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Melatonin and *Phoenix dactylifera modulates* Bisphenol A induced antioxidant and androgen toxicity in Male Wistar Rats

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ABSTRACT

Bisphenol A (BFA), which is one of the most widely used industrial chemicals in the production of plastic has been implicated with male infertility and achieved this through increased in reactive oxygen species and oxidative stress. Melatonin and *phoenix dactylifera* (date palm) have been shown to have many beneficial medicinal properties, including antioxidant effects. The aim of the study to was evaluate the effects of melatonin and *Phoenix dactylifera* on antioxidant enzyme and hormones (androgens) associated with bisphenol-A induced testicular toxicity in male Wistar rats. Twenty-five (25) adults' male Wistar rats with a weight range of 120–150 g were randomly divided into five groups of five rats each. Group 1 received distilled water, orally, Group 2 received BFA only; 4 mg/kg orally, Group 3 received BFA; 4 mg/kg plus melatonin; 10mg/kg, orally, Group 4 received BFA; 4mg/kg plus Phoenix dactilyfera; 500mg/kg, orally and Group 5 received BFA; 4mg/kg plus melatonin; 10mg/kg and Phoenix dactilyfera; 500mg/kg, orally. The administration was done for 14 days. The animals were humanely sacrificed using chloroform 24 hours after last administration. Blood samples were taken by cardiac puncture for hormonal assay and biochemical analysis. The data were presented as mean ± SEM and analyzed by ANOVA using the SPSS package version 25 software. Differences were considered significant at $p<0.05$. Bisphenol A administration caused a significant increase in the testosterone and follicle-stimulating hormones and increased MDA levels with a corresponding decrease in SOD and MDA levels. However, administration of melatonin and *phoenix dactylifera* showed significant attenuation of adrogenous hormones and intracellular antioxidant enzymes. Melatonin and *Phoenix dactylifera* showed powerful modulating effects against environmental pollutant Bisphenol A induced infertility in Male Wistar rats.

KEYWORDS: Bisphenol A (BFA), melatonin, Phoenix dactylifera (date palm), Wistar rats, superoxide dismutase (SOD), malondialdehyde (MDA), glutathione peroxidase (GPX), testosterone, Follicle stimulating hormone., *spermatocytes and toxicity.*

INTRODUCTION

Infertility is a global health issue affecting millions of people of reproductive age worldwide[1]**.** Available data indicates that 48 million couples and 186 million persons are subjects of infertility globally[2]. Infertility is a disease of the reproductive system defined by the lack of ability of a non-contraceptive couple to conceive after more than 12 months of regular unprotected sexual intercourse. Primary infertility is the inability to have any pregnancy, while secondary infertility is the inability to have a pregnancy after a successful conception[1]. Infertility may occur due to many factors including male factors, female factors, or a combination of both or unexplained causes[3]. Males and females apart other causes like, environmental and lifestyle factors such as smoking, excessive alcohol intake, obesity, exposure to environmental pollutants and industrial chemicals like Bisphenol A (BPA) which is an endocrine-disrupting chemical, have been associated with low fertility[4].

Bisphenol A (BFA) is one of the most widely used industrial chemicals in the production of plastic materials[5]; prolonged exposure to BFA is said to have harmful effects on the male reproductive system due to increased reactive oxygen species level and oxidative stress (OS)[6]. Several research groups and pharmacological approaches in the past years have focused their investigations on preventing and reverting male infertility[7].

Melatonin is an endogenous hormone derived from tryptophan, which was first found in the vertebrate pineal gland[8]**.** It regulates many critical biological functions, including sleep, circadian rhythm, reproduction, immunity, and oncostatin processes [9,10,11]. Melatonin exerts an antioxidant effect on organs and an anti-apoptotic effect on cells[12] and can quickly move across the cell membrane blood–brain barrier, protecting various biomolecules by detoxifying Reactive oxygen species (ROS) and reactive nitrogen species (RNS) [13]. It enhances the antioxidant defense system by increasing the expression of antioxidant enzymes [14]. The role of melatonin in reproduction in many species is related to seasonal reproductive cycles. In man, it has been demonstrated that changes in the pineal gland's melatonin secretion can modulate the reproductive neuroendocrine axis [15]**.**

Phoenix dactylifera (Date palm) fruit is one of the plants suggested in traditional medicine to improve fertility potential. Date palm fruits were traditionally claimed to be aphrodisiacs and fertility enhancers and have been used in the Middle East as a natural medicine for the treatment of male infertility and promoting fertility in women [16]. The Iranian traditional medicine reveals that date palm has refreshing and nutritional value and is beneficial for treating infertility in both males and females. However, it is widely used to treat male infertility [17]. Date palm fruit extract (DPFE) has been shown to contain a high concentration of antioxidant compounds [18] and may be a suitable and cost-effective alternative to conventional drugs for the protection of fertility potential [19]. BPA widespread presence in several daily-used products and its detection in several human tissues and body fluids have raised many concerns about its potential association with human disorders such as cancer, cardiovascular diseases, obesity, diabetes, and reproductive disorders [20,21]**.** Although BPA may be toxic to other organs, attention has been paid to its reproductive and endocrine-disrupting effects [22,23,6]**.** Therefore, the study aimed at evaluating the effects of melatonin and *Phoenix dactylifera* (Date palm) on hormone and antioxidant enzymes associated with bisphenol A induced testicular and androgen toxicity in Male Wistar rats.

MATERIALS AND METHODS

The following materials were used in the study; Plastic Cages, Spectrophotometer auto analyzer, blood sample containers, Centrifuge, Temperature controlled refrigerator, Microwave oven, humidity chamber, , MRC spectrophotometer.

Bioactive compounds and drugs used in this study were: Melatonin M5250-1G (Sigma Aldrich, USA), Bisphenol A (Sigma Aldrich, USA), Haematoxylin and Eosin Stain (H&E), and Periodic Acid Schiff stain were used in the study.

Source of Animal and Management: Twenty-five male Wistar rats weighing 120-150g, were obtained from the Animal Housing Facility, Faculty of Basic Medical Sciences College of Medical Sciences Rivers State University Port Harcourt, Nigeria. They were acclimatized for two weeks before starting the experiment. During this period, the rats were housed in metal cages at room temperature, maintained under a 12-hour light/dark cycle, and had free access to standard rat chow and water until the end of the experiment. All rats were handled according to the standard guide for the care and use of laboratory animals.

Acquisition and extraction of *Phoenix dactilyfera* fruits: Fresh fruits of Phoenix dactilyfera (date palm) was purchased from Mile Three (3) market, Port-Harcourt. The fruits were washed, air dried, minced and powdered using laboratory mortar. 1000g of the powdered leaves was extracted in 1.5 liters of 80% ethanol using a soxhlet extractor. These were further filtered using a Whatman filter paper (24mm). The filtrate was dried in a laboratory water bath set at 68°C and total yield of (55.8g) was obtained per 1000g of the powdered fruits.

Animal Grouping and treatment procedure: Twenty-five rats were randomly divided into five groups, as shown in table 1.0 below:

Table 1: Animal grouping and treatment protocol

GROUPS	TREATMENT
1	Control group (distilled water, orally for 14 days).
2	Bisphenol A group (BFA; 4 mg/kg orally for 14 days).
3	Treatment group I BFA; 4 mg/kg + Melatonin; 10 mg/kg, orally for 14 days.
4	Treatment group II (BFA; 4mg/kg + Date palm; 500mg/kg, orally for 14 days).
5	Treatment group III (BFA; 4mg/kg + Melatonin; 10mg/kg + Date palm; 500mg/kg, orally for 14 days).

N= five rats per group, the treatment duration is once daily for 14 days.

Sample collection: The administration was by gavage using oral metal cannulas. The body weights of the rats were measured just before administration, after seven days of administration, and after 14 days. The rats were sacrificed after 14 days of administration. At the end of the experiment, the animals were humanely sacrificed using chloroform, 24 hours after the last administration. Blood samples were taken by cardiac puncture from all groups for hormonal assay (androgens) and biochemical assay (antioxidants/enzymes).

Estimation of serum testosterone and follicle stimulating hormone: The serum samples obtained were analyzed to determine the concentration of testosterone and follicle stimulating hormone. Analysis was carried out via the tube-based enzyme immunosorbent assay (EIA). The protocol used in hormone testing followed the method described by the kit manufacturers (Immunometric Limited UK) and met the WHO research program standards for reproductive studies.

Estimation of antioxidant enzymes (Malondialdehyde (MDA), Superoxide Dismutase (SOD) and Glutathione peroxidase (GPx): The enzyme activity of glutathione peroxidase (GPX), super peroxide dismutase (SOD), and serum level of malondialdehyde (MDA) was assayed using spectrophotometry respectively. The protocol used in biochemical/enzyme assay followed the method described by the kit manufacturers (Immunometric Limited UK) and met the WHO research program standards for reproductive studies.

Statistical analysis: The data were presented as mean values ± SEM. ANOVA was carried out on the Statistical Package for the Social Sciences (SPSS version 17), and we checked for significant differences between the results. Differences were considered significant at $p<0.05$.

RESULTS

Effect of melatonin and *Phoenix dactylifera* on testosterone and follicle stimulating hormone serum levels of bisphenol-induced toxicity in Wistar rats: The results obtained in table 2 showed that serum testosterone and follicle-stimulating hormone levels were significantly increased ($p<0.05$) with treatment with Bisphenol. At the same time, bisphenol treatment with melatonin and date palm extract treatment showed a significant reduction in ($p<0.05$) the testosterone and follicle-stimulating hormone. Co-administration of Bisphenol treatment with melatonin and date palm extract showed a normalized level of testosterone and follicle-stimulating hormone (figure 1, 2).

Table 2: Effect of Melatonin and *Phoenix dactylifera* on Testosterone and Follicle Stimulating Hormone Serum Levels of Bisphenol induced Toxicity in Wistar Rats.

GROUP	TET (MIU/ML)	FSH (mIU/ML)
1	1.26 ± 0.03	0.24 ± 0.02
2	1.69 ± 0.34*	1.35 ± 0.21*
3	1.44 ± 0.26	0.82 ± 0.17
4	1.45 ± 0.04	0.55 ± 0.12
5	1.29 ± 0.06	0.42 ± 0.06

Values expressed as mean ± SEM; n = 5
*Denote significant variation ($p<0.05$) from control.

Effect of Melatonin and *Phoenix dactylifera* on the Serum Lipid Peroxidation Biomarker (Malondialdehyde) Levels of Bisphenol induced Toxicity in Wistar rats: The result obtained indicate that serum level of Malondialdehyde was significantly increased (P<0.05) upon administration of bisphenol (table 3). While treatment with melatonin and date palm extract showed a slight increase but not significantly different (P<0.05) from the control group (figure 3).

The effect of Melatonin and *Phoenix dactylifera* on the Serum antioxidant enzyme Levels of Bisphenol induced Toxicity in Wistar rats: The result showed that serum levels of GPX and SOD significantly decreased (P>0.05) upon administration of Bisphenol (table 3).

In comparison, treatment with melatonin and date palm showed an increase in serum GPX significantly different from (P<0.05) in the bisphenol group. Co-administrative therapy with melatonin and date palm extract caused an increase in GPX levels quite different (P<0.05) from the control group (figure 4). Treatment with melatonin and date palm extract showed an increase in the serum level of SOD, which is not significantly different (P<0.05) from the control group. Co-administrative treatment with melatonin and date palm extract showed an increase in SOD levels insignificantly different

(P<0.05) from the control group (figure 5).

Table 3: Mean Serum GPX, SOD and MDA Levels of Wistar Rats Treated with Bisphenol A, Melatonin and *Phoenix dactylifera*

GROUP	GPX (U/L)	SOD (U/ML)	MDA (UMOL/L)
1	0.64 ± 0.01	0.35 ± 0.03	0.51 ± 0.06
2	0.38 ± 0.01*	0.24 ± 0.05*	0.91 ± 0.07*
3	0.52 ± 0.08	0.32 ± 0.06	0.57 ± 0.03
4	0.61 ± 0.01	0.33 ± 0.08	0.48 ± 0.04
5	0.72 ± 0.00	0.37 ± 0.05	0.45 ± 0.05

Values expressed as mean ± SEM; n = 5; for free radicals and antioxidants of Wistar rats.
*Denote significant variation ($p<0.05$) from control.

DISCUSSION

The findings showed that the serum levels of testosterone and follicle-stimulating hormone (FSH) increased significantly in the group treated with bisphenol A compared to the control group. This finding disagrees with [24,26]. They further reported that BFA caused a reduction in testosterone and follicle-stimulating hormone, which contributed to the defects in spermatogenesis and sperm maturation. A decrease in serum testosterone levels has also been reported following the exposure of adult male Wistar rats to environmentally relevant doses of BFA in the studies by [25]. They stated that BFA induced modulations in Leydig's steroidogenetic functions, thereby inhibiting testosterone production. However, there was significant elevation in FSH levels following treatment with bisphenol A in this study**.**

They clearly showed that BFA exhibited anti-androgenic actions in the treated rats. Significant over secretion of testosterone and follicle stimulating hormones has been associated with testicular damage and spermatogenesis abnormalities [27]. The normalization of serum testosterone and follicle stimulating hormone levels following administration of melatonin is consistent with the findings of [28,29]**,** they further explained that melatonin regulates many neuroendocrine functions and inhibits testosterone, follicle-stimulating hormone secretion by acting on the hypothalamo-pituitary secretion axis. There is a functional relationship and regulation between the pineal gland and the testes. Melatonin has also been shown to normalise testosterone levels of Wistar rats following bisphenol A induced toxicity in the study conducted by [30]. Testosterone plays a key role in the process of spermatocyte meiosis, it is suggested that modulation of testosterone level by melatonin protects the meiosis of spermatocytes and the production of normal sperm quality, and this effect might be attributed to its capacity to control cellular redox state [30,31]. Date palm administration significantly improved the testosterone serum levels compared to the bisphenol A treated group. This corresponds with the studies by [32] who revealed that the steroids and flavonoid content of the extract might be the cause of this normalisation, as steroids are reported to decrease testosterone levels. Flavonoids are also reported to decrease plasma testosterone levels in Wistar rats [33,34] This also indicates that the extract could affect spermatogenesis as testosterone is necessary for the normal development of spermatogenic cells [35]**.**

The results obtained from the enzyme assay showed a significant increase in Malondialdehyde (MDA) activities causing a reduction in antioxidant enzymes such as Superoxide dismutase (SOD) and Glutathione peroxidase (GPX) in the Bisphenol A group showed increase in lipid peroxidation and reactive oxygen species (ROS), which leads to oxidative stress in testicular tissue. These clarifications are in accordance with the studies reported by [7,26,36]. It could be inferred that SOD converted the superoxide anion radicals into hydrogen peroxide, which accumulated in the testes due to its reduced elimination. Elevated testicular MDA levels have been suggested to be responsible for the pathologic lipid peroxidation of the spermatozoa membrane and the reduction of sperm motility [26]**.**

The preventive antioxidant-promoting effects of Melatonin were reflected in the treatment groups, which maintained the antioxidant enzyme activities, thus lessening BPA-induced oxidative stress in the testicular tissue of Wistar rats. This finding tallies with that of [37,38] as melatonin successfully enhanced the antioxidant defence system by increasing the expression of antioxidant enzymes, which prevented the deterioration of cellular membranes and reduced mitochondria lipid peroxidation in the rats treated with bisphenol A.

Antioxidant enzyme activities were also maintained in the treatment groups after the administration of date palm. This agrees

with the report by [39]. Date fruit extract was found to significantly inhibit lipid peroxidation and protein oxidation and exhibit potent superoxide and hydroxyl radical scavenging activity [39]. This present study confirmed the free radical scavenging activity of date palms and suggested their usefulness in solving male fertility problems.

CONCLUSION

Melatonin and *Phoenix dactylifera* have shown powerful modulating effects against environmental pollutant Bisphenol A induced infertility in Male Wistar rats.

REFERENCES

1. World Health Organization. WHO? International Classification of Diseases, 11th Revision (ICD- 11) Geneva. 2018.
2. Mascarenhas M.N, Flaxman S.R, Boerma T, Vanderpoel S, Stevens GA. National, Regional, and Global Trends in Infertility Prevalence Since 1990: A Systematic Analysis of 277 Health Surveys. PLoS Med., 2012; 9(12): e1001356. doi: 10.1371/journal.pmed.1001356.
3. Chowdhury, S.H, Cozma A.L, Chowdhury J.H. Infertility, Essentials for the Canadian Medical Licensing Exam: Review and prep for Medical Council of Canada Qualifying Exam Part 1. 2nd edition. Wolters Kluwer. Hong kong. 2017.
4. Kumar, M, Sarma DK, Shubham S, Kumawat M, Verma V, Prakash A and Tiwari R. Environmental Endocrine-Disrupting Chemical Exposure: Role in Non-Communicable Diseases. Front. Public Health. 2020; 8:553850. doi: 10.3389/fpubh.2020.553850.
5. Tomza, Marciniak, A.; Ste˛pkowska, P.; Kuba, J.; Pilarczyk, B. Effect of bisphenol A on reproductive processes: A review of in vitro and epidemiological studies. *Journal Applied Toxicology.* 2018, *38*: 51–80.
6. Radwan, M.; Wielgomas, B.; Dziewirska, E.; Radwan, P.; Kałuzny, P.; Klimowska, A.; Hanke, W.; Jurewicz, J. Urinary Bisphenol A˙ Levels and Male Fertility. *American Journal of Men's Health.* 2018; *12*: 2144–2151.
7. Santiago, J.; Silva, J.V.; Santos, M.A.S.; Fardilha, M. Fighting Bisphenol A-Induced Male Infertility: The power of Antioxidants. Antioxidants. 2021; 10: 289.
8. Auld, F, Maschauer E.L, Morrison I, Skene D.J, Riha R.L. August. "Evidence for the efficacy of melatonin in the treatment of primary adult sleep disorders" (PDF). Sleep Medicine Reviews. 2017; 34: 10–22. Doi: 10.1016/j.smrv.2016.06.005.hdl:20.500.11820/0e890bda-4b1d-4786-a907-a03b1580fd07. PMID 28648359.
9. Guerrero, J.; Reiter, R.J. Melatonin-immune system relationships. Current Topics in Medicinal Chemistry. 2002; 2: 167–179.
10. Hardeland, R, Tan D.X, Reiter R.J (2009). Kynuramines, metabolites of melatonin and other indoles: The resurrection of an almost forgotten class of biogenic amines. Journal of Pineal Research 47: 109–126. DOI 10.1111/j.1600-079X.2009.00701x
11. Voiculescu, S.E, Zygouropoulos N, Zahiu CD, Zagrean AM. Role of melatonin in embryo fetal development. Journal of Medicine and Life. 2014; 7: 488–492.
12. Aysun Hacışevki and Burcu Baba. An Overview of Melatonin as an Antioxidant Molecule: A Biochemical Approach, Melatonin - Molecular Biology, Clinical and Pharmaceutical Approaches, Cristina Manuela Drăgoi and Alina Crenguţa Nicolae, IntechOpen, 2018. DOI: 10.5772/intechopen.79421. Available from: https://www.intechopen.com/chapters/62672.
13. Kratz, E.M.; Piwowar, A. Melatonin, advanced oxidation protein products

and total antioxidant capacity as seminal parameters of prooxidant-antioxidant balance and their connection with expression of metalloproteinases in context of male fertility. Journal of Physiology and Pharmacology. 2017; 68: 659–668.

14. Loren, P.; Sánchez, R.; Arias, M.-E.; Felmer, R.; Risopatron, J.; Cheuquemán, C. Melatonin Scavenger Properties against Oxidative and Nitrosative Stress: Impact on Gamete Handling and in Vitro Embryo Production in Humans and Other Mammals. International Journal of Molecular Science. 2017; 18: 1119.
15. Anderson, R.A, Lincoln G.A, Wu, F.C.W. Endocrinology: Melatonin potentiates testosterone-induced suppression of luteinizing hormone secretion in normal men, Human Reproduction. 1993; 8: 1819–1822, https://doi.org/10.1093/oxford*journals of human reproduction.* a137940.
16. Arias-Jime´nez, E J. Date Palm Cultivation. Rome, Italy: Food and Agriculture Organization of the United Nations. 2002. https://www.fao.org/3/Y4360E/y4360e00.htm
17. Zargari, A. Medical Plants. Tehran, Iran: University of Tehran. 1999; 3: 33-40.
18. Akunna, G, Saalu C, Ogunmodede O, Ogunlade B, Bello A. Aqueous Extract of Date Fruit (*Phoenix Dactylifera*) Protects Testis against Atrazine-induced Toxicity in Rat. *World Journal of Pharmaceutical and Life Sciences*. 2012; 2: 100.
19. Taouda, H, Chabir R, Errachidi F, Aarab L. Comparison of antioxidant activities and phenolic content of Moroccandate fruits. International Journal of Innovative Research in Science, Engineering and Technology. 2014; 3: 16120–16126.
20. Balabanic, D., Rupnik, M.; Klemencˇicˇ, A.K. Negative impact of endocrine-disrupting compounds on human reproductive health. *Reproduction, Fertility and Development.* 2011; *23*: 403–416.
21. Rochester, J.R. Bisphenol A and human health: A review of the literature. *Reproductive Toxicology.* 2013; *42*: 132–155.
22. De Toni, L.; Ponce, M.D.R.; Petre, G.C.; Rtibi, K.; Di Nisio, A.; Foresta, C. (2020). Bisphenols and Male Reproductive Health: From Toxicological Models to Therapeutic Hypotheses. *Frontiers in. Endocrinology. 11*, 1–9.
23. Cariati, F., D'Uonno, N., Borrillo, F. "Bisphenol a: an emerging threat to male fertility". *Reproductive Biology and Endocrinology*. 2019; 17: 6. https://doi.org/10.1186/s12958-018-0447-6.
24. Wisniewski, P.; Romano, R.M.; Kizys, M.M.; Oliveira, K.C.; Kasamatsu, T.; Giannocco, G.; Chiamolera, M.I.; Dias-Da-Silva, M.R.; Romano, M.A. Adult exposure to bisphenol A (BPA) in Wistar rats reduces sperm quality with disruption of the hypothalamic–pituitary–testicular axis. *Toxicology.* 2015; *329*, 1–9.
25. Dhanabalan, S, Mathur P.P, Latha P. Tetrachlorodibenzodioxin (TCDD) and corticosterone on testicular steroidogenesis and antioxidant system of epididymal sperm in rats. Toxicology and Industrial Health. 2013; 31,811-822.
26. Olukole, Samuel Gbadebo, Olo-Davies, Eunice Olufunke, Lanipekun, Damilare Olaniyi and Oke, Bankole Olusiji. "Chronic exposure of adult male wistar rats to bisphenol A causes testicular oxidative stress: Role of gallic acid" Endocrine Regulations, 2020; 54(1): 14-21. https://doi.org/10.2478/enr-2020-0003.
27. Leslie, S.W, Siref L.E, Soon-Sutton T. L. (2021) August 12. Male Infertility.https://www.ncbi.nlm.nih.gov/books/NBK562258/
28. Hanan, Farouk Aly and Maha Zaki Rizk. "Melatonin and its indisputable

effects on health state". 2018; Doi: 10.5772/intechopen.79817.
29. Bayram Yilmaz, Selim Kutlu, Rasim Mogulkoc, Sinan Canpolat, Suleyman Sandal, Berrin Tarakci, Haluk Kelestimur. Neuro Endocrinology Letters., 2000; 21(4):301-306.
30. Othman, A.I.; Edrees, G.M.; El-Missiry, M.A.; Ali, D.A.; Aboel-Nour, M.; Dabdoub, B.R. Melatonin controlled apoptosis and protected the testes and sperm quality against bisphenol A-induced oxidative toxicity. *Toxicology and. Industrial Health.* 2016; *32*, 1537–1549
31. Jin, P, Wang X, Chang F. Low dose bisphenol a impairsspermatogenesis by supreesing reproductive hormone production and promoting germ cel apoptosis in adult rats. *Journal of Biomedical Research.* 2013; 27: 135-144.
32. Karila, T. A. M., Karjalainen, J. E., Mäntysaari, M. J., Viitasalo, M. T., & Seppälä, T. A. Anabolic androgenic steroids produce dose-dependent increase in left ventricular mass in power athletes, and this effect is potentiated by concomitant use of growth hormone. International journal of sports medicine, 2003; 24(05): 337-343.
33. Cline, M. J, Franke A.A, Register T.C. Effects of dietary Isoflavone Aglycones on the reproductive tract of male & female Mice. Toxicology and pathology, 2004; 32:91-99.
34. Weber, K.S, Setchell K.D, Stocco D.M. Dietary Soy-phytoestrogen decrease testosterone level & prostate weight without altering LH, Prostate 5-alpha reductase or testicular steroidogenic acute regulatory peptide in adult male Sprague-dawley rats. Journal of Endocrinology, 2001; 170:591-599.
35. Junqueiro, L.C, Caneiro J. Basic Histology: Text & Atlas 11thedition, McGraw-Hill Co Incorporated. 2005.
36. Kamel, A.H., Foaud, M.A. & Moussa, H.M. The adverse effects of bisphenol A on male albino rats. *Journal of Basic and Applied Zoology.* 2018; 79:6. https://doi.org/101186/s41936-018-0015-9.
37. Olukole, S.G.; Lanipekun, D.O.; Ola-Davies, E.O.; Oke, B.O. Maternal exposure to environmentally relevant doses of bisphenol A causes reproductive dysfunction in F1 adult male rats: Protective role of melatonin. *Environmental Science and Pollution Research.* 2019; *26*: 28940–28950.
38. El-Missiry, M.A.; Othman, A.I.; Al-Abdan, M.A.; El-Sayed, A.A. Melatonin ameliorates oxidative stress, modulates death receptor pathway proteins, and protects the rat cerebrum against bisphenol-A-induced apoptosis. *Journal of Neurological Science.* 2014; 347: 251–256.
39. Vyawahare, N, R Pujari, A Khsirsagar, D Ingawale, M Patil, V Kagathara. Ph*oenix dactylifera*: An update of its indegenous uses, phytochemistry and pharmacology. The Internet Journal of Pharmacology. 2008; 7(1)