The Protective Role Of Sesame Oil Against Bisphenol- A Induced Cardiotoxicity :Histological And Immunohistochemical Study

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Abstract

Bisphenol- A (BPA), an estrogenic compound, is used in manufacture of polycarbonate plastics and epoxy resins. Sesame oil is a potent antioxidant dietary source for human health.

Aim of the work: The present study is conducted to estimate the protective effects of sesame oil (SO) against bisphenol-A (BPA) induced Cardiotoxicity.

Material 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 10mL/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 sacrificed animal was subjected to histopathological examination by H&E, masson's and INos stain. In addition, blood was collected for biochemical assessment of the enzymes.

Results: Administration of high dose BPA (50mg/kg b wt) significantly increased the weight of rats, several histopathological alterations in cardiac tissue and elevation in MDA , CK-MB and GST and reduction of GSH and catalase when compared to the control. Low dose BPA (25mg/kg b wt) produced mild histoapatholgical effect on the heart , On the contrary, oral gavages of sesame oil with BPA was effective in the reduction of weight, amelioration of histopathological alterations, and in the reduction of the MDA , CK-MB and GST levels and elevation of GSH and catalase activity when compared to high dose BPA's treated rats.

Conclusion: The present study provided clear evidence that sesame oil possesses a promising protective activity against the cardiotoxic effects of bisphenol

Keywords: Bisphenol-A, Cardiotoxicity, Sesame oil, Histopathological alterations

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, acidic and basic situation increase penetration of BPA to the food or environment [3].

The health hazard of BPA is mainly due 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 over 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]

Studies revealed that oxidative stress can induce many kinds of negative effects including membrane peroxidation and DNA strand breakages, which could lead to myocytes 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 changed blood pressure in rodents. The underlying mechanisms may involve alteration of cardiac Ca^{2+} handling, ion channel inhibition/activation and oxidative stress [8].

Sesame oil is one of the major cooking oil used in the diet and has antioxidant components. Sesame oil, found in the seeds of Sesamumindicum [9]Sesame seeds contain flavonoids and other phenolic compounds that can act as antioxidants [10]. 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 [11].Studies from experimental models showed it could protect the heart injury [12].

Recently found that chronic administration of sesame oil enhances the endogenous antioxidants in ischemic myocardium [9].

Materials and methods

Materials

A- Animals

The present study was carried out on thirty two adult male Sprague Dawley rats aged 8-12 weeks, weighing 1500–1800 g , were used in this study. They were obtained from the Animal house, Faculty of Veterinary Medicine, Benha University, Egypt. The rats werehoused 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±1ºC, 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.

-Group I (Control group): the rats received no medications and left to survive for 4 weeks.
-Group II (BPA 25) treated group: Each rat received bisphenol -A in a dose 25 mg/kg via gavage once a day, 5 times per week, for 4 weeks**(13).**
-Group III (BPA 50)treated group: Each rat was received bisphenol -A in a dose 50 mg/kg via gavage once a day, 5 times per week, for 4 weeks**(13).**
-Group IV (BPA 50 - Sesame) treated group: Each rat received bisphenol- A in a dose 50 mg/kg via gavage once a day, 5 times per week, for 4 weeks plus 10 ml/kg Sesame oil via gavage once a day for four weeks[14] .

B- Drugs:

Bisphenol-A :

Bisphenol-A (BPA) (≥ 99 %) was purchased from Sigma-Aldrich Company (St. Louis, MO, 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 2 different doses 1) 25 mg/kg BPA treated group and 50 mg/kg BPA treated group , BPA was administrated via gavage once a day, 5 times per week, for 4 weeks [13]

Sesame oil:

Commercial sesame oil was purchased from EL Captin Company (Al Obour City, Cairo, Egypt). And given in a dose (10 ml/kg) with the 50 mg/kg BPA treated group administrated via gavage once a day, 7 times per week, for 4 weeks [14]

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 from tail vein 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

Biochemical parameters

Measurement of MDA (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 minutes at 4°C (POLYTRON-PT 10-35, Kinematica, Switzerland) in 1.15% KCl in order to provide a 10% homogenate. MDA

levels were determined according to the method of Fernández et al. Data are expressed as nmole/g wet.wt [15].

Myocardial reduced glutathione (GSH)

GSH was estimated by the method of [16] .The reaction mixture contained 0.1 mL of supernatant, 2.0 mL of 0.3 M 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 [17]. 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

Glutathione-S-transferase activity was assayed by the method of which measures the conjugation of 1-chloro-2, 4-dinitrobenzene with reduced glutathione. This conjugation is accompanied by an increase in absorbance at 340 nm, the rate of increase being directly proportional to GST activity[18] .

Measurement of Creatine Phosphokinase-MB

The commercial colorimetric kit (Biosystem, Spain) was used to measure The creatine Phosphokinase-MB (CK-MB) in serum by an auto analyzer (Tokyo Boeki Prestige).

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: using hematoxylin and eosin (H & E) for routine histological examination and masson's Trichrome stains for studying the collagen fiber distribution [19].

Immunohistochemical staining: for iNos antigens using the avidin-biotin peroxidase complex technique [20] .
The sections were collected on poly-L-lysine coated slides. Non-specific 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 mm 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, then blocked with 5% normal goat serum for 30 min at room temperature. Sections were incubated with the primary antibodies for 30 min, iNOS (inducible nitric oxide synthase) rabbit polyclonal antibody IgG (ab15323, Abcam, Cambridge,UK) . Section were incubated with a biotinylated goat anti-polyvalent 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, CA) 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 non-immune mouse serum. The sections were studied and photographed using a Canon digital camera attached to an IBM computer system.

Image analyzer study
The mean area % of iNOS immuno-expression was quantified in five images from five non-overlapping 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 non-overlapping fields of each rat using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA).

Statistical analysis

All the data collected from the experiment was recorded and analyzed using IBM SPSS Statistics software for Windows, Version 19 (IBM Corp., Armonk, NY, USA). One-way analysis of variance (ANOVA) with Post Hoc LSD test was used to compare differences among the groups. In each test, the data was expressed as the mean (M) value, standard deviation (SD) and differences were considered to be highly significant at $P \leq 0.01$, significant at $P \leq 0.05$ and non-significant at $P > 0.05$. [21]

Results:

Animal body weights

The effect of BPA on rat body weights at different doses revealed that body weight of treated rat group with bisphenol A 50 mg/kg (group III) was significantly increased when compared to (group I , II & IV) ($P < 0.01$),while coadministration of sesame oil with high dose in (BPA 50–sesame) caused significant decrease in comparable to (BPA 50) group **.Table (1) & histogram (1).**

Biochemical parameters :

The following biochemical parameters were studied in the heart homogenate:

MDA (malondialdehyde)

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

Myocardial reduced glutathione (GSH)

GSH level in group III (BPA 50) was significantly decreased as compared to groups(I , II & IV) ($P < 0.01$) .While GSH level was significantly increased in (PBA –sesame) group IV compared to(BPA 50) group III and its level raised to near normal . **Table (2) & histogram (2)**

Serum creatinine phosphokinase-MB:(CK- MB)

Measurement of serum CK-MB activity revealed significant increase in (BPA 50) group III as compared to groups(I , II & IV) ($P < .001$). However significant increase in (CK- MB) activity in(BPA -50) group compared to (BPA- 25) group

Co administration of sesame oil and 50 mg/kg of BPA (group IV) decreased serum CK-MB activity as compared with the (group III) ($P < .001$) and its level raised to near normal. **Table (3), histogram (3).**

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 & IV) ($p<0.01$) and significant increase in catalase activity in (PBA – sesame)group IV compared to (BPA 50) group .**Table (3), histogram (3).**

Glutathione-S-transferase activity (GST)

GST activity was significantly increase in (group III) BPA 50 ($p<0.01$). as compared to groups (I , II & IV) .Co administration of sesame oil and 50 mg/kg of BPA in (group IV) led to significant decrease in GST activity compared to BPA 50(group III) . **Table (3), 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. 1,2).**

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 with had dark cytoplasm, pyknotic nuclei & area of fibers loss **(Fig. 3,4).**

The histological pattern of Group III (BPA 50 mg/kg treated group): showed area of marked distortion and fragmentation of cardiac muscle fibers. Some fibers with dark cytoplasm &pyknotic nuclei are seen.Focal lytic area of sarcomere. mononuclear cellular infiltrations surround the wall of blood vessels and in between the sarcomere also disorganized sarcomeric structure with massive infiltration with inflammatory cells and connective in between sarcomere **(Figs. 5,6).**

The histological pattern of Group IV (BPA 50 mg/kg + 10 ml/kg Sesame oil 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 Flat dark nuclei of fibroblast of connective tissue endomysium was noted **(Figs. 7, 8).**

Morphometric results

Masson's Trichrome stain

The mean area % of collagen deposition for all groups was represented in **table (4) and histogram (4).** There was insignificant increase in mean area% of collagen deposition ($P>0.05$) in groups II & IV as compared with control group. But area % of collagen deposition was highly significantly increased in group III as compared to groups I, II & IV groups ($P<0.01$).

Immunohistochemistry

The mean area % of iNOS immuno-expression for all groups was represented in **table (5) and histogram (5)** . There was insignificant increase in iNOS immuno-expression ($P>0.05$) in group II as compared with control group. But area % of iNOS immuno- reactivity was highly significantly increased in groups III as compared to groups I, II &IV ($P<0.01$). Also, area % of iNOS immuno-expression was insignificantly increased in group IV as compared to control ($P>0.05$).

Table (1) showing mean values of BW ± SD in the 4 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 & IV	With group III	

Histogram(1) showing mean values of BW in the 4 groups.

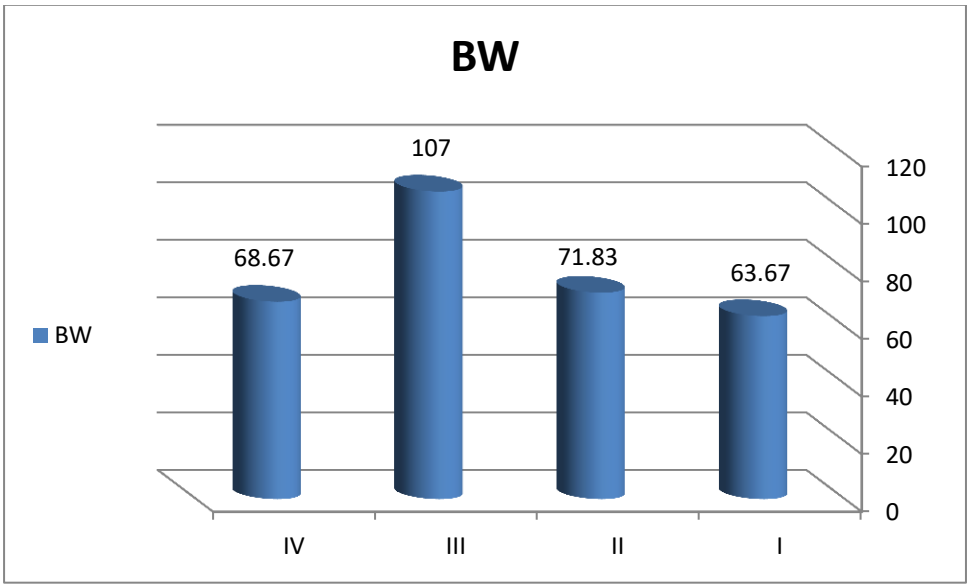


Table (2) showing mean values of MDA, GSH ± SD in the 4 groups

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 & IV	With group III	

Histogram(2) showing mean values of MDA & GSH in the 4 groups

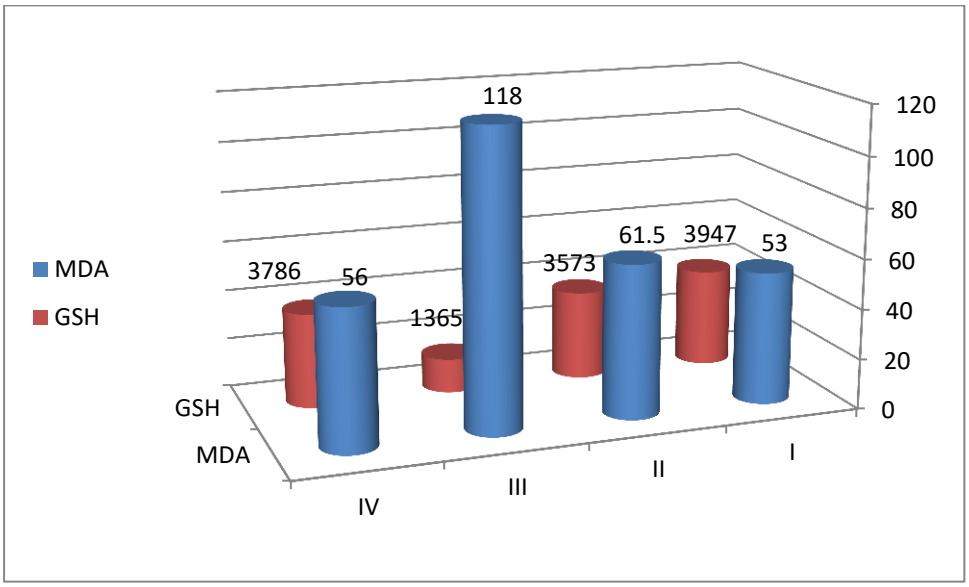


Table (3) showing mean values of CK-MB, GST & Catalase ± SD in the 4 groups

CK-MB (U/I)	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 & IV	With group III	

Histogram (3) showing mean values of CK-MB, GST & Catalase in the 4 groups

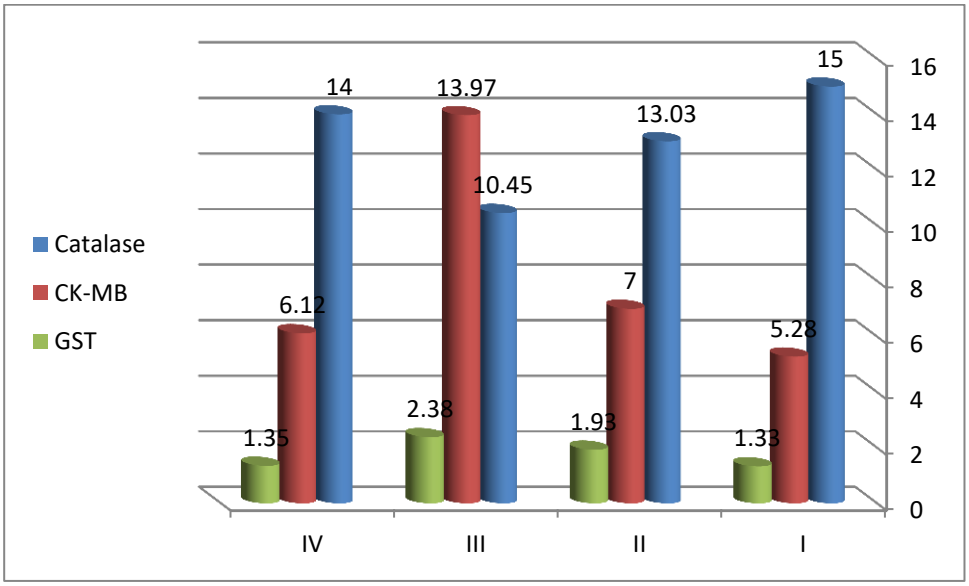


Table (4) : Showing the mean area %, SD of collagen fibers deposition in groups I, II, III and IV with comparison between all groups by Post Hoc LSD test.

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 & IV	With group III		

Histogram (4) Showing the mean area % of collagen fibers deposition in groups I, II, III and IV.

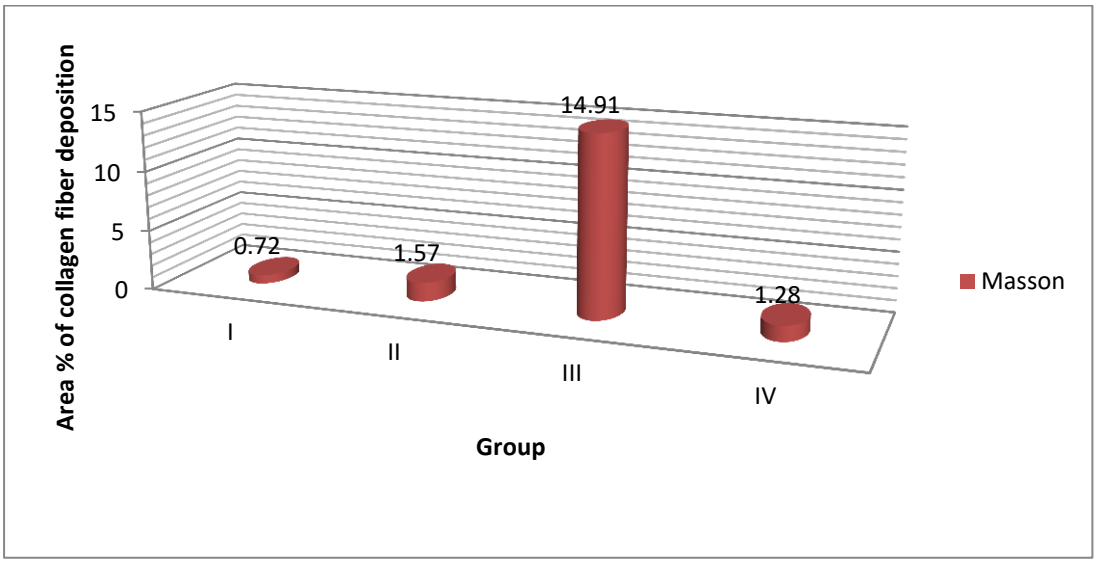
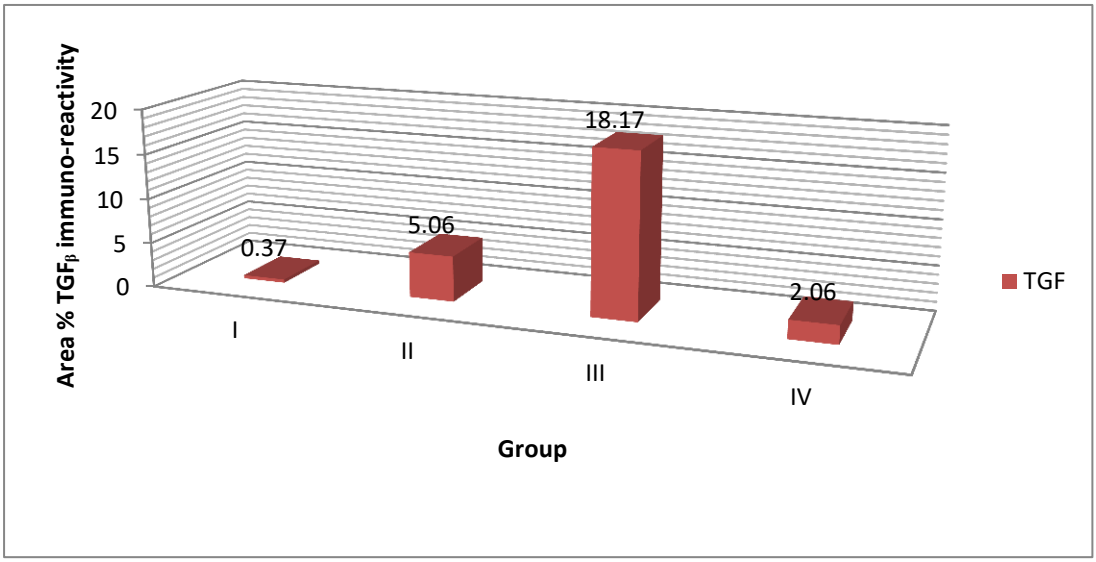


Table (5) showing mean values of area % immunoreactivity of iNos ± SD in the 4 groups:

Mean % ± SD	Group I	Group II	Group III	Group IV	F test	P value
iNos immune reactivity	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 & IV	With group III		

Histogram (5) showing mean values of area % iNOS immuno-reactivity in the 4 groups.



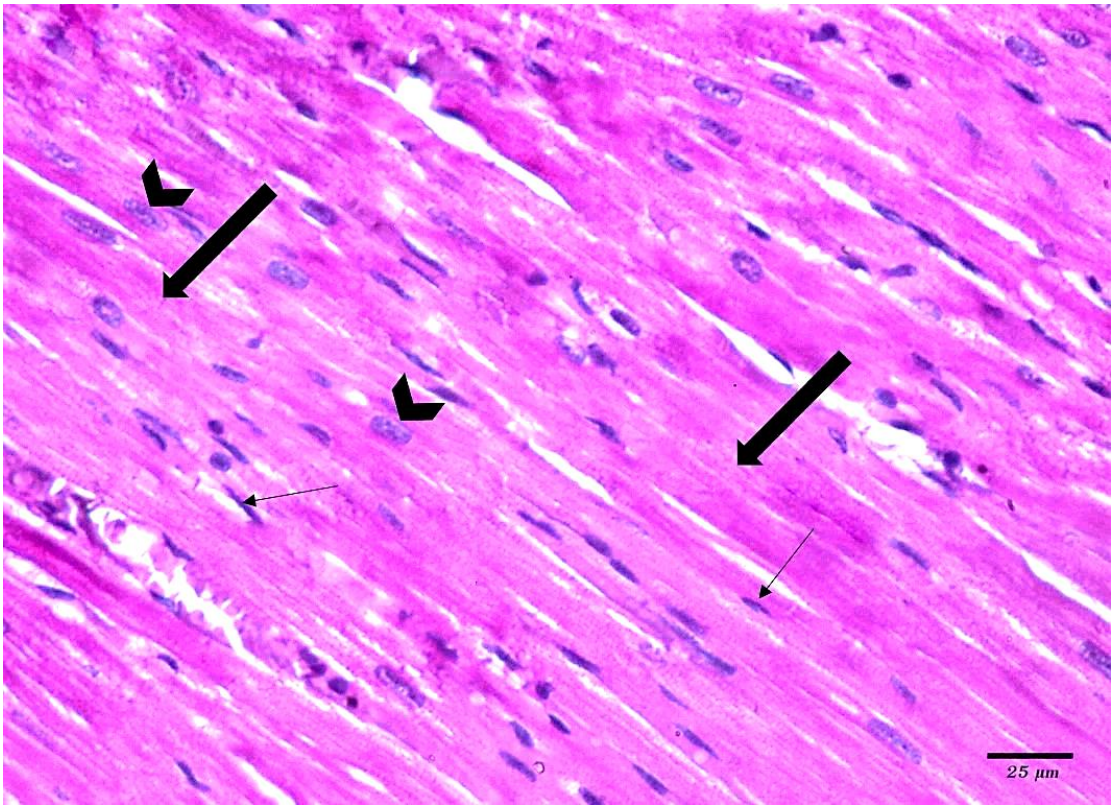


Fig.1. A photomicrograph of a section of myocardium of control group I rat showing: branching and anatomizing longitudinal cardiac muscle fibers (thick arrow) with acidophilic sarcoplasm and central elongated vesicular nuclei (arrow head). Flat dark nuclei of fibroblasts (thin arrow) of connective tissue can be seen. (H&E x 400)

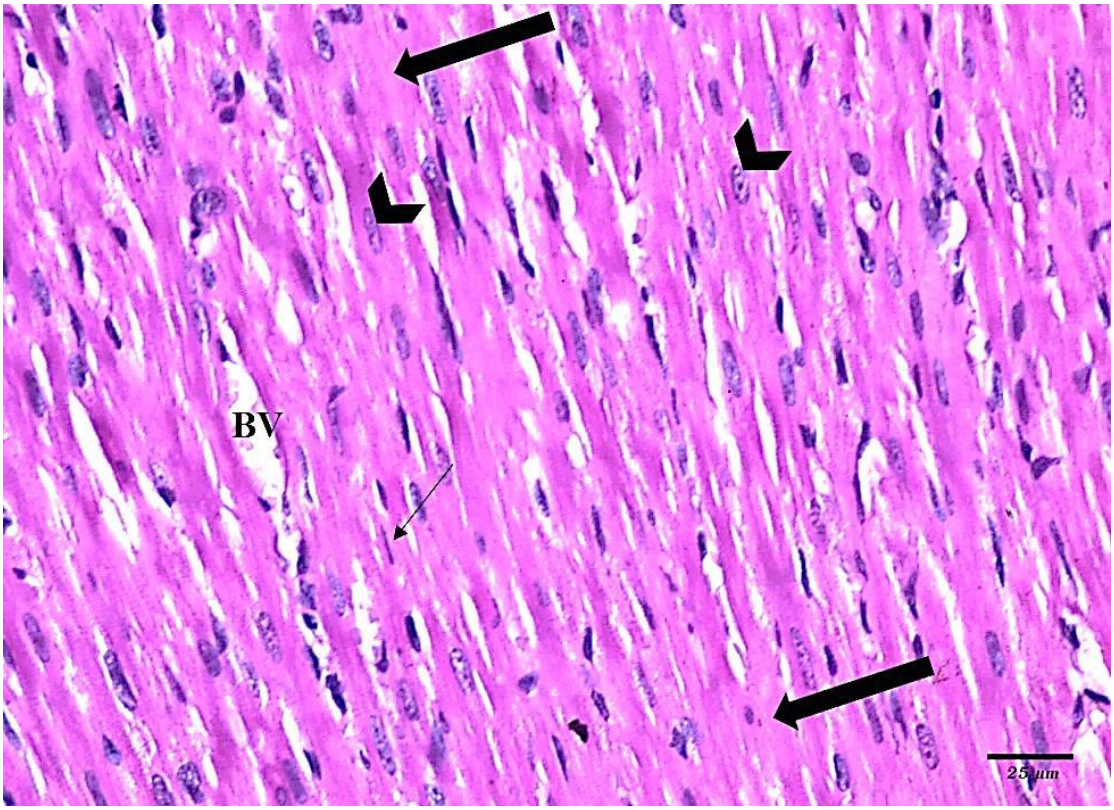


Fig.2. A photomicrograph of a section of cardiac muscles of control group I rat showing: longitudinally arranged cardiac muscle fibers (thick arrow) with acidophilic sarcoplasm and central, vesicular and oval nuclei (arrow head). The fibers are branching and anastomose with each other. Notice: connective tissue cells with dense flattened nuclei (thin arrow) and elongated blood vessel (BV) can be observed. (H&E x 400)

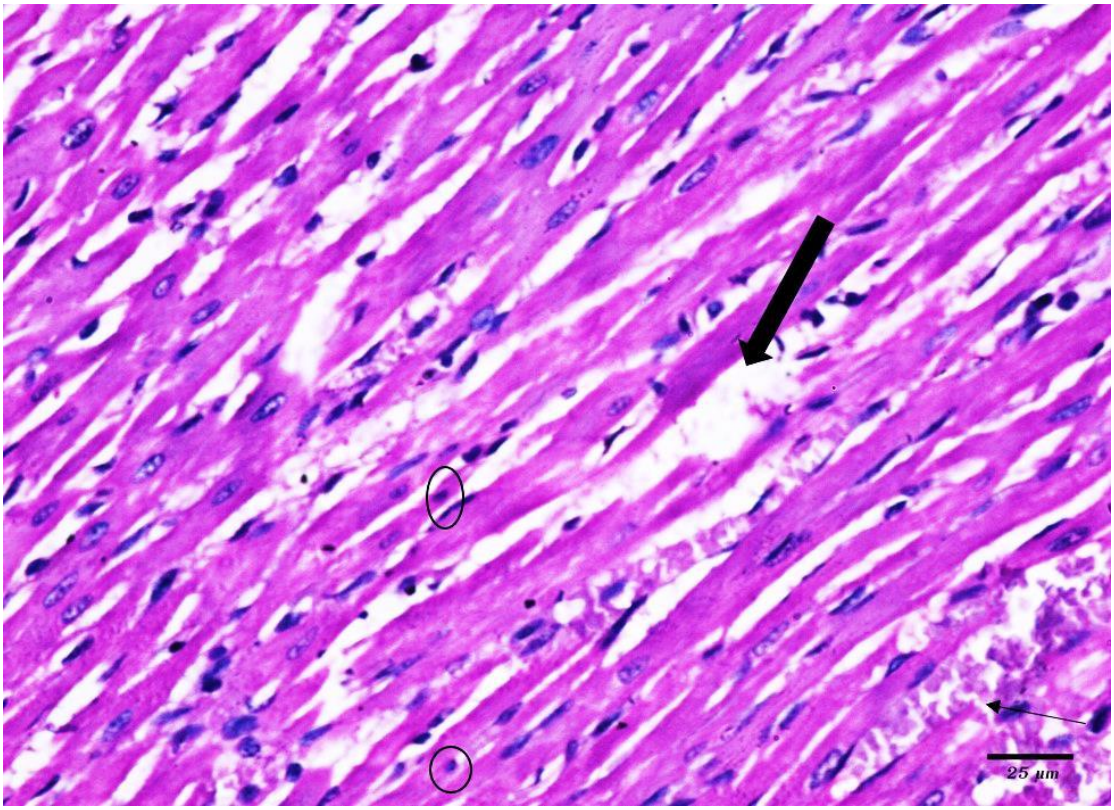


Fig.3. A photomicrograph of a section of rat's myocardium of treated group II (BPA 25) rat showing: area of fibers loss (thick arrow). Some fibers with dark cytoplasm &pyknotic nuclei (circle) are seen. Rupture the wall of blood vessels (thin arrow) is detected. (H&E x 400)

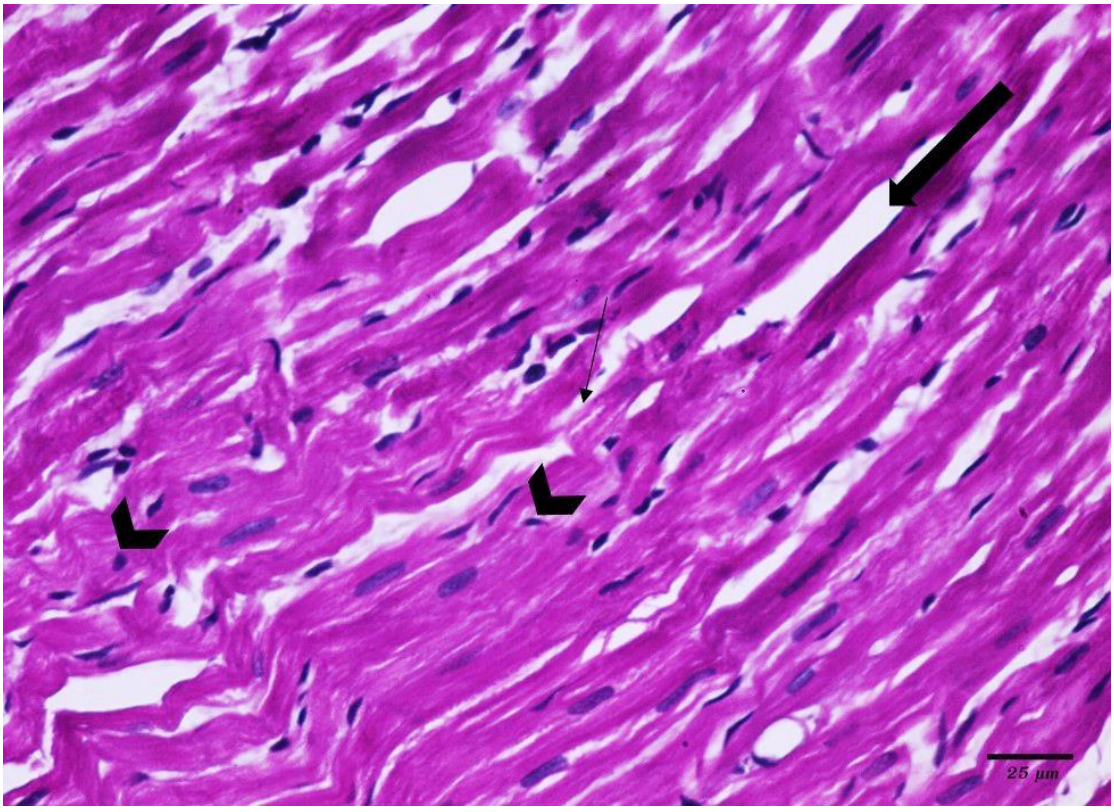


Fig.4. A photomicrograph of a section of rat's myocardium of treated group II (BPA 25) rat showing: area of fibers loss (thin arrow). Some fibers with dark cytoplasm &pyknosis of some nuclei are seen (arrowheads).Destruction of some fibers (thick arrow). (H&E x 400)

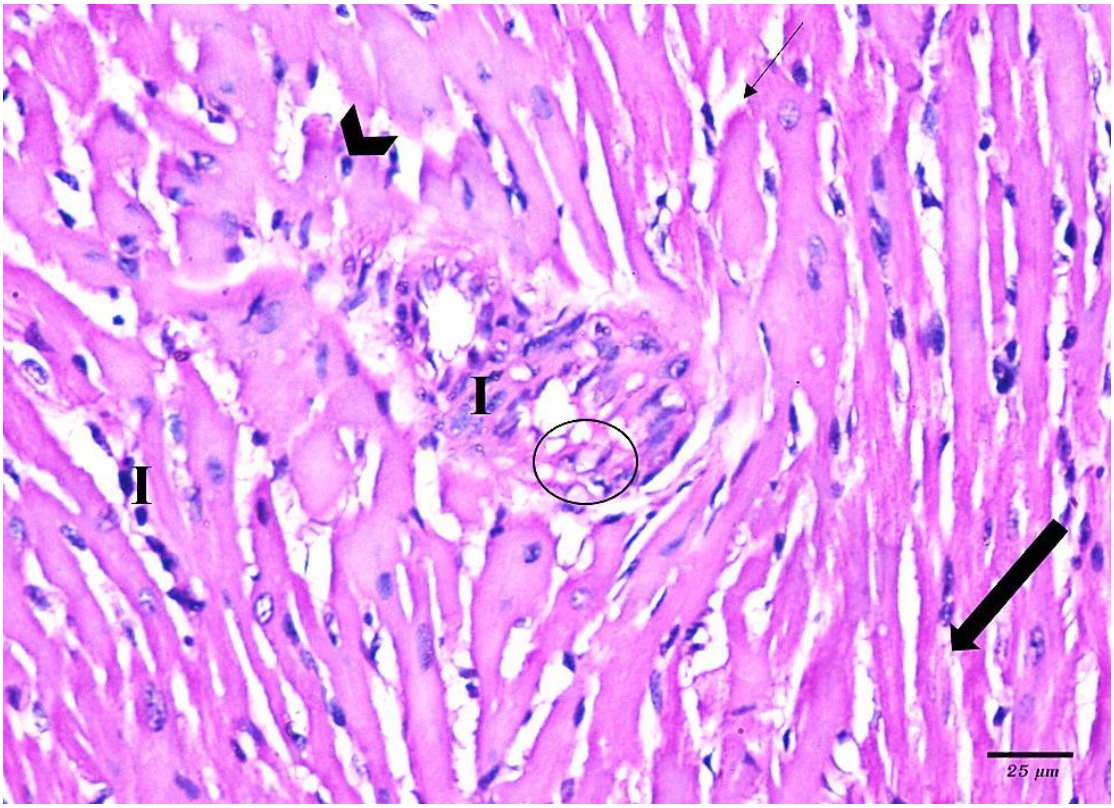


Fig.5. A photomicrograph of a section of rat's myocardium of treated group III (BPA 50) rat showing: loss of striations &area of fibers loss (thin arrow). Some fibers with dark cytoplasm &pyknotic nuclei are seen (arrowheads). Focal lytic area of sarcomere (thick arrow) . Mild mononuclear cellular infiltrations (I) surround the wall of blood vessels and in between the sarcomere. Notice: vacuoles in the wall of blood vessels (circle). (H&E x 400)

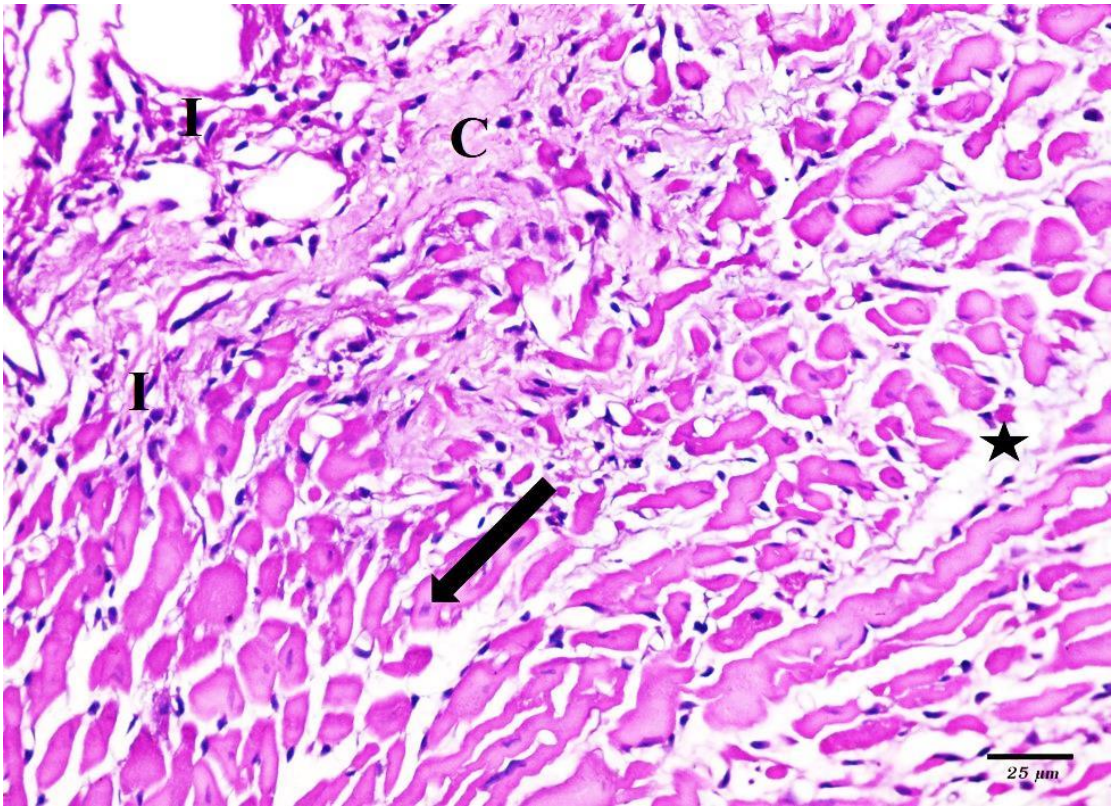


Fig.6. A photomicrograph of a section of rat's myocardium of treated group III (BPA 50) rat showing: disorganized sarcomeric structure with massive infiltration with inflammatory cells (I) and connective fibers (C) in between sarcomere. Cross section of cardiac muscle fibers with pyknotic nuclei (thick arrow).There is increase in interstitial connective tissue (astric). (H&E x 400)

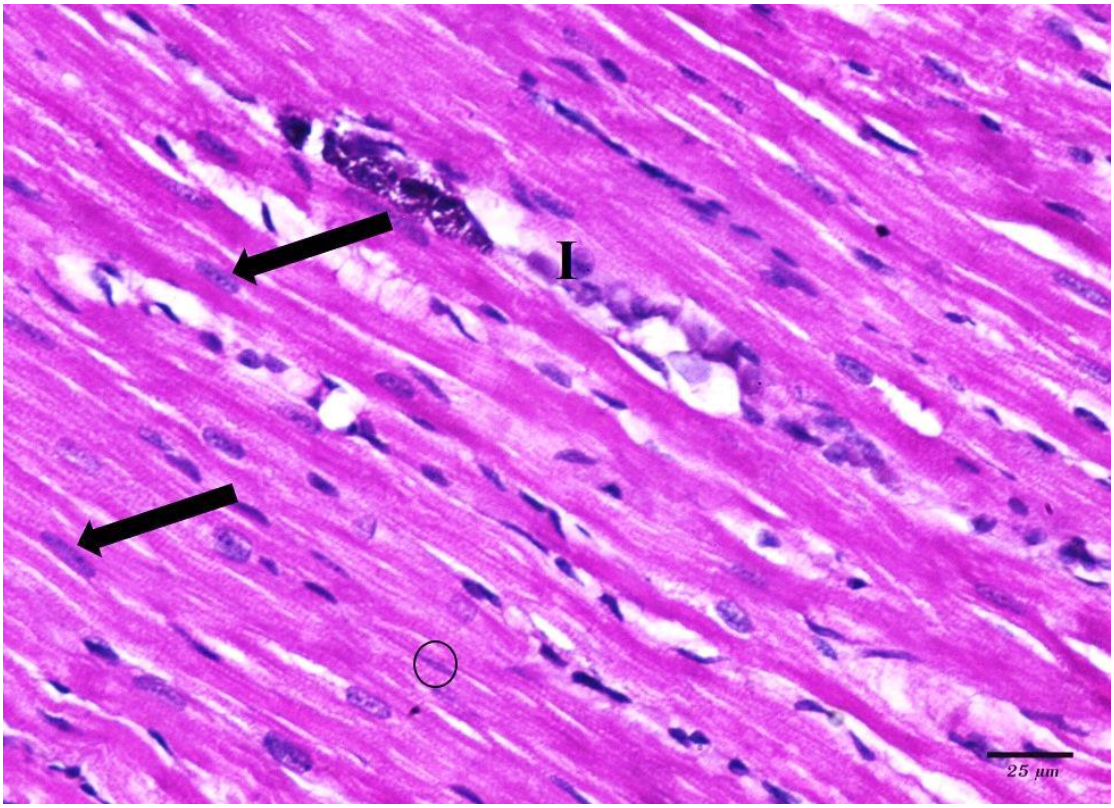


Fig.7. A photomicrograph of a section of rat's myocardium of treated group IV (PBA –sesame) showing: the cardiac muscle fibers with appearance more or less similar to control. Note: vesicular oval nuclei of cardiac muscle fiber (thick arrow)& Flat dark nuclei of fibroblast of connective tissue endomysium(circle) are seen. Also slightly cellular infiltration with inflammatory cells (I) can be observed. (H&E x 400)

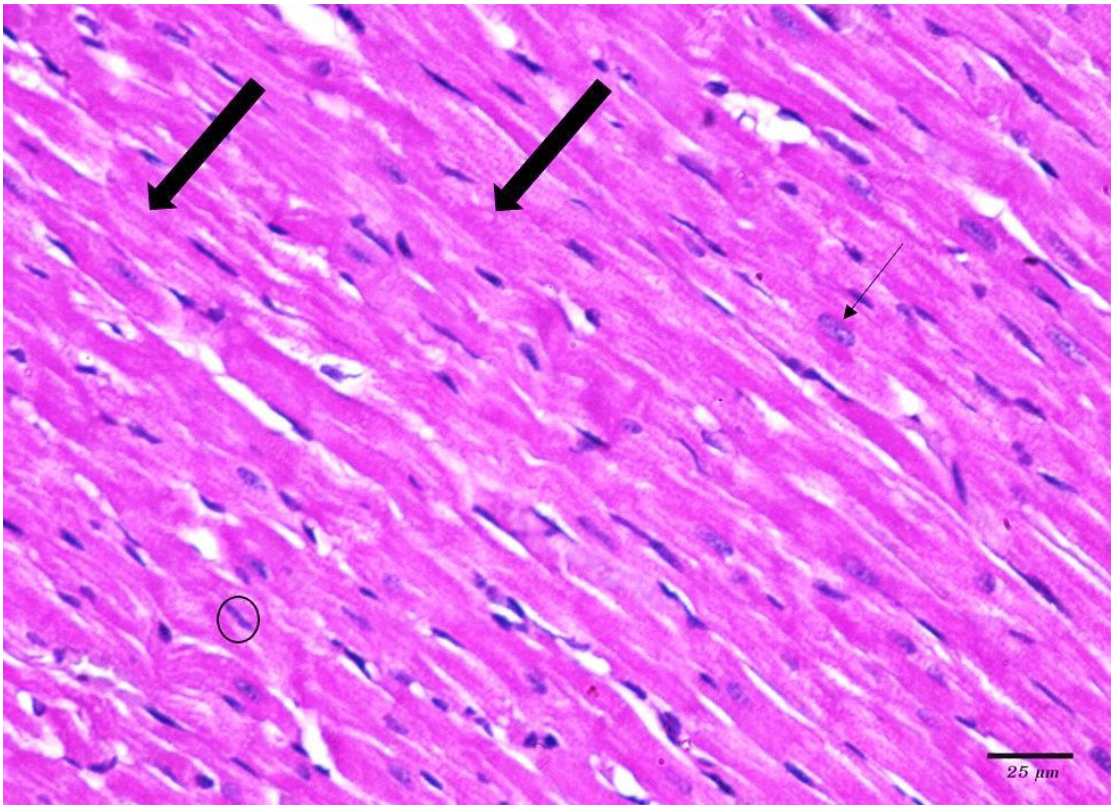


Fig.8. A Photomicrographs of sections of rat myocardium from protected group IV (PBA –sesame) rat showing: more or less normal branching and anastomosing longitudinal muscle fibers (thick arrow) with central oval nuclei (thin arrow). Flat dark nuclei of fibroblast of connective tissue endomysium (circle) are seen. (H&E x 400)

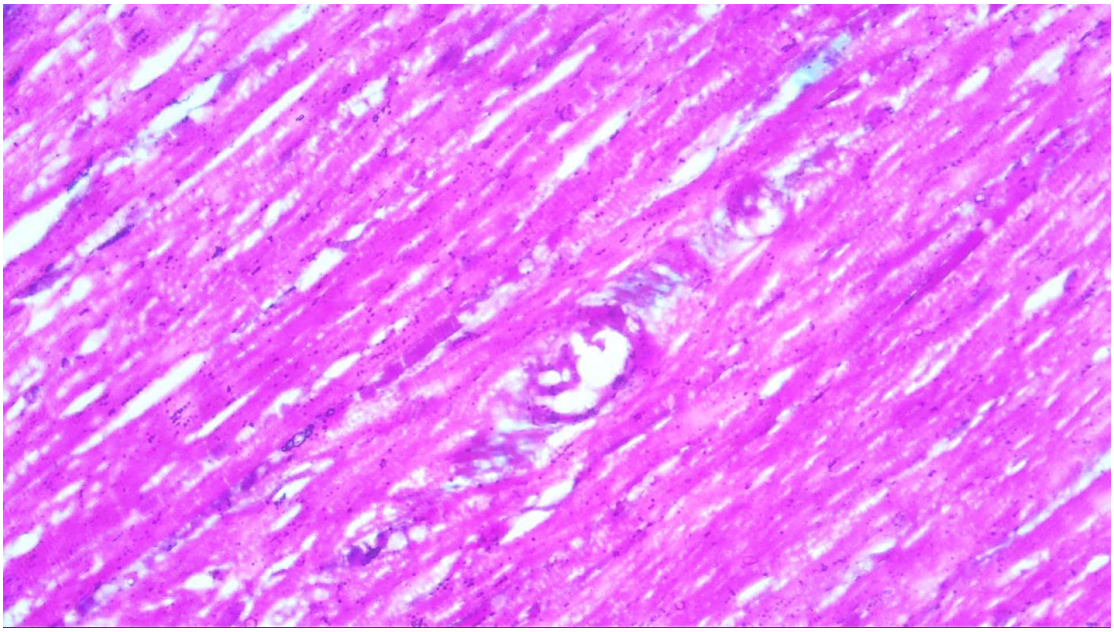


Fig.9. A photomicrograph of a section of the myocardium of group I (control) showing minimal collagen fibers deposition between cardiac muscle fibers.

(Masson's Trichrome×400).

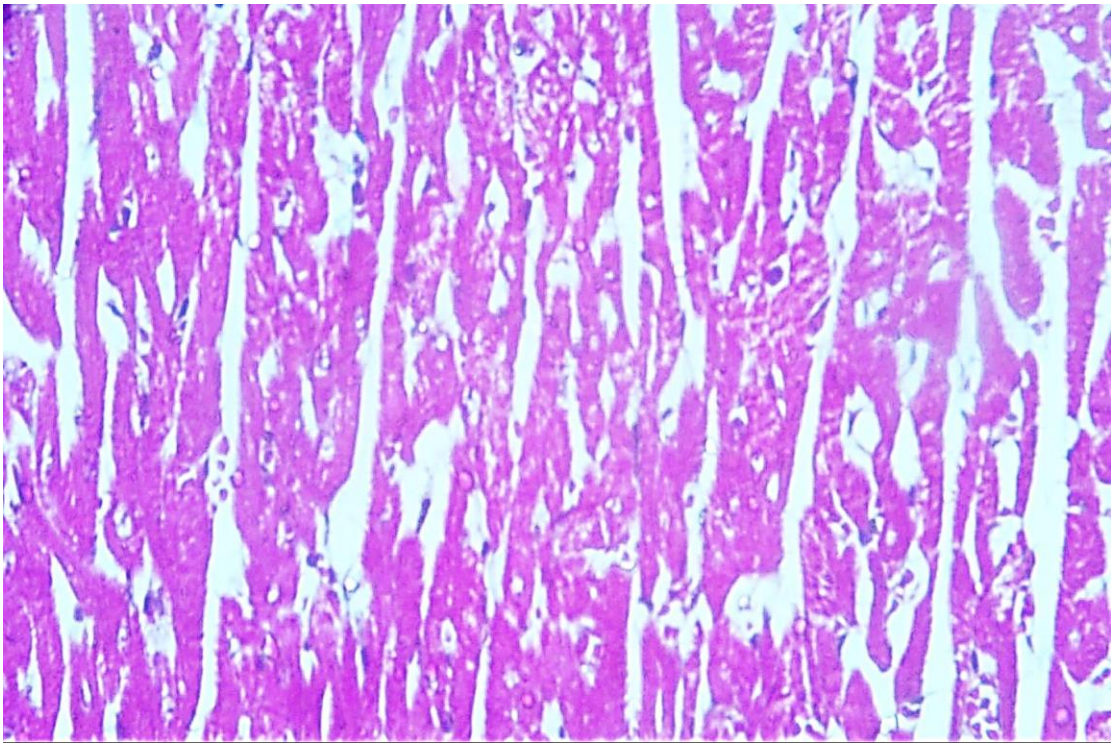


Fig.10. A photomicrograph of a section of the myocardium of (BPA 25) treated group II rat showing: few collagen fibers deposition between cardiac muscle fibers (Masson's Trichrome×400).

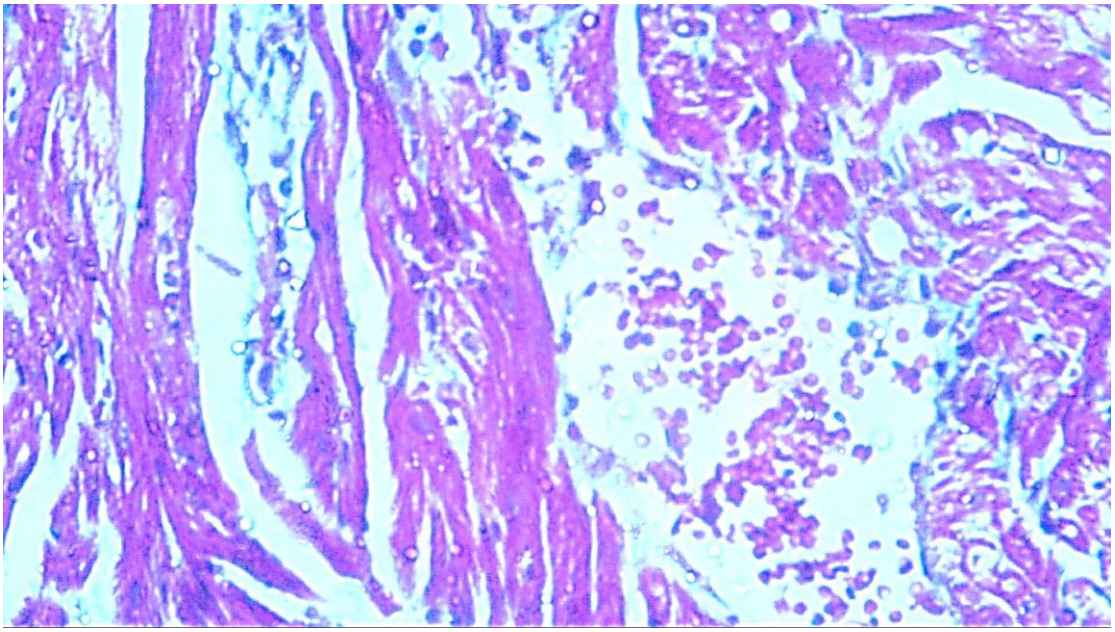


Fig.11.A photomicrograph of a section of the myocardium of (BPA 50) treated group III rat showing :heavy collagen fibers deposition between cardiac muscle fibers

(Masson's Trichrome×400).

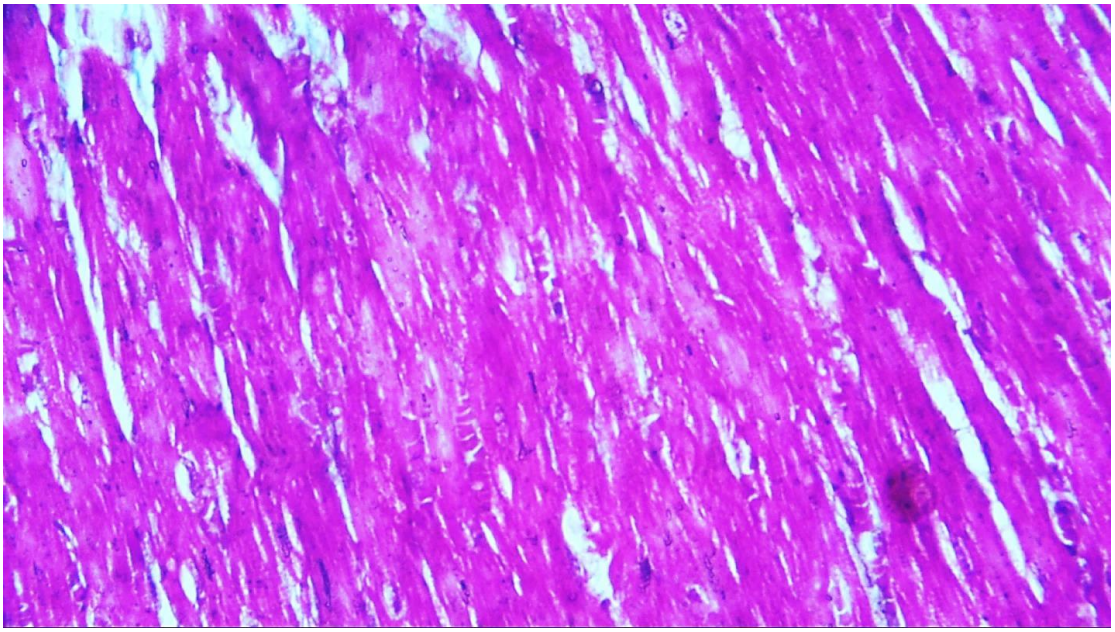


Fig.12. A photomicrograph of a section of the myocardium of (PBA –sesame) protected group IV rat showing: minimal collagen fibers deposition between cardiac muscle fibers (Masson's Trichrome×400).

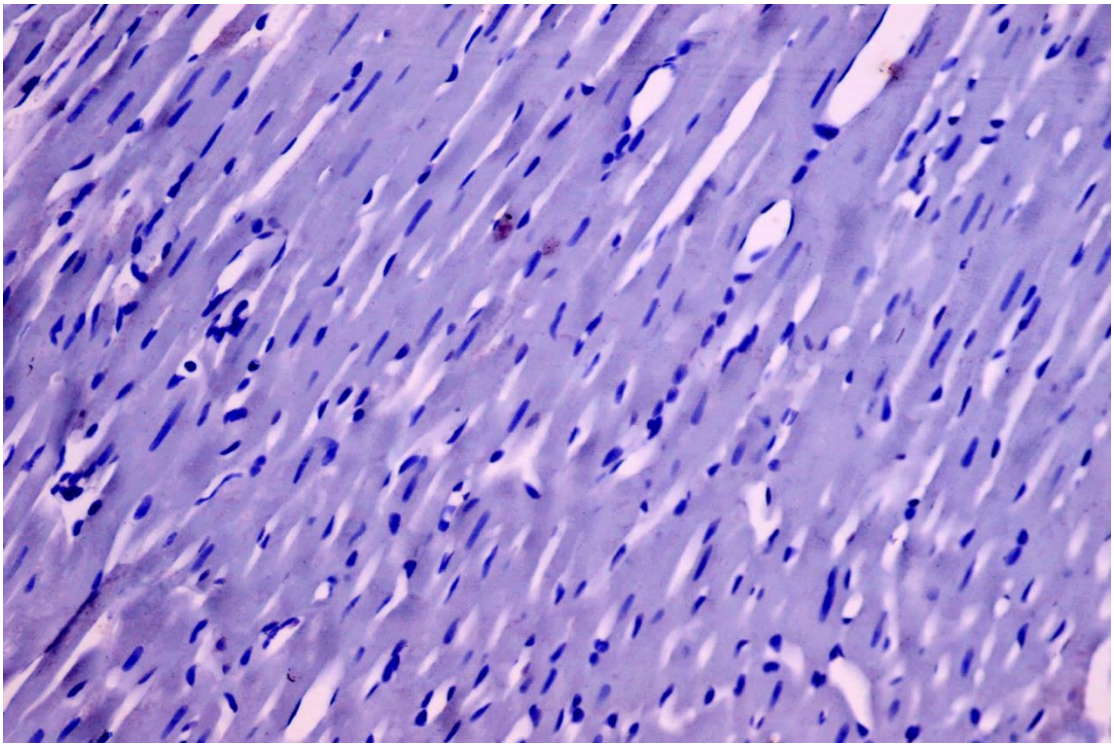


Fig.13. A photomicrograph of section of cardiac muscles of (control) group I rat showing :no expression of iNOS. (iNOS x 400)

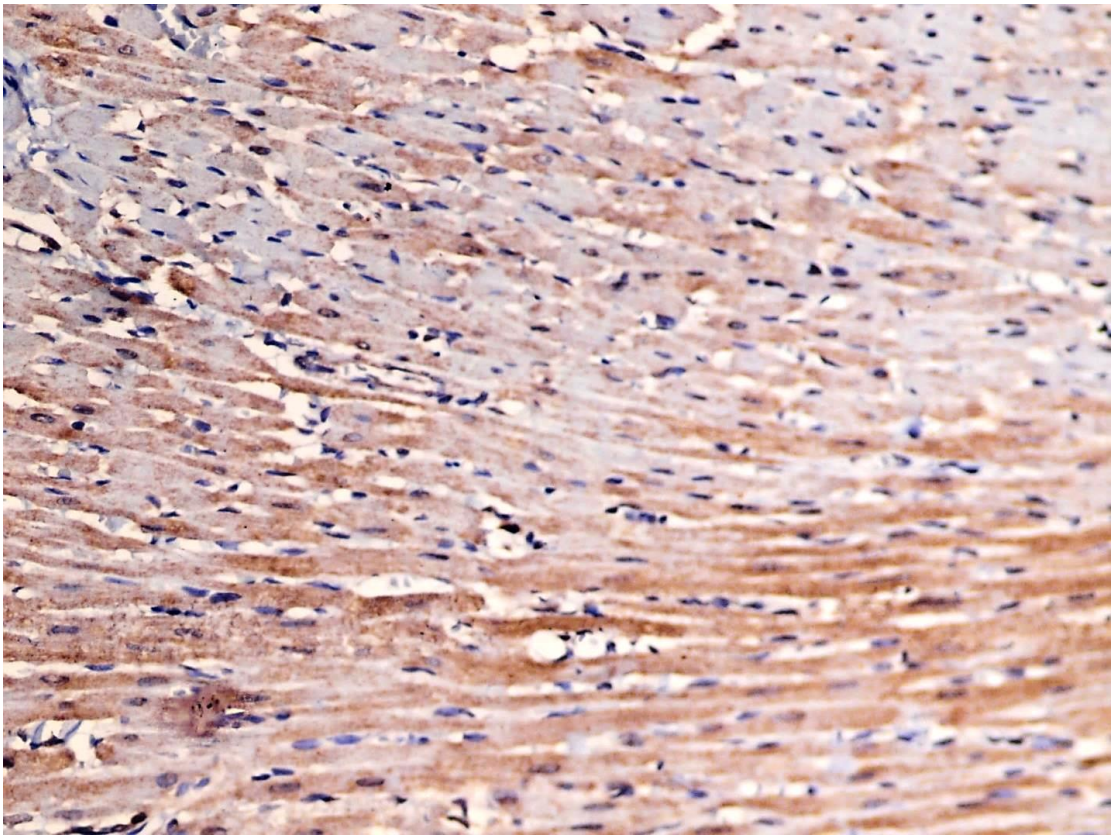


Fig.14. A photomicrograph of section of cardiac muscles of (BPA 25) treated group II rat showing mild to moderate expression of iNos. (iNos x 400)

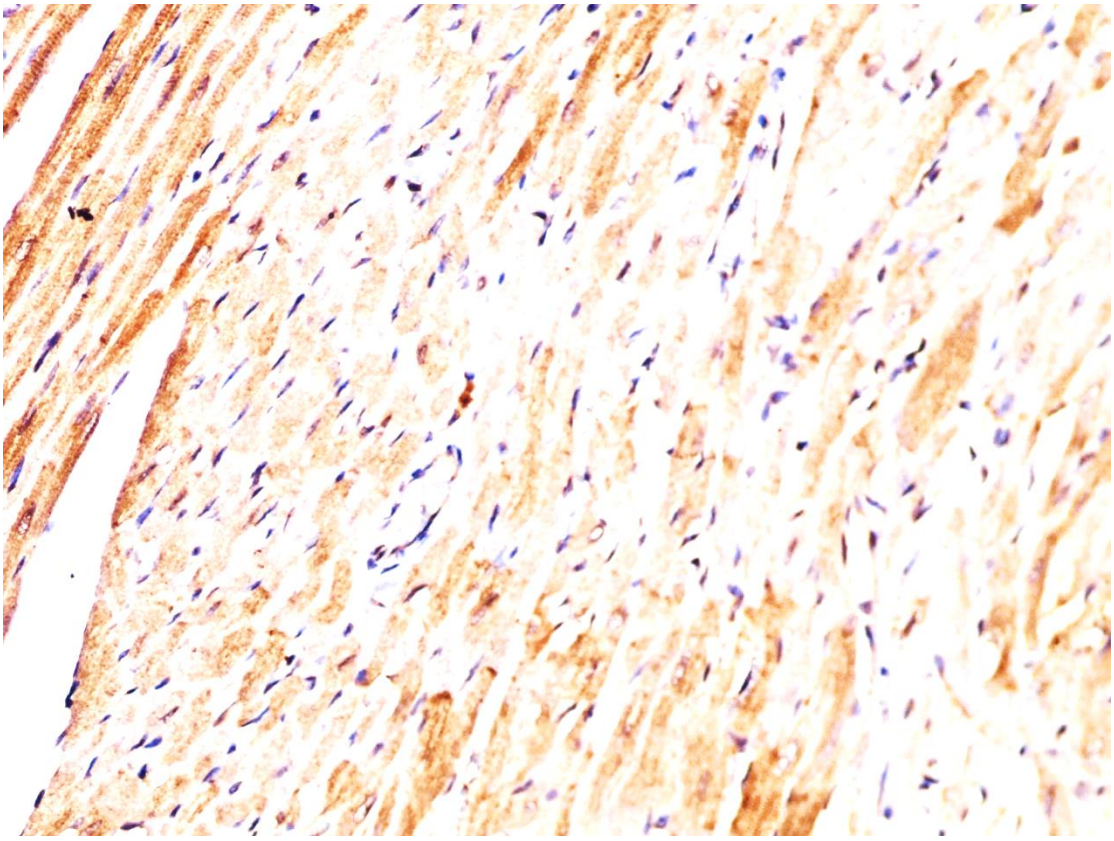


Fig.15. A photomicrograph of section of cardiac muscles of (BPA 50) treated group III rat showing high expression of iNos. (iNos x 400)

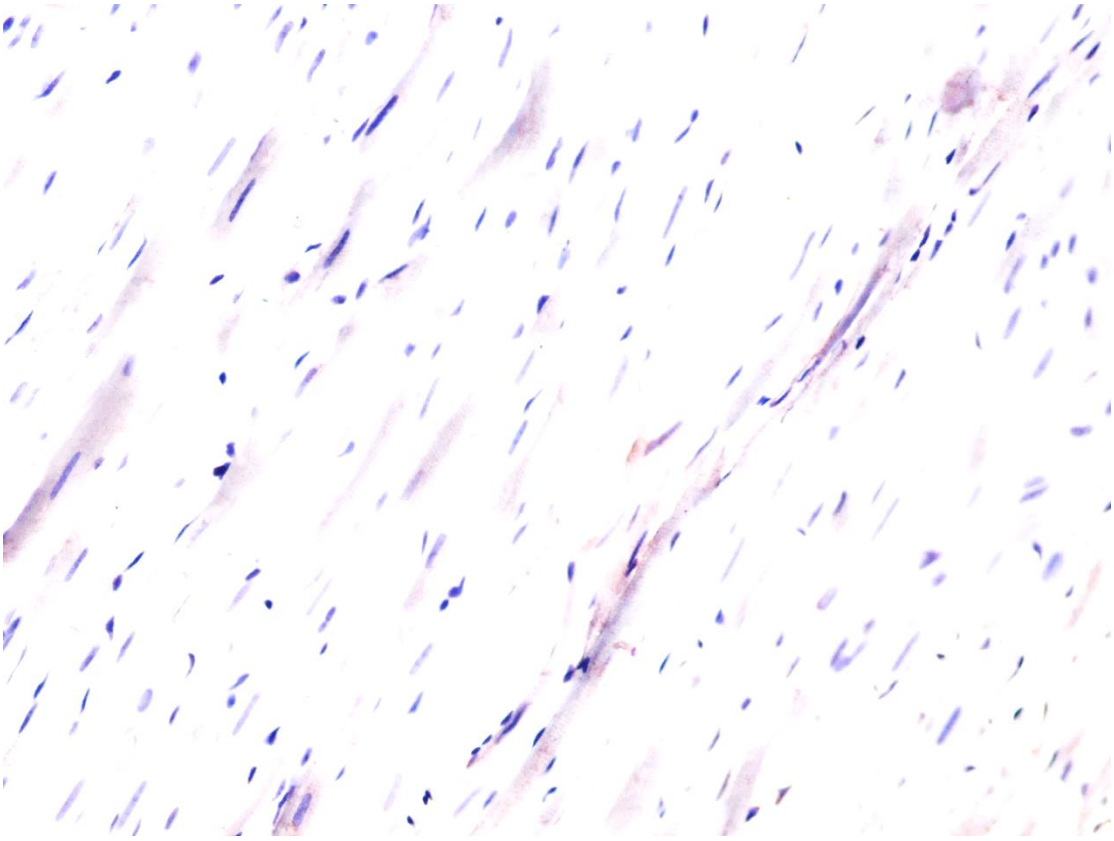


Fig.16. A photomicrograph of section of cardiac muscles of (PBA –sesame) protected group IV rat showing very low expression of INos (iNos x 400)

Discussion:

Our data stated that the oral administration of BPA at the two tested doses (25, 50 mg/kg b. wt) for 4 weeks to rats led to increase of the body weight ,moreover the body weight gain of animals treated with the high dose (50mg/kg) significantly increased in relative to the control and low dose group rats. Similarly, previous studies showed that body weight of offspring of Sprague-Dawley rats were exposed to BPA treatments (1 , 1.2 mg BPA/kg BW/day) were increased on postnatal and continued into adulthood, dose and gender-dependently [22,23] in their studies on Sprague-Dawley female rats. In addition, several investigators confirmed that BPA has a role in weight gain and the development of obesity [24,25,26].

This effect of BPA could be explained by the report of Hugo et al. (2008)[27] 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 [28](Wade and Schneider 1992). so 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 [29].

However , Kwon et al failed to observe significant differences in body weight after perinatal exposure to much higher levels of BPA than levels used in the present study [30]. Another study [31] 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, delayed the puberty in the offspring [32, 33,34].

According to the results of the current study concomitant administration of sesame oil 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 and this was in accordance with the study of [35] who found that administration of sesame oil only not affect the body weight .But on contrary of the study of [36,37] suggested that the fluid extract of sesame leaves significantly increase the weight of rats, and was explained by that the sesame seed and giving this 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 to the control values, decrease in GSH levels and catalase activity below the control values However, 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 non-monotonic dose–response curves that have been reported for many actions of BPA (27, 38). And this in accordance with the study of [39] that revealed increase of both MDA, GST level and decrease of GSH levels and catalase activity in both low and high doses of BPA (25,10 mg/kg for 6 weeks) ,and considered with the study of [13]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 due to the exhaustion of the enzyme in attempting to eliminate the

hydrogen peroxide generated after the exposure to BPA. This may also be due to enzyme inactivation caused by excess ROS production in mitochondria and microsomes [40].

The increased oxidative stress may play a role in the BPA's potential adverse impaction on the CV system and explained by BPA exposure resulted in a decrease in nitric oxide (NO) which may cause vasoconstriction and decreased blood supply to the heart .Another mechanisms by which BPA might increase the risk of cardiovascular disease, including, altered vascular reactivity to endothelin-1, and inflammation [41].

In the current study, administration of sesame oil at dose of 10mg/ kg with the high dose 50mg/kg significantly lowered the activities of marker enzymes (MDA, CK-MB) and significantly increased the activities of GST, catalase ,GSH, which ally the free radical formation during myocardial array as previous reports proved the antioxidant activity of sesame oil in oxidative cardiac assault conditions (14, 42,43)

Histopatological changes of cardiac tissue of the rats received BPA at a dose(50mg/kg for 4 weeks)reveled 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 and these changes coincided with other similar studies, mentioned signs were observed by five doses of BPA ranging from 2.5 to 25000 μg/kg/day to offspring at postnatal day 21, 90, and 180 Sprague-Dawley rats [44] , another study at a dose of 50 mg/kg BPA both 20 and 30 days in Sprague-Dawley male rat[45]and the study of [13]who uses a dose BPA (50 mg/kg, for 4 weeks).
However , only mild changes in the myocardial structure were observed in the low dose of BPA (25 mg/kg) in the form of only some fibers had dark cytoplasm&pyknotic nuclei are seen. Rupture the wall of blood vessels was observed and this finding not agreed with the study of [13] who didn't observe any

pathological changes in the myocardium at a dose BPA (25 mg/kg, for 4 weeks) even the myocardium resembled the normal.
The current study revealed marked increase of the myocardial fibrosis after exposure the high dose BPA but mild increase in the low dose and finding detected in the study of [46] 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 [47] or as a result of increased density and/or activation of cardiac mast cells [48,49].

Drugs which 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 [50]. The study [51] reported that the beneficial effect of daily intake of sesame oil in endothelial dysfunction in hypertensive men. Recently several researchers reported the antioxidant role of sesame oil in experimental models and also protect the heart via eliminating the risk factor [12, 52].

The current study revealed that administration of sesame oil reverses most of pathological changes and myocardial tissue appear relatively normal and this could be an evidence for antioxidant mechanism of sesame oil as cardioprotective agent. This was noticed in other previous studies like [9] that showed a protective action of chronic oral administration of sesame oil 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 sesame oil is due to the phenolic hydroxyl group present in the sesame and this finding confirmed in the study of [50]who stated that

administration of three different doses of sesame oil (50, 100 and 200 mg/kg of body weight) for 7 days for isoproterenol induced heart damage in Wister rats improve all histopathological changes of ISO- induced cardiotoxicity [42]. .So it is advisable to intake of sesame oil as it lowers the harmful effects of BPA on the heart .

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