Research Article

Genotoxic and Infertility Effects of Bisphenol A on Wistar Albino rats

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ABSTRACT

The plastic monomer and plasticizer bisphenol A (BPA) is one of the highest volume chemicals produced worldwide. It is used in the production of polycarbonate plastics and epoxy resins used in many consumer products. The study was designed to determine genotoxic and infertility effect of BPA. Adult male wistar albino rats were orally administered with various doses of BPA (5 µg, 50 µg and 100 µg/100g bw) and vitamin E (4 mg/100g bw) once a day for 90 days. Decline in testicular protein and LDH level and increases in the frequency of micronucleus in polychromatic erythrocytes and normochromatic erythrocyte were observed at various dose treated groups as compared to control and vitamin E intervention groups. Study concludes that, BPA exposure partly inhibit the reproductive function in male rat and cause genotoxicity whereas, supplementation of vitamin E during BPA exposure have certain protective effect on reproductive inhibition caused by BPA exposure.

Keywords: Bisphenol -A, LDH, protein, micronuclei, genotoxicity.

INTRODUCTION

Thousands of anthropogenic chemicals are present in the environment, and mounting evidence indicates that some have endocrine-disrupting effects in a variety of organisms. Of particular concern are chemicals that act as agonists or antagonists on vertebrate estrogen or androgen receptors. One such compound is bisphenol A (BPA), a synthetic monomer used in production of polycarbonate plastics, epoxy resins, food packaging, dental sealant and lacquers for food cans[1] Human beings are exposed to BPA, as it leaches from the inner lining of tin cans and microwave containers during heating into the food materials[2], from dental sealant into saliva[3] and into beverages from the polycarbonate bottles due to the repeated usage or contact with any acidic/alkaline contents[4]. BPA concentration has been detected in human serum and in 95% of the urine samples obtained from a reference population in the USA[5]. The presence of BPA has been reported in maternal and fetal plasma[6], placental tissue[7] and in the milk of lactating mothers[8].Higher levels of urinary BPA has been correlated with cardiovascular disease and diabetes and may be associated with increased risk of miscarriages with abnormal embryonic karyotype[9, 10]. All these reports confirm that indeed human beings are getting exposed to BPA. This has raised a great concern regarding human health and environmental exposure to BPA[11] .Various animal models of BPA exposure have revealed multiple effects on the male and female reproductive system Mendiola *et al.*[12] reported that exposure to environmental dose of BPA decreased the plasma level of testosterone in adult male rats Tohei *et al.*[13] also reported the similar effect on testosterone level. In another study oral administration of low dose of BPA (2µg/kg) for consecutive 14 days in adult rats also impairs spermatogenesis[14]. Exposure of adult male rats with BPA resulted in decrease in sperm count and motility and also affected sperm morphology[15,16] .The oral exposure of pregnant mice to BPA at doses of 2–20µg/kg/day caused enlargement of prostate gland, decrease in size of seminal vesicle and epididymis, and daily sperm production in male offsprings[17].BPA has been associated with declined semen quality and increased sperm DNA damage among men from an infertility clinic[18]. Our own studies in rats confirmed that BPA is an endocrine disruptor and at low dose it causes infertility by affecting spermatogenesis which cause decrement in sperm production[19]. The above mentioned reports are also supporting the fact of germ cell toxicity potential of BPA.

BPA was shown to induce aneuploidy and DNA adducts formation in Syrian hamster embryo cells[20]. BPA has been observed to induce micronuclei formation in organisms like mussel gills[21] and fish erythrocytes[22] but evidences for this effect in *in-vivo* studies is inconsistent and inconclusive. Therefore, it is important to study the genotoxic activity of BPA in an *in-vivo* mammalian system. Currently there are few *in-vivo* genotoxicity studies carried out in bone marrow cells of mice upon BPA exposure at different time interval (one to five days), which document that BPA failed to induce micronuclei formation [23, 24].Based on a few *in-vivo* studies it is not possible to draw a definite conclusion about genotoxic activity of BPA as it is estrogenic in nature.

Previous studies have reported the occurrence of oxidative stress after BPA exposure in rats and mice[25, 26] .A state of oxidative stress in the testes disrupts both spermatogenesis[27]. 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[28]. Vitamin E has also been shown to suppress lipid peroxidation in testicular microsomes and mitochondria[29,30] and is absolutely vital for the maintenance of mammalian spermatogenesis. Aim of the present study was to assess the possible genotoxic effects of BPA exposure by measuring the frequency of micronucleus (MN) in polychromatic erythrocytes (PCEs) in bone marrow cells. Hormonal and biochemical study also perform to find the reason behind the spermatogenesis disruption and infertility and to explore the effect of vitamin E on reproductive function, if it will be supplement with BPA.

MATERIALS 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 libitam*. The animals were maintained under perfect veterinary supervision and accordance to the guidelines of CPCSEA [31].

Test Chemical

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) (<99% pure) 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) was purchased from medical store and diluted in olive oil to obtain final concentration 4mg/100gm body weight of the animals.

Experimental Design

Animals were divided into seven groups with ten in each and olive oil was used as vehicle.

Group I: Control (vehicle treated)

Group II: Oral administration of 5µg BPA/100 g/bw

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

Group IV: Oral administration of 50 µg BPA/100 g/bw

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

Group VI: Oral administration of 100 µg BPA/100 g/bw

Group VII: Oral administration of 100 µg BPA/100 g/bw +4mg/100g/bw Vitamin E

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

Biochemical studies

Lactate dehydrogenase (LDH) estimation

The portion of testis was used for the analyses of androgen sensitive biochemical marker lactate dehydrogenase (LDH)[32]. Activity of LDH was determined spectrophotometrically. Elevated levels showed cellular damage.

Protein estimation

The portion of testis was used for the assay of protein[33]. Briefly, tissue homogenate was prepared in ddH_2O. Further to this Tri carboxilic acid (TCA) was added and centrifuged for 10 min at 2000 rpm, precipitate will be collected and 1N NaOH added and kept in boiling water for 5 min. The solution was incubated with reagent D (0.1 N NaOH, sodium tartrate and $CuSO_4$) for 10 min. Finally Folin reagent (FC) added and absorbance was estimated on spectrophotometer.

Toxicology tests

Micronucleus test

Bone marrow cells from sacrificed animals were collected in fetal albumin serum (FBS) and after washing smeared on to glass slide. Further, slides were stained with May-Gruenwald and Giemsa stain[34]. For positive control animals were injected with mitomycin C (3µg/gram body weight) before 48 hours of scarification.

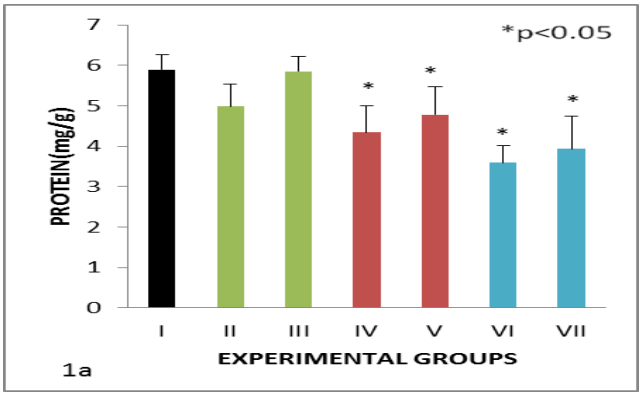
Statistical analysis

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 (ANVOA) test. P<0.05 was considered as significant.

RESULTS

Effect on testicular protein and LDH

The levels of testicular protein were significantly lower in the BPA treated rats than those in the control rats and vitamin E intervention groups at all doses 5µg BPA/100 g/bw, 50 µg BPA/100 g/bw and 100µg BPA/100 g/bw.(figure 1a)



Testicular LDH level increased in the BPA treated rats than those in the control rats and vitamin E intervention groups at all doses. (figure 1b)

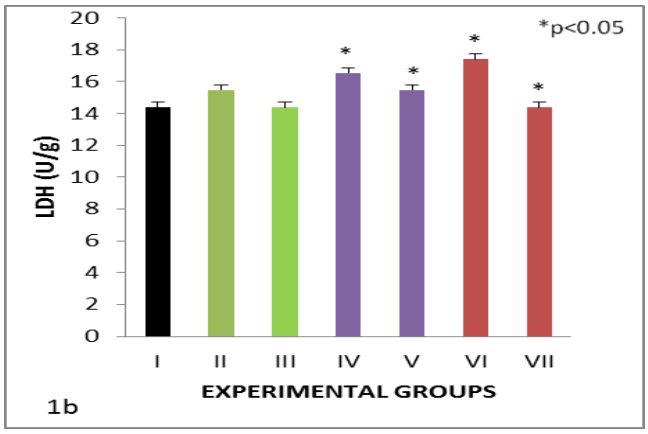


Figure 1b: Effect of BPA and vitamin E on (a) testicular protein (b) LDH level. I. vehicle control, II: 5µg BPA/100 g/bw, III: 5µg BPA/100 g/bw + 4mg/100g/bw Vitamin E, IV:50µg BPA/100 g/bw, V:50µg BPA/100 g/bw+ 4mg/100g/bw Vitamin E, VI: 100µg BPA/100 g/bw, VII: 100µg BPA/100 g/bw+ 4mg/100g/bw Vitamin E

Micronucleus test

The stained slides were analyzed at 100X magnification using oil immersion, the results of the study showed micronuclei in polychromatic erythrocytes (PCEs), normochromatic erythrocyte (NCE) as shown in figure 2 and the effect of BPA on frequency of micronucleus in these cells and the PCE/NCE ratio in rat bone-marrow cells are presented in table 1. An increase in number of MN-PCEs was observed in male rats treated with BPA at various doses compared to vitamin E intervention group and control group. However, the increase in frequency of MN-PCEs observed in positive control group was significantly higher than that of vehicle control and treated group demonstrating the sensitivity of the test system. The cytotoxic effect of BPA on bone marrow cell was tested by assessing polychromatic PCE/NCE ratio. As far as PCE/NCE ratio is concerned it remained well within the acceptable range and the ratio was comparable with the vehicle control.

DISCUSSION

The present study documents the genotoxic activity of BPA using a micronucleus test. The data obtained from this assay clearly showed an increase in the frequency of MN in bone marrow cells of adult male rats which were exposed to various doses of BPA. These results demonstrate genotoxic effect of BPA in *in-vivo* systems and it affects the proliferative activity of bone marrow. The induction of MN following oral administration in rats observed in our study is in agreement with the data published, wherein nitrosylated BPA could induce MN in the bone marrow cells of mice[35] .Further, these observations also confirm the earlier *in vitro* studies, where BPA induced micronuclei in cell types like hamster V79 cells[36] human MCL-5 cells[37] human lymphoblastoid cell lines AHH- 1, MCL-5 and Chinese hamster V79 cell lines[38] respectively. One of the possibilities of the presence of micronuclei could be due to the aneugenic effect of BPA[39, 40]. Earlier study also demonstrated that exposure of BPA for six consecutive days in adult male and female rats at 5mg even at 10µg showed micronuclei formation which consistent with our study[41]. Significant increase in micronucleus frequency in Chinese hamstar ovary cells clearly demonstrated that BPA exhibit genotoxic effect[42] and confirmed its genotoxicity.

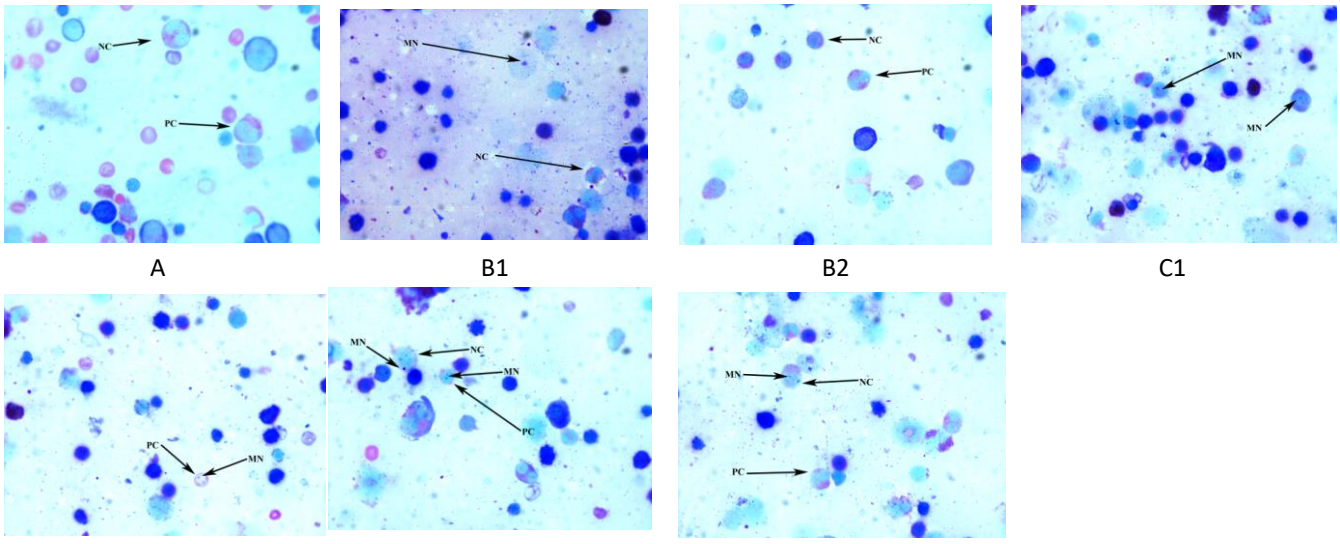


Figure 2: Photomicrograph of erythrocytes from bone marrow of rats showing micronuclei in erythrocyte population. A. vehicle control B1: 5µg BPA/100 g/bw, B2: 5µg BPA/100 g/bw + 4mg/100g/bw Vitamin E, C1:50µg BPA/100 g/bw, C2:50µg BPA/100 g/bw+ 4mg/100g/bw Vitamin E, D1: 100µg BPA/100 g/bw, D2: 100µg BPA/100 g/bw+ 4mg/100g/bw Vitamin E, polychromatic erythrocytes (PCEs), normochromatic erythrocyte (NCE) micronuclei (MN)

Table 1: Micronucleus record of male albino rats following BPA and vitamin E treatment

Groups	Dose	Micronuclei in P.E (%)	Micronuclei in N.E. (%)	Micronuclei in Erythrocyte (%)	P.E. /N.E (%)
I(control)	Olive oil	4/2362 (0.16%)	5/9635 (0.05%)	9/12047 (0.07%)	2362/9635 (24.51 %)
II	5µg (BPA)	6/2452 (0.24%)	7/9818(0.07%)	13/12270 (0.10%)	2452/9818 (24.97%)
III	50µg (BPA)	8/2510 (0.31%)	9/9365 (0.09%)	16/11875 (0.13%)	2510/9365 (26.80%)
IV	100µg (BPA)	12/2456 (0.48%)	11/9712 (0.11%)	23/12168 (0.18%)	2456/9712 (25.05%)
V	5µg (BPA +Vit.E)	5/2453 (0.20%)	4/9526 (0.04%)	9/11979 (0.07%)	2453/9526 (25.75%)
VI	50µg (BPA +Vit.E)	6/2351(0.25%)	5/9634 (0.05%)	11/11985 (0.09%)	2351/9634 (24.40%)
VII	100µg (BPA +Vit.E)	6/2451(0.24%)	7/9625 (0.07%)	13/12076 (0.10%)	2451/9625 (25.46%)
Positive control	Mitomycin C 3µg/g bw	48/2008 (2.39%)	41/9891 (0.41%)	89/11899 (0.74%)	2008/9891 (20.30%)

Furthermore our study also demonstrates that BPA also cause adverse effect on testicular tissue by observing the alteration in the level of protein and LDH. The LDH plays an important role in the cell metabolism. It is located mainly in the interstitial tissue of both foetal and adult rat testis[43]. It has been shown that the normal adult testis of[44, 45] rat, rabbit, dog, guinea pig, bull and pigeon[46] contain usual isoenzymes of LDH isoenzymes designated as LDH-X. The LDH-X is correlated with the onset of sexual maturation[47] sperm cell number and motility[48]. Appreciable changes in the concentration of LDH indicate that secretory activity of the testis got affected. Level of LDH get elevated in dose depended BPA groups as compare to control and vitamin E intervention groups.

The most profound general metabolic action of androgen is the promotion of protein anabolism which induces nitrogen retention in the form of tissue protein[49]. The testicular protein synthesis in the hypophysectomized adult mice was stimulated by gonadotropines[50]. The LH has been shown to stimulate protein synthesis in the adult rabbit testis in vitro. The effect of LH on steroidogenesis is secondary to its stimulatory action on testicular protein synthesis[51].The FSH has relatively little effect on protein synthesis in spermatids. However, it has been reported that FSH stimulates protein synthesis in rat Sertoli cells[52].Testicular necrosis following increased temperature stimuli to scrotal sac[53] and antispermatogenic drug administration[54] lowers the testicular protein contents. It would seem, that the decreased protein contents in testes were mainly due to the fact that later stages of spermatogenesis were absent, because the total protein contents depend upon the number of spermatozoa present in the testes[55, 56]. No considerable change showed normal spermatogenesis in the testes but in BPA treated groups we observed decreased level of protein in testis compare to vehicle treated and group a supplemented vitamin E with BPA.

CONCLUSION

The results reported herein demonstrated that BPA exposure for consecutive 90 days in adult male albino rats at dose 5, 50 and 100µg cause disruption in spermatogenesis decrement in testicular protein and elevation in LDH level. It also has genotoxic activity which was confirmed by the formation of micronuclei in BPA treated groups. The results of our study also suggest that simultaneous administration of vitamin E may ameliorates the adverse effects of BPA on reproductive function. Our obtained results indicate that BPA cause harmful effect on human health and further research on vitamin E intervention and genotoxic activity of BPA is needed.

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