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Anti-infertility significance of aqueous extract of *Ipomoea batatas* (L.) Lam. against exposure of bisphenol A (BPA) promoted testicular toxicity in male Sprague Dawley rats

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ABSTRACT

Objective: To investigate the *in vitro* antioxidant activity of *Ipomoea batatas* (*I. batatas*) and anti-infertility efficacy of aqueous extract of *I. batatas* against Bisphenol A (BPA) induced testicular toxicity in experimental animals. **Methods:** The experimental rats were divided into four groups and each group consisted of six animals. Group II – Bisphenol A diluted in propylene glycol at a dose of 200 mg/kg body weight was administered orally for 30 days. Reproductive toxicity encouraged animals illustrate notable alterations in the activites of hormones like FSH and LH and also in the levels of adenosine triphosphatase. **Results:** The hypo-osmotic swelling test display damaged sperm membrane integrity and histopathological examination of BPA intoxicated animals display a stuctural and functional alterations of reproductive organs that would interfere with its function and contribute to infertility. **Conclusion:** Aqueous extract of *I. batatas* at the concentration of 400 mg/kg body weight/day administered orally for 45 days prevent biochemical changes and structural alterations of testis and epididymis due to bisphenol A administration.

1. Introduction

Infertility primarily refers to the biological inability of a person to contribute to conception[1]. Infertility has been recognized by the WHO as a problem affecting between 15% and 20% of couples in developed countries[2]. Infertility affects both men and women. Approximately half of the infertility cases are related to men[3]. There are several potential causes of male infertility. More than 90% of male infertility cases are due to low sperm counts, poor sperm quality, or both. Sperm abnormalities can be caused by a range of factors including environmental pollutants, exposure to high heat for prolonged periods, genetic abnormalities, heavy use of alcohol, marijuana, or cocaine, smoking, hormone deficiency or taking too much of a hormone[4]. In industrialized societies, humans are exposed to a wide spectrum of man-made chemicals. Endocrine Disrupting Chemicals (EDCs) are important class of chemicals that interfere with the production, release, transport, metabolism, binding, action, or elimination of natural hormones in the body that are responsible for the maintenance of homeostasis and the regulation of developmental processes. These chemicals in the environment have estrogenic activity[5]. Some of the common EDCs include bisphenol A (BPA), phthalates, and certain pesticides[6].

Bisphenol A (2, 2-bis (4-hydroxyphenyl) propane) is

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one of the highest volume chemicals produced worldwide with molecular formula $C_{15}H_{16}O_2$, with over six billion pounds produced each year. BPA, an estrogenic endocrine disrupting chemical with two unsaturated phenol rings, is used in the production of polycarbonate plastics, epoxy resins used to line metal cans, and in many plastic consumer products including toys, water pipes, drinking containers, eyeglass lenses, sports safety equipment, dental monomers, medical equipment and tubing, and consumer electronics. Oral, dietary exposure is currently considered a major route of human exposure to BPA[7]. BPA has been shown to alter endocrine function through multiple pathways, and a number of animal studies have reported adverse reproductive effects in males exposed to low levels of BPA in early life or in adulthood[8]. Sakaue *et al*. [9] have reported that BPA even at a low dose affects spermatogenesis in the adult rat.

Asian traditional medicine emphasizes the importance of prevention of illnesses and development of natural resistance to disease, and thus believes in the promotion of general well-being. Asian traditional medicine predominates in the Asian countries, and it is used for the treatment of various physical and mental illnesses. It has also been used for the treatment of sexual dysfunction in men[10].

2. Materials and methods

2.1. Plant material and authentication

The fresh tubers of *Ipomoea batatas* (*I. batatas*) were purchased from the local market in Chennai, Tamilnadu, India in the month of January 2011. The tuber was authenticated by the chief botanist Jayaraman, plant anatomy and research centre (PARC), Chennai, India. A voucher has been deposited in the Department of Pharmacology and Environmental Toxicology, Dr. ALM Post Graduate Institute of Basic Medical Science, University of Madras, Taramani, Chennai-600 113.

2.2. Preparation of the aqueous extract of I. batatas

The tubers of *I. batatas* were shade dried and then coarsely powdered. A known weight of the powder was soaked in 100 mL of distilled water and kept at room temperature for 12 h. Then it was filtered three times using Whatmann filter paper. The filtrate was poured on petridishes and concentrated to obtain a semisolid residue by using water bath at 66 ℃. The yield of the total aqueous extract was 19% and then the extract was stored in the refrigerator for further studies.

2.3. In vitro antioxidant studies

Scavenging of 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical was assayed[11]. Measurement of superoxide anion scavenging activity of aqueous extract of *I. batatas* was based on the method[12] with slight modifications[13]. The protective role of aqueous extract of *I. batatas* on the inhibition of free radical mediated DNA-sugar damage was assayed[14]. Total antioxidant activities of the aqueous extract of *I. batatas* were estimated by two methods *i.e.* Ferric thiocyanate (FTC) and Thiobarbituric acid method (TBA).

2.4. In vivo studies

2.4.1. Animals

Healthy adult male Sprague Dawley rats at the age group of 45-48 days weighing between 140-160 g were procured from the Central Animal House Facility, Dr.ALM PG IBMS, University of Madras, Taramani, Chennai, Tamilnadu, India. The rats were handled as per the guidelines from the Institutional Animal Ethics Committee (IAEC No. 05/02/2011). The rats received a standard rat pellet diet and water *ad libitum*. The rats were housed under conditions of controlled temperature (26±2 ℃) with 12 h light and dark cycle throughout the experimental period.

2.4.2. Acute toxicity studies and dose fixation

The animals were divided into different groups of animals. The first group served as control group. *I. batatas* was administered orally to different groups at the dose levels of 100, 250, 500, 1000, 2000, 4 000, 5 000 mg/kg/ body weight. All the animals were observed for toxic symptoms and mortality for 72 h. The absence of any adverse effects and mortality on administrations of acute dose of 5 000 mg of the aqueous extract of *I. batatas*/kg body weight indicates the non-toxic nature of the plant extract. Toxicologists agree that any substance that is not lethal when administered acutely at a concentration of 5 000 mg/kg body weight is non-toxic. Therefore a concentration of 400 mg/kg body weight was selected for this present investigation.

2.4.3. Experimental design

Adult male Sprague Dawley rats were divided into four groups and six animals in each. Group I - Control animals. Group II - Bisphenol A diluted in propylene glycol at a dose of 200 mg/kg body weight was administered orally for 30 days. Group III - Aqueous extract of *I. batatas* at the concentration of 400 mg/kg body weight/day was administered orally for 45 days after Bisphenol A treatment. Group IV -Aqueous extract of *I. batatas* at the concentration of 400 mg/kg bodyweight/day was administered orally for 45 days.

2.4.4. Collection of samples

After the experimental period, the animals were fasted overnight and sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected and the serum was separated by centrifugation at 2 000 rpm at 4 ℃ for 10 minutes stored at -20 ℃ until further analysis. The abdominal region was wiped with normal saline, scrotum was dissected to expose the testes, epididymides and extraneous connective tissues were trimmed. The dissected organs (right and left) from each rat in the experimental groups were weighed. The right testis and epididymis were fixed with buffered 6% formaldehyde solution for histological evaluations. The dissected organs were washed 2 to 3 times with saline and known weight of testis was homogenized in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was subjected to differential centrifugation and were used for the biochemical assays.

2.4.5. Biochemical estimation

The HOS test was performed[15]. FSH ELISA kit is a sandwich enzyme imunnoassay for the measurement of rat FSH in serum, plasma and other biological fluids. LH ELISA kit is a sandwich enzyme immunoassay for the measurement of rat LH in serum, plasma and other

biological fluids. Enzyme immunoassay kit was used for the quantitative determination of serum testosterone. Adenosine triphosphatase catalyse the conversion of adenosine triphosphate in to adenosine diphosphate. During the conversion, one molecule of phosphate is liberated. The inorganic phosphate was estimated[16]. Na^+/K^+– ATPase was estimated[17]. The activity of Ca^{2+}– ATPase was assayed[18]. The activity of Mg^{2+} – ATPase was assayed[19].

2.4.6. Histological studies

Testis was fixed with buffered 6% formaldehyde solution, embedded in paraffin, sectioned and stained routinely with hematoxylin and eosin, and was observed microscopically. The appropriate fixative, embedment and stain for testis are Bouin's, glycol methacrylate and periodic acid Schiff/ hematoxylin, but obvious and severe injuries can be easily detected by formalin fixation which has been used in most toxicity tests[20].

2.4.7. Statistical analysis

Data are presented as the mean ± standard deviation (SD). One way analysis of variance (ANOVA) followed by Tukey's multiple comparison method was used to compare the means of different groups of by using SPSS 12.5 student's versions. Comparisons were made between group II and IV with group I and III for animal studies. $P<0.05$ was considerable statistically significant in all cases.

3. Results

3.1. DPPH free radical trapping activity

The free radical trapping activity of the aqueous extract of *I. batatas* by DPPH method (Figure 1) shows the free radical trapping at various concentrations from 100, 200, 300, 400 and 500 μg/mL. Interestingly the *I. batatas* exhibits nearly 50% excellent activity at the concentration of 300 μg/mL, when nearly compared with the standard Vitamin C.

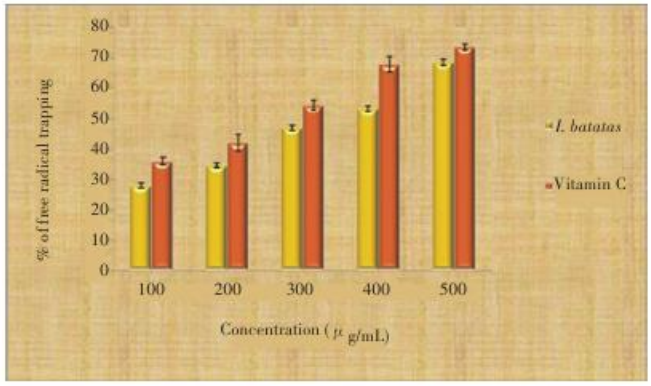

Figure 1. Free radical trapping of aqueous extract of *I. batatas* by DPPH method.

Values are mean ± SD of triplicate determinations.

3.2. Nitric oxide trapping activity

Figure 2 represents the trapping of the Nitric oxide radicals by various concentrations (100, 200, 300, 400 and 500 μg/mL) of the aqueous extract of *I. batatas*. The extract showed strong free radical inhibiting activity when compared with the standard Vitamin C. From these results it is inferred that the extract might be a potent peroxide inhibitor.

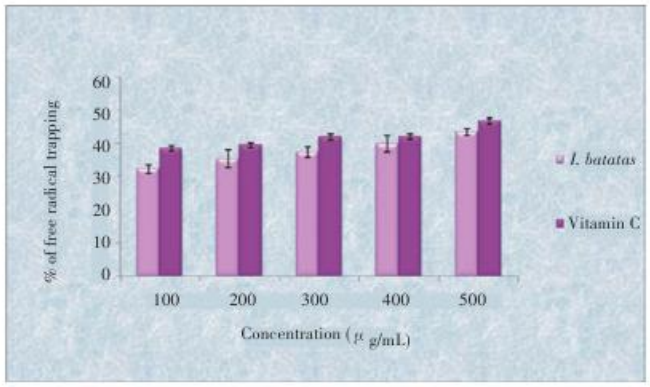

Figure 2. Nitric oxide radical trapping activity of aqueous extract of *I. batatas*.

Values are mean ± SD of triplicate determinations.

3.3. Super oxide anion trapping activity

In the present investigation, the dose dependent inhibition of super oxide radicals by the aqueous extract of *I. batatas* was performed and the results obtained were presented in Figure 3. The results showed the aqueous extract of *I. batatas* significantly inhibited the super oxide radicals at various concentrations of 100, 200, 300, 400 and 500 μg/mL. A remarkable inhibition of super oxide was observed in the concentration of 300 μg/mL with more than 50% of inhibition and this was comparatively well with the standard vitamin C.

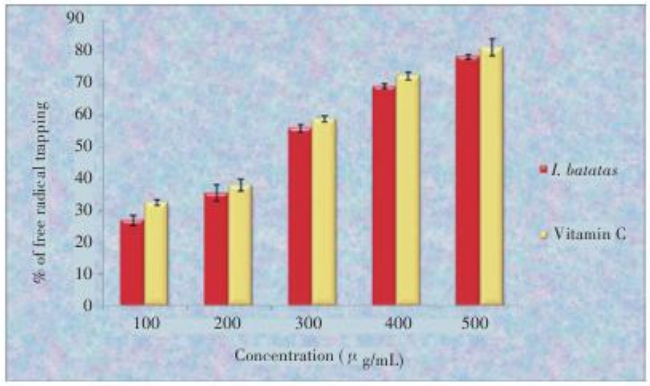

Figure 3. Superoxide anion radical trapping activity of aqueous extract of *I. batatas*.

Values are mean ± SD of triplicate determinations.

3.4. Protective effect on DNA sugar damage

The protective role of DNA sugar damage was presented in Figure 4. From Figure 4, it was observed that the extract of the *I. batatas* extraordinarily prevented the free radical mediated DNA sugar damage at dose dependent manner.

3.5. Total antioxidant activity

Figure 5 and 6 shows the antioxidant activity of aqueous extract of *I. batatas* by FTC and TBA method. In this study, the extract of *I. batatas* markedly inhibited the oxidation of linoleic acid for the period of 6 days with nearly 50% when compared with the standard Vitamin E.

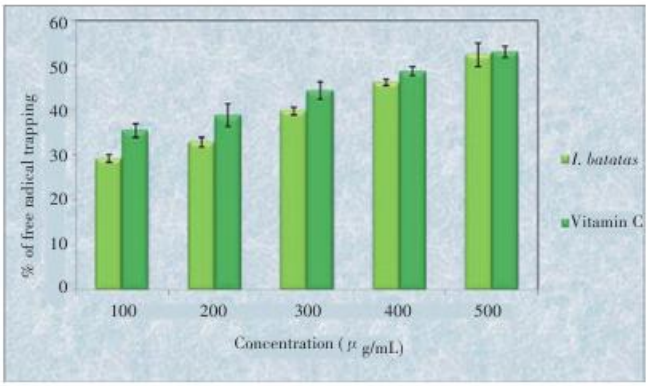

Figure 4. Protective effect of aqueous extract of *I. batatas* on DNA sugardamage.

Values are mean ± SD of triplicate determinations.

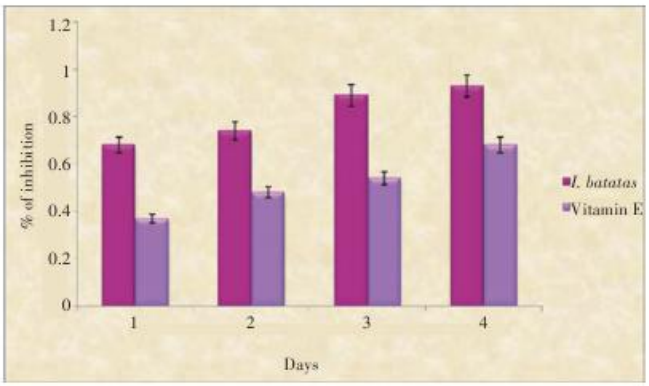

Figure 5. Antioxidant activity of aqueous extract of *I. batatas* by FTC method.

Values are mean ± SD of triplicate determinations.

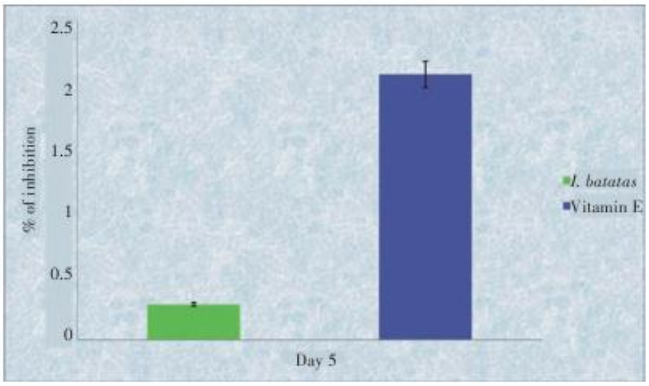

Figure 6. Antioxidant activity of aqueous extract of *I. batatas* by TBA method.

Values are mean ± SD of triplicate determinations.

3.6. Sperm characteristic and hormonal parameters

Table 1 illustrates the effect of *I. batatas* on sperm characteristics such as concentration, motility, morphology, vitality–HOS (Figure 7) and viability of control and experimental animals. The sperm characteristics was found to be significantly decreased ($P<0.05$) in group II bisphenol A induced toxicity animals when compared to the control. Upon administration of aqueous extract of *I. batatas* in group III animals, the sperm characteristics were brought back to near normal comparable to that of group II animals ($P<0.05$). Drug control group IV animals did not show much variation when compared to control group I animals.

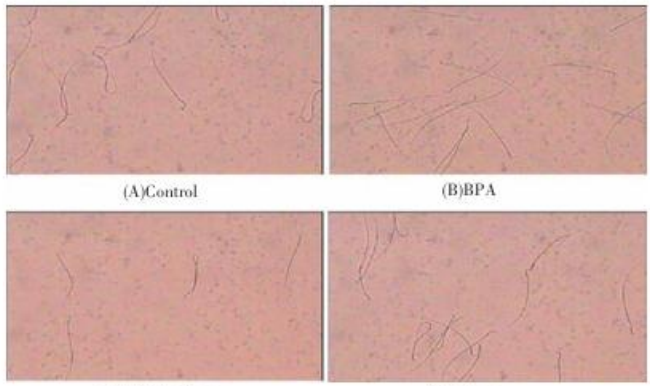

Figure 7. Hypo-osmotic swelling test in control and experimental animals.

The effect of *I. batatas* on hormonal levels in serum of control and experimental animals demonstrated in Table 2. Due to toxicity induced in group II animals the levels of hormones like FSH, LH and Testosterone were declined when compared with group I animals. After treatment with *I. batatas* extract these hormones levels were elevated in group III animals when compared with group II. However, there were no significant changes in this parameter observed in group IV animals when compared with group I control animals.

3.7. Total protein

Table 3 explains the effect of *I. batatas* on the level of protein in testes and serum of control and experimental animals. Considerable decrease in total protein level in serum and testes were observed in group II when compared with group I (serum and testes $P<0.05$). Additionally, the levels of total protein was drastically increased ($P<0.05$) in serum and testes of group III. There was no significant change in group IV animals when compared with group I animals.

3.8. Adenosine triphosphatases

Table 4 portray the activities of Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases in testes of control and experimental animals. Bisphenol A induced toxicity group II animals shows a significant decline in the levels of Na^+/K^+, Ca^{2+} and Mg^{2+} ATPases ($P<0.05$) when compared with control animals. These levels were found to be significantly increased on aqueous extract of *I. batatas* treatment ($P<0.05$) in group III animals when compared to group II animals. On the other hand, there was no significant variations in group IV *I. batatas* alone treated animals when compared with group I control animals.

3.9. Histopathology

Light microphotographs of testes of the control group and experimental animals are presented in Figure 8. The histopathological studies of the testis revealed the following changes. Group II toxicity bearing animals shows small and focally atrophic seminiferous tubules, impaired spermatogenesis when compared with the control animals. Group III animals were normalized with treatment of *I. batatas*. There were no changes found in Group IV animals. Microphotograph of epididymis of the control group and experimental animals are presented in Figure 9. The histopathological studies of the epididymis in Group II toxicity revealed decreased spermatozoa inside the lumen when compared to the control animals. In group III animals the production of spermatozoa was improved by the ameliorative effect of *I. batatas*. Group IV shows normal architecture of epididymis indicating no changes in *I. batatas* treated animals.

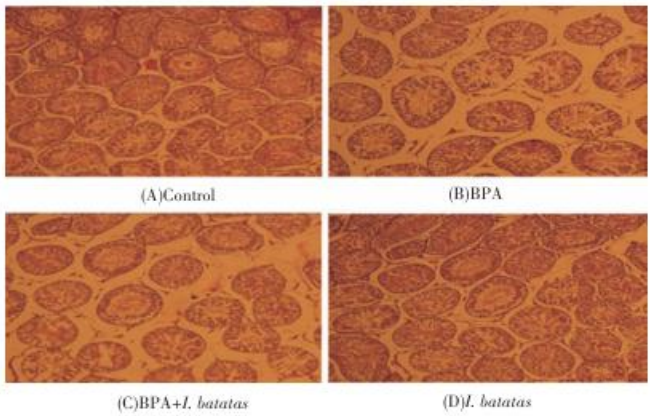

Figure 8. Histopathological sections of testis of control and experimental animals (100×).
(A) Group I control animals shows normal architecture showing regular seminiferous tubules, normal intertubular gaps and consisting of germ cells that mature to form sperms in the lumen. (B) Group II toxicity bearing animals shows small and focally atrophic seminiferous tubules, impaired spermatogenesis. (C) Group III shows the lesions induced by Bisphenol A were substantially normalized by treatment with *I. batatas*. (D) Group IV shows normal architecture of testis indicating no changes in *I. batatas* treated animals.

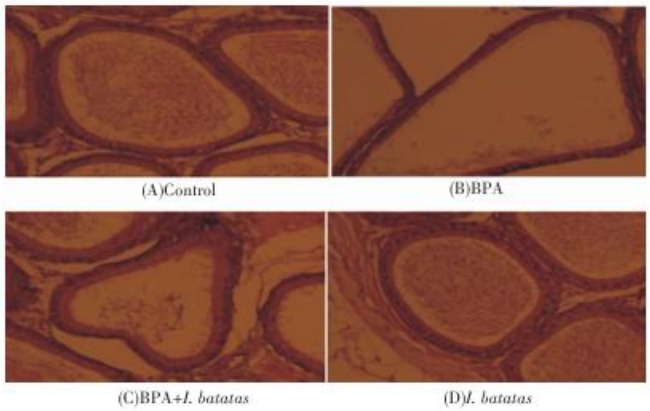

Figure 9. Histopathological sections of epididymis of control and experimental animals.
A Group I control animals shows normal architecture of epididymis. B Group II toxicity bearing animals shows decreased spermatozoa inside the lumen. C Group III animals induced by Bisphenol A were substantially normalized by treatment with *I. batatas*. D Group IV shows normal architecture of epididymis indicating no changes in *I. batatas* treated animals.

4. Discussion

Active oxygen species such as hydrogen peroxide, hydroxyl radical, and superoxide anion radical, are readily generated in many cells by metabolic processes such as respiration, ischemia/reperfusion, and oxidation of fatty acids. They are highly toxic to cells by damaging such components as DNA, lipids, and enzymes[21]. Antioxidants act as reducing agents which prevent oxidative damage to cellular components such as DNA, proteins and lipids by reactive oxygen species (ROS) produced in cells. Reactive oxygen species including superoxide (O), hydrogen peroxide (HO), nitric oxide (NO) and hydroxyl (OH) exert oxidative stress in the cells of human body rendering each cell to face about 10 000 oxidative hits per second[22]. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other constituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather

Table 1
Effect of *I. batatas* on sperm concentration of control and experimental animals.

Parameters	Group I (Control)	Group II (BPA)	Group III (BPA + *I. batatas*)	Group IV (*I. batatas*)
Sperm concentration (million)	67.33±0.12	40.24±1.25[a]	66.50±2.30[a,b]	69.55±1.01[b,c]
Sperm motility (%)	70.21±1.13	29.15±1.20[a]	63.38±2.13[a,b]	70.45±2.30[b,c]
Sperm morphology (%)	59.33±0.14	27.56±3.10[a]	51.60±2.00[a,b]	59.45±1.11[b,c]
Vitality (HOS) (%)	79.45±0.01	45.65±1.31[a]	60.27±3.20[a,b]	79.60±1.15[b,c]
Dye exclusion test (%)	71.66±1.30	40.90±1.00[a]	56.37±2.12[a,b]	71.80±2.14[b,c]

Values are expressed as mean ± SD for six animals in each group, a – Group I *vs.* Group II, III and IV, b – Group II *vs.* Group III and IV c – Group III *vs.* Group IV. The significance at the level of $P<0.05$.

Table 2

Efficacy of *I. batatas* on hormonal levels in serum of control and experimental animals.

Parameters	Group I (Control)	Group II (BPA)	Group III (BPA + *I. batatas*)	Group IV (*I. batatas*)
FSH (pg/mL)	30.31±1.22	14.44±0.95[a]	20.68±1.33[a,b]	29.99±0.03[b,c]
LH (pg/mL)	20.46±0.18	11.77±1.27[a]	15.39±0.45[a,b]	21.00±1.33[b,c]
Testosterone (ng/mL)	6.30±1.28	2.80±0.30[a]	3.90±1.24[a,b]	6.30±0.21[b,c]

Values are expressed as mean ± SD for six animals in each group, a – Group I *vs.* Group II, III and IV, b – Group II *vs.* Group III and IV, c – Group III *vs.* Group IV. The significance at the level of $P<0.05$.

Table 3

Effect of *I. batatas* on the level of protein in testes and serum of control and experimental animals.

Parameters	Group I (Control)	Group II (BPA)	Group III (BPA + *I. batatas*)	Group IV (*I. batatas*)
Protein (Testes) (mg protein/g tissue)	950.15±2.45	643.20±1.15[a]	856.66±0.12[a,b]	952.31±1.23[b,c]
Protein (Serum) (g/dL)	6.90±2.48	5.10±1.71[a]	5.90±0.95[a,b]	7.00±3.66[b,c]

Values are expressed as mean ± SD for six animals in each group, a – Group I *vs.* Group II, III and IV, b – Group II *vs.* Group III and IV, c – Group III *vs.* Group IV. The significance at the level of $P<0.05$.

Table 4

Activities of *I. batatas* on membrane bound ATPases in testes of control and experimental animals.

Parameters (μ mol of inorganic phosphate liberated/mg protein/min)	Group I (Control)	Group II (BPA)	Group III (BPA + *I. batatas*)	Group IV (*I. batatas*)
Na^+/K^+ ATPase	1.10±1.47	0.54±1.36[a]	0.86±2.00[a,b]	1.1±1.87[b,c]
Ca^{2+} ATPase	35.44±2.13	21.64±2.98[a]	26.34±2.17[a,b]	35.99±2.10[b,c]
Mg^{2+} ATPase	0.19±2.19	0.09±1.30[a]	0.14±1.65[a,b]	0.19±1.87[b,c]

Values are expressed as mean ± SD for six animals in each group, a – Group I *vs.* Group II, III and IV, b – Group II *vs.* Group III and IV, c – Group III *vs.* Group IV. The significance at the level of $P<0.05$.

than from synthetic sources[23]. Therefore, there is a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants[24]. One such method, that is currently popular is based upon the use of the stable free radical DPPH[25]. DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging activity measures hydrogen-donating ability of antioxidants[26]. This activity is measured as the relative decrease in absorbance of DPPH as it reacts with the antioxidant[27]. In the present study, the aqueous extract of *I. batatas* showed concentration dependant increase in the trapping of free radicals. This might due to the various active phytochemical constituents present in the aqueous extract of *I. batatas*.

Nitric oxide is an essential bioregulatory molecule required for several physiological processes like neural signal transmission, immune response, control vasodialation and control of blood pressure[28]. Overproduction of NO- and ONOOH, the product of a rapid reaction between O_2^-, and NO-, has been associated with chronic inflammation and may be associated with the etiology and pathology of a number of chronic diseases. During inflammation, the activation of mast cells, macrophages, eosinophils, and neutrophils generate O_2^-, with NADPH oxidase playing important role in vascular complications[29]. The plant products may have the property to counteract the effect of NO formation and in turn may be of considerable interest in preventing the ill effects of excessive NO generation *in vivo*. The aqueous extract of *I. batatas* reduced the generation of NO *in vitro* in a concentration dependent manner.

Superoxide anion is a reduced form of molecular oxygen created by receiving one electron. Superoxide anion is an initial free radical formed from mitochondrial electron transport systems. Mitochondria generate energy using 4 electron chain reactions, reducing oxygen to water. Some of the electrons escaping from the chain reaction of mitochondria directly react with oxygen and form superoxide anions[30]. The superoxide anion plays an important role in the formation of other reactive oxygen species such as hydrogen peroxide, hydroxyl radical, or singlet oxygen in living systems[31]. The superoxide anion can react with nitric oxide (NO•) and form peroxynitrite ($ONOO^-$), which can generate toxic compounds such as hydroxyl radical and nitric dioxide. These toxic compounds can induce damage in lipids, protein and DNA. In the present study, it is inferred that aqueous extract of *I. batatas* exhibits excellent superoxide quenching activity. This may be due to the phenolic content present in the extract of *I. batatas*[32]. Free radicals could damage macromolecules in cells, such as DNA, protein and membrane lipids. Oxidative damage to DNA represents an early stage of cancer and provides a valuable marker of overall oxidative stress. In the present investigation, the aqueous extract of *I. batatas* tubers inhibits DNA damage by quenching free radicals. This may be due to the existence of components of the extract containing isoflavonoids, anthocyanins, phenolic compounds and some proteins that have antioxidant activities[33].

The total antioxidant activity of aqueous extract of *I. batatas* tubers was estimated by the FTC method and compared with the TBA method. The ferric thiocyanate method measures the ability of antioxidants to scavenge peroxyl radicals, which react with polyunsaturated fatty acids, through hydrogen donation[34]. The ferric ion produced from the reaction of peroxide with ferrous ion reacts with thiocyanate to form a red-colored complex, the absorbance of which is monitored every 24 h until the reaction is complete. The TBA method measures free radicals present after peroxide oxidation. The present investigation indicated a high level of antioxidant activity of the aqueous extract of *I.*

batatas. This suggests the presence of active phytochemical constituents that contribute to antioxidant activity, such as ascorbic acid and carotenoids, both of which are present in sweet potato.

The assessment of sperm vitality is one of the basic elements of semen analysis, and is especially important in samples where many sperm are immotile, to distinguish between immotile dead sperm and immotile live sperm [35]. The hypo-osmotic swelling (HOS) test was used for evaluating the functional integrity of human spermatozoal membranes. HOS test is based on the semi-permeability of the intact cell membrane, which causes spermatozoa to "swell" under hypoosmotic conditions, when an influx of water results in an expansion of cell volume[36]. It is reported that the HOS test was used to detect physiological integrity of the sperm membrane[37]. This assay assesses the sperm membrane's functional integrity by evaluating its reaction under hypoosmotic conditions. In the present investigation exposure to Bisphenol A induced a complete degeneration of epididymal epithelium with reduction in the number of spermatozoa. The epididymis and spermatozoa are highly rich in polyunsaturated fatty acids and thus susceptible to damages induced by ROS[38]. The extract of *I. batatas* stabilize and re-establish the normal membrane potential. This may be because of *I. batatas* extract displayed a significant protection against free radicals mediated plasma membrane damage.

Leutinizing hormone (LH), follicle stimulating hormone (FSH) and testosterone are required for normal spermatogenesis[39]. Luteinizing hormone (LH) is a glycoprotein released from the anterior pituitary that stimulates testosterone production[40]. Testosterone is the main male gonadal hormone produced by the interstitial cells of the Leydig in the testis. It is also the major index of androgenicity. Certain concentration of androgens is required for the initiation and maintenance of spermatogenesis and for the stimulation of growth and function of the prostate and seminal vesicles[41]. Luteinizing hormone is produced in the anterior pituitary called gonadotrophs. In the testes, luteinizing hormone binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone. FSH has key roles in the development of a normal complement of functional sertoli, in the maturation of sertoli cells at puberty and the maintenance of their cytoskeleton and cell junctions, and in the maintenance of spermatogonial development. Together, FSH and testosterone support meiosis, exhibit an anti-apoptotic action on spermatocytes and spermatids, and act co-operatively to promote spermatid maturation and sperm release[42]. The sertoli cells produce FSH responsive protein factors that have some regulatory effects on leydig cell steroidogenesis. FSH synergizes with testosterone in aiding synthesis of androgen receptor, enhancing transport and localizing testosterone within sertoli cells[43]. A reduction in testosterone level could be the primary cause of induction of infertility induced by the compound, but other possibilities may not be excluded[44]. Dysfunction of Leydig cells will disturb the normal testosterone levels[45]. In this connection, Pectasides *et al*. [46] have reported that LH concentration was increased and testosterone levels were decreased during the complete failure of leydig cells. However, hormonal production may be reduced in rats due to the seminiferous tubular damage. An elevation in circulating levels of inhibin, a glycoprotein of primarily sertoli cell origin which inhibits FSH synthesis and secretion by the pituitary[47], could account for the observed decrease in serum FSH level in the present study which was confirmed histopathologically by degeneration and atrophy of seminiferous including leydig and sertoli cells. FSH stimulates the sertoli cells of the seminiferous tubules to produce androgen binding protein, probably moves via the sertoli cells to other germ cells and to the epididymis where the testosterone is released to exert its physiological effects in sperm maturation[48]. In the present study the administration of Bisphenol A decreases the FSH and LH levels, due to the influences of environmental toxicant on sertoli cells, leydig cells and seminiferous tubule. The toxicity induced rats with treatment of *I. batatas* significantly decreased the toxic effects of BPA and the level of FSH, LH and testosterone were greater than before. This might be due to the presence of the active constituents such as flavonoids, triterpenes, alkaloids and saponins which directly or indirectly scavenge the oxidative damage to various cells and organs while normalizing their functions. It has been reported that flavanoids and antioxidants remarkably reduce oxidative damages in the cells [49].

Protein content of testicular tissue is considered as a marker of tissue injury, damage and rewound healing[50]. The testicular fluid contains both stimulatory factors as well as inhibitory factors that selectivity alters the protein secretions[51]. Thus, the changes in protein suggested that there is a reduction in the synthetic activity in testes. It is reported that periportal infiltration, inflammation, testicular toxic has been affected due to the disturbance in protein metabolism[52]. Protein metabolic perturbations may also favour toxic levels in the tissues[53]. The present results illustrate considerable decrease in testicular protein content in animals treated with bisphenol A compared to control group, another indication to the oxidative damage induced by toxicant on testicular tissue. Aqueous extract of *I. batatas* was able to re-establish testicular protein and serum total proteins. Our results indicated significant decrease in relative weights of testes in animals treated with bisphenol A compared to control. However, in both prophylactic and curative groups, plant extract alleviated the toxicity and the weight of these tissues reached the control values. This may be due to the collective effect of various phytochemicals present in the extract. In view of this Wang *et al*.[54] have reported that the active constituents of the plants suppress the lipid peroxidation reaction.

ATPases are membrane bound enzymes complexes/ion transporters that combine ATP synthesis and/or hydrolysis with the transport of protons across a membrane[55]. ATPases can harness the energy from a proton gradient, using the flux of ions across the membrane via the ATPase proton channel to drive the synthesis of ATPI[56]. The Na^+, K^+-ATPase commonly known as sodium pump, is responsible for coupled extrusion and uptake of Na^+ and K^+ ions across the plasma membranes of most eukaryotic organisms[57]. The pump drives three sodium ions out of the cell and two potassium ions into the cell against substantial concentration gradient. The activity of this enzyme is required for diverse functions like maintenance of cellular osmotic balance, generation of neuronal membrane potentials and renal as well as intestinal handling of solutes. The activity of Na^+ K^+ ATPase can also be regulated by hormones, protein and second messengers. It is reported that lipid peroxidation was also associated with the inhibition of Na^+ K^+ ATPase activity in proximal tubule cell lysate and this occurs secondary to mitochondrial injury[58]. Direct inhibiton of Na^+ K^+ ATPase within the cell membrane may reflect peroxidation of surrounding membranes.

Ca^{2+} is considered a prime regulator of sperm motility, capacitation and in the initiation of the acrosome reaction processes [59-61]. In addition, Feng and coworkers found that calcium plays a role in cell growth and differentiation during spermatogenesis [62]. Whereas Zn is thought to be one

of the factors that affect spermatozoa motility in seminal plasma, it exerts this effect by controlling Ca^{2+}–Mg^{2+}–ATPase enzyme activity[63]. Calcium plays a predominant role in regulating many functional processes of spermatogenesis and fertilization including acrosome reaction and motility[64]. Ca^{2+} gradients across the plasma membrane, required for Ca^{2+} homeostasis and signaling, are maintained in part by plasma membrane Ca^{2+}– ATPase activity. In somatic cells, Ca^{2+}–ATPase activity was found to be affected by Bisphenol A due to its toxicity[65].

Mg^{2+} ATPase plays a role in endogenic process in addition to ion transport. The ion sensitive Mg^{2+}–ATPase utilizes a part of ATP that is not directly related to the change in free energy for sodium transport. It is reported to regulate the flow of potential energy from the mitochondria and from the cytoplasm [66]. In the present investigation, it was observed that the level of ATPases was inhibited in erythrocyte membrane in Bisphenol A administered animals may be due to the peroxidation of the membrane lipids by the toxicant which initiates the loss of membrane integrity and enzyme activity. In this connection, it was reported that peroxidative damage in membrane alters the Ca^{2+} ion concentration leading to membrane rupture. However, *I. batatas* treatment showed a mild increase in activities of ATPases. This may be due to the protective role of membrane integrity of the seed extract through its antioxidant activity. Since these membranes bound enzymes are thiol group containing enzymes, that are lipid dependant and hence the restoration of the activities of ATPase enzymes suggest the ability of *I. batatas* to protect the thiol group from oxidative damage through inhibition of lipid peroxidation.

The histological changes in testes of rats treated with BPA showed severe damage within the seminiferous tubules and vascular degeneration on the spermatogenic and sertoli cells cytoplasm. The germinal epithelium of the seminiferous tubules was thinner in places and spermatids were almost absent; sperm numbers was low and there were no sperm in the lumen. Histopathologically, focal atrophy, germ cell exfoliation and retention of elongated spermatids were observed in seminiferous tubules of the BPA treated group in accordance with findings of Feng *et al.* [67]. On the contrary the administration of *I. batatas* significantly reverted back the altered structures to near normal this might be due to the radical scavenging activity of the tubers extract.

From the present investigation, it can be concluded and strongly put forward that *I. batatas* has tremendous antioxidant property and is proficient to hold back male fertility. In the present study, treatment with aqueous extract of *I. batatas* suggests that alterations in the male reproductive organs induced by bisphenol A were recovered due to its anti-infertility efficacy of the plant extract.

Declare of interest statement

We declare that we have no conflict of interest.

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