the metal ions accumulated in tissues and cells from cell culture, will be submitted to analysis by induction coupled plasma – mass spectrometry.

DISCUSSION TO THE FUTURE RESULTS

The results obtained will show that the experimental set-up combined with the FE numerical technique can be very useful for detecting and quantifying changes in the complex dielectric conductivity and permittivity with high interest to the human and Public Health. The findings will show the possible existence of the synergism between the accumulation of heavy metal ions of pollution and with the presence of electromagnetic fields. Therefore, the study proposed in this project should provide good knowledge on the physiological and biochemical mechanisms with incidence to possible tissue alterations in human health, and also with the possible incidence of the free radicals and oxidative stress in view to neurological and degenerative disorders.

Hypothesis:

In the human body, the electric impulse is possible thanks to the presence of electron movement in chemical reactions. Parallel to a football stadium (human body), the wave (electric impulse), which is possible thanks to the people (electrons) moving successively. Electrons induce electric impulse in the human body. The physiological response of a stimulus from the feet to the brain and the nervous response was first empirically proposed by René Descartes (1596-1650), hundred years afterwards Galvani experimentally describe the electric impulse and consequent response (1596–1650).

The presence in the human body of heavy metal ions alone have an affinity to the enzymatic thyolic groups: Hg, Pb, Cd binds to these –SH groups. On the other hand ELF-MF (Extremely low frequency magnetic fields) induce to vibrations and to the release of metallic ions from the enzymatic and protein molecules, bound by thyolic groups. It is known that ELF-MF induced also to the permeability of the cell membrane (8,9,18,19). Heavy metal ions have great affinity for thyolic groups and its binding is very stable by this way they have a long biological half-life and bound to metallothionein, they are excreted slowly (17-21).

There are known in pathology several human diseases with overload named the saurismosis, Pneumoconiosis) of inorganic and organic molecules (22). This fact justifies our proposal because metal ions accumulation modifies the electric properties of the human tissues, and also the defense peptides and protein molecules, like metallothionein's and metallothionein like molecules.

The accumulation of mineral elements, like an excess of ingestion, induce the generation of free radicals. Similarly, the excess in tissues of toxic metal ions, induce an excess of electrons and free radicals which are toxic to the human body. The 80% of proteins in the human body are metalloproteins, whose cofactors, activities and biological function depend on the essential metal ions as trace elements (17). Those essential metal ions are substituted by the toxic heavy metal ions and its molecules lose its biochemical activity, blocked and suppressed its physiological signification. The metallic charge or overload in the human body can be healthy in the case of essential trace elements but also pathological by accumulation.

We must wait to know if magnetic fields could have a pathological role in humans, alone as hypersensitivity, and together with overload or higher than normal concentrations in the human body, or by intoxications with heavy metal ions. Simply by hypersensitivity because not all human beings answer equally after a stimulus e.g. electrical or chemical stimulus (13,17). Human hypersensitivity to magnetic fields could depend, not only from the toxic charge of heavy metal ions but also from the chemical products as medicaments or pesticides also present in food (19).

Units' explanation:

Electromagnetic fields (EMFs) are part of the non-ionizing radiation spectrum, existing as static or time varying electric and magnetic fields. EMFs are characterized by their wavelength (λ) and frequency (f), expressed in the units of a meter (m) and Hertz (Hz), respectively. These two quantities are related by the velocity of light (c): $f = c/\lambda$. Einstein related the energy and the movement with the mass.

Magnetic fields are associated with electric current and exist only if electric charges are in motion. For magnetic fields, two quantities are used: magnetic field strength (H) in Ampere per metre (Am^{-1}), and magnetic flux density (B) expressed in Tesla (T). B and H are related by magnetic permeability of the medium (μ): $B = \mu H$. In air, H can be calculated from B by equation: $H = 1.26 \times 10^{-6}$ B. 1 μT (microTesla) = 0.8 Amperes per meter (Am^{-1}). The current density (J) expressed in Ampere per square meter (Am^{-2}) is proportional to E: $J = \sigma E$. Where σ is the conductivity of the medium in Siemens per meter (Sm^{-1}).

Static fields represent 0 Hz frequency. (Magnetic resonance images) Time-varying frequencies: 1 to 300 GHz. Electrified railways, welding (1-300 Hz). Electric power systems in Europe: 50 Hz. and in the USA): 60 Hz.

REFERENCES

1. Gabriel C.; Gabriel S. and Corthout E. (1996). The dielectric properties of biological tissues: I. Literature Survey. *Phys. Med. Biol.* 41: 2231-2249.

2. Peyman A.; Rezazadeh AA. and Gabriel C. (2001). Changes in the dielectric properties of rat tissue as a function of age at microwave frequencies. *Phys. Med. Biol.* 46 (6):1617-1629.

3. Mudryi IV. (1997). Heavy metals in the soil plant man system (a review). *Gigiena. I. Sanitariya.* 1:14-17.

4. Dietz R.; Riget F.; Cleemena M.; Aarkog A.; Johansen P. and Hansen JC. (2000). Comparison of contaminants from different trophic levels and ecosystems. *Sci. Total. Environ.* 245(1-3):221-231.

5. Peyman A.; Holden SJ. and Gabriel C. (2007). Dielectric Properties of Porcine Cerebrospinal Tissues at Microwave Frequencies; In-vivo, In-vitro and Systematic Variation with Age. *Phys. Med. Biol.* 52: 2229-2245.

6. Peyman A.; Gabriel C.; Grant EH.; Vermeeren G. and Martens L.(2009). Variation of the dielectric properties of tissues with age: the effect on the values of SAR in children when exposed to a walkie–talkie devices. *Phys. Med. Biol.* 54:227–241.

7. Christ A.; Gosselin MC.; Christopoulou M.; Kühn S. and Kuster N. (2010). Age-dependent tissue-specific exposure of cell phone users *Phys. Med. Biol.* 55: 1767-1783.

8. Morrow H. (1997). Cadmium, metals and minerals annual review, The Mining Journal Ltd. London, UK.
9. Langford N. and Ferner R. (1999). Toxicity of mercury. *J. Hum. Hypertens.* 13(10):651-656.
10. Gabriel S.; Lau RW. and Gabriel C. (1996a). The dielectric properties of biological tissues: II. Measurements in the frequency range 10 Hz to 20 GHz. *Phys. Med. Biol.* 41: 2251-2269.
11. Gabriel S.; Lau RW. and Gabriel C. (1996b). The dielectric properties of biological tissues: III. Parametric models for the dielectric spectrum of tissues. *Phys. Med. Biol.* 41:2271-2293.
12. Ribas-Ozonas B.; Pérez-Calvo M.; González-Rodríguez J.; Vizán-Suaña E. and Novelli ELB. (2004). Enzymatic activities in hypothalamus-thalamus of mice exposed to magnetic field (ELF-MF). In: Macro and Trace Elements (Anke M, *et al.* eds) Editorial: Schubert-Verlag, Leipzig, Germany. Vol. II, 1327-1331.
13. Ribas B. (1989). Toxicología de oligoelementos, Monografías Técnicas. Ministerio de Sanidad y Consumo 8:35-53.
14. Ribas B.; Castro Gonzalez MD.; García Arribas y. and Nuñez M. (2001). Metalotioneina y medio ambiente, Debates sobre la salud en el siglo XXI, Pablo Gil Loyzaga ed. *Fundación General de la UCM* 4:351-357.
15. Ribas B.; García Arribas O.; Sebastián JL. and Muñoz SM. (2001). Determinación de la permitividad y conductividad eléctricas a la frecuencia de microondas de tejidos de ratas tratadas con cadmio. *An. Real. Acad. Farmacia.* 67(1):15-25
16. Ribas-Ozonas B.; García-Arribas O. and Pérez-Calvo M. (2001). Some data on the comparison between the eukaryotic cell electric activity and the electromagnetic hypersensitivity syndrome. In: Proceedings of the XIII Conference Naukowo-Techniczna: ELSAF, 12-14 September 2001, Wroclaw, Poland. Pp.:148-155.
17. Ribas-Ozonas B. (2004). Metallothioneins. Chapter 6, In: Elements and their Compounds in the Environment. (Merian E, Anke M, Ihnat M, Stoeppler M, eds). 2nd ed. Serie. vol 1. Wiley-VCH, Weinheim, Germany, 391-400.
18. Mathiessen T.; Ellingsen DG. and Kjuus H. (1999). Neuropsychological effects associates with exposure to mercury vapor among former chloralkali workers. *Scand. J. Work. Environ. Health.* 25 (4): 342-350.
19. Rossini SR.; Reimao R.; Lefevre BH. and Medrado-Faria MA. (2000). Chronic insomnia in workers poisoned by inorganic mercury: psychological and adaptative aspects. *Arq. Neuropsiquiatria*. 58(1): 32-38.
20. Polec K.; García Arribas O.; Pérez Calvo M.; Szpunar J.; Ribas Ozonas B. and Lobisnki R. (2000). Identification of cadmium-bioinduced ligands in rat liver using parallel HPLC-ICP-MS and HPLC-electrospray MS. *J. Anal. At. Spectrom.* 15:1363-1368.
21. Polec K.; Pérez Calvo M.; García Arribas O.; Szpunar J.; Ribas Ozonas B. and Lobisnki R. (2002). Investigation of metal complexes with metallothionien in rat tissues by hyphenated techniques. *J. Inorg. Biochem*. 88:197-206
22. Okazaki Y.; Namikawa K. and Minami T. (2000). Studies of metals and metallothionein in tissues. *Yakugaku. Zasshi* 120(3):282-289.

Prevalence of Amoebic Dysentery in Kirkuk City-Iraq

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ABSTRACT

Amoebic dysentery is an epidemic disease in Iraq. Serious steps are required in order to reduce the infection ratio and to prohibit any problems caused by this disease. This study was conducted on children Pediatric Hospital in Kirkuk city for a period from the beginning of November until the end of April 2018, who suffered from amoebic dysentery. Children's ages ranged between two months to 12 years old. A stool sample was examined by direct microscope method to investigate the prevalence of *Entamoeba histolytica*.
The total percentage of infection with *E. histolytica* were 586 out of 2888 (20.29%). The epidemiology survey based on microscopic examination revealed that there were no significant differences in the level of gender who infected with *E. histolytica*. While according to age groups the highest rate of infection recorded among two months -2 years 48.80% while the lowest percentage was 11-12year 2.04%. The higher rate of infection was in urban areas 60.93%, while in a rural area was 29.69%. The highest percentage of infection was during April 27.18% and the lowest percentage was during January 15.29%. The highest percentages of infection were found in bad living conditions 61.09%, compared to 15.29% and 15.38% in good hygienic areas. The highest percentage was found in patients with the blood group O 50% and the lowest in the patients with blood group AB 4%. There was no association between parasitic infection and Rhesus factor (Rh) for blood groups.

Keywords: *Entamoeba histolytica*, Entamoeba histolytica, prevalence, blood group.

INTRODUCTION

E.histolytica is an enteric protozoan parasite that causing amoebic dysentery (1). The third leading cause of death from parasitic diseases worldwide after Malaria and schistosomiasis (2, 3). It is estimated that it affects nearly 50 million people worldwide, leading to the deaths of approximately 40,000 to 100,000 people annually (4,5). The *E.histolytica* is prevalent throughout the world, being most prevalent in tropical and subtropical regions (6-8) especially in developing countries (9,10).There are more than 20 species of *Entamoeba*, with varying disease-causing potential and host specificity (11).Unfortunately, many species of *Entamoeba* are morphologically indistinguishable from *E. histolytica*, including the potentially-pathogenic but rare *E. nuttalli* (12). As well as many non-pathogenic species of *Entamoeba*, including E. *dispar*, *E.moshkovskii* and *E. bangladeshi* (11,13).

The current study aimed to determine the prevalence of *E. histolytica* in the children attending the Pediatric hospital in Kirkuk city , the effect of the gender, age, the location of their residence, the condition of their families, and their blood groups and Rh, on parasite proliferation as well as the seasonal influence on infection during the months of study.

MATERIALS AND METHODS

Collection of samples:

A total of 2888 stool sample of children were collected randomly suffering from severe diarrhea who admitted into Pediatric hospital in Kirkuk city governorate at the interval from the beginning of November until the end of April 2018. Each stool sample was placed in a clean plastic container. The sample was examined within 30 minutes to ensure the presence of *E. histolytica* trophozoite or cyst stage using a direct wet mount. A questionnaire was designed to collect information including age, gender, living condition, a period of study, area of residence, blood groups and Rh factor.

Specimens' examination:

The stool samples were examined using by two direct smears method were done, one in normal saline 0.9%, and the other one in lugol's iodine 1% to screening on *E. histolytica* trophozoites and cysts stage, respectively (14). A test was conducted to determine the blood groups and Rh factor of 100 people with amoebic dysentery by using ABO kit produced by Plasmatec Company- U.K.

Statistical Analysis:

The statistical analysis system (SAS 2010) used statistical analysis of the studied data to study the effect of the different factors in the studied traits. The differences between the mean were compared with the least significant difference of LSD, and the percentages were compared with the Chi-square test.

RESULTS AND DISCUSSION

The consistency of stools was varied from solid to semi-solid to diarrhea (watery or bloody and mucus together). Out of 2888 stool samples taken from children's attending the pediatric hospital in Kirkuk city, 586 were positive for *E. histolytica* 20.29% (table 1).The percentage of the prevalence of *E. histolytica* similar to what obtained by Al-Janabi (15) Baghdad city, where the incidence of infection with parasite was 18.72, and in the city of Kirkuk recorded (16) rate infection amounted to 21.67%. While other investigators in Tikrit city and, in Zako city found 9.7% and 23.78% respectively (17,18).The difference in parasitic infection in the present study and some of the above studies may be due to several factors, including the total number of samples examined, geographical location and age groups taken, as well as the methods used in diagnosis, study period, climate effects, and awareness Cultural and personal hygiene, population density, and differences in the level of sanitation services. The similarity between infection rates close to the percentage we have found in our present study may be due to similarities in terms of cultural, social and health level (19).

Table (1): Infection rate of *E. histolytica* of Pediatric Hospital in Kirkuk city

Total number	2888
Number of positive samples%	586(20.29%)
Number of negative samples%	2302(79.71%)

Table (2) shows that the rate of *E. histolytica* was higher in males 333(20.48 %) than females 253 (20.05 %). Statistical analysis was performed, and there was no significant difference between males and females in parasitic infection.

Table (2): Infection rate of *E. histolytica* of children according to gender

Gender	Total number	Number of positive samples%	Number of negative samples%
Male	1626	333 (20.48%)	1293 (79.52%)
Female	1262	253 (20.05%)	1009 (79.95%)
Total summation	2888	586 (20.29%)	2302 (79.71%)

These results agreed with that obtained from other studies in Iraq. In the province of Tikrit city infection rate in males amounted to 17.5%, and in females 20%, and recorded (20), in the city of

Kirkuk infection rate in males amounted to 20.8% and females 23.07%.(16). These results did not consistent with the results of (17) in Tikrit, He found that male infestation was 13%, while in females, 6.7%.And in the city of Kirkuk, where it was found that the proportion of male infection parasite 54.47%, while in females 45.5%, and recorded (21), in the city of Baghdad male infection rate of parasites 63% and in females 37%.(15).

The results of this study are consistent with previous studies due to equal opportunities for males and females to obtain food or drinks contaminated with the parasite, especially those children of these ages of both genders have a tendency to eat exposed foods from peddlers. Consistent with our current study, the susceptibility of male infestation to parasites more than females was attributed to several factors including the presence of genetic or physiological factors related to sex, the high numbers of male patients, the social habits that give males greater attention than females, the increased effectiveness of males and their contact with the of females compared.

The results showed that there was a high incidence of *E.histolytica* infection in the two-month and 2-year age groups with a rate of 48.80%, while the lowest rates in the age group 11-12 years were 2.04% Statistical analysis There were significant differences in parasitic infection among age groups at $P \leq 0.05$ (table 3).

Table (3): Infection rate of *E.histolytica* of children according to age group

Age groups/year	No. examined	Positive percentage %
2 mouths-2 years	286	48.80%
3-4 years	58	9. 89%
5-6 years	123	20.98%
7-8 years	60	10.23%
9-10 years	47	8.02%
11-12 years	12	2.04%

These results were in agree with (21) in Kirkuk and (20) in Tikrit, which recorded the highest incidence of parasitic infection in children aged between 1-10 years, and agreed with (22) and (16) in the city of Kirkuk, which recorded the highest infection rate of parasitic in children between the ages of 1_5 years and with the results (23), which recorded the highest rate of infection with parasites in children aged 4-6 years. The reason for the high incidence of infection in these categories may be due to several factors, including incomplete immune system and thus less resistance to diseases.

Too, increased friction of these ages to the environment and non-adherence to the rules of hygiene, such as nail care and washing hands after playing with dirt and others, eating by hands and eating (24). For miscellaneous foods from outside the home, these foods may be contaminated with parasite bags, other people at home carrying infectious parasite bags, and direct contact with pets. The incidence of parasitic infection was high in the 2 months to 2 year age group at 48.80%. This may be due to the lack of breastfeeding for the infant. It is known that the mother's milk contains IgA antibodies that have the potential to kill many germs and child protection from diseases, as well as due to the lack of correct methods in sterilization of milk bottles and other utensils used in preparation, leading to infection with bacteria.

As well as diminishing care from parents (23), they may be attributed to the social and economic level, and to their living in crowded areas(25).and the lowest infection rate was recorded in the age group between 11-12 years of 2.04%, and This is due to the development of their immune system compared to the smaller groups Increasing health and cultural awareness in this category.

The study showed that the site has an effect on the percentage of *E. histolytica* parasites. A significant increase was observed in parasitic infection among children who are urban dwellers and from areas with poor sanitation and service levels within the city. 60.93%. The judiciary was 29.69%, while the lowest percentage among those from the governorates was 9.38% (table 4).

Table (4): Infection rate of *E. histolytica* according to residency

Areas of Residence	Number	Percentage
Urban	357	60.93%
Rural	174	29.69%
Other governorates	55	9.38%

These results were in agreement with (20) in the city of Tikrit, where the percentage of infection in the city was 52.5% while in the countryside 47.0% and also were in agree with (15) in the city of Baghdad, where recorded infection rate in the city of 70% and in the countryside 30% and(26) where recorded infection rate in the city 41.3%while in the countryside 27.3%. The difference in the incidence of infection between the rural population and the city can be attributed to our choice of one hospital, the hospital location in the city, Most of the people in the study are in the city center, while the rest are from different rural areas, easy access to the hospital from all areas of the city, and water pollution due to the untreated industrial wastewater. Other contributing factors are the low socio-economic situation, community and personal hygiene, and the increased density of displaced persons in the city of Kirkuk, especially after the recent events in 2014.

The results showed that *E. histolytica* parasites had the highest rates of bad-living environment 61.09% compare with the incidence of living conditions was moderate at 21.85% and the lowest number of cases in the case of persons with good living, which amounted to 17.06%. This result is similar to that of (21) in the city of Kirkuk, where the rate of injury was 50.0% in cases below the average and 27.9% of the average living conditions, while the lowest number of injuries with good living, which amounted to 22.02% (table 5).

Table (5): Distribution of *E. histolytica* parasites by family living status

Living situation	Number	Percentage
Good	100	17.06%
Bad	358	61.09%
Medium	128	21.85%

The reason for the recent security problems in the country led to the migration of large numbers of residents of the governorates and the affected districts for Kirkuk governorate, where most of them lack housing and income. This has led to a decline in the standard of living as it is difficult to provide the necessities of life. In the food consumed has a role in the resistance of the body to parasitic infections and others, and characterized most of the components of this category according to the results of the questionnaire of the infected living in the popular neighborhoods and on the outskirts of the city, where the health conditions are low.

Table (6) shows the incidence of *E.histolytica* parasites in the pediatric hospital for each month of the study. The highest percentage was in April 27.18%, followed by March 24.53% and then the months of November and February, while the lowest percentage was recorded during December The second and the first by 15.29% and 15.38%, respectively.

Table (6): Infection rate of *E.histolytica* during the period of study

Months	Total examinees	Positive samples%	Negative samples%
November	666	146(21.92%)	520(78.08%)
December	689	106(15.38%)	583(84.62%)
January	412	63(15.29%)	349(84.71%)
February	308	60(19.48%)	248(80.52%)
March	379	93(24.53%)	286(75.47%)
April	434	118(27.18%)	316(72.82%)

A study conducted by (27) recorded percentage a 43.7% infection with parasitic infection in April, and were in agree with (28) where it recorded the highest percentage during April of 40% and the lowest in February and January respectively. While did not were in agree with (29), where recorded the highest infection rate of parasitic infection in July amounted to 15.6%, while the lowest rate in December 3.1%, and each (30) recorded the highest rate of infection with parasites during the month of October 18.42%, and the lowest rate was in June 5.71% and (31) recorded the highest rate of infection with parasites during the month of January15.85% while the lowest rate was during the month of May was 9.10%.

The results of the current study indicate that the increase in the incidence was during the month of April and may be due to the availability of environmental conditions suitable for the growth and prevalence of parasite as intestinal parasites are more prevalent in the tropical and hot areas also due to the increase in drinking water consumption during the months and the increase in the likelihood of eating water contaminated with the cysts of intestinal parasites, and the chances of human infection in *E. histolytica* the condition of the tissue increases significantly during the hot months due to the proliferation of insects carrying disease and prevalence, domestic housefly (Musca domestica) which is a mechanical vector of parasitic and eggs of some tapeworms and cylindrical.

Table (7) shows the relationship between the infection *E. histolytica* parasites and blood groups compared with control groups. The highest percentages were found in the O group of patients with amoeba tissue parasites by 50%, followed by blood group A and by infection 25%, then blood group B by 21%, while the lowest percentage in the blood group AB by 4%.

Table (7): Infection rate of *E. histolytica* according to blood groups

Blood groups	positive samples%
A	25(25%)
B	21 (21%)
AB	4 (4%)
O	50 (50%)

These results were in agreement with (21), which recorded the highest percentage of patients in the blood groups O and A by 36% and 34%, respectively, and blood group B by 20%, while the lowest proportion in the blood group AB by 10% %. And also agreed with (32) who had the highest percentage of blood group O and lowest in AB group (33) which had the highest rates of infection among the A blood group. The reason for the dominance of O and A is due to the fact that most of the population groups of the blood group (32) are identical.

In the primary Rh, the number of people with the positive Rh was 94, while the number of people with the negative Rh was 6, as shown in table (8). This discrepancy in the number showed that there was no association between the incidence and the Rh for blood groups as reported in the studies (33, 34).

Table (8): Infection rate of *E. histolytica* according to Rhesus factor (Rh)

Rh+		Rh-	
Number	Percentage	Number	Percentage
94	94%	6	6%

Several studies have dealt with the relationship between blood groups and infectious diseases including parasitic diseases. These studies dealt with several main aspects of this relationship, namely the adhesion of pathogens in the cells that produce substances, blood groups, change in the response of antibodies, Genetic factors play a major role in protecting the body against parasitic pathogens, so the importance of these genetic factors in the intestinal parasite is widely recognized (32,35).

CONCLUSION

The results of the present study showed there was no significant difference in the level of gender in the susceptibility of infection *E.histolytica* parasites, and the highest infection rate *E.histolytica* parasites in the age range between recorded among two months -2 years and children with a bad living condition, the study has the highest infection of parasites *E.histolytica* in April, in children who are residents of the city. The current study showed that there was no relationship between parasitic cases and between different blood groups. The highest rates of infection were found in O and A groups.

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REFERENCES

1. Ali IKM. (2015). Intestinal amebae. *Clin. Lab. Med.* 35(2):393-422.

2. Mortimer L. and Chadee K. (2010). The immunopathogenesis of *Entamoeba histolytica*. *Exp. Parasitol.* 126: 366–380.

3. Ghasemi E.; Rahdar M. and Rostami M. (2015). Prevalence of *Entamoeba histolytica/dispar* in drinking water in the city of Shush, Khuzestan Province in 2011. *Int. J. Curr. Microbiol. App. Sci.* 4:582–588.

4. Inam A.; Mittal S.; Rajala MS.; Avecilla F. and Azam A. (2016). Synthesis and biological evaluation of 4-(2-(dimethylamino) ethoxy) benzohydrazide derivatives as inhibitors of Entamoeba histolyica. *Euro. J. Med. Chem.* 124:445-455.

5. Pineda E. and Perdomo D. (2017). *Entamoeba histolytica* under oxidative stress: what countermeasure mechanisms are in place? Cells. 6(4): 44.

6. Al-Areeqi MA.; Sady H.; Al-Mekhlafi HM.; Anuar TS.; Al-Adhroey AH.; Atroosh WM. and Lau YL. (2017). First molecular epidemiology of *Entamoeba histolytica, E. dispar* and *E. moshkovskii* infections in Yemen: different species-specific associated risk factors. *Trop. Med Int. H.* 22(4): 493-504.

7. Yimer M.; Zenebe Y.; Mulu W.; Abera B. and Saugar JM. (2017). Molecular prevalence of *Entamoeba histolytica/dispar* infection among patients attending four health centres in north-west Ethiopia. *Trop. Dr.* 47(1):11-15.

8. Sastry AS. and Bhat S. (2018). Essentials of medical parasitology. Jaypee Brothers. 2nd ed., Medical Publishers Pvt. Limited.

9. Peterson KM.; Singh U. and Petri Jr WA. (2011). Enteric amebiasis. In Tropical Infectious Diseases: Principles, Pathogens and Practice, WB Saunders. Pp.: 614-622.

10. Gwairgi MA. and Ghildyal R. (2018). Nuclear transport in *Entamoeba histolytica*: knowledge gap and therapeutic potential. *Parasitol.* 1-10.

11. Nozaki T. and Bhattacharya A. (2015). Amebiasis: Biology and Pathogenesis of Entamoeba. Springer Ltd. P.:260.

12. Levecke B.; Dorny P.; Vercammen F.; Visser LG.; Van Esbroeck M.; Vercruysse J. and Verweij JJ. (2015). Transmission of Entamoeba nuttalli and Trichuris trichiura from nonhuman primates to humans. *Em. Inf. Dis. J.* 21(10):1871.
13. Jirků-pomajbÍkov ÁK.; Čepička I.; Kalousov ÁB.; Jirků M.; Stewart F.; Levecke B. and Petrželkov ÁKJ. (2016). Molecular identification of Entamoeba species in savanna woodland chimpanzees (Pan troglodytes schweinfurthii). *Parasitol.* 143(6):741-748.
14. Cheesbrough M. (2005). District Laboratory Practice in Tropical Countries. 2nd ed. Cambridge University Press. Pp.:200-202.
15. Al-Janabi MAB.(2014). Effect of caffeine, water and alcoholic extracts of green tea on the experimental infection of *Entamoeba histolytica* in laboratory mice with an epidemiological study of the parasite in the Central Child Hospital in Baghdad. Master thesis , University of Tikrit.
16. Lazar LTY.(2012). Epidemiological study on *Entamoeba histolytica* in Kirkuk city and its relationship with free radicals in blood serum of infected persons. Master thesis, University of Tikrit.
17. Kadir MA.; El-Yassin ST. and Ali AM. (2018). Detection of Entamoeba histolytica and Giardia lamblia in children with diarrhea in Tikrit city. *Tikrit. J. Pur. Sci.* 23(6):57-64.
18. Mero WM.; Jameel AY. and Amidy KSK. (2015). Microorganisms and viruses causing diarrhea in infants and primary school children and their relation with age and sex in Zakho city, Kurdistan Region, Iraq. *Int. J. Res. Med. Sci.* 3(11):3266-3273.
19. Long KZ.; Rosado JL.; Montoya Y.; de Lourdes Solano M.; Hertzmark E.; DuPont HL. and Santos JI. (2007). Effect of vitamin A and zinc supplementation on gastrointestinal parasitic infections among Mexican children. *Pediat.* 120(4):e846-e855.
20. Al-Somaeday IGAB.(2012). Determination of Giardia lamblia assemblagesand *Entamoeba histolytica/Entamoeba dispar* complex by molecular diagnosis (PCR) in patient in Tikrit. PhD. Thesis. University of Tikrit.
21. Bahaa Al-Deen EI.(2018). The epidemical, immunological and biochemical study in patients infected with intestinal parasitic protozoa in Kirkuk city. Master thesis. College of Science, University of Kirkuk.
22. Ibraheem MR.(2008). Study on prevalence of *Entamoeba histolytica* among children in Kirkuk city and the efficiency of some chemotherapeutic drugs and medicinal herbs extracts in vitro culture. Master thesis. College of Science, University of Tikrit.
23. Al- Nasiry MA. (2007). An epidemiological study of *Entamoeba histolytica* for children in Baiji, with effect for some extracts plants on it. Master thesis. College of Education, University of Tikrit.
24. Raddam KK. and Hasson AJ. (2008). The epidemiological aspects of infection with *Entamoeba Histologica* in acute diarrhea in Thi-Qar Governorate during the Year 2006. *Kufa. Med. J.* 11(1):15-24.
25. Hammadi KA. (2012). Study for intestinal parasites among children in Al-Mahmoudyia area / Baghdad province. *Biol. J. Al-Kufa Univ*. 4(1): 271-274.
26. Al-Ammash MSJ. (2016). Study on prevalence of *Entameoba histolytica* and *Giardia lamblia* in Samarra city. *Kufa. J. Vet. Med. Sci.* 6(2):194-204.
27. Al-Masoudi HKM. (2009). The effect of some plants extracts and drugs on immobilized cysteine proteinase of *Entamoeba histolytica* isolated from diarrhea in Al-Exandaria District, Babylon province. PhD. Thesis. College of Science, University of Baghdad.
28. Al-Safou FH. (2012). Epidemiological study of *Entamoeba histolytica /dispar* and the other intestinal parasites and microorganisms associated and the effect of parasite infection on laboratory investigation of children in Tikrit City. Master thesis. College of Education, University of Tikrit.
29. Al-Kahfaji MSAB.(2010). Some immunological criteria for patient infected with *Entamoeba histolytica* and histological changes in white mice. Master thesis. College of Science for Women, University of Babylon.
30. Al-Bayati ShAH.(2011). Study of some intestinal protozoan parasites among peoples in Al-Touz town with study of Giardiasis on infected tissue in laboratory mice. Master thesis. College of Education, University of Tikrit.
31. Atiyah AH. and Al-Mwsawi HQH.(2016). The most detective protozoal infections in correlation with radiological findings in Wasit Governorate Hospitals during 2015. *Med. J. Babylon*. 13(4):862-873.
32. Koyee QM. (2017). Intestinal parasitosis in relation to ABO blood groups among some asymptomatic Turkish, Bangladesh and Kurdish cleaners in Hospitals of Erbil city. *J. Koya Univ.* 21:97-108.
33. Mandefro A.; Kelel M. and Wessel G. (2014). Association of Abo Blood Group and Rh factor with malaria and some gastrointestinal infectious disease in a population of Adet and Merawi, Ethiopia. *Glob. J. Biotechnol. Biochem.* 9(4):137-142.
34. Bakare AA.; Azeez MA. and Agbolade JO. (2006). Gene frequencies of ABO and rhesus blood groups and haemoglobin variants in Ogbomoso, South–West Nigeria. *Afr. J. Biol.* 5(3):224-229.
35. Berger SA.; Young NA. and Edberg SC. (1989). Relationship between infectious diseases and human blood type. *Eur. J. Clin. Microbiol. Inf. Dis.* 8(8):105-109.

Seasonal variation of steroid hormone estradiol, progesterone in follicular fluid and blood serum of buffaloes at Basrah province-Iraq

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ABSTRACT

Physiology of ovary is controlled by endogenous and exogenous factors, including the endocrinological and biochemical alterations that occur in the follicular fluid during the estrus cycle. The aim of this study was to determine and compare the concentrations of some hormonal composition in peripheral circulation and follicular fluid of buffaloes during season and out season for this purpose 100 sample of ovaries was collected from adult buffaloes immediately after slaughter, and blood samples were also collected from the same buffaloes before slaughter in Basrah abattoir. Serum was separated and stored for further analysis. The follicle classified according to diameter, small (3–5 mm), medium (6–9 mm) and large (10–20 mm). Follicular fluid was aspirated and stored at -4°C for further analysis. Results of the present study revealed that the estradiol hormone in follicular fluid were found to be higher in season (667.31 ± 13.08) than that in out season (563.42± 14.29). The blood serum were found to be lower in out season (35.20 ± 1.3) than that in season (55.26 ± 1.7).The progesterone levels of follicular fluid were found to be lower in out season (21.6 ± 0.48) in compared to season (26.27±0.26).The progesterone concentration of blood serum were found to be significantly ($P < 0.01$) lower in outside season (0.58 ± 0.02) than that during season (0.91 ± 0.09).

Keywords: Estradiol, Progesterone, Follicular Fluid, Blood, Buffaloes

INTRODUCTION

Buffaloes are one of the most important livestock since ancient times all over the world; these animals are classified to be part of *mammalia's* class, order of *Artiodactyla* and suborder of *Ruminantia* in the family of *Bovidae* (1). Buffaloes (*Bubalus bubalis*) play prominent role in rural livestock production, particularly in Iraqi contributing high quality animal protein, both milk and meat. However, the buffalo is classified according to where it is spread to two main species (Syncerus caffer) with 52 chromosomes and the water buffalo (Bubalus Bubalis), which is classified into two other species, the swamp buffalo 48 and the river buffalo 50 chromosomes, and although chromosomal differences mating can occur between them and obtain births (2). The buffaloes of Iraq are classified as Bubalus Bubalis (3).

Ovarian follicular fluid is the environment of oocyte maturation is a viscous and straw colored solution with above pH 7. FF is could be mixed secretion of follicular cells and transudate of plasma. The constituents of follicular fluid are considered as a regulating factor in follicular development and steroidogenesis (4). The increase in size of follicles from early antral to graafian stage is due to the accumulation of follicular fluid in antral cavities (5). Follicular fluid is partially composed of locally produced substances, which are related to the metabolic activity of follicular cells (6). The current study was aimed to measure the level of estradiol and progesterone in follicular fluid and blood serum during season and out season.

MATERIALS AND METHODS

One hundred ovaries from sexually mature buffaloes were collected within 30 minutes after slaughter in the abattoir, they were transported within 20-30 minutes of slaughterhouse to the laboratory in physiological normal saline (0.9% NaCl) containing antibiotics (100 µg/ml streptomycin sulfate and 100 IU/ml penicillin) and preserved in cool boxes at 4-8°C. Upon arrival at the laboratory, foreign tissue was removed from the ovaries. Then, the ovaries were washed with 70% ethanol to control contamination, rinsed 3 times in normal saline (0.9% NaCl), and finally dried with sterilized paper towels (7).

Blood samples were collected immediately from jugular vein before slaughtering in plain vacationer tube. All samples placed in isolated box on ice bags and transported within 20-30 minutes to the laboratory.

Follicular dimension was measured by employing a caliper and follicles were classified into 3 classes: small (3–5 mm), medium (6–9 mm) and large (10–20 mm) according to (8). Cystic ovaries were removed from this study.

Sampling:

1. Follicular Fluid: The contents of the sex gland follicles of various size (small, medium and large) were aspirated employing a ten metric capacity unit syringe hooked up to an eighteen gauge needle and centrifuged at 3000 rate for 15 min for separation of the fluid from the cell fraction.

2. Blood: Collected samples throughout slaughtering for serum separation after centrifugation at 3000 rpm for 15 minutes. Collected serum samples were kept at -20 °C until analysis (9).

Measured Parameters:

Levels of estradiol and progesterone were determined by Enzyme Linked Immuno Sorbent Assay (ELISA) by using of Elisa reader type BioElisa ElX800, Germany.

1. Estradiol: Estradiol-17β concentrations were analyzed by using kits from Accu-Bind -USA

2. Progesterone: Progesterone concentrations were analyzed by using kits from Accu-Bind –USA.

Statistical analysis:

The significance of variations between two groups by using ANOVA test analyzed using stander t-test ($P < 0.05$). The results were expressed as Mean ± Standard error (M± SE), the experiments were analyzed by using independent t-test by using SPSS (special program for statistical system), the level of significant set on ($P < 0.05$) (10).

RESULTS

The result of crossly examination of ovaries revealed that the ovaries were normal and in estrous cycle. Stage of estrous cycle (follicular or luteal) was identified according to the presence or absence of the corpus luteum on the ovary according to (11).

Tables (1 and 2) showed that the overall means of estradiol in follicular fluid and blood serum is higher significantly ($P <0.001$) in season than that in out season. The Progesterone concentration in follicular fluid and blood serum as indicated in tables (1and 2) are significantly ($P <0.01$) lower in out season than in season.

Table (1): Hormone Parameters of blood serum in buffalo in season and out season, Values are in Mean ± SE

Hormone Parameters	M ± SE in season	M ± SE Out season
Estradiol (pg/ml)	55.26 ± 1.7 b	35.20 ± 1.3 b
Progesterone (ng/ml)	0.91 ± 0.09 a	0.58 ± 0.02 a

a. Means having significant variation (P <0.01)
b. Means having significant variation (P <0.001)

Table (2): Hormone parameters of follicular fluid in buffalo in season and out season, values are in Mean ± SE

Hormone Parameters	M ± SE in season	M ± SE out season
Estradiol (pg/ml)	667.31 ± 13.08 b	563.42± 14.29 b
Progesterone (ng/ml)	26.27 ± 0.26 a	21.6 ± 0.48 a

a. Means having significant variation (P <0.01)
b. Means having significant variation (P <0.001)

DISCUSSION

In the current study the estradiol hormone in follicular fluids were found to be significantly (P <0.001) higher in season (667.31 ± 13.08) than that in out season (563.42± 14.29). This higher level can be attributed to the effect of sexual activity in buffalo with the availability of green fodder, the appropriate temperature of the atmosphere as well as the short duration of light, as all these factors affect the ovarian activity and the occurrence of estrus and these were in agreement with (12) and (13).

There was decreased in blood serum estradiol levels (P<0.001) during summer (35.20 ± 1.3) as compared with those in winter (55.26 ± 1.7) this recorded during this study, this decrease in serum estradiol levels because that the heat stress act directly on the ovary to decrease the sensitivity of the ovary to gonadotrophin stimulation and this agree with (14) and (15) in cows when observed that lower estradiol levels in serum during summer as compared with those in winter.

In the current study, the progesterone levels of follicular fluid were found to be significantly (P <0.01) lower in out season (21.6 ± 0.48) in compared to season (26.27 ± 0.26).During hot months progesterone levels may be related to the adversely effect on luteal function during the summer season and all the samples were taken during from follicular phase in summer season. These results are in agreement with (16) and (17).

Since all buffaloes included in the present study were non-pregnant, expected to be low according to (18).

The progesterone concentration of blood serum in outside season (0.58 ± 0.02) as recorded in this study is lower than that during season (0.91 ± 0.09) this result agrees with(19), (20) These results are in disagreement with (21) and (22) they have reported increased blood concentrations of this hormone during summer in dairy cows.

REFERENCES

1. Bruford MW.; Bradley DG. and Luikart G. (2003). DNA markers reveal the complexity of livestock domestication. *Nature. Rev. Gen.* 4(11):900.

2. Hafez ESE. and Hafez B. (2000). Folliculogenesis, Egg maturation and Ovulation, Chapter 5: In: Reproduction in farm animal. 7th ed. Lippincott Williams and Wilkins, London and New York. P.:604.

3. Al-Obaidi S.; Al-FartosiK. and Fazaa NA. (2007). Water Buffalo in Iraqi marshes. Nature Iraq Status Report.

4. Thakur RS.; Chauhan RAS. and Singh BK. (2003). Studies on biochemical constituents of caprine follicular fluid. *Indian Vet. J.* 80:160-162.

5. Ola SI. and Sun Q. (2012). Factors influencing biochemical markers for predicting mammalian oocyte quality. *J. Reprod. Develop.* 58(4): 385-392.

6. Gerard M.; Chaubey A. and Malhotra BD. (2002). Application of conducting polymers to biosensors. *Biosen. Bioelectr.* 17(5):345-359.

7. Warriach HM. and Chohan KR.(2004). Thickness of cumulus cell layer is a significant factor in meiotic competence of buffalo oocytes. *J. Vet. Sci*.5:247-251.

8. Kruip TAM. and Dieleman SJ. (1982). Macroscopic classification of bovine follicles and its validation by micro morphological and steroid biochemical procedures. *Reprod. Nutri. Dev.* 22: 465- 473.

9. Srirman PK.(2009).Veterinary laboratory diagnosis. Lippincotts. London.

10. Steel RG.; Torrie JH. and Dickey DA. (2006). Principles and Procedures of Statistics. A biometrical approach. 3rd ed. McGraw Hill Co. New York, USA. Pp.:36-37.

11. Mondal S.; Kumar V.; Reddy IJ. and Khub S. (2004). Progesterone and nucleic acid content of buffalo corpus luteum in relation to stages of estrus cycle. *Indian. J. Anim. Sci.* 74:710-712.

12. Ronchi B.; Stradaioli G.; Supplizi AV.; Bernabucci U.; Lacetera N.; Accorsi PA. and Seren E. (2001). Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17β, LH, FSH, prolactin and cortisol in Holstein heifers. *Livestock. Prod. Sci.* 68(2-3): 231-241.
13. Borghese A. (2005). Buffalo production and research. *REU Techn. Ser.* (67):101-110.
14. Megahed GA.; Anwar MM.; Wasfy SI. and Hammadeh ME. (2008). Influence of heat stress on the cortisol and oxidant-antioxidants balance during oestrous phase in buffalo-cows (Bubalus bubalis): thermo-protective role of antioxidant treatment. *Reprod. Domes. Anim.* 43(6):672-677.
15. Ronchi B.; Stradaioli G.; Supplizi AV.; Bernabucci U.; Lacetera N.; Accorsi PA. and Seren E. (2001). Influence of heat stress or feed restriction on plasma progesterone, oestradiol-17β, LH, FSH, prolactin and cortisol in Holstein heifers. *Livestock Prod. Sci.* 68(2-3): 231-241.
16. Rao LV. and Pandey RS. (1983). Seasonal variations in oestradiol-17β and luteinizing hormone in the blood of buffalo cows (Bubalus bubalis). *J. Endocrinol.* 98(2):251-255.
17. Qureshi MS.; Habib G.; Nawab G.; Siddiwqui MM.; Ahmad N. and Samad H. (2000). Milk progesterone profiles in various reproductive states in dairy buffaloes under field conditions. Proceedings of the National Science Council, Republic of China. Part B. *Life Sci.* 24(2):70–75.
18. Cristofori P.; Aria G.; Seren E.; Bono G.; Aaden AS. and Nur HM. (1986). Somalia: endocrinological aspects of reproduction in the female camel. *World Anim. Rev. (FAO)*.
19. Roth Z.; Meidan R.; Shaham-Albalancy A.; Braw-Tal R. and Wolfenson D. (2001). Delayed effect of heat stress on steroid production in medium-sized and preovulatory bovine follicles. *Anim. Reprod. Sci.* 121:745-751.
20. Megahed GA.; Anwar MM.; Wasfy SI. and Hammadeh ME. (2008). Influence of heat stress on the cortisol and oxidant-antioxidants balance during oestrous phase in buffalo-cows (Bubalus bubalis): thermo-protective role of antioxidant treatment. *Reprod. Domes. Anim.* 43(6):672-677.
21. Trout JP.; McDowell LR. and Hansen PJ. (1998). Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. *J. Dairy Sci.* 81(5): 1244-1250.
22. Vaught LW.; Monty DW. and Foote WC. (1977). Effect of summer heat stress on serum LH and progesterone values in Holstein-Frisian cows in Arizona. *Am. J. Vet. Res.*38:1027-1032.