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Full Length Research Paper

Hypoglycemic, hypolipidemic and kidney protective potential of combined formulation of *Tribulus terrestris* and *Andrographis paniculata* in alloxan induced mice

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The study aims to evaluate the effect of *Tribulus terrestris* and *Andrographis paniculata* on body weight, blood glucose, lipid profile and renal protective capability in alloxan induced type-1 diabetic mice. Six groups of five male Swiss Albino mice each, at four weeks of age were used in this experiment. Group-X were fed saline water and Group-Y with combined herbal formulation at a high dose (1 g/kg bwt) to assess toxicity. The remaining four groups were designated into healthy control, diabetic control, diabetic + Amaryl[®] 800 µg/kg, Diabetic + Formulation 200 mg/kg bwt. The combined formulation improved body weight loss and lowered blood glucose levels significantly ($P < 0.001$) compared to diabetic control group after 8 weeks of treatment. The total cholesterol and plasma triglyceride were decreased at a significant level ($P < 0.01$) compared to diabetic control. However, High density lipoproteins (HDL) and Low density lipoproteins (LDL) levels remained unchanged by combined formulation compared to diabetic control. The combined formulation also lowered the plasma creatinine levels in the formulation treated group compared to diabetic control. Histo-pathological evaluation revealed that combined formulation partially improved renal glomerular sclerosis and hypertrophy, tubular damage and pancreatic β -cells damage. The combined herbal formulation may have antidiabetic and renal protective capability in alloxan induced type-1 diabetic mice.

Key words: *Tribulus terrestris*, *Andrographis paniculata*, blood glucose, lipid profile, renal injury, antidiabetic effects.

INTRODUCTION

Diabetes is a heterogeneous metabolic disorder characterized by altered carbohydrate, lipid and protein metabolism which causes hyperglycemia resulting from insufficient insulin secretion, insulin action or both (Mutalik et al., 2003; Joseph and Jini, 2011). With an

estimated 371 million affected adult people worldwide, diabetes mellitus is one of the most widespread diseases that caused 4.8 million deaths in 2012 (IDF, 2013). The most prevalent form globally is the non insulin dependent diabetes mellitus (NIDDM type-2) which is associated

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with elevated post-prandial hyperglycemia. The classic symptoms of untreated diabetes are weight loss, polyuria, polydipsia and polyphagia (Cooke and Plotnick, 2008). Serious long-term complications include cardiovascular disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes (Kitabchi et al., 2009). Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in diabetic patients (Xie et al., 2003). Management of diabetes without any side effects is still a challenge in the medical field, as presently available drugs for diabetes have one or more adverse effects (Bohannon, 2002). The base of healthcare system has been founded by medicinal plants worldwide since the primitive stage of humanity and plant products are still a major source of drug/formulation for the treatment of various diseases (Kamboj, 2000). The active principles present in medicinal plants have been reported to possess various activities such as pancreatic β -cell regeneration, stimulating insulin release from β -cells, showing insulin-like action, fighting the problem of insulin resistance, and reducing the uptake, absorption and utilization of glucose (Wadkar et al., 2008).

Tribulus terrestris L, is commonly known as puncture vine, caltrop, yellow vine, goat head and devil's horn. Saponins in the extracts of *Tribulus* species showed hypoglycemic and hypolipidemic effects in diabetic rats (El-Tantawy and Hassanin, 2007). *T. terrestris* significantly reduced the level of serum glucose, serum triglyceride and serum cholesterol. (Chhatre et al., 2008). The decoction of *T. terrestris* showed inhibition of gluconeogenesis in mice (Amin et al., 2006). Thus, *T. terrestris* could be beneficial in the treatment of diabetes by lowering blood glucose, lipid levels, and by protecting kidney and pancreas.

Andrographis paniculata plant has been effectively used in traditional Asian medicines for centuries. Andrographolide appears to dose-dependently reduce plasma glucose concentration in streptozotocin induced diabetic rats and normal rats. The hypoglycemic effect of *A. paniculata* is due to insulin release from pancreatic β -cells through ATP-sensitive potassium channels, is similar to other insulin tropical antidiabetic agents (Wibudi et al., 2008). However, the uses of these two plants extract combinedly in diabetes to ameliorate its complications such as diabetic nephropathy and pancreatic injury are not yet evaluated. This research was conducted to evaluate the effects of *Tribulus terrestris* and *A. paniculata* on body weight, blood glucose, lipid profile that is, total serum cholesterol (TC), serum triglyceride (TG), serum HDL cholesterol, serum LDL cholesterol, serum creatinine, diabetic nephropathy, and pancreatic injury in alloxan induced diabetic mice.

MATERIALS AND METHODS

The experiment was conducted in the Department of

Pharmacology, Bangladesh Agricultural University, Mymensingh. Experimental protocols and animal care were performed according to the guidelines for the care and use of animals established and approved by the Animal Welfare and Ethical Committee, Bangladesh Agricultural University, Mymensingh.

Collection and acclimatization of mice

A total of 30 healthy adult male Swiss albino mice were collected from International Centre for Diarrheal Diseases Research and Rehabilitation, Bangladesh (ICDDR) Mohakhali, Dhaka. All the mice were acclimatized to the new environmental condition for a period of two weeks. The cages were kept in well ventilated room at $28 \pm 2^\circ\text{C}$ and a relative humidity of 70 to 80% with natural day and light. The normal body weight and normal fasting glucose level of each animal was measured by electric balance (Camry, EK3052) and Glucometer (Omron, E-OHS-BD), respectively.

Experimental design

Study 1

Two groups (Group-X and Group-Y) each group containing 5 mice were used, mice of Group-X were fed normal chaw and administered saline water orally, and the mice of Group-Y were supplied combined formulation at a dose of 1 gm/kg body weight. The body weight and blood glucose level were calculated after 7 days. The result was taken to check whether there is any adverse effect on glucose level and body weight gain in healthy animals.

Study 2

After being sure about the promise of the formulation on normal mice, the 2nd step of the experiment began to evaluate the effects of combined formulation on diabetic mice model. For this, twenty (20) mice of one month age were used in this study. The mice were randomly divided into four (4) groups, each group containing five (5) mice. The mice of Group-A were administered with saline water orally and fed normal diet. This group served as Healthy Control group. Alloxan Monohydrate injection was given at a dose rate of 120 mg/kg in intra-peritoneal route to each mouse to induce diabetes in the remaining three groups. The mice were fed normal diet and given water *ad-libitum*. During that period at week 0, 2, 4 and 8 the body weights and blood glucose levels were measured. One group served as Diabetic Control group. Combined formulation was given orally to alloxan induced mice at a dose of 200 mg/kg body weight for 8 weeks to another group. This group served as Diabetic + Formulation to find the effect of formulation as antidiabetic drug. Tablet Amaryl[®] was administered at a dose rate of 800 $\mu\text{g}/\text{kg}$ bwt for 8 weeks. This group served as Diabetic + Amaryl[®].

Collection of drugs and herbal combined formulation

A bottle of 25 g Alloxan Monohydrate (SIGMA- ALDRICH Company, UK) was purchased from the market. The combined herbal formulation of *T. terrestris* and *A. paniculata* was a kind gift of Kabiraz (Unani Ayurvedic Doctor) Kazi Shazzad Hossain, Proprietor of Janani Chikitsalaya, Barishal, Bangladesh. The plants were authenticated with the help of Kabiraz Kazi Shazzad Hossain and the Voucher samples were stored in the department of Pharmacology, Bangladesh Agricultural University. The Glimperide tablet (Amaryl[®]) was collected from Local Market, Bangladesh

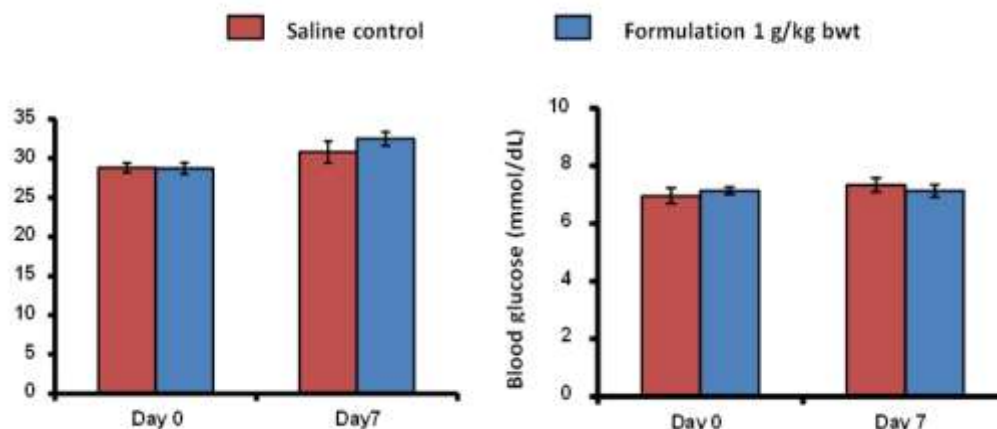


Figure 1. Effect of *Tribulus terrestris* and *Andrographis paniculata* on body weight (g), and blood glucose level (mmol/dL) in healthy mice. Data are shown as Mean±Standard Error. Mean of n=5 samples per group.

Agricultural University, Mymensingh.

Collection of sample

The mice were anaesthetized under sodium pentobarbital (65 mg/kg, i.p.) anesthesia and sacrificed and blood was collected directly from the heart. Kidneys were perfused with an isotonic saline and removed with pancreas. Both kidney and pancreas were stained with Haematoxylin and Eosin (H & E) stain.

Biochemical analysis for oxidative stress

Assay of plasma lipid peroxidation

For measuring the rate of thiobarbituric acid-reactive substances (TBARS), an index of lipid peroxidation was used. Plasma samples were mixed with Tricarboxylic acid (TCA) (20%) and the precipitate was dispersed in H_2SO_4 (0.05 M). TBA (0.2% in sodium sulfate 2M) was added and heated for 30 min in boiling water bath as described previously (Rafiq et al., 2012). TBARS adducts were extracted by *n*-butanol and the absorbance was measured at 532 nm. This reaction is formed in acidic pH and high temperature, and the maximum absorption is a pink complex in 532 nm (Alam and Fareed, 2016).

Statistical analysis

All data were expressed as Mean ± SEM, and differences among the groups of animals were compared using one-way ANOVA with post-hoc Bonferroni test. The preliminary control data from study-1 was compared by using Student's t-test.

RESULTS

Study 1

No significant difference was observed in glucose level

between the groups on day 0 and day 7. This data from study-1 suggests that even in high dose herbal combination formulation have no significant effect on body weight and blood glucose level in healthy mice (Figure 1).

Study 2

After induction of diabetes in mice, there was a significant ($P<0.001$) reduction in body weight of alloxan induced type-1 diabetic mice from 32.1 ± 1.51 g to 28.4 ± 0.85 g. In treated groups a significant ($P<0.001$) increase in body weight was observed compared to diabetic control group, which was almost close to healthy control group (Figure 2).

In diabetic control group after alloxan administration the blood glucose levels increased to 14.42 ± 0.9 mmol/L at week 2 which was further increased to 16.3 ± 1.1 at week 8. As well as, combined herbal formulation powder was found to significantly ($P<0.001$) reduce blood glucose levels from 15.34 ± 0.9 mmol/kg to 12.06 ± 0.7 mmol/L at week 2 to week 8 respectively, comparing with the diabetic control group (Figure 3).

There was no significant changes in HDL and LDL values after treating them with Amaryl[®] or combined herbal formulation (200 mg/kg bwt) but formulation treatment prevent the further worsening in cholesterol level in alloxan induced diabetic mice (Figure 4). After 8 weeks of treatment, average triglyceride (TG) level of diabetic control group elevated significantly ($P<0.001$) to 221.25 ± 8.7 mg/dL. On the other hand, at week 8 of treatment, combined formulation treated group also showed lower triglyceride (TG) level 185.5 ± 4.7 mg/dL comparing to diabetic control mice. There was no significant changes in plasma creatinine values after treating them with Amaryl[®] or combined herbal

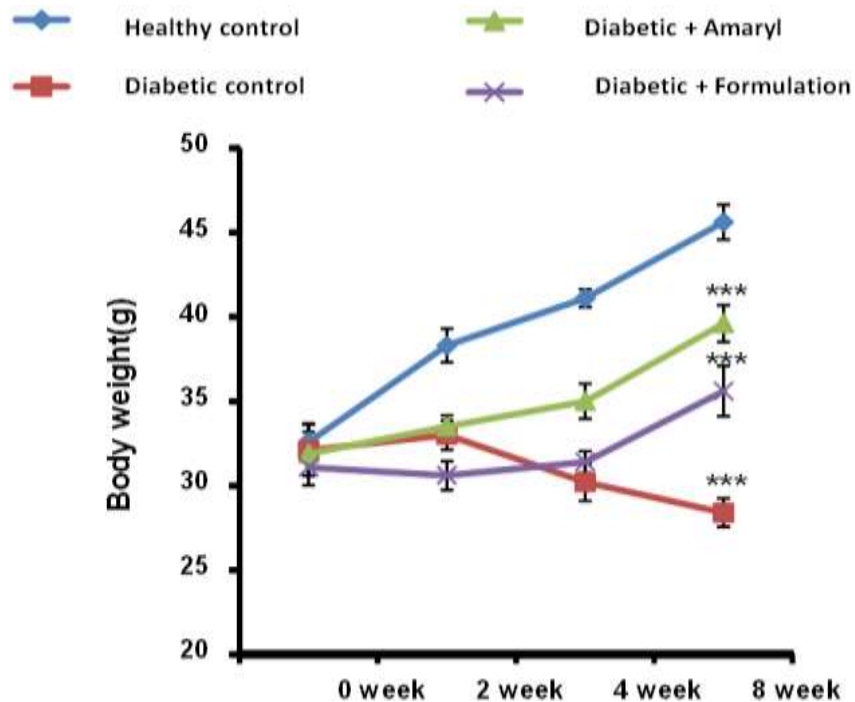


Figure 2. Effect of *T. terrestris* and *A. paniculata* on body weight (g) in alloxan induced type-1 diabetic mice. Data are shown as Mean±Standard Error Mean of n=5 samples per group. *Significant at 5 percent level ($P<0.05$); **Significant at 1 percent level ($P<0.01$); ***Significant at 0.1 percent level ($P<0.01$) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

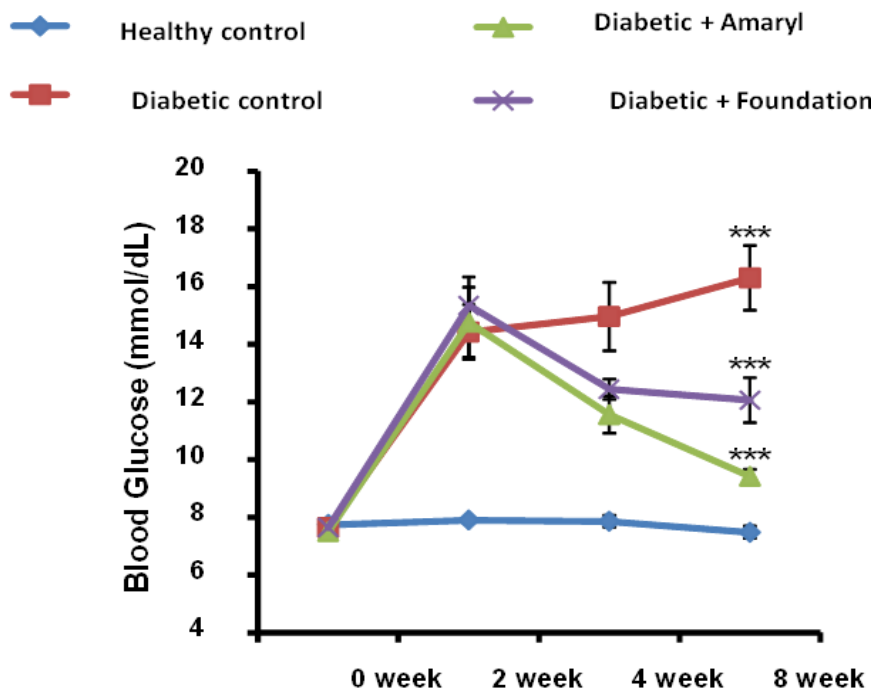


Figure 3. Effect of *T. terrestris* and *A. paniculata* on blood glucose level (mmol/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean ± Standard Error Mean of n=5 samples per group. *Significant at 5 percent level ($P<0.05$); **Significant at 1 percent level ($P<0.01$); *** Significant at 1 percent level ($P<0.01$) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

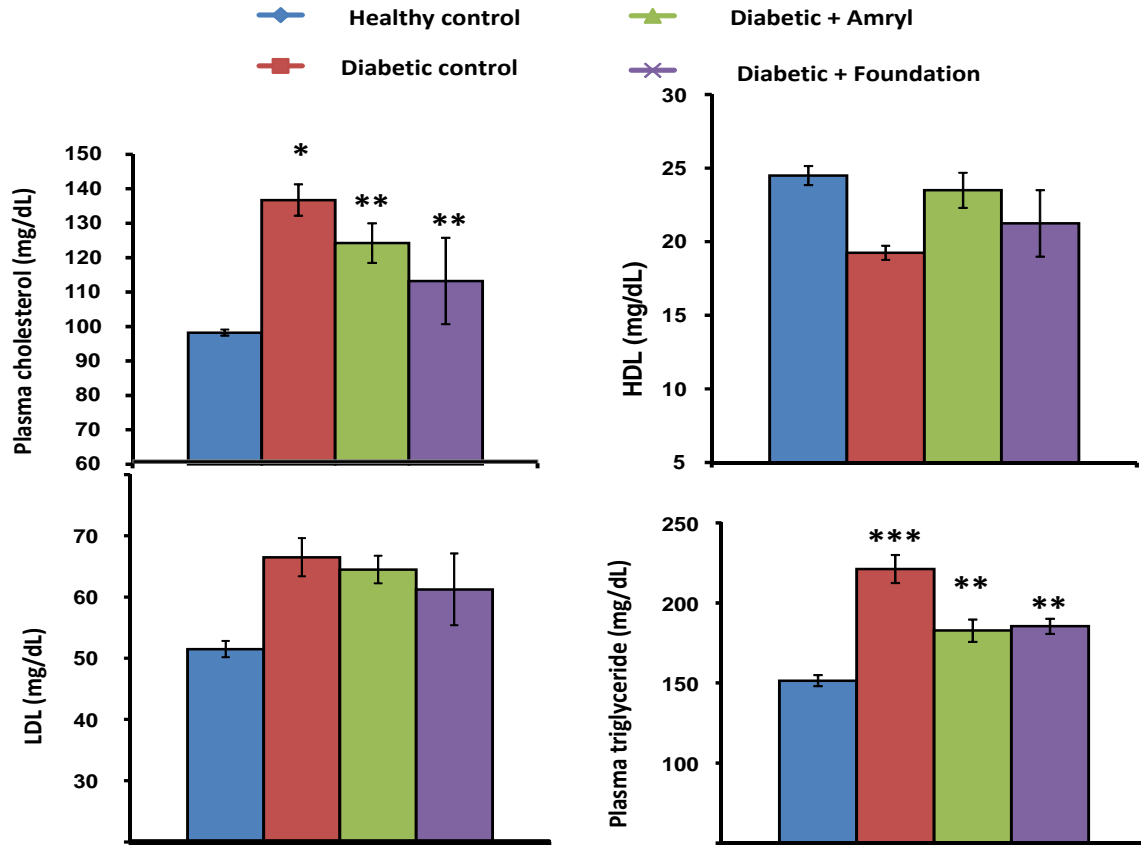


Figure 4. Effect of *T. terrestris* and *A. paniculata* on lipid profile (mg/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean \pm Standard Error Mean of n=5 samples per group. *Significant at 5 percent level ($P < 0.05$); **Significant at 1 percent level ($P < 0.01$); *** Significant at 0.1 percent level ($P < 0.001$) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

formulation (200 mg/kg bwt) but formulation treatment prevent the further increase in creatinine level of alloxan induced type-1 diabetic mice (Figure 5).

Induction of diabetes with alloxan was associated with marked histological changes in the kidney over a period of 8 weeks as revealed by tubular epithelial damage, glomerulo sclerosis, glomerular atrophy, abnormal renal corpuscles, interstitial fibrosis, (B). The healthy section showed normal architecture of the renal glomerulus and tubules (A). Treatment of diabetic mice with the formulation afforded significant protection from renal damage (D) whereas tubular damage was also pronounced in mice treated with Amaryl[®] (C) (Figure 6). In the untreated diabetic mice atrophy and degeneration were observed mostly in the β -cells specially in the central portion of the islets of langerhans. Treatment of diabetic mice with combined formulation and Amaryl[®] led to normalization of the affected β -cells (Figure 7).

Alloxan induced mice showed increased plasma lipid peroxidase level indicating systemic oxidative stress. At 8 week, treatment with combined formulation decreased plasma lipid peroxidase level in diabetic mice (Figure 8).

DISCUSSION

The combined formulation of *T. terrestris* and *A. paniculata* was administered at a high dose (1 g/kg) to find out any toxic effect in comparison to saline control animal. The finding suggests that the body weight and blood glucose level did not have any significant variation between the 0 day and 7 day in saline water and formulation fed mice. It implies that the newly derived herbal formulation has no adverse effect on blood glucose level of healthy mice. The basal body weight before this treatment was similar between the groups. Alloxan @ 120 mg/kg significantly decreased the body weight in mice. Body weight was elevated by the treatment with both combined formulation and Amaryl[®] treatment for 8 weeks.

During 8 weeks of treatment period, administration of combined preparation in alloxan induced diabetic mice showed a significant improvement in body weight loss when compared to diabetic control mice. Alloxan induced elevated blood glucose levels were reduced by the treatment with both combined formulation and Amaryl[®]

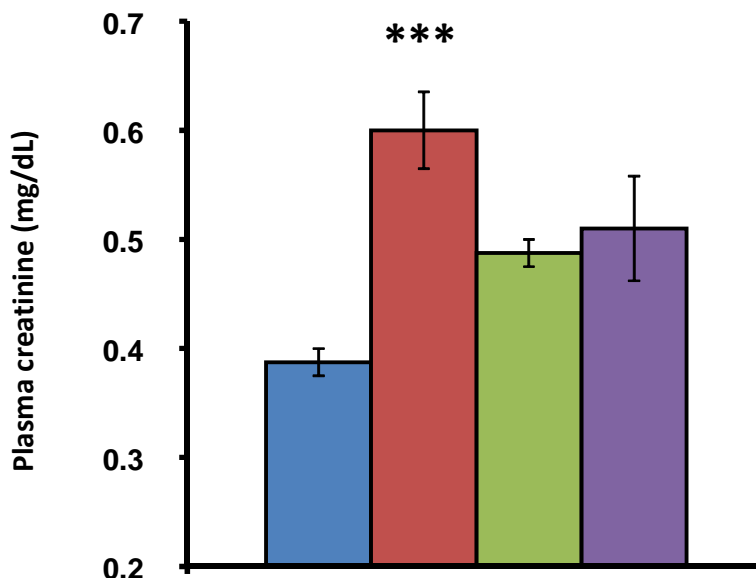


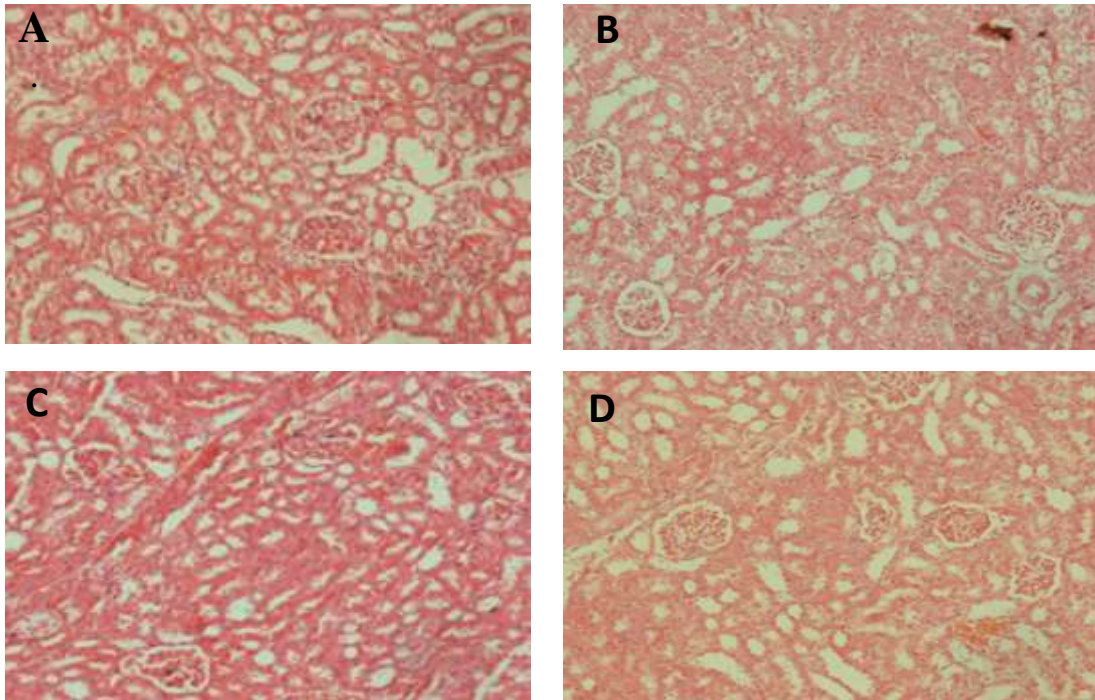
Figure 5. Effect of *Tribulus terrestris* and *Andrographis paniculata* on plasma creatinine level (mg/dL) in alloxan induced type-1 diabetic mice. Data are shown as Mean \pm Standard Error Mean of n=5 samples per group. *Significant at 5 percent level ($P<0.05$); **Significant at 1 percent level ($P<0.01$); *** Significant at 0.1 percent level ($P<0.001$) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

treatment for 8 weeks. There was no significant difference between combined formulation and Amaryl[®] treated groups. Previous study (El-Tantawy and Hassanin, 2007) showed that feeding of diabetic rats with 50 mg/kg of alcoholic extract of *T. terrestris* significantly decrease the blood glucose level after 2, 4 and 6 hour of treatment as compared to untreated diabetic rats. Nugroho et al. (2014) expressed that administration of the purified extract of *A. paniculata* (Burm. f.) for 5 days significantly decreased ($P<0.05$) preprandial and postprandial blood glucose level in high-fat-fructose-fed rats in a dose-dependent manner.

During 8 weeks of treatment period, administration of combined preparation in alloxan induced diabetic mice significantly lowered the total cholesterol level when compared to diabetic control mice. Sivakumar and Rajeshkumar (2015) elaborated that the levels of serum cholesterol was found to be elevated in streptozotocin induced diabetic rats when compared to normal. During 8 weeks of treatment period, administration of combined preparation in alloxan induced diabetic mice showed a significant improvement in HDL-cholesterol loss when compared to diabetic control mice. This result partially

matched with Nugroho et al. (2014) who found that *A. paniculata* has mild lowering effect on HDL- cholesterol levels from pre to post. On the other hand, Sen et al. (2011) found that treatment with *Tribulus terrestris* significantly increased serum HDL level and decreased elevated LDL level when compare to diabetic control. Administration of combined preparation in alloxan induced diabetic mice lowered the LDL level when compared to diabetic control mice. Sen et al. (2011) also exposed the LDL lowering potential of *T. terrestris* at a level of 25.7% compared to diabetic control. Lakshmia et al. (2014) studied the LDL lowering potential of *A. paniculata* to a significant level ($P<0.01$) in contrast to cholesterol treated animal.

The plasma triglyceride level was significantly ($P<0.01$) decreased in formulation and Amaryl[®] treated groups in contrast to diabetic control groups. This result partially matches with the evaluation from Sen et al. (2011) describing that, elevated serum TG level was decreased in extract and glibenclamide treated group. Formulation and Amaryl[®] both decrease serum creatinine but was not so significant. Premanath et al. (2015) described that diabetic control mice exhibited higher plasma creatinine,



A. Healthy control B. Diabetic control C. Diabetic+ Amaryl D. Diabetic + Formulation

Figure 6. Photomicrographs of histopathological studies of Kidney sections of normal and experimental diabetic mice. Paraffin embedded sections of renal cortex were stained with hematoxylin and eosin (H&E). Representative light micrographs (20 X) from each mice groups are shown. (HE-20X).

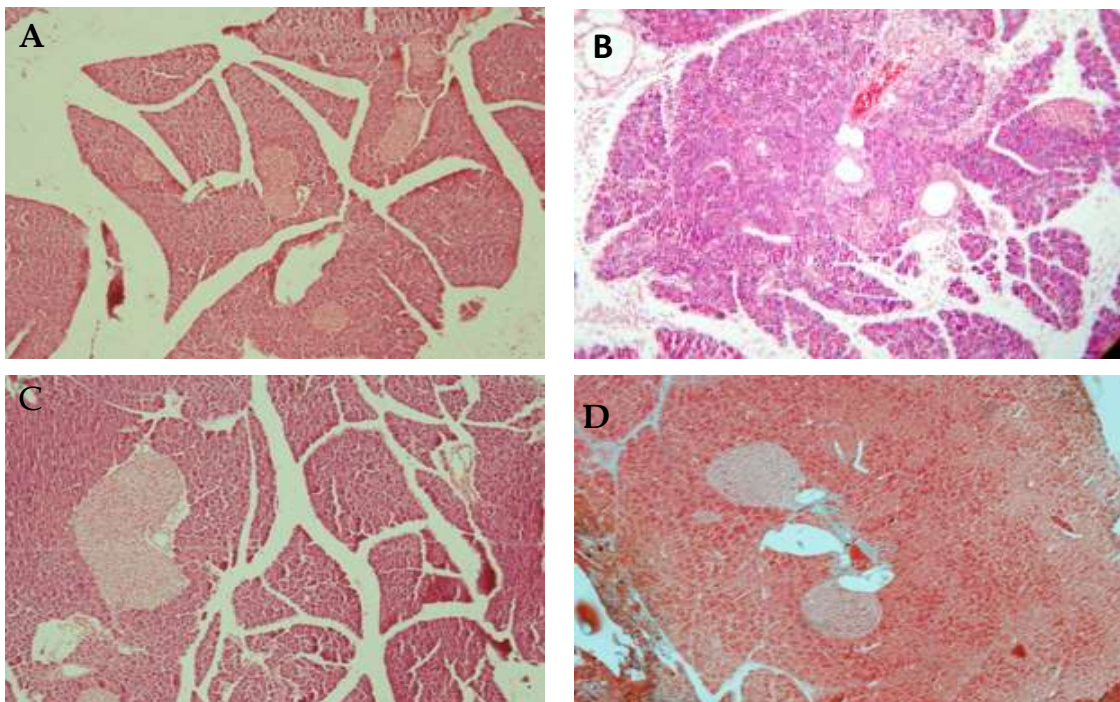


Figure 7. Representative histopathological profiles on β -cells (arrow show one islet) Paraffin embedded sections of pancreatic tissue were stained with hematoxylin and eosin (H&E). Representative light micrographs (20 X) from each mice groups are shown. (HE-20X).

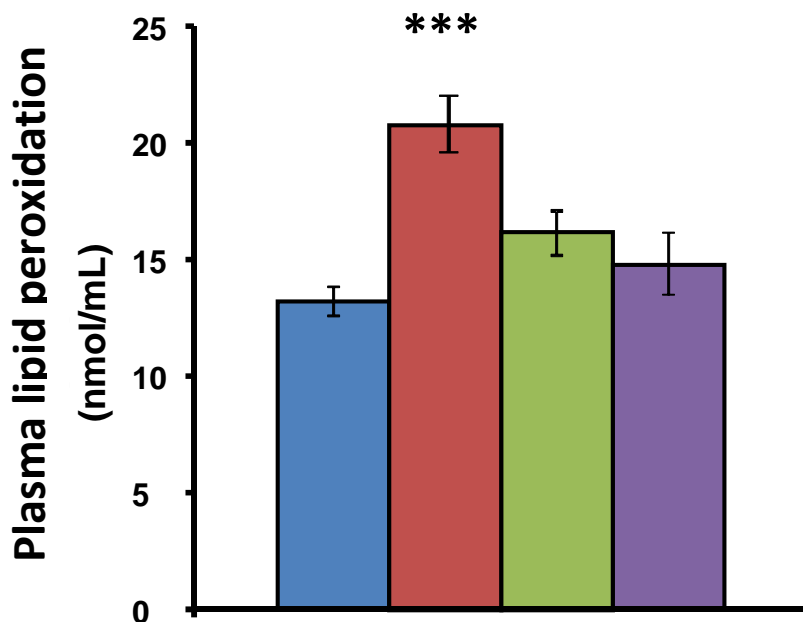


Figure 8. Effect of *T. terrestris* and *A. paniculata* on plasma Lipid peroxidation (nmol/mL) in alloxan induced type-1 diabetic mice. Data are shown as Mean \pm Standard Error Mean of n=5 samples per group. *Significant at 5 percent level ($P < 0.05$); **Significant at 1 percent level ($P < 0.01$); *** Significant at 0.1 percent level ($P < 0.001$) compared with Healthy Control vs Diabetic Control and Diabetic Control vs Treatments.

urinary creatinine and serum urea levels compared to those of normal mice. Combined formulation of *T. terrestris* and *A. paniculata* as well as Amaryl[®] significantly increased the number and size of islet cells especially in the β -cell region. Atrophy and degeneration of the β -cells of the central zone of the islets of Langerhans of untreated mice were improved in mice treated with formulation of *T. terrestris* and *A. paniculata* as well as Glimpiride.

Oxidative stress leads to the onset and subsequent complications of type 2 diabetes mellitus, neural damage, vascular dysfunction, cognitive decline and renal injury. In this regards previous study showed that a dramatic loss of learning and memory function was observed in mice with large increases in brain oxidative stress, whereas an antioxidative treatment almost completely reversed the behavioral changes (Liu et al., 2003). There is also clinical evidence of increased oxidative damage in subjects with mild cognitive impairment has been highlighted (Keller et al., 2005).

Dobrian et al. (2003) reported that high salt intake induces increased vascular oxidative stress in rats. Other clinical studies also highlighted that increased oxidative stress induced by hyperglycemia may contribute to the pathogenesis of diabetic complications including nephropathy (Abe et al., 2011; Goodarzi et al., 2010).

In contrast, present study demonstrated that alloxan induced mice showed argumentation of plasma lipid peroxidation. The increase in TBARS, an index of lipid peroxidation in the diabetic mice might be due to increased levels of oxygen free radicals. In animal studies, tea polyphenol administration was shown to decrease serum TBARS level due to its potential antioxidant activity (Sharifzadeh et al., 2017). Along with previous finding, these results suggest that argumentation of oxidative stress plays an important role in the pathogenesis of pancreas and renal injuries in alloxan induced diabetic mice. In addition, the mechanism of the synergistic or beneficial effects of combination preparation is not yet clear; however, these

studies have highlighted the potential roles of their antioxidative properties (Abe et al., 2011; Goodarzi et al., 2010; Sharifzadeh et al., 2017).

Conclusion

These data suggested that the hypoglycemic, hypolipidemic and kidney protective potential of combined formulation of *T. terrestris* and *A. spaniculata* in alloxan induced mice may be due to their antioxidative properties. Further studies are needed to isolate the active ingredients in combination formulation.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Full Length Research Paper

Antioxidant activity of selenium on bisphenol-induced apoptosis and testicular toxicity of rats

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Bisphenol A (BPA) is an industrial chemical widely used to make polycarbonate plastics for packaging and epoxy resins. This study sought to examine how selenium (Se) affects BPA toxicity in terms of albino rats' histological structure, antioxidant enzymes, sexual hormones, and reproductive organs (seminiferous tubule (coiled tubule) diameter, epithelial height and sperm count). Adult male rats were divided into four experimental groups: Group 1: The control; Group 2: Orally administered sodium selenite (10 µg/kg bw/day) for 4 weeks; Group 3: Orally administered BPA (10 µg/kg bw/day) for 4 weeks; Group 4: Treated daily with BPA (10 µg/kg bw/day) followed by Se (orally administered, 10 µg/kg bw/day) for 5 times per 4 weeks. BPA exposure caused changes in the testicular histological structure, which included apoptosis, and led to changes in several biochemical markers: Malondialdehyde, catalase, superoxide dismutase, and glutathione peroxidase. Additional changes occurred in the plasma testosterone, luteinizing hormone, and follicle-stimulating hormone levels, body and reproductive organ weights, and sperm counts. However, these BPA-induced effects were significantly ameliorated in rats treated with BPA-plus-Se. This protective effect of Se is attributable to its ability to remove potentially damaging oxidizing agents in living organisms. The results confirmed that Se countered the oxidant effects and increased the BPA-induced stress response in rats. Hence, Se promotes the healthy growth and development of mammals by protecting them from oxidative stress.

Key words: Bisphenol A, selenium, testis, histology, oxidative stress, rats.

INTRODUCTION

Bisphenol A (BPA: 2, 2-bis (hydroxyphenyl) propane) is an environmental contaminant that comes from the industrial manufacturing of polycarbonate plastics. BPA is widely used in the lining of food and beverage packaging to protect the perishable contents from contamination and to extend their shelf life (Vandenberg et al., 2009; Rochester et al., 2013). However, BPA may be released by food containers, plastic bottles, and beverage containers; moreover, it can leach from production sites and landfills (vom Saal et al., 2007). The toxicity and side

effects of BPA have been investigated through experimental animal studies, and high BPA concentrations are now associated with a variety of adverse human health issues (Wang et al., 2013). For example, in mice, BPA reportedly affects the male reproduction system, by causing noticeable structural changes in the histological architecture of the testes (Aikawa et al., 2004; Zang et al., 2016). Even a low-dose exposure to BPA may lead to a reduction in spermatogenesis of male rats (Akingbemi et al., 2004;

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Jin et al., 2013), while other research suggested that BPA can adversely affect the quality and quantity of sperm and is responsible for abnormal male fertility (Akingbemi et al., 2004; Liu et al., 2013; Li et al., 2016).

BPA has been shown to cause a reduction in the activity of several key antioxidant enzymes (superoxide dismutase, SOD; catalase, CAT; and glutathione peroxidase, GPx) in rat livers. Furthermore, recent work showed that BPA significantly reduced both SOD and CAT system activity as well as oxidised glutathione (H₂O₂) generation and lipid peroxidation (Eid et al., 2015). More recently, the plasma testosterone levels and testis weight were found decreased by BPA in association with morphological alterations and changes in sperm count and motility (Zang et al., 2016).

Antioxidant molecules play a vital role in maintaining the health and defense mechanisms of biological tissues and organs. The human body has several mechanisms to counteract oxidative/nitrosative stress via the production of antioxidants (Kurutas, 2016). In particular, Selenium (Se) is an essential trace element for humans and animals (Tuormaa et al., 2000; Saito et al., 2003) that can prevent the formation of reactive metabolites induced by many toxicants (Atif et al., 2008; Djefal et al., 2015). Selenium is well known for its effective antioxidant influence against unfavorable residues and chemicals; furthermore, Se decreases the risk organ injury, such as intestines, heart, lungs, and kidneys (Parízek, 1990; Benstoem et al., 2015) and it exhibits protective effects against cadmium-induced testicular toxicity (Li et al., 2010). Recently, Dkhil et al. (2016) and Ullah et al., (2016) reported that Se protects testicular tissue from oxidative injury and has beneficial effects on the reproduction of experimental animals.

Therefore, in this context, this study's objective was to investigate the effects of Se as an antioxidant agent against bisphenol A-induced toxicity in the testes of albino rats, with respect to their histological structure, germ cell apoptosis, antioxidant enzyme activities, sexual hormone levels and reproductive organs.

MATERIALS AND METHODS

Chemicals

Bisphenol A (BPA) (2,2 Bis-4- hydroxyl phenyl propane) was obtained from Sigma Chemicals Co. (Sigma, St. Louis, USA). BPA was suspended in water and orally administered to the treated animals at a dose of 10 µg/kg bw/day for 4 weeks (Takahashi and Oishi, 2003). Selenium (Se) was used in the form of sodium selenite (Na₂ SeO₃). This compound was obtained from British Drug Houses Ltd. (Laboratory Chemicals Division, UK) and dissolved in distilled water for administration to the treated animals at a dose of 10 µg/kg bw/day for 4 weeks.

Animals

All experiments were performed according to the local ethics committee of Umm Al-Qura University. Eighty adult male albino

rats, each weighing 240 to 280 g, were caged and randomly assigned to four treatment groups (20 rats each). For 2 weeks, these groups were fed rodent food and water *ad libitum* and maintained in a controlled environment (21 ± 24°C, 40-60% relative humidity, 12-h light-dark cycles). The animals were divided as follows:

Group 1: Served as the control. This group was used as a positive control to compare the anti-oxidative damage induced by BPA.

Group 2: Received sodium selenite (10 µg/kg bw/day; administered orally for 4 weeks).

Group 3: Received BPA (10 µg/kg bw/day; administered orally for 4 weeks).

Group 4: Received BPA (10 µg/kg bw/day) followed by Se (10 µg/kg bw/day). Both administered orally for 4 weeks.

Histological study

From each group of animals, a subset (n = 5 rats) was sacrificed at the experiment's end (that is, after 4 weeks of the treatment) and their testes excised for histological observations. The collected testes were fixed in Bouan's solution for 24 h and washed five times with alcohol. The testes were then dehydrated in ascending grades of ethyl alcohol, cleared in xylene, and infused with paraplast for later partitioning. Serial sections 5-µm thick were cut and later colored with hematoxylin and eosin stains.

Morphometrics (body and reproductive organ weights and sperm counts)

Rat body weights were recorded at 0, 2 and 4 weeks during treatment in all four groups. At each time point, five rats from each group were sacrificed. The weights of their reproductive organs, namely testes, epididymis, seminal vesicle, and prostate gland, were recorded. To determine their sperm count, the epididymis was removed and dissected in 10 mL of 0.1 M phosphate buffer previously incubated at 37°C. The sperm numbers per volume were tallied using a hemacytometer.

Germ cell preparation for flow cytometry

Germ cell isolation

Testicular tissues of all groups (n = 3 tissues from 3 rats per group) were prepared and used for flow cytometry, as described by Urriola-Munoz et al. (2014). Briefly, the testes from the control rats and those rats treated with Se, BPA, and BPA-plus-Se were removed, decapsulated, and placed in Phosphate-buffered saline (PBS) containing 0.1 mg/mL of collagenase (Sigma). The tubules were washed several times in a PBS. Tubule cell isolation was performed via disaggregation in the presence of 0.1 mg/mL DNase (Sigma), to reduce viscosity resulting from any DNA released from damaged cells during the harvesting, by using a 21G needle applied to different segments of the seminiferous tubules (previously isolated in PBS). Then, the solutions were passed through a mesh with a 200-µm pore diameter, and filtered again using a 70-µm pore diameter. A small drop of the filtrate was observed under a microscope to check the number and integrity of released germ cells before fixing in ice-cold 70% ethanol (Sigma) at 4°C overnight. The suspensions were centrifuged at 1500 rpm for 10 min and re-suspended in a cell cycle buffer (PBS) containing 0.1% of sodium citrate, 0.3% of Triton X-100 (Sigma), 50 mg/mL of propidium iodide (PI), (Sigma), and 50 µg/mL of RNase A (Invitrogen, Carlsbad, USA). The cells were immediately used for analysis by flow cytometry.

Flow cytometric analysis of cell cycle apoptosis

Germ cell apoptosis was analyzed using a fluorescence-activated cell sorting (FACS) flow cytometer (Becton Dickinson, Sunnyvale, USA) equipped with a 15 mW air-cooled 488 nm argon-ion laser. The FL2 (PI) signals were detected through a 585/42 nm band-pass filter. A total of 20 000 events were recorded in list mode and analyzed in Cell Quest Pro software (Becton Dickinson, New Jersey, USA). The cell population was gated; assuming the linear forward scatter (FSC) and side scatter (SSC) properties. Fluorescence excitation at 512 nm, with a relatively large Stokes shift, emits at a maximum wavelength of 617 nm. Apoptosis was indicated by the percentage of cells in the M1, sub G₁ phase of the cell cycle.

Biochemical assays

The testes of all groups were rapidly isolated at two time points (2 and 4 weeks) during treatment ($n = 5$ from each group) and washed with ice-cold isotonic saline (0.9%), then stored at -80°C until they were homogenized in a 50 mM phosphate buffer (pH 7.4) using an electronic homogenizer to prepare the 10% w/v homogenate. The homogenate was then divided into aliquots by volume. The level of lipid peroxidation measured as malondialdehyde (MDA) and the activity of CAT, SOD, and GPx were determined using kits bought from Randox Laboratories Ltd (Antrim, UK).

Analysis of glutathione peroxidase (GPx) activity

GPx activity was estimated with a GPx detection kit as described by manufacturer's instructions. The reduction in absorbance was measured spectrophotometrically (S2000 UV model; WPA, Cambridge, UK) against a blank at 340 nm. One unit (U) of GPx was defined as the μmol of oxidized hydrogen peroxide (NADPH) per min per mg of tissue protein. The GPx activity level was expressed as milliunits per mg of protein (that is, mU mg^{-1} protein).

Analysis of superoxide dismutase (SOD) activity

SOD activity was evaluated with a SOD detection kit as described by manufacturer's instructions. SOD activity was then measured by the degree of inhibition of this reaction. One unit (U) of SOD caused a 50% inhibition of the rate of reduction of INT under the conditions of the assay. The SOD activity level was measured spectrophotometrically at 505 nm, and expressed as U mg^{-1} protein.

Analysis of catalase (CAT) activity

Tissue CAT activity was evaluated as described by Aebi (1984). The reaction was initiated by adding hydrogen peroxide to the reaction mixture and the enzyme's activity level was quantified spectrophotometrically as the rate at which tissue catalase decomposed the hydrogen peroxide; this was done, by monitoring the decrease in absorbance at 240 nm against a blank containing a phosphate buffer instead of the substrate (S2000 UV model). The $\log A1/A2$ value for a measured interval was used to define a unit given the first-order reaction of the enzyme. One unit of CAT is the amount of enzyme that decomposes 1.0 nM of hydrogen peroxide per minute at pH 7.0 and 25°C .

Analysis of lipid peroxidation

Lipid peroxidation MDA level was measured by the content of MDA in the testis. Tissue-level MDA was determined by using the

thio-barbituric acid reactive substance assay, as described by Buege and Aust (1978). The absorbance of the clear supernatant was determined spectrophotometrically (S2000 UV model) at 535 nm, and the MDA concentration calculated using $1.56 \times 10^5 \text{ mol}^{-1} \text{ cm}^{-1}$ as molar absorbance coefficient. MDA results were expressed as nmol per gram of wet tissue.

Hormonal assays

Five blood samples from each group were collected (one sample/rat) at 2 and 4 weeks from the treatment (that is, $n = 5$ rats from each group). A 1-ml blood sample from each animal was collected from the dorsal pedal vein, and the blood plasma was separated by centrifuging at 2000 g for 10 min in a refrigerated centrifuge (Eppendorf 5804R, Hamburg, Germany) and used immediately for the testicular hormone analysis. Plasma testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were assayed at two time points (2 and 4 weeks), using an enzyme immunoassay kits (Alfa Scientific Designs, California, USA), and their levels analyzed on an absorbance microplate reader (ELx808, USA). This assay is based on the competitive binding technique.

Statistical analysis

Data are expressed here as mean \pm SEM. The significance of differences among the four group means was evaluated by multiple two-way ANOVAs, in SPSS v19 for Windows; multiple comparisons between group means were then done using the Tukey-Kramer test. Differences at $P < 0.01$ were considered as highly statistically significant, while those at $P < 0.05$ as statistically significant.

RESULTS

Histological observations

Figure 1A shows a section of the rat testis from the control group. The histological arrangement appeared healthy, with normal functional and structural seminiferous tubules. Each tubule contained multiple spermatogenic stages that included spermatogonia, primary and secondary spermatocytes, spermatids, spermatozoa, and Sertoli cells. The histological examination of the Se treated group (Figure 1B) revealed no histological or structural differences from the control group. By contrast, the testes section of BPA-treated rats (Figure 2A) showed clear alterations in their seminiferous tubules and interstitial tissue; noteworthy is the reduced spermatogenic cell size with an abnormal arrangement. The interstitial tissue appeared degenerated with signs of intertubular hemorrhaging. The blood vessels of the BPA-treated section were also enlarged and congested with interstitial edema (Figure 2B). Necrotic germ cells with pyknotic nuclei were exfoliated in the center of the tubule lumens of the section treated with BPA (Figure 2C). Figure 2D shows the degeneration of seminiferous tubules and the inhibition of spermatogenesis. However, the histological structure of testes of rats given BPA-plus-Se showed fewer pathological changes, and displayed intact histological structures, similar to the healthy

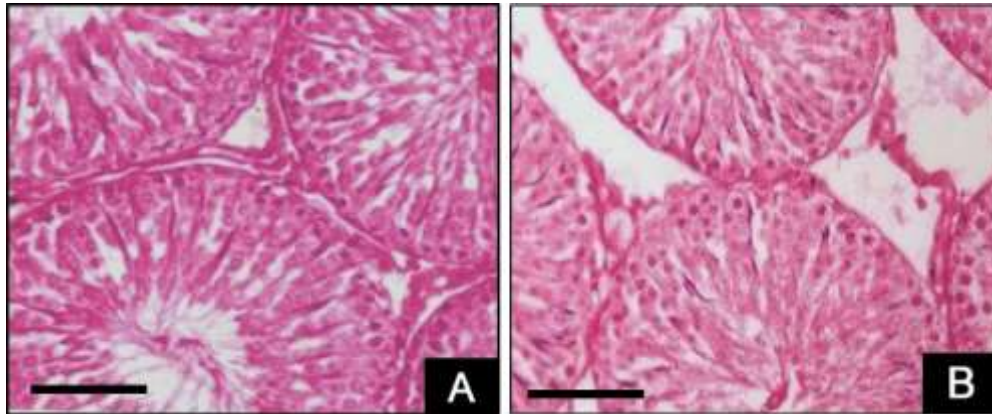


Figure 1. Photomicrograph sections of rat testes treated for 4 weeks. **(A)** Testis of control rat showing normal histological structure and healthy arrangement of seminiferous tubules. **(B)** Section of rat testis treated with Se showing normal Seminiferous tubules with various stages of sperm development.

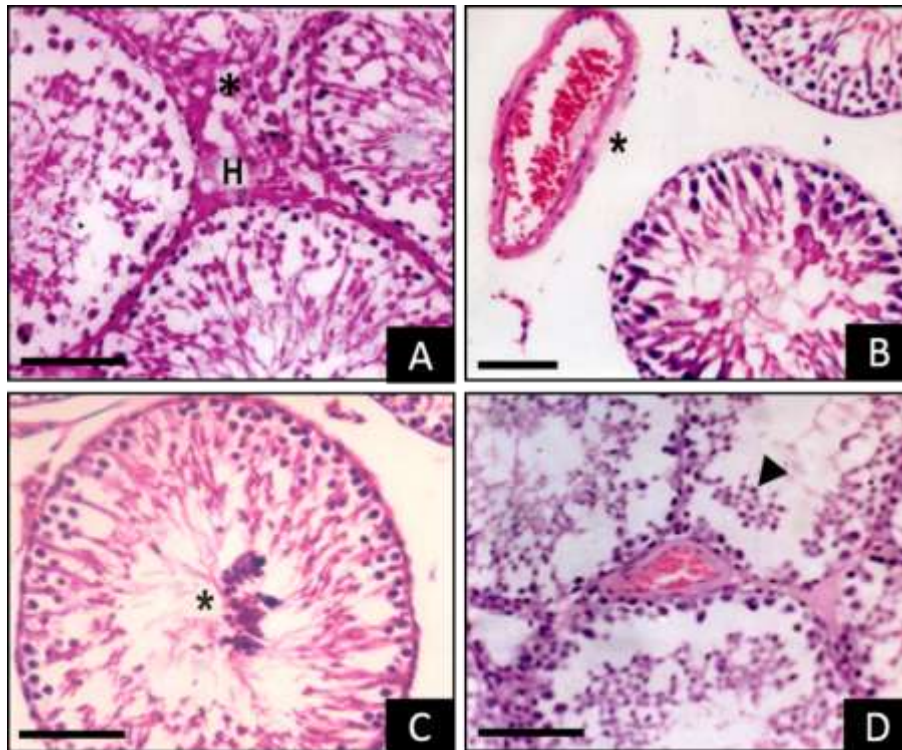


Figure 2. Photomicrograph sections of rat testes treated with BPA for 4 weeks. **(A)** Seminiferous tubules of rat testis showing degenerated interstitial tissue (*) and haemorrhage (H); **(B)** Section of rat testis showing oedema and congested blood vessel and disorganized structure; **(C)** Section of rat testes showing degenerated germ cells (-) exfoliated in the centre of seminiferous tubules; **(D)** Section of rat testis showing degeneration of seminiferous tubules and inhibition of spermatogenesis (arrow-head).

arrangement observed in both the control and Se sections. Most of the seminiferous tubules apparently contained normal spermatogenic cells, with normal

interstitial tissue (Figure 3A). Figure 3B shows the normal seminiferous tubules with numerous active germ cells, spermatocytes, spermatids, and Leydig cells (Figure 3C).

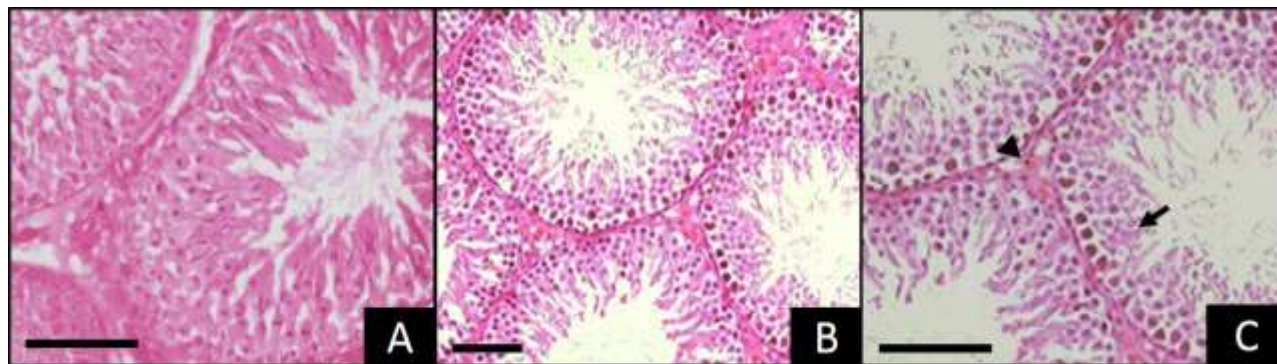


Figure 3. Photomicrograph sections of rat testes treated with BPA plus Se for 4 weeks. **(A)** Section of rat testis showing a significant improvement of the germ cells and regeneration of spermatogenesis, and normal seminiferous tubule formation; **(B)** Section of rat testis showing normal seminiferous tubules with numerous active germ cells; **(C)** Magnified photomicrograph of rat testis showing normal seminiferous tubules (arrow), spermatocytes, spermatids and Leydig cells (arrow-heads).

Table 1. Effect of BPA and selenium on seminiferous tubules (coiled tubules of the testis) diameter, epithelial height and sperm count of male rats.

Time (weeks)	Seminiferous tubules (μm)	Epithelial height (μm)	Sperm count ($\times 10^5/\text{Epididymis}$)
Control			
2	252 \pm 4.8	110 \pm 5.3	21 $\times 10^5 \pm 3.1$
4	260 \pm 5.6	115 \pm 6.7	22.2 $\times 10^5 \pm 3.7$
BPA			
2	160 \pm 4.3**	70.5 \pm 5.2**	9.2 $\times 10^5 \pm 3.8$ **
4	130 \pm 6.8**	53.4 \pm 6.9**	5.5 $\times 10^5 \pm 3.4$ **
Selenium			
2	262 \pm 6.4	111 \pm 4.6	20 $\times 10^5 \pm 3.2$
4	267 \pm 8.5	108 \pm 5.7	22.7 $\times 10^5 \pm 2.9$
BPA + Selenium			
2	230 \pm 5.4***	90.1 \pm 7.2**	17.4 $\times 10^5 \pm 2.7$ ***
4	220 \pm 5.3***	84.5 \pm 7.8***	15.3 $\times 10^5 \pm 4.6$ ***

The results are expressed as mean \pm SEM, $n = 5$ rats/group. **,***: Significant in comparison with the control group at $P < 0.05$ and $P < 0.01$, respectively; +,***: Significant in comparison with the BPA group at $P < 0.05$ and $P < 0.01$, respectively

Morphometrics

A morphometric investigation was done to obtain quantitative information on the seminiferous tubules, epithelial height, and sperm count of all four experimental groups. Changes occurred in the seminiferous tubule dimensions in those rats given BPA for 4 weeks: they had a significantly decreased diameter of the seminiferous tubules (coiled tubules) at 2 and 4 weeks relative to the control and Se-treated group. However, the group treated with BPA-plus-Se for 2 and 4 weeks showed an increase in the diameter of the seminiferous tubules when compared that of the BPA-treated group (Table 1). Hence, treating the rats with both BPA and Se caused their seminiferous tubule diameter to enlarge.

With respect to the epithelial height per unit length, at 2 weeks it was higher in the control and Se groups. Compared with these, treating rats with BPA for 2 and 4 weeks, led to decreases in epithelial height, whereas rats in the group treated with BPA-plus-Se for 2 and 4 weeks showed increases in epithelial height over the BPA-treated group (Table 1). The epididymal sperm count was remarkably reduced in the BPA-treated rats: Compared with control, a significant reduction was already evident at 2 and 4 weeks. By contrast, those rats receiving BPA and Se showed higher sperm counts at 2 weeks and 4 weeks (Table 1).

The weights of the testes, epididymis, vas deference, seminal vesicle, and prostate gland were significantly reduced ($P < 0.01$) in rats treated with BPA at two time

Table 2. Effect of BPA and selenium on weight (g) of gonadal organs of male rats.

Time (weeks)	Testes	Vas deferens	Epididymis	Seminal Vesicle	Prostate gland
Control					
2	1.37 ± 0.06	0.10 ± 0.003	0.28 ± 0.01	0.41 ± 0.04	0.27 ± 0.02
4	1.25 ± 0.09	0.08 ± 0.002	0.28 ± 0.01	0.45 ± 0.005	0.31 ± 0.006
BPA					
Time	Testes	Vas deferens	Epididymis	Seminal Vesicle	Prostate gland
2	0.65 ± 0.02**	0.07 ± 0.004**	0.16 ± 0.004**	0.21 ± 0.004**	0.16 ± 0.005**
4	0.37 ± 0.005**	0.05 ± 0.004**	0.11 ± 0.004**	0.10 ± 0.003**	0.10 ± 0.006**
BPA and Selenium					
Time	Testes	Vas deferens	Epididymis	Seminal Vesicle	Prostate gland
2	1.16 ± 0.07**	0.09 ± 0.003**	0.29 ± 0.02**	0.38 ± 0.002**	0.25 ± 0.005**
4	1.10 ± 0.06***	0.06 ± 0.002**	0.21 ± 0.004***	0.30 ± 0.001***	0.20 ± 0.007***

The results are expressed as mean ± SEM; *n* = 5 rats/group. *, **: Significant in comparison with the control group at *P* < 0.05 and *P* < 0.01, respectively. +, ++ : Significant in comparison with the BPA group at *P* < 0.05 and *P* < 0.01, respectively.

Table 3. Effect of BPA and selenium on body weight (g) of male rats relative to time Zero.

Time	Animal group			
	Control	Selenium	BPA	BPA + Selenium
Zero	250.70 ± 10.80	257.30 ± 7.50	260.10 ± 8.60	265.10 ± 8.30
2 weeks	274.35 ± 5.30	270.28 ± 7.40	263.55 ± 10.40	289.50 ± 9.65
Change %	9.43	5.04	1.32	9.20
4 weeks	312.30 ± 5.43	314.44 ± 10.50	284.64 ± 11.20	305.80 ± 8.75
Change %	24.57	22.20	9.43	15.3

The results are expressed as mean ± SEM; *n* = 5 rats/group.

points (2 and 4 weeks) relative to the control group. However, compared with the BPA-treated rats, those given BPA-plus-Se showed a remarkable increase (*P* < 0.01) in the weight of their gonadal organs (Table 2). In addition, the body weight gains of rats treated with BPA were lower than those of the control group (Table 3).

Flow cytometry of apoptosis

Cell cycle analysis was evaluated via the flow cytometry of PI-stained germ cells. No significant changes were detected in the control and Se-treated rats in all phases of the cell cycle. There was a significant increase (*P* < 0.05) in the percentage of germ cell apoptosis (M1, sub G₁) in the testes of rats treated with BPA for 2 and 4 weeks, but a decrease in other phases of the cell cycle (M₂, M3, M4) in comparison with the control and Se

groups. At 2 and 4 weeks, the respective mean percentage of apoptosis in BPA-plus-Se group was 1.7- and 1.8-fold that of the BPA group (Figures 4 and 5).

Biochemical results

Changes in the lipid peroxidation marker and antioxidant enzymes

Compared with the control, those rats in the BPA-treated group exhibited a significant increase in their MDA level (Table 4), as well as a significant decrease in the antioxidant enzyme activity of CAT, SOD, and GPx. However, rats treated with BPA-plus-Se had relatively greater SOD, CAT, and GPx activity levels but these were still less than those of the control group (Table 4), whereas, the level of MDA was significantly decreased.

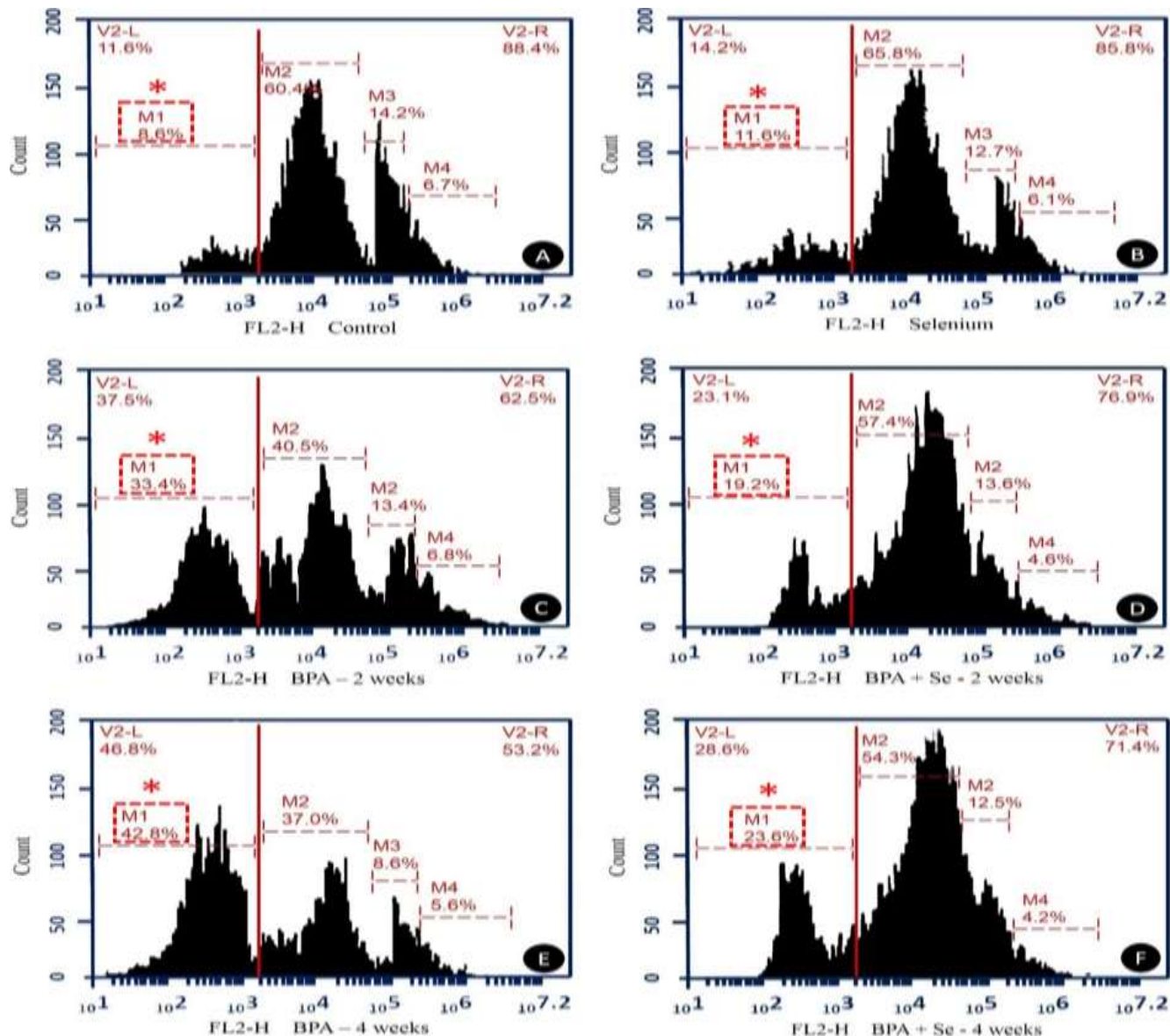


Figure 4. Effect of selenium (Se) against BPA oxidative stress. Flow cytometric of DNA cycle analysis, location of apoptotic cells (Ap) on DNA content histograms. The presence of apoptotic cells in individual experimental groups stained with **Propidium iodide (PI)**. (M1=apoptosis % (*), M2=G0/1, M3=S % and M4=G2/M). (A) Control group, (B) Group treated with selenium, (C) Group treated with BPA for 2 weeks, (D) Group treated with BPA and Se for 2 weeks, (E) Group treated with BPA for 4 weeks, (F) Group treated with BPA and Se for 4 weeks.

Changes in plasma testosterone, LH and FSH

Treating rats with BPA significantly decreased their plasma testosterone and LH levels, but significantly increased their FSH level (Table 5). In comparison, treatment with BPA-plus-Se caused a significant increase in both the plasma testosterone and LH while reducing FSH hormones (that is, the rat response to added Se in the presence of BPA). The rats in the control and Se-treated groups showed similar responses at both time points (Table 5).

DISCUSSION

Bisphenol A (BPA) is being increasingly studied due to its widespread use and accumulation in various human body fluids and tissues. This investigation demonstrates that BPA can induce histopathological alterations and inhibit spermatogenesis in the testes of albino rats. Administration of BPA significantly induced germ cell apoptosis and decreased the weights of testes and other reproductive organs in male rats. This result may be due to the inhibition of spermatogenesis, and decreased

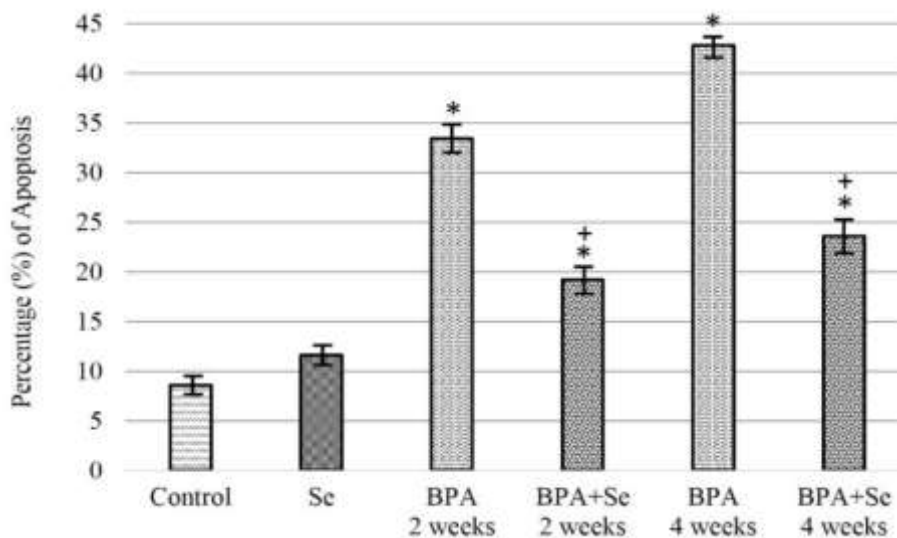


Figure 5. Changes in germ cells apoptosis activity among different animal groups. The group treated with BPA showed a significant increase in apoptotic activity compared with control and Se groups. The group treated with BPA and Se showed a positive effect, leading to a reduction in the apoptotic level compared to the BPA treated group. *, **: Significant in comparison with the control group, $p < 0.05$, $p < 0.01$; +, ++: Significant in comparison with the BPA group, $p < 0.05$, $p < 0.01$.

Table 4. Effect of BPA and selenium on malondialdehyde (MDA), catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) level of male at 2 and 4 weeks of treatment.

Time (weeks)	MDA (nmol/g ⁻¹ tissue)	CAT U mg ⁻¹ tissue	SOD U mg ⁻¹ tissue	GPx mU mg ⁻¹ tissue
Control				
2	52.5 ± 0.05	3.6 ± 0.02	44.7 ± 0.02	108.8 ± 0.04
4	53.1 ± 0.07	3.5 ± 0.03	44.5 ± 0.03	123.6 ± 0.005
Selenium				
2	53.4 ± 0.02	3.51 ± 0.004	44 ± 0.02	112.2 ± 0.04
4	56.7 ± 0.05	3.59 ± 0.04	45.2 ± 0.04	131.8 ± 0.03
BPA				
2	60.2 ± 0.07*	2.81 ± 0.04*	29.7 ± 0.05**	80 ± 0.003*
4	71.3 ± 0.06***	2.4 ± 0.005**	23.5 ± 0.03**	64 ± 0.001**
BPA + Selenium				
2	52.5 ± 0.04 ⁺	3 ± 0.04 ⁺	36.4 ± 0.06***	94 ± 0.04 ⁺
4	58.3 ± 0.04***	3.15 ± 0.03***	40.4 ± 0.03***	92.3 ± 0.06***

The results are expressed as mean ± SEM, $n = 5$ rats/group. *, **: Significant in comparison with the control group at $P < 0.05$ and $P < 0.01$, respectively; +, ++: Significant in comparison with the BPA group at $P < 0.05$ and $P < 0.01$, respectively.

elongated spermatids and steroidogenic enzyme activity. It has been shown that administering BPA to male mice reduced their sperm count and quality, and impaired spermatogenesis by decreasing the levels of reproductive hormones (Li et al., 2010; Jin et al., 2013; Tamilselvan et al., 2013). Takahashi and Oishi (2003) and Abdel-Halim et al. (2016) reported that administering BPA decreased

the number of spermatogenic cells in the seminiferous tubules within the intertubular spaces, reduced the sperm count and testes weight, and caused a marked decline in steroidogenic enzyme activity in mice. Additionally, sperm abnormalities were found to be caused by DNA strand breaks in the germ cells of testis tissue that resulted from BPA-induced apoptotic activity (Eid et al., 2015).

Table 5. Effect of BPA and Selenium on plasma testosterone, Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) level of male at 2 and 4 weeks of treatment.

Time (weeks)	Plasma testosterone (ng/ml)	Plasma LH (mIU/ml)	Plasma FSH (mIU/ml)
Control			
2	3.3 ± 0.06	2.9 ± 0.04	3 ± 0.04
4	3.6 ± 0.05	2.86 ± 0.06	3.13 ± 0.06
Selenium			
2	3 ± 0.02	2.8 ± 0.003	2.79 ± 0.04
4	3.3 ± 0.05	2.6 ± 0.06	2.8 ± 0.07
BPA			
2	1.8 ± 0.07**	0.9 ± 0.05**	3.83 ± 0.05*
4	0.8 ± 0.06**	1.3 ± 0.007**	5.95 ± 0.07**
BPA + Selenium			
2	2.8 ± 0.05*	2.7 ± 0.008***	3.4 ± 0.008**
4	2.1 ± 0.004***	2.1 ± 0.003***	4.7 ± 0.006***

The results are expressed as mean ± SEM, $n = 5$ rats/group. *, **: Significant in comparison with the control group at $P < 0.05$ and $P < 0.01$, respectively; +, ++: Significant in comparison with the BPA group at $P < 0.05$ and $P < 0.01$, respectively.

Kitraki et al. (2015) found that exposure to BPA can lead to a considerable increase in the numbers of necrotic and apoptotic cells in the testes and semen of rats, and hypothesized that BPA may induce oxidative stress, which would cause germ cells to undergo apoptosis. The present study's results clearly indicated that exposure to even a low dose of BPA disrupted spermatogenesis by decreasing the activity of antioxidant enzymes and the levels of key reproductive hormones. The results also showed that BPA could significantly inhibit the seminiferous epithelium height, seminiferous tubule diameter, and sperm count.

Concerning the biochemical results, exposure to BPA led to significant changes in the activity of antioxidant enzymes in rats. Specifically, BPA caused an elevation in the lipid peroxidation marker, testicular malondialdehyde (MDA) and a reduction of the CAT, SOD, and GPx antioxidant enzymes in rat testes. These results confirmed that BPA-induced oxidative stress and changes in SOD and CAT levels in testes, reduce the epididymal sperm count, and cause abnormal reactions with radical oxygen in several biological systems (Aitken et al., 2008). As such, antioxidant enzymes can become inhibited with an increase in the lipid peroxidation products such as MDA (Obata and Kubota, 2000).

Endocrine disrupting chemicals are defined as any exogenous agents that can interfere with the synthesis, metabolism, and action of endogenous hormones (Phillips et al., 2008). Zang et al. (2016) reported that BPA affects the functioning of the reproductive system and leads to infertility in male mice. The present study's results indicated that exposing rats to BPA caused a significant abnormality in their sexual hormone levels. Nonetheless, few reports have discussed the underlying

effects of BPA on sexual functions. Notably, Zhou et al. (2013) found that BPA reduced the testosterone levels in mice and inhibited their sexual behaviors. In rats, the results here indicated that plasma testosterone and LH levels were both significantly lowered, whereas the FSH level was markedly increased by BPA. Moreover, the results showed that BPA was able to reduce the body and gonadal weights of the male rats. A plausible explanation is that BPA caused cellular damage which affected the antioxidant enzymes' activity and sexual hormones of male rats.

Many clinical and experimental trials have used antioxidant agents to protect spermatogenesis from exposure to toxicants (Aitken et al., 2008). Selenium is one of the elements classified within the group of micronutrients that play an important role in maintaining the health and performance of human and animals (Mehdi and Dufrasne, 2016). Selenium is more commonly known for its antioxidant capacity, which protects cells from free radicals (Parizek, 1990; Mistry et al., 2012). In the present study, Se was used as an antioxidant agent against the side effects and toxicity of BPA in male rats. Se was able to reduce germ cell apoptosis in the rat testes and had a positive countervailing influence against the histological, biochemical, and morphometrical damages caused by BPA. Se led to a significant increase in spermatogenesis, seminiferous epithelium height, seminiferous tubule (coiled tubules of the testis) diameter, sperm count, body and reproductive organ weights, as well as elevating antioxidant enzymes (MDA, CAT, SOD and GPx) activity and the levels of sexual hormones (plasma testosterone, LH, and FSH). During spermatogenesis cycle, germ cell apoptosis removes most abnormal spermatogenic cells

and thereby maintains a normal quantity and quality of sperm (Print and Loveland, 2000). Abnormal apoptosis of germ cells is a critical determining factor for oligozoospermia and azoospermia, which causes detrimental histological and physiological changes in testes (Tesarik et al., 1998; Saygin et al., 2015).

The obtained results revealed a marked improvement in the testicular structure in the group given BPA with Se when compared with group given only BPA. Further, inclusion of Se had a positive effect on the body and reproductive organ weights of rats and increased their sperm count. It has been reported that sodium selenite protects testicular gametogenic and spermatogenic disorders against carbimazole and prevents testicular oxidative stress by increasing the antioxidant status (Long et al., 2016). Recently, Erkekoglu et al. (2012) reported that supplementation of Se to di(2-ethylhexyl) phthalate-treated rats lowered the activity of testicular germ cell apoptosis. In another study, a notable decrease in the apoptotic index of spermatogenic cells was recorded after scrotal hyperthermia in Se-supplemented mice (Kaur and Bansal, 2015).

The results also showed that co-administration of Se led to a decrease in MDA and an increase in the activities of CAT, SOD, and GPx. These effects are likely linked to a suppression of *GPx1* gene expression, and *GPx4*, leading to a disruption in protein oxidation (Fátima et al., 2013). Ahmed et al. (2012) reported that changes in antioxidant enzymes' activities (CAT, SOD) are related to the overexpression of the selenoprotein iodothyronine deiodinase, thus leading to the high-level production of reactive oxygen species (ROS). Some studies also suggest that the stimulation of antioxidant activity by Se may be related to the effects of GH and IGF *in vitro* (Aksu et al., 2013), which would promote oxidation and diminish GPx activity when they act as somatic growth inductors. Therefore, Se and GPx, among other selenoproteins, may thus play a key role in biological development, namely by preventing concomitant oxidation.

This study's results also confirmed that Se has positive effects against BPA-induced abnormal testicular hormones and body and gonadal weights, while confirming that it provides cellular protection against oxidative stress. Selenium's positive effect against abnormalities of the gonadal hormones and sperm count as caused by cadmium has been documented (Alhazza, 2005). Said et al. (2012) reported that Se enhanced folliculogenesis in rats via ovarian granulosa cells' expansion, estradiol and FSH discharge, and GPx movement, while diminishing both lipid peroxidation and oxidative anxiety. Selenoprotein P is the most common Se-binding protein and it is important for supplying Se to organs, especially the testis and kidney (Swathy et al., 2006; Said et al., 2012). Selenoprotein synthesis is therefore highly dependent on Se, and there is a hierarchy of selenoprotein expression in mammals when

Se is limited (Said et al., 2012; Xi et al., 2012). Selenoproteins include enzymes such as the glutathione peroxidases (GPxs), thioredoxin reductases, deiodinases, selenophosphate-synthetase 2, and selenoprotein H (Said et al., 2012). Furthermore, when and how Se is active in animal systems is mainly determined by such selenoproteins. Hence, it seems reasonable to posit that the action of Se against BPA toxicity in testes may be attributable to the presence of selenoprotein P (Schomburg et al., 2009).

Conclusion

The results obtained from this investigation demonstrate that selenium provides cellular protection against oxidative stress induced by BPA. Moreover, the reduced percentage of apoptosis in the BPA-plus-Se group compared with BPA-treated group represents the protective effect of Se against BPA toxicity, which led to a relatively healthier histological arrangement, antioxidant enzyme activity, sexual hormone levels, and body and reproductive organ weights. This experimental study confirms Se plays a crucial role as an antioxidant agent by ameliorating or even eliminating the negative effects and toxicity of other components, including BPA, thus suggesting Se possesses effective therapeutic countervailing properties against BPA toxicity. In summary, Se can promote the healthy growth and development of animals and protect them from oxidative stress.

ABBREVIATIONS

Bisphenol A, [BPA, 2, 2-bis (hydroxyphenyl) propane]; **Se**, Selenium; **MDA**, Malondialdehyde; **SOD**, Superoxide dismutase; **GPx**, glutathione peroxidases; **GPx1**, Glutathione Peroxidase 1; **GPx4**, Glutathione Peroxidase 4; **GSH**, superoxide dismutase; **CAT**, catalase; **LH**, luteinizing hormone; **FSH**, Follicle stimulating hormone; **EDC**, endocrine disrupting chemicals; **ROS**, reactive oxygen species; **GR**, oxidised glutathione; **H₂O₂**, hydrogen peroxide; **NADPH**, Nicotinamide adenine dinucleotide phosphate; **FZCS**, fluorescence activated cell sorting; **PBS**, Phosphate buffered saline.

ETHICS APPROVAL

Animal care and experimental procedures were performed per the Umm Al-Qura University, Faculty of Applied Sciences regulations.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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