The Professional Medical Journal
www.theprofesional.com

DOI: 10.29309/TPMJ/2019.26.12.4051

ORIGINAL PROF-0-4051

HISTOPROTECTIVE EFFECTS OF CURCUMIN ON BISPHENOL-A INDUCED TESTICULAR TOXICITY IN ADULT ALBINO RATS.

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Article received on:
16/08/2019
Accepted for publication:
15/10/2019

ABSTRACT: Endocrine Disrupting chemicals including bisphenols have contaminated the environment significantly and is known to damage spermatogenesis via increase in oxidative stress. The anti-oxidant properties of naturally occurring substances like curcumin have been well established. **Objectives:** To evaluate the protective effects of curcumin against bisphenol-A induced testicular damage. **Study Design:** Experimental Study. **Setting:** Department of Anatomy, University of Health Sciences, Lahore. **Period:** From March 2015 to December 2015. **Material & Methods:** Thirty six rats were divided into four groups A, B, C and D of 9 animals each. Group A (control) was given 5ml/kg/day of corn oil orally for 10 days. Group B (Bisphenol-A) was given 100mg/kg/day of Bisphenol-A dissolved in corn oil orally for 10 days. Group C (recovery) was given 100mg/kg/day of BPA dissolved in corn oil orally for 10 days and left untreated for 10 days. Group D (Curcumin + BPA) was given 100mg/kg/day of BPA and 100mg/kg/day of Curcumin, dissolved in corn oil orally for 10 days. Rats of group A, B and D were sacrificed on day 11 and those of group C on day 21. After weighing the euthanized rats, testes were removed, processed and tissue sections were stained with H&E for Johnson scoring and with PAS stain for assessment of basement membrane integrity. **Results:** Bisphenol-A administration caused a significant decrease in weight of animals, a significantly low Johnson score of seminiferous tubules and high frequency of disrupted basement membranes of the tubules in Group B as compared to control. The weight gain of animals improved in the with-drawl group C while no self-recovery was observed in other parameters. Curcumin co-administration improved the body weight gain of animals, increased the Johnson scoring of tubules significantly and partially restored the basement membrane integrity in group D, comparable to the control group. **Conclusion:** The results of this study indicate that co-administration of a potent antioxidant curcumin causes a significant antagonism of the histo-toxicity of testis produced by Bisphenol-A in albino rats.

Key words: Bisphenol-A, Basement Membrane, Curcumin, Johnson Score.

Article Citation: Toor RS, Irshad F, Asif S. Histoprotective effects of Curcumin on Bisphenol-A induced testicular toxicity in adult albino rats. Professional Med J 2019; 26(12):2206-2215. **DOI:** 10.29309/TPMJ/2019.26.12.4051

INTRODUCTION

Infertility affects 15% of couples worldwide with male attribution of 20-30% solely, females of 50% and 20-30% due to both sexes combined according to data collected from infertility clinics of developed countries.[1] An emerging group of pollutants called Endocrine disruptors (EDs) are now well known for being a significant cause of deterioration of reproductive health.[2]

Bisphenol-A (BPA), one of the xenoestrogenic EDs, is a synthetic organic substance produced in large amounts every year.[3] It is used in production of plastic goods and lining resin of food cans. Through these daily-use products, it is absorbed in human bodies via oral as well as cutaneous route.[4] Human exposure to BPA was quantified as significantly high by measuring urinary BPA concentration.[5] BPA was also found to be present in all environmental and personal media of pre-school children like food, toys etc.[6]

BPA is now known to bind to estrogen receptors (α and β) and mimic the actions of endogenous estrogen.[7] Animal based studies showed that BPA can also increase oxidative stress in various tissues and may damage vital organs like liver, kidneys and gonads.[8] Deteriorative changes in

weight, behavior, sexual maturation, mammary gland, testis and prostate have been reported in various studies emphasizing the hazardous effect of the pollutant on reproductive health.[9]

Most *in vivo* studies showed detrimental effects of BPA on spermatozoa especially during their developmental period via DNA damage[10], DNA methylation[11], increased oxidative stress[12] and increased ER (endoplasmic reticulum) stress.[13]

Curcumin, an active polyphenolic yellow-colored component of an ancient spice Turmeric, has been a part of aboriginal medicaments especially in South Asian countries.[14] Studies showed therapeutic effects of curcumin as anticancer, anti-inflammatory and antioxidant remedy.[15,16] As BPA has been associated with increased oxidative stress, employing the use of a potent antioxidant like curcumin may prove beneficial. The present study has, therefore, been designed to evaluate the protective effect of curcumin against BPA-induced reprotoxicity in male albino rats.

MATERIALS AND METHODS

Animals

The study was experimental and included thirty two rats of age 6 to 8 weeks and weight 180-200gm were procured from the Animal house of University of Health Sciences, Lahore. The initial body weight was noted while marking the animals with numbers and randomly dividing them in four groups of nine rats each. The animals were allowed to acclimatize for 2 days before conducting experiment. Free access to water and food was provided at room temperature ($24^{o}\pm5^{o}$) and normal conditions of humidity (45% ±5%). The dosage was administered at 10 am daily.

Group A (control): rats were given 5ml/kg/day of corn oil daily for 10 days by oral gavage.

Group B (BPA): rats were given 100mg/kg/day of BPA dissolved in corn oil daily for 10 days by oral gavage.

Group C (BPA with-drawl): rats were given 100mg/kg/day of BPA dissolved in corn oil daily for 10 days by oral gavage and left untreated for 10 days.

Group D (BPA & curcumin): rats were given 100mg/kg/day BPA and 100mg/kg/day of curcumin, each dissolved in corn oil daily for 10 days by oral gavage.

DISSECTION

At the end of experimental duration, the animals were weighed and then euthanized. The pair of testes of each rat was dissected out, weighed and fixed in Buoin's solution. Tissue processing was done in automated processor and 4μm thick sections were cut. H&E stained sections were examined to assess the Johnson score of tubules. PAS staining was done to examine the basement membranes of seminiferous tubules.

Statistical Analysis

The data was entered and analyzed using SPSS version 21. Quantitative parameters like body weights and Johnson scoring were analyzed using one way ANOVA or Kruskal Wallis test among groups and pair-wise comparison was done by using post-hoc Turkey and Mann Whitney U tests. The qualitative parameters like basement membrane integrity was analyzed using Fischer's Exact test.

Frequencies and percentages were given for the parameter of basement membrane and mean with standard deviation or standard error of means were given for the body weight and Johnson score. P-value $\geq$ 0.05 was considered statistically significant.

RESULTS

The rats were assessed for behavior, appetite and growth. Animals of group A were healthy with normal feed and behavior. Group B and C rats showed irritability and decreased appetite but in group C, the appetite of animals started improving after with-drawl of BPA. Group D rats showed irritability but due to normal appetite, they remained healthy throughout the experiment.

Body Weight (gm) of Rats at the Start and end of Experiment

Mean body weight (gm) at the beginning of

experiment was 180.22 ± 5.86, 185.22 ± 5.98, 182.00 ± 4.18 and 185.94 ± 9.20 among groups A, B, C and D respectively (Table-I and bar chart in Figure-1). There was no statistically significant difference among groups (p-value = 0.06)

Mean body weights (gm) of rats at 11th day of groups A, B, C and D were 198.71 ± 22.13, 177.78 ± 10.55, 191.5 ± 10.08 and 212.06 ± 08.68 respectively. One way ANOVA showed statistically significant difference in body weights of animals with p-value = 0.001 (Table-II and Figure-2).

Multiple comparison of mean final body weights of animals among groups was done by using post-hoc Tukey HSD test (Table-III). The statistical difference observed between group A and B was significant (p-value = 0.016), between group B and D was very highly significant (p-value = 0.001) and between group C and D was significant (p-value = 0.018). There was insignificant result between group A and C (p-value = 0.693), between group A and D (p-value = 0.197) and between group B and C (p-value = 0.178).

Johnson's Score

The scoring of seminiferous tubules observed in groups A, B, C and D was 9.40 ± 0.15, 5.25 ± 0.12, 6.42 ± 0.13 and 8.29 ± 0.11 respectively. One way ANOVA showed significant difference among groups (p-value = 0.001) as shown in Table-IV and bar chart given in Figure-3.

Multiple comparison was done by post-hoc Tukey HSD test. The statistical difference was very highly significant between groups A and B (p-value = 0.001), groups A and C (p-value = 0.001), groups A and D (p-value = 0.001), groups B and C (p-value = 0.001), groups B and D (p-value = 0.001) and groups C and D (p-value = 0.001). All data and observations are shown in Table-V, Figure-4 for group A, Figure-5 for group B, Figure-6 for group C and Figure-7 for group D.

Basement Membrane Integrity

Frequencies and percentages of the three grades of basement membrane integrity was calculated and data was represented as bar chart as shown in Figure-8 and Table-VI.

In group A, Out of 225 seminiferous tubules, 150 tubules showed normal basement membrane, 54 tubules showed ruffled basement membranes and 17 tubules showed disrupted basement membranes (Figure-8 and 9)

In group B, Out of 225 seminiferous tubules, 64 tubules showed normal basement membranes, 83 tubules showed ruffled basement membranes and 78 tubules showed disrupted basement membranes (Figure-8,10-11).

In group C, out of 225 seminiferous tubules, 73 tubules showed normal basement membranes, 94 tubules showed ruffled basement membranes and 58 tubules showed disrupted basement membranes (Figure-8,12).

In group D, 90 tubules showed normal basement membranes, 76 tubules showed ruffled basement membranes and 59 tubules showed disrupted basement membranes. (Figure-8,13).

Groups	A	B	C	D	P-Value
Mean ± SD	180.22 ± 5.86	185.22 ± 5.98	182.00 ± 4.18	185.94 ± 9.20	0.06
Median (IQR)	179.00 (177.00-180.75)	185.00 (180.75-187.75)	184.00 (177.75-185.00)	183.00 (180.50-187.25)	

Table-I. Comparison of mean body weight (gm) of rats at the start of experiment among groups (n=9) *p ≤ 0.05 as statistically significant value

Parameter	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	Group D (Mean ± SD)	P-Value
Mean body weight (gm) at the 11th day	198.71 ± 22.13	177.78 ± 10.55	191.5 ± 10.08	212.06 ± 08.68	0.001*

Table-II. One way ANOVA showing comparison of mean body weight (gm) of rats at the 11th day of experiment among group A, B, C and D (n=9) *p ≤ 0.05 as statistically significant value

Group I	Group J	Mean Difference	Standard Error of Mean	P-value
A	B	20.933	6.563	0.016*
	C	7.211	6.563	0.693
	D	-13.344	6.563	0.197
B	A	-20.933	6.563	0.016*
	C	-13.722	6.563	0.178
	D	-34.278	6.563	0.001*
C	A	-7.211	6.563	0.693
	B	13.722	6.563	0.178
	D	-20.556	6.563	0.018*
D	A	13.344	6.563	0.197
	B	34.278	6.563	0.001*
	C	20.556	6.563	0.018*

Table-III. Multiple comparison of mean weight (gm) of rats at the 11th day of experiment among groups A, B, C and D
***p-value ≤ 0.05 as statistically significant**

Parameter	Group A (Mean ± SD)	Group B (Mean ± SD)	Group C (Mean ± SD)	Group D (Mean ± SD)	p-value
Mean Johnson's score of seminiferous tubules	9.40 ± 0.15	5.25 ± 0.12	6.42 ± 0.13	8.29 ± 0.11	0.001*

Table-IV. One way ANOVA showing comparison of mean Johnson score among all groups (n=9)
***p-value ≥ 0.05 considered as significant**

Group (I)	Group (J)	Mean Difference (I-J)	Standard Error of Mean	P-Value**
A	B	4.16	0.06	0.001*
	C	2.98	0.06	0.001*
	D	1.11	0.06	0.001*
B	A	-4.16	0.06	0.001*
	C	-1.18	0.06	0.001*
	D	-3.05	0.06	0.001*
C	A	-2.98	0.06	0.001*
	B	1.18	0.06	0.001*
	D	-1.87	0.06	0.001*
D	A	-1.11	0.06	0.001*
	B	3.05	0.06	0.001*
	C	1.87	0.06	0.001*

Table-V. Multiple comparison of mean Johnson score among all groups
***p-value ≤ 0.05 as statistically significant ** Tukey's post-hoc test**

Groups	Basement membrane Integrity			Total Tubules	P-Value*
	Normal n (%)	Ruffled n (%)	Disrupted n (%)		
A	154 (68.4%)	54 (24.0%)	17 (7.6%)	225 (100%)	0.001*
B	64 (28.4%)	83 (36.9%)	78 (34.7)	225 (100%)	
C	73 (32.4%)	94 (41.8%)	58 (25.8%)	225 (100%)	
D	90 (40.0)	76 (33.8%)	59 (26.2%)	225 (100%)	
	381	307	212	900	

Table-VI. Number and percentages of seminiferous tubules showing normal, ruffled and disrupted basement membranes *p-value ≤ 0.05 considered as significant

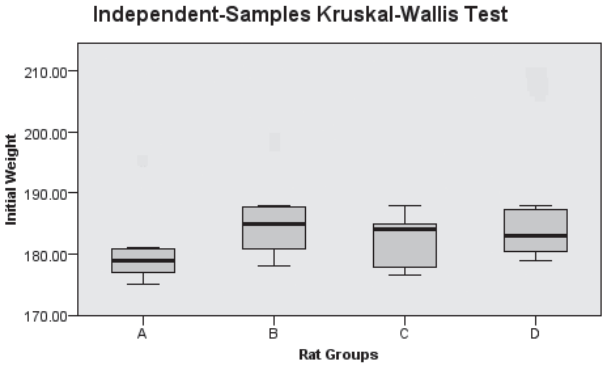

Figure-1. Box plot showing comparison of mean body weight (gm) of animals at the start of experiment as median and interquartile range among all groups (n=9)

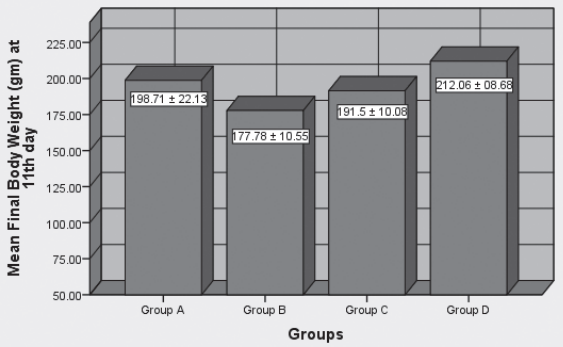

Figure-2. Bar chart showing comparison of mean weight (gm) of rats at the end of 11th day of experiment among groups A, B, C and D (n=9)

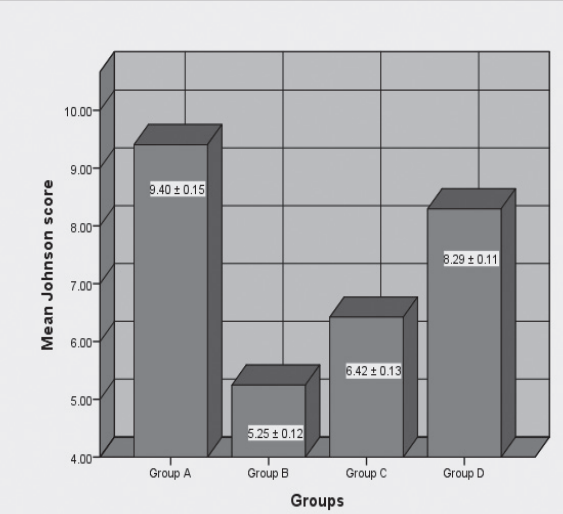

Figure-3. Bar charts showing comparison of mean Johnson score among groups A, B, C and D (n=9)

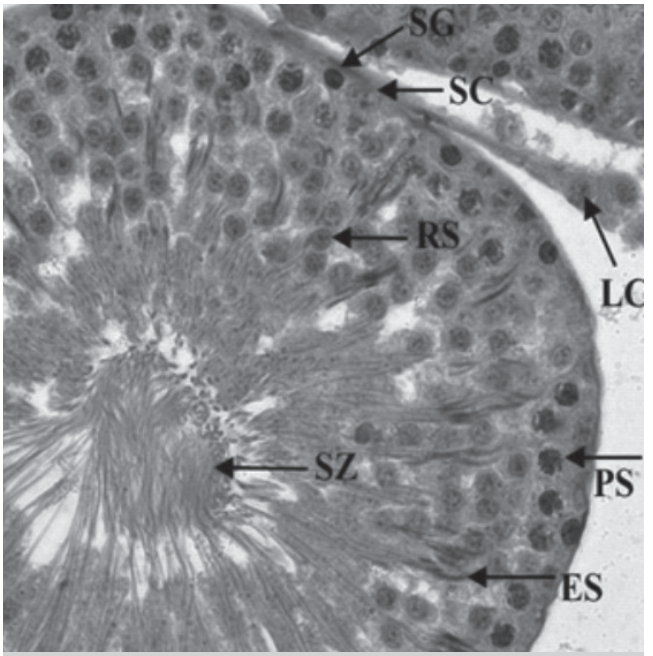

Figure-4. Photomicrograph of testis from group A showing a Johnson score of 10. Spermatogonia (SG), Primary Spermatocytes (PS), Round Spermatids (RS), Elongated Spermatids (ES) and Spermatozoa (SZ) are different stages of spermatogenesis seen in addition to Sertoli Cells (SC). H&E stain. X400

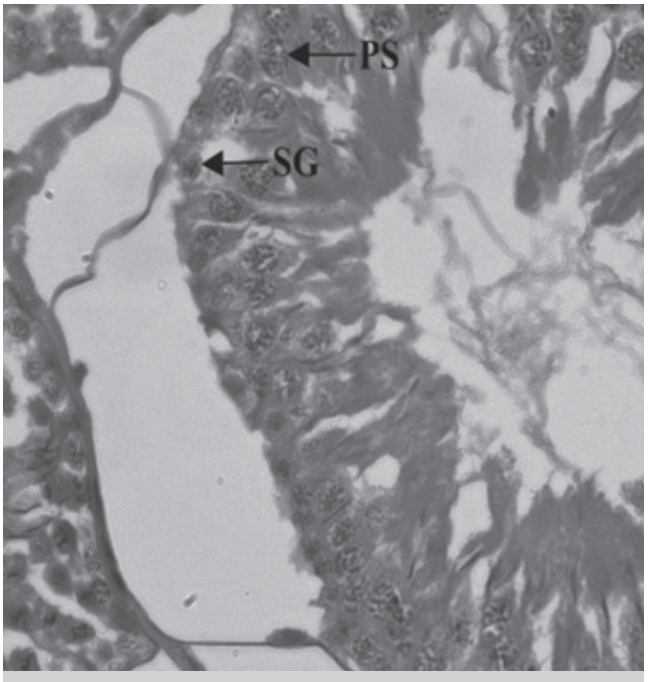

Figure-5. Photomicrograph of testis from group B showing a Johnson score of 5. Spermatogonia (SG) and Primary Spermatocytes (PS) are seen along with sloughing and disruption of seminiferous epithelium. H&E stain. X400

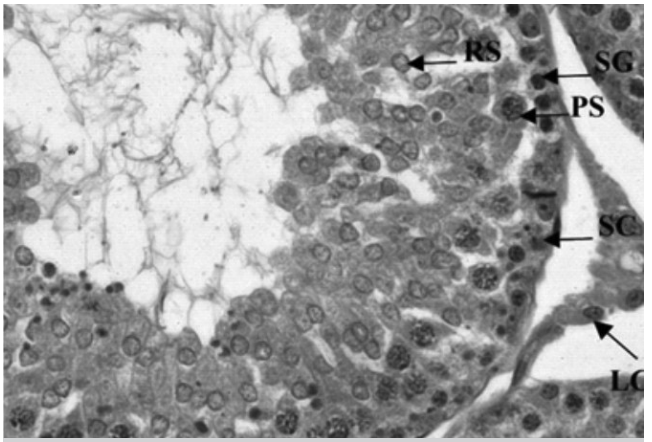

Figure-6. Photomicrograph of testis from group C showing a Johnson score of 7. Sertoli cells (SC), Spermatogonia (SG), Primary Spermatocytes (PS), Round Spermatids (RS) are identified in this section. H&E stain. X400

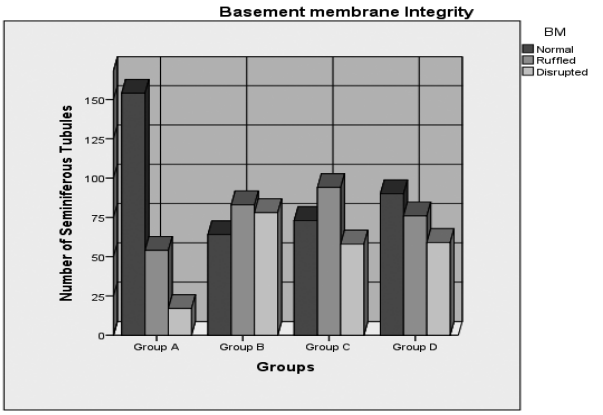

Figure-8. Bar Chart showing number of seminiferous tubules of rats in each group with normal (blue bars), ruffled (green bars) and disrupted (off-white bars) basement membrane of seminiferous tubule.

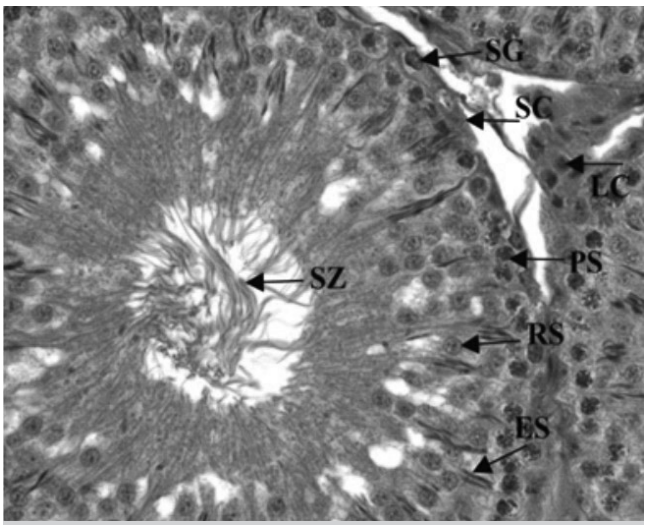

Figure-7. Photomicrograph of testis from group D showing a Johnson score of 9. All stages of spermatogenesis are seen along with Sertoli cells (SC). Spermatogonia (SG), Primary Spermatocytes (PS), Round spermatids (RS) and Elongated spermatids (ES) are seen in the epithelium. Spermatozoa (SZ) are seen in lumen. H&E stain. X400

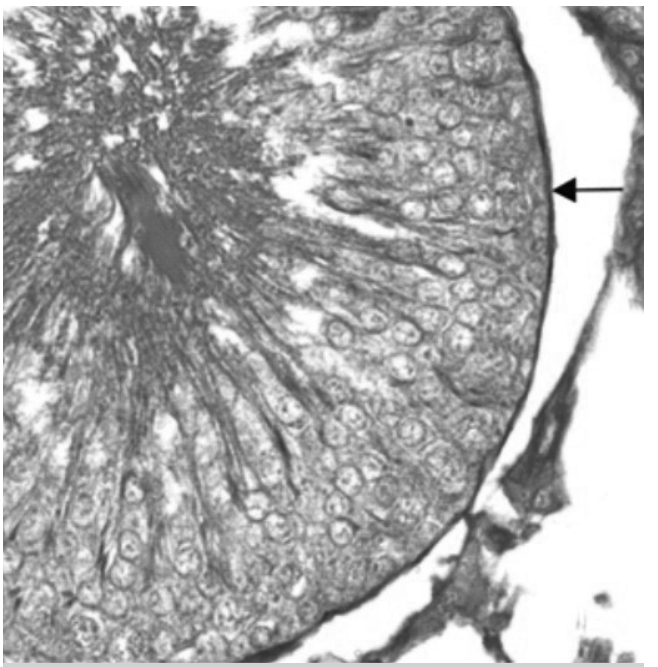

Figure-9. Photomicrograph of seminiferous tubule from testis of group A rat showing regular, normal basement membrane (arrow). PAS stain. X400

DISCUSSION

Male infertility has increased worldwide.[17] BPA, a synthetic carbon based plasticizer is now well known for its hazardous effects on male fertility.[18] The current study was designed to assess the acute high dose effects of BPA on testicular tissue of rodents.

The body weight of rats did not differ from each other in the beginning of the experiment. Ten days after the start of the experiment when the feeding of BPA and BPA with curcumin was terminated, the animals were weighed again and the mean weights of the animals among groups were significantly different ($p<0.001$).

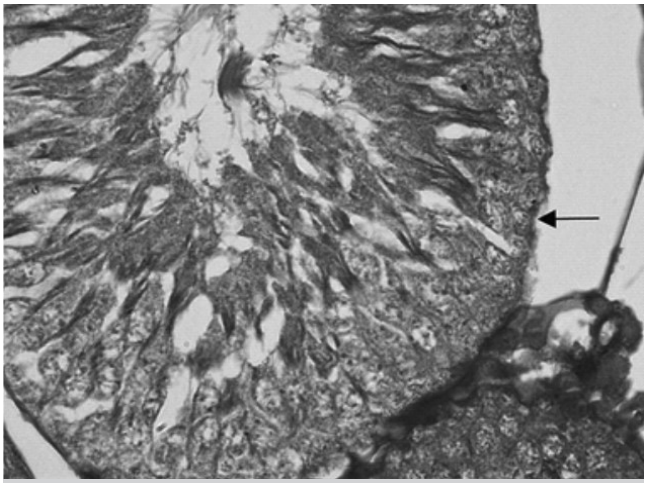

Figure-10. Photomicrograph of seminiferous tubule from testis of group B rat showing disrupted basement membrane (arrow). PAS stain. X400

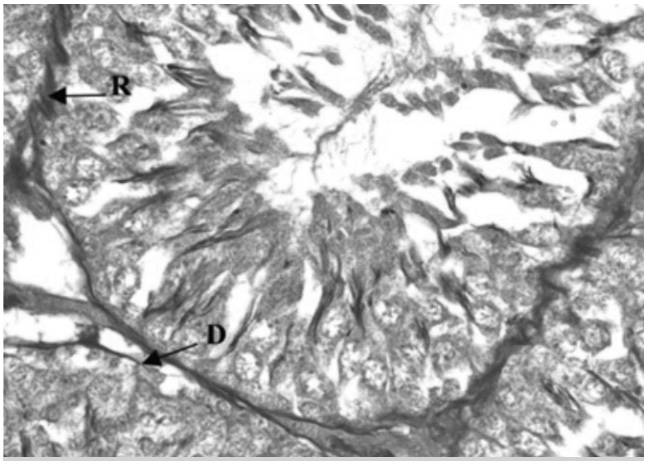

Figure-12. Photomicrograph of seminiferous tubule from testis of group C rat elucidating ruffled (R) and disrupted (D) basement membrane (arrows). PAS stain. X400

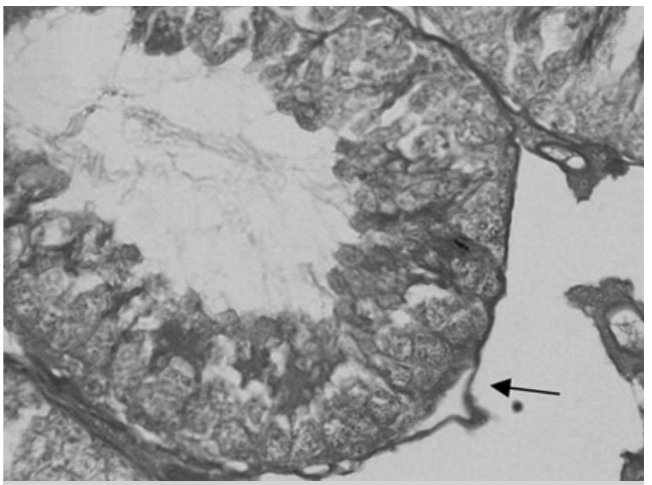

Figure-11. Photomicrograph of seminiferous tubule from testis of group B rat elucidating ruffled basement membrane (arrow). PAS stain. X400

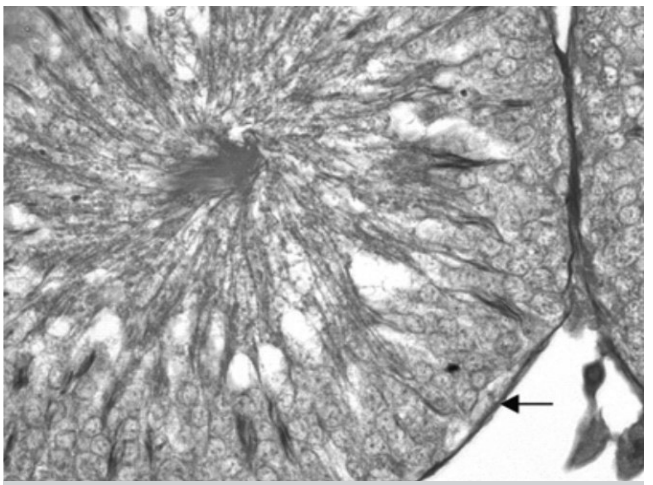

Figure-13. Photomicrograph of seminiferous tubule from testis of group D rat elucidating regular basement membrane (arrow). PAS stain. X400

Observations in this study were suggestive of two reasons, the slower growth of animals given BPA only and faster growth of animals given BPA with curcumin. When growth per day was calculated for all the groups during 10 days of the experiment, it was; 1.02, 0.402 and 1.41 g/day in groups A, B and D. These figures clearly showed that BPA slows down gain in body weight. Curcumin protected the organism from the negative effects of BPA as the increase in weight per day (1.412 g/day) was the highest in the group D. Improved weight gain by intake of curcumin in rats after toxic injury was similar to other studies, the results were better than the control group as in the present study.[19] Kazemi et al. (2016) showed that BPA, in doses as low as 5, 25 and 125μg given for 35 days in adult albino rats, decreased the body weights of animals significantly and disrupts the morphology of testes. The decrease in weight was associated with decrease appetite in these animals.[20]

The group C was allowed to continue for another 10 days in order to know the withdrawal effects of BPA. The weight data points out that rats grew quite faster than the previous 10 days when they were having BPA. It also points to the fact that BPA impacts negatively on rat weight and brings a faster increase of weight gain per day afterwards owing to the fact that the appetite of the animals improved after with-drawl.

Spermatogenesis was greatly affected in group B animals given BPA as established by decreased Johnson scoring in which is similar to the work of other studies. It was also established that free radicals produced by BPA cross blood testis barrier and also effect the stages of spermatogenesis directly.[21] Disruption of seminiferous epithelium was also seen at low dose of BPA (5mg/kg/day) when given to prenatal mice. There was associated down-regulation of the expression of those genes in testis which predict repro-toxicity of this low dose of BPA.[22]

In the present study, the group D which was given BPA and curcumin together, showed improved Johnson score showing that curcumin protected all stages of spermatogenesis. Some improvements were also seen in group C after withdrawal of BPA however, these improvements were considerably low as compared to the effect of adding of curcumin. These results suggested that the damage caused by the direct and indirect effect of increased oxidative stress of this plasticizer can be controlled via adding antioxidants like curcumin. Similar protective effect of curcumin on spermatogenesis was observed in sodium arsenite-induced testicular damage.[23]

BPA treated rat testis showed damage and disruption of the basement membrane around seminiferous tubules and Curcumin treated rat testis showed a significant improvement in this parameter. Similar disruptive effects on basement membranes were seen by Di-octyl phthalate (a xenobiotic like BPA and restoration of the membranes was observed in animals fed on vitamin E (a potent anti-oxidant) together with the phthalate.[24]

Oxidative stress is one of the mechanism by which BPA produce significant damage to male reproductive organs and germ cells.[25] All cellular membranes are damaged in increased oxidative stress mainly via phosphorylation and it may be considered the main mechanism through which BPA had caused deleterious effects on basement membranes of seminiferous tubules and this effect was counteracted by the antioxidant properties of curcumin.

CONCLUSION

The testicular histology was markedly deteriorated by exposure to the high dose of BPA as shown by significant decrease in Johnson score and increased disruption in basement membranes of the seminiferous tubules. Hence, it is concluded that high dose exposure of BPA given for short duration causes testicular toxicity in albino rats. Co-administration of curcumin restored the parameters emphasizing its antioxidant effect as mechanism of protection.

The study can further be investigated at cellular and genetic levels to understand the effects of BPA on male gonad.

REFERENCES

1. Agarwal A, Mulgund A, Hamada A, Chyatte MR. **A unique view on male infertility around the globe.** *Reprod Biol Endocrinol,* 2015; 13: 37.

2. Rehman S, Usman Z, Rehman S, AlDraihem M, Rehman N, Rehman I, Ahmad G. **Endocrine disrupting chemicals and impact on male reproductive health.** *Transl Androl Urol,* 2018; 7(3): 490-503. doi: 10.21037/tau.2018.05.17.

3. Norazit A, Mohamad J, Razak SA, Abdulla MA, Azmil A, Mohd MA. **Effects of soya bean extract, Bisphenol A and 17-β estradiol on the testis and circulating levels of estradiol among peripubertal juvenile male Sprague Dawley rats.** *Sains Malaysiana,* 2012; 41(1): 63-9.

4. Liao C, Kannan K. **Concentrations and profiles of Bisphenol A and other Bisphenol analogues in food stuffs from the United States and their implications for human exposure.** *J Agric Food Chem,* 2013; 61: 4655-62. doi: 10.1021/jf400445n.

5. Huang RP, Liu ZH, Yuan SF, Yin H, Dang Z, Wu PX. **Worldwide human daily intakes of Bisphenol A estimated from global urinary concentration data (2000-2016) and its risk analysis.** *Environ Pollut* 2017; 230: 143-52. doi: 10.1016/j.envpol.2017.06.026.

6. Myridakis A, Chalkiadaki G, Fotou M, Kogevinas M, Chatzi L, Stephanou EG. **Exposure of preschool-age Greek children (RHEA cohort) to bisphenol A, parabens, phthalates and organophosphates.** *Environ Sci Technol,* 2016; 50: 932-41. doi: 10.1021/acs.est.5b03736.

7. Yoon K, Kwack SJ, Kim HS, Lee BM. **Estrogenic endocrine disrupting chemicals: Molecular mechanisms of actions on putative human diseases.** *J Toxicol Environ,* 2014; 17(3): 127-74. doi:10.1080/10937404.2014.882194.

8. Mourad IM, Khadrawy YA. **The sensitivity of liver, kidney and testis of rats to oxidative stress induced by different doses of Bisphenol A.** *Int J Life Sci Pharma Res,* 2012; 2(2): 19-28.

9. Helal EGA, Badawi MMM, Soliman MG, Abdel-kawi NA, Fadel HAE, Abozaid NMG. **Physiological and Histopathological studies on Bisphenol A compound as xenoestrogen in male albino rats.** *Egyptian J Hosp Med,* 2013; 50: 127-36.

10. Tiwari D, Vanage G. **Mutagenic effect of Bisphenol A on adult rat male germ cells and their fertility.** *Reprod Toxicol,* 2013; 40: 60-8.

11. Doshi T, D'souza C, Vanage G. **Aberrant DNA methylation at Igf2-H19 imprinting control region in spermatozoa upon neonatal exposure to Bisphenol A and its association with post-implantation loss.** *Mol Biol Reports,* 40:4747-57.

12. Hulak M, Gazo I, Shaliutina A, Linhartova P. ***In vitro* effects of Bisphenol A on the quality parameters, oxidative stress, DNA integrity and adenosine triphosphate content in starlet (Acipenser ruthenus) spermatozoa.** *Comp Biochem Physiol Part C: Toxicol Pharmacol,* 2013; 158:64-71.

13. Jiang X, Chen HQ, Cui ZH, Yin L, Zhang WL, Liu WB. **Low dose and combined effects of oral exposure to Bisphenol A and diethylstilbestrol on the male reproductive system in adult Sprague-Dawley rats.** *Environ Toxicol Pharmacol* 2016; 43: 94-102.

14. Daily JW, Yang M, Park S. **Efficacy of turmeric extracts and curcumin for alleviating the symptoms of joint arthritis: A systemic review and meta-analysis of randomized clinical trials.** *J. Med. Food* 2016; 19: 717-29.

15. Sahebkar A, Serbanc MC, Ursoniuc S, Banach M. **Effects of curcuminoids on oxidative stress: a systemic review and meta-analysis of randomized controlled trials.** *J Funct Food,* 2015; 18: 898-909. doi: 10.1016/j.jff.2015.01.005.

16. Panahi Y, Alishiri GH, Parvin S, Sahebkar A. **Mitigation of systemic oxidative stress by curcuminoids in osteoarthritis: results of a randomized controlled trial.** *J Diet Suppl,* 2016; 13: 209-20. doi: 10.3109/19390211.2015.1008611.

17. Kumar N, Singh AK. **Trends of male factor infertility, an important cause of infertility: A review of literature.** *J Hum Reprod Sci,* 2015; 8(4): 191-6. doi: 10.4103/0974-1208.170370.

18. Rahman MS, Kwon WS, Lee JS, Yoon SJ, Ryu BY, Pang MG. **Bisphenol A affects male fertility via fertility related proteins in spermatozoa.** *Sci Reprod* 2015; 5: 9169.

19. Bulmus FG, Sakin F, Turk G, Sonmez M, Servi K. **Protective effect of curcumin on antioxidant status, body weight gain and reproductive parameters in male rats exposed to subchronic 2,3,7,8-tetrachlorodibenzo-p-dioxin.** *Toxicol Environ Chem,* 2013; 95(6): 1019-29. doi: 10.1080/02772248.2013.829061.

20. Kazemi S, Feizi F, Aghapour F Joorsaraee GA, Moghadamnia AA. **Histopathology and histomorphometric investigation of Bisphenol A and nonyl-phenol on male rat reproductive system.** *N Am J Med Sci,* 2016; 8(5): 215-21.

21. Yousuf B, Amina, Liu G, Wang R, Qadir A, Ali MU, Kanwal Q, Munir B, Asmatullah, Abbas Z. **Bisphenol A exposure and healing effect of *Adiantum capillus-veneris* L. plant extract (APE) in Bisphenol A induced reproductive toxicity in albino rats.** *Environ Sci Pollution Res,* 2016; 23(12): 11645-57.

22. Tainaka H, Takahashi H, Umezawa M, Tanaka H, Nishimune Y, Oshio S, Tekada K. **Evaluation of the testicular toxicity of pre-natal exposure to Bisphenol A based on microarray analysis combined with MeSH annotation.** *J Toxicol Sci,* 2012; 37(3): 539-48.

23. Mehranjani MS, Hatamikia N, Noreini SN. **Curcumin protects mice testicular tissue against the adverse effects of sodium arsenite: A stereological study.** *J Kerman Uni Med Sci,* 2016; 23(6): 714-26.

24. Habib N, Tahir M, Lone KP, Latif W. **Protective effect of vitamin E on phthalate induced toxicity on spermatogenesis and testosterone level.** *Biomedica,* 2016; 32(2): 83-7.

25. Yin L, Dai Y, Cui Z, Jiang X, Liu W, Han F. **The regulation of cellular apoptosis by the ROS-triggered PERK/EIF2α/chop pathway plays a vital role in Bisphenol A-induced male reproductive toxicity.** *Toxicol Appl Pharmacol* 2017; 314: 98-108.

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Rabia Sajjad Toor	Contributed in study design, data collection, data analysis, interpretation of result.	
2	Faiza Irshad	Worked out in introduction, Literature reveiw and discussion of the study.	
3	Sania Asif	Contributed in proof-reading, preparing manuscript according to TPMJ criteria and helped in citation of references according to Vencover's style.	