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ROLE OF PUMPKIN SEED OIL ON SOME CARDIOVASCULAR AND RENAL ASPECTS IN ADULT MALE ALBINO RATS

By

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ABSTRACT

Background: Obesity, a sort of dietary imbalance, is one of the most frequently encountered medical problems associated with many complications as hypertension, hyperlipidemia, and seems to make morphological alterations of kidney too. Pumpkin seeds oil (PSO) is rich in unsaturated fatty acids, antioxidants and fibers. **Objective:** Evaluation of the efficiency of using PSO on regulation of arterial blood pressure, cardiac and renal health in high fat diet (HFD)-treated rats. **Materials and methods:** Thirty adult male albino rats were divided into three equal groups: Group I (control group), Group II (HFD-treated group), Group III (HFD & PSO 100 mg/kg-treated group). At the end of the experiment body weight, mean arterial blood pressure (MABP) and electrocardiogram (ECG) were determined for all groups. These were beside measuring serum levels of lipid profile (TC, TG, LDL and HDL), urea, creatinine, nitric oxide (NO), malondialdehyde (MDA), reduced glutathion (GSH), interleukin-6 (IL-6), tumor necrosis factor alpha (TNF-α) and vascular cell adhesion molecule 1 (VCAM1). The effect of different doses of PSO (75-1200 ?g/ml) on norepinephrin-induced contractions of isolated rabbit aortic spiral strips was recorded also. Kidney samples were taken and processed for light and electron microscopic examination too. **Results:** PSO significantly decreased body weight, blood pressure, heart rate and kidney function tests in HFD rats. Serum cholesterol, triglycerides and LDL significantly decreased, while HDL significantly increased. Furthermore, PSO suppressed the increase in MDA, IL-6, TNF-α and VCAM1 levels and elevated GSH level significantly. Histological examination revealed that PSO improved the kidney structure as it decreased the extent of renal tissue damage and interstitial fibrosis in HFD rats. Also it decreased the norepinephrin-induced contraction in aortic strip of rabbit. **Conclusion:** PSO possessed anti-atherogenic action by its ameliorative effect on the lipid profile, kidney functions, inflammatory markers, kidney structures and its effect on aortic strip. Thus, Pumpkin may be a novel strategy for prevention and treatment of atherosclerosis and hypertension.

Key words: Pumpkin, High fat diet, Isolated Aortic Spiral Strips, Arterial blood pressure, kidney.

INTRODUCTION

There was an evidence suggesting that obesity increases the risk of chronic kidney diseases (CKD) by four folds and accounts for 25% of all chronic renal failure patients ***(Reddy and Natarajan, 2015).*** Hyperlipidemia induces changes in renal lipid metabolism due to an imbalance between lipogenesis and lipolysis in the kidneys, and subsequent renal lipid accumulation and renal injury ***(Kume et al., 2008).*** The glomerular and tubulointerstitial lesions associated with chronic glomerulopathy ***(Pinhal et al., 2013),*** nephrotic syndrome ***(Kim and Vaziri, 2009),*** chronic renal failure ***(Kim et al.,***

2009), diabetic nephropathy, obesity-associated renal disease and aging nephrosclerosis ***(Ishigaki et al., 2007).*** Excess body weight gain may raise blood pressure which increases renal blood flow and increases the glomerular size ***(Decl`eves and Sharma, 2015)***.

Essential fatty acids (EFAs) have been considered as functional food and nutraceuticals. A lot of research studies have documented their significant roles in many biochemical pathways resulting in cardioprotective effect because of their considerable antiatherogenic, antithrombotic, anti-inflammatory, antiarrhytmic, hypolipidemic effects. Beside complex influence on concentrations of lipoproteins, fluidity of biological membranes, function of membraned enzymes and receptors, modulation of eicosanoids production, blood pressure regulation, and finally, on the metabolism of minerals ***(Mi?urcov? et al., 2011 and Mobraten et al., 2013).***

Pumpkin plant of the Cucurbitaceae family is a native of Asia. However, it is now grown extensively in many of temperate and warm climates of the world ***(Al-Okabi et al., 2014).*** It has been considered as beneficial to health because it contains various biologically active components such as polysaccharides, para-aminobenzoic acid, fixed oils, sterols, proteins and peptides ***(Abuelgassim and Al-showayman, 2012)***. Pumpkin seed oil (PSO) is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acid-omega 6, omega 9, vitamin A and vitamin E, carotenoids, tocopherols, phytoestrogens, phytosterols, polyphenols and selenium ***(Al-Okabi et al., 2014)***.

The aim of this study was to explore the possible ameliorative role of PSO on some of the cardiovascular system and kidney aspects.

MATERIAL AND METHODS

Materials:

- Commercial chow diet (balanced diet) containing 67% carbohydrates, 10% fat, and 23% protein as the energy sources (overall calories: 3.6 kcal/g), was purchased from Al-Gomhorya Company (Cairo, Egypt).
- High fat diet (HFD) consisting of 88% of standard pellet animal diet, 10% natural fat and 2% cholesterol was prepared and used to induce hyperlipidemia ***(Xu et al., 2010 and Hussain et al., 2016).*** The HFD was composed of the following energy sources: 52% carbohydrates, 30% fat and 18% protein Overall calories 4.8 kcal/g ***(Hussain et al., 2016).***
- Natural cold pressed pumpkin oil was purchased from Imtenan Company, (Cairo, Egypt).

Experimental animals and design:

Thirty adult male albino rats (7-8-weeks old, weighing 130-150 g) were purchased from the Nile Pharmaceuticals Company (Cairo, Egypt). They were housed in laboratory standard cages (5 rats/ 25X 30X30 cm cage), under specific pathogen-free conditions in facilities maintained at 21-24°C with a 40-60% relative humidity and normal light/dark cycle. All animals have free access to chow diet and water *ad libitum*, and were acclimated for one week prior to initiation of the experiment in the laboratory of Physiology, Faculty of Medicine AI Azhar University. All

procedures were approved by the Animal Care Committee. The Principles of laboratory animal care were followed, as well as specific national laws were applicable.

The rats were divided into 3 equal groups:

- ***Group I:*** Rats were assigned to control group and given normal balanced chow and supplemented with saline using a gastric gavage tube for 8 weeks.
- ***Group II:*** Rats were given HFD for 8 weeks ***(Xu et al., 2010*** and ***Hussain et al., 2016).*** The rats also were supplemented with saline using a gastric gavage tube.
- ***Group III:*** Rats received HFD for 4 weeks, and then received pumpkin seed oil orally in addition to the HFD at a daily dose of 100 mg/kg for further 4 weeks ***(El-Mosallamy et al., 2012)***.
- To study the effect of PSO on isolated aortic spiral strips, six rabbits were used. PSO was applied at different doses ranging from 75 to 1200 ?g/ml to test its effects against norepinephrin-induced contraction (***Furchgott and Bhadarkom, 1984).***

The body weight of each rat was measured and recorded weekly for all groups. To confirm hyperlipidemia, serum lipid profile was done by the end of the 4th week of HFD supplementation.

At the end of the experiment, after overnight fasting, rats were anesthetized in the morning, and blood samples were collected from retro-orbital venous plexus by capillary tubes under light phenobarbitone anesthesia. Blood samples were collected from retro-orbital venous plexus by capillary tubes. 0.2 ml of the blood was hemolyzed by addition of 1.8 ml H_2O, and the hemolysate was used for assessment of GSH level. The rest of the blood was then centrifuged at 3000 rpm for 15 minute for serum collection. Serum was separated in aliquots in Eppendorf tubes and stored frozen at -80°C until analysis. Blood pressure and ECG were recorded. Both kidneys were removed and took specimens from it and processed for light and transmission electron microscopic (TEM) examination.

I- Biochemical analysis:

1. The total serum cholesterol and HDL were measured by quantitative enzymatic colorimetric determination of total and HDL cholesterol in serum using biomed diagnostic assay kits ***(MacLachlan et al., 2000).***
2. Serum triglycerides were measured by quantitative enzymatic colorimetric determination of triglycerides in serum using Cayman colorimetric assay kit ***(Cole et al., 1997)***
3. Serum LDL cholesterol was calculated from the values of total cholesterol (TC), HDL and triglycerides using Friedewald equation: LDL (mg/dl) = TC - HDL –$(TG/5.0)^2$***(Ahmadi et al., 2008).***
4. Serum nitric oxide (NO) was evaluated by nitrite reductase method using Total Nitric Oxide Kit (Beyotime, Haimen, China, S0023) ***(Cortas and Wakid, 1990).***
5. Serum urea was measured according to modified Berthelot - Searcy method ***(Henry, 1991).***

6. Serum creatinine activity was determined using the application of Jafe reaction ***(Wilson and Walkes, 2000).***
7. Serum malondialdehyde (MDA) was measured by using free-SH groups estimation method ***(Janero, 1990).***
8. Serum reduced glutathione (GSH) was measured by the protocol described previously by, using glutathione peroxidase assay kit (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instruction. ***(Ceballos-Picot et al., 1992).***
9. Serum tumor necrosis factor alpha (TNF-α) level was measured by commercial ELISA kits (Ray Bio® Rat, Ray Biotech, Norcross, GA, USA) according to manufacturer's protocol. The level of sensitivity of the kit was less than 25 pg TNF-α/ml ***(Engelmann et al., 1990).***
10. Serum interleukin-6 (IL-6) level was measured by commercial ELISA kits (Ray Bio® Rat, Ray Biotech, Norcross, GA, USA) according to manufacturer's protocol. The level of sensitivity of the kit was less than 30 pg IL-6/ml ***(Ferrari et al., 2003).***
11. Vascular cell adhesion molecule1 (VCAM1) was determined by commercially available ELISA and standards (R&D System Europe Ltd). The level of sensitivity of the kit was less than 3.9 ng/ml ***(Pigott et al., 1992).***

II- Pharmacological study:

1. ***Effect of PSO on arterial blood pressure*** (***Ordodi et al., 2005)*** and ***Parasuraman et al. (2010):*** The effect of PSO 100mg/kg-orally on arterial blood pressure of pentobarbitone anesthetized rats was studied using Lab power Chart. Comparison study between groups was also done.
2. ***Effect of PSO on heart rate by Electrocardiogram (ECG):*** ECG was recorded by an electrocardiograph (Cardiopen 531) Philips, using lead II. The effect of pumpkin seed oil (100 mg/kg-orally) on heart rate of pentobarbitone anesthetized rats was studied. Comparison study between groups was also done.
3. ***Effect of PSO (75 - 1200 ?g/ml) on isolated rabbit aortic spiral strips: (Furchgott and Bhadarkom, 1984):*** The effect of different doses of PSO dissolved in dimethylsulfoxide (DMSO)], on nor-epinephrine-induced contractions was tested after 30 minutes incubation.

III- Histopathological Studies:

1. ***Light microscopic examination***: We rapidly immersed the kidney specimens in neutral buffered formalin for 36 hours, and dehydrated in ascending grades of ethyl alcohol. The specimens were then cleared in benzene followed by impregnation and finally embedded in paraffin wax. The blocks were sectioned by a rotatory microtome at 5 microns (um) thickness. Sections were mounted, stained with Hematoxylin and Eosin (H&E) for studying the general structure, Masson’s trichrome stain for staining the collagen fibers, orcein stain for staining the elastic fibers ***(Drury and Wallington, 1980),*** and Periodic Acid-Schiff reaction (PAS) for demonstration of glycogen ***(Bancroft and Stevens, 1996)***.

2. ***Electron microscopic Examination (TEM):*** 1mm³ kidney specimens were immediately fixed in 5% glutaraldehyde in 0.1M sodium cacodylate buffer, at 0-4 C° and pH 7.3. The specimens were washed for 1.5 hour with 3 changes of the same buffer. Post fixation was carried out in 1% osmium tetraoxide in the same cacodylate buffer for 2 hours at 0-4ºC ***(Robinson et al., 1987).*** This was followed by washing in the same buffer, and dehydration in ascending grades of ethanol up to absolute alcohol. The specimens were cleared with propylene oxide, impregnated in epoxy resin, finally embedded in pure resin, and left for 48 hours in the oven at 60° C for resin polymerization ***(William and Carter, 1996)***. Semithin sections 1 um thickness using LKB ultra microtome were cut, picked up on glass slides and stained with toluidine blue. The ultrathin sections were cut (80 nm) and picked up on formvar coated 200 mesh copped grid (***Hajibaghari, 1999),*** stained with uranyl acetate ***(Johanneseen, 1978)***, followed by lead citrate ***(Reynolds, 1963 and Robinson et al., 1987).*** Sections were examined and photographed by JEOL 1010 transmission electron microscope (Jeol; Tokyo, Japan), at the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University.
3. ***Morphometrical measurements:*** They were carried out at Pathology Department, Faculty of Dentistry, Al-Azhar University, using a computerized image system composed of a Leica Microsystems DM 2500 image analyzer which was connected to a Leica microscope, and is calibrated automatically to convert the measurement units (pixels) produced by image analyzer program into actual micrometer units. It was used for evaluation of the area percentage of collagen fiber in Masson's trichrome stained sections, and the optical density of PAS treated sections in five randomly selected non overlapping fields in each group specimens using magnification (200 X) by light microscopy transferred to the screen. Mean value and standard error were obtained from each specimen.

Statistical Analysis: All the data were expressed as mean ±standard error of mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni post hoc multiple comparison test using the program Statistical Package for the Social Sciences (SPSS), IBM SPSS Statistics (version 18). The values of $P < 0.05$ were considered significant.

RESULTS

Effect of PSO on body weight of HFD treated rats (Table 1): There were significant differences on comparing all groups with each other in body weight ($P<0.001$). HFD feeding for eight weeks significantly increased the body weight when compared to normal control rats. When PSO was given orally after inducing hyperlipidemia. The body weight significantly decreased versus HFD rats.

Table (1): Effect of pumpkin on body weight (g) of HFD rats (Mean ± SEM).

Groups / Parameters	Group I (Control group)	Group II (HFD group)	Group III (PSO group)	ANOVA P Value
Body weight (g)	214.9 ± 0.9	303.4 [a]± 3.4	218.1[b]±1.6	P<0.001

Number of sample in each group = 10
a = Significant values versus group I (control).
b = Significant values versus group II (HFD group).

There were significant differences on comparing all groups with each other in serum concentration of total cholesterol and triglycerides respectively. HFD for eight weeks significantly increased the fasting serum total cholesterol level in HFD group versus control rats. In comparison to HFD group, PSO treatment showed significantly lower total cholesterol and triglycerides.

There were significant differences on comparing all groups with each other in serum concentration of LDL and HDL respectively**.** Similarly, HFD supplementation increased the serum LDL in HFD rats compared to control rats and PSO treatment significantly lowered LDL level. On the other hand, a significant reduction in serum HDL was observed on the 8th week of high fat diet supplementation when compared to control rats. PSO-treated group significantly elevated HDL level when compared to HFD rats.

Table (2): Effect of PSO on lipid profile of HFD treated rats (Mean ±SEM).

Groups / Parameters	Group I (Control)	Group II (HFD)	Group III (HFD&PSO)	ANOVA P value
Serum cholesterol (mg/dl)	100.1 ± 0.8	186.0 [a] ± 2.0	106.3[b]± 2.5	P<0.001
Serum triglyceride (mg/dl)	81.1± 0.9	150.5[a] ± 3.4	88.5[b]±4.1	P<0.001
Serum LDL (mg/dl)	51.5 ± 0.6	117.0[a] ± 1.6	54.2[b] ±5.6	P<0.001
Serum HDL (mg/dl)	59.8 ± 1.0	25.1 [a] ± 1.2	57.9[b] ±3.3	P<0.001

Number of sample in each group = 10
a = Significant values versus group I (control).
b = Significant values versus group II (HFD group).

Effect of PSO on No, MDA and GSH of HFD group (Table 4): There were significant differences on comparing all groups with each other in serum concentration of NO, MDA and GSH respectively.

After 8 weeks of high-fat diet supplementation, NO production in the HFD model was significantly abolished compared to control rats, indicating that hyperlipidemia might impair endothelial function. PSO administration significantly increases its level when compared to HFD group.

Serum MDA level as a marker of lipid peroxidation was extremely high with HFD supplementation for eight weeks compared to normal rats. A significant decrease of serum MDA concentration was observed in PSO treated group when compared to HFD group. The increased MDA indicated enhanced lipid peroxidation in the HFD group.

Daily feeding of HFD for 8 weeks to rats significantly decreased the level of serum GSH compared to control group. In comparison to HFD treated rats, GSH level showed significant elevation with PSO treatment.

Table (4): Effect of PSO treatment on Serum Nitric oxide (NO) (?mol/L), Malondialdehyde (MDA) (nmol/ml), and Glutathione (GSH) (nmol/ml) on HFD rats (Mean ± SEM).

Groups / Parameters	Group I (Control)	Group II (HFD)	Group III (HFD&PSO)	ANOVA P value
Serum NO (?mol/L)	8.2 ± 0.9	4.5[a] ± 0.1	7.9 [b] ± 0.5	P<0.001
Serum MDA (nmol/ml)	1.9 ± 0.1	6.7[a] ± 0.2	2.1[b] ±3.1	P<0.001
Serum GSH (nmol/ml)	61.6 ± 1.2	32.3 [a] ± 0.9	58.2[b] ± 5.4	P<0.001

Number of sample in each group = 10
a = Significant values versus group I (control).
b = Significant values versus group II (HFD group).

Anti-inflammatory effects of PSO on HFD group (Table 5): There were significant differences on comparing all groups with each other in serum concentration of ***TNF-α***, ***IL-6*** and ***VCAM1*** respectively**.**

HFD supplementation for eight weeks up regulated both TNF-α and IL-6 by ~3 and ~4-fold, respectively, compared to control rats. A significant down-regulation of serum TNF-α and IL-6 concentrations was observed in pumpkin treated group when compared to HFD group.

Assessment of VACM1 concentration could be useful to detect the risk for atherosclerotic lesions in HFD-treated rats and to estimate the effect of pumpkin supplementation. HFD supplementation

for eight weeks to normal rats induced significant elevation in serum VCAM1 compared to control rats. When PSO was used after the HFD supplementation, there was a complete reduction of the level of VACM1, and it became not different from control rats.

Table (5): Effect of PSO treatment on Serum Tumor Necrosis Factor Alpha (TNF- α) (pg/ml), Interlukin-6 (IL-6) (pg/ml) and Vascular Cell Adhesion Molecule 1 (VCAM1) (ng/ml) on HFD rats (Mean ± SEM).

Groups / Parameters	Group I (Control)	Group II (HFD)	Group III (HFD&PSO)	ANOVA P value
Serum TNF-α (pg/ml)	35.2 ± 0.7	101.7[a] ± 1.3	37.5[b] ±0.9	P<0.001
Serum IL-6 (pg/ml)	1.9 ± .0.1	8.1[a] ± 0.1	2.1[b] ±1.1	P<0.001
Serum VCAM1 (ng/ml)	37.2 ± 0.4	151.2[a] ± 0.7	36.5[b] ± 0.9	P<0.001

Number of sample in each group = 10
a = Significant values versus group I (control).
b = Significant values versus group II (HFD group).

***Effect of PSO on mean arterial blood pressure of HFD rats* (Table 6 & Figure 1):** Pumpkin seed oil (100 mg/kg orally) caused significant reduction on mean arterial blood pressure of HFD treated rats. It was reduced from 254 ± 16.0 to 162 ± 3.0. While it was 155 ± 4.0 in the control group.

***Effect of PSO on heart rate of HFD rats* (Table 6 & Figure 2):** On the ECG record, PSO (100 mg/kg orally) induced significant reduction in heart rate from 650 ±1.9 to 390 ±2.5 in HFD treated group. Significant increase was also observed in HR from 495 ±2.1 to 650 ±1.9 in control rats treated with HFD.

Effect of pumpkin seed oil (75-1200 µg/ml) on isolated rabbit aortic strips: Pumpkin seed oil (75-1200 µg/ml) caused significant reduction on nor-epinephrine (0.2µg/ml) induced contraction. The Mean % reduction was ranged from 4.13±2.25 to 58.51±2.72 **(Table 7 & Figure 3**).

Table (6): Effects of PSO (100 mg/kg orally) on mean arterial blood pressure and heart rate of HFD rats (Mean ± SEM).

	Group I (Control)	Group II (HFD)	Group III (HFD&PSO)	ANOVA P value
MABP (mmHg)	155±4.0	254[a] ± 16.0	162[b] ± 3.0.	P<0.001
HR (beat/minute)	495±2.1	650[a]±1.9	390[b]±2.5	P<0.001

Number of sample in each group = 10
a = Significant values versus group I (control).
b = Significant values versus group II (HFD group).

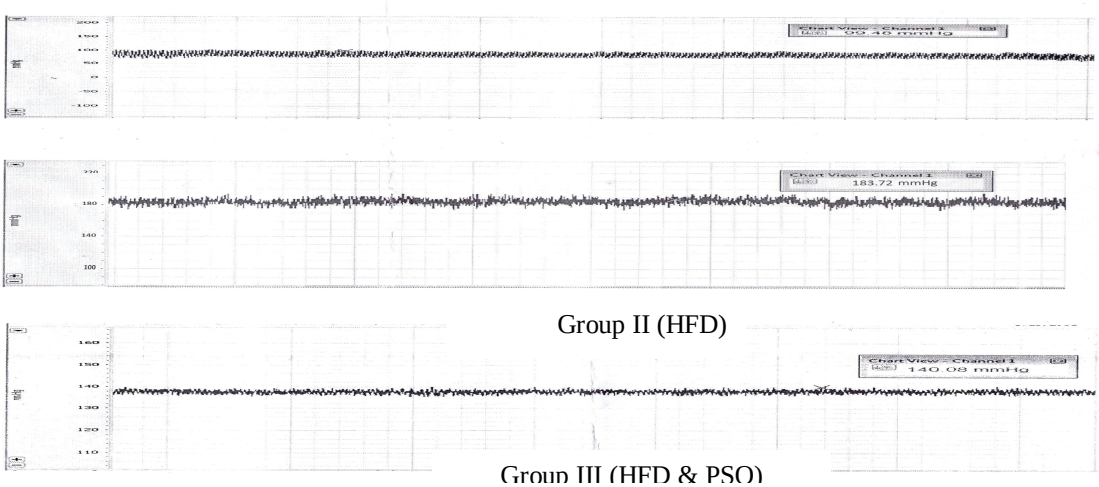
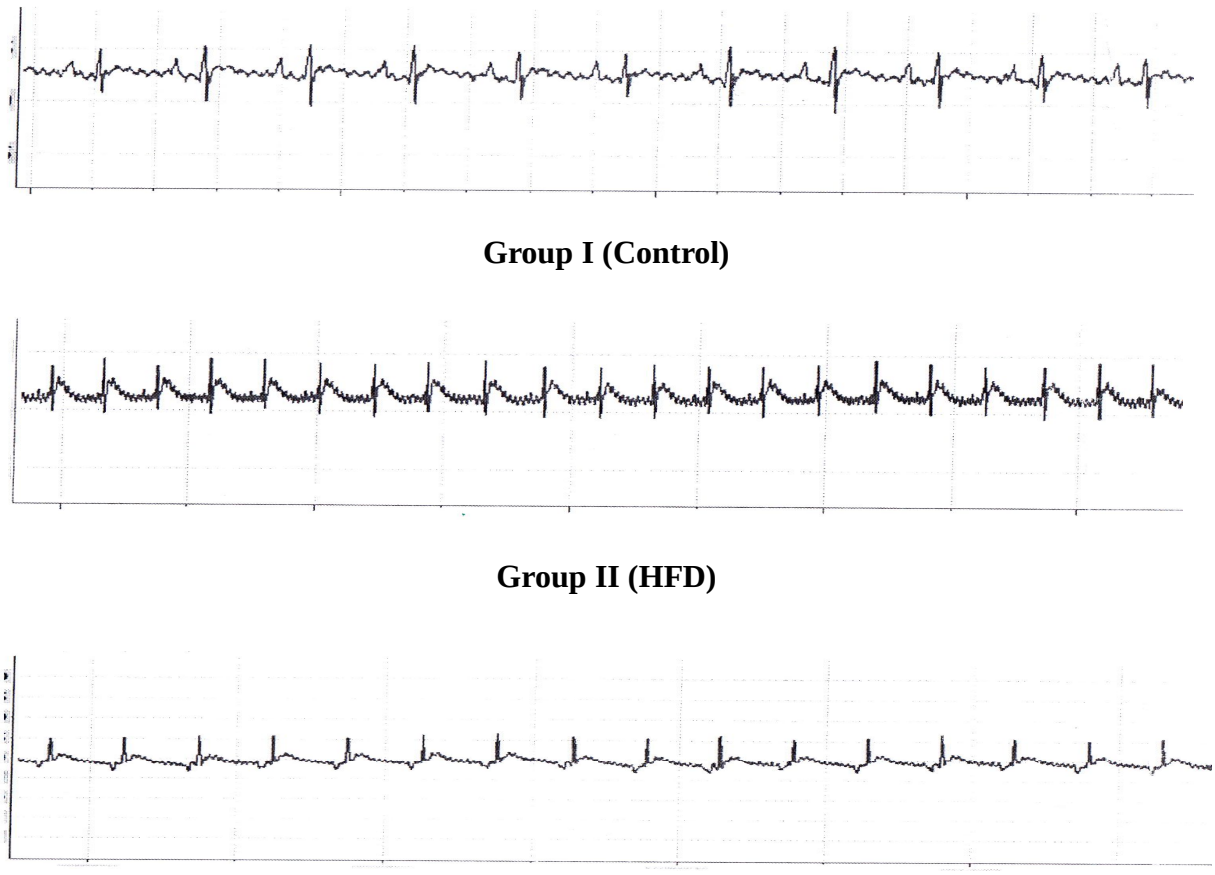


Figure (1): Effect of PSO (100 mg/kg-orally) on MABP (mmHg) of HFD group



Group I (Control)

Group II (HFD)

Group III (HFD&PSO)

Figure (2): Effect of PSO (100 mg/kg-orally) on heart rate (beats/minute) of HFD group

Table (7): Effect of PSO (75-1200 µg/ml) on nor-epinephrine (0.2µg/ml) induced-contractions of isolated rabbit aortic spiral strips on HFD rats (Mean ± SEM).

Doses (µg/ml)	75	150	300	600	1200
Mean % reduction change	4.13±2.25	10.6±2.43	23.84±3.14	40.71±3.93	58.51±2.72
P	<0.01	<0.001	<0.001	<0.001	<0.001

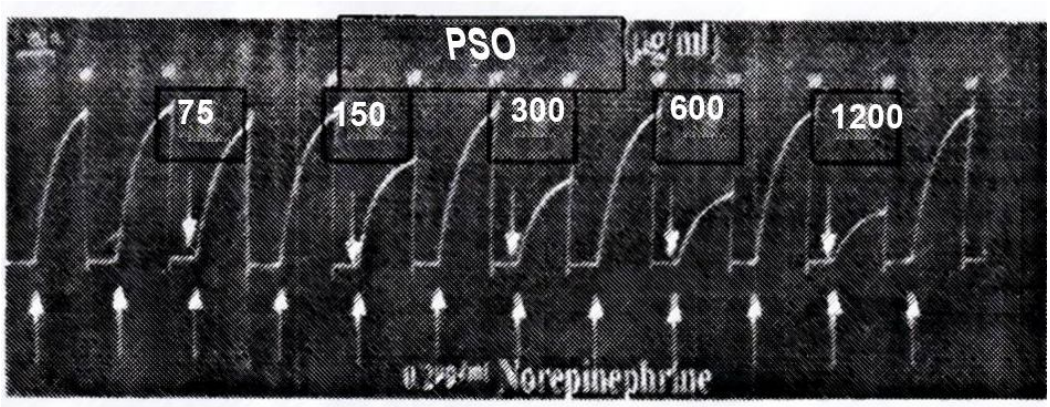


Figure (3): Effect PSO (75-1200 µg/ml) on nor-epinephrine (0.2µg/ml) induced-contractions of isolated rabbit aortic spiral strips.

Results of light microscopic examination:

Group I: Histological examination of control adult male rat kidney sections stained with H&E revealed clear tubular and glomerular structures of the cortex. It has the renal (Malpighian) corpuscles formed of Bowman's capsule surrounding the glomerulus (capillary tuft), the proximal convoluted tubules (PCT) and the distal convoluted tubules (DCT). The Bowman's capsule had two layers: parietal and visceral layer. PCT had a narrow lumen and lined by high cubical epithelium with apical brush border and deep acidophilic cytoplasm, while DCT had a wider lumen and lined by cubical epithelium with less acidophilic cytoplasm and no brush border. The main blood vessels were formed of tunica intima, media and adventitia **{Plate 1 A (1-4)}**. All basement membranes of the glomeruli and the tubules, in addition to the apical brush border of PCT gave positive PAS reaction. In addition, mild PAS positive reaction was noted in the wall of the main blood vessels **{Plate 2 A (1-2)}**. Minimal collagen fibers in between the nephrons and in the wall of the main blood vessels were detected with Masson's trichrome stain. Also, intact elastic fibers were seen with orcein stain **{Plate 3 A (1-3)}**.

Group II: H&E kidney stained sections of rats fed on HFD showed dilatation in the glomerular space, pyknotic nuclei in some PCT & DCT, congested blood vessels, mononuclear cell infiltration and apparently slight thickening in the wall of the blood vessels with some desquamated parts in the intima **{Plate 1 B (1-4)}**.

Compared with the control, HFD led to an increase in the density of PAS positive reaction in the glomerular and tubular structures in addition to the wall of the main blood vessels **{Plate 2 B (1&2)}**. Increased the amount of collagen fibers in the interstitium of the kidney and in the wall of the main blood vessels with many subintimal and medial vacuolations in between the elastic fibers were noted in Masson's trichrome and orcein stained sections respectively **{Plate 3 B (1-3)}**.

Group III: The structure of the kidney improved in the rats treated with PSO with HFD if compared with HFD treated rats. Less dilatation in the glomerular space, minimal cellular infiltration and congestion was noticed with nearly normal appearance of the main blood vessels. **{Plate 1C (1-4)}**. Reduction in the PAS positive reaction, to appear more or less similar to the control, was also observed **{Plate 2 C (1&2)}**. Decrease in the interstitial fibrous tissue of the kidney and wall of the main blood vessels was also noticed in Masson's trichrome stained sections with nearly normal appearance of the elastic fibers in orcein stained sections **{Plate 3 C (1-3)}**.

Electron microscopic results:

Group I: Electron microscopic examination of control rats' kidney revealed the glomeruli containing several capillary loops, lined with fenestrated endothelial cells with its bulging nuclei into the capillary lumen. Podocytes lined the visceral layer of Bowman's capsule with their primary and secondary processes resting on the glomerular basement membrane. Messangeal cells and Messangeal matrix were present in between Podocytes **{Plate 4 A (1&2)}**. PCT showed apical long microvilli, basal infolding containing elongated mitochondria, multiple lysosomes and pinocytotic vesicles **{Plate 5 A (1&2)}**. DCT showed its wide lumen, more cells per cross-section, its nuclei bulging into the lumen with apical few short microvilli, Less obvious basal infolding that containing less numerous mitochondria **{Plate 6 A (1&2)}**.

Group II: Electron microscopic examination of kidney of rats fed on HFD showed mesangial matrix expansion**,** irregular thickening of glomerular basement membrane and secondary foot processes effacement in the glomeruli **{ Plate 4 B (1&2)}**. PCT showed distorted nucleus, loss of basal infolding and multiple swollen mitochondria with irregular cristea **{Plate 5 B (1-3)}.** As regarding DCT distorted nucleus, loss of basal infolding, multiple vacuoles in the apical parts and slight decrease in mitochondria were seen. There were more fibrous tissue and many dilated inter cellular spaces **{Plate 6 B (1&2)}**.

Group III: The structure of the kidney was improved in the rats treated with PSO and HFD if compared with HFD treated rats as; in the glomeruli there was decrease in mesangial matrix, thin secondary foot process, but glomerular basement membrane was still apparently thick. **{Plate 4 C (1&2)}**. As regarding PCT& DCT, their nuclei appeared more regular in shape, with nearly normal basal infolding and mitochondria **{Plate 5 (C) & 6 (C)}** respectively.

Effect of PSO on area percentage of collagen fibers and optical density of PAS reaction in HFD treated rats (Table 8):

There were significant differences on comparing all groups with each other in

mean area % of collagen fibers, in Masson's trichrome stained sections, and optical density of PAS reaction sections. HFD feeding for eight weeks significantly increased area % of collagen fibers and optical density of PAS when compared to normal control rats. PSO caused significant decrease in these parameters when compared to HFD treated rats.

Table (8): Area % of collagen fibers and optical density of PAS reaction (Mean ± SEM).

Groups / Parameters	Group I (Control)	Group II (HFD)	Group III (HFD&PSO)	ANOVA P value
Area % of collagen fibers	26.611±1.59	37.904 [a]±2.299	30.073 [b]±0.81	P<0.001
Optical density of PAS reaction	5.026± 0.905	11.198 [a] ± 0.603	9.046 [b]± 0.812	P<0.001

Mean examined in five randomly selected non-overlapping fields
a = Significant values versus group I (control).
b = Significant values versus group II (HFD).

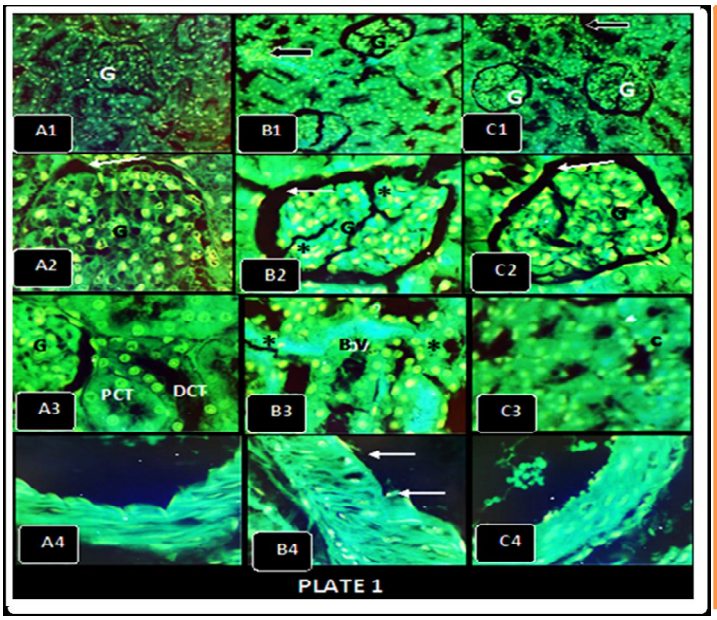


Plate (1): Kidney sections of **{A (1-4)} (GI)** note the normal histological structure; well–developed glomeruli (G), glomerular space (black arrow), distal convoluted tubules (DCT) and proximal convoluted tubules (PCT). Note also the normal appearance of the blood vessels with intact endothelial cells **{B (1-4)}. (GII)** showed mononuclear cell infiltration (white arrow) a dilatation in the glomerular space (black arrow), pyknotic cells (*) in the glomeruli &some tubules and congested blood vessels (BV). Note also the slight thickening in the wall of the blood vessels with some desquamated intima (black arrows) in B4. **{C (1-4)} (G III)** showing minimal cellular infiltration (white arrow), less dilatation in the glomerular space (black arrow) and congestion (c). Note also the normal thickening in the wall of the blood vessel with normal intima in C4. **{(H&E stain) (1 X200) - (2, 3 &4 X 400)}.**

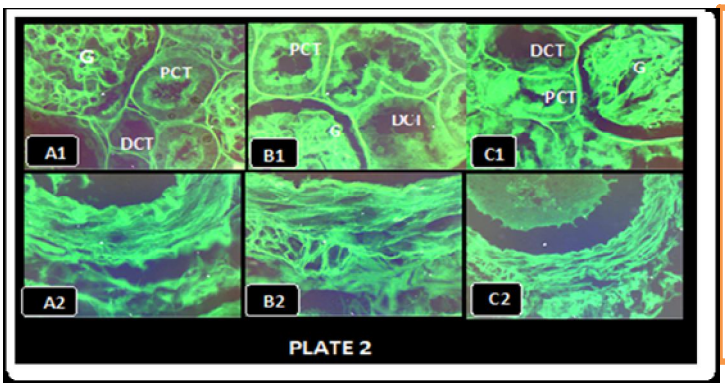


Plate (2): Kidney sections of {**A (1&2)} (GI)** revealed positive PAS reaction inside the glomeruli (G), the basement membranes, apical parts of the proximal convoluted tubules (PCT) and in the wall of the main blood vessels, while apical parts of the distal convoluted tubules (DCT) gave no reaction. **{B (1-3)} (GII)** gave marked positive reaction. {**C (1-3)} (G III)** gave moderate positive reaction **{PAS reaction, All X 400}.**

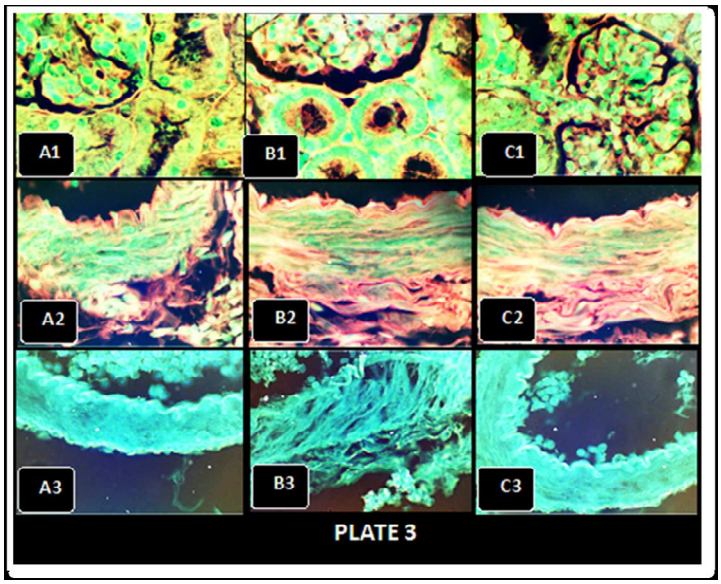


Plate (3): Kidney sections of **{A (1-3)} (GI)** revealed the minimal delicate connective tissue with minimal collagen fibers surrounding the glomeruli, the tubules and in the wall of the main blood vessels(1&2) with intact elastic fibers in (3)**. {B (1-3} (GII)** showed mild increase in the amount of collagen fibers in the interstitium and the wall of the main blood vessels(1&2) many subintimal and medial vacuolations in between elastic fibers in (3)**. {C (1-3)} (G III)** showing slight decrease in the fibrous tissue with nearly normal appearance of the elastic fibers. **{(1&2) Masson's trichrome and (3) Orcein stain, All X 400)}**

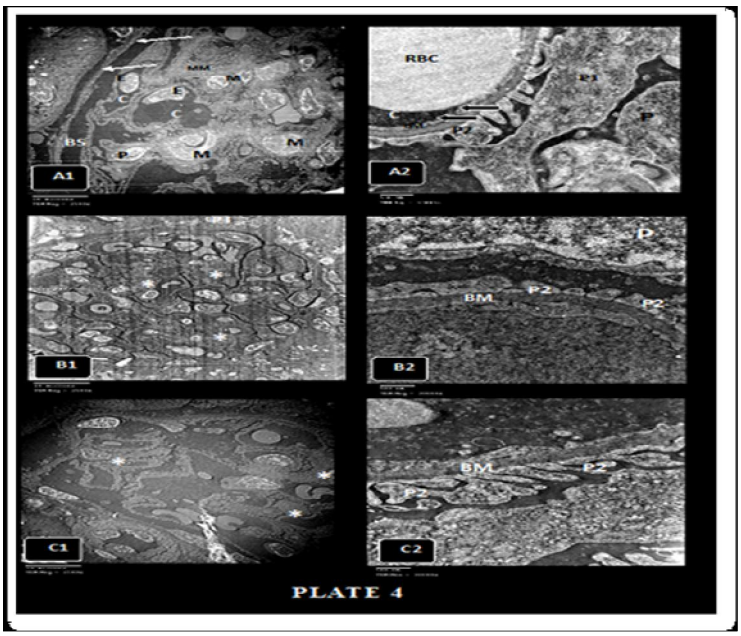


Plate (4): Electron micrographs of sections in the glomeruli: **{A (1&2)} (GI)**: **(A1)** the Bowman's capsule (black arrow), Bowman's space (BS) capillary lumen (C), lined with fenestrated endothelial cells (E) with its bulging nuclei. Mesangial cells (M), mesangial matrix (MM) and the podocytes (P). **(A2)**: Note the fenestrations of the endothelial cell (white arrows) the capillary lumen(C) and a red blood corpuscle (RBC), primary (P1) and secondary (P2) cell processes of the podocyte (P) resting on the glomerular basement membrane (BM). {**B (1&2)} (GII): (B1)** Mesangial matrix expansion (*); **(B2)** irregular thickening of glomerular basement membrane (BM) and secondary foot process effacements (P2). **{C (1&2)} (G III)** showing **(C1)** decrease in mesangial matrix (*), **(C2)** thin secondary foot process (P2) but glomerular basement membrane (BM) still apparently slightly thick. **Original magnification: (all 2) 30.000, (A1&C1) 5,000 and (B1) 2500**

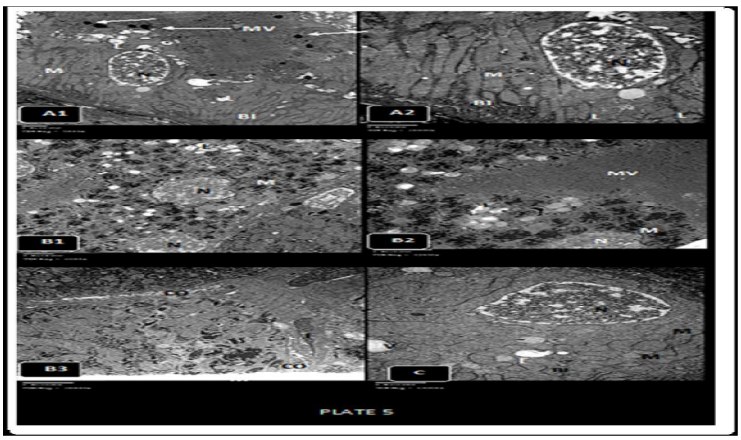


Plate (5): Electron micrographs of sections in PCT: **{A (1&2)} (GI)** revealed the basal infolding (BI) containing numerous elongated mitochondria (M), multiple lysosomes (L) and pinocytotic vesicles (black arrows). The cell apex has abundant long microvilli (MV). {**B (1-3)} (GII)** showed distorted nucleus (N), loss of basal striations, multiple swollen mitochondria with irregular cristea (M), increased amount of interstitial tissue and collagen fibers surrounding it (CO). Note also the fibroblast (F). **{C} (G III)** showing improvement in the shape of the nucleus (N), nearly normal basal infolding (BI) and mitochondria (M). **Original magnification: {A1 5,000, B1 6000, B2 12000, A2, B3 & C 10,000}.**

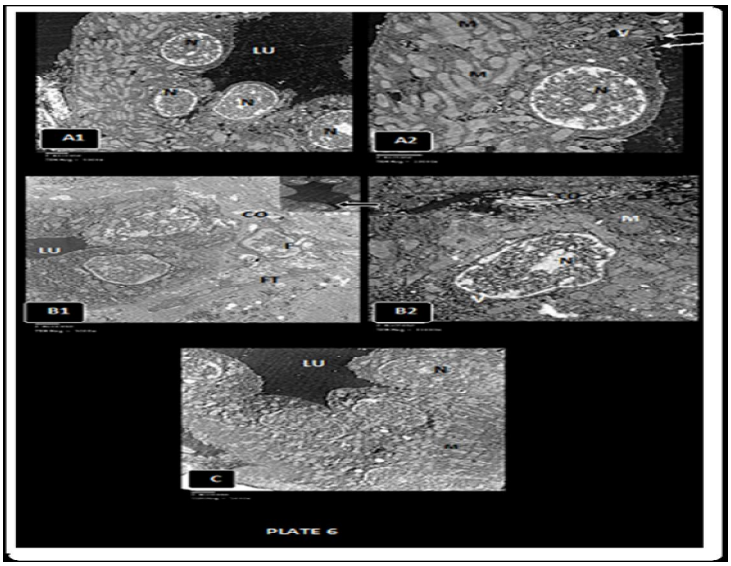


Plate (6): Electron micrographs of sections in DCT: **{A (1&2)} (GI):** Note a larger more clearly defined lumen(LU), more nuclei per cross-section bulging into it, less obvious basal infolding (BI) and less numerous mitochondria (M). The cell apex has few short microvilli (black arrows).{**B (1&2)} (GII):** Note distorted nucleus (N), loss of basal striations, multiple vacuoles in the apical parts (V) and slight decrease in mitochondria (M), fibroblast (F) and collagen fibers (CO). Note also, the dilated inter cellular space (black arrows) in the insert of B2. **{C}(G III)**: Note the improvement in the shape of the nucleus (N), nearly normal basal infolding and mitochondria (M). **Original magnification: {(A1, B1& C) 5,000 and (A2& B2) 10,000.**

DISCUSSION

Lipids are considered as one of the most elemental nutrients for humans. Lipid metabolism generates many bioactive lipid molecules which are fundamental mediators of multiple signaling pathways and they are also indispensable compounds of cell membranes. Any kind of changes in lipid metabolism can result in modification of membrane composition and subsequently in changing its permeability which leads to disruption of signaling network. It could be associated with some pathological states such as cancer, cardio-vascular, neurodegenerative, metabolic and inflammatory complications ***(Huang & Freter,* 2015 *and Simmons et al., 2015)***. Vegetable oils represent an attractive target for intervention, as besides potential health effects conferred by the type of fatty acids Other minor components, as phytochemicals, may also have health benefits ***(Morrison et al., 2015***). The aim of the present study was to offer a possible natural cardiovarscular and renal curative agent which can effectively ameliorate the pathological changes induced by HFD.

In the present study, HFD for 8 weeks induced obesity, elevation of blood pressure and ECG changes in the form of tachycardia in rats. The significant increase of body weight was supported by the study of ***Yang et al. (2015)*** who mentioned that a diet high in saturated fats is considered to be one of main contributors to overweight and obesity because fat is digested into mono-glycerides and fatty acids by lipase and absorbed fat is accumulated in adipose tissue through excessive adipocyte differentiation***. Dodd et al. (2015)*** attributed this increase to the hyperphagia and consequently high energy intake induced by adipocyte-derived leptin hormone secretion.

Regarding to the elevated blood pressure and heart rate in the present study, ***Laiali et al. (2016)*** explained this elevation to the changes in gene expres-sion of neuropeptides within autonomic nuclei in the hypothalamus and brainstem, which could be contributed to the

development of obesity-associated sympathetic hyperactivity and hypertension. Another explanation was reported by ***Zhuang et al. (2016)*** that hyperlipidemia and atherosclerosis induced hypertension through decrease of nitric oxide (NO) levels, which is a ubiquitous, naturally occurring molecule found in a variety of cell types and organ systems. It is considered an important determinant of basal vascular tone, prevents platelet activation, limits leukocyte adhesion to the endothelium, and regulates myocardial contractility in the cardiovascular system. This hypothesis was supported by the results of the present work that showed significant reduction of NO levels. Atherosclerotic renovascular disease is another explanation for this hypertension as reported by ***Decl`eves & Sharma (2015)*** and ***Dongdong et al. (2016)*** that obesity increases the kidney tubular sodium reabsorption, which in turn cause renal vasodilation and glomerular hyperfiltration. So the glomerular filtration rate increases to induce hypertension

Pumpkin seed oil (PSO) administration, after induction of hyperlipidemia, significantly decreased the body weight and blood pressure. Significant reduction of body weight was on line with previous studies of ***Amin et al. (2014)*** and ***Galaly et al. (2014)***. They explained this reduction to the presence of number of anti-oxidants and a high amount of tryptophan, an essential amino acid involved in the synthesis of a key brain chemical called serotonin, which is involved in mood, sleep and appetite regulation. The reduction of blood pressure and heart rate with PSO supplementation was supported by the study of ***El-Mosallamyet al. (2012)*** that PSO exhibits an antihypertensive and cardioprotective effects through a mechanism that may involve generation of NO. ***Kwon et al. (2007)*** explained this reduction to its phenolic antioxidant-enriched dietary contents that has the potential to reduce cellular oxidation stress and hypertension. The bradycardia observed with PSO was also supported by the study of ***Gorlov et al. (2015)*** on a model supplemented with food rich in cholesterol, carbohydrates and fats, and subjected to cardiac distress with intramuscular injection of epinephrine. They found that pumpkin can be used to correct metabolic disorders of lipids, reducing the risk of cardio pathology in rats. ***Costa (2011)*** reported that PSO or amlodipine treatment significantly reduces the elevation of blood pressure and normalize the ECG changes (prolongation of P-R interval and increased P wave duration) induced by L-NAME. The same author reported that PSO modulates the effect of felodipine and captopril in spontaneously hypertensive rats. PSO can modify the potency of the calcium antagonist felodipine or angiotensine converting enzyme inhibitor captopril in modulating the biochemical derangement in blood, heart, kidney, blood pressure and heart rate.

The antihypertensive effect of PSO was confirmed in this study by application of PSO in different doses to aortic strips which showed reduced the norepinephrin-induced contraction. ***Phelps and Peuler (2010)*** reported that concomitant administration of PSO succeeded to cause marked reduction of aortic contractile response on high cholesterol-fed rabbits. The relaxing effect observed in this study may be attributed to the increase of NO level by

PSO. This was explained by the study of ***Hove et al. (2009)*** and ***Liu et al. (2012)*** who reported that NO induced relaxation in mouse aorta via two pathways: the first is the non cGMP dependent stimulation of sarcoplasmin reticulum Ca^{++} ATPase, causing Ca^{++} reuptake into the sarco-plasmic reticulum and was prominent when intracellular Ca^{++} was mobilized and the second is involvement of soluble guanylate cyclase and formation of cGMP, causing relaxation without changing Ca^{++} but desensitizing the contractile apparatus.

The current study showed marked increase in the lipid profile (total cholesterol, triglycerides and LDL) in the high fat diet group, while serum HDL significantly decrease. These results were consistent with the studies indicating altered lipid metabolism associated with HFD intake reported by ***Galaly et al.(2014) and Li et al.(2015).*** The cholesterol level in plasma and liver of hyperlipidemic rats increased due to the increased uptake of exogenous cholesterol and subsequent its deposition in addition to the decreased cholesterol catabolism as evidenced by a reduction in bile acid production and turnover of bile acids ***(Barakat and Mahmoud, 2011)***. The alteration of lipid profile was supported by the histological changes of the kidney vessels which showed slight increase in the thickness of the blood vessels and its collagen fibers in addition to subintimal and medial vaculatioin between its elastic fibers with some desquamated parts in its media, which may indicates atherosclerosis. This vascular change was in agreement with ***Amin, et. al. (2014)*** who mentioned that atherosclerotic renovascular diseases might augment deterioration of renal function and ischemic nephropathy.

PSO administration improved all the lipid profile to return nearly to the normal levels. This was supported by the study of ***Abuelgassim and Al-Showayman (2012)*** who observed that atherogenic rats supplemented with pumpkin seed showed a significant decrease in their serum concentration of TC and LDL-C. They attributed this decrease to the Pumpkin seeds high concentration of phytosterols which inhibit cholesterol absorption in the small intestine. ***Agatemor (2006)*** and ***Al-Masri (2015)*** explained the hypolipidemic effect of PSO to the presence of unsaturated fatty acids which play a crucial role in reducing blood cholesterol in humans and rats which might be related to reduction of cholesterol synthesis and /or increased cholesterol catabolism in the liver. ***Sedigheh et al. (2011)*** attributed this lipid lowering effect of pumpkin to its fibers contents, as the dietary fibers reduce plasma LDL cholesterol levels by inhibiting the absorption of bile acids and cholesterol, and increases the activity of LDL receptors. ***Gossell-Williams et al. (2008)*** reported that the lipid lowering effect related to the presence of phytoestrogens in PSO, especially secoisolariciresinol, a lignin that has antioxidant, lipid lowering and cardiovascular benefits.

Regarding to serum urea and creatinine, HFD induced significant elevation of their levels. This was confirmed by our histological results that revealed alteration in glomerular structure, increased thickening in the glomerular and

tubular basement membranes, which might relate to increased deposition of glycoproteins. This was supported by ***Tziomalos (2009)*** who reported that the decrease in the glomerular filtration rate retain toxins to the circulation and lead to elevation of serum urea and creatinine**.** ***Marieb (2006)*** stated that there is part of body creatinine and other body toxins excreted via the convoluted tubules. So, the tubular affection and cytoplasmic vaculation, loss of basal infolding and multiple swollen mitochondria in some PCT and DCT reported in this study led to the changes in ions and water movement through the membranes. Toxic tubular necrosis could be another explanation for increased serum urea and creatinine as mentioned by ***Deji et al.(2009).*** Treatment with PSO showed an announced significant improvement of renal structures with decrease in levels of serum urea and creatinine as compared with HFD group. ***Amin et al. (2014)*** explained this decrease to decreased renal acidity (toward alkalinity) due to its high phosphorus content. So, renal histological changes improved**.**

In the present study, we assessed a major compounds involved in the down regulation of substances formed during oxidative stress (MDA and GSH) and pro-inflammatory cytokines, like TNF-α and IL-6, in order to investigate the effect of pumpkin on hyperlipidemia-induced inflammation in rats. Our results showed significant increase of MDA, TNFα, IL-6, and decrease of GSH after 8 months old HFD supplementation. The results with oxidative stress, inflammatory markers and endothelial dysfunctional markers were consistent with the studies of ***Peairs et al. (2011)*** and ***Herieka & Erridge (2014)*** who reported that HFD stimulates oxidative stress, impairs endothelial function and causes a rise in circulating inflammatory factors as soluble intercellular adhesion molecule-1 (ICAM-1), TNF-α and C-reactive protein (CRP). Also, ***Shi et al. (2005)*** found that the high TNF-α response, which are mainly produced by macrophages and monocytes, were the result of acute responses to the high fat diet as these cytokines normally return to baseline concentration once the acute phase response is attenuated. The histological changes of this study give another support that revealed dilation in the glomerular space, mononuclear cell infiltration, congestion, pyknotic nuclei in the glomeruli with apparently slight thickening and vaculation in the wall of the blood vessels and the more fibrous tissue in between the tubules and the wall of main blood vessels as proved also by image analysis. These changes were consistent with ***Altunkaynak (2008) and Szalay el al. (2015).*** The studies of ***Liu (2006) and Halberg et al. (2009)*** attributed this fibrosis of the kidney after HFD treatment to stabilization and transcription of hypoxia-inducible factor 1α (HIF-1α), a key mediator of hypoxia and an important driving force to induce fibrosis. An excessive metabolic challenge in adipose tissue induces hypoxia in the tissue that leads to the initiation of inflammation and, in turn, to fibrosis (glomerulosclerosis and tubulointerstitial fibrosis). Another study of ***Decl`eves and Sharma (2015)*** explained this fibrosis to the induction of TGF-β in the kidney in response to diet induced obesity, which is the major driver of matrix synthesis, inhibition of matrix degradation and stimulator of myofibroblast activation,

and has been considered as the major mediator of chronic fibrosis in kidney disease. This factor is formed in association with upregulation of extracellular matrix (ECM) molecules, including fibronectin, type IV and type I collagens.

In present research, a pronounced lower concentrate of MDA and higher level of GSH was observed in PSO-treated rats than those on HFD. These results suggested that pumpkin mediated prevention of atherosclerosis was closely related with inhibition of oxidative stress. ***Pastori et al. (2014)*** pointed out that acute and chronic overproduction of reactive oxygen species (ROS) under pathophysiologic conditions, was integral in the development of atherosclerosis**.** Also, inflammatory markers have a key role in mediating inflammatory cascades and promoting atherosclerosis formation. The positive impact of PSO on plasma levels of VCAM-1, TNF-α, IL-6 were observed in this research supported that better function in atherosclerosis prevention and treatment. ***Sedigheh et al. (2011)*** and ***Al-Okbi et al. (2014)*** reported that supplementation of rats with pumpkin seed powder and oil respectively revealed a significant reduction in CRP and TNF-α levels. The hypolipidemic anti-inflammatory effects of this plant are related to its anti-oxidant compounds such as flavonoids. ***El-Mosallamy et al. (2012)*** explained the anti-inflammatory effects of PSO to its high contents of linoleic and oleic unsaturated fatty acids. Linoleic and linolenic acids compete with arachidonate for oxidative enzymes, thereby reducing the production of arachidonate cyclooxygenase products. It has been shown that a diet rich in linolenic acid has actions similar to nonsteroidal anti-inflammatory agents in reducing the production of prostaglandin E2 and leukotriene B4 generated during inflammation. Another mechanism was reported by ***Fink et al. (2015)*** that pumpkin seeds containing selenium, and the administration of selenium diminished ICAM-1 and VCAM-1 mediated monocyte adhesion induced by microparticles of resuscitated patients, suggesting that selenium has anti-inflammatory effect.

CONCLUSION

Pumpkin PSO possessed significant weight reduction, anti-hyperlipidemic, anti-inflammatory and anti-hypertensive properties. Pumpkin may contribute to reduce hypertension by improving plasma antioxidant defenses, lipids profiles and kidney functions. The medicinal value of this plant may be attributed to the bioactive components established to be present in this plant. However, further studies are needed to evaluate the potential value of this oil for the management of hypertension, and the adverse effects of them for being used as anti-atherogenic drugs in human being.

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دور زيت بذرة قرع العسل علي بعض خواص القلب والأوعية الدموية والكلي في الجرذان البيضاء البالغة

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خلفية البحث: تعتبر السمنة نوعا من الغذاء الغير المتوازن . وهي من أكثر الأمراض شيوعا. حيث أنها ترتبط بأمراض عديدة مثل إرتفاع ضغط الدم وإرتفاع نسبه الدهون في الدم. و قد تؤثر في التركيب الدقيق للكلية أيضاً. ويعتبر زيت بذرة قرع العسل من المصادر الغنية بالدهون غير المشبعة، مضادات الاكسدة والألياف.

الهدف من البحث: دراسة تأثير تناول زيت بذرة قرع العسل علي ضبط ضغط الدم، والجهاز الدوري، و الكلي في الجرذان البيضاء الذكور البالغة و المعالجة بوجبات عالية الدهون .

مواد وطرق البحث: تم تنفيذ الدراسة الحالية على 30 من الجرذان البيضاء الذكور البالغة، تم تقسيمهم إلى ثلاث مجموعات متساوية: **المجموعة الأولى** (المجموعة الضابطة): تغذت علي غذاء متوازن بالإضافة إلي تناول محلول ملحي و لم تخضع لأي معالجات. **المجموعة الثانية** (المجموعة المتغذية على الغذاء الغني بالدهون) : تغذت هذه المجموعة علي غذاء متوازن لمدة أربعة اسابيع و تم إستبدال هذا الغذاء بغذاء عالي الدهون لمدة ثمانية اسابيع، بالإضافة إلي تناول محلول ملحي عن طريق أنبوبة المعدة المغذية. **المجموعة الثالثة** (مجموعة معالجة بزيت بذرة قرع العسل بجانب الغذاء الغني بالدهون): تغذت علي غذاء عالي الدهون لمدة ثمان أسابيع، و بعدها تم إعطائها زيت بذرة قرع العسل (100 مليليتر/كجم) لمدة أربعة اسابيع عن طريق أنبوبة المعدة المغذية.

فى نهاية التجربة تم تحديد التغيرات في وزن الجسم، ومتوسط ضغط الدم وضربات القلب ورسم القلب، و تأثير جرعات مختلفة من زيت بذرة قرع العسل من 75 الي 1200 ميكروجرام/مل علي الإنقباض الأورطي المستحدث بالنورإيبينفرين و ذلك في الشرائط الحلزونية المعزولة من الأرنب البلدي. هذا بالإضافة إلي قياس مستويات دهنيات الدم (نسب الكوليستيرول الكلي و الكوليستيرول في البروتين الدهني منخفض الكثافة و الكوليستيرول في البروتين الدهني عالي الكثافة والدهون الثلاثية)، ومضادات الاكسدة (المانولدهيد والجلوتاثيون)، وأوكسيد النيتريك ، واليوريا والكرياتينين، ودلالات الإلتهابات (عامل نخر الورم ألفا والإنترليكون-6) وجزيئات الإلتصاق بخلايا الأوعية الدموية). كما تم أخذ عينات من الكلى لتحضيرها وفحصها بالمجهر الضوئي والإلكتروني.

النتائج: تتلخص نتائج البحث الحالي في أن الاستخدام العلاجي لزيت بذرة قرع العسل في الفئران عالية الدهون أدي إلي خفض وزن الجسم وضغط الدم و ضربات القلب بدلالات إحصائية، وأيضا إلي تثبيط التقلصات الناتجة من دواء النورإيبنيفرين و كان هذا التأثير ذو دلالة إحصائية. كما أدي لإنخفاض مستويات الكوليستيرول الكلي و الدهون الثلاثية و والكوليستيرول في البروتين الدهني منخفض الكثافة إنخفاضا له دلالة إحصائية، بينما إرتفع الكوليستيرول في البروتين الدهني عالي الكثافة و أكسيد النيتريك. هذا بالاضافة إلي إنخفاض ذو دلالة إحصائية في مستويات مضادات الاكسدة (المانولدهيد)، ووظائف الكلي (اليوريا والكرياتينيين)، وعامل نخر الورم ألفا، والانترليكون 6، بالإضافة إلي جزيئات الإلتصاق بخلايا الأوعية الدموية -1، مع زيادة ملحوظة في مستويات عامل الاكسدة الجلوتاثيون. . وأظهر الفحص النسيجي أن زيت حبوب قرع العسل أدى إلى تحسن التركيب النسيجي للكلية، كما إنخفض مدى تلف الأنسجة الكلوية والتليف البيني في الفئران التي تغذت على الغذاء الغني بالدهون.

الإستنتاج: إستخدام زيت بذرة قرع العسل قد يلعب دورا هاما في تخفيض نسبة الدهون الضارة في الجسم، وضغط الدم، وضربات القلب ، ووظائف الكلي، وبعض دلالات الإلتهاب. هذا بالإضافة الي تأثيره علي الشريان الأورطي، وإحتمالية إلتصاق خلايا الدم بجدار الأوعية الدموية و أيضا إلي الإقلال من الإصابات الكلوية. و علي ذلك يمكن التوصية بإستخدام هذا الزيت لعلاج العديد من أمراض القلب و الأوعية الدموية وأمراض الكلي بشكل عام، وتصلب الشرايين بشكل خاص.