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EXPLOITATION OF NANO-SELENIUM PARTICLES AND IONIZED SELENIUM FOR ATTENUATION THE HEPATOTOXICITY AND NEPHROTOXICITY INDUCED BY BISPHENOL A IN MALE ALBINO RATS

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Abstract- The present study was undertaken to evaluate the protective effect of ionized selenium (ISe) and selenium Nano-particles (SeNps) against bisphenol A (BPA) induced hepatotoxicity and nephrotoxicity in experimental rats. The selected way of application for the study was oral gavages for 8 consecutive weeks. Adult male albino rats were randomly divided into six equal groups. The first one was served as a control and administrated the corresponding dose of corn oil, whereas the remaining groups were respectively treated with bisphenol A (150mg/Kg. b.wt), bisphenol A plus ISe (3mg/kg. b.wt), bisphenol A plus SeNps (2mg/Kg. b.wt), ISe and SeNps. The exposure of rats to BPA induced hepatotoxicity and nephrotoxicity resulted in increased transaminases (AST, ALT) and alkaline phosphatase (ALP)activities. The concentrations of total cholesterol, blood urea nitrogen and creatinine were also elevated. BPA induced significant decrease in the GSH level and catalase activity while lipid peroxidation (LPO) was significantly increased in both liver and kidney homogenates. In the same time, BPA caused significant increase in DNA fragmentation in liver and kidney homogenates of exposed rats. Marked histopathological alterations were revealed in both liver and kidney tissues. The co-administration of ISe and SeNps attenuated the biochemical parameters cited above as well as the changes in DNA fragmentation. Liver and kidney histopathological studies have confirmed the changes observed in biochemical parameters and proved the beneficial role of selenium preparations. In conclusion, the use of SeNps appeared to be more significant than ISe in attenuating the undesirable effects of BPA.

Keywords- Bisphenol A, Nano-selenium particles, ionized selenium, hepatotoxicity, nephrotoxicity and DNA.

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Introduction

Bisphenol A (BPA) is a high-volume production chemical used to make epoxy resin and polycarbonate plastic products, including certain water bottles, baby bottles, as well as food storage and heating containers. It is also used in the epoxy resin lining of metal food cans, dental sealants and in carbonless paper [1] and as an additive to certain plastics used in children's toys [2]. Additionally, bisphenol A is widely distributed in the environment and commonly found in dust particles, surface water and drinking water, as over 6 billion pounds are produced worldwide each year [3]. Production of bisphenol A releases approximately two hundred thousand pounds of the chemical into the atmosphere annually [4]. Because of the extensive use of Bisphenol A (BPA), the general population may be exposed to BPA via inhalational, dermal and oral contact through foods and beverages as well as air, drinking water, dust, soil and personal care products [5]. BPA exhibits estrogenic activity and consequently disrupt endocrine balance and may lead to reproductive disorder [6]. It seems that the early stage of mammalian life is the most sensitive period of the live to BPA effects [7]. Recently, it was suggested that BPA disrupts the balance of the oxidant and anti-oxidant system. BPA may show its toxicity by increasing hydrogen peroxide in mice [8]. These authors suggested that, exposure to BPA throughout embryonic/fetal life and during infancy induce tissue oxidative stress response, ultimately leading to underdevelopment of the brain, kidney and testis in mice [9]. BPA shows potential acute, short-term, and subchronic toxicity [10]. Most studies of the health effects of BPA have focused on well-documented estrogenic activity, but reports have highlighted additional modes of action including liver damage [11], kidney damage [10], disrupted pancreatic β-cell function [12] and thyroid hormone disruption, [13]. BPA acts as a biomarker for renal insufficiency and exhibits nephrotoxicity. BPA increases estrogen metabolism in the kidney and up-regulates cytochrome p-450 aromatase activity by means of steroidogenesis. The highly water-soluble major BPA metabolite is BPA-monoglucuronide which is formed in the gut wall and liver and is rapidly removed from the blood by the kidneys [14]. Antioxidants are scavengers by preventing cell and tissue damage that could lead to cellular damage and disease [15]. BPA can cause liver, kidneys, brain, and other organs injury by forming ROS. The liver has a range of antioxidant defense system. ROS are scavenged by the endogenous antioxidant defense system, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) [9,11]. The liver is a target organ of xenobiotic or toxicants so; BPA could induce liver damage, affecting oxidant/antioxidant balance in rat liver [16]. Selenium is a trace element that is essential at small amounts, but can be toxic at larger amount. Humans and animals require selenium for the function of a number of selenium-dependent enzymes, also known as selenoprotiens. During selenoproteins synthesis, it is incorporated into a very specific location in the amino acid sequence in order to form a functional protein. At least two types of selenoproteins are necessary for each animal cell, the first form is the family of GSH-peroxidase (GSH-PX) and the second form is the family of deiodinases. GSH-peroxidases are the most powerful antioxidant enzymes, which defend the cell against oxidative damage and thus oxidative stress related diseases and disorders such as cardiovascular disease, malignancies, bacterial or viral diseases, muscle dystrophy, arthropathy, arterial plaques [17]. GSH-PX has many other regulatory

functions, such as regulation of biosynthesis of prostaglandins, prostacycline, leukotrienes, and thromboxane [18]. A different selenium form with significantly lower toxicity and higher efficacy would make it more possible to use. In this regard nano- particles of elemental selenium (SeNPs) have been recently produced. All of the comparative toxicity and efficacy studies on (SeNPs) confirm its efficacy on synthesis of selanoprotein with lower toxicity and acceptable bioavailability [19,20]. Therefore, the present study was conducted to investigate (1) the biochemical alterations, oxidative stress and DNA fragmentations induced in liver and kidney of male albino rats treated with Bisphenol A, (2) the role of nano-selenium and ionized selenium as antioxidants in alleviating negative effect of bisphenol A and (3) the effect of nano-selenium and ionized selenium alone on the tested biochemical parameters as protective supplements.

Materials and Methods

Chemicals:

Bisphenol A (BPA, 4,4-dihydroxy-2,2-diphenylpropane, CAS No: 80-05-7 in 99.9% purity) was purchased from Sigma–Aldrich company (Los Angeles, USA). It was dissolved in corn oil (vehicle). Sodium selenite (Na2SeO3) was also obtained from sigma Aldrich Company. Red dispersed solution of selenium nanoparticles (SeNPs) of diameter 20-60nm was obtained from Chemical Department, Faculty of Science Beni-Suef University. SeNPs Size was detected by transmission electron microscopy (Philips Em400T/FEG). Commercial kits for serum ALT, AST, Alkaline Phosphatase, Cholesterol, urea, creatinine were obtained from Biodiagnostic Company Egypt. Also, diagnostic kits for assaying MDA, GSH and catalase in liver and kidney homogenates were purchased from Biodiagnostic Company, Giza, Egypt.

Animals:

Sixty mature male Wister rats of the same age (8weeks) and weighing150-180g were used in this study. Animals were obtained from the Egyptian holding company for biological products and vaccines, Cairo, Egypt. Animals were maintained under standard conditions of ventilation, temperature (25±2◦C), humidity (60-70%) and light /dark condition (12/12h). The rats were housed in stainless steel cages and provided with free access to food and drinking water. After two weeks of acclimatization, animals were orally administrated their respective dose by gavage every day throughout the study. The local committee approved the design of the experiments, and the protocol complied with guidelines of the National Institutes of health (NIH, USA).

Experimental design:

Animals were divided into 6 groups of 10 animals each. Group 1 (control): rats were orally administrated corn oil (vehicle for BPA) once per day for 8 weeks. Group 2 (Bisphenol A) rats were orally administrated Bisphenol A (150 mg/kg b.wt equal to 1/20LD50 [10]. Group 3 (Bisphenol A+ sodium selenite, Naseo3) rats were orally administrated Bisphenol A with the same dose as in group 2, beside 3 mg/kg b.wt. Naseo3 [21]. Group 4 (Bisphenol A + Nanoselenium particles) rats were orally administrated Bisphenol A with the same dose, beside 2 mg/kg b.wt of Se-NPs [22]. Group 5 (sodium selenite, Naseo3) rats were orally administrated sodium selenite 3 mg/kg. Group 6 (selenium nanoparticles) rats in this group orally intubated with 2 mg/kg b.wt of Se-NPs only. The experiment was lasted for 8 weeks. Oral administration of sodium selenite and selenium Nano-particles was carried out 2 hours before oral administration of Bisphenol A in groups 3 and 4, respectively. At the end of the experiment, the animals were fasted overnight then anaesthetized with diethyl ether and sacrificed for samples collection. Blood samples were collected, left to clot then centrifuged at 3000rpm for 15 minutes. Serum was stored at -80c for evaluating the biochemical parameters. Liver and kidney of each rat were collected and divided into 3 parts. One part used for estimation of lipid peroxidation and antioxidants, the second part used for evaluation of DNA fragmentation, estimation of liver and kidney function; for serum ALT and AST activity were measured using the colorimetric method of [23], alkaline phosphatase [24], total cholesterol [25], urea and creatinine [26,27], while the third part used for histopathological study.

Preparation of Se-NPs

One ml 25 mM sodium selenite was mixed with 4 ml 25 mM GSH containing 20 mg bovine serum albumin (BSA). The mixture was adjusted pH to 7.2 with 1.0 M sodium hydroxide, instantly formed red elemental se and oxidized glutathione (GSSG). The red solution was dialyzed against double distilled water for 96 hours, the water changes every 24 hours to separate GSSG from Se-NPs. The final solution containing Se-NPs and BSA was lyophilized and stored at room temperature. X- ray photoelectric energy spectra (XPS) showed the binding energy of Se 3d was 55.3 eV indicating Se0. Transmission Electron Microscopy (TEM) showed the size of red elemental Se was 20˜60 nm [Fig-1]

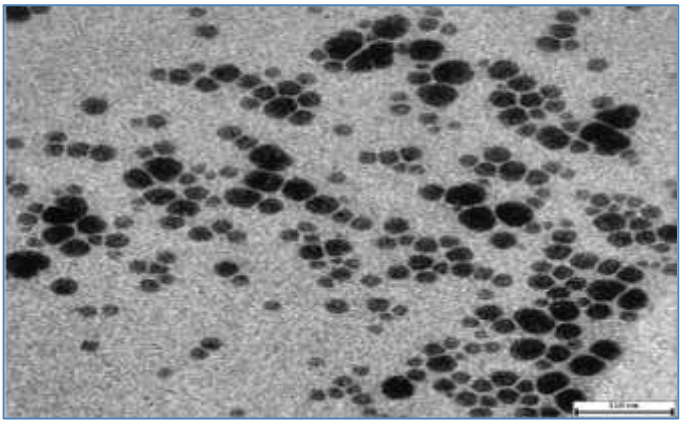


Fig-1 High-resolution transmission electron microscopy image of Se NPs; the bar indicates 110 nm. The sizes of elemental Se particles distribute from 20 to60 nm with the average size in 36 nm.

Liver and kidney tissues:

Liver and kidney tissues were minced and homogenized (10% w/v) in tris- sucrose buffer (50 mm Tris-Hcl, 0.25M sucrose, pH 7.2-7.4) by using a tissue homogenizer. The homogenate was centrifuged at 5000 rpm for 20 minutes at 4◦C, and the supernatant was used for determination of different antioxidant enzymes activity and thiobarbituric acid reactive substances (TBARS). Glutathione (GSH) and catalase (CAT) activity were estimated in liver and kidney tissue homogenates according to the methods of Beutler *et al.* (1963) and Aebi (1984)[25] respectively. Lipid peroxidation product (MDA levels) was determined in liver and kidney tissue homogenates according to the method adopted by [28].

DNA fragmentation:

Liver and kidney tissues were lysed in 1 ml buffer (10mMTris-Hcl, pH 7.4, 10 mM EDTA, 0.5% TRITON X-100) by the method of Sellins and Cohen (1987) [29].

Agarose gel electrophoresis of fragmented DNA

For electrophoretic analysis of fragmented DNA, total nuclear DNA was isolated from the tissues according to the method of Kuo *et al*., (2005) [30].

Histopathological examination:

Liver and kidney tissue samples of rats in different groups were taken and fixed in 10% formalin, prepared and stained according to Banchroft *et al.,* (1996) [31] for histopathological examination.

Statistical analysis:

The data were analyzed using one-way analysis of variances (ANOVA) followed by Duncan TEST using SPSS 11.0 statistical software (Spss, Inc, Chicago, IL.USA). They were expressed as the mean ± SE. and $P<0.05$.

Results

Biochemical analysis

The data recorded in [Table-1] indicates that AST,ALT and alkaline phosphatase (ALP) activities were significantly increased ($P<0.05$) in rats received Bisphenol A either alone or in combination with nano-selenium and ionized selenium (groups

3&4) when compared with the control group (group 1). However, administration of SeNPs or ISe in combination with Bisphenol induced significant decreased activity of the tested enzymes when compared with Bisphenol group. The same parameters in rats received Se NPs or ISe alone showed similar level as that of control group.

Results in [Table-2] shows significant increase in total cholesterol level in groups treated with Bisphenol A alone or combined with SeNPs or ionized Se compared to the control group. Meanwhile, there was a significant decrease in total cholesterol level in-group (3&4) that received (SeNPS) or ISe in combination with Bisphenol A compared to Bisphenol A alone. Furthermore, Bisphenol A treatment led to a significant increase ($P<0.05$) in the urea and creatinine level in comparison with the control group, while co-administration of SeNPs or ISe showed a significant recovery in these parameters ($P<0.05$) by reducing their concentration in serum in comparison with Bisphenol group. Also, administration of SeNPs or ISe alone induced similar level of these parameters as that of control group.

Table-1 *Serum activities of total cholesterol, AST, ALT and alkaline phosphatase in male albino rats in control and treated groups*

Parameters / Groups	Cholesterol(mg/L)	AST(U/ml)	ALT(U/ml)	Alkaline phosphatase(IU/L)
Control	120.61±5.59	132.6±5.03	60.4±1.5	209.85±28.46
Bisphenol	180.58±13.62[a]	157±2.37[a]	99.6±4.12[a]	477.12±31.72[a]
Bisphenol+ Nano Se	140.48±2.62[ba]	140.6±6.92[b]	84.4±4.84[ba]	327.76±22.58[ba]
Bisphenol+ Ionized Se	141.74±5.03[ba]	139.3±13.44[b]	88±7.32[ba]	332.68±11.82[ba]
Nano Se	119.82±1.73[b]	128.8±12.03[b]	64.4±6.12[b]	204.52±9.82[b]
Ionized Se	119.15±1.47[b]	130.56±11.23[b]	65.6±5.36[b]	198.43±18.46[b]

[a]Significant different from control group at p<o.o5
[b]Significant different from Bisphenol group at p<o.o5

Table-2 *Serum level of urea and creatinine in male albino rats in control and treated groups*

Parameters / Groups	Urea(mg/dL)	Creatinine(mg/dL)
Control	30.89±1.26	1.352±0.007
Bisphenol	51.61±1.59[a]	2.71±0.007[a]
Bisphenol+ Nano Se	35.29±3.42[ba]	1.85±0.002[ba]
Bisphenol+ Ionized Se	40.12±7.43[ba]	1.89±0.001[ba]
Nano Se	30.15±1.97[b]	1.29±0.004[b]
Ionized Se	31.11±1.58[b]	1.34±0.001[b]

[a]Significant different from control group at p<o.o5
[b]Significant different from Bisphenol group at p<o.o5

Hepatic and kidney oxidative stress (antioxidant defense system):

Significant decrease in hepatic and nephro-GSH concentration and CAT activity of BPA (group 2) exposed animals was observed in comparison with the control group. SeNPs and ISe pretreatment 2h before Bisphenol (groups 3,4) administration attenuated the undesirable effect of bisphenolin comparison with Bisphenol A group, but these levels were significantly lower than the level of control one. Oral administration of SeNPS and/ or ISe alone induced desirable effect on the previous parameters and their values became similar to that of control group.

Tissue lipid peroxidation product MDA.

The obtained results in [Table-3] revealed that liver and kidney MDA level was significantly increased ($P<0.05$) in BPA (group 2) compared to the control one. Oral administration of SeNPs and/ or ISe 2h before BPA induced significant decrease in MDA level in these organs compared to the BPA group. Administration of SeNPs or ISe alone (group 5,6) showed no effect on MDA level than that of control group.

Table-3 *Catalase activity, GSH level and LPO concentration in liver and kidney tissues homogenate of albino male rats in control and treated groups*

Groups	Liver homogenate			Kidney homogenate		
	Catalase (U/g.)	GSH(ug/g.)	LPO (nmol/g)	Catalase(U/g)	GSH (ug/g.)	LPO (nmol/g)
Control	0.764±0.008	0.754±0.002	23.5±1.5	0.624±0.007	0.589±0.003	18.5±1.26
Bisphenol	0.298±0.005a	0.285±0.003a	43.5±5.9a	0.213±0.005a	0.234±0.004a	32.6±3.2a
Bisphenol+ Nano Se	0.499±0.004ab	0.416±0.007ab	30.6±2.7ab	0.498±0.004ab	0.429±0.002ab	24.9±3.6ab
Bisphenol+ Ionized Se	0.502±0.006ab	0.431±0.001ab	28.8±0.73ab	0.503±0.004ab	0.417±0.003ab	25.8±2.7ab
Nano Se	0.741±0.007b	0.657±0.004b	21.2±1.16b	0.624±0.006b	0.621±0.004b	19.13±1.5b
Ionized Se	0.718±0.003b	0.643±0.008b	22.8±0.003b	0.615±0.003b	0.601±0.002b	20.3±1.3b

[(a)]Significant different from control group at P<0.05
[(b)]Significant different from Bisphenol group at P<0.05

DNA fragmentation assay

Results of DNA fragmentation assay were illustrated in [Table-4] and [Fig-2&3]. They revealed that BPA induced highly significant percentage of DNA fragmentation in liver and kidney of BPA treated rats in comparison with the control group. Oral administration of SeNPs and ISe to BPA induced marked protective effect in DNA fragmentation and the results denoted significant inhibition in percentage of DNA fragmentation in groups (3&4) in comparison with BPA group (group 2) while oral administration of SeNPs and ISe alone showed no changes in DNA fragmentation percentage than that of control group.

Table-4 *Mean values of DNA fragmentation % in liver and kidney of experimental bisphenol and treated groups*

Groups	Liver	kidney
Control	21.86±1.54	18.66±1.47
Bisphenol	49.59±0.65 [a]	43.77±0.7[a]
Bisphenol+ Nano Se	26.4±1.04 [ab]	22.65±0.66 [ab]
Bisphenol+ Ionized Se	25.76±3.11 [ab]	21.3±0.44 [ab]
Nano Se	21.6±1.24 [b]	17.49±1.37 [ab]
Ionized Se	23.7±1.16 [ab]	19.7±0.83 [ab]

[a]Significant different from control group at p<o.o5
[b]Significant different from Bisphenol group at p<o.o5

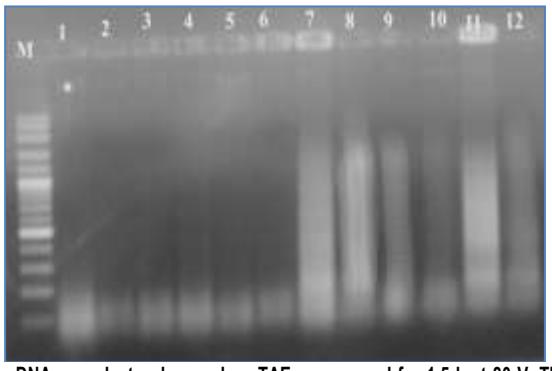


Fig-2 The DNA was electrophoresed on TAE agarose gel for 1.5 h at 80 V. The DNA fragments were visualized with ethidiumbromide staining. Samples are Liver. Lane 1,2: control group; lane 3,4: ionized se group; lane 5,6: Nano Se group; lane 7,8: bisphenol group; lane 9,10: bisphenol with ionized se group; lane 11,12: bisphenol with Nano Se group; M: 100 bp

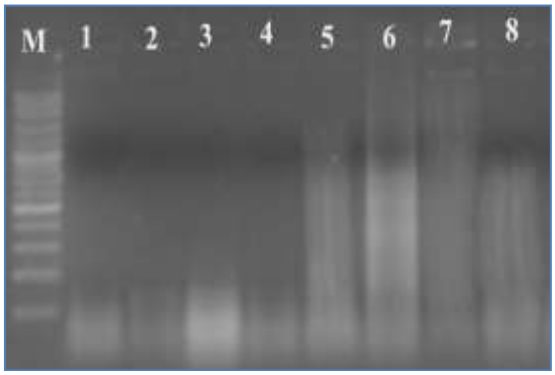


Fig-3 The DNA was electrophoresed on TAE agarose gel for 1.5 h at 80 V. The DNA fragments were visualized with ethidiumbromide staining. Samples are kidney. Lane 1,2: control group; lane 3: Nano Se group; lane 4: ionized se group; lane 5,6: bisphenol group; lane 7: bisphenol with Nano Se group; lane 8: bisphenol with ionized se group; M: 100 bp

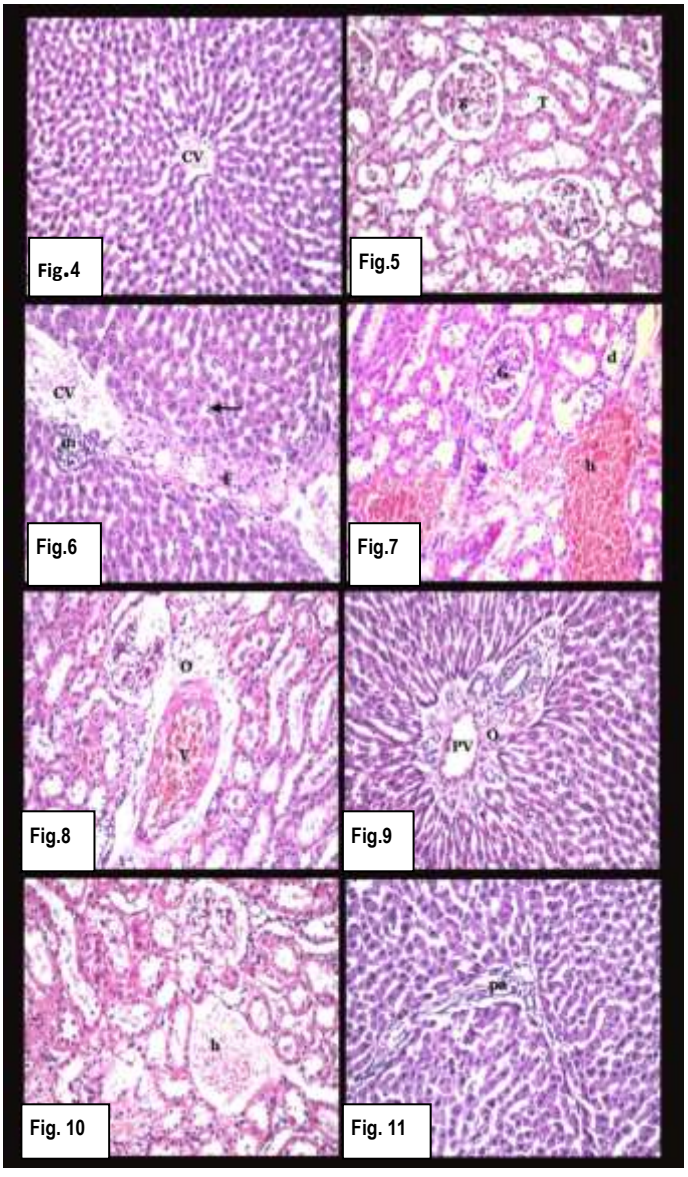
Histopathological finding

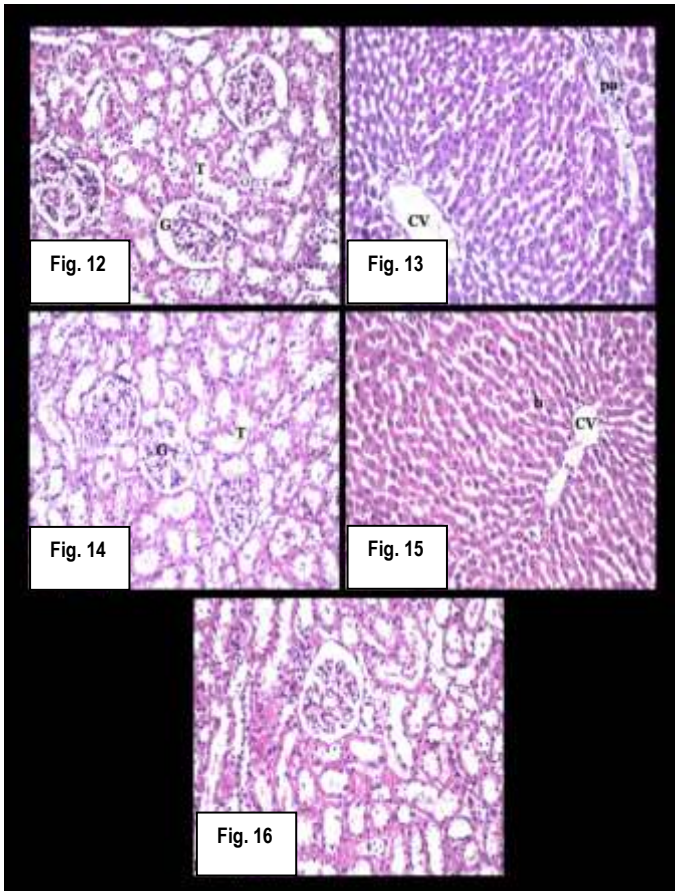
Histopathological examination of liver tissue sections of rats exposed to BPA showed abnormalities as compared to the control rats [Fig-.4]. Liver sections from the control (group, 1), SeNPs (group, 5) and ISe (group, 6) treated rats showed normal histological structure of the central vein and surrounding hepatocytes in the hepatic parenchyma [Fig-4,13,15]. In contrast, the exposure of BPA to rats induced focal inflammatory cells infiltration as well as focal area of fibroblastic cells proliferation in the hepatic parenchyma associated with dilatation and congestion in the central vein and diffuse proliferation of the Kupffer cells in between the hepatocytes [Fig-6]. Co-administration of SeNPs with BPA (group, 3) showed mild dilatation in the portal vein with oedema in the portal area [Fig-9] while co-administration of ISe (group, 4) recorded no histopathological alteration and the liver tissue almost normal [Fig-11]. Light microscopic evaluation of the kidneys of the control group, SeNPs (group 5)and ISe (group 6) revealed normal histologic structure of the glomeruli and tubules at the cortex [Fig-5,14,16]. However, there were focal haemorrhagic areas were detected in between the degenerated tubules and congested glomeruli at the cortex [Fig-7], in addition to congestion in the cortical blood vessels associated with perivascular oedemain rats treated with BPA [Fig-8]. Co-administration of SeNPs with BPA ameliorated the toxic effects of the BPA and histopathological findings in the kidney appeared as a focal hemorrhage in between the tubules at the cortex [Fig-10] while co-administration of ISe, showed no histopathological alteration in the kidney [Fig-12].

Discussion

Bisphenol A (BPA) is an environmental chemical that has been widely used in the manufacture of polycarbonate plastics and expoy resin for many years. Due to its major applications in the production of plastic containers used in food or beverage and the coating of food cans, people of different ages are inevitable exposed to BPA in daily life [32]. Bisphenol has known to induce abnormalities in the liver and kidney of rats and mice. The mechanism of action of BPA on liver and kidney is not clear. The aim of this study was to investigate if BPA induces a stress in the liver and kidney of rats and if co-administration of selenium nanoparticles (SeNPs) and/or ionized selenium (ISe), as antioxidants can ameliorate stress effect of BPA. Liver and kidney functions generally refer to blood tests that evaluate the presence of liver and kidney damage or disease. These tests may include ALT, AST, ALP, total cholesterol, urea and creatinine. In the present study, the serum ALT, AST and ALP levels increased in BPA group when compared to control group. The rise in both ALT and AST levels is one of the most familiar indicators of hepatocellular damage. These observations are similar to the previously reported data [12,34].

They indicate that exposure of male and female rats to octylphenol induced potential toxic effects on liver and kidney tissues. Also, our results are similar to findings reported by Hassan *et al.* (2012)[21] who mentioned that 50mg /kg BPA to male rats significantly increased the biochemical levels of ALT, AST, ALP and cholesterol. Compared study reported increase in the serum ALT and AST levels in BPA groups compared to the control one [16]. Also, Yasmaski *et al*., (2002) [35] reported an increase in AST activity in treated male rats with BPA ≥200 mg/kg/day and an increase in ALP activity in males treated with 600 mg/kg/day. In the current study, marked increase in the concentration of total cholesterol was found in BPA treated group compared to the control group, which may reflect the impairment of the liver function. This result is similar to data obtained by Eman, (2012) [34] which revealed significant increase in the levels of serum total lipids, total cholesterol, and triglyceride in octylphenol treated group.





In control group, [Fig-4] The normal histological structure of the central vein (CV) and surrounding hepatocytes in the hepatic parenchyma. [Fig-5] The normal histological structure of the glomeruli (G) and tubules (T) at the cortex. In BPA treated group, [Fig-6] Focal inflammatory cells infiltration as well as focal area of fibroblastic cells proliferation were observed in the hepatic parenchyma associated with dilatation and congestion in the central vein and diffuse proliferation of the kupffer cells in between the hepatocytes. [Fig-7] Focal haemorrhage was detected in between the degenerated tubules (d) and congested glomeruli (G) at the cortex. [Fig-8]. There was congestion in the cortical blood vessels(V) associated with perivascular oedema (O). In SeNPs with BPA treated group, [Fig-9] The portal area showed mild dilatation in the portal vein (PV) with oedema in the portal area(O). [Fig-10] Focal haemorrhage was detected in between the tubules at the renal cortex. In ionized Se with BPA treated group, [Fig-11,12] there was no histopathological alterations in liver and kidney tissue sections of rats, respectively. In SeNPs treated group [Fig-13, 14] there was no histopathological alterations in liver and kidney tissues, respectively. In ionized Se treated group [Fig-15,16] there was no histopathological alteration in liver and kidney tissue sections, respectively.

In the present study, nephrotoxicity was manifested by inhibition of kidney function as indicated by increased serum creatinine and urea levels in BPA treated group compared to the control group. The nephrotoxic effect of BPA was supported by the present histopathological changes in kidney of treated rats.

Similar results was recorded by Eman (2012) [34] who manifested nephrotoxicity of 4-tert-octylphenol (OP) in rats dosed with 100mg/kg and indicated by increase serum creatinine and urea. Also, Morgan *et al*., (2014) [36] recorded elevated urea and creatinine levels in BPA and OP treated rat groups. Antioxidants reduce the damage resulting from interaction between lipid, protein and DNA molecules and ROS. Regardless of the presence of this antioxidant system, an over or unbalanced production of ROS due to contact with chemicals may result in a number of clinical disorders. BPA can cause oxidative stress by distributing the redox status in cells [37]. The levels of non-enzymatic antioxidant GSH and enzymatic antioxidant (CAT) activity were measured to evaluate the stability of ROS production in liver and kidney. GSH acts directly as an antioxidant and also participates in catalytic cycles of several antioxidant enzymes such as glutathione peroxidase and glutathione reductase. The reduction of GSH shows the failure of primary antioxidants system to act against free radicals [16]. The current results in the present study show that GSH concentration and CAT activity in liver and kidney of BPA treated rats were decreased. Similar to our results, data recorded by Hassan *et al.,* (2012) [21] which revealed significant decrease in the level of reduced glutathione and catalase activity in liver tissue of BPA treated rats. Also, Hussein and Eid (2013) [38] reported decreased activity of CAT in liver tissue of mice treated with different doses of BPA.

Our finding of the decreased activity of CAT in liver and kidney of BPA treated rats is correlated with results of [39]. Reduction in the activity of catalase may reflect inability of liver or kidney mitochondria and microsomes to eliminate hydrogen peroxide produced after exposure to BPA [11]. Lipid peroxidation (LPO) is widely used as a marker of oxidative stress and cell membrane damage which result in gradual loss of membrane fluidity, decrease membrane potential and increased permeability to ions [15]. The present results revealed increased LPO in the liver and kidney of BPA treated rats. It has been reported that ROS were induced in the brain, livers, kidney and tests of mouse when BPA was administrated throughout the embryonic fetal life during infancy [8,9,40,].

In addition, [41] have been reported that absorption of large amount of BPA through the skin causes extensive damage to liver, kidney and other organs in humans. It has been known that increased TBARS levels and decreased level of GSH and CAT activity indicate an increase of ROS, which cause lipid peroxidation in the liver and kidney [42]. In our study, the BPA treated rats showed significant increase in LPO concentration in liver and kidney. Similarly, previous studies showed increased in the LPO levels in liver, kidney, brain and testes of treated rats exposed to BPA [16,21,38]. BPA has been investigated for genotoxicity in a variety of test system, both in vitro and in vivo, but the results were controversial [43]. It has been shown to bind DNA in a cellular system, in which BPA reacts with DNA after metabolic activation [44,45], in cultured mammalian cells [46] and in the livers of both rats [47] and mice [45] treated in vivo. The results in this study revealed that BPA in a dose of 150mg/kg b.wt for 8 weeks induced significant increase in DNA fragmentation in both liver and kidney tissues of treated rats. Several investigations studied the mutagenic effect of BPA. [48] provided evidence that BPA is able to form DNA adducts in cultured human prostatic cells in vitro and to induce a variety of alteration in the cells of rats receiving BPA in drinking water. Also, Eman (2012) [34] demonstrated that liver DNA in OP treated rats was found to be greatly degraded compared to control animals.

Moreover Izzotti *et al*., 2009 [45], demonstrated that administration of BPA with drinking water resulted in the formation of DNA adducts and proteome alteration in the mammary tissue of mice. In humans and in experimental animals, BPA is metabolized to its glucuronide and hydroxylated derivatives, mainly 3-hydroxy-BPA, (3-OH-BPA or BPA catechol), which is then oxidized to its ortho quinine i.e. BPA-3,4Quinone (BPAQ) (Edmonds *et al*., 2004). It is a noteworthy that oxidation of catechols to semiquinones or quinones is a mechanism of tumor initiation for regulation for endogenous estrogen as well as far synthetic estrogens such as diethylstilbestrol [49]. In the present results, the oxidative stress markers in the liver and kidney tissues and liver and kidney function tests of treated groups were confirmed by the histopathological evaluation of liver which had focal inflammatory cells infiltration as well as focal area of fibroblastic cell proliferation. Congestion of central vein and diffuse proliferation of Kupffer cells were observed. Similar lesions were reported by Moon *et al.* (2012) and Mourad and Khadrawy (2012).

Light microscopic examination revealed signs of inflammatory cellular infiltration, vacuolated hepatocytes, congested blood vessels and increase in number of Kupffer cells. These changes were demonstrated by Boshra and Moustafa (2011), Royal *et al.* (2011) and Hassan *et al.*(2012) [21]. These observations may be explained by BPA induced peroxidation of membrane lipids in the liver. In addition, BPA treated rats showed focal hemorrhage in between the degenerated tubules and congested glomeruli of the cortex. Congestion in the cortical blood view vessels (perivascular) oedema was noted. Similar lesions were detected by Morgan *et al.* (2014) [50] who revealed that kidney section of BPA – exposed rats showed glomerular congestion and hypercellularity.

Recently, elemental nano-Se has attracted a wide spread attention due to its high bioavailability, low toxicity and antioxidant activity. In our experiment, co-administration of NSeP Or ISe with BPA tended to alleviate plasma transaminases and ALP as demonstrated in the current result and by other studies [21,44,51,52]. The present data also indicated that Co-administration of NSeP and /or ISe to BPA treated group induced significant reduction of serum urea and creatinine levels. These results may indicate that NSeP or ISe can attenuate the renal damage. Similar observation was reported by [50,53]. The Co-treatment of Se (NSeP or ISe) improved the histological alterations induced by BPA, which could be attributed to that antiradical /antioxidant and metal chelating efficacy of selenium. Moreover, these results are good accordance with those obtained by other studies which have postulated the protective role of Se on biochemical and histopathological changes of rat's tissue [21,50]. BPA has a pro-oxidant character and its administration is associated with induction of oxidative stress by the generation of free radicals. The obtained data revealed that Co-administration of NSeP and/ or ISe prevented the BPA- induced lipid peroxidation and also modulated enzyme antioxidant activity and resorted the antioxidant activity of CAT and GSH, respectively, near the control level. Improvement of lipid peroxidation, CAT activity and level of GSH in BPA treated rats by Se preparation was demonstrated by Bhattacharjee *et al.* (2014) [52] against cyclophosphamide, AbouZaid *et al*.(2014) [53] against Benzo(a), pyrene, Mohamed and Safwat(2013) [54] against acetaminophen, Rezvanfar *et al.* (2013) [22] against cisplatin, Hassanin *et al.(*2013) [38] against chromium and Heikal *et al.* (2010) [21] against chloropyrifos. Also, our results are in good accordance with those obtained by El-demerdash (2004) and Abou-Zaid *et al.* (2014) [44,53] who found that Se maintained the levels of antioxidants, membrane bound enzymes, and the activities of antioxidant enzymes near normal levels. The protective role of Se may be explained by Ognjanovic *et al*. (2012) by the important role of Se in preventing hydroxyl radicals formation and in protecting the integrity and the function of tissues. The important finding of the present study is that NSeP or ISe significantly inhibits BPA – induced DNA damage in liver and kidney of the treated rats, which clearly indicated the potential role of Se in protecting DNA damage. Our data was correlated with finding of Bhattacharjee *et al.* (2014) [52] who demonstrated that Nano -Se significantly decreased DNA damage in peripheral lymphocyte cells and also in bone marrow cells of mouse treated with the chemotherapeutic drug cyclophosphamide. In the present study Co-administration of NSeP with BPA induced more significant protective effect on the liver and kidney function, oxidative stress and DNA damage than that of ISe. This observation was correlated with the finding of Zhang *et al.* (2001) who mentioned that nano-elemental Se has attracted wide spread attention due to its high bioavailability and low toxicity because nanometers particulates exhibit novel characteristics, such as great specific surface area, high surface activity, a lot of surface active centers, high catalytic efficiency and strong adsorbing ability and the characters of low toxicity of routine Se. Also, our results are in agreement with Zhang *et al.* and Wang *et al* (2007,)[19,20] who reported that Nano- Se is less toxic than ISe in short term /large dose treatments.

Conclusion

This study clearly indicates that BPA has a pro-oxidant characters and its administration is associated with induction of oxidative stress by the generation of free radicals that affect anti-oxidative system as well as biochemical parameters, in addition to significant increase in DNA fragmentation and marked histopathological alterations in both liver and kidney tissues of exposed rats. Co-administration of ionized selenium (ISe) and/ or selenium nano- particles (SeNps) ameliorates these disturbances and reduces the damage resulting from interaction between ROS and lipid, protein and DNA molecules. These results could be attributed to their antiradical /antioxidant efficacy. Moreover, the data showed that the use of SeNps appeared to be more effective than ISe in attenuating the undesirable effects of BPA and it has attracted a wide spread attention due to its high bioavailability, low toxicity and antioxidant activity.

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