**The Protective Role Of Sesame Oil Against Bisphenol- A Induced Cardiotoxicity :Histological And Immunohistochemical Study Eman El Bana & Kamal M. Kamal,Nehal Shaheen and Sahar M Anatomy &Embryology Department Faculty of Medicine, Benha University .**

**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 Ca2+ 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 (pH8.4), 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 avidinbiotin 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 nonimmune 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≤ 0.01, significant at P≤ 0.05 and non-significant at P>0.05. [21]