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RESEARCH ARTICLE

POSSIBLE PROTECTIVE EFFECT OF VITAMIN E ON BISPHENOL –A INDUCED CHANGES IN THE MALE REPRODUCTIVE SYSTEM OF ADULT ALBINO RATS: HISTOLOGICAL STUDY.

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Key words:-

BPA, accessory reproductive organs,
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Abstract

Background: Bisphenol A (BPA) is an abundantly used xenoestrogenic chemical, a contaminant of polycarbonate plastics, resin-based dental composites and sealants, which cause various reproductive disorders.

Objective: The aim of the study is to evaluate the adverse effect of BPA, on accessory reproductive organs (epididymis, vas deferens, seminal vesicle and prostate) of male reproductive system. Protective effect of Vitamin E on deleterious outcomes of BPA also evaluated.

Material and Methods: Adult male Wistar albino rats aged 3 weeks were randomly divided into ten groups: control group (olive oil treated) BPA group (dose 5, 50,100 µg/100g BW) and Vitamin E intervention group (dose 5, 50,100 µg/100g BPA+ Vitamin E 4mg/100gbw) and recovery group. Animals were sacrificed 3 months later, blood and tissue samples were collected.

Results: A significant reduction in the epididymis and seminal vesicle weight was observed whereas prostate showed weight significant increment. A degenerative change in epithelium of epididymis was observed whereas; other reproductive organs dose not showed any adverse histopathological change in the BPA group as compared with the control and Vitamin E intervention group. All these changes were attributed to disrupted spermatogenesis that would interfere with sperm production which is confirmed by decreased sperm count. Root cause behind all these effects is decrement in testosterone level by BPA treatment. No changes were observed in recovery group animals.

Conclusion: BPA exposure causes various fertility problems by effecting hormonal level, sperm count, weight and histology of reproductive organs. On the other hand supplementations of Vitamin E have certain protective effect on reproductive dysfunction caused by BPA. Thus, it could have a protective role in improving male fertility.

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Introduction:-

In recent years, scientists have increasingly reported evidences that certain pollutants in the environment plays important roles in contributing causes of fertility problems. A wide range of compounds, including components from pesticides to plastics, from detergents to cosmetics registered as pollutant. Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (BPA), is a chemical found in polycarbonate plastics, leaches from plastic containers during normal usage,

and detectable amounts can be found in many commercial food products and dental sealant (1,2). BPA is present ubiquitously in the environment and ingested routinely by humans (3). Reports say, that 10-20 µg of BPA in canned food is leached from the lacquer lining (4). 20-30 µg of BPA/mL was detected in the saliva of patients who had been treated with a dental sealant (5). The European Food Safety Authority (EFSA) established 50 µg/kg b.wt./d as the tolerable daily intake (TDI) of BPA based on non-observable adverse effect level (NOAEL) of 5000 µg/kgbw/d (6). However, recently EFSA experts has reduced the previously settled TDI to 4 µg/kg bw/d as a temporary base due to the latest refined risk assessment and the uncertainties over the mechanism of BPA action to produce its adverse effects.

It is chronically ingested by humans, 95% of adults and children have detectable amount of urinary BPA (7,8). BPA has also been measured in maternal serum and ovarian follicular fluid, as well as in fetal plasma and amniotic fluid, indicating passage across the placenta (9,10). It has both estrogenic and anti-androgenic effect (11, 12). Toxicological studies (13) have pointed out that rodents exposed to BPA during the prenatal period show a large variety of adverse reproductive outcomes, including decreased epididymal weight and daily sperm production, increased sloughing from seminiferous epithelium (14,15,16) and increased prostate weight (17). Postnatal exposure disrupt blood testis barrier by this germ cell cannot develop into mature sperm and also increases the activation of caspase-3 which cause germ cell apoptosis (18). Hence, there is a significant risk of BPA exposure during critical development period that are particularly sensitive to changes in estrogenic environment (19). During adult exposure changes in sperm morphology like abnormalities in acrosomal cap, vesicle and deformed nuclei were observed in Wistar and Swissrat at 20µg/kg/ day (20). Studies showed that the level of testosterone decrease in rats when exposed to different dose level of BPA (21,22,23). Previous studies have reported the occurrence of oxidative stress after BPA exposure in rats and mice (20, 24). A state of oxidative stress in the testes disrupts both spermatogenesis and the production of testosterone (25). Vitamin E (α-tocopherol) is a powerful lipophilic, antioxidant present in particularly high amounts in Sertoli cells and pachytene spermatocytes and to a lesser extent in round spermatids (26). Vitamin E has also been shown to suppress lipid peroxidation in testicular microsomes and mitochondria (27,28) and is absolutely vital for the maintenance of mammalian spermatogenesis.

Reviewing to all these studies the present study was planned to determine whether exposure of BPA to different dose level affects or cause any histological changes in accessory reproductive organs, if so, what is the mechanism associated with observed effects.

Material and Methods:-

Adult male Wistar albino rats (*Rattus norvegicus*), 3 months old, weighing 150-200 grams, were used in present investigation. The animals were maintained in the Departmental Experimental Facility with light and dark (12h: 12h) schedule in individual polypropylene cage (size 43×27×15cm). Animals were fed with rat pellet diet and water *ad libitum*. The animals were maintained under perfect veterinary supervision and accordance to the guidelines of CPCSEA (29).

Ethical Approval:-

The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC), Department of Zoology, University of Rajasthan, Jaipur.

Test Chemical:-

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (<99% pure)(CAS no.#80-05-7) was purchased from sigma Aldrich. This compound was diluted in olive oil to obtain final concentration of the 5, 50 and 100µg/100gm body weight of the animals respectively.

Vitamin E-(α-tocopherol) capsules (Evion) of 400 mg was purchased from medical store and diluted in olive oil to obtain final concentration of 4mg/100gm body weight of the animals.

Experimental Design:-

Experiments were carried out in two phase's treatment and recovery phase.

Treatment phase (n=10):-

Animals were divided into groups.

Group I:- Control (vehicle treated)

Group II:- Oral administration of 5µg BPA/100 gmbw

Group III:- Oral administration of 5 µg BPA/100 gmbw + 4mg/100gmbw Vitamin E

Group IV:- Oral administration of 50 µg BPA/100 gmbw

Group V:- Oral administration of 50 µg BPA/100 gmbw +4mg/100gm bw Vitamin E

Group VI:- Oral administration of 100 µg BPA/100 gmbw

Group VII:- Oral administration of 100 µg BPA/100 gmbw +4mg/100gmbw Vitamin E

Doses were given for consecutive 90 days. On 91th day of experiment, animals were sacrificed by overdose of anesthetic ether.

Recovery phase (n=5):-

Following completion of treatment schedule, all animals were drawn from doses of BPA. Tests were carried out to assess the recovery pattern for a period of 45 days.

Reproductive organ weight:-

The weights of all accessory reproductive organs of all animals were obtained at the time of sacrifice schedule of each group.

Sperm Concentration:-

The sperm concentration was calculated by diluting the epididymal fluid in sperm diluting fluid. A drop of this dilution was observed in Neubauer's haemocytometer under light microscope and numbers of sperms were counted. (30)

Histopathology:-

A portion of epididymis, vas deferens, and seminal vesicle, prostate was fixed in 4% paraformaldehyde, dehydrated in ethanol, cleared in Xylene and embedded in paraffin wax. Five micron thick sections were stained with haematoxylin and eosin for light microscopic observation.

Hormone analyses:-

Circulatory levels of testosterone in all BPA group, Vitamin E intervention group, control group and recovery group of rats assayed by ELISA kit.

Statistical analysis:-

The mean values were compared using respective standard deviations followed by statistical comparison between control and test groups for evaluation of significant changes in values by one way analysis of variance (ANOVA) test. $P < 0.05$ was considered as significant.

Results:-

Reproductive Organ Weight:-

The caudaepididymal and seminal vesicle weight in BPA treated group animals, was found to be lower and prostate weight was found significantly higher. Similar results were observed in Vitamin E intervention group too but better than BPA alone group as compared to control group at 50 µg BPA/100 g b.wt. and 100µg BPA/100 g b.wt. doses. Whereas at 5 µg BPA/100 g b.wt. dose, no significant alternation in weight was observed. The weight of corpus epididymis also decreased but non significantly. No alternations in weight of vas deferens were observed. No changes were observed in recovery group animals (Table. 1)

Table 1:-Reproductive organs Weight (mg/100gm b.wt.) following oral administration of BPA and Vitamin E in rats

Groups	Reproductive Organ Weight (mg/100gm body weight)				
	Corpus epididymis	Cauda epididymis	Vas deferens	Seminal vesicle	Ventral prostate
Group I	138.3± 4.87	398.2± 37.93	152.6±51.38	657.2±124.84	94.2±20.5
Group II	128.2 ±12.83 ^{ns}	361.3±93.86 ^{ns}	149±36.58	592.1±72.69 ^{ns}	128±44.92 ^{ns}
Group III	136.1± 3.10 ^{ns}	395.2±34.31 ^{ns}	151.6±50.46	648.3±121.24 ^{ns}	96.4±12.86 ^{ns}
Group IV	120.3±13.27 ^{ns}	335.4±97.32 [*]	147.8±53.85	497.2±53.34 [*]	191.2±73.34 [*]
Group V	134.3 ±3.09 ^{ns}	393.2±30.26 [*]	150±49.46	645.2±108.61 [*]	98.4±11.6 [*]
Group VI	105.6±25.25 ^{ns}	252.8±73.37 [*]	146.4±34.51	424.1±69.31 [*]	254.4±81.92 [*]
Group VII	133.8 ±3.58 ^{ns}	390.2±31.24 [*]	150.6±49.73	642.2±54.11 [*]	99.8±22.03 [*]
Recovery Group					
Group VIII (5µg/100g b.wt /day)	130.2± 9.39	365± 75.02	149.9± 8.21	591.5± 57.15	134± 45.94
Group IX (50µg/100g b.wt /day)	120.3 ±13.28	338.1± 93.19	148± 51.08	495± 74.12	194± 76.18
Group X (100µg/100g b.wt./day)	107.6 ± 25.7	277.1± 71.66	145.4 ±35.3	392.1± 54.11	274± 78.02

(Mean± S.D.)

Ns=non significant

*=Significant

Group II, III, IV,V,VI,VII compared with Group I

Group VIII, IX, X compared Group II,IV,VI

p<0.05 significance level

Group I:-vehicle treated control; **Group II:-**5µg BPA/100g b.wt./day; **Group III:-**5µg BPA/100g b.wt./ day+ 4mg Vitamin E;**Group IV:-**50µg BPA/100g.b.wt/day; **Group V:-** 50µgBPA/100g b.wt./ day+ 4mg Vitamin E; **Group VI:-** 100µg BPA/100g b.wt./ day; **Group VII:-** 100µg/100g b.wt./ day+ 4mg Vitamin E; **Recovery Group;** **Group I:-** 5µg BPA/100g b.wt./day**Group II:-**50µg BPA/100g b.wt./day; **Group III:-** 100µg BPA/100g.b.wt/day**Histopathology of Epididymis:-**

Histologically group II, IV and VI animals showed degenerative changes as observed by altered epithelium and almost nil sperms in the treated animals of group VI whereas in group III,V,VII animals these changes were not observed and epididymal structure was comparable to control. No recovery was observed in recovery group animals when compare to treated group (Fig.: 1a, 1b, 1c &2a,2b,2c)

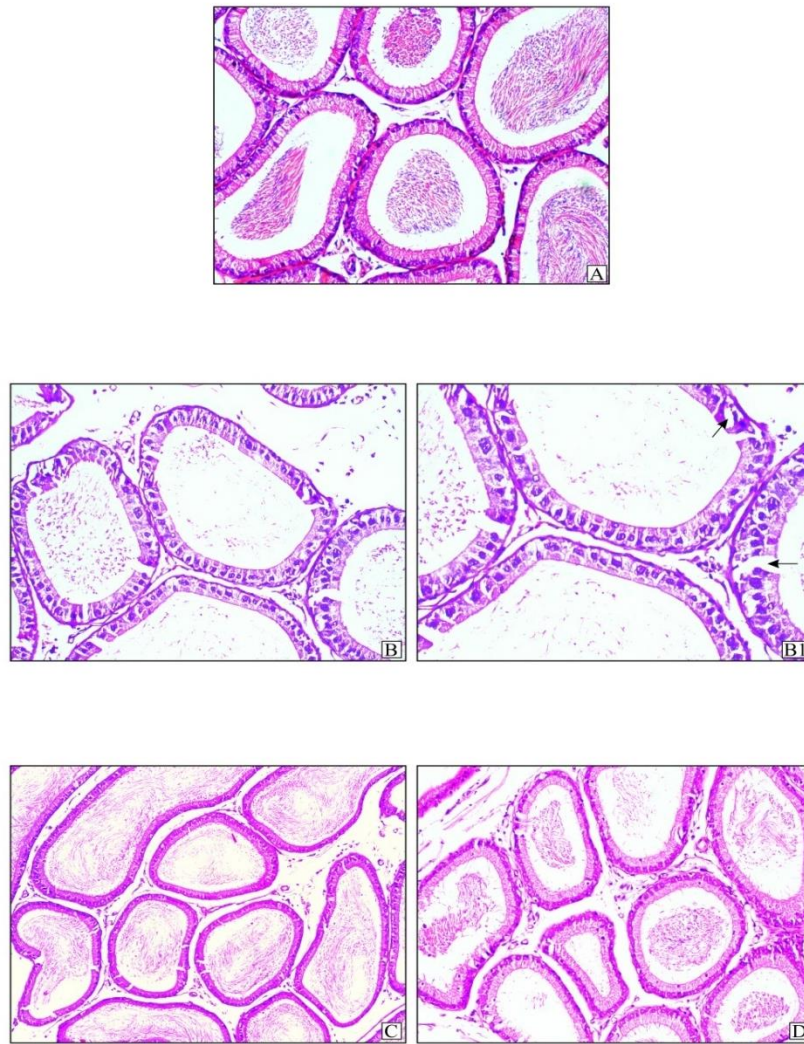


Figure 1a:-Photomicrograph showing histological changes in corpus epididymis of adult male albino rat after 5µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 5µg BPA/100 g b.wt., **B1:** 5µg BPA/100 g b.wt. (40X) **C:** 5µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group. 20X. Epithilium degeneration indicated by arrow (←)

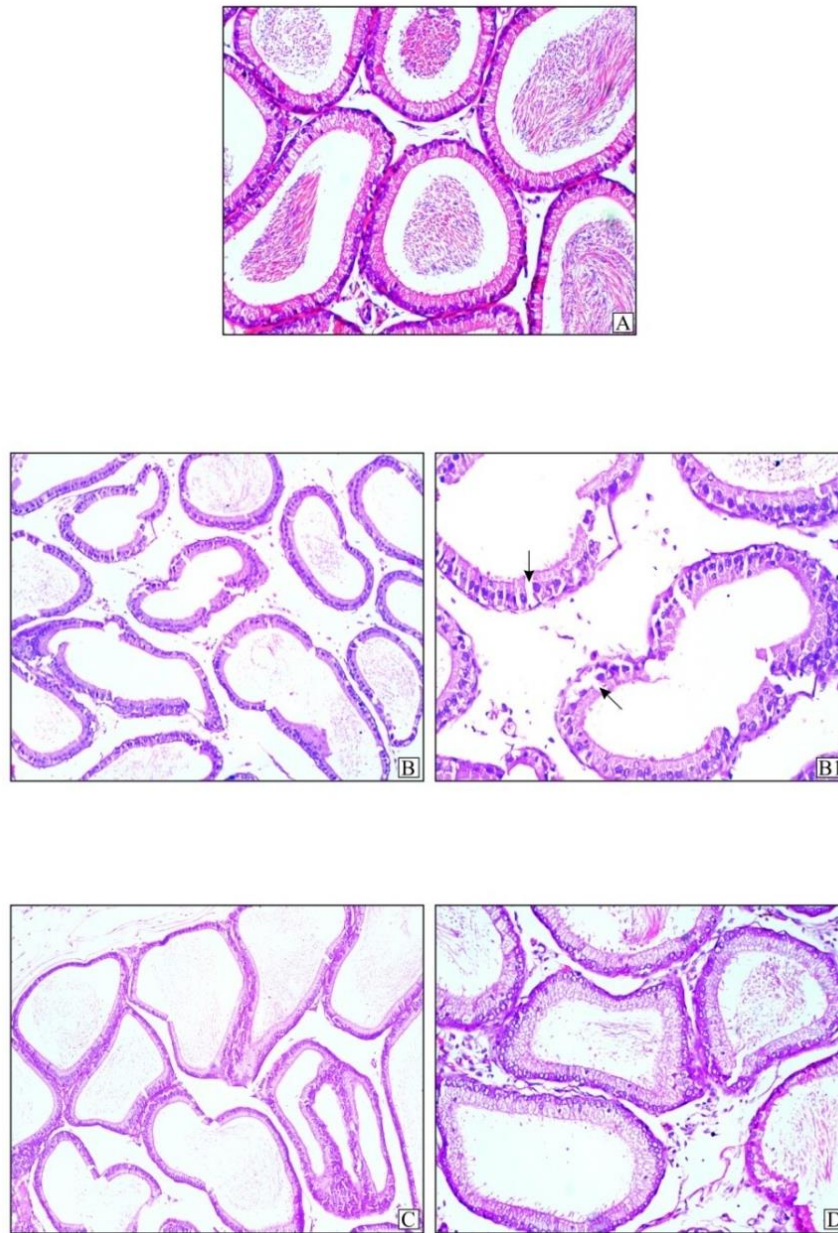


Figure 1b:-Photomicrograph showing histological changes in corpus epididymis of adult male albino rat after 50µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 50µg BPA/100 g b.wt., **B1:** 50µg BPA/100 g b.wt. (40X) **C:** 50µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group. 20X. Epithilium degeneration indicated by arrow (←)

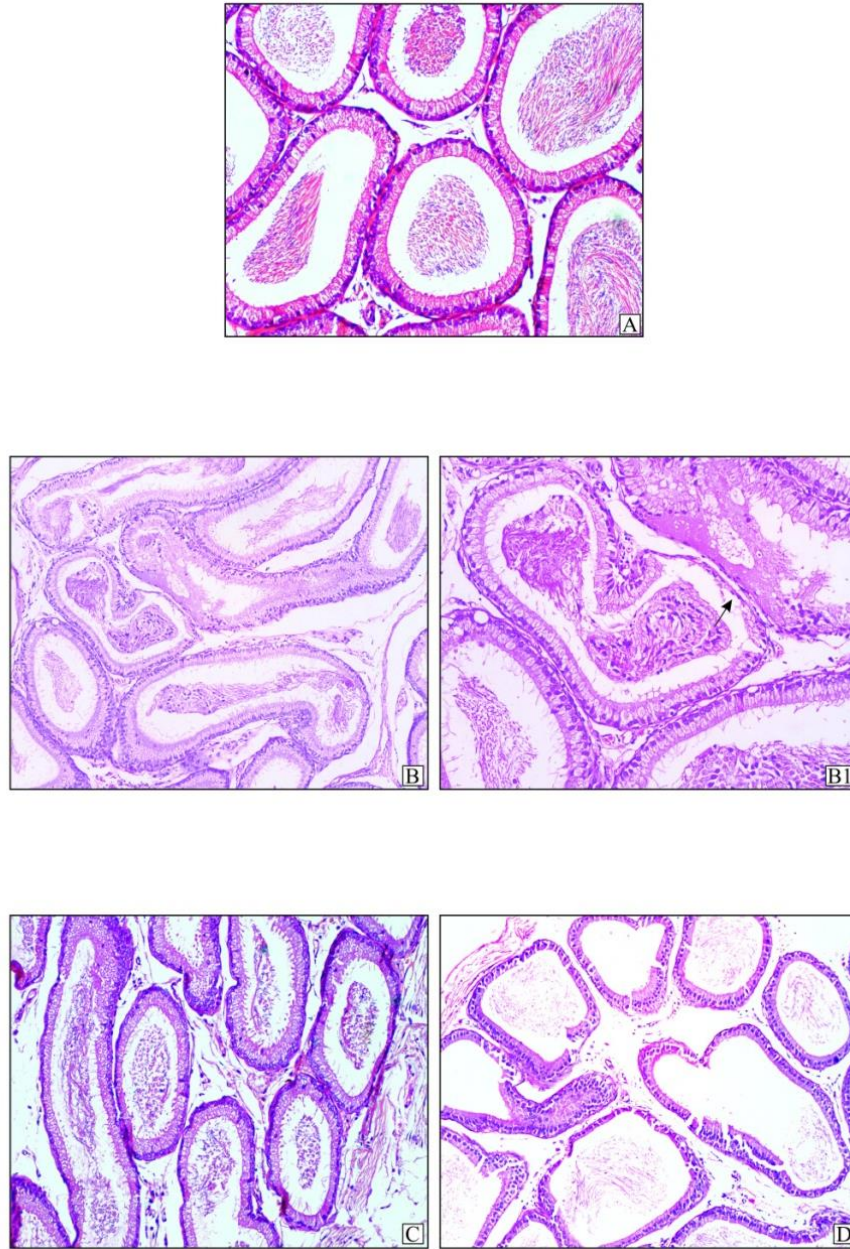


Figure 1c:-Photomicrograph showing histological changes in corpus epididymis of adult male albino rat after 100µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 100µg BPA/100 g b.wt., **B1:** 100µg BPA/100 g b.wt. (40X) **C:**100µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group. 20X. Epithilium degeneration indicated by arrow (←)

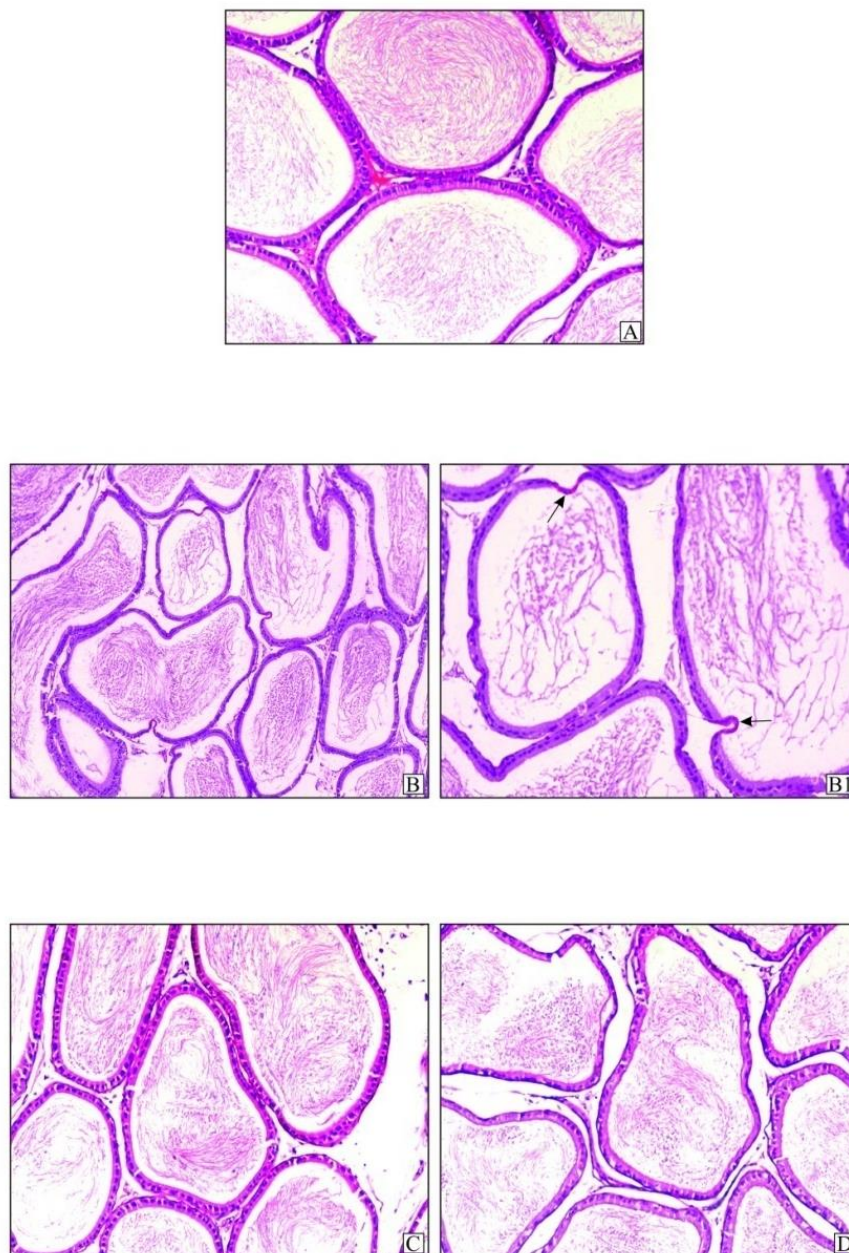


Figure 2a:-Photomicrograph showing histological changes in cauda epididymis of adult male albino rat after 5µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 5µg BPA/100 g b.wt., **B1:** 5µg BPA/100 g b.wt. (40X) **C:** 5µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group. 20X. Epithilium degeneration indicated by arrow (←)

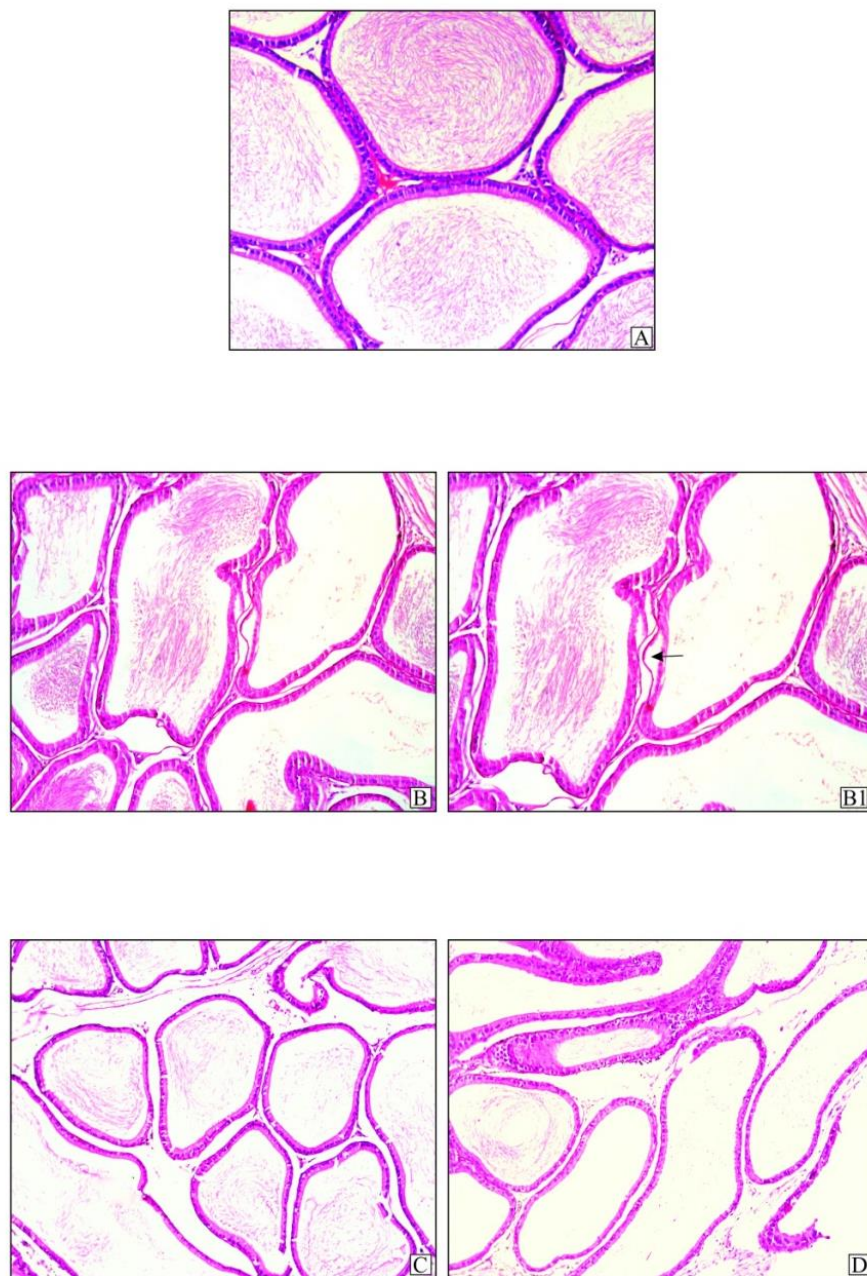


Figure 2b:-Photomicrograph showing histological changes in cauda epididymis of adult male albino rat after 50µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 50µg BPA/100 g b.wt., **B1:** 50µg BPA/100 g b.wt. (40X) **C:** 50µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group .20X. Epithilium degeneration indicated by arrow (←)

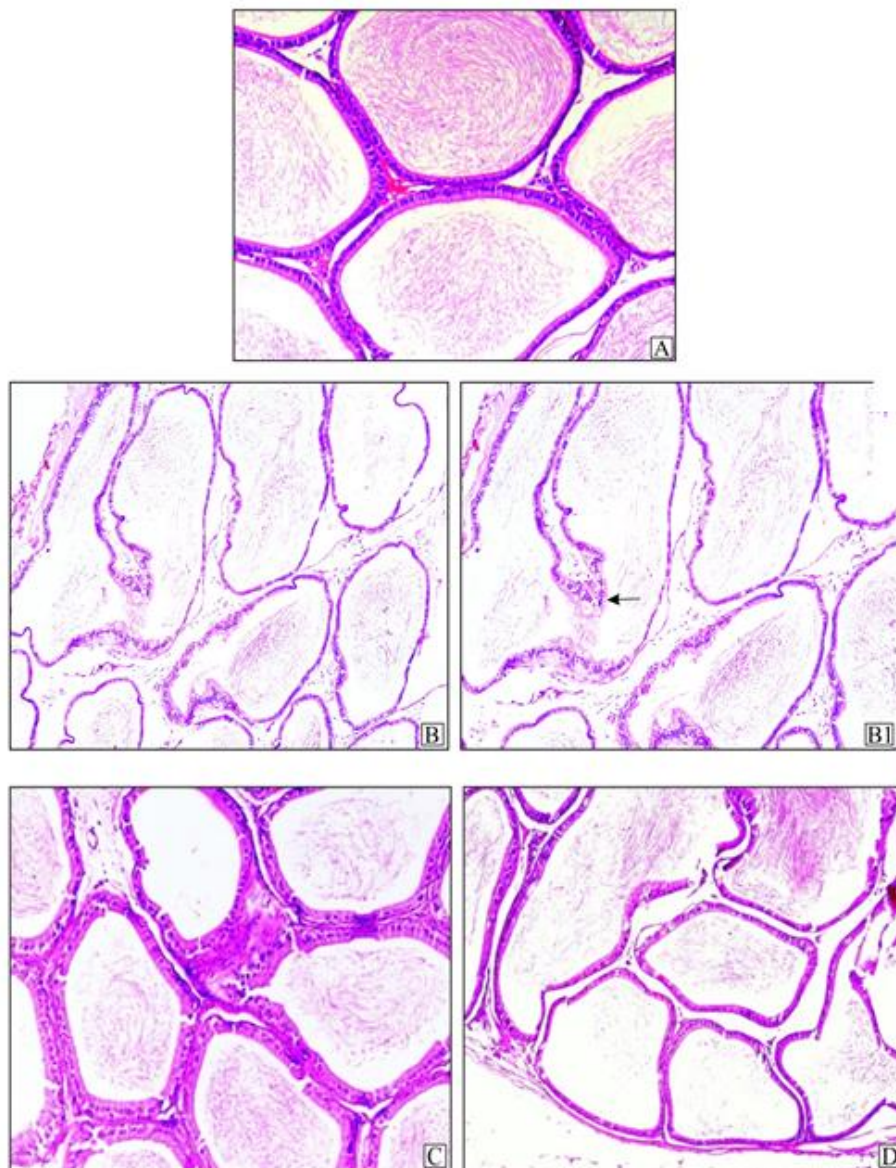


Figure 2c:-Photomicrograph showing histological changes in cauda epididymis of adult male albino rat after 100µg BPA/100 g b.wt. BPA treatment **A:** Control, **B:** 100µg BPA/100 g b.wt., **B1:** 100µg BPA/100 g b.wt. (40X) **C:** 100µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** Recovery group. 20X. Epithelium degeneration indicated by arrow (←)

Histopathology of remaining reproductive organs:-

No appreciable changes were observed in histology of vas deferens, seminal vesicle and prostate of BPA treated animals as compared to control. (Fig.3,4,5)

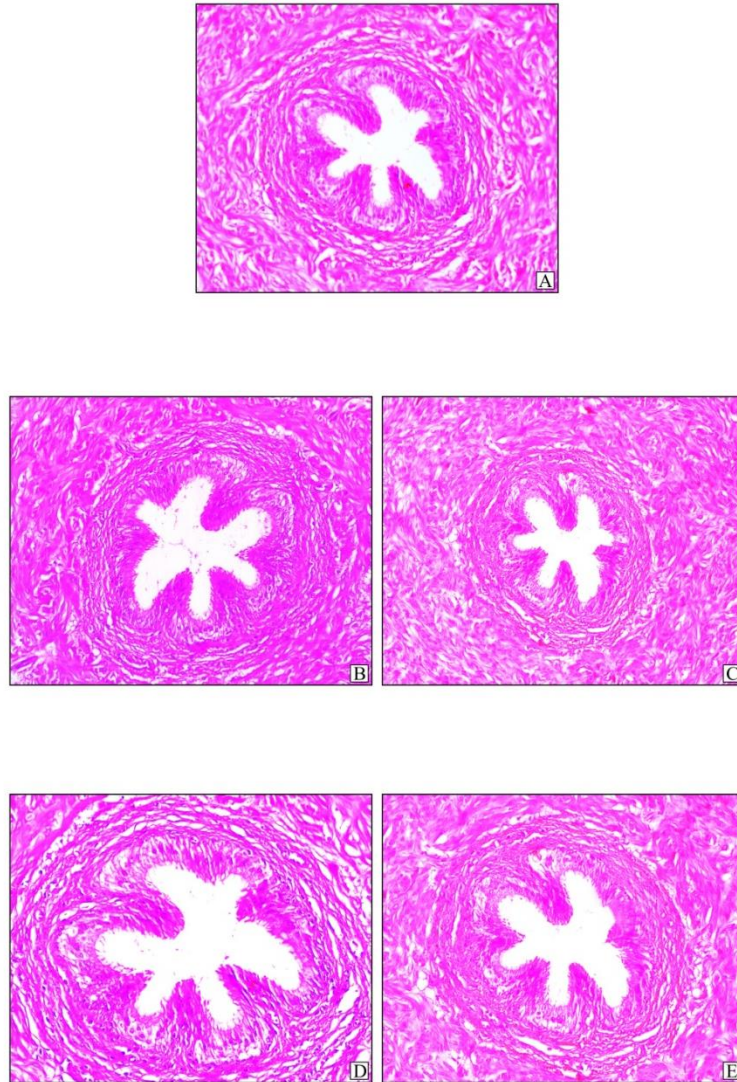


Figure 3:-Photomicrograph showing histological changes in vas deferens of adult male albino rat after BPA treatment **A:** Control, **B:** 5µg BPA/100 g b.wt., **C:** 50µg BPA/100 g b.wt. **D:** 100µg BPA/100 g b.wt., **E:** Vitamin E treated. 20X

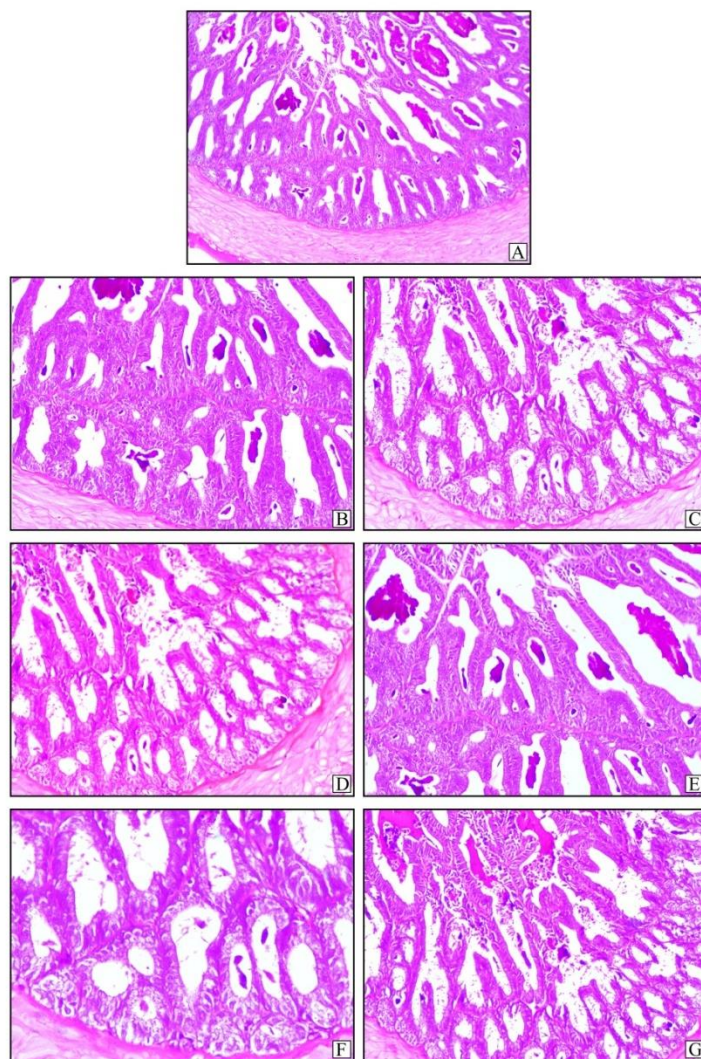


Figure 4:-Photomicrograph showing histological changes in seminal vesicle of adult male albino rat after BPA treatment **A:** Control, **B:** 5µg BPA/100 g b.wt., **C:** 5µg BPA/100g bw + 4mg/100g b.wt. Vitamin E, **D:** 50µg BPA/100 g b.wt., **E:** 50µg BPA/100g bw + 4mg/100g b.wt. Vitamin E, **F:** 100µg BPA/100 g b.wt., **G:** 100µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E. 20X

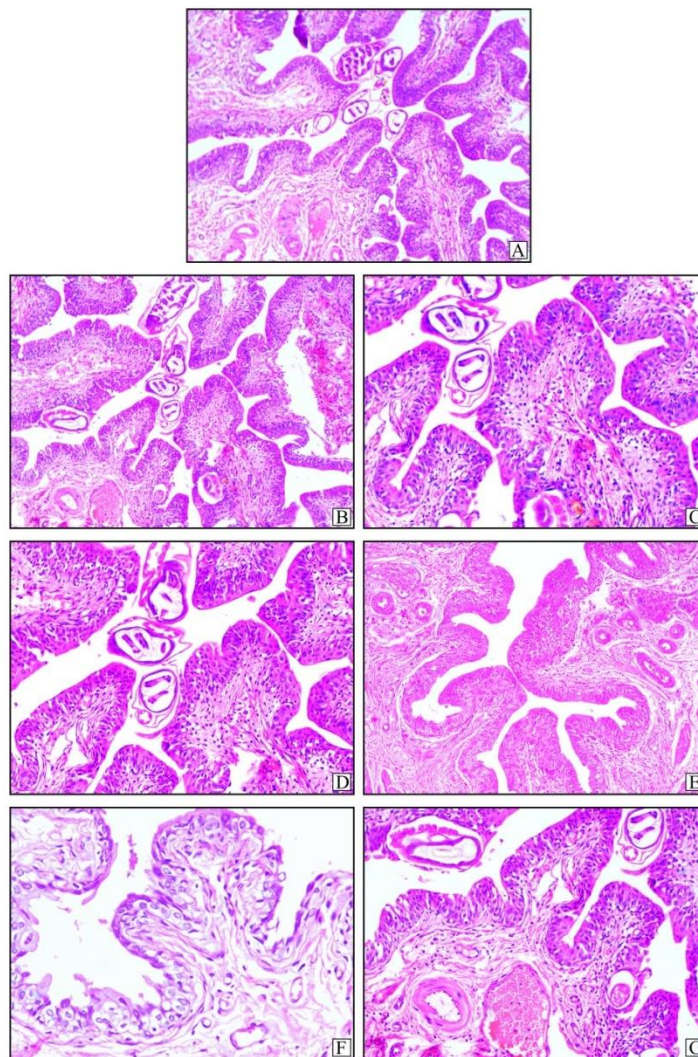


Figure 5:-Photomicrograph showing histological changes in prostate of adult male albino rat after BPA treatment
A: Control, **B:** 5µg BPA/100 g b.wt., **C:** 5µg BPA/100g b.wt. + 4mg/100g b.wt. Vitamin E, **D:** 50µg BPA/100 g b.wt., **E:** 50µg BPA/100g b.wt. + 4mg/100g b.wt. Vitamin E, **F:** 100µg BPA/100 g b.wt., **G:** 100µg BPA/100 g b.wt. + 4mg/100g b.wt. Vitamin E. 20X.

Sperm Concentration:-

The sperm count was significantly low in the BPA exposed rats than those in the control group animals and Vitamin E intervention group animals at 50 µg BPA/100 g b.wt. and 100µg BPA/100 g b.wt. doses. Non significant decline was observed in 5 µg BPA/100 g b.wt. dose treated animals. Recovery group does not show any recovery. (Fig. 6)

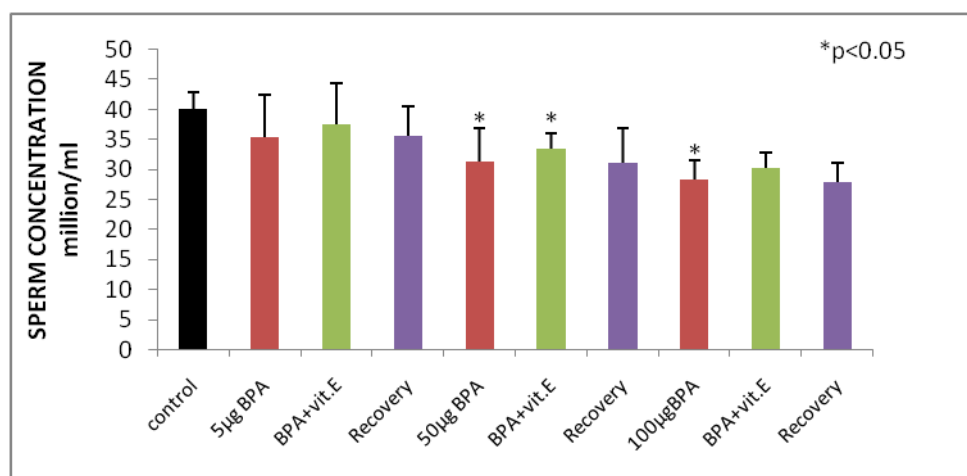


Figure 6:-Effect of BPA and Vitamin E on epididymal sperm count in adult male albino rat.

Testosterone level:-

Serum testosterone levels decreased significantly at 50 µg and 100µg BPA/100 g b.wt. doses, whereas at 5 µg BPA/100 g b.wt. dose a non significant decline was observed as compared to control group, and supplementation of Vitamin E with BPA checked the decline but not as comparable to control group. No recovery was observed in recovery group. (Fig.7)

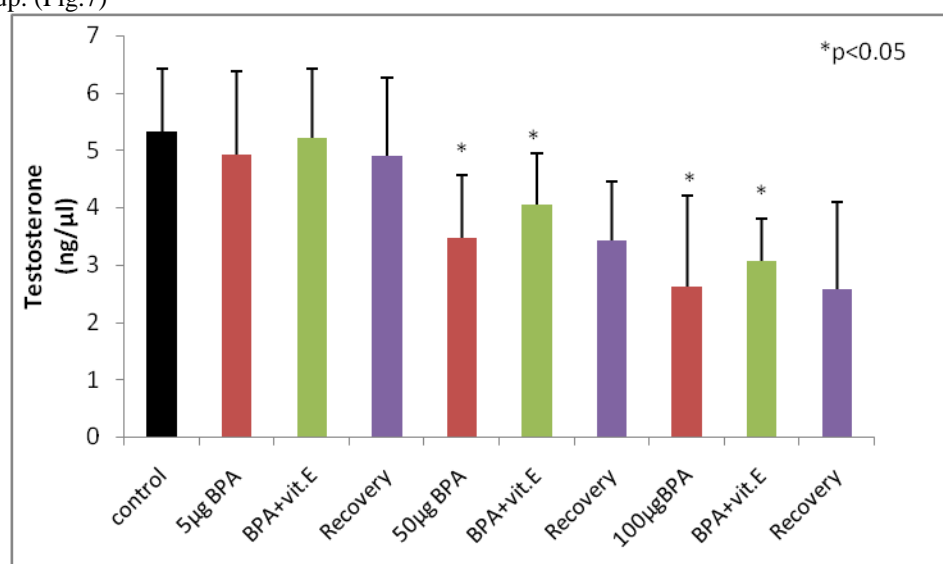


Figure 7:-Effect of BPA and Vitamin E on serum testosterone level in adult male albino rats.

Discussion:-

BPA ingestion at different doses reduces testicular sperm counts and its efficiency of sperm production. Degenerative changes in epithelium were seen in epididymis and lesser amount of sperms were observed in the lumen which suggests that BPA cause adverse effect on sperm concentration. The documented action of BPA confirms too the said result with orally ingested 25 and 100 µg/kg BPA for 28 days (31). Reduced sperm count and epididymal epithelium degeneration in Wistar rat at 20µg/kg /day dose level for 60 days exposure study was observed. (20). The present study also confirms similar results at 5 µg, 50 µg and 100µg/100gm bw/day dose of BPA in wistar albino rats. The main cause behind this was found to be spermatogenesis impairment (32).Jin (33) also observed the similar event at the 2µg/kg bw dose of BPA, when administrated for consecutive 14 days in adult rats. Spermatogenesis is highly sensitive to fluctuations, particularly hormones like testosterone, which is required in large concentration to maintain the process, which is achieved via the binding of testosterone by androgen binding protein present in seminiferous tubules. Testosterone produced by interstitial cells, also known as leydig cells, which reside adjacent to the seminiferous tubule. In the present study decrement in serum testosterone level was also

observed in rats exposed to BPA in a dose dependent manner which indicates spermatogenesis disruption. Wisniewski (23) also reported that exposure of BPA at 5mg and 50mg/kg/day dose level for 60 days decreased the plasma level of testosterone in adult male rats. Histological study done following BPA treatment on the various reproductive organs found no change in vas deferens showed that its structure remains unaltered. The vas deferens also called ductus deferens, transport sperm from the epididymis to the ejaculatory ducts. No changes have been reported in it as also in the present finding.

Seminal vesicles composed of tubular alveoli and the mucosa thrown into an intricate system of folds with the epithelium overlaying the lamina propria (34), no changes were observed. Secretion of the seminal vesicles constitutes the main (50%) and the last fraction of the ejaculates. The growths of seminal vesicles are highly dependent on androgen (35, 36). In rats, any increase in serum testosterone or treatment with androgens were found to be associated with increased secretory activity of the seminal vesicles (37,38) and increased seminal vesicle weight (39) but as hormonal level decrease weight of seminal vesicle also decreased, as also observed in the present study.

Normal Histology of prostate in rat showed several alveoli containing tubular and cryptic epithelium and lumen containing eosinophilic secretory material. No structural changes were evident in the present study except the increased weight of prostate in dose dependent manner in BPA treated rats. Treatment with 10 µg/kg BPA resulted in increased animal weight and prostate epithelial height compared with the controls. These results indicated that environmental exposure to low doses of BPA may induce proliferation of prostate in adult rats which cause weight increment (40). As observed too in the present study where weight of prostate increased with the dose.

Vitamin E an antioxidant present particularly in high amounts in Sertoli cells and pachytene spermatocytes and to a lesser extent in round spermatids (26) and is absolutely vital for the maintenance of mammalian spermatogenesis. Supplement dose of Vitamin E during BPA exposure in the study overcome the adverse effect of BPA to some extent. The results showed that more amounts of sperm observed in Vitamin E intervention groups and the level of testosterone also increased as compared to BPA treated groups though not comparable to the control group. Histological structure of epididymis of Vitamin E intervention group animals were found as same as control group animals. Studies says that rats gavaged with 0.5 mg/kg bw of BPA and 150mg/kg bw Vitamin E showed recovery from BPA effects (41) which is similar with the present finding.

In our study we also observed that if BPA was not exposed to male wistar rats for short duration after 90 days of its exposure then adverse effects caused by it can be recovered or not. For this after completion of 90 days of treatment phase we kept all BPA treated animals on 45 days of recovery phase. After completion of 45 days of recovery phase we observed no recovery.

Conclusion:-

The present study concludes that BPA exposure for consecutive 90 days in adult male albino rats at dose 5, 50 and 100µg adversely affects the normal reproductive functioning of the males by mainly altering the testosterone levels. Whereas, supplementation with Vitamin E resists these effects, therefore the study suggests a further investigation in this direction in finding solution to this problem. We also evaluate that the damaging effect of BPA remains in reproductive organs and cannot be recovered if even after BPA exposure was stopped.

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Conflict of interest:-

No

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