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Microscopic Assessment on the Effects of Date Palm *(Pheonixdactilyfera)* Methanol Fruit Extract on Lead Acetate Induced Testicular Toxicity in Adult Male Wistar Rats.

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ABSTRACT

This study assessed the effects of methanol fruit extract of *Pheonix dactilyfera* and lead acetate on histology of the testes. This work was essential in providing information on interactions between the date palm extract and lead acetate in rat testes. Twenty five male Wistar rats were grouped into five (1–5; n=5). Group 1 (control) received distilled water (2 ml/kg). Group 2 received lead acetate (30 mg/kg). Groups 3, 4 and 5 received methanol fruit extract of *Pheonix dactilyfera* (75, 150 and 300 mg/kg respectively) followed by lead acetate (30 mg/kg) concurrently for 42 days via oral route. Histological examinations of tissues were done using Haematoxylin and Eosin, and Periodic Acid Schiff stains. The tubular diameter and germinal epithelium height were measured using the Image J software (v2). Findings revealed degenerative changes like pyknosis, karyorrhexis in all groups except Group 1 in varying degrees. Group 4 rats displayed little changes and had tubular diameters and epithelium heights similar to that of the Control. Results suggest protective potentials of methanol fruit extract of *Pheonix dactilyfera* at dose 150 mg/kg when compared to other treatment groups.

Key words: degeneration, epithelium, lead, spermatids, tubular

INTRODUCTION

Patients described as primarily infertile by many authorities are so called if they are unable to achieve successful conception of pregnancy after a year of regular unprotected intercourse[1]. Environmental and occupational exposure to a large number of chemicals occurs at various stages throughout human life. Lead as a point of focus, has been a widespread public concern for decades[2]. Lead exposure results in anemia and small increases in blood pressure, mostly in middle aged and older people [3]. Severe damage to the nervous system and kidneys in adults and children ultimately result in death due to exposure to high lead levels. In pregnant women, miscarriages can also occur due to high levels of exposure to lead[4]. Numerous studies have depicted the ability of lead exposure in adversely affecting the endocrine function as well as the spermatogenesis capability of the testis[5]; occupational exposure of lead to men decreases their fertility[6], chronic high level exposure has been shown to reduce fertility in males[7].

The testes are the gonads-paired ovoid male reproductive glands that produce the male germ cells and male hormones, primarily testosterone and it is suspended in the scrotum by the spermatic cord[8]. It is 3.7cm in length, 2.5 cm wide from before backwards and 1.8 cm thick from side to side and weight of 10-15g in the adult[9].

On a global level, the role traditional medicines play in the solutions of health problems is invaluable [10]. According to certain conditions, there are huge variations in the shape, size, quality and consistency of fleshy portions of date palms [11]. Date palm is known plant globally, a few of these names include: Hausa: Dabino; Yoruba: labidun; Arabic: Nakhla; English; Date; Greek: Phoinix[12]. Proteins, dietary fibres, less fat and some sugars such as glucose, sucrose and fructose can be found in the fleshy portions of date palms[13]. Vitamins such as folic acid, ascorbic acid and minerals such as calcium, iron, phosphorus, zinc, copper, manganese, potassium, sodium which are vital to the nourishment of the human body are also found in date pulps[13].

The date palm phytochemical constituents such as alkaloids, anthocyanins, procyanidins, flavonoids, and steroids serve as hepatoprotective, nephroprotective, anti-inflammatory, radical scavengers, and fertility agents[14 15]. Oral administration of date palm fruit suspensions at doses of 120 and 240mg/kg improved the

sperm count, motility, morphology, and DNA quality with a concomitant increase in the weights of the testis and the epididymis [11].
Moreover, date extracts have been shown to increase sperm count in guinea pigs and to enhance spermatogenesis and increase the concentrations of testosterone, follicle stimulating hormone (FSH) and Luteinizing hormone (LH) in rats[16]. These studies suggest its usefulness in solving infertility problems in males.

MATERIALS AND METHOD

Experimental Animals: Twenty-five apparently healthy male Wistar rats (107g-197g) were obtained from the Animal House of Faculty of Medicine, Bayero University, Kano, Nigeria and were housed in Animal House of Department of Human Anatomy, Faculty of Basic Medical Sciences, Ahmadu Bello University, Zaria and acclimatized for a period of two weeks. The animals were provided with standard pelletized growers feed and water *ad libitum*. The rats were then categorized into control and treatment groups.

Plant Materials: The date palm fruits were obtained in a local (Samaru) market, Sabon Gari Local Government of Kaduna state, Nigeria. The date palm fruits were then taken to the Department of Biological Sciences, Faculty of Life Sciences, Ahmadu Bello University, Zaria and were identified in its Herbarium Unit as date palm fruits.

Drug: Lead (II) acetate was obtained and used for the experiment as the reproductive toxicant. It was manufactured by Best of Chemical Science, New York with the batch number CAS 304-55-2. The Lead (II) acetate compound was then taken to the Department of Chemistry, Faculty of Physical Science, Ahmadu Bello University, Zaria for verification and identification.

Extract Preparation: The crude methanolic fruit extract was prepared by the method of maceration [17]. The extract preparation was done in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria.

Experimental Procedure: Adult Male Wistar rats were grouped into 5 groups of 5 each. The groupings were as follows:
Group 1 (Control group) was given 2 ml/kg distilled water per body weight.
Group 2 (lead acetate group only) was given 30 mg/kg (5% of LD_{50}) of lead acetate (LD_{50} = 600 mg/kg [18] per rat body weight for 42 days;
Group 3 was administered with 75 mg/kg body weight of methanol extract of date palm fruit and 30 mg/kg of lead acetate for 42 days;
Group4 was administered with 150 mg/kg of the date palm extract and 30 mg/kg of lead acetate for 42 days;
Group 5 was co-administered 300 mg/kg (LD_{50} = 6000 mg/kg[19] of the methanolic extracts of date palm fruit and 30 mg/kg of lead acetate for 42 days. It should be noted that administration was done via the oral route.

Animal Sacrifice and Collection of Tissues: On the 43rd day, the animals were humanely sacrificed after an overnight fast. Chloroform was the choice of anesthesia. Testes of the animals were removed by dissection. After weighing, one of each testis was immersed in Bouin's fluid for fixation while the other was prepared as a tissue homogenate.

Histological Staining: The tissues were then processed into thin slices of 5µ diameter and were stained using Haematoxylin and Eosin (H & E) and Periodic Acid Schiff (PAS) stains. This was done in the Histology Lab of Human Anatomy Department, Ahmadu Bello University, Zaria.

Sterological Techniques: Five randomized sections were picked after every 40th section using systematic random sampling. The seminiferous tubular diameter was measured as the length of a straight line from a point on the basement membrane of the seminiferous tubules through the center of the tubule to an opposite point on the basement of the tubule. The germinal epithelium height was measured as the length of the seminiferous epithelium measured from the basement membrane of seminiferous tubules to the beginning of the luminal compartment of the tubule. Both were expressed in µm and were done using the Image J software v2.

Data Collection: All the data were expressed as mean ± Standard Error of Mean (SEM) and were statistically analyzed using Statistical Package for Social Sciences (SPSS) version 22 (IBM, Incorporation, NY). Comparisons between control values and those obtained in treated groups of rats were performed with one way analysis of variance (ANOVA). Post hoc test using Tukey's comparative test were carried out to find the level of significance where differences exist. A p-value <0.05 was considered significant.

RESULTS

Histological Examination: Histological examination of the seminiferous tubules of the Adult Wistar rats revealed the following: There was intact germinal epithelium and basement membrane noted in the Control group (Group 1) of animals (Plate I and VI). Sections of the testes taken from the Group 2 rats (lead acetate only) revealed cells undergoing karyorrhexis and vacuolation with a general lack of the spermatid population (Plate II). There was also detachment of the basement membrane with an empty lumen noted when stained with PAS (Plate VII). Sections of the tissues of Group 3 rats revealed few mature sperm cells in the lumen and also a detached basement membrane (Plates III and VIII). Few cells with pyknotic nuclei and a fairly intact germinal epithelium and basement membranes were observed in the sections of the tissues of the Group 4 rats (Plates IV and IX). Sections of the tissues of Group 5 rats showed cells with complete dissolution of the nuclear material and vacuolations with a slightly detached membrane (Plates V and X).

From table 1 below, the results indicated a statistical significant increase in the seminiferous tubule diameter (STD) in the control group when compared to Groups 2, 3 and 5. There was also a significant decrease in the diameter of the seminiferous tubules of Group 2 when compared to Groups 3 and 4. As regards the germinal epithelium height (GEH), Group 4 had a significant increase when compared to Groups 2 and 5.

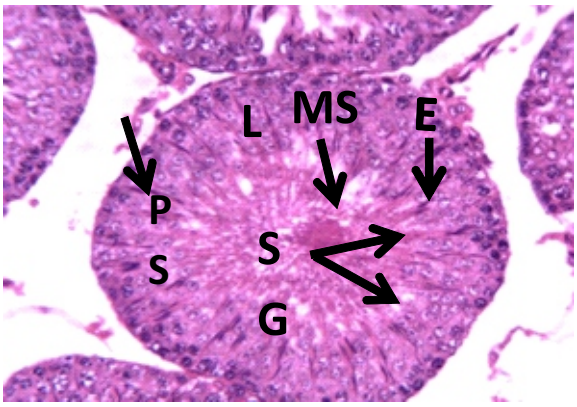

Plate I: A section of the testes in Group 1 (2 ml of Distilled water) showing normal histology; PS: pachytene spermatocytes, MS: Mature Sperms, L: Lumen, SG: Spermatogonia, LC: Leydig cells. (Haematoxylin and Eosin x250)

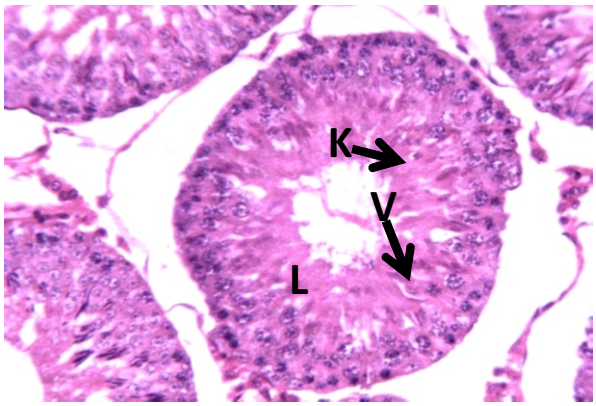

Plate II: A section of the testes in Group 2 (30 mg/kg Lead acetate) showing vacuolation (v), karyorrhexis (k) and a general lack of spermatids and essentially sperm cells in the lumen (L) (Haematoxylin and Eosin x250)

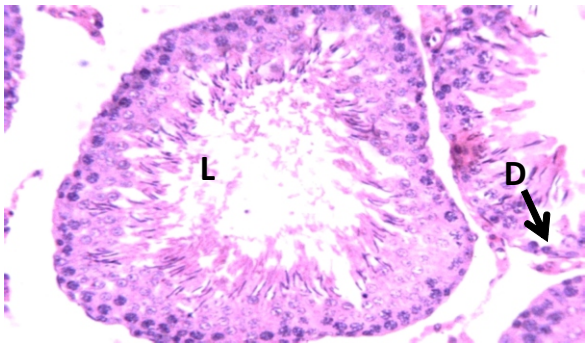

Plate III: A section of the testes in Group 3 (75 mg/kg MFPD + 30 mg/kg Lead acetate) showing degeneration of the seminiferous tubules with few mature sperm cells in the lumen and uneven epithelium height (Haematoxylin and Eosin x250)

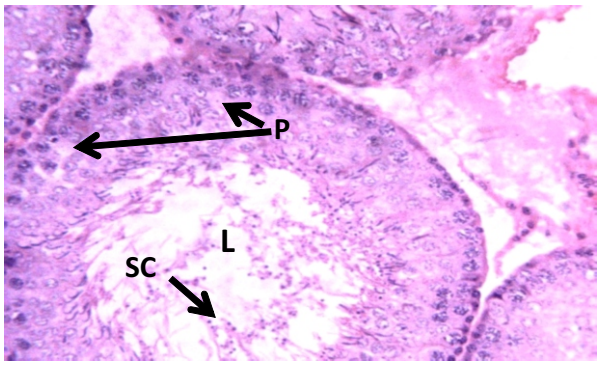

Plate IV: A section of the testes in Group 4 (150 mg/kg MFPD + 30 mg/kg Lead acetate) showing few cells with pyknotic nuclei, lumen (L) with moderate amounts of mature sperm cells (SC) (Haematoxylin and Eosin x250)

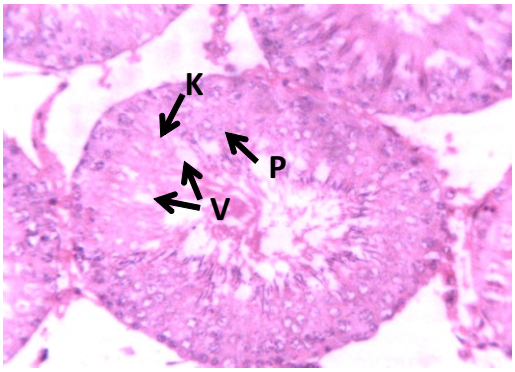

Plate V: A section of the testes in Group 5 (300mg/kg MFPD + 30mg/kgleadacetate) showing karyolysis (k), pyknotic nuclei (p) and numerous vacuolated cells (v) (Haematoxylin and Eosin x250)

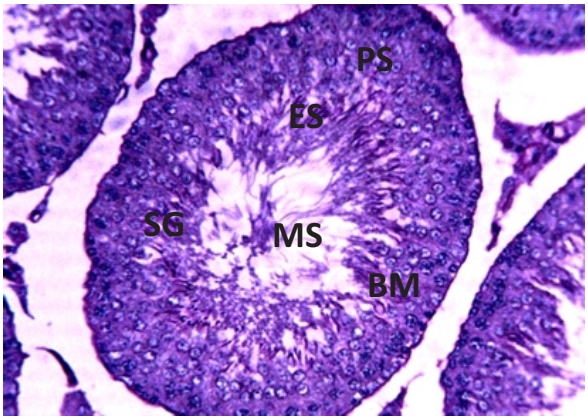

Plate VI: A section of the testes in Group 1 (2 ml Distilled water) showing normal histology and intact basement membrane (BM), MS: Mature sperms, ES: Elongating spermatids, SG: Spermatogonia, PS: Pachytene spermatocyte (PAS stain x250)

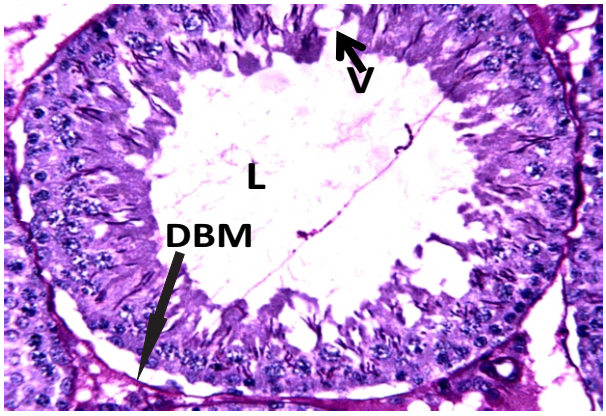

Plate VII: A section of the testes in Group 2 (30 mg/kg Lead acetate) showing vacuolation (v), an almost empty lumen (L), with a noticeable detached basement membrane (DBM) (PAS stain x250)

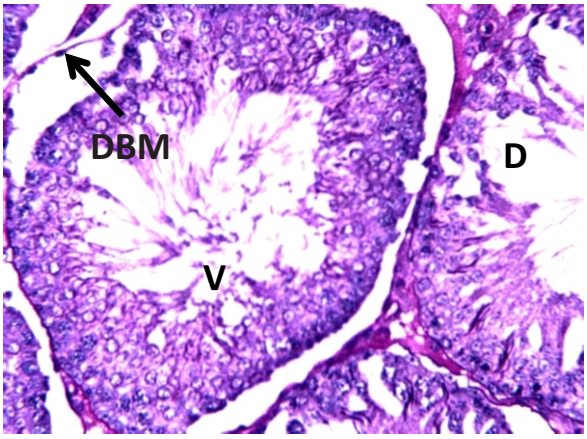

Plate VIII: A section of the testes in Group 3 (75 mg/kg MFPD + 30 mg/kg Lead acetate) showing vacuolation (v), degeneration of seminiferous tubule (D) and a detached basement membrane (DBM) (PAS stain x250)

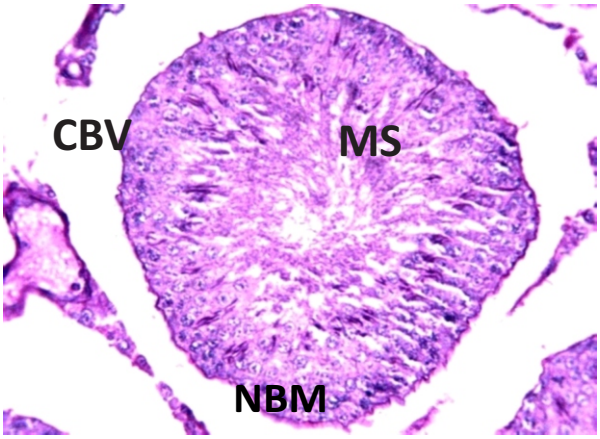

Plate IX: A section of the testes in Group 4 (150 mg/kg MFPD + 30 mg/kg Lead acetate) showing normal basement membrane (NBM), mature sperms (MS), congested blood vessel (CBV) (PAS stain x250)

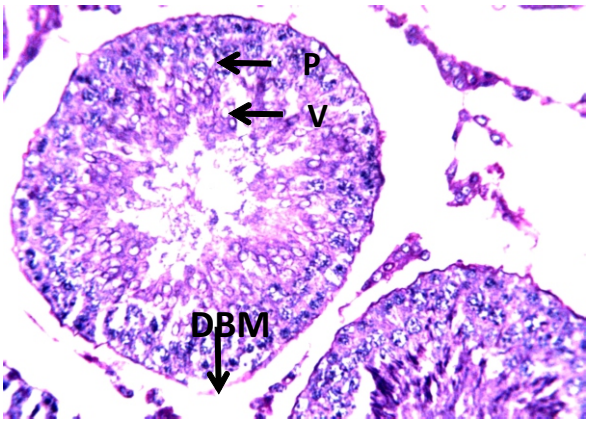

Plate X: A section of the testes in Group 5 (300 mg/kg of MFPD + 30 mg/kg lead acetate) showing numerous vacuolated cells (v), pyknosis (p) and a detached basement membrane (DBM) (PAS stain x250)

Table 1: Microscopic Morphometry of Wistar Rats (μm)

Groups	Doses	STD	GEH
1	DW (2 ml/kg)	872.20±2.44	186.27±4.09
2	LA (30 mg/kg)	725.20±16.79[1, 3, 4]	160.93±14.00[1, 4]
3	75 mg/kg MFPD + LA (30 mg/kg)	797.00±18.70[1, 2]	174.79±5.47
4	150 mg/kg MFPD + LA (30 mg/kg)	852.60±21.94[5]	188.91±6.69[2, 5]
5	300 mg/kg MFPD + LA (30 mg/kg)	765.80±34.58[1]	160.11±3.18 [1]

n = 5; Mean ± SEM; $p < 0.05$; DW: Distilled water, LA: Lead acetate, MFPD: Methanolic fruit extract of *Pheonix dactilyfera,* STD: Seminiferous tubule diameter, GEH: Germinal epithelium height
Superscript letters refer to the groups which are statistically significant to that particular group of interest (where $p < 0.05$).

DISCUSSION

The testicular histopathology evaluation in this research showed that lead acetate can alter the structure and integrity of the seminiferous tubule. Groups II through V which received 30 mg/kg of lead acetate experienced several cyto-architectural distortions in varying degrees with Groups II and V having the most of these distortions. These changes include degenerations of the epithelium, low population of spermatids and spermatozoa in the lumen, karryohexis (degeneration of the nucleus), pyknosis (shrinking of the nuclear materials), vacuolations, karyolysis (complete dissolution of the nuclear material) and detached basement membrane (except in Group 4).

This work is in consistence with previous studies who reported histo-architectural distortion as a result of lead administration in animal models[20, 21, 22]. Oxidative stress which plays an important role in lead poisoning is caused by imbalance in the production of free radicals and compromising the system's ability to readily detoxify the body of those radials resulted in cellular damage[23]. Nearly all cellular components including lipid membranes are affected by the generation of free radicals [24, 25, 26]. Also, a reduced glutathione (GSH) dependent dehydroascorbate reductase, which is abundant in the testes maintains the level of Vitamin C in a reduced state[27]. With the ability of lead to suppress the activity of GSH, deficiency of vitamins C thereby occurs in the testis which disrupts the spermatogenesis process [28]. Group 4 which received date palm fruit extract 150 mg/kg + 30 mg/kg Lead acetate had the most protective effects as it had considerably less degenerative changes when compared to the control and other treated groups. It also had an intact basement membrane and germinal epithelium as close to that of the control group.

The microscopic measurements obtained in this research show that lead acetate significantly affect the seminiferous tubular diameter and germinal epithelium height when compared to the control and other treated groups. This is in line with a research conducted in 2013[29] which recorded similar decreases in these parameters and such decreases were restored when treated with *Fumaria parviflora*. On the other hand, date palm especially at the dose of 150 mg/kg was able to maintain the tubular diameter and epithelium height as close to that of the control group as possible. This could be attributed to the presence of phenolic compounds. The antioxidant activity of phenolic compounds is a result of their redox properties, which can play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides [30].This is in line with a research [31] which also noted restoration of the epithelium height after cadmium induced testicular toxicity.
Therefore, at this dose (150 mg/kg), date palm offered more layers of protection against lead acetate intoxication than any other dosages administered in this research.

CONCLUSION

From this study, it can be concluded that at a dose of 150 mg/kg, date palm proved to be most protective against lead acetate induced damage on the testes. Lead acetate induced damage was observed by various cellular necrotic changes (karyolysis, karryohexis and pyknosis) and loss of the integrity of the basement membrane. From the observations in the present study, it can also be concluded that interactions between higher doses of the methanolic date palm fruit extract and lead acetate may in fact result in more injuries than expected to the testicular tissue as seen in the highest dose (300 mg/kg MFPD + 30 mg/kg Lead acetate) of this experiment.

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REFERENCES

1. Stephen, S.F. Male infertility overview (Assessment, diagnosis and treatment), Department of Surgery, University of North Carolina School of Medicine; 2007.
2. Basha, R. Reddy, G.R. Developmental exposure to lead and late life abnormalities of nervous system. *Indian Journal of Experimental Biology* 2010. 48: 636–641.
3. Barbosa, JR, F., Tanus-Santos, J.E., Gerlach, R.F. Parsons, P.J. "A Critical Review of Biomarkers Used for Monitoring Human Exposure to Lead: Advantages, Limitations, and Future Needs". *Environmental health perspectives.* 2005; 113(12): 1669–1674.
4. Dart, R.C., Hurlbut, K.M. Boyer-Hassen, L.V. "Lead". In Dart, RC. Medical Toxicology (3rd ed.). Lippincott Williams & Wilkins. 2004; ISBN 0-7817-2845-2.
5. Grant, L.D. "Lead and compounds". In Lippmann, M. Environmental Toxicants: Human Exposures and Their Health Effects (3rd ed.). Wiley-Interscience. 2009.
6. Landrigan, P.J., Schechter, C.B., Lipton, J.M., Fahs, M.C. Schwartz, J. "Environmental pollutants and disease in American children". *Environmental health perspectives.* 2002; 110(7): 721–728.
7. Golub, M.S. "Summary". Metals, fertility, and reproductive toxicity. Boca Raton, Fla.: Taylor and Francis. 2005. ed. p. 153.
8. Moore, K.L. Dalley, A.F. Abdomen, Pelvis and Perineum. *Clinically oriented anatomy.* 5th ed. Lippincott Williams and Wilkins. 2006.
9. Chaurasia, B.D. Testes in Pelvis and Perineum. Human anatomy regional and applied. 4th ed. Satish Kumar Jair for CBS publishers. 2004; vol 20.
10. Krentz, A.J. & Bailey, C.J. Oral antidiabetic agents: current role in type 2 diabetes mellitus. *Drugs*, 2005; 65: 385 - 411.
11. Bahmanpour, S., Talaei, T., Vojdani, Z., Panjehshahin, M.R., Poostpasand, L.A., Zareei, S. Ghaeminia, M. Effect of Phoenix dactylifera pollen on sperm parameters and reproductive system of adult male rats. *International Journal of Molecular Sciences.* 2006; 31(4): 208-211.
12. Ahmad, A., Soni, D.S., Singh, K.V. Maurya, K.S. *Pheonix dactilyfera* Linn: A review, Department of Dravyaguna, Faculty of Ayurverda, Banaras Hindu University, Varanasi India. 2013.
13. Al-Farsi, M. Lee, C. Nutritional and Functional Properties of Dates: A Review. *Critical reviews in food science and nutrition.* 2008. 48: 877-887.
14. Allaith, A.A. In vitro Evaluation of Antioxidant Activity of Different Extracts of *Phoenix dactylifera* L. fruits as functional foods. *Deutsche LebensmittelRundschau.* 2005; 101: 305-308.
15. Baliga, M.S., Baliga, B.R.V., Kandathil, S.M., Bhat, H.P. Vayalil, P.K. A review of the chemistry and pharmacology of the date fruits (Phoenix dactylifera L.). *Food Research International* 2011, 44: 1812–1822.
16. Elgasim, E.A., Alyousif, Y.A. & Homeida, A.M.): Possible hormonal activity of date pits and flesh fed to meat animals. *Food Chemistry*, 1995; 52: 149–150.
17. Agbon, A.N., Kwanashie, H.O. Hamman, W.O. Antidiarrheal activity of aqueous fruit extract of Phoenix dactylifera L. (date palm) in Wistar rats. *British Journal of Pharmacology and Toxicology*. 2013. 4(3): 121- 127
18. Sujatha, K., Srilatha, C., Anjanyulu, Y., Amarawathi, P.. "Lead acetate induced neurotoxicity in Wistar albino rats; A pathological, immunological, and ultrastructural studies;" *Journal of Pharmacology and bioscience.* 2011; 2: 459-462.
19. Okwuosa, N.C., Theophilus, K.U., Joshua, E.U., Augustine, C.O., Innocent, C.A. Romanus, E.M. Hepatoprotective effects of Methanolic fruit extracts of Pheonix dactilyfera on Thioacetamide induced Liver Damage in rats. *American Journal of Phytomedicine and Clinical Therapeutics*. 2014; 2(3): 290-300.
20. Hamadouche, A., Slimani, M., Merad-Boudia, B. Zaoui, C. Reproductive toxicity of lead acetate in adult male rats. *American Journal of Scientific Research*. 2009; 3: 38-50.
21. El-Shafai, A., Zohdy, N., El-Mulla, K.H., Hassan, M. Morad, N. Light and electron microscopic study of the toxic effect of prolonged lead exposure on the seminiferous tubules of albino rats and the possible protective effect of ascorbic acid. *Food and Chemical Toxicology.* 2011.49: 734-743.
22. Jegede, A.I., Offor, U., Azu, O. Akinloye, O. Red Palm Oil Attenuates Lead Acetate Induced Testicular Damage in Adult Male Sprague-Dawley Rats. *Evidence- Based Complementary and Alternative Medicine*. Hindawi Publishing Corporation, 2015; Article ID 130261, 7 pp.

23. Flora, S.J. Arsenic-induced oxidative stress and its reversibility. *Free Radical Biology and Medicine*. 2011; 51(2): 257-281.
24. Agarwal, A. Saleh, R.A. Role of oxidants in male infertility: rationale, significance, and treatment. *Urology Clinic of North America.* 2002.29: 817–827.
25. Marchlewicz, M., Wiszniewska, B., Gonet, B., Baranowska-Bosiacka, I., Safranow, K., Kolasa, A., Glabowski, W., Kurzawa, R., Jakubowska, K. Rac, M.E. Increased lipid peroxidation and ascorbic acid utilization in testis and epididymis of rats chronically exposed to lead. *BioMetals.* 2007; 20: 13-19.
26. Ademuyiwa, O., Agarwal, R., Chandra, R. Behari, J.R. Lead induced phospholipidosis and cholesterogenesis in rat tissues. *Chemico-Biological Interactions.* 2009; 179: 314–320.
27. Paolicchi, A., Pezzini, A., Saviozzi, M., Piaggi, S., Andreuccetti, M. Chieli, E. Ocalization of a GSH-dependent dehydroascorbatereductase in rat tissues and subcellular fractions. *Archives of Biochemistry and Biophysics.* 1996; 333: 489–95.
28. Johnson, F.C. The antioxidant vitamins. CRC. *Critical Reviews in Food Science and Nutrition.* 1979; 1: 217–309.
29. Dorostghoal, M., Seyyednejad, S.M. Jabari, A. Protective effects of Fumaria parviflora L. on lead-induced testicular toxicity in male rats. *Andrologia.* 2013; pg: 1-10.
30. Shahidi, F., Janitha, P.K. Wanasundara, P.D. Phenolic antioxidants. Critical Reviews of *Food Science & Nutrition.* 1992; 32(1): 67–103.
31. El-Neweshy, M.S., El-Maddawy, Z.K. El-Sayed, Y.S. Therapeutic effects of date palm (Phoenix dactylifera L.) pollen extract on cadmium-induced testicular toxicity. *Andrologia.* 2012. 45: 369-378.