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Ameliorative Potential of Selenium against Bisphenol A- Induced Hepatotoxicity in Rats

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ABSTRACT

Background: environmental pollutants affect various tissues. Bisphenol A, a compound used in making epoxy resins and polycarbonate plastics**,** induces many hazardous effects. **Aim of the work:** this work was designed to test the ameliorative potential of selenium against hepatotoxicity caused by bisphenol A. **Materials and Methods:** male rats were divided into four groups. Group 1 served as control, group 2 given sodium selenite, group 3 was administered with suspension of bisphenol A that is dissolved in corn oil. Rats of group 4 were administered with selenium plus bisphenol A. Liver specimens and blood samples were inspected after 3 and 6 weeks of treatment.

Results: there was no statistical difference between control and selenium -administered rats in all parameters. Rats treated with bisphenol A suffered significant depression in weight whereas selenium administration decreased the effect on rat's weight. Bisphenol A administration induced blood vessels congestion, inflammatory infiltration, bile duct proliferation, cytoplasmic vacuolization and macrosteatosis while selenium administration improved liver histopathological criteria either after 3 or 6 weeks. Bisphenol A treatment elevated nuclear PCNA and caspase-3 expression in the cytoplasm and liver function enzymes (serum AST and ALT) and bilirubin. Again, selenium ameliorated these changes. In **conclusion**, bisphenolA exerted deleterious impact on rats' hepatocytes and serum biochemical parameters in a time-dependent manner. Selenium supplementation provides an extent of amelioration against bisphenol A- induced hepatotocixity.

Keywords: Bisphenol A**;** Selenium**;** Histology; Immunohistochemistry; Biochemistry.

INTRODUCTION

Environmental chemical contaminants might alter some metabolic processes accordingly cause injuries to body organs. Such contaminants disturb the hormonal regulation and affect other body's mechanisms [1]. Of these pollutants is the estrogenic compound bisphenol A (BPA) applied in a range of products as reusable drink and food containers and other products [2]. Under basic, acidic conditions or high temperature, BPA is hydrolyzed thus leaches into these containers [2]. A 95% BPA was found in human urine samples in a United States' reference population [3] , in plasma of mothers and fetuses and in placental tissue at delivery [4] and in human colostrums [5]. BPA affects rat's spermatogenesis [6], decreasing the sperm production efficiency in male rats [7] and injures kidneys, brain, liver and other organs via formation of reactive oxygen species [8, 9]. Moreover, association between elevated levels of free active BPA and obesity, diabetes and cardiovascular diseases and low spermatozoa count was recorded [10] and to rats brain damage [11]. *Hassan et al.* [12] found generation of reactive oxygen species and decreased expression of antioxidant genes in the liver by BPA. Selenium (Se), a nutritional supplement is an important component of antioxidant defense system in cells and is engaged in the modulation of intracellular redox specially glutathione peroxidases and thioredoxin reductases [13]. Se protected liver against Cd [14], aluminum [15], mercury chloride- induced rats liver and kidney damage [16] and efficiently inhibiting AFB1- induced liver carcinogenesis [17]. This study aims at evaluation of the protective capability of selenium in combating BPA hepatic toxicityin albino rats by histopathological inspection, PCNA and caspase-3 immunoexpression and by measuring serum ALT, AST and bilirubin.

MATERIALS AND METHODS

Animals and experimental design

Adult male albino rats (*Rattus norvegicus*), weighing 130±10 g were obtained from the National Institute of Serum and Vaccination, Cairo, Egypt. Rats were kept under laboratory conditions of 22–25^{o}C and 12 h light/

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dark cycle in special plastic rodent cages and fed on standard chow rodent diet and water *ad libitum* for one week. Animals were handled according to the protocol of care of Laboratory Animal of Faculty of Science, Menoufia University, Egypt. Following one week adaptation, rats were randomly divided into four groups; 6 rats from each group. Group 1, normal control rats receiving standard diet and water *ad libitum* throughout the whole experimental period. Group 2 rats were orally administered aqueous sodium selenite (Se) (10 µg/kg b.w/day) according to Sakr *et al.* [18] (obtained from British Drug Houses LTD, Laboratory Chemicals Division, England). Group3 was orally administered suspension of 20 mg BPA / kg b.wt/day in accordance of Takahashi and Oishi [19] (BPA from Sigma Chemicals Co. St. Louis, MO, USA). It is dissolved in corn oil. Group4 received BPA (10 µg/kg b.w) followed by Se (10 µg/kg b.w) daily. After 3weeks half the rats of each group were sacrificed and after 6 weeks of daily administration the other half were sacrificed. Liver specimens and blood samples were collected.

The study was approved by the Ethics Board of Menoufia University.

Histological preparations

Liver specimens from all groups were fixed in 10% neutral buffered formalinfor 24 hours, washed in tap water, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax (melting point 52-56^{O}C). Sections of 5µm thickness were cut by rotary microtome, stained with haematoxylin and counterstained with eosin [20].

Immunohistochemical study

For detection of PCNA and caspase 3, avidin biotin complex immunoperoxidase technique was performed on paraffin-embedded sections. Deparaffinized slides were blocked with 1.75% methanolic hydrogen peroxide for 20 minutes then antigen retrieval using Biogenex Antigen Retrieval Citra solution in 90^{O}C water bath for 30 minutes was done. Slides were left to cool for 20 minutes then blocked by normal horse serum for 5 minutes at 37^{O}C. Sections were incubated overnight at 4^{O}C with the antibodies against PCNA (polyclonal rabbit-anti-human (A3533 Ig fraction; DAKO, Glostrup, Denmark) andcaspase-3 (rabbit polyclonal antibody at dilution 1:200, Thermo Scientific, Ab-4). The immunohistochemical reaction was then developed and stained with diaminobenzidinechromogen solution "DAB" (Sigma). Sections were counterstained with hematoxylin and finally mounted with DPX. For negative controls, PBS was applied instead of the primary antibody.

Image analysis

Digital images were analyzed by a semi-quantitative scoring system (Image J software, Java based application for analyzing images). The positive- stained immunohistochemical expressions of PCNA- and caspase 3 in cells were measured by counting the number of positively- stained cells in five high power fields selected randomly at magnification X400.

Biochemical assessment

Samples of blood were clotted at room temperature then sera were separated by 20 minutes centrifugation at 3000 rpm and stored at -18 to -20^{O}C until further analysis. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured following Reitman and Frankel method [21] using commercial kit supplied by Randox Co. For total bilirubin measurement, the method of Walter and Gerade [22] was followed.

Statistical analysis

Data were presented as the mean ± standard deviation (SD). Analysis was performed utilizing the Statistical Package for the Social Sciences (SPSS software version 16). Student t- test was performed for assessment of the significance of differences between groups and control at $P \leq 0.05$.

RESULTS

1. Change in body weight

Treating rats with BPA either for 3 or 6 weeks caused significant depression in body weight especially after 6 weeks compared to control rats. When Se was given, slight change in body weight was recorded. Additively, Se with BPA administration caused noticeable elevation in body weight when compared with BPA group especially after 3 weeks while it still significantly decreased after 6 weeks (Fig.1).

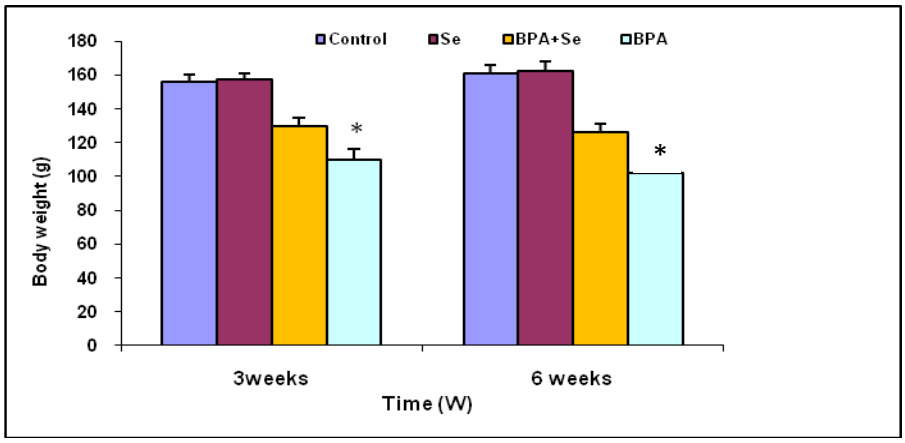


Fig. 1: Effect of BPA and Se on body weight

2. Histological results

Liver sections from control and Se-administered rats showed normal structure (Fig.2a). Liver sections of BPA- exposed rats for 3 weeksrevealed blood vessel congestion, cytoplasmic vacuolization and necrosis (Fig.2b), inflammatory cellular infiltration and proliferation of bile duct (Fig.2c) and loss of architecture (Fig.2d). After 6 weeks, clumps of leucocytic infiltration and congested blood vessels were evident (Fig.3a). Micro- and macrosteatosis were noted (Fig. 3b). Administration of Se wit BPA reduced the deleterious impact of BPA where hepatocytes were arranged around central veins and minimal number of inflammatory leucocytes was observed (Fig. 3c).

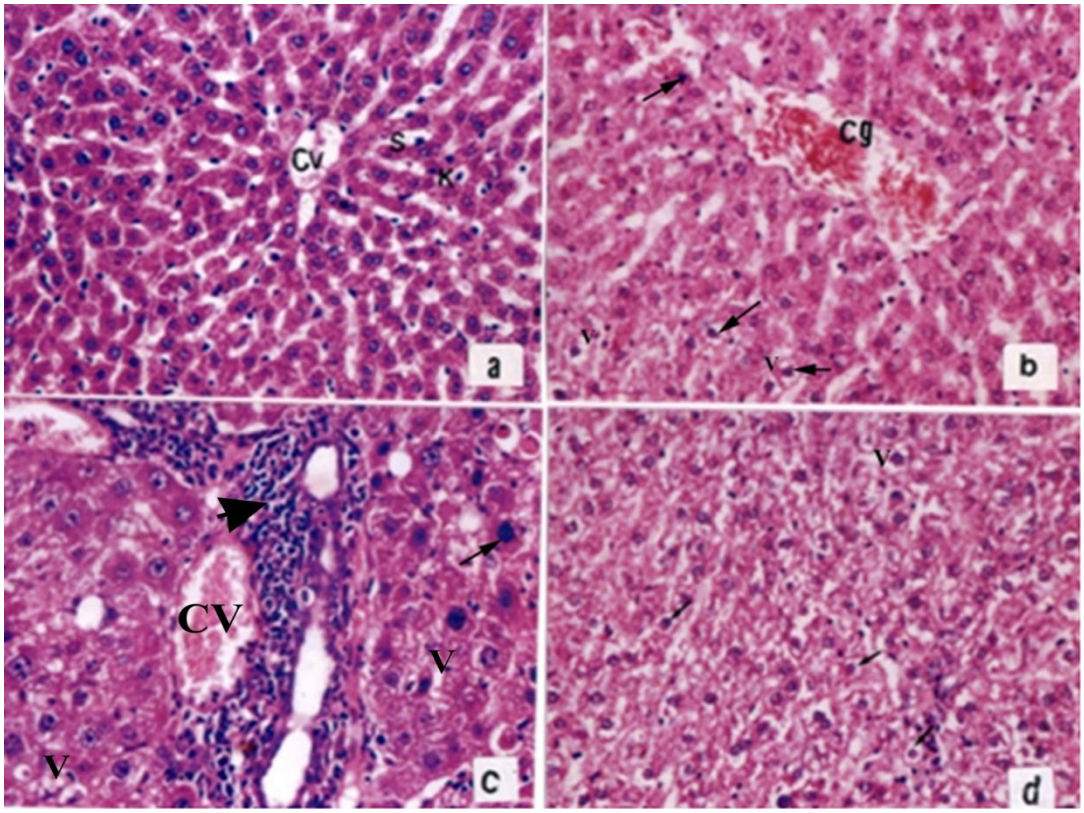


Fig. 2: a: Liver section of a control rat showing normal structure where hepatocytes radiating from the central vein (CV), normal spaced - sinusoids (S) and kupffer cells (K).

Fig.2 b, c and d: Liver sections of rats exposed to BPA for 3 weeks; **2b**: Showing congested central vein (Cg), cytoplasmic vacuolization (V) and pyknotic nuclei (arrows). **2c:** Showing cytoplasmic vacuolization (V), pyknotic nucleus (arrow), congested portal veins (CV) and massive leucocytic infiltration (arrow head). **2d**: Still disruption of hepatic architecture, intensive vacuolization (V) and nuclear pyknosis (arrows). H&E. X200

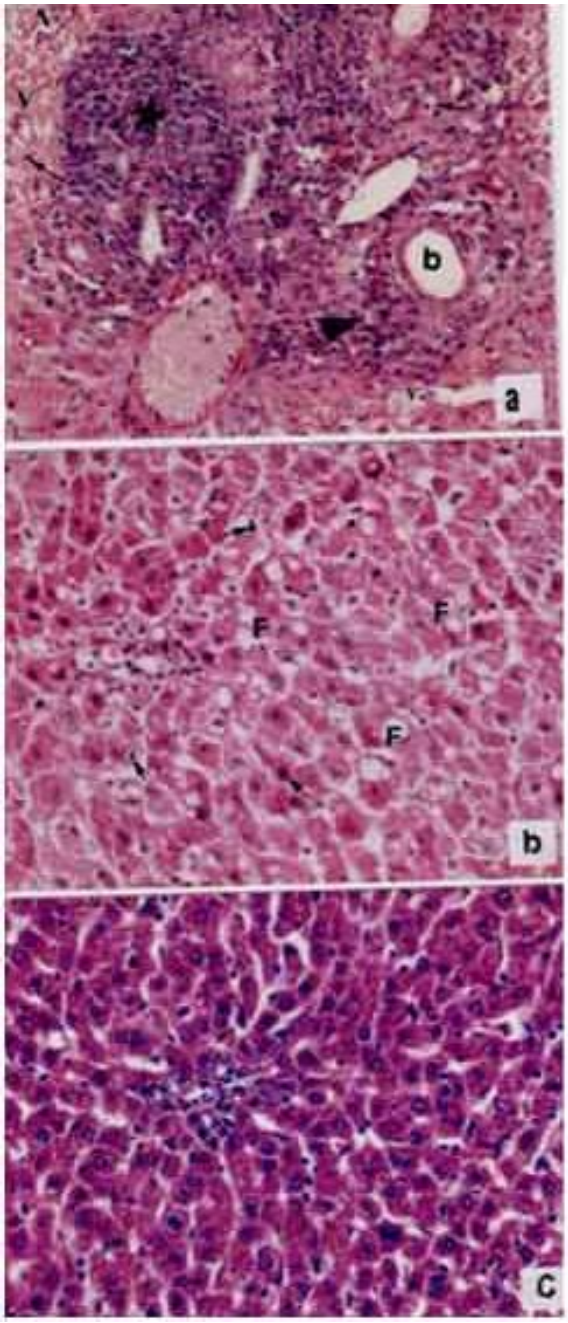


Fig.3. a & b: Liver sections of rats exposed to BPA for 6 weeks.

3a: Showing proliferating bile ductule (b) surrounded by lymphocytic infiltration (arrow head), huge mass of leucocytic inflammation (star), cytoplasmic vacuolization (V) and pyknotic nuclei (arrows).

3b: indicating presence of pyknotic nuclei (arrows) and fatty infiltration (F).

Fig. 3c: Liver of rats treated with BPA and Se manifesting improvement in the histological architecture.

H&E. X200

3. Immunohistochemical results

A weak expression of PCNA in a few number nuclei of control liver could be demonstrated as brown immunohistochemical staining (Fig.4a). BPA increased PCNA expression in hepatocytes of 3 weeks-exposed animals to become more striking after 6 weeks in a large number of nuclei (Fig.4b). In BPA and Se- treated rats a reduced PCNA expression in a comparatively less number of nuclei of hepatocytes was recorded (Fig.4c). Accordingly, image analysis of PCNA immunoreactivity in BPA-exposed animals resulted in a significant increase ($P \leq 0.05$) as compared to that of controls. However, Se lowered PCNA expression as compared to that of BPA alone (Fig.5).

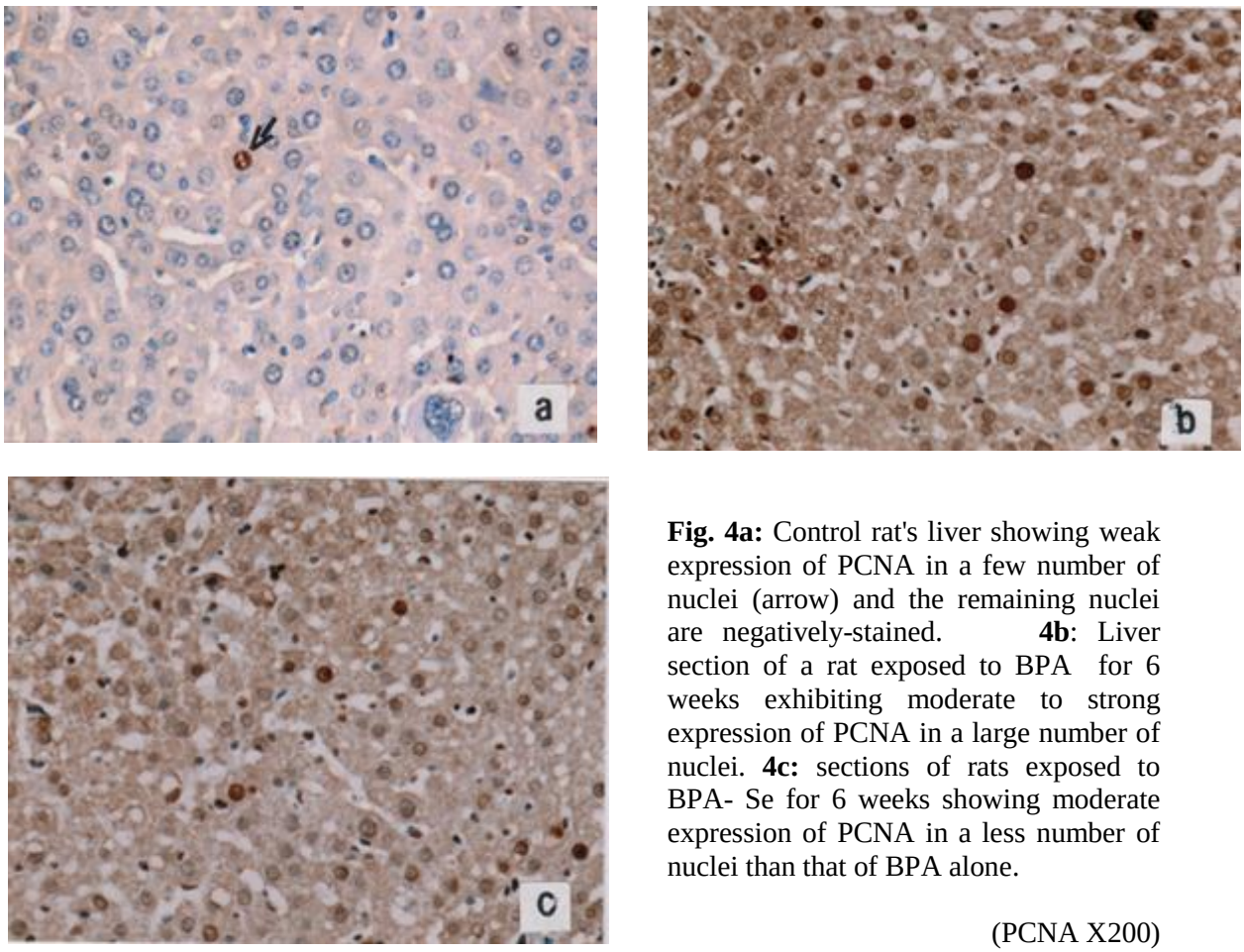


Fig. 4a: Control rat's liver showing weak expression of PCNA in a few number of nuclei (arrow) and the remaining nuclei are negatively-stained. **4b**: Liver section of a rat exposed to BPA for 6 weeks exhibiting moderate to strong expression of PCNA in a large number of nuclei. **4c:** sections of rats exposed to BPA- Se for 6 weeks showing moderate expression of PCNA in a less number of nuclei than that of BPA alone.

(PCNA X200)

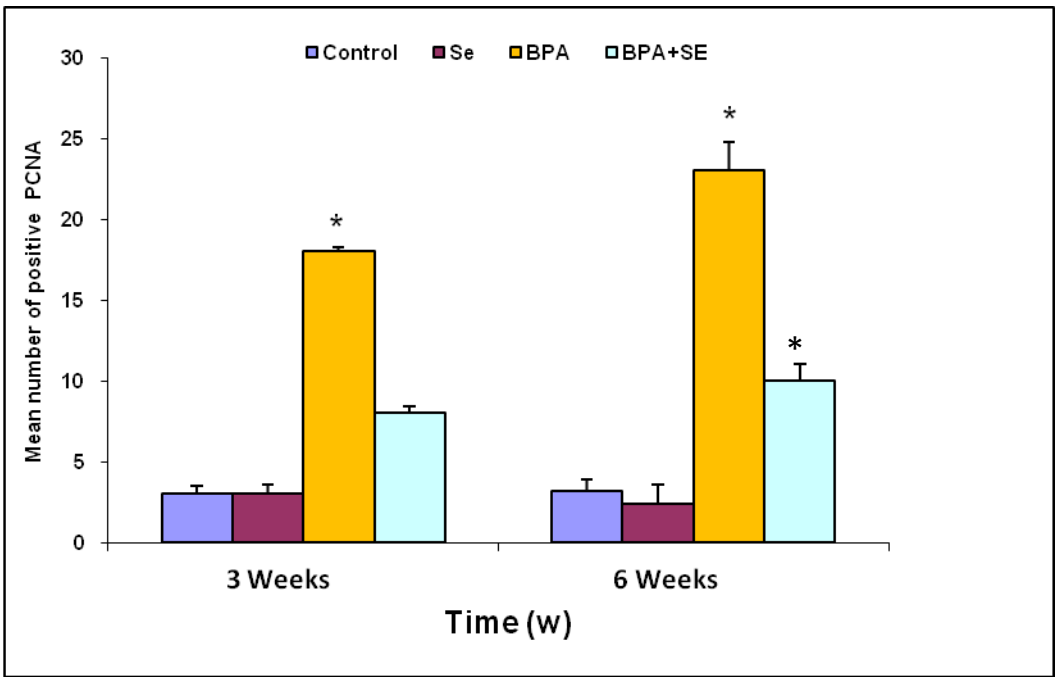


Fig. 5: Mean number of PCNA- positive immune-stained nuclei by image analysis

Liver sections of control rats showed weak brown staining of caspase-3 expression in the cytoplasm of few hepatocytes (Figs.6a). More hepatocytes demonstrated strong caspase-3 immunoreactivity in BPA-treated rats (Fig.6b). Moderate caspase-3 immunoreaction was encountered in cytoplasm of less number of hepatocytes in animals given BPA and Se (Fig.6c). By image analysis, the reaction of caspase-3 in liver recorded a significant elevation in BPA-exposed animals for 3 weeks which becomes more increased after 6 weeks as compared to that of either control or BPA and Se-treated animal.

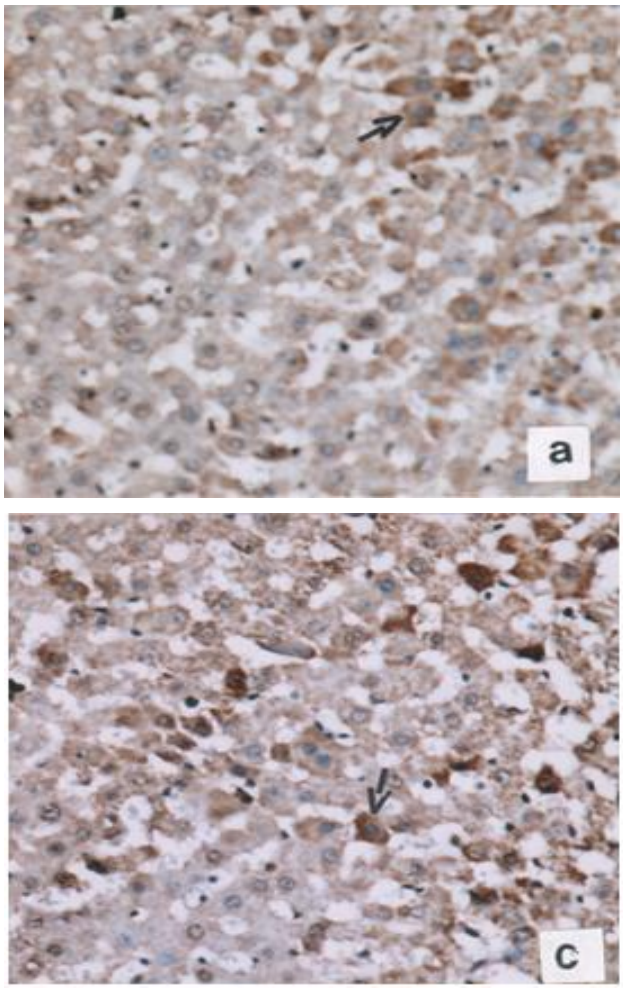


Fig. 6: Caspase-3 immunostaining expression in cytoplasm of hepatocytes. **6a:** Weak expression in few numbers of hepatic cells (arrow) of a control rat. **6b:** Strong Caspase-3 immunostaining in liver cells (arrows) of a rat exposed to BPA for 6 weeks. **6c:** Moderate caspase-3 immunostaining in liver cells (arrow) of a rat treated with BPA and Se for 6 weeks.
Caspase-3 X 200

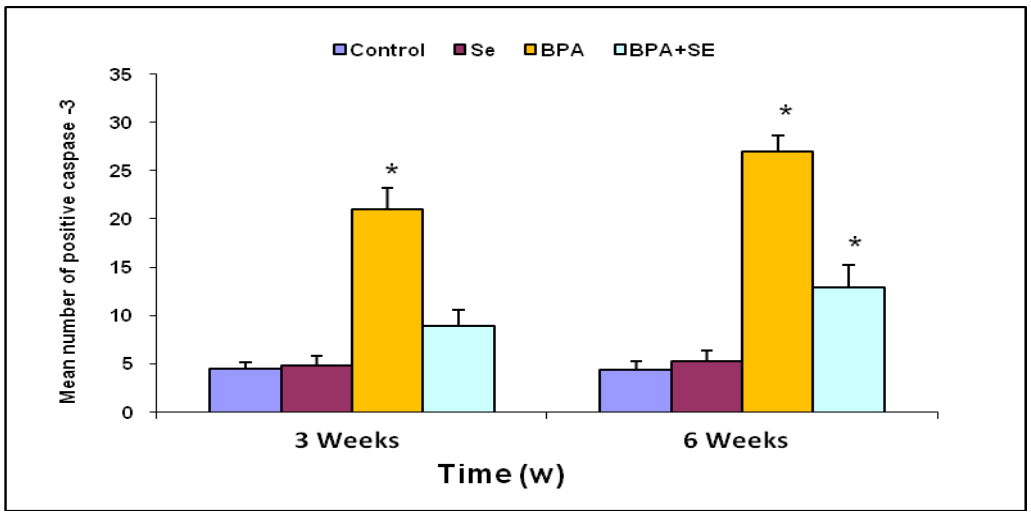
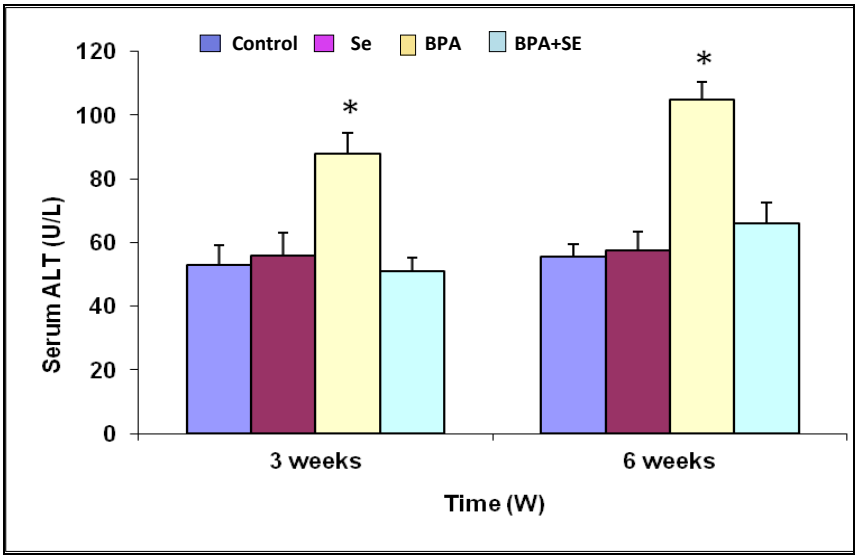


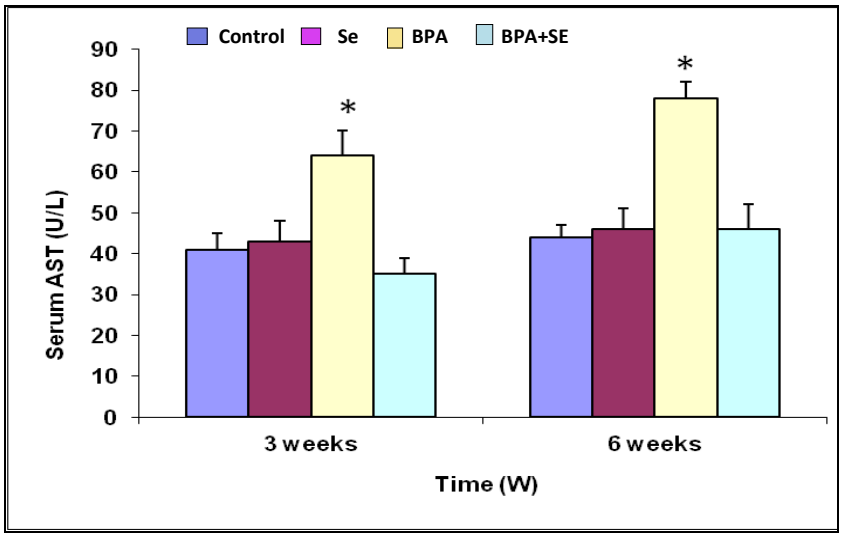
Fig. 7: Image analysis of mean number of Caspase-3 immune-stained hepatocytes.

4. Biochemical results

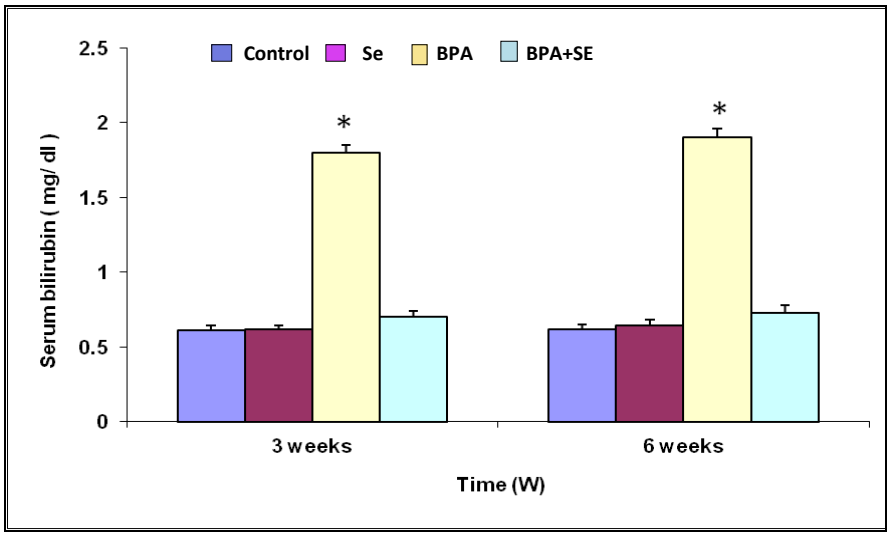
Serum ALT and AST levels were significantly elevated in BPA rats compared to control or Se administered- rats ($P<0.05$). In addition, Se induced significant depression in enzyme activity in rats given BPA (Figs. 8 and 9). Insignificant difference in bilirubin was found between control and Se group while significant elevation detected in BPA-treated rats compared to control or Se group. Se with BPA treatment significantly declined bilirubin levels (Fig.10).



Figs. **8**: Serum ALT in different treated groups showing significant elevation in the BPA- exposed animals ($P\leq 0.05$) both after 3 and 6 weeks while selenium supplementation improved the condition.



Figs. **9:** Serum AST in different treated groups indicating significant increase ($P\leq 0.05$) in rats exposed to BPA especially after 6 weeks.



Figs. **10**: Bilirubin in serum of various treated groups showing significant elevation (P≤ 0.05) in the BPA- exposed rats as compared to other groups.

DISCUSSION

An increasing alert towards the application of environmental pollutants that impede human and animals' health is adopted by the scientific community. Bisphenol A (BPA) is an environmental synthetic monomer toxicant used in the production of food or beverage plastic containers, water storage tanks and baby bottles [23]. BPA is largely absorbed via skin causing extensive injury to kidney and liver [24]. The liver is principally responsible for BPA metabolism [25], thus, it could be highly targeted by BPA and more susceptible to lower doses than other organs [26].

The present results indicated significant loss in body weight by BPA treatment. Similar results were demonstrated by *Yamasaki et al.* [27] while *Tamilselvan et al.* [28] recorded loss of body and testis weight in rats.

The obtained results indicated hepatotoxicity of BPA. Livers suffered intrahepatic vessels congestion, cytoplasmic vacuolization, leucocytic infiltrations and fatty degeneration. These changes were accompanied by elevation in serum ALT, AST and bilirubin together with the increased CAN and caspase-3 expressions. The cytoplasmic vacuolization is a reversible injury indicated stress that may proceed to necrosis. These changes reflected by increase in serum AST and ALT. The increase in caspase- 3 expression indicated increase in apoptotic rate while the increase in PCNA is an indicator of liver to replace the damaged cells. The BPA effect of on liver was studied by several investigators. BPA causes cellular infiltration and necrosis [29], vacuolation of hepatocytes [30], incidence of multinucleated giant liver cells [31], central vein dilation and congestion, dilated sinusoid and lymphocytic infiltration [32, 33] and pyknotic nuclei, blood vessels congestion and necrosis in liver of female rat offspring [33]. *Kourouma et al.* [34] found that apoptosis, increased the activities of liver ALT and enhanced ROS and LPO by BPA exposure. The present study confirms the findings of Alkalby [35] who recorded dilatation of the central vein, nuclei enlargement and vacuolization in liver of BPA – exposed rats. Recently, *Faheem et al.* [36] indicated that BPA caused central vein congestion, inflammation, edema, degeneration and necrosis of hepatocytes of major carp, *Catlacatla*.

Biochemical results of this work clearly demonstrated increase in serum AST, ALT activities and bilirubin level in BPA treated rats . Coincide with these findings are results of Alkalby [35] and *Korkmaz et al.* [37] who recorded significant rise in AST and ALT level in rats. Moreover, Eshak and Osman [38] recorded rise in AST, ALT, ALP and bilirubin following BPA injection. Recently, *Rahimi et al.* [39] indicated that doses of 10, 50 and 100mg /kg bw of BPA significantly elevated ALT and AST.

In the current study, overexpression of PCNA was recorded in BPA-exposed rats' livers. Similarly, DeBenedictis [40] demonstrated higher PCNA but lower caspase- 3 expression in a sex-specific manner in mouse fetuses' livers

following prenatal exposure to BPA. Morphological changes observed in apoptotic cells are attributed to caspase-3 [41]. In this work, caspase 3 expression was increased in liver of rats exposed to BPA. Recently, *Abd El Dayem et al.* [42] demonstrated significant caspase-3 increase with significant depression in the antiapoptotic protein Bcl2 in rats' liver by BPA. BPA dose over 100lM caused apoptosis in mouse hippocampal HT-22 cells via elevating reactive oxygen species and intracellular calcium and activating caspase-3 [43]. Recently, *Elswefy et al.* [44] indicated liver fibrosis by BPA administration as reflected by caspase-3 increase and decreased numbers of BCL2-immunopositive hepatocytes.

BPA was found to inhibit cellular antioxidant activity and increase oxidative stress [37, 12]. From current findings, it is speculated that BPA is an oxidative stress inducer resulting in hepatotoxicity as was confirmed by the increase in PCNA, caspase- 3 expression in liver cells and elevated AST and ALT in serum.

Interestingly, the current results confirmed attenuation of BPA hepatotoxicity by Se. Se treatment with BPA improved body weight, liver histology, down regulated the PCNA and caspase-3 expression and decreased serum ALT, AST and bilirubin. The down regulation of PCNA could be explained on the fact that selenium reduced cell injury so the dividing capacity of the liver is restrained as reflected by the apoptotic indicator caspase-3. Hepatotoxicity is supposed to result from generating free radicals, hence, antioxidants supplementation exert an ameliorative potential. Moreover, it is believed that the antioxidant power in cells is strengthened via supplementing dietary antioxidants from natural resources [45]. Among antioxidants is selenium. The present results agreed with other investigators who studied the protective role of Se. The improvement in body weight offered by Se is consistent with results of Alarcon and Vique [46] who found growth, activation and development following selenium intake whereas *Heikal et al.* [47] indicated improvement of rats' liver and body weights against CPF toxicity.

Selenium protected rats liver against BPA toxicity. Reports regarding Se hepatoprotection against a wide range of xenobiotics were documented. Coincides with this, *Sakr et al.* [18] recorded significant liver protection by selenium against carbimazole. In addition, *Shen et al.* [48] demonstrated decreased liver fibrosis induced by CCl_4 in rats by supplementation of diet contains vitamin E and selenium. *Yu et al.* [49] indicated inhibition of oxidative stress and apoptosis resulted from excess fluoride in rats' kidney by selenium as well as significant reduction in serum ALT and AST in rats by administration of selenium combined with HgCl2 [16]. *Heikal et al.* [47] recorded decreased plasma transaminases (ALT and AST), alkaline phosphatase and lactate dehydrogenase and increased liver glutathione peroxidase, superoxide dismutase, catalase and lactate dehydrogenase by selenium in chlorpyrifos-induced rat hepatotocity. *Elgaml et al.* [50] indicated decrease in AST, ALT and increase in SOD and glutathione GSH by selenium against the toxic impact of lead acetate. Moreover, selenium and vitamins administration regressed the significant increased PCNA labeling index of the alveolar cells induced by amiodarone drug in rats [51]. *Liao et al.* [52] found that Se alleviated Aflatoxin B1 (AFB1)-induced increased expression level of Bax, caspase-3, and p53 in the liver of ducklings. Added, *Zhang et al.* [53] revealed that Se significantly alleviated Cd-induced apoptosis in liver of chickens confirmed by decrease in apoptotic cell numbers, and mRNA and protein expression levels of caspase-3, Bax in addition to Cyt-c. *Viezeliene et al.* [15] confirmed that Se protected mice liver against aluminium- produced oxidative stress.

In conclusion**,** Bisphenol A was indicated to be hepatotoxic. However, selenium supplementation exerted undeniable ameliorative role against such hepatotoxicity. Accordingly, persisting use of bisphenol- A must be continued with great caution, so that an important decision should be taken to mitigate the deleterious action on human and his domestic animals. Supplementation of natural antioxidative materials has to be encouraged to combat deleterious impacts of environmental toxicants.

Authors' contributions: All authors contributed equally in producing this manuscript from sample collection to writing the final version of the manuscript.

Conflict of interest disclosure

The authors disclose no conflict of interest.

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