The protective role of sesame oil against bisphenol A-induced cardiotoxicity: a histological and immunohistochemical study

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Introduction
Bisphenol-A (BPA), an estrogenic compound, is used in the manufacture of polycarbonate plastics and epoxy resins. Sesame oil (SO) is a potent antioxidant dietary source for human health.
Aim
The present study was conducted to estimate the protective effects of SO against BPA-induced cardiotoxicity.
Materials and methods
Thirty two adult rats were divided into 4 equal groups eight rat for each; Control group, 2 Treated group, one group received BPA (25 mg/kg b wt) orally 5 times/weak for 4 weeks and other group rats received (50 mg/kg b. wt) orally 5 times /weak for 4 weeks. Protected group received sesame oil orally at a dose 10 mL/kg b wt orally daily for 4 weeks to the rat group which received the high dose of BPA. After the end of treatments, the heart of each killed animal was subjected to histopathological examination by hematoxylin and eosin, Masson's, and NOS stain. In addition, blood was collected for biochemical assessment of the enzymes.
Results
Administration of high-dose BPA (50 mg/kg b. wt) significantly increased the weight of rats. Several histopathological alterations in cardiac tissue and elevation in malondialdehyde, creatine phosphokinase-MB, and glutathione-S-transferase activity and reduction of glutathione and catalase occurred when compared with the control. Low-dose BPA (25 mg/kg b. wt) produced mild histopathological effect on the heart. On the contrary, oral gavages of SO with BPA was effective in the reduction of weight, amelioration of histopathological alterations, and in the reduction of the malondialdehyde, creatine phosphokinase-MB, and glutathione-S-transferase activity levels and elevation of glutathione and catalase activity when compared with high-dose BPA-treated rats.
Conclusion
The present study provided clear evidence that SO possesses a promising protective activity against the cardiotoxic effects of BPA.

Keywords:
bisphenol-A, cardiotoxicity, histopathological alterations, sesame oil

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Introduction

Bisphenol-A (BPA) is an organic synthetic compound used mainly in the production of polycarbonate plastics and epoxy resins [1].

BPA-based products are tough, versatile, and water resistant and are used in various consumer goods such as food containers, baby bottles, beverage and food can linings, as well as for industrial purposes such as water pipes [2].

The hydrolysis of the ester bonds between BPA molecules under high temperature and acidic and basic situation increase penetration of BPA to the food or environment [3].

The health hazard of BPA is mainly owing to the incomplete polymerization reaction that leaves some unbound monomer BPA molecules in the products. These unbound monomers can be released into food or beverage over time, especially under heat, acidic, or basic environmental conditions [4].

Multiple human exposure assessment studies have shown that BPA is present at detectable levels in more than 90% of individuals examined in various populations. Mean/median urinary BPA concentrations in the low μg/l range have been reported in various human exposure [5].

BPA exposure could evoke hypertension, heart attack, vascular diseases, and atherosclerosis [6].

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Studies revealed that oxidative stress can induce many kinds of negative effects including membrane peroxidation and DNA strand breakages, which could lead to myocyte necrosis, apoptosis, and cancer [7].

Experimental studies have established that acute BPA exposure promotes the development of arrhythmias in female rodent hearts. Chronic exposure to BPA has been shown to result in cardiac alteration, atherosclerosis, and change in blood pressure in rodents. The underlying mechanisms may involve alteration of cardiac Ca^{2+} handling, ion channel inhibition/activation, and oxidative stress [6].

Sesame oil (SO) is one of the major cooking oil used in the diet and has antioxidant components. SO is found in the seeds of *Sesamum indicum* [8].

Sesame seeds contain flavonoids and other phenolic compounds that can act as antioxidants [9]. Sesamin, one of the major ligands in sesame seeds, possesses a wide range of pharmacological functions, including antioxidative, antihyperlipemic, and antihypertensive properties in animal models [10].

Studies from experimental models showed it could protect the heart injury [11].

Saleem *et al.* [8] recently found that chronic administration of SO enhances the endogenous antioxidants in ischemic myocardium.

Materials and methods

Materials

Animals

The present study was carried out on 32 adult male Sprague-Dawley rats aged 8–12 weeks, weighing 1500–1800 g. They were obtained from the Animal House, Faculty of Veterinary Medicine, Benha University, Egypt. The rats were housed in separate clean cages under standard environmental conditions approved by the Animal Use and Care Committee, under controlled light cycle (12 h light/12 h dark). The rats were housed in uniform husbandry conditions at a temperature of 25±1°C, with a relative humidity of 50 ±10%. The rats were freely supplied with sterilized diet consists of milk, vegetables, and bread feed and water ad libitum. All rats were kept under the same circumstances throughout the experiment. The rats were divided into four groups of eight rats each.

(1) Group I (control group): the rats received no medications and were left to survive for 4 weeks.
(2) Group II (BPA 25-treated group): each rat received BPA in a dose 25 mg/kg via gavage once a day, five times per week, for 4 weeks.
(3) Group III (BPA 50-treated group): each rat was received BPA in a dose 50 mg/kg via gavage once a day, five times per week, for 4 weeks.
(4) Group IV (BPA 50–sesame-treated group): each rat received BPA in a dose 50 mg/kg via gavage once a day, five times per week, for 4 weeks plus 10 ml/kg. SO via gavage was used once a day for 4 weeks.

Drugs

BPA (≥99%) was purchased from Sigma-Aldrich Company (St. Louis, Missouri, USA). BPA was dissolved in absolute ethyl alcohol (95%) and diluted with corn oil [1 : 20 alcohol: corn oil (vehicle)] to obtain a final concentration of BPA. It was freshly prepared before use and given in two different doses: first, 25 mg/kg BPA-treated group; and second, 50 mg/kg BPA-treated group. BPA was administrated via gavage once a day, five times per week, for 4 weeks [12].

For SO, commercial SO was purchased from EL Captin Company (Al Obour City, Cairo, Egypt) and given in a dose of 10 ml/kg to the 50 mg/kg BPA-treated group, administrated via gavage once a day, seven times per week, for 4 weeks [13].

Body weight measurement

The body weight of the control and treated animals was measured at the beginning of the study followed by weekly measurement. The body weight change of each animal was calculated every week.

Biochemical blood tests

At the end of the experiment, the fasted animals (overnight, 10–12 h) were decapitated, and the thorax blood was collected into the gel and clot-activated tube. After 15 min standing in the RT, the tubes were centrifuged at 3500 rpm for 15 min. The serum was collected in tubes and stored at −70°C for further analysis. The serum samples were analyzed for measurement of malondialdehyde (MDA), glutathione (GSH), catalase, and glutathione-S-transferase (GST).

Biochemical parameters

Measurement of malondialdehyde

At the end of the study period (4 weeks), the heart tissues were removed and washed in normal saline. To measure MDA, an important marker of oxidative stress, the right piece of heart tissues of different groups was homogenized for 2 min at 4°C (POLYTRON-PT 10–35, Kinematica, Switzerland) in 1.15% KCl to

provide a 10% homogenate. MDA levels were determined according to the method of Fernández *et al.* [14] Data are expressed as nmole/g wet wt.

Myocardial glutathione reduction

GSH was estimated by the method of Ellman [15]. The reaction mixture contained 0.1 ml of supernatant, 2.0 ml of 0.3 mol/l phosphate buffer (pH: 8.4), 0.4 ml of double distilled water, and 0.5 ml of DTNB (5,5 dithiobis-2-nitrobenzoic acid). The reaction mixture was incubated for 10 min, and the absorbance was measured at 412 nm. Data are expressed as nmole/g wet wt.

Determination of enzyme activities

Catalase activity

Catalase activity was measured using the Biodiagnostic Kit No. CA 25 17 (Giza, Egypt), which is based on the spectrophotometric method described by Aebi [16]. Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3, 5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm.

Glutathione-S-transferase activity

GST activity was assayed by the method that measures the conjugation of 1-chloro-2, 4-dinitrobenzene with reduced glutathione. This conjugation is accompanied by an increase in absorbance at 340 nm, with the rate of increase being directly proportional to GST activity [17].

Measurement of creatine phosphokinase-MB

The commercial colorimetric kit (Biosystem, Barcelona, Spain) was used to measure the creatine phosphokinase-MB (CK-MB) in serum by an autoanalyzer (Tokyo Boeki Prestige 24i Biolis 24i Chemistry Analyzer, China).

Light microscopic study

Parts of the myocardium of the left ventricle were kept in 10% formaldehyde solution (as a fixative) for 72 h. Tissues were then embedded in paraffin blocks. Sections of 5 μm thicknesses were obtained from the paraffin blocks and subjected to the following techniques.

Histological examination

It was done using hematoxylin and eosin for routine histological examination and Masson's trichrome stains for studying the collagen fiber distribution [18].

Immunohistochemical staining

It was done for iNOS antigens using the avidin–biotin–peroxidase complex technique [19].

The sections were collected on poly-l-lysine-coated slides. Nonspecific endogenous peroxidase activity was blocked by treatment with 0.9% hydrogen peroxide in absolute methanol for 10 min. Then, antigen retrieval was done by heating the sections in 10 mmol/l sodium citrate buffer, in a water bath at 95–100°C for 30 min Sections were rinsed two times in PBS Tween 20 for 2 min, and then blocked with 5% normal goat serum for 30 min at room temperature. Sections were incubated with the primary antibodies for 30 min, that is, iNOS rabbit polyclonal antibody IgG (ab15323; Abcam, Cambridge, UK).

Section were incubated with a biotinylated goat antipolyvalent secondary antibody for 60 min at room temperature. Immunodetection was carried out with the horseradish peroxidase–avidin–biotin complex method using a VECTASTAIN1 Elite ABC kit (Vector Laboratories Inc., Burlingame, California, USA), and DAB was applied as the chromogen. Localization was detected with DAB and counter-stained in Meyer's hematoxylin, dehydrated, and mounted. Negative control sections were done with the same procedure stated before except that the primary antibody was replaced with a nonimmune mouse serum. The sections were studied and photographed using a Canon digital camera attached to an IBM computer system (Armonk, New York, USA).

Image analyzer study

The mean area % of INOS immunoexpression was quantified in five images from five nonoverlapping fields of each rat using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

The mean area percentage of collagen deposition was quantified in five images from five nonoverlapping fields of each rat using Image-Pro Plus program version 6.0 (Media Cybernetics Inc.).

Statistical analysis

All the data collected from the experiment were recorded and analyzed using IBM SPSS Statistics software for Windows, Version 19 (IBM Corp., Armonk, New York, USA). One-way analysis of variance with post-hoc least significant difference test was used to compare differences among the groups. In each test, the data were expressed as the

mean value, and SD, and differences were considered to be highly significant at *P* less than or equal to 0.01, significant at *P* less than or equal to 0.05, and nonsignificant at *P* greater than 0.05 [20].

Result

Animal body weights

The effect of BPA on rat body weights at different doses revealed that body weight of treated rat group with BPA 50 mg/kg (group III) was significantly increased when compared with groups I, II, and IV ($P<0.01$), whereas coadministration of SO with high dose of BPA (BPA 50–sesame group) caused significant decrease in rat body weight compared with BPA 50 group (Table 1 and Histogram 1).

Biochemical parameters

The following biochemical parameters were studied in the heart homogenate.

Malondialdehyde

As shown in Table 1, MDA level in animals receiving 50 mg/kg of BPA (group III) showed a significant increase ($P<0.01$) when compared with group I (control), group II (BPA 25), and to group IV (BPA–sesame). However, MDA level was significantly decreased in BPA–sesame group compared with BPA 50 group, and its level increased to near normal (Table 2 and Histogram 2).

Myocardial glutathione reduction

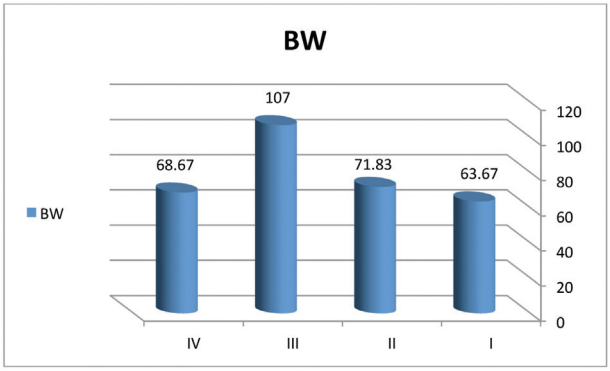
GSH level in group III (BPA 50) was significantly decreased as compared with groups I, II, and IV ($P<0.01$). However, GSH level was significantly increased in group IV (BPA–sesame) compared with group III (BPA 50) and its level increased to near normal (Table 2 and Histogram 2).

Serum creatinine phosphokinase-MB

Measurement of serum CK-MB activity revealed significant increase in group III (BPA 50) compared with groups I, II, and IV ($P<0.001$). However, a significant increase in CK-MB activity in BPA 50 group compared with BPA 25 group.

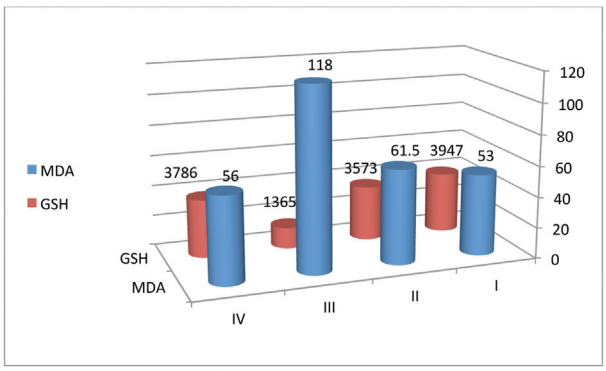
Co-administration of SO and 50 mg/kg of BPA (group IV) decreased serum CK-MB activity as compared with the group III ($P<0.001$), and its level increased to near normal (Table 3 and Histogram 3).

Histogram 1



Mean values of BW in the four groups. BW, body weigh.

Histogram 2



Mean values of malondialdehyde and glutathione in the four groups.

Table 1 Mean values of BW±SD in the four groups

Mean±SD	Group I	Group II	Group III	Group IV	*P* value
BW (g)	63.67±3.14	71.83±1.17	107±8.74	68.67±1.2	0.000
Significance ≤0.01	With group III	With group III	With groups I, II, and IV	With group III	

Table 2 Mean±SD values of malondialdehyde and glutathione in the four groups

Mean±SD	Group I	Group II	Group III	Group IV	*P* value
MDA (nmol/g)	53±2.6	61.5±1.87	118±6.45	56±1.78	0.000
GSH (nmol/g)	3947.17±168.7	3573.33±162.8	1365.83±140.0	3786.67±114.5	0.000
Significance ≤0.01	With group III	With group III	With groups I, II, and IV	With group III	

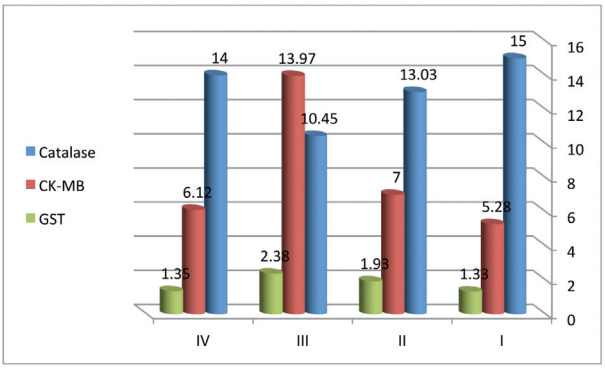
GSH, glutathione; MDA, malondialdehyde.

Table 3 Mean±SD values of creatine phosphokinase-MB, glutathione-S-transferase activity, and catalase in the 4 groups

CK-MB (U/l)	528.33±60.55	700±60	1397±181.6	611.67±58.8	0.000
GST (U/g)	1.33±0.54	1.93±0.42	2.38±0.58	1.35±0.57	0.007
Catalase (U/g)	15±2.37	13.03±2.46	10.45±1.2	14±1.9	0.007
Significance ≤0.01	With group III	With group III	With groups I, II, and IV	With group III	

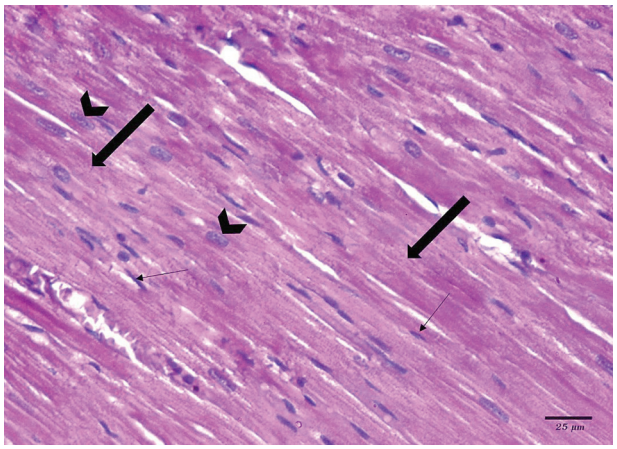
CK-MB, creatine phosphokinase-MB; GST, glutathione-S-transferase activity.

Histogram 3



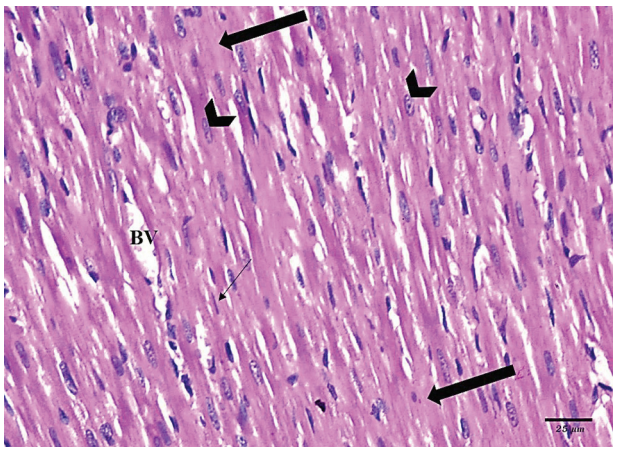
Mean values of creatine phosphokinase-MB, glutathione-S-transferase activity, and catalase in the four groups.

Figure 1



A photomicrograph of a section of the myocardium of a control group (group I) rat, showing branching and anatomizing longitudinal cardiac muscle fibers (thick arrow) with acidophilic sarcoplasm and central elongated vesicular nuclei (arrow head). Flat dark nuclei of fibroblasts (thin arrow) of connective tissue can be seen (hematoxylin and eosin, ×400).

Figure 2



A photomicrograph of a section of the cardiac muscles of a control group (group I) rat showing longitudinally arranged cardiac muscle fibers (thick arrow) with acidophilic sarcoplasm and central, vesicular, and oval nuclei (arrow head). The fibers are branching and anastomosed with each other. Notice: connective tissue cells with dense flattened nuclei (thin arrow) and elongated blood vessel can be observed (hematoxylin and eosin, ×400).

Determination of enzyme activities

Catalase activity

Measurement of serum catalase activity showed significant decrease in catalase activity in group III as compared with groups I, II, and IV ($P<0.01$), and a significant increase in catalase activity in group IV (PBA–sesame) compared with BPA 50 group (Table 3 and Histogram 3).

Glutathione-S-transferase activity

GST activity was significantly increase in group III (BPA 50) ($P<0.01$) compared with groups I, II, and IV. Co-administration of SO and 50 mg/kg of BPA in group IV led to significant decrease in GST activity compared with (group III) (BPA 50) (Table 3 and Histogram 3).

Histopathological examination

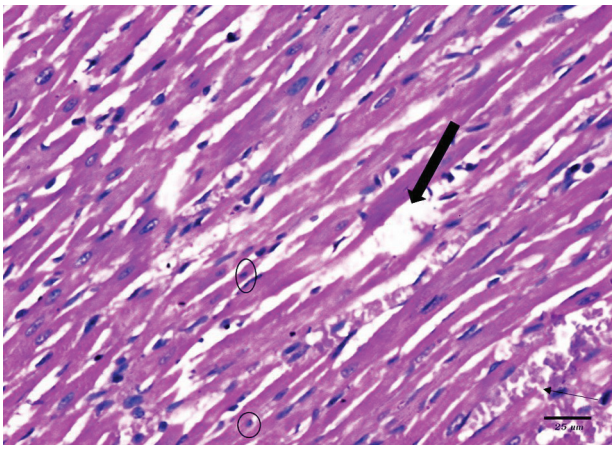
Hematoxylin and eosin-stained sections

The cardiac myocytes in the left ventricles of the control group I (control group) showed normal histological architecture with longitudinally striated branching and anastomosing muscle fibers with acidophilic sarcoplasm and central elongated vesicular oval nuclei. Flat dark nuclei of fibroblasts of connective tissue were evident. Blood capillaries were apparent in the intercellular spaces (Figs 1 and 2).

Examination of the ventricular sections of rats of group II (BPA 25 mg/kg-treated group) showed minimal changes of myofibrillar structure with striations and low level of inflammation. Some cardiac muscle fibers had dark cytoplasm, pyknotic nuclei, and area of fiber loss (Figs 3 and 4).

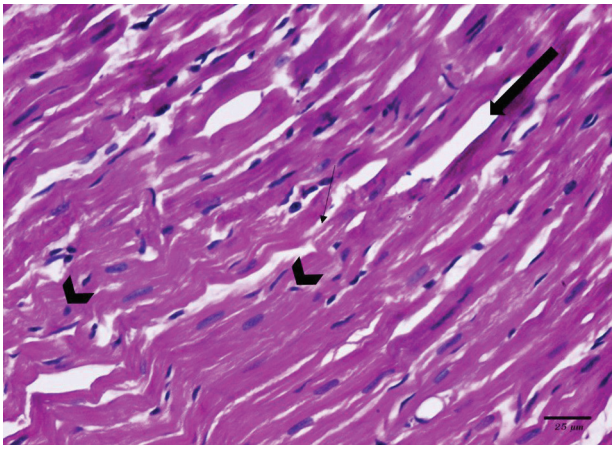
The histological pattern of group III (BPA 50 mg/kg-treated group) showed area of marked distortion and

Figure 3



A photomicrograph of a section of rat's myocardium of a treated group II (BPA-A 25) rat, showing area of fibers loss (thick arrow). Some fibers with dark cytoplasm and pyknotic nuclei (circle) are seen. Rupture of the wall of blood vessels (thin arrow) is detected (hematoxylin and eosin, ×400).

Figure 4

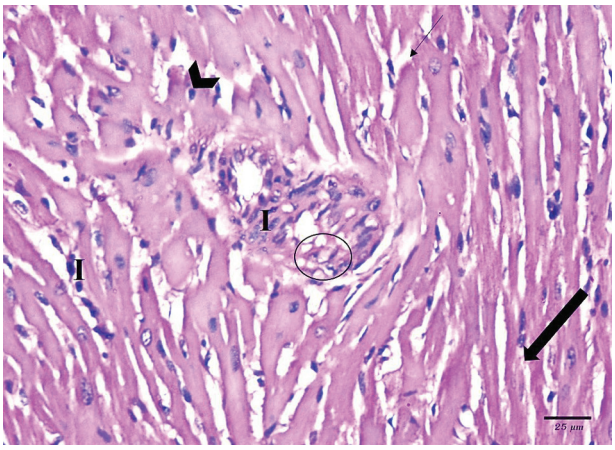


A photomicrograph of a section of rat's myocardium of a treated group II (BPA-A 25) rat, showing an area of fiber loss (thin arrow). Some fibers with dark cytoplasm and pyknosis of some nuclei are seen (arrowheads). Destruction of some fibers (thick arrow) (hematoxylin and eosin, ×400).

fragmentation of cardiac muscle fibers. Some fibers with dark cytoplasm and pyknotic nuclei are seen. Focal lytic area of sarcomere is seen, and mononuclear cellular infiltrations surround the wall of blood vessels and in between the sarcomere, along with disorganized sarcomeric structure with massive infiltration with inflammatory cells and connective in between sarcomere (Figs 5 and 6).

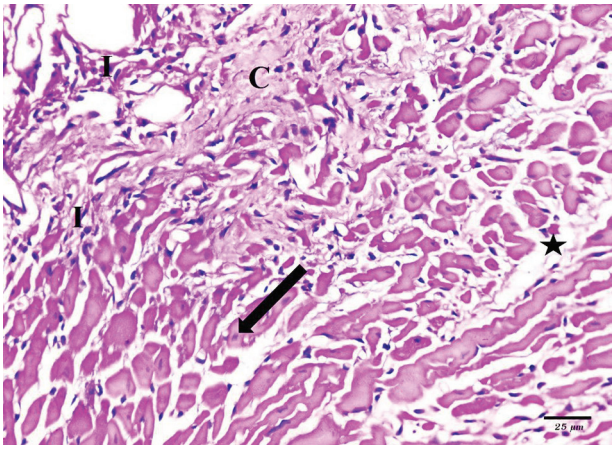
The histological pattern of group IV (BPA 50 mg/kg +10 ml/kg SO –treated group) showed marked improvement as the cardiac muscle fibers are more or less normal branching and anatomizing longitudinal muscle fibers with central oval nuclei. Appearance of

Figure 5



A photomicrograph of a section of rat's myocardium of a group III (bisphenol-A 50) rat showing loss of striations and areas of fiber loss (thin arrow). Some fibers with dark cytoplasm and pyknotic nuclei are seen (arrowheads). Focal lytic area of sarcomere (thick arrow). Mild mononuclear cellular infiltrations (I) surround the wall of blood vessels and in between the sarcomere. Notice: vacuoles in the wall of blood vessels (circle) (hematoxylin and eosin, ×400).

Figure 6



A photomicrograph of a section of rat's myocardium of a treated group III (BPA-A 50) rat showing disorganized sarcomeric structure with massive infiltration with inflammatory cells (I) and connective fibers (C) in between sarcomere. Cross-section of cardiac muscle fibers with pyknotic nuclei (thick arrow). There is increase in interstitial connective tissue (astric) (hematoxylin and eosin, ×400).

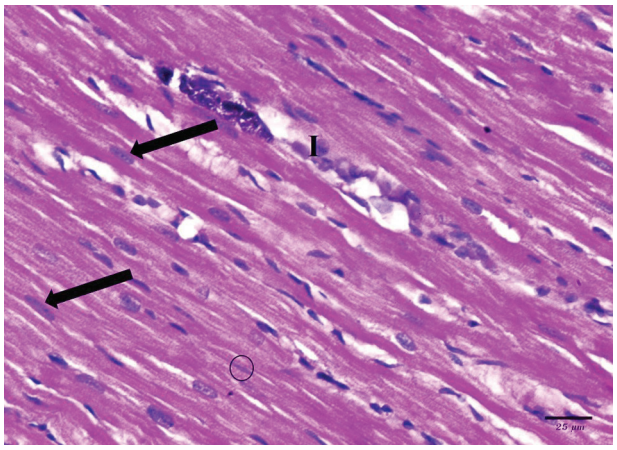
flat dark nuclei of fibroblast of connective tissue endomysium was noted (Figs 7 and 8).

Morphometric results

Masson's trichrome stain

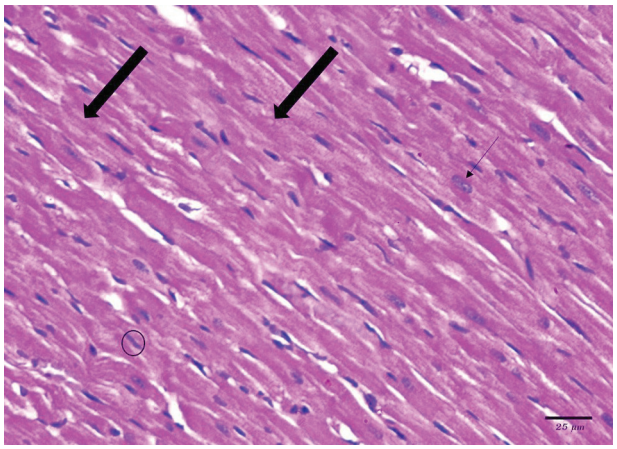
The mean area % of collagen deposition for all groups is represented in Table 4 and Histogram 4. There was insignificant increase in mean area% of collagen deposition ($P>0.05$) in groups II and IV as compared with control group. However, area % of collagen deposition was highly significantly increased

Figure 7



A photomicrograph of a section of rat's myocardium of a treated group IV (PBA – sesame) rat showing the cardiac muscle fibers with appearance more or less similar to control. Note: vesicular oval nuclei of cardiac muscle fiber (thick arrow) and flat dark nuclei of fibroblast of connective tissue endomysium (circle) are seen. Moreover, slightly cellular infiltration with inflammatory cells (I) can be observed (hematoxylin and eosin, ×400).

Figure 8

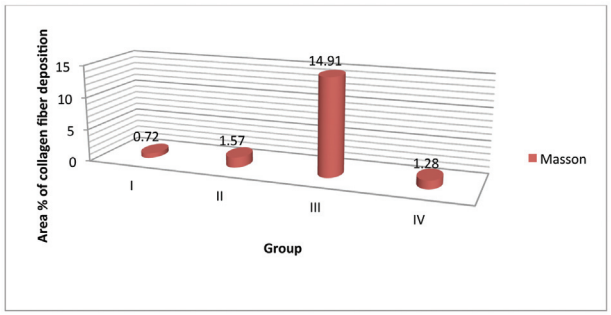


A photomicrographs of sections of rat's myocardium from a protected group IV (PBA – sesame) rat showing more or less normal branching and anastomosing longitudinal muscle fibers (thick arrow) with central oval nuclei (thin arrow). Flat dark nuclei of fibroblast of connective tissue endomysium (circle) are seen (hematoxylin and eosin, ×400).

Table 4 The mean±SD area%, of collagen fibers deposition in groups I, II, III, and IV with comparison between all groups by post-hoc least significant difference test

Mean±SD	Group I	Group II	Group III	Group IV	*F* test	*P* value
Masson	0.72 ±0.64	1.57 ±0.78	14.91 ±7.14	1.28 ±0.72	10.79	0.003
Significance ≤0.01	With group III	With group III	With groups I, II, and IV	With group III		

Histogram 4

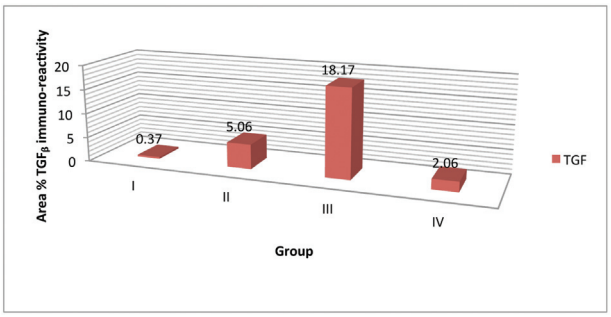


The mean area % of collagen fibers deposition in groups I, II, III, and IV.

Table 5 Mean±SD values of area % immunoreactivity of iNOS ±SD in the four groups

Mean±SD	Group I	Group II	Group III	Group IV	*F* test	*P* value
iNOS immunoreactivity	0.37 ±0.57	5.06 ±1.6	18.17 ±7.58	2.06 ±0.66	12.89	0.002
Significance ≤0.05	With group III	With group III	With groups I, II, and IV	With group III		

Histogram 5



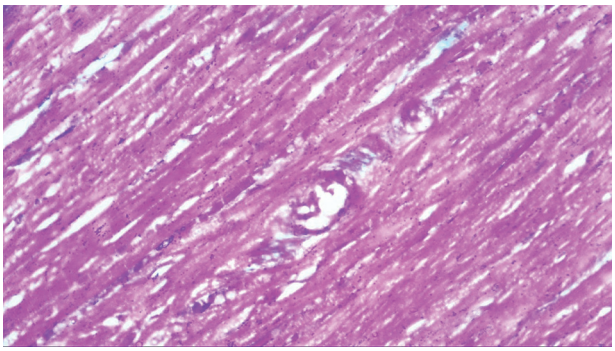
Mean values of area % iNOS immunoreactivity in the four groups.

in group III as compared with groups I, II, and IV groups ($P<0.01$).

Immunohistochemistry

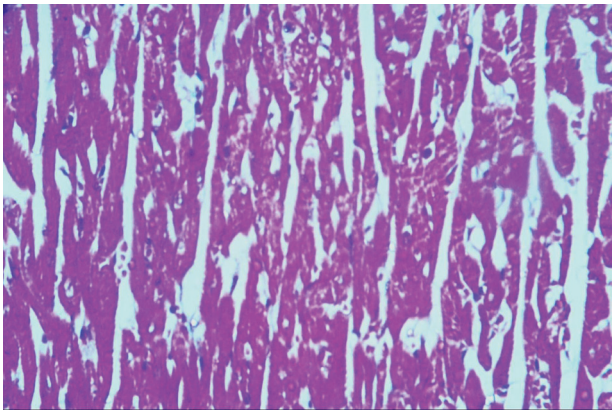
The mean area % of iNOS immunoexpression for all groups is represented in Table 5 and Histogram 5. There was insignificant increase in iNOS immunoexpression ($P>0.05$) in group II as compared with control group. However, area % of iNOS immunoreactivity was highly significantly increased in groups III as compared with groups I, II, and IV ($P<0.01$). Moreover, area % of iNOS immunoexpression was insignificantly increased in group IV as compared with control ($P>0.05$) (Figs 9–16).

Figure 9



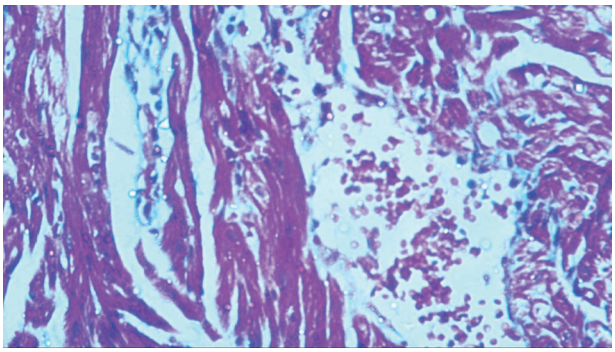
A photomicrograph of a section of the myocardium of group I (control) showing minimal collagen fiber deposition between cardiac muscle fibers (Masson's trichrome, ×400).

Figure 10



A photomicrograph of a section of the myocardium of (BPA 25) group II rat showing few collagen fiber deposition between cardiac muscle fibers (Masson's trichrome, ×400).

Figure 11

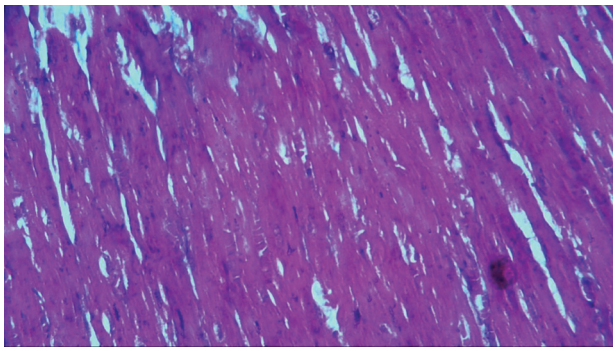


A photomicrograph of a section of the myocardium of (BPA 50) group III rat showing: heavy collagen fibers deposition between cardiac muscle fibers (Masson's trichrome, ×400).

Discussion

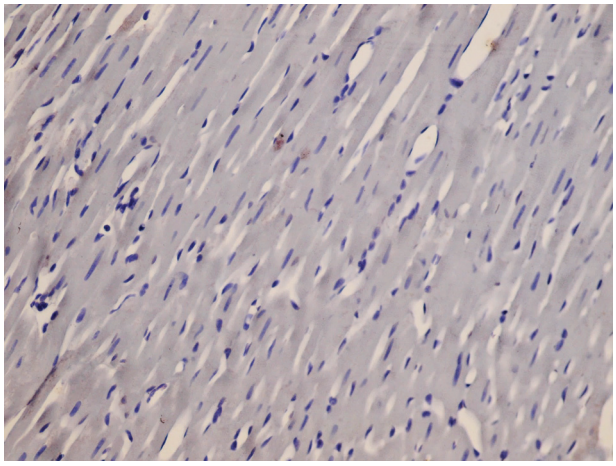
Our data state that the oral administration of BPA at the two tested doses (25 and 50 mg/kg b. wt) for 4 weeks to rats led to increase of the body weight.

Figure 12



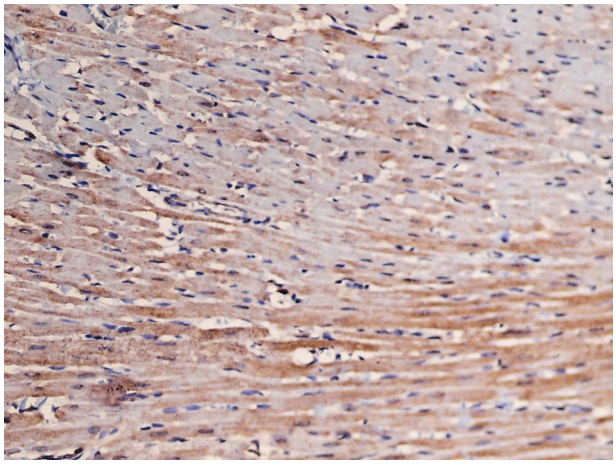
A photomicrograph of a section of the myocardium of (PBA –sesame) protected group IV rat showing minimal collagen fiber deposition between cardiac muscle fibers (Masson's trichrome, ×400).

Figure 13



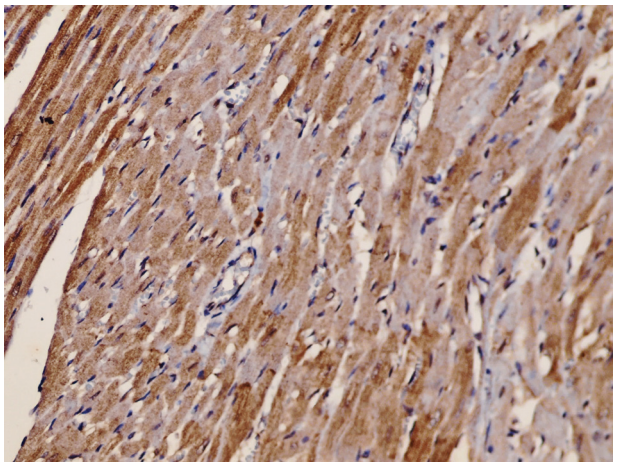
A photomicrograph of a section of cardiac muscles of (control) group I rat showing no expression of iNOS (iNOS, ×400).

Figure 14



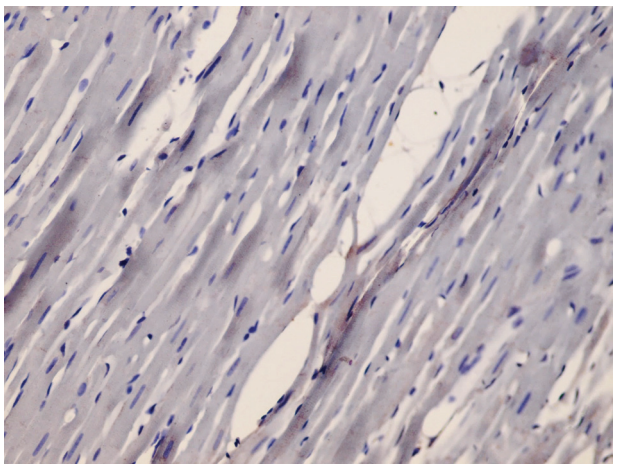
A photomicrograph of section of cardiac muscles of (BPA 25) group II rat showing mild to moderate expression of iNOS (iNOS, ×400).

Figure 15



A photomicrograph of section of cardiac muscles of (BPA 50) group III rat showing high expression of iNOS (iNOS, ×400).

Figure 16



A photomicrograph of section of cardiac muscles of (PBA –sesame) protected group IV rat showing very low expression of iNOS (iNOS, ×400).

Moreover, the body weight gain of animals treated with the high dose (50 mg/kg) significantly increased in relative to the control and low-dose group rats. Similarly, previous studies showed that body weights of offsprings of Sprague-Dawley rats exposed to BPA treatments (1 and 1.2 mg BPA/kg bw/day) were increased postnatally and continued into adulthood, dose and sex dependently [21,22]. In addition, several investigators confirmed that BPA has a role in weight gain and the development of obesity [23–25].

This effect of BPA could be explained by the report of Hugo *et al.* [26] who showed the ability of low levels of BPA to decrease adiponectin release from human adipose tissue explants. Adiponectin is known to play a positive role in cardiovascular health. Another possible explanation of enhanced weight gain in BPA-exposed animals is an increase in food intake as the estrogenic action of BPA can affect neuronal circuits that control appetite by acting on the hypothalamus [27]. Therefore, the increase in body weight gain emphasizes the ability of BPA to promote obesity which in turn could exacerbate many of the metabolic and cardiovascular disorders reported after BPA exposure [28].

However, Kwon and colleagues failed to observe significant differences in body weight after perinatal exposure to much higher levels of BPA than levels used in the present study [29]. Another study [30] stated that the body weights did not significantly change in rats exposed to any concentration of BPA by inhalation methods compared with the control group.

On the contrary, previous reports have indicated that perinatal or neonatal BPA exposure caused a reduction of the birth weight, slowed the growth, decreased the survival rate, and delayed the puberty in the offspring [31–33].

According to the results of the current study, concomitant administration of SO with the high dose of BPA 50 mg/kg to the experimental rats did not show any significant increase in body weight and its value was little above the control group. This was in accordance with the study of [23] who found that administration of SO only not affect the body weight. However, this effect in contrary to the studies of other researchers of Mahabadi *et al.* [34] and Shittu *et al.* [35], which suggested that the fluid extract of sesame leaves significantly increases the weight of rats, and it was explained by that the sesame seed-administered diet for a long time may increase body weight in this study.

The current study revealed that daily oral BPA administration (50 mg/kg for 4 weeks) induced a state of oxidative stress in the heart of rats as evident by a significant increase ($P<0.05$) in MDA, GST, and CK-MB activity levels when compared with the control values, and a decrease in GSH levels and catalase activity below the control values. However, a mild change was observed in these values with the low dose (25 mg/kg for 4 weeks). It was evident from percentage differences of these values that BPA (50 mg/kg) for 6 weeks produced a larger effect than BPA (25 mg/kg) for 4 weeks. This pattern is typical of the nonmonotonic dose-response curves that have been reported for many actions of BPA [26,36]. This is in accordance with the study of Pigeolet *et al.* [37] which revealed increase of both MDA and GST levels and

decrease of GSH levels and catalase activity in both low and high doses of BPA (25 and 10 mg/kg for 6 weeks), and is consistent with the study of Valokola *et al.* [12], who stated that administration of BPA (50 mg/kg, 4 weeks) has adverse effect on myocardial enzymes, as it increases MDA levels and reduces GSH levels.

Increased lipid peroxidation may indicate an increased oxygen-free radical generation. BPA induces ROS production and significantly compromises mitochondrial function. The reduction in the activity of catalase may be owing to the exhaustion of the enzyme in an attempt to eliminate the hydrogen peroxide generated after the exposure to BPA. This may also be owing to enzyme inactivation caused by excess ROS production in mitochondria and microsomes [38].

The increased oxidative stress may play a role in the BPA's potential adverse effect on the CV system. This can be explained by BPA exposure resulted in a decrease in nitric oxide, which may cause vasoconstriction and decreased blood supply to the heart. Other mechanisms by which BPA might increase the risk of cardiovascular disease include altered vascular reactivity to endothelin-1 and inflammation [39].

In the current study, administration of SO at a dose of 10 mg/kg with the high-dose BPA of 50 mg/kg significantly decreased the activities of marker enzymes (MDA and CK-MB) and significantly increased the activities of GST, catalase, and GSH, which allay free radical formation during myocardial array; this is consistent with previous reports, which have proved the antioxidant activity of SO in oxidative cardiac assault conditions [13,40,41].

Histopathological changes of cardiac tissue of the rats that received BPA at a dose of 50 mg/kg for 4 weeks revealed marked alternation in the structure of the myocardium in the form of marked distortion of cardiac muscle fibers, massive infiltration with inflammatory cells, and connective tissue deposition in between sarcomere. These changes coincided with other similar studies, which mentioned signs were observed by five doses of BPA, ranging from 2.5 to 25 000 μg/kg/day, to offspring at postnatal days 21, 90, and 180 Sprague-Dawley rats [42], another study at a dose of 50 mg/kg BPA for both 20 and 30 days in Sprague-Dawley male rat [43], and the study of Valokola *et al.* [12], who used a dose of BPA of 50 mg/kg for 4 weeks.

However, only mild changes in the myocadial structure were observed in the low dose of BPA (25 mg/kg) in the form of only some fibers had dark cytoplasm and pyknotic nuclei are seen. Rupture of the wall of blood vessels was observed, and this finding did not agree with the study of Valokola *et al.* [12], who did not observe any pathological changes in the myocardium at a dose of 25 mg/kg of BPA for 4 weeks, as even the myocardium resembled normal.

The current study revealed marked increase of the myocardial fibrosis after exposure to high-dose BPA, but there was mild increase in the low-dose BPA group. This finding was detected in the study of Bahey *et al.* [44] on the effect BPA at a dose 1.2 mg/kg/day, intraperitoneally for 3 weeks.

The fibrosis could be a consequence of the increased proliferation of cardiac fibroblasts and enhanced collagen production [45] or as a result of increased density and/or activation of cardiac mast cells [46,47].

Drugs that enhance the endogenous antioxidant enzymes to protect the heart from stress have been paid more interest. Natural antioxidants play a major role to reduce the oxidative stress by scavenging the excess free radicals [48]. The study by [49] reported the beneficial effect of daily intake of SO in endothelial dysfunction in hypertensive men. Recently, several researchers reported the antioxidant role of SO in experimental models and also protection of the heart via eliminating the risk factor [11,50].

The current study revealed that administration of SO reverses most of the pathological changes, and myocardial tissue appears relatively normal, and this could be an evidence for antioxidant mechanism of SO as a cardioprotective agent. This was noticed in other previous studies like [8], who showed a protective action of chronic oral administration of SO at different doses (5 and 10 ml/kg) orally for thirty days against the myocardial tissue damage during ischemic reperfusion injury via enhancing the myocardial endogenous antioxidant system in the ischemic heart.

It was thought that this antioxidant property of SO is owing to the phenolic hydroxyl group present in the sesame, and this finding is confirmed in the study of [48] who stated that administration of three different doses of SO (50, 100, and 200 mg/kg of body weight) for 7 days for isoproterenol-induced heart damage in Wister rats improved all histopathological changes of ISO-induced cardiotoxicity [40].

So it is advisable to intake SO, as it lowers the harmful effects of BPA on the heart.

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Conflicts of interest
There are no conflicts of interest.

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