Cucumeropsis mannii seed oil protects against bisphenol A-induced hepatotoxicity by mitigating inflammation and oxidative stress in rats

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All authors reviewed the results and approved the final version of the manuscript.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not for-profit sectors.

Conflict of interest Authors declare no conflicts of interest in this publication.

Data availability: There is no data to share

ABSTRACT

OBJECTIVES: This study looked at how CMSO affected male Wistar albino rats' liver damage caused by bisphenol A.

METHODS: The standard HPLC method was used to assess the CMSO's phenolic content. Then, six (n = 8) groups of forty-eight (48) male Wistar rats (150 20 g) each received either CMSO or olive oil before being exposed to BPA for 42 days. Groups: A (one milliliter of olive oil, regardless of weight), B (BPA 100 mg/kg body weight (BW)), C (CMSO 7.5 mg/kg BW), D (CMSO 7.5 mg/kg BW + BPA 100 mg/kg BW), E (CMSO 5.0 mg/kg BW + BPA 100 mg/kg BW), and F (CMSO 2.5 mg/kg BW + BPA 100 mg/kg BW).

KEY FINDINGS: A surprising abundance of flavonoids, totaling 17.8006 10.95 g/100 g, were found in the HPLC data. Malondialdehyde, liver enzymes, reactive oxygen species, total bilirubin, and direct bilirubin levels were all significantly elevated by BPA (p 0.05). Additionally, nuclear factor-B, interleukin-6, interleukin-1, tumor necrosis factor, and histological alterations were all considerably (p 0.05) caused by BPA. The altered biochemical markers and histology were, however, noticeably recovered by CMSO to a level that was comparable to the control.

CONCLUSION: Due to the abundance of flavonoid components in the oil, CMSO protects the liver from BPA-induced hepatotoxicity by lowering oxidative stress and inflammatory reactions.

Keywords: Bisphenol A, Hepatotoxicity, Oxidative stress, Anti-inflammation, Antioxidant, *Cucumeropsis mannii*, Flavonoids profile.

INTRODUCTION

The recent increase in environmental pollution poses a serious health challenge. According to [1], bisphenol A [BPA; 2, 2-bis (4-hydroxyphenyl) propane] is a well-known environmental contaminant and is becoming more prevalent globally. BPA is an endocrine disruptor with estrogenic and thyroid hormone-like effects [2]. Several studies have found that BPA exposure promotes hepatotoxicity and causes oxidative damage via various mechanisms [3,4]. Other studies have connected BPA exposure to an increased risk of thyroid dysfunction, cardiovascular disease, obesity, and type II diabetes [5,6]. Over the years, several studies have shown that BPA is toxic even at low doses [7]. BPA has been shown in studies to cause liver, kidney, brain, and epididymal sperm damage in rodents and other organ damage by forming reactive oxygen species (ROS) [8, 9]. ROS plays a crucial role in pathological defense mechanisms. However, excessive production of free oxygen radicals can damage tissues and proteins, resulting in structural changes and functional inactivation of many enzymes and receptor proteins involved in cell signaling [10]. However, epidemiological studies of BPA continue to be controversial. Because of growing concerns about the safety of BPA, its use in plastic bottles for infants was prohibited by various health agencies, including Health Canada in 2009, the European Union in 2011, and the US Food and Drug Administration (FDA) in 2012, and completely banned in food containers in France in 2015 [11].

Cucumeropsis mannii (African Melon), popularly known as "egusi" [12], is the true indigenous egusi of West Africa, and its common names include "Egusi" in Igbo, "Elegushi" in Yoruba, "Agushi" in Hausa, and "Ashi" in the Izzi dialect of Ebonyi State. In English, it is called Mann's cucumeropsis and white-seed melon. In some Nigerian savanna belts, African melon, a cucurbit crop with a fibrous and shallow root system, is typically planted alongside early maize and yam [13]. *C. manni* seed is consumed in different ways by different people. A traditional soup known as "Egusi soup" in Cameroon, Nigeria, and Benin and "Pistachio soup" in Côte d'Ivoire is thickened in Sub-Saharan Africa using C. mannii [14, 15]. The seed of egusi (*Cucumeropsis mannii*) is also a rich source of protein (31.4%), essential amino acids, fat (52.5%), essential fatty acids, minerals, and vitamins [16, 17]. The fatty acids that it contains in abundance are linoleic (62.42%), oleic (15.90%), palmitic (10.27%), and stearic (10.26%) [18]. Seed oils are one of the sources of nutritional oil with industrial and pharmaceutical importance [19]. Interestingly, there is evidence that *Cucumeropsis mannii* contains high levels of polyunsaturated fatty acids (PUFAs) and essential amino acids, implying that the seed could be a promising nutritional supplement repository [16, 17, 18]. The therapeutic potential of PUFAs and phospholipids in maintaining erythrocyte membrane integrity, function, and regenerative capabilities [19, 20, 21, 22, 23] could be plausible in the maintenance of liver function. Therefore, the use of *Cucumeropsis mannii* seed oil (CMSO) can be invaluable in the search for better treatment for liver diseases. According to [24], the seeds of *C. mannii* are highly valued as food in Africa. Furthermore, due to their chemical composition, *C. mannii* has many important medicinal properties and can be a source of nutraceuticals whose large-scale production will be ideal for the economic growth of a country. As a result, the current study investigates the effects of CMSO on bisphenol A-induced hepatotoxicity in male Wistar albino rats.

Material and Method

Materials

Equipment and Instruments

The major instrument and equipment used for this study were Hitachi 911 Chemistry Analyzer (Roche Hitachi), Centrifuge (Pic, England), Local wooden Mortar and Pistol (Aba,Nigeria,) Grinder (Corona, Nigeria), Refrigerator (Kelvinator, Germany), pH meter (Pye, Unicam 293, England), Electronic balance (Model mp 300, USA), BS-2025T Biological Microscope (New York Microscope Company), Centrifuge machine (C-866-346-6800, Chicago corporation Hammond), spectrophotometer (UV- 1601PC, Shimadzu Europe), Fluorescence spectrometer (HITACHI, Model No F7000, equipped with a FITC filter), Elmer LS-50B luminescence fluorescence spectrophotometer (New York Microscope Company, Ultrasound centrifuge machine (Pic, England), Dounce glass homogenizer (Pic, England), Bright-Line® Hemacytometer (Pic, England), Electronic weighing balance (Pic, England), Incubator (Pic, England), Water bath (Pic, England), Micro-pipette of various sizes (Pye, Unicam 293, England), etc.

Chemicals and Reagents

The chemicals and reagents used were of good analytical grades. They include: 2-[4-(2 hydroxyethyl) piperazin-1-yl] ethanesulfonic acid (HEPES), 2'-7'dichlorofluorescein (DCF), 2'-7'dichlorofluorescin diacetate (H_2DCF-DA), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 5-thionitrobenzoic acid (TNB), 5-5'-dithiobis [2-nitrobenzoic acid] (DTNB), Benzylamine solution (Sigma), Bisphenol-A (Riedel-De HaenAGSeelze-Hannover, Germany via Bristol Scientific) (Sigma), Bovine serum albumin (BSA), Copper(II) tetraoxosulphate (VI) pentahydrate ($CuSO_4.5H_2O$) (Sigma), Cyclohexane (Hi-Media), Cytochrome C solution (SRL), Dihydrorhodamine 123 (DRH-123) (Sigma), Disulfde glutathione solution (GSSG), Ethanol, Ethyl acetate (Sigma), Ethylene diamine tetraacetic acid (EDTA, Sigma), Ethylene glycol bis(2-aminoethylethyl) tetraacetic acid (EGTA) (Sigma), Glucose solution, Glutathione peroxidase (GPx) (Sigma), Glutathione reductase (GR) (Sigma), Hydrochloric acid (HCl) (Sigma), Hydrogen peroxide solution (H_2O_2) (Sigma), Manganese superoxide dismutase (MnSOD) (Sigma), Bottle olive oil (Randolf pharmacy Ltd, Abakaliki, Ebonyi State), Thiobarbituric acid (TBA) (Sigma), Trichloroacetic acid (TCA) (Sigma), Tris buffer at pH 8.5 (SRL).

Biological Materials

The biological materials used for the study were male Wistar Albino rats and *Cucumeropsis mannii* seed oil.

Methods

Experimental Animals

The experimental animals used were Wister albino rats purchased from the Animal House of Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The rats were kept in stainless steel rats cages in a well-ventilated animal house of the Biochemistry Department, Ebonyi State University, Abakaliki. They were acclimatized for seven days under good laboratory conditions. They were also allowed free access to standard rodent chow (Vital feed®, Grand Cereals Ltd, Jos, Nigeria) and water *ad libitum.* The procedures for

experimental studies were performed consistent with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80□23, revised in 1996). The Department of Biochemistry Ethical Review Committee, Ebonyi State University, Abakaliki, Nigeria, approved the study and assigned an Approval Number (**EBSU/BCH/ET/20/002**).

Collection, Classification, and Authentication of Plant Material

The plant material used in this study was *Cucumeropsis mannii* Seed purchased from a market woman farmer in Nkwegu market, Abakaliki Local Government Area, Ebonyi state. The *Cucumeropsis mannii* specimen was classified with Voucher **No: 42485/HNC** and deposited at the Cameroon National Herbarium in Yaounde [24]. However, it was authenticated by Mr. O. E. Nwankwo, a plant taxonomist in the Department of Applied Biology of Ebonyi State University, Abakaliki, Nigeria.

Extraction of *Cucumeropsis mannii* seed oil (CMSO)

The *Cucumeropsis mannii* seeds were extracted using the methods of [25], with modifications. The CMSO was peeled and then ground using a manual grinder (Corona). The oil from ground seeds was extracted locally by mechanical press using mortar and pestle. During extraction, warm water was added in drops at intervals to enhance the release of the oil since water will help to rupture the cells. The extracted oil was left to stand undisturbed to sediment in a corked bottle for 5-7 days before separation by decantation method to obtain a pure form of the oil which was stored in a separate bottle.

Acute Toxicity of CMSO

According to OECD/OCDE Guidelines no. 425, the acute toxicity study was carried out using the limit dose up and down method. Male albino Wistar rats (aged 2 months) were used in the experiment, and they were acclimatized to the laboratory condition for seven days before starting. A male rat was given 5 ml/kg of CMSO orally after an overnight fast. Following CMSO administration, the animal was closely monitored for the first 30 minutes for physical or behavioral changes, then for the next 24 h, and then every day for the next 14 days. Food was given after 3 h of CMSO administration. Since the first rat survived, four more male rats were recruited and fasted for 4 h. They were then given the same dosage of CMSO and subjected to the same stringent monitoring for the next 14 days for any signs of toxicity [26, 27, 28, 29]. Within the 24 h and 14-day testing periods, the rats did not exhibit any signs of gross physical or behavioral modifications such as hair erection, decrease in eating, or motor movements at the limit-test dose of 5 ml/kg. For this reason, based on OECD guideline No 425, 10 % of the limit dose (5 ml/kg) was chosen as the middle/intermediate dose, half of it (2.5 ml/kg) as the lower dose, and 1.5 times the middle dose (7.5 ml/kg) as the higher dose [29].

Quantitation of flavonoids profile using high-performance liquid chromatography (HPLC)

Cucumeropsis mannii seed oil sample was used for flavonoids profile assay [30]. High-performance liquid chromatography (HPLC) analysis for quantitation of the individual flavonoids was carried out on Waters 616/626 HPLC. The sample was separated isocratically on a reversed-phase LC 18 column at 5 mM (25 cm x 4.6 mm) (Supelco Inc. USA). The mobile phase consisted of 40 nm sodium phosphate dibasic heptahydrate and 20%

acetonitrile (v/v), (pH 6.5, adjusted with 85% phosphoric acid). The mobile phase was filtered through a 0.22 mm pore membrane filter and degassed before use. The column was equilibrated at 25^{o}C at a flow rate of 0.85 mL/min. The injection volume was 25 mL. Standard solutions of the various flavonoid compounds were also prepared. Calibration standard samples were prepared from stock. Concentrations of the flavonoid compounds in the samples were determined by the Waters HPLC application pack [30, 28].

Experimental Design

A total of forty-eight (48) male albino Wistar rats were randomly assigned into six (6) experimental groups of A, B, C, D, E, and F with eight (8) rats in each group. Groups A, B, and C are control groups while groups D, E, and F are the treatment groups. BPA in the formed pellet was dissolved into a solution of 5 g/100 ml olive oil.

Group A: Normal control received 1 ml of olive oil

Group B: Negative control (BPA intoxicated group) received 100 mg/Kg body weight

Group C: Positive control (CMSO control) received 7.5 ml/kg body weight

Group D: Treatment group 1 pre-administered 100 mg/kg body weight of BPA and treated with 7.5 ml/kg body weight of CMSO.

Group E: Treatment group 2 pre-administered 100 mg/kg body weight of BPA and treated with 5 ml/kg body weight of CMSO.

Group F: Treatment group 3 pre-administered 100 mg/kg body weight of BPA and treated with 2.5 ml/kg body weight of CMSO.

Administration of both BPA and CMSO were concurrently by oral intubation once every day and the weights of animals across the group were also measured every seven (7) days for six weeks.

Tissue Sample Collection

After the trial, the animals were sacrificed by cervical dislocation under mild anesthesia. Blood samples were collected via the femoral vein's cut. The liver of each member of the group was collected and placed in specimen bottles stored in ice and transferred to the laboratory within 3-6 h after the sacrifice for analysis.

Determination of Biochemical Parameters

Determination of liver enzyme activities in the rat liver

ALP activity was assayed by colorimetric methods as described by [31]. The activity of ALT was assayed according to the method of [32].

Principle: The principle of this test was based on the hydrolysis of p-nitrophenyl phosphate to yield a phosphate and p-nitrophenol catalyzed by ALP.

P-nitrophenyl phosphate + H_2O $\xrightarrow{ALP}$ phosphate + p-nitrophenol

Procedures: Exactly 0.01 ml and 0.5 ml of the sample and reagents respectively were added to the micro-cuvette. The mixture was properly mixed and the initial absorbance was read again after 1, 2, and 3 minutes to get the changes in absorbance.

Calculation: ALP (u/L) = 2760 x ΔA nm/minutes.

Determination of Total Bilirubin Concentration

Total bilirubin concentration was determined according to the method described by [33].

Principle: The principle was based on the colorimetric method whereby total bilirubin is determined in the presence of caffeine, which releases albumin-bound bilirubin by the reaction with diazotized sulphanilic acid.

Procedure: Two cuvettes were prepared and arranged, the sample blank and the sample cuvette. Exactly 4 drops (200 µl) of reagent 1 were added to the two cuvettes followed by the addition of 1 drop (50 µl) of reagent two only to the sample cuvette. After that, 1000 µl and 200 µl of reagent three and sample (serum) respectively were added to the two cuvettes. The cuvette was properly mixed and incubated for 10 minutes at 20-25^{0}C. Finally, 1000 µl of reagent four was added to both sample blank and sample cuvette, mixed, and incubated for a further 5–30 minutes at 25^{0}C and the absorbance of the sample against sample blank was read at the wavelength of 578 nm.

Calculation

Total Bilirubin (mg/dl) = 10.8 x A_{TB}

Determination of oxidative stress markers levels in the rat liver

The ROS level in the liver homogenate was determined using Dichlorohydrofluorescein Diacetate (DCFDA) which was converted to DCF fluorescence by cell peroxides. Fluorescence levels were measured using a fluorimeter [34, 35]. Reduced glutathione (GSH) level in the liver homogenate was quantified by the method of [35]. Lipid peroxidation in the liver homogenate was determined by measuring thiobarbituric acid reactive substances (TBARS) expressed in terms of malondialdehyde (MDA) content using the method of [36]. Catalase (CAT) (EC. 1.11.1.6) activity in the liver homogenate was assayed by monitoring the decomposition of H_2O_2 at 240 nm as described by [37, 38]. Superoxide dismutase (SOD) (EC. 1.15.1.1) in the liver homogenate was determined by the method of [38]. The activity of glutathione peroxidase (GPx) (EC. 1.11.1.9) in the liver homogenate was determined using the method of [39].

Determination of inflammatory mediator levels in rat liver

Liver interleukin-1 (IL-1), interleukin-6 (IL-6) (ERA31RB), tumor necrosis factor-α (TNF-α) (SL0722Ra), and nuclear factor-kappa B (NF-κB) (SL0537Ra) levels were determined using enzyme-linked immunosorbent assay (ELISA) kits (rat IL-6; NF-κB ELISA rat kit; rat TNF-α ELISA kit) purchased from Sunlong Biotechnology, Shanghai, China and EBIOSCIENCE, Inc., San Diego, California, USA following the manufacturer's protocols. In summary, the control standards and liver homogenate supernatant samples were pipetted into pre-coated wells with primary antibodies specific for rat cytokines and NF-κB. The immobilized primary antibody was allowed to react with the respective sample protein. Thereafter, wells were washed to remove the unbound substance. After washing, the enzyme-linked polyclonal

antibody was added to each well, followed by washing to remove any unbound antibody-enzyme reagent. The wells were then filled with a substrate solution for the linked enzyme to allow color development. Following that, the levels of cytokine and NF-κB were calculated using the color intensity determined in an ELISA plate reader (BRIO POMEZIA, Rome, Italy) by spectrophotometry.

Determination of Caspase-3 activity level in rat liver

The level of Caspase-3 activity in liver tissue lysate was determined using rats' assay kits following the manufacturer's protocols.

Histopathological examination of rat liver

The liver was placed in the Bouin's fluid for histopathological examination according to the methods of [35]. The liver was immediately removed and stored in 10% neutral buffered formalin for 48 hours before being dehydrated in ethanol and embedded in paraffin for a blind histopathological examination. The tissue was sectioned longitudinally with a microtome and stained for microscopic histopathological alterations with hematoxylin and eosin (H and E). A light microscope was used to examine the stained slides.

Statistical Analysis

Results were expressed as means ± standard deviation (SD). Data were analyzed using a one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison post hoc test using SPSS statistical software, version 23.0 (SPSS Inc., Chicago, Illinois, USA). Differences at $p < 0.05$ were considered significant. Graphs were created using GraphPad Prism 5 software (GraphPad Software, California, USA).

Effect of CMSO on Plasma Liver Function Parameters in BPA–induced hepatotoxicity in rats

Results showed that the activities of ALP, ALT, AST, levels of total bilirubin, and direct bilirubin were significantly ($p < 0.05$) increased in the blood after BPA administration in rats with a reduction in the level of albumin (Figure 1). However, a significant ($p < 0.05$) reduction in the activities of ALP, ALT, AST, levels of total bilirubin, and direct bilirubin were observed in rats that were co-administered BPA and CMSO with a significant ($p < 0.05$) elevation in albumin level as shown in Figure 1.

Figure 1(a-f): Effect of CMSO on Liver function parameters in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Effect of CMSO on oxidative stress markers in Liver in BPA–induced hepatotoxicity in rats

BPA administration in rats significantly ($p < 0.05$) elevated the levels of ROS and MDA in the liver homogenates as shown in Figures 2 (e and f). Besides, BPA administration significantly ($P < 0.05$) decreased activities of CAT, SOD, GPx, and level of GSH in liver homogenates in rats Figure 2 (b-d). However, the levels of ROS and MDA in liver homogenate were significantly ($P < 0.05$) lowered when BPA and CMSO were co-administered to the rats as shown in Figures 2 (e and f). Also, there was a significant ($p <$

0.05) increase in the level of GSH with co-administration of BPA and CMSO Figure 2(a). Activities of CAT, SOD, and GPx were significantly ($p < 0.05$) elevated when BPA and CMSO were co-administered in rats as shown in Figure 2 (c and d). No significant difference ($p < 0.05$) was observed among the co-administered groups.

Figure 2(a-f): Effect of CMSO on Oxidative stress markers in BPA-induced hepatotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil)

Effect of CMSO on inflammatory mediators' levels such as interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), nuclear factor-kappa B (NF-κB), and caspase-3 activity in Liver in BPA–induced Hepato-toxicity in rats

BPA administration in rats significantly ($p < 0.05$) up-regulated the liver expression of nuclear factor-kappa B (NF-κB) and caspase-3 activity along with increased levels of interleukin-1β (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-α (TNF-α) but the co-administration of BPA+CSMO in rats significantly ($p < 0.05$) lowered the levels of these inflammatory markers and activity of apoptotic marker in rat's liver (Figure 3).

Figure 3 (a-e): Effect of CMSO on inflammatory markers and Caspase- 3 in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Effect of CMSO on Liver glycogen level and Bodyweight in BPA–induced hepatotoxicity in rats

BPA administration in rats significantly ($p < 0.05$) decreased liver glycogen storage but the co-administration of BPA+CSMO in rats significantly ($p < 0.05$) increased the level of glycogen in rat liver (Figure 4a). BPA administration in rats significantly ($p < 0.05$) decreased the weights of the rats (Figure 4b). The rats' weights significantly ($p < 0.05$) increased after co-administration of BPA + CMSO in the treatment groups (Figure 4b).

Figure 4 (a & b): Effect of CMSO on glycogen level and Bodyweight in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Effect of CMSO on Histology of the liver in BPA–induced hepatotoxicity in rats

Photomicrograph of the liver induced with BPA (x400) (H/E) shows marked degeneration with severe intrahepatic inflammation (HI) the overall features are consistent with (chronic hepatitis) (Plates 1-6).

Plate 1: Photomicrograph of Group 1 section of the liver (x400) (H/E) shows well perfuse normal hepatic architecture with the central vein (CV) and well-outlined hepatocyte(H).

Plate 2: Photomicrograph of Group 2 section of liver induced with BPA only (x400) (H/E) shows severe degeneration with severe intrahepatic inflammation (HI) the overall features are consistent with (chronic hepatitis).

Plate 3: Photomicrograph of Group 3 section of liver administered with 7.5 ml/kg b.w of CMSO only (x400) (H/E) shows mild congestion of the central vein (CCV) otherwise normal.

Plate 4: Photomicrograph of Group 4 section of liver induced with BPA and treated with7.5ml/kg of CMSO (x400) (H/E) shows moderate regeneration with mild fatty change (FC) and mild intrahepatic infiltration of the inflammatory cell (IIC).

Plate 5: Photomicrograph of Group 5 section of liver induced with BPA and treated with 5.0 ml/kg of CMSO (x400) (H/E) shows mild to moderate regeneration with a mild focal area of intrahepatic hemorrhage (IHH) and moderate focal aggregate inflammatory cell (FAIC).

Plate 6: Photomicrograph of Group 6 section of liver induced with BPA and treated with 2.5 ml/kg of CMSO (x400) (H/E) shows mild regeneration with moderate focal aggregate Intra (FAI) and portal inflammatory cell (AIPC).

Discussion

In the current investigation, the flavonoid profile of *Cucumeropsis mannii* seed oil revealed an intriguing result (Table 1) showing a myriad of nutraceuticals with strong antioxidant effects perhaps attributed to the mitigations of the BPA-induced hepatotoxicity. Earlier studies on the impact of bisphenol A (BPA) on human health concluded that the toxic effects of BPA may be due to increased oxidative stress [41,42]. We established BPA-induced hepatotoxicity in a rat model by administering 100 mg/kg body weight of BPA for 42 days. The presence of liver damage or disease was determined using liver function tests such as ALT, AST, ALP, bilirubin, and albumin. BPA causes oxidative stress and lipid peroxidation by increasing hepatic damage and disrupting the integrity of cellular membranes, which leads to cytoplasmic liver enzyme leakage [43]. According to the current study, BPA significantly raised the levels of ALT and AST, ALP, plasma total bilirubin, and plasma direct bilirubin. These results are congruent with the previous results by [44] that showed BPA treatment increased the activities of ALT, AST, and ALP and caused significant defects in liver morphology. Additionally, patients with liver disease have higher BPA levels than healthy people [45] This finding raises the possibility that BPA exposure and liver health are related. The findings of the histopathological analysis conducted in the current study corroborate those of the biochemical tests (Plates 1-6). BPA treatment significantly altered the architecture of the liver in comparison to control livers, causing cellular infiltration, the development of large cytoplasmic vacuoles and hepatic sinusoids, as well as an increase in Kupffer cells. Hepatic lipid accumulation and oxidative stress, followed by liver injury and inflammation, are pathogenic events for non-alcoholic steatohepatitis [46]. Interestingly, our research showed that administering CMSO to rats has strong hepatoprotective effects against BPA-induced liver damage. Furthermore, CMSO improved the secretory function and structural integrity of liver cells. The serum ALT and AST activities markedly decreased and prominently decreased the total bilirubin level, producing results lower than the control and BPA-treated groups (Figure 1). These results are consistent with those emphasized by [46], who demonstrated that sesamin effectively reduced serum ALT and AST and alleviated hepatic histological changes. The hepatoprotective effects of CMSO may be due to its anti-inflammatory activities. *C. manni* seed is a known inhibitor of arachidonic acid synthesis [18]. It reduces oxidative stress, pro-inflammation, and apoptosis [47, 48, 49, 50, 51]. CMSO has general anti-inflammatory effects, which are demonstrated by its capacity to lower

inflammatory mediators. The phenolic compounds in CMSO (Table 1), which contribute to its hepatoprotective potential, may be responsible for the antioxidative effect of CMSO and its effects on lowering lipid peroxidation. Thus, the pathogenic events linked to BPA-induced steatohepatitis may decline in tandem with the decrease in hepatic lipid accumulation.

In the current study, BPA caused hepatic oxidative stress and decreased the secretory function and integrity of the liver, as indicated by increased hepatic malondialdehyde (MDA) level and decreased antioxidant system. BPA significantly reduced glutathione, glutathione peroxidase (GPx), glutathione reductase (GR), and superoxide dismutase (SOD) activities. These findings are in line with those made by [52], who found that BPA exposure damages human red blood cells by inducing oxidative stress. Another previous study found that BPA increased lipid peroxidation and decreased the activity of antioxidant defense enzymes produced in rat livers [53]. The reduction in glutathione (GSH) levels caused by BPA could be attributed to its conjugation with BPA toxic metabolites and oxidation to oxidized glutathione [54]. According to [55], some lipid peroxidation end products, MDA and 4-hydroxynonenal, can alter the mitochondrial enzyme activity and deplete the glutathione pool. Reduced GSH levels may lead to decreased GPx activity [56]. Reduced GPx activity is associated with an increase in hepatic hydrogen peroxide (H_2O_2) as well as the direct inhibition of SOD activity [57]. BPA reacts with oxygen radicals, decomposing them into several reactive metabolites with high oxidant activity [58]. These metabolites increase reactive oxygen species (ROS) production. They also increase H_2O_2 levels and reactive thiobarbituric acid substances, which inhibit antioxidative enzyme activity [58]. The ROS caused by BPA may accelerate peptide chain cleavage and amino acid cross-linking in enzymes, resulting in a change or loss of enzyme activity [59]. Thus, the mechanism of BPA-induced oxidative damage may be primarily caused by the inhibition of the antioxidant enzyme system, leading to an increase in ROS content.

In contrast, the current study indicated that CMSO attenuated BPA-induced oxidative stress by decreasing MDA, increasing the specific activities of GPx, CAT, and SOD, and increasing the level of GSH in the livers of the treated rats. Similarly, the present study also revealed that CMSO attenuated measured parameters to a level similar to the control (Figure 2). This observation may be due to its free radical scavenging properties. Our findings are consistent with those of [60], who discovered that sesame lignans increased GPx and GR activity while decreasing MDA levels in rats treated with LPS. In addition, [61-62] reported that sesamin decreased ROS and MDA production in the liver extract of CCL4-treated mice. The authors concluded that the augmented SOD activity induced by sesame lignans enhances the ability of hepatic cells to decompose superoxide anions produced by BPA into H_2O_2, preventing the further generation of free radicals. Subsequently, GPx broke down H_2O_2, which uses GSH as a reducing agent. Therefore, the increased GPx activity and GSH levels observed in the present study show that CMSO protects liver tissue against oxidative damage.

Robust evidence confirms the contribution of oxidative stress to inflammation, a biochemical response of the body towards cell injury [62]. In the current study, BPA administration increased the levels of inflammatory markers such as interleukin-1 (IL-1), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), and nuclear factor-kappa B (NF-B). However, co-administration of BPA with CMSO decreased the levels of these inflammatory markers except for interleukin-6 (Figure 3). These findings are consistent with reports in the literature indicating a link between oxidative stress, proinflammation, and apoptosis [63, 64, 65, 66, 67, 68]. Ordinarily, nuclear factor-kappa κB (NF-κB) is a redox-sensitive transcription factor; its expression is inducible in all cells and regulates many genes involved in inflammatory

responses (Nna *et al*., 2017). BPA-induced oxidative stress may have triggered the expression of the inhibitor of kappa B kinase (IKK) for degradation of the inhibitor of kappa B (IκB) to increase the DNA-binding affinity of NF-κB. This observation may be because NF-κB was activated for translocation from the cytoplasm to the nucleus to regulate the expression of multiple target genes, such as TNF-α, IL-2β, and IL-6β [69]. Moreover, triggering the activation of the NF-κB signaling pathway plays a critical role in several liver diseases, including hepatitis, liver fibrosis, cirrhosis, and hepatocellular carcinoma [70]. Consequently, NF-κB is activated for translocation from the cytoplasm to the nucleus to regulate the expression of multiple target genes, such as TNF-α, IL-2β, and IL-6β [71]. This result has justified studies that hypothesized that inhibition of the NF-κB signaling pathway could block excess IL-6β, TNF-α, IL-1β, and NO level [72, 73, 74, 75, 76].

Caspases are crucial mediators of programmed cell death (apoptosis). Among them, caspase-3 is a frequently activated death protease, catalyzing the specific cleavage of many cellular proteins. In the present study, BPA administration in rats increased the level of caspase-3 activity, but co-administration of BPA with CMSO lowered the level of caspase-3 expression in a dose-dependent manner (Figure 4a). However, BPA activation of caspase-3 was due to oxidative stress and cytokine (IL-6β, TNF-α, and IL-1β) overexpression. CMSO administration prevented oxidative stress and activation of NF-κB, which suppressed the expression of pro-inflammatory cytokines. Hence, CMSO reduced the expression of caspase-3 in hepatocytes. Our findings correlate with previous studies, which suggest that BPA induces apoptosis via the elevation of caspase-3 in mice [77] and goat testis Sertoli cells [78]. Similarly, the present study is consistent with [72], which reported that fenugreek (Trigonella foenum-graecum) modulated caspase-3 on bisphenol-A induced testicular damage in mice. Thus, CMSO exhibits antioxidant, anti-inflammatory, and antiapoptotic properties in rats by suppressing NF-B/IL-2/IL-6/TNF-α and caspase-3.

Glycogen is a branched polymer of glucose, primarily stored in the liver and skeletal muscle, which provides glucose to the bloodstream during fasting periods, as well as to muscle cells during muscle contraction [70]. BPA modulates glucose utilization in muscles and interferes with liver tissue function. In addition to peripheral tissues, previous research has shown that BPA affects neuroendocrine regulation of glucose metabolism via the Central Nervous System, promoting glucose metabolism dysfunction such as glucose intolerance and insulin resistance. As a result, BPA exposure appears to be a significant risk factor for obesity and metabolic syndrome [60]. In the present study, BPA administration in rats decreased liver glycogen storage, but CMSO treatment in rats increased glycogen levels in the liver of rats (Figure 4a). Our result is consistent with a study by [61] that reported the effects of Bauhinia forficata on glycemia, lipid profile, hepatic glycogen content, and oxidative stress in rats exposed to Bisphenol-A. Evidence suggests that BPA exposure perturbs insulin signaling and glucose transport in the brain; therefore, it might be a risk factor for brain insulin resistance [62].

Surprisingly, BPA administration also decreased the body weight of the rats in the present study. In parallel, the body weight of the rats increased after co-administration of BPA and CMSO in the treated groups (Figure 4b). Findings in the current study correlated with previous reports on the effects of phytocompounds from natural extracts and seed oil on body weight in BPA-induced testicular toxicity. Naringin (a flavonoid) [63], *Cordyceps militaris* [64], *Eruca Sativa* [65], *Trigonella foenum-graecum* [66], Lycopene (carotenoid) [67], Lespedeza cuneata [66], and Aloe vera [66] all improved the body weight in vivo and in vitro. However, in vivo studies have shown that fetal exposure to BPA at levels equal to or

lower than the established daily human safe dose (50 µg BPA/kg BW/day) increases body weight and postnatal growth [67]. In another study, pregnant rodents given 10 mg/L of BPA orally had tissue levels of 10–25 ng/g of BPA, which is comparable to human samples. Rats exposed to BPA caused offspring obesity, adipocyte hypertrophy, and increased adipogenic and lipogenic factors [68]. In vitro incubation of pre-adipocytes from rats with BPA increased their number, adipogenic transcription factors, and TNF-α [69]. In a similar study, rats exposed to 0.5 µg/kg BW/day of BPA orally from gestational day 3.5 to postnatal day 22, an amount 8–10 times lower than the European Food and Safety Authority's daily tolerable dose, had higher plasma triglyceride concentrations and inguinal WAT adipocyte density in offspring [70]. Therefore, the decrease in body weight after BPA intoxication in the current study could be a feedback response to tissue proliferation via mechanistic elevation of apoptotic factors such as inflammatory markers and caspase-3. Important molecules, such as proteins in rats, were protected from damage by CMSO. Hence, CMSO improved the body weight of rats by suppressing pro-inflammatory cytokines through antioxidant activities and the regenerative potentials of the essential lipids present.

CONCLUSIONS

The present study demonstrates the ability of *Cucumeropsis mannii* seed oil (CMSO) to protect rats from BPA-induced hepatotoxicity. Our findings support previous reports on BPA-induced oxidative stress and inflammation. However, we demonstrate for the first time that CMSO's antioxidant potential can help to mitigate liver damage by lowering the production of inflammatory mediators. Therefore, CMSO could be useful in the clinical management of BPA-induced hepatotoxicity in patients with liver diseases.

List of Abbreviations

BPA: Bisphenol A

*CMSO***:** *Cucumeropsis mannii* seed oil

OECD: Organization for Economic Co-operation and Development

HPLC: High-performance liquid chromatography

ALT: Alanine transaminase

ALP: Alkaline phosphatase

DCFDA: Dichlorodihydrofluorescein diacetate

GSH: Reduced glutathione

TBARS: Thiobarbituric acid reactive substance

MDA: Malondialdehyde

CAT: Catalase

SOD: Superoxide dismutase

IL: Interleukin

TNF: Tumor necrosis factor

ELISA: Enzyme-linked immunosorbent assay

NF: Nuclear factor

ANOVA: Analysis of variance

SD: Standard deviation

AST: Aspartate aminotransferase

ROS: Reactive oxygen species

GPx: Glutathione peroxidase

GR: Glutathione reductase

LPS: Lipopolysaccharide

PUFAs: Polyunsaturated fatty acids

Availability of data and material

The datasets used and/or analyzed during the current study are available on request.

Competing interests

The authors declare that there is no conflict of interest in reports in this study.

Funding

This research received no form of external funding

Acknowledgments

We appreciate the management of the Department of Biochemistry Institutional Research Ethics Committee, Ebonyi State University, Abakaliki, Nigeria.

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Figure 1(a-f): Effect of CMSO on Liver function parameters in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Figure 2(a-f): Effect of CMSO on Oxidative stress markers in BPA-induced hepatotoxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil)

Figure 3 (a-e): Effect of CMSO on inflammatory markers and Caspase- 3 in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Figure 4 (a & b): Effect of CMSO on glycogen level and Bodyweight in BPA-induced hepato-toxicity in albino rats. Data are shown as mean ± S.D (n = 6). Mean values with the different signs are significantly different at $p < 0.05$. BPA (Bisphenol A), CMSO (*Cucumeropsis mannii* Seed Oil).

Plate 1: Photomicrograph of Group 1 section of the liver (x400) (H/E) shows well perfuse normal hepatic architecture with the central vein (CV) and well-outlined hepatocyte(H).

Plate 2: Photomicrograph of Group 2 section of liver induced with BPA only (x400) (H/E) shows severe degeneration with severe intrahepatic inflammation (HI) the overall features are consistent with (chronic hepatitis).

Plate 3: Photomicrograph of Group 3 section of liver administered with 7.5 ml/kg b.w of CMSO only (x400) (H/E) shows mild congestion of the central vein (CCV) otherwise normal.

Plate 4: Photomicrograph of Group 4 section of liver induced with BPA and treated with7.5ml/kg of CMSO (x400) (H/E) shows moderate regeneration with mild fatty change (FC) and mild intrahepatic infiltration of the inflammatory cell (IIC).

Plate 5: Photomicrograph of Group 5 section of liver induced with BPA and treated with 5.0 ml/kg of CMSO (x400) (H/E) shows mild to moderate regeneration with a mild focal area of intrahepatic hemorrhage (IHH) and moderate focal aggregate inflammatory cell (FAIC).

Plate 6: Photomicrograph of Group 6 section of liver induced with BPA and treated with 2.5 ml/kg of CMSO (x400) (H/E) shows mild regeneration with moderate focal aggregate Intra (FAI) and portal inflammatory cell (AIPC).

Effect of CMSO on Plasma Liver Function Parameters in BPA–induced hepatotoxicity in rats

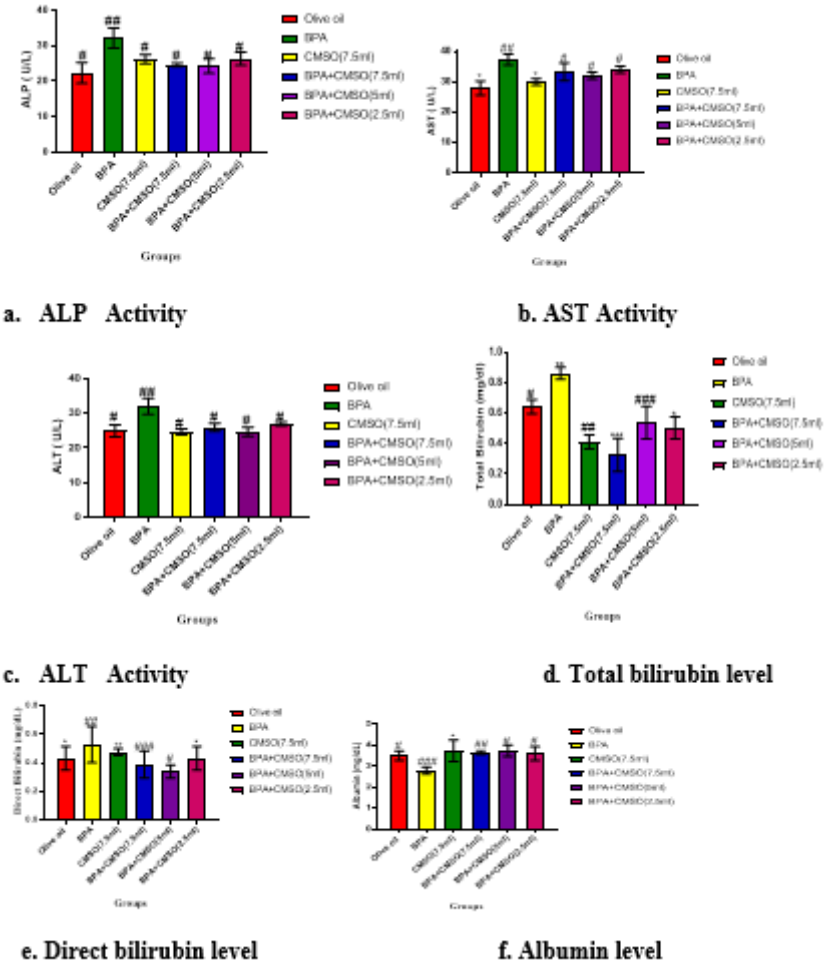
Results showed that the activities of ALP, ALT, AST, levels of total bilirubin, and direct bilirubin were significantly ($p < 0.05$) increased in the blood after BPA administration in rats with a reduction in the level of albumin (Figure 1). However, a significant ($p < 0.05$) reduction in the activities of ALP, ALT, AST, levels of total bilirubin, and direct bilirubin were observed in rats that were co-administered BPA and CMSO with a significant ($p < 0.05$) elevation in albumin level as shown in Figure 1.

Table 1: Flavonoids Profile of CMSO (*Cucumeropsis mannii* Seed Oil)

S/N	Flavonoids	Concentration
1	Hesperidin	0.0299 ± 0.01
2	Nanirutin	0.4975 ± 0.24
3	Neoriocitin	0.3235 ± 0.12
4	Poncirin	1.5138 ± 0.72
5	Didymin	0.0886 ± 0.04
6	Eriocitrin	0.0357 ± 0.02
7	Rhoifolin	0.1480 ± 0.07
8	Diosmin	0.0643 ± 0.03
9	Nobiletin	1.4255 ± 0.64
10	Acacetin	0.1772 ± 0.08
11	Raxifolin	0.0160 ± 0.07
12	Sinerisetrin	0.0355 ± 0.02
13	Tangeretin	0.5906 ± 0.27
14	Neodiosmin	0.0462 ± 0.03
15	Naringin	0.5885 ± 0.42
16	Naringinenin	0.0329 ± 0.02
17	Quercetin	0.0133 ± 0.01
18	Eriodictyol	0.0553 ± 0.04
19	Myricetin	0.0239 ± 0.02
20	Kaempferol	0.5314 ± 0.38
21	Apigenin	0.0660 ± 0.01
22	Isorharmnetic	0.0060 ± 0.00
23	Luteolin	0.0132 ± 0.01
24	Daidzein	0.2202 ± 0.16
25	Genistein	0.1881 ± 0.20
26	Glycitein	2.3906 ± 2.50
27	Anthocyanine	0.1343 ± 0.14
28	Catechin	0.0538 ± 0.06
29	Epicatechin	0.2241 ± 0.23
30	Thearflarins	0.0973 ± 0.01
31	Thearubigins	2.1581 ± 2.26
32	Epigallocatechin	0.2681 ± 0.28
33	Epicatechin gallate	0.0243 ± 0.03
34	Epigallocatechin gallate	0.0535 ± 0.06
35	Proanthocyanidins	0.8944 ± 0.94

36	Hesperetin	0.3335 ± 0.20
37	Rhamnazin	4.239 ± 2.50
38	Taxifolin	0.2378 ± 0.06
39	Fisetin	0.0955 ± 0.06
40	Total Flavonoids	17.8006 ± 10.95

Figure 1



a. ALP Activity

b. AST Activity

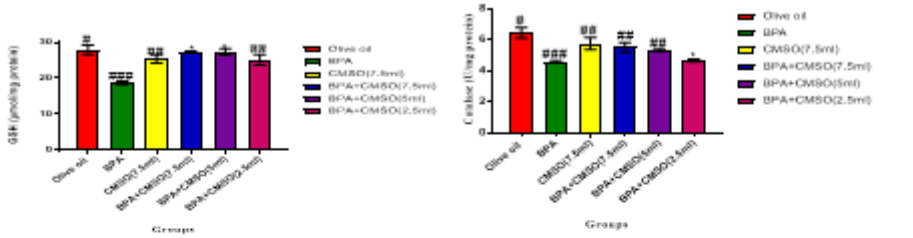
c. ALT Activity

d. Total bilirubin level

e. Direct bilirubin level

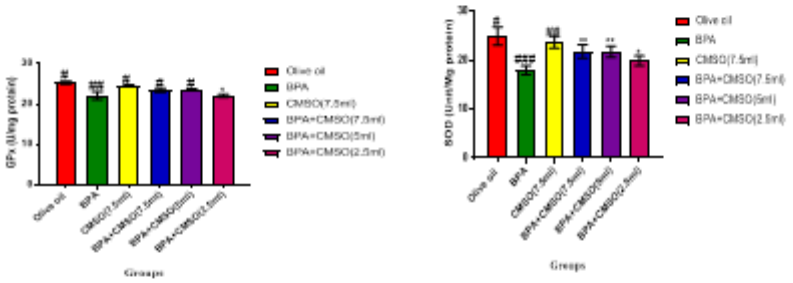
f. Albumin level

Figure 2



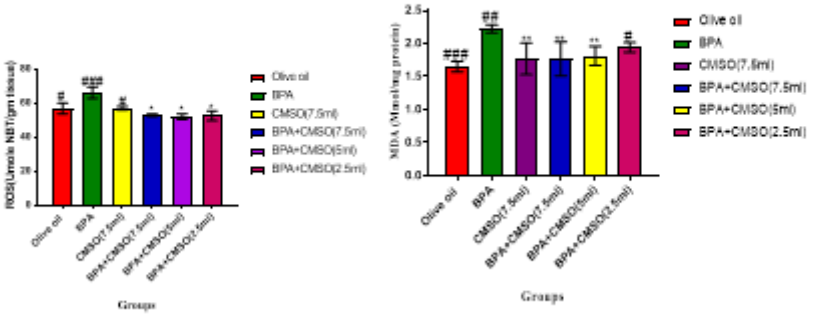
a. GSH level

b. Catalase Activity



b. GPx Activity

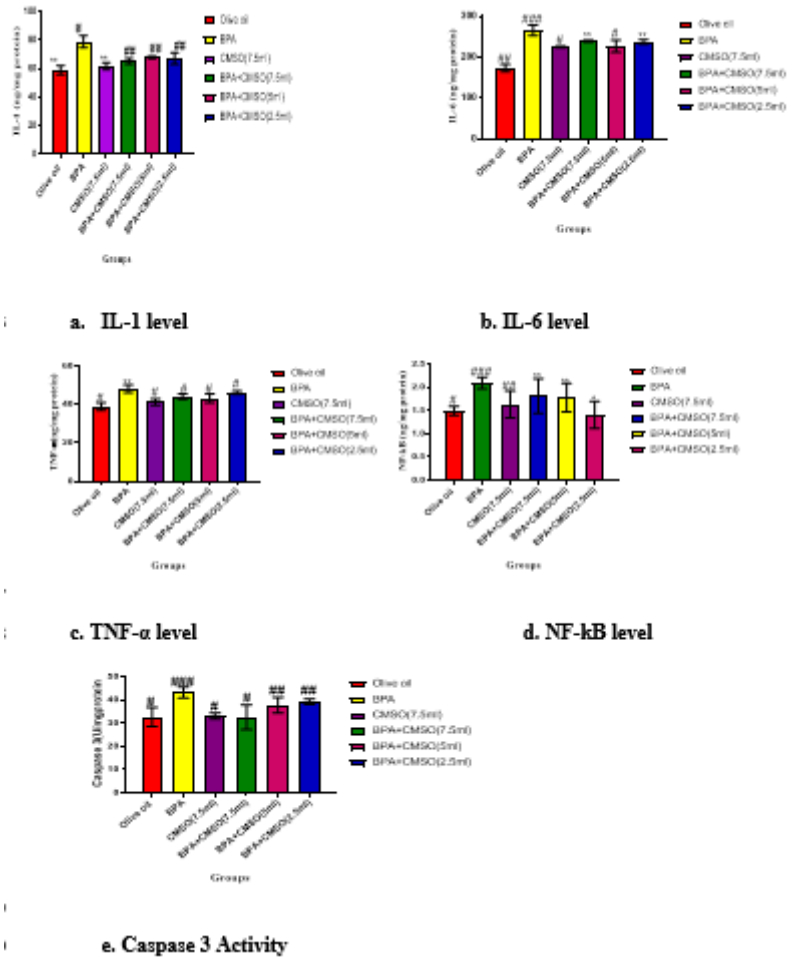
d. SOD Activity



e. ROS level

f. MDA level

Figure 3



a. IL-1 level

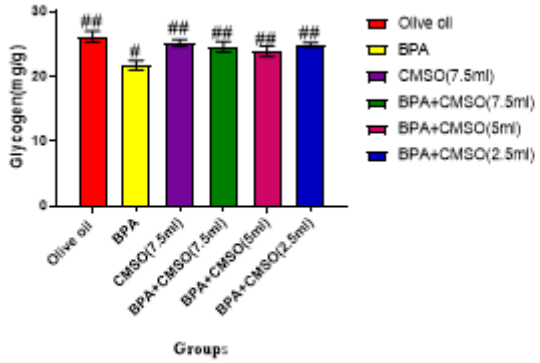
b. IL-6 level

c. TNF-α level

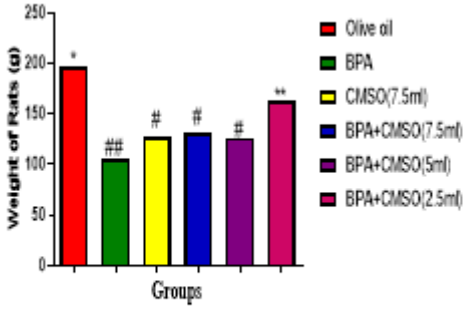
d. NF-kB level

e. Caspase 3 Activity

Figure 4



a. Glycogen level



b. Bodyweight of the rats