Original Article

Glutamine Supplementation Attenuates Bisphenol A-Induced Testicular Toxicity in Rats

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Abstract

Background: The induction of testicular toxicity by bisphenol A (BPA) may involve oxidative stress. Glutamine (Gln) is a rate limiter for the synthesis of glutathione (GSH), which inhibits oxidative stress. **Aim:** This study assessed the potential of BPA to preserve testicular structure and function in BPA-treated albino rats. **Study Settings and Design:** Thirty-two adult male albino rats (210–250 g) were randomly allocated to 4 (A-D) of 8 rats per group with the approval of the Research Ethics Committee. **Materials and Methods:** Groups B-D were orally treated with Gln (20 mg/kg body weight), BPA (50 mg/kg body weight) and Gln (20 mg/kg body weight) + BPA (50 mg/kg body weight) daily for 65 days, respectively. Group A (control) was orally treated with normal saline (0.2 mL) daily for 65 days. At the termination of treatment, the rats were weighed and anaesthetized blood samples were collected and evaluated for gonadal hormones. The testes were weighed and evaluated for sperm parameters, oxidative stress markers and histology. **Statistical Analysis Used:** The data were analysed using one-way analysis of variance and Bonferroni *post hoc* test. **Results:** BPA caused a significant ($P < 0.001$) decrease in testis and body weights, sperm count, volume, motility and normal morphology when compared to the control. BPA significantly ($P < 0.001$) decreased serum testosterone, follicle-stimulating hormone, luteinising hormone, testicular catalase, superoxide dismutase, GSH and GSH peroxidase levels relative to control. Significantly ($P < 0.001$) increased serum prolactin, estradiol and testicular malondialdehyde levels occurred in BPA-treated rats relative to control. The testes of BPA-treated rats showed sloughing and coalescence of germ cells. However, Gln supplementation prevents BPA-induced testicular toxicity. Gln supplementation restored testis histology. **Conclusion:** Based on the observation in this study, Gln seems effective against BPA-induced testicular toxicity.

Keywords: *Bisphenol A, glutamine, protection, rat, testis, toxicity*

Introduction

Toxicology of the male reproductive system has received increased interest due to reports of falling sperm counts and rising reproductive disorders in humans.[1] In recent years, there have been documented concerns on the deleterious effects of chemicals on the male reproductive system.[2] These chemicals include various classes of therapeutic drugs, industrial chemicals, solvents, agrochemicals and food additives.[3] Among these chemicals, the impact of endocrine-disrupting chemicals (EDCs) on the male reproductive system has gained serious attention.[4] Early-life exposures to EDCs have been associated with developmental

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abnormalities.[5] In adult human males, reproductive disorders such as testicular cancer and reduced sperm counts may be due to exposure to EDCs.[6]

Bisphenol A (BPA) is an EDC widely used for the production of refillable drinking containers, dental sealants, plastic utensils, linings of metal cans and food packaging materials.[7] It mimics the activity of endogenous oestrogen, a discovery that caused great concern in recent years.[8] The primary route of human exposure to BPA is speculated to occur via diet, when it leeches from food and drink containers.[9] Data from investigation showed that 93% of tested people in the US had detectable levels of BPA in their urine.[10] Studies in occupationally exposed workers correlated cumulative BPA exposure with a possible risk of male sexual dysfunction.[11] In animal models, investigations demonstrated testicular toxicity marked by disruption in the functions of reproductive hormones, decreased sperm quality and testis weight due to BPA exposure.[12,13] Oxidative stress characterized by the generation of reactive oxygen species (ROS), decreased antioxidants and lipid peroxidation (LPO) in the testes are noteable features observed in animal studies.[14]

Glutamine (Gln) is an amino acid that plays several biological functions, including cell proliferation, energy production, glycogenesis and maintenance of acid–base balance.[15] In most cells, it can serve as a substrate for nucleotide synthesis, nicotinamide adenine dinucleotide phosphate and other biosynthetic pathways involved in the maintenance of cellular integrity and function.[16] Glutamate resulting from Gln is a substrate for glutathione (GSH) synthesis one of the most important antioxidants.[16] GSH acts as an antioxidant that inhibits oxidative stress either directly by interacting with ROS and electrophiles or by operating as a cofactor for various enzymes.[17] Gln supplementation can increase antioxidants syntheses, thereby upregulating their activities.[18] It has a number of immunomodulatory functions including the upregulation of the functions of anti-inflammatory mediators.[19] The capacity of Gln to provide protective and resistance responses to injuries have been documented in animal models. These include protection against cisplatin-induced nephrotoxicity,[20] cadmium-induced testicular dysfunction[21] and BPA-induced intestinal injury in mice.[22] This study, therefore, assessed the protective effect of Gln on BPA-induced testicular dysfunction in albino rats.

Study Setting and Design

Thirty-two adult male albino rats (210 – 250g) were used. The rats were placed in cages and were acclimated for 2 weeks with access to feed and water *ad libitum*. The rats were kept under temperature 28°C and 12 h light/12 h dark cycle. The rats were handled in accordance with the Guide for the Care and Use of Laboratory Animals.[23] BPA (Loba Chemie Pvt. Ltd, India) and L-Gln (Qualikems Fine Chem Pvt Ltd, India) were used. Ethical approval (PHARM/2021/023) was obtained from the Research Ethics Committee of the Department of Pharmacology/Toxicology, Madonna University, Elele, Rivers State.

Materials and Methods

Thirty-two adult male albino rats were allocated to 4 groups (A-D) of n = 8/group and were orally treated daily for 65 days as follows: Group A, vehicle (normal saline [0.2 mL]); Group B, Gln (20 mg/kg/body weight)[24] in vehicle; Group C, BPA (50 mg/kg body weight)[25] in vehicle; and Group D, Gln (20 mg/kg body weight) + BPA (50 mg/kg body weight). After treatment, the rats were anaesthetized and blood samples were collected from the heart and assessed for gonadal hormones. The rats were dissected and the testes were collected and weighed. The testes were assessed for sperm parameters, oxidative stress markers and histology.

Hormonal assay

The blood samples from each group were centrifuged (2000 g for 10 min) in a refrigerated centrifuge (Eppendorf 5804R, Hamburg, Germany) and serum samples were collected. Serum samples were analysed for testosterone, luteinising hormone (LH) and follicle-stimulating hormone (FSH), prolactin and estradiol using an enzyme immunoassay kits (Alfa Scientific Designs, California, USA).

Evaluation of sperm parameters

The caudal epididymis was dissected using a surgical blade and the seminal content was obtained and squeezed in a sterile clean watch glass. The seminal content was diluted 10 times with sodium citrate dihydrate solution (2.9%) and thoroughly mixed. It was used for the estimation of progressive motility, sperm count, normal morphology, volume and viability.

Evaluation of oxidative stress markers

The testes were collected and washed with ice-cold isotonic saline (0.9%). The testes were homogenised in a 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer and 10% w/v homogenates were prepared. The homogenates were decanted and the supernatants collected. The supernatants were assessed for malondialdehyde (MDA) as reported by Buege and Aust,[26] superoxide dismutase (SOD) as described by Sun and Zigman,[27] catalase (CAT) as reported by Aebi,[28] GSH as explained by Sedlak and Lindsay[29] and

glutathione peroxidase (GPx) according to the method explained by Rotruck *et al*.[30]

Histological study

The testes were collected weighed and fixed in Bovine's solution for 24 h. The testes were then dehydrated in ascending grades of ethyl alcohol. Serial sections (3-μm thick) were cut and stained with hematoxylin and eosin. The stained sections were examined with the aid of a light microscope.

Statistical analysis

Data were expressed as mean ± standard error of the mean. The group means were evaluated using one-way analysis of variance and Bonferroni *post hoc* test. Significance was set at $P < 0.001$

Results

Effects of glutamine on body and testis weights of bisphenol A-treated rats

Significantly ($P < 0.001$) decreased body and testis weights were observed in BPA-treated rats when compared to the control [Table 1]. However, Gln supplementation restored body and testes weights significantly ($P < 0.001$) when compared to BPA-treated rats [Table 1].

Effect of glutamine on sperm parameters of bisphenol A-treated rats

Significantly ($P < 0.001$) decreased sperm count, motility, normal morphology, volume and viability occurred in BPA-treated rats when compared to the control. However, Gln supplementation significantly ($P < 0.001$) increased the aforementioned sperm parameters when compared to BPA-treated rats [Table 2].

Effect of glutamine on gonadal hormones of bisphenol A-treated rats

Serum testosterone, FSH and LH levels decreased significantly ($P < 0.001$) in BPA-treated rats when compared to the control. However, Gln supplementation significantly ($P < 0.001$) increased serum testosterone, FSH and LH levels when compared to BPA-treated rats [Table 3]. BPA significantly ($P < 0.001$) increased serum prolactin and estradiol levels when compared to control. On the other, Gln supplementation significantly decreased ($P < 0.001$) serum prolactin and estradiol levels when compared to BPA-treated rats [Table 3].

Table 1: Effects of glutamine on body and testes weights of bisphenol A-treated rats

Treatment (mg/kg)	Final body weight (g)	Testis weight (g)	Relative testis weight (%)
Control	356.62±19.51	3.50±0.03	0.98±0.12
Gln 20	344.54±18.73	3.48±0.14	1.01±0.24
BPA 50	210.75±19.66[a]	1.22±0.05[a]	0.58±0.10[a]
Gln 20 + BPA 50	322.85±17.48[b]	3.01±0.06[b]	0.93±0.18[b]

[a]$P<0.001$ Significant difference when compared to control, [b]$P<0.001$ Significant difference when compared to BPA (ANOVA followed by Bonferroni *post hoc* test). Data as mean±SEM (n=8). Gln=Glutamine, BPA=Bisphenol A, SEM=Standard error of mean, ANOVA=Analysis of variance

Effect of glutamine on testis oxidative stress makers of bisphenol A-treated rats

BPA significantly ($P < 0.001$) decreased testes GSH, GPx, CAT and SOD levels and significantly ($P < 0.001$) increased testes MDA levels when compared to control [Table 4]. However, Gln supplementation significantly ($P < 0.001$) increased testes SOD, CAT, GPx and GSH and significantly ($P < 0.001$) decreased testes MDA levels when compared to BPA-treated rats [Table 4].

Effect of glutamine on testis histology of bisphenol A-treated rats

The control rat treated with vehicle [Figure 1a] and rat treated with Gln [Figure 1b] showed seminiferous tubules containing germ cells and normal spermatozoa. However, rats treated with BPA showed sloughing and coalescence of germ cells [Figure 1c]. On the other hand, Gln supplemented rats showed seminiferous tubules containing normal gern cells and normal spermatozoa [Figure 1d].

Discussion

The global quantity of BPA consumed due to its applications was estimated to be 7.7 million metric tons in 2015 and could be 10.6 million metric tons in 2022.[31] Due to its applications, there is increased attention on the effect of BPA on the health of humans. Studies in animal models showed a possible correlation between BPA level and testicular toxicity.[32] Gln is an amino acid, which showed promising cytoprotective activity in animal studies.[22] This study examined the protective effect of Gln on BPA-induced testicular toxicity in rats. Body and organ weights are important indicators of the adverse effects of xenobiotics and are considered important in toxicity studies. In this study, the body and testes weights of BPA-treated rats decreased significantly. This is consistent with the observation in rats treated with BPA (50 mg/kg/day) for 30 days.[25] The observed decrease in body weight may be attributed to the appetite suppression whereas decrease in testis weight may be due the inhibition of hypothalamic–pituitary–gonadal (HPG) axis by BPA. However, supplementation with Gln restored body and testes weights appreciably. Sperm parameters, including motility, normal morphology, volume viability and counts are frequently and routinely used as

Table 2: Effect of glutamine on sperm parameters of bisphenol A-treated rats

Treatment (mg/kg)	Sperm count (×10⁶)	Sperm motility (%)	Normal morphology (%)	Semen volume (mL)	Viable sperm (%)
Control	70.88±7.67	82.32±7.45	80.21±8.12	0.73±0.07	84.65±7.11
Gln 20	74.64±8.68	88.43±2.07	83.33±9.11	0.80±0.05	87.68±8.93
BPA 50	35.79±4.62[a]	38.78±3.41[a]	41.22±4.17[a]	0.34±0.08[a]	42.20±6.31[a]
Gln 20 + BPA 50	65.33±6.31[b]	70.65±8.03[b]	72.33±8.19[b]	0.69±0.10[b]	72.95±8.60[b]

[a]P<0.001 Significant difference when compared to control, [b]P<0.001 Significant difference when compared to BPA (ANOVA and Bonferroni *post hoc* test). Data as mean±SEM (n=8). Gln=Glutamine, BPA=Bisphenol A, SEM=Standard error of mean, ANOVA=Analysis of variance

Table 3: Effect of glutamine on gonadal hormones of bisphenol A-treated rats

Treatment (mg/kg)	Testosterone (ng/mL)	FSH (ng/mL)	LH (ng/mL)	Prolactin (ng/mL)	Estradiol (pg/mL)
Control	7.68±0.67	13.32±1.45	9.97±0.17	500.66±30.7	5.54±0.17
Gln 20	7.97±0.68	14.43±1.07	10.02±1.05	465.54±33.3	5.66±0.20
BPA 50	2.62±0.62[a]	5.78±0.41[a]	3.33±0.38[a]	974.20±40.3[a]	12.43±2.11[a]
Gln 20 + BPA 50	7.08±0.31[b]	11.65±1.23[b]	8.74±1.10[b]	442.95±25.6[b]	6.75±1.12[b]

[a]P<0.001 Significant difference when compared to control, [b]P<0.001 Significant difference when compared to BPA (ANOVA and Bonferroni *post hoc* test). Data as mean±SEM (n=8). Gln=Glutamine, BPA=Bisphenol A, SEM=Standard error of mean, ANOVA=Analysis of variance, FSH=Follicle-stimulating hormone, LH=Luteinising hormone

Table 4: Effect of glutamine on testis oxidative stress makers of bisphenol A-treated rats

Treatment (mg/kg)	GSH (µmol/g protein)	GPx (U/mg protein)	CAT (U/mg protein)	SOD (U/mg protein)	MDA (nmol/L)
Control	13.88±1.67	25.32±2.45	17.11±1.17	34.66±3.11	5.54±0.17
Gln 20	14.01±1.68	26.43±2.07	18.74±1.35	36.54±3.93	8.13±0.17
BPA 50	4.22±0.62[a]	10.78±0.41[a]	7.33±0.38[a]	14.20±2.31[a]	17.43±1.11[a]
Gln 20 + BPA 50	11.33±0.31[b]	23.65±2.03[b]	15.73±1.10[b]	30.95±4.60[b]	6.75±0.12[b]

[a]P<0.001 Significant difference when compared to control, [b]P<0.001 Significant difference when compared to BPA (ANOVA and Bonferroni *post hoc* test). Data as mean±SEM (n=8). Gln=Glutamine, BPA=Bisphenol A, SEM=Standard error of mean, ANOVA=Analysis of variance, GSH=Glutathione, GPx=GSH peroxidase, CAT=Catalase, SOD=Superoxide dismutase, MDA=Malondialdehyde

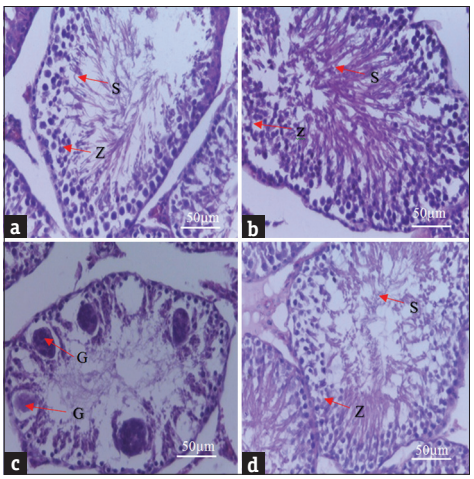

Figure 1: D showed the testes of the control rats and rats in the experimental groups. (a): Control, (b): Treatment with glutamine (20 mg/kg body weight), (c): Treatment with bisphenol A (50 mg/kg body weight) (d): Treatment with glutamine (20 mg/kg body weight) + bisphenol A (50 mg/kg body weight). G: Sloughing and coalescence of germ cells. S: Normal seminiferous tubules containing germ cells. Z: Normal spermatozoa × 400 H and E

determinants for reproductive toxicity.[33] Studies have correlated appreciable decreased concentrations of the aforementioned sperm parameters with reproductive toxicity.[34] The current study observed low levels of sperm motility, normal morphology, volume, viability and counts in PBA-treated rats. Karnam *et al*.[35] reported similar findings in rats treated with BPA (50–600 mg/kg/day) for 30 days. Spermatogenesis is a highly complex process mainly regulated by testosterone and inhibin B hormones released by Leydig and Sertoli cells, respectively. Any disturbance in hormonal levels may compromise the spermatogenic process, resulting in abnormal sperm parameters and reduced fertility. BPA might have decreased sperm parameters by inhibiting androgen production and Sertoli cells function.[32] Studies in animal models also showed that BPA directly acts on Leydig cells, reducing their proliferation and impairing normal steroidogenesis.[32,36,37] However, supplementation with Gln restored the levels of sperm motility, normal morphology, volume, viability and counts. LH, FSH and testosterone are essential for spermatogenesis. LH is secreted from the pituitary gland and triggers testosterone production. Testosterone is required for sperm production and maturity[38] whereas, FSH in collaboration with testosterone stimulates spermatid growth and sperm release.[39] Studies have shown that impaired levels of the aforementioned hormones can arrest spermatogenesis.[40] In the present study, treatment with BPA decreased LH, FSH and testosterone hormone

and increased progesterone and estradiol hormones. Similarly, Alboghobeish *et al*.[41] reported decreased LH, FSH and testosterone hormone in BPA (50 mg/kg/day)-treated rats for 30 days. The altered levels of reproductive hormones observed in this study may be due to the oestrogenic activity of BPA via its inhibitory effect on HPG axis.[32] HPG axis regulates testicular function via the production of LH, which stimulates the production of testosterone. Studies have showed that BPA can indirectly suppresses the synthesis and release of LH from the pituitary through aromatase upregulation in testes, activating the mechanisms of negative hormonal feedback.[42]

Oxidant-antioxidant balance is paramount for cellular function and the wellbeing of biological systems. Under physiological conditions, antioxidant defence such as SOD, CAT, GPx and GSH eliminate the excess oxidative activity of ROS.[43] However, exogenous compounds as pro-oxidants could disrupt oxidant–antioxidant equilibrium causing a remarkable surge in ROS activity above the surmountable capacity of antioxidant defence system. This scenario depletes antioxidant defence causing damage to cellular components as a consequence of oxidative stress.[34] In the current study, BPA remarkably decreased testicular antioxidants (SOD, CAT, GSH and GPx). Similarly, Othman *et al*.[44] reported decreased antioxidants in the testes of BPA (50 mg/kg)-treated rats. The decreased antioxidants may be due to the capacity of BPA to induce testicular oxidative stress through the generation of ROS.[44] Oxidation of poly unsaturated fatty acids is termed LPO. MDA is often used as a yardstick for LPO.[45] This study observed elevated levels of MDA in the testes of BPA-treated rats. This supports the elevated level of MDA in the testes of BPA (25 mg/kg/day)-treated rats reported by Kalender *et al*.[46] However, Gln supplementation decreased testicular MDA levels. In toxicity studies, the histological assessment of reproductive organs is considered as one of the most sensitive endpoints for evaluating testicular injuries in animals.[46] This study showed sloughing and coalescence of germ cells to form multinucleated giant cells in the testes of BPA-treated rats. Similar observations were reported by Kalender *et al*.[46] However, Gln supplementation restored testicular histology.

Conclusion

This study showed that Gln supplementation protects testis weight, gonadal hormones, oxidative stress markers and testicular histology of BPA-treated rats. Gln may have clinical use in BPA-associated testicular toxicity.

Limitation of the study

The mechanism by which Gln protects against BPA-induced testicular toxicity in rats was not fully elucidated, which can be evaluated in further studies.

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Conflicts of interest

There are no conflicts of interest.

Data availability

Data are available on request from the corresponding author.

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