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Antiatherogenic potency of canola oil and/or wheat germ oil in association with the expression of some inflammatory markers in rats

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Abstract

The hypolipidemic and antiatherogenic potency of canola oil and/ or wheat germ oil and their effect on the expression of some inflammatory genes in liver and heart tissues were assessed. Forty male Wistar rats were divided into five groups fed on: standard diet, high fat diet, high fat diet +20% canola oil, high fat diet +20% wheat germ oil, high fat diet +20% mixture of canola and wheat germ oils. After forty five days, induced hyperlipidemia by high fat diet resulted in atherosclerosis as manifested by the significant change in lipid profile parameters, elevated serum butyrylcholine esterase activity and atherogenic index, in addition to the significant body and liver weight gain. It produced functional and structural disturbance in liver and heart tissues with the progression of oxidative stress as indicated by increased liver and heart lipid peroxidation, enzyme activities, serum uric acid level, and over expression of C-reactive protein, serum amyloid p component and interlukin-6 genes with the reduction of total antioxidant capacity and total proteins. Conversely, 20% of canola or wheat germ oil protects against hyperlipidemia and atherosclerosis by attenuating all the biochemical parameters and down regulating the expression of the inflammatory genes. Wheat germ oil was found to have more profound effect than canola oil. Mixing both oils has the lowest protective effect. Histological findings of heart and liver tissues verified the biochemical data. Our results recommend the use of canola or wheat germ oil as a strategy of healthy diet against atherosclerosis.

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Introduction

Cardiovascular diseases (CVDs) remain the leading cause of death in modern societies. The primary cause of dramatic clinical events of CVDs, such as unstable angina, myocardial infarction and stroke, is the atherosclerotic process (Charo and Taub, 2011; Szostak and Laurant, 2011).

Several studies have shown that an increased dietary intake of cholesterol results in hypercholesterolemia, which is known to eventually generate atherosclerosis and enhance the risk of coronary heart disease (CHD), fatty liver disease and cancer associated with hydroxyl radical formation (Anderson and Hana, 1999; Festi *et al.*, 2004) Moreover, studies using animal models of atherosclerosis have documented that reactive oxygen species (ROS), which are produced and used by all plaque constituents, serve as one of the drivers of the atherosclerotic process (Madamanchi *et al.*, 2005; Gutierrez *et al.*, 2006). Indeed, lesion formation is associated with a collection of events that are regulated by ROS: accumulation of lipid peroxidation products (Pratico *et al.,* 1997; Martinet and Kockx, 2001), induction of inflammatory/inflammation-related genes (Liao *et al.*, 1994), inactivation of nitric oxide (NO) leading to endothelial dysfunction (Keaney *et al.*, 1995), activation of matrix metalloproteinases (Rajagopalan *et al.*, 1996) and increased smooth muscle cell growth (Griendling *et al.*, 1994).

Thus, dietary modulation with emphasis on the composition of dietary lipids could be a therapeutic option in the prevention of thrombosis and coronary infarctions and in the treatment of various diseases including heart diseases to improve the quality of arterial walls and vascular patency (Proust *et al.*, 2014). In the past few years, nutritionists have recommended vegetable oils as an important part of a healthy diet due to their high contents of fatty acids (FAs) besides their traditional sources (Mišurcová *et al.*, 2011; Maehre *et al.*, 2014). Also, Natural antioxidants from plants are reported to provide substantial protection that slows down the process of oxidative damage caused by ROS (Jacob and Burri, 1996).

Canola (low erucic acid rapeseed) oil (CO) [22:1(n-9)] is considered favorable dietary oil. It is widely used as a cooking and salad oil, in table spreads, for baking and in a variety of other prepared foods. Canola oil is an important vegetable oil in the world, compared to other common vegetable oils as it contains the lowest concentration of saturated fatty acids, is a good source of omega-3 fatty acids (α-linolenic acid), and, after olive oil, has the highest amount of monounsaturated fatty acids (oleic acid) 63%.

Moreover, there are some important minor nutrients in canola oil, including phytosterols, tocopherols, and phenolic components (Lin *et al.,* 2013).

Wheat germ oil (WGO) has a number of nutritional and health benefits such as reducing plasma and liver cholesterol levels, improving physical endurance/fitness, and possibly helping to delay effects of aging (Tong and Lawrence, 2001). Also, it can reduce oxidative stress, improve lipid metabolism (Singh *et al.*, 2006). These effects are attributed to the high concentration of bioactive compounds present in the oil. Wheat germ oil is an excellent source of polyunsaturated fatty acids, the total unsaturated and polyunsaturated fatty acid (PUFA) content of WGO is about 81 and 64%, respectively making it processing very challenging. In addition, hexane extracted WGO consists of about 56% linoleic acid (18:2 n6), which is an essential fatty acid (Dunford and Zhang, 2003). Also WGO is a rich source of phytosterols, policosanols (POC), carotinoids, ceramide, thiamine, riboflavin, and niacin (Atwell, 2001).

Moreover, it is one of the richest natural sources of α-tocopherol, the type of tocopherol with the greatest vitamin E activity (Leenhardt *et al.,* 2008). It was reported that wheat germ oil intake results in a rapid increase in the content of vitamin E in different rat tissues and a change in the intensity of lipid peroxidation processes (Paranich *et al.,* 2000). One physiological role of vitamin E is its ability to react with and quench free radicals in cell membranes and other lipid environments, thereby preventing polyunsaturated fatty acids (PUFA) from oxidation.

The main objective of the current study was to evaluate the potency of canola oil and/or wheat germ oil and their combination as a strategy of a healthy diet in the protection against hyperlipidemia and consequent atherosclerosis.

Materials and methods

Preparation of standard and high fat diet

Standard and high fat diets were prepared according to the modified AIN-76 diet (Table 1) (American Institute of Nutrition, 1977).

Animals

A total of 40 healthy adult male Wistar rats weighing 160-180g obtained from the breeding unit of the Medical Research Center (Faculty of Medicine, Ain Shams University, Cairo) were used throughout this study. Rats were housed in steel mesh cages (3/cage) on wood-chip bedding and maintained on standard diet and tap water for one week before the start of the experiment as an acclimatization period. All guidelines for the care and use of animals were followed (Research Ethics Committee, Faculty of Science, Ain Shams University).

Chemicals

Canola (100%) pure oil was purchased from hypermarket in Dubai, UAE, wheat germ oil was purchased from Elcaptain (Cap pharm Co) for oil and herbs extraction, Azhar, Cairo, Egypt. Cholesterol, Choline chloride and sodium cholate were from El Gomhoreia Co, Cairo, Egypt.

Induction of hyperlipidemia and atherosclerosis

Experimental atherosclerosis was induced by feeding rats with a high fat diet containing 1% cholesterol, 10% lard, 5% corn oil and 0.5% sodium cholate for 45 days (Table 1).

Table 1. Composition of the standard and high fat diet (% w/w).

Ingredients	Standard diet	High fat diet
Casein	20	20
DL-methionine	0.3	0.3
Corn starch	29	17.5
Sucrose	40	40
Cellulose	1	1
Corn oil	5	5
Lard	-	10
Mineral mix	3.5	3.5
Vitamin mix	1	1
Choline chloride	0.2	0.2
Cholesterol	-	1
Sodium cholate	-	0.5

Experimental design

In total, 40 rats were randomly allocated to 5 groups of 8 rats each. Group I. normal control (NC): rats were fed on standard diet and tap water. Group II. High fat diet (HFD): rats were administered a high fat diet for 45 days. Group III. High fat diet+ canola oil (HFD+CO): rats were administered high fat diet supplemented with 20% canola oil for 45 days. Group IV. High fat diet + wheat germ oil (HFD+WO): rats were administered a high fat diet supplemented with 20% wheat germ oil for 45 days. Group V. High fat diet + Mix (HFD+M): rats were administered a high fat diet supplemented with 10% canola oil and 10% wheat germ oil for 45 days.

The amount of food consumed was recorded every day and the animals were weighed once every week.

Blood collection and tissue sampling

At the end of the experiment and after a fast of 12h, the animals were weighed, anesthetized under light ether anesthesia and blood was withdrawn from the abdominal aorta. Serum was separated from the clotted blood samples after centrifugation at 5,000rpm for 5min then aliquoted. For RNA extraction, 100mg of liver and heart tissues were immediately cut on ice, placed in 1ml BIOZOL Bio Flux™ Reagent and stored at -80°C. A part of liver and heart tissues was preserved in 10% phosphate buffered formalin (pH 7.2) at 4°C for histological examination.

The rest of liver and heart tissues were dissected out, rinsed in ice cold saline, weighed and stored in physiologic saline at -20°C until biochemical analyses.

Preparation of tissue homogenates

Parts of heart and liver tissues were weighed then homogenized in ice-cold phosphate buffered saline (pH 7.4) using a glass homogenizer to prepare a 10% (w/v) whole tissue homogenate. Aliquots of the whole tissue homogenates were centrifuged at 10,000rpm for 15min at 4°C to obtain the cytosolic supernatants. The supernatants were separated and preserved at -20°C until biochemical analyses.

Biochemical assays

Lipid profile

Serum total cholesterol level and high density lipoprotein-cholesterol (HDL-c), phospholipids, triglycerides, and total lipids were determined using kits provided from Biodiagnostic (Giza, Egypt) according to the methods of Wadehra *et al.* (1985). Zilversmit and Davis (1950) and Foster and Dunn (1973), respectively. Serum low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) levels were calculated (Friedewald *et al.,* 1972). The atherogenic index (AI) was calculated from the formula (AI=TC-HDL-C/HDL-C) (Wilson *et al.,* 1980).

Biochemical parameters of oxidative stress and serum uric acid

Lipid peroxides were assessed colorimetrically in liver and heart homogenates as thiobarbituric acid-MDA adduct concentration using a commercial assay kit (Biovision, USA). Total antioxidant capacity (TAC) was assessed in serum by the method of Koracevic *et al.* (2001) using a kit purchased from Biodiagnostic (Giza, Egypt).

Total protein concentration was determined in the supernatant of liver and heart homogenates as well as in serum according to Lowry *et al.* (1951). Uric acid was determined in serum using bio-dignostic kit (Giza, Egypt).

Enzymes assays

Serum butyrylcholine esterase (BuChE) activity was determined using an assay kit provided from Biodiagnostic (Giza, Egypt) with butyrylthiocholine as a substrate. Serum lactate dehydrogenase (LDH) and creatine kinase MB subunit (CK–MB) activities were determined for assessing heart function using assay. Kits provided from Biodiagnostic (Giza, Egypt). Also, serum gamma glutamyl transferase (GGT) activity was assayed as an index for hepatic problems using a commercial assay kit (Biodiagnostic, Giza, Egypt).

RNA extraction and semi-quantitative RT-PCR analysis

RNA was extracted from rat livers and hearts according to the method of (Marko, 2004) using BIOZOL BioFlux™ Reagent (BioFlux, South San Francisco, USA).

The extracted total RNA was then reverse transcribed with Oligo d (T) primer according to Revert Aid First Strand cDNA Synthesis Kit™ (Fermentas Life Science Co., Invitrogen Corporation, Van Allen Way, Carlsbad, Canada).

Positive and negative control reactions were used to verify the results of the first strand cDNA synthesis steps. GAPDH gene RNA was used as positive control.

The resulting cDNA was subjected to PCR for 35 cycles with respective primers designated from the sequence of the C-reactive protein (CRP), serum amyloid P component (SAP) and interlukin-6 (IL-6) genes (Table 2) using primer premier 5.0 software and were purchased from Invitrogen Corporation (Van Allen Way, Carlsbad, Canada).

Dream Taq™Green PCR Master Mix (Invitrogen Corporation, Van Allen Way, Carlsbad, Canada) was used in the PCR. Products of PCR were then displayed on an appropriate agarose gel (2%) and examined for yield and specificity. Analysis of gel images was done using Gel analyzer Pro (version 3.1) software.

Table 2. Sequences of the 5' and 3' synthetic primers used in PCR.

Gene & its accession number	Primers (sense and antisense 5'→3')	Annealing Temperature
CRP M83176	Sense: 5'-CGA AGC TTC AGC ATC TTC TC-3' Antisense: 5'-CTG CAT TGA TCT GTT CTG GAG-3'	46.5℃
SAP NM_017170	Sense 5'-CTC AGA CAG ACC TCA ATC AG-3' Antisense 5'-TCA GCA ATA CCA GAG GAG GA-3'	41.1℃
IL-6 M26745.1	Sense: 5'-CCA GCC AGT TGC CTT CTT GGG A-3' Antisense: 5'-GGCATA GCA CACTAG GTTTGCCGA-3'	58℃
GPDH NM_002046.5	Sense: 5'-CAAGGTCATCCATGACAACTTTG-3' Antisense: 5' -GTCCACCACCCTGTTGCTGTAG-3'	58℃

Histological investigations

Fixed Liver and heart Specimens were processed for paraffin embedding following the standard microtechnique (Banchroft *et al.*, 1996). Sections (5µm) of liver and heart stained with hematoxylin and eosin, (H&E) were mounted in neutral disterene dibutyl phthalate xylene (DPX) medium and evaluated for histopathological changes under a light microscope.

Statistical analysis

Results were expressed as means ±SD of 8 rats in each group and were statistically analyzed using one way analysis of variance (ANOVA). In case of significance, post hoc Bonferroni test for multiple comparisons was done using SPSS (version 14.0) (Chicago, USA). Differences were considered significant at ρ value less than 0.05.

Results

Biochemical analyses

Effect of high fat diet, wheat germ oil and/or canola oil on food intake, body, liver and heart weight

There was a significant increase in the amount of food intake, body and liver weights of rats fed with high fat diet compared to standard diet fed group with a percentage change of (90, 23.5 & 116.6%) respectively. Addition of canola oil or wheat germ oil to the high fat diet significantly decreased body and liver weights by (9.35% &40.8%) and (12.9% & 44.2%) respectively in Gr III and Gr IV compared to HFD fed group. On the other hand, there was no significant effect of administrating high fat diet containing a mixture of both canola oil and wheat germ oil on the amount of food intake, body, heart and liver weights compared to the high fat diet fed group Table (3).

Table 3. Effect of high fat diet, canola oil and/or wheat germ oil on food intake, body, liver and heart weight in all studied groups.

Groups	Food intake (g/day)	Initial BW (g)	Final BW (g)	BW gain (g)	Liver weight (g)	Heart weight (g)
GI (NC)	10±2.1^{a}	181±11^{a}	225±28^{a}	44±10.6^{a}	5.54±1.8^{a}	0.66±0.07^{a}
GII (HFD)	19±3.4^{b}	191± 6.5^{a}	278±59^{b}	88±11^{b}	12±3.8^{b}	0.97±0.11^{a}
Change% from NC	90	5.52	23.5	100	116.6	47
GIII (HFD+CO)	14±1.6^{ab}	189± 5.6^{a}	252±60^{c}	62±13.2^{a}	7.1±2.1^{a}	0.7±0.14^{a}
Change% from NC	40	4.41	12	41	28.2	6.1
Change %from HFD	-26.3	-1.04	-9.35	-29.5	-40.8	-27.8
G IV (HFD+WO)	12±4.2$^{a\,b}$	190±3.8^{a}	242±44$^{a\,c}$	52±9.2^{a}	6.7±1.6^{a}	0.67±0.2^{a}
Change % from NC	20	4.97	7.6	18.2	21	1.5
Change % from HFD	-36.8	-0.52	-12.9	-41	-44.2	-31
GV (HFD+M)	18±5.4^{b}	189±6.7^{a}	269±37^{b}	80±21^{b}	10.4±2.4^{b}	0.87±0.21^{a}
Change %from NC	80	4.41	19.6	81.8	87.7	31.8
Change % from HFD	-5.3	-1.04	-3.2	-9.1	-13.3	-10.3

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at ρ<0.05.Groups sharing the same superscripts is not statistically different.

Effect of high fat diet, wheat canola oil and /or wheat germ oil on serum BuChE activity, lipid profile and atherogenic index

A significant increase in BuChE activity (81.3%), serum total cholesterol (149%), LDL-C (465.6%), VLDL (98.5%), phospholipids (55.1%), triglycerides (104.4%), total lipids levels (82.2%) and atherogenic index (455.6%) with a significant decrease in HDL-C level (17.86%) was observed upon supplementing rats with high fat diet compared to the standard fed diet group. Adding 20% of canola oil or wheat germ oil to the high fat diet produced a significant decrease in BuChE activity (29.8 &36.5%), TC level (48 &52.8%), LDL-C level (69.6 & 77.9%), VLDL (31&40.3%), total lipids (27.2& 36.8%) and atherogenic index (70.7&79.1%) in Gr III and Gr IV respectively. A significant increase in HDL-C level was observed in the above two groups with a percentage change of (26.1 & 34.8%) respectively compared to the high fat diet administrated group and (3.57 & 10.7%) respectively compared to standard fed diet group, Table (4).

The presence of 20% Wheat germ oil in the high fat diet significantly lowered the levels of TG (40.4%) and phospholipids (25.6%) compared to HFD fed group, while 20% of canola oil decreased TG levels by (30.8%) and phospholipids by (20.1%) from HFD group. Mixing both oils with high fat diet caused a significant decrease in most of the foregoing parameters with respect to HFD group, although they were higher than in Gr I, Gr III and Gr IV except for HDL-C levels that showed a non-significant change among the other groups and for VLDL, TG and phospholipids levels which showed a non-significant decrease with respect to HFD group, Table 4.

Table 4. Effect of high fat diet, canola oil and/ or wheat germ oil on serum butyrylcholine esterase activity, lipid profile and AI in all studied groups.

	GI (NC)	GII (HFD)	GIII (HFD+CO)	GIV (HFD+WO)	GV (HFD+M)
S BuCE (U/L)	316±22.9^{a}	573±24.1^{b}	402±26.9^{c}	364±23.7^{c}	467±16.7^{d}
change % from NC		81.3	27.2	15.2	47.8
change% from HFD			-29.8	-36.5	-18.5
Total Cholesterol (mg/dl)	102±5.3^{a}	254±13.4^{b}	132±7.5^{c}	120±7.3^{c}	202±11^{d}
change% from NC		149	29.4	17.6	98
change% from HFD			- 48	-52.8	-20.5
LDL-c (mg/dl)	32±3.1^{a}	181±8^{b}	55±5.8^{c}	40±4.2^{a}	127±10.7^{d}
change% from NC		465.6	71.9	25	296.9
change% from HFD			-69.6	-77.9	-29.8
HDL-c (mg/dl)	56±3.03^{a}	46±4.8^{b}	58±4.7^{a}	62±2.4^{a}	49±4.8^{ab}
change% from NC		-17.86	3.57	10.7	-12.5
change% from HFD			26.1	34.8	6.5
VLDL (mg/dl)	13.6±1.3^{a}	27.8±1.2^{b}	19.2±0.74^{ab}	16.6±0.62^{a}	26±1.5^{b}
change% from NC		98.5	41.2	22.1	91.2
change% from HFD			-31	-40.3	-6.47
Triglycerides (mg/dl)	68±6.3^{a}	139±6.1^{b}	96.2±3.7$^{a\ b}$	82.8±3.1^{a}	130±7.6^{b}
change% from NC		104.4	41.5	21.8	91.2
change% from HFD			-30.8	-40.4	-6.5
Total lipids (mg/dl)	635±21.1^{a}	1157±114.7^{b}	842±30.9^{c}	731±17.1^{a}	918±33.5^{c}
change% from NC		82.2	32.6	15.1	44.6
change% from HFD			-27.2	-36.8	-20.7
Phospholipids (mg/dl)	79.3±12.7^{a}	123±16^{b}	98.3±18$^{\ a\ bc}$	91.5±12.3^{ac}	100 ±20.7^{bc}
change% from NC		55.1	24	15.4	26.1
change% from HFD			-20.1	-25.6	-18.7

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at $\rho<0.05$.Groups sharing the same superscripts are not statistically different.

Effect of high fat diet, wheat germ oil and/or canola oil on biochemical parameters for lipid oxidation and serum uric acid

Induction of hyperlipidemia produced a significant increase in the level of lipid peroxidation in both liver and heart tissues which was manifested in the elevated levels of MDA (68.2 & 98.9%) in both tissues respectively also, it increased the level of serum uric acid (161.5%) and these increases were accompanied by a significant decrease in the total antioxidant capacity (55.6%) compared to the standard diet fed group. The presence of canola or wheat germ oils in the high fat diet significantly lowered the level of lipid peroxidation in liver and heart tissues and ameliorated the induced increase in uric acid (39.1 & 47.9%) compared to HFD group also, it alleviated the induced suppressive effect on total antioxidant capacity (97.8 & 52.1%) in Gr III and Gr IV respectively, Table (5). On using a mixture of both oils with HFD, group V reported non-significant decrease in serum uric acid and liver MDA levels. Decrease in heart MDA level (10.4%) followed by a significant increase in total antioxidant capacity (30.4%) were recorded for the same group compared to high fat diet Gr II although these biochemical parameters were not returned to the normal levels and still significantly higher than those in GI.

Table 5. Effect of high fat diet, canola oil and/ or wheat germ oil on the level of serum uric acid and oxidative stress markers.

Groups	MDA (nmol/mg protein)		Uric acid (mg/dl)	TAC (mmol/L)
	Liver	Heart		
GI (NC)	0.88 ± 0.06^{a}	0.92 ± 0.05^{a}	2.78 ± 0.42^{a}	2.07 ± 0.07^{a}
GII (HFD)	1.48 ± 0.04^{b}	1.83 ± 0.05^{b}	7.27 ± 0.66^{b}	0.92 ± 0.09^{b}
Change% from NC	68.2	98.9	161.5	-55.6
GIII (HFD+CO)	1.04 ± 0.04^{c}	1.2 ± 0.06^{c}	4.43 ± 0.49^{c}	1.4 ± 0.21^{c}
Change% from NC	18.2	30.4	59.4	-32.4
Change %from HFD	-29.7	-34.4	-39.1	52.1
G IV (HFD+WO)	$0.94\pm0.06^{a,c}$	0.89 ± 0.03^{a}	3.79 ± 0.199^{c}	1.82 ± 0.10^{a}
Change % from NC	6.8	3.26	36.3	-12.1
Change % from HFD	-36.5	-51.4	-47.9	97.8
GV (HFD+M)	1.39 ± 0.05^{b}	1.64 ± 0.04^{d}	6.95 ± 0.48^{b}	1.2 ± 0.21^{c}
Change %from NC	58	78.3	150	-42
Change % from HFD	-6.1	-10.4	-4.4	30.4

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at $\rho<0.05$.Groups sharing the same superscripts is not statistically different.

Effect of high fat diet, canola oil and /or wheat germ oil on liver and heart functions

Administration of high fat diet significantly affected liver and heart functions, this was observed in the marked increase in serum GGT (142.5%), LDH (115.7%) and CK-MB (150%) activities accompanied by a marked decrease in serum total proteins to reach (34.8%) from the standard diet fed group. On the other hand, the presence of either canola oil or wheat germ oil with high fat diet in Gr III & Gr IV preserved liver and heart functions, this was indicated by the significant decrease in the activities of GGT (40.7 & 50.6%), LDH (29.4 & 46.8%) and CK-MB (37.1 & 53.7%) also, it normalized total proteins to reach (26.7 & 35.6%) for each of Gr III and Gr IV respectively from HFD (Gr II). Although there was insignificant change in the above parameters on mixing both oils with high fat diet compared to HFD group, Table (6).

Molecular analyses

Expression of C-reactive protein (CRP), serum amyloid p component (SAP) and interleukin 6 (IL-6) genes in liver and heart tissues

CRP, SAP and IL-6 genes were highly expressed by hepatocytes and cardiomyocytes in HFD group (Gr II). In both tissues the bands molecular weight on agarose gel were 433, 331bp and 582 bp for CRP, SAP and IL-6 respectively (Fig. 1A, B & C).

While, there was no expression for the studied genes in the liver and heart tissues of standard diet fed group. A significant decrease in the expression level of CRP, SAP and IL-6 genes was observed in liver tissues of Gr III and Gr IV compared to Gr II, this reduction in the expression level of inflammatory genes was higher in WGO group than in CO group. On the other hand, no expression was reported for the studied genes in the heart tissue of both groups.

Decrease in the expression levels of the studied genes compared to Gr II although they were higher than in Gr III and Gr IV. GADPH gene expression was used as positive control in examining all the studied genes. Rat studied gene/GAPDH gene ratio was determined for each gene by densitometry which was performed by measuring the photo stimulated luminescence values using gel analyzer pro version software (Fig. 2 A, B, & C).

Table 6. Effect of high fat diet, canola oil and/or wheat germ oil on liver and heart functions.

Groups	GGT (U/L)	Total proteins (g/dl)	LDH (U/L)	CK-MB (U/L)
GI (NC)	29.2±1.9^{a}	6.9± 0.6^{a}	357±17.4^{a}	270.3±19^{a}
GII (HFD)	70.8±4.0^{b}	4.5 ± 0.27^{b}	770±20.3^{b}	676±15^{b}
Change% from NC	142.5	-34.8	115.7	150.1
GIII (HFD+CO)	42±4.6^{c}	5.7 ±0.28^{c}	544±48^{c}	425±26.6^{c}
Change% from NC	43.8	-17.4	52.4	57.4
Change %from HFD	-40.3	26.7	-29.4	-37.1
G IV (HFD+WO)	35±3.4^{a}	6.1 ± 0.22^{ac}	410±22.5^{a}	313.2±10.3^{a}
Change % from NC	19.9	-11.6	14.8	15.9
Change % from HFD	-50.6	35.6	-46.8	-53.7
GV (HFD+M)	73±4.2^{b}	4.8 ± 0.2^{b}	748±26.6^{b}	664±24.4^{b}
Change %from NC	150	-30.4	109.5	146
Change % from HFD	3.1	6.7	-2.9	-1.8

Values are represented as mean ± SD of 8 rats. Each value is considered statistically significant at $\rho<0.05$.Groups sharing the same superscripts is not statistically different.

Histological examination

Histological examination of liver and heart tissues sections of standard fed diet control group showed normal structure of hepatocytes and myocardium (Fig. 3 Aa & Ba) respectively while, deleterious fatty change was observed in the hepatocytes of high fat diet fed group at the periphery of the hepatic lobules as well as surrounding the portal area (Fig. 3Ab).

Also there was fat deposition in between the chambers of the heart associated with oedema and inflammatory cells infiltration in the subpericardium and myocardium in focal manner (Fig. 3Bb). Moderate fatty change was observed in the hepatocytes at the periphery of the hepatic lobules and mild congestion was noticed in the myocardial blood vessels of canola oil treated group, Fig. (3Ac & Bc) respectively. Fig. 3Ad shows mild fatty change in the hepatocytes of wheat germ treated group located at the periphery of the hepatic lobules, while no histopathological alteration was observed in the heart of this group, (Fig.3Bd). Sever fatty change in massive manner in the hepatocytes located surrounding the portal area associated with congestion in the portal vein, and fat deposition between the cardiac. Chambers associated with moderate congestion in the myocardial blood vessels were detected in MO+HFD treated group (Fig. 3Ae & 3Be) respectively.

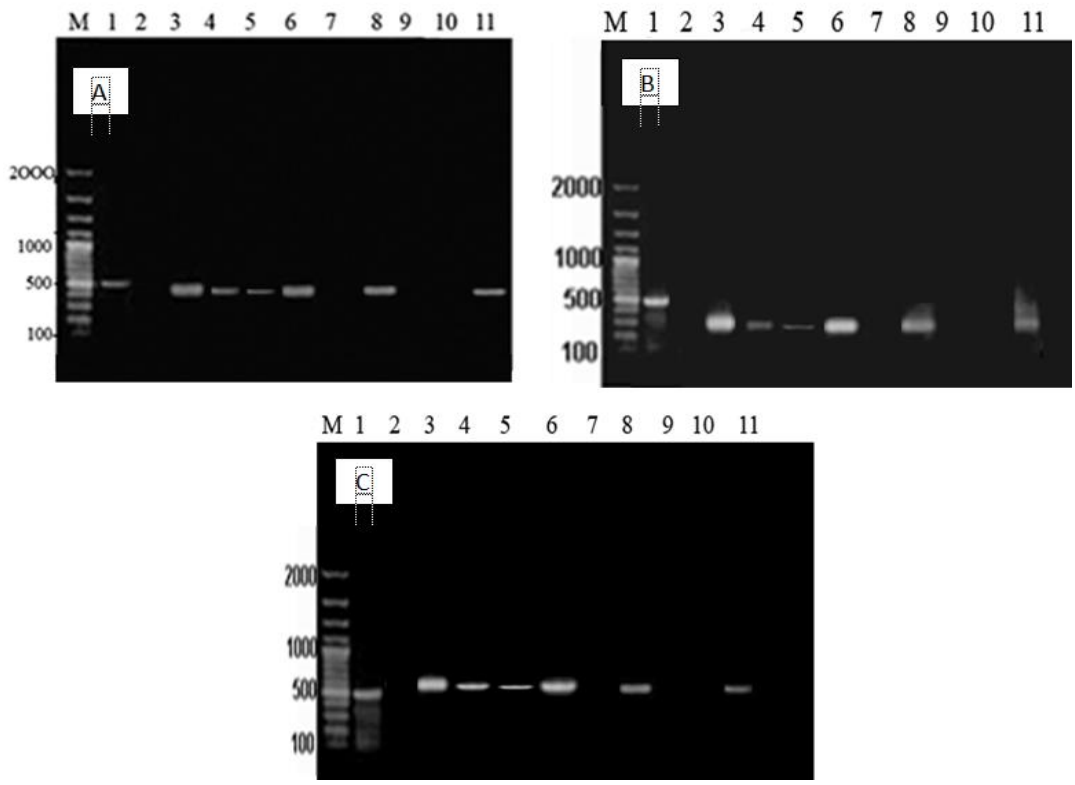
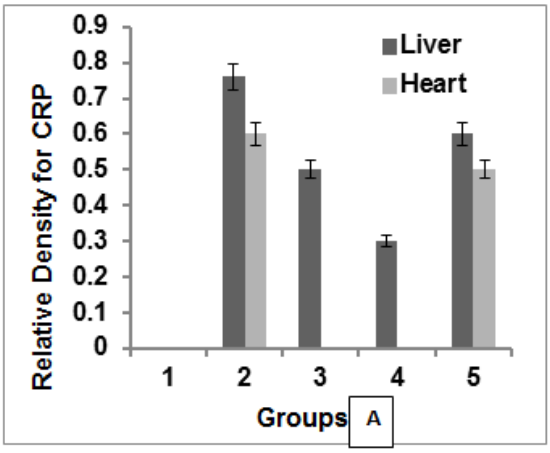
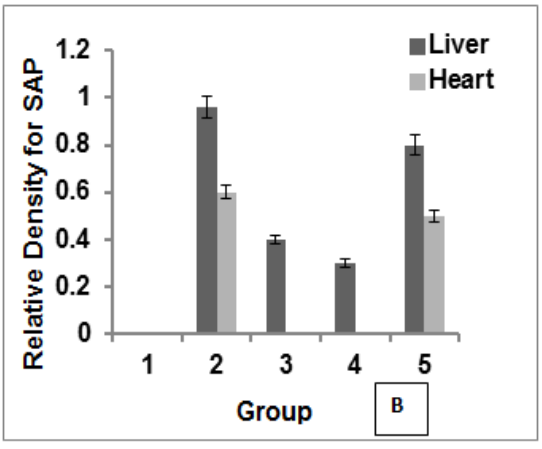


Fig. 1. Gene expression of (A) C-reactive protein (CRP), (B) serum amyloid p component and (C) interlukin-6 (IL-6) in accordance to GAPDH (lane: 1) obtained by semi quantitative RT-PCR in liver (lanes: 2, 3, 4, 5 &6) & heart (lanes: 7 ,8 , 9, 10 & 11) tissues, M: DNA marker.





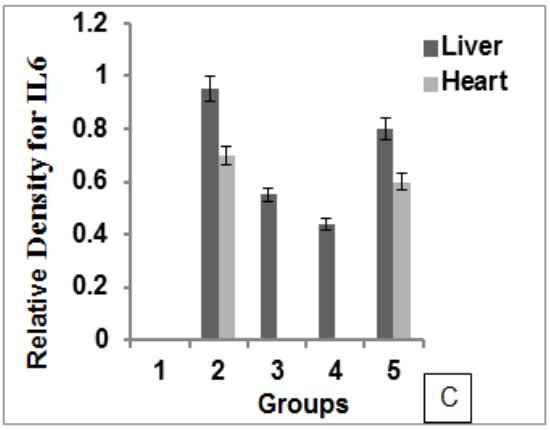


Fig. 2. The relative density of expressed rat, [A) C-reactive protein (CRP), B) serum amyloid p component and (C) interlukin-6 (IL-6)] genes bands in liver and heart tissues.

Fatty changes at the periphery of hepatic lobules (Ac) and pericardial oedema (o) with congestion of myocardial blood vessels (Bc) in CO and HFD fed rats (x80), Mild fatty change at the periphery of hepatic lobules of WGO and HFD administrated group (arrow) (Ad) with intact normal histological structure

of the myocardium (Bd) (x80), congestion in the portal vein with massive fatty change in the hepatocytes (Ae) and fat cuts in between heart chambers associated with congestion of the myocardium blood vessels (Be) in mixed oils fed animals (x80).

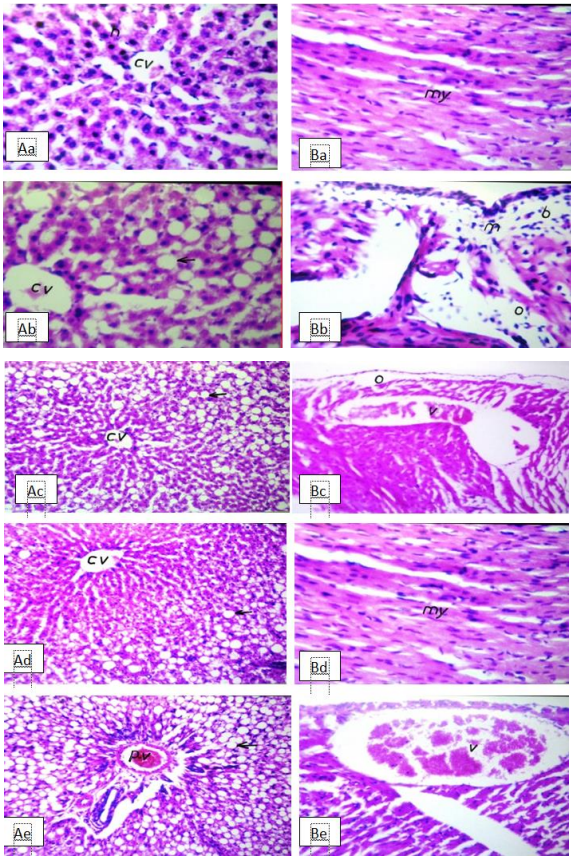


Fig. 3. Photomicrographs of rat liver and heart sections stained with hematoxylin and eosin showing normal histological structure of the central vein (cv) and surrounding hepatocytes (h) (Aa) with normal myocardium (Ba) of standard diet fed group (x80). Sever fatty changes at the periphery of hepatic lobules as well as surrounding portal vein (Ab) with oedema and inflammatory cells infiltration in the sub-pericardium and myocardium (Bb) of animals fed on high fat diet (x80).

Discussion

Dyslipidemia is considered as one of the crucial contributors to cardio- vascular diseases. It has dramatically increased worldwide mostly due to the modern lifestyle and increase in consumption of high-fat diets. Adisakwattana *et al.* (2010) reported that changes in dietary pattern, especially in dietary oil, are among the crucial components of controlling the plasma lipid and lipoprotein levels. An increasing evidence of hypocholesterolemic effects of vegetable oils rich in Polyunsaturated Fatty Acids (PUFAs) was recorded (Ranjbar-Zahedani *et al.*, 2015).

In the present study, the hypolipidemic and antiatherogenic potency of canola and wheat germ oils either individually or in combination were investigated. Induction of atherosclerosis using high fat diet produced a significant increase in body and liver weights of the animals compared to standard diet control group. This observation is in accordance with that of Xu *et al.* (2005) who related this increase to the increased food intake which was also indicated in our results. Moreover, Schrauwen-Hinderling *et al.* (2005) found that high fat diet feeding was accompanied by molecular adaptations that favour fat storage in muscle rather than oxidation. The presence of either CO or WGO in the diet attenuates the increase in whole body and liver weights regardless the increase in the amount of food intake.

Butyrylcholine esterase (BuChE; E.C. 3.1.1.8) is a α-glycoprotein nonspecific esterase hydrolysing other esters as well as choline esters. It takes part in the protidosynthetic pathway and regulates the degradation of butyrylcholine, an intermediate of lipid metabolism. It is found in plasma, pancreas, liver, brain, smooth muscle cells, adipose tissue, intestinal villi, kidney tubules, and macrophages in a soluble form (Silver, 1974). BuChE activity was positively associated with serum concentrations of cholesterol and triglycerides and with measures of overweight, obesity, and body fat distribution (Das, 2012). Administrating high fat diet, group II showed a significant increase in butyrylcholine esterase activity compared to standard diet fed group. This result agrees with that of Vijaya *et al.* (2015) who indicated a positive association between BuChE activity and serum cholesterol and triglyceride concentrations. Kalman *et al.* (2004) reported that high serum lipid concentrations may induce stereoscopic alteration in the enzymatic configuration that modifies BuChE activity or alter expression of the enzyme-encoding gene that determines BuChE concentration and activity.

On the other hand, BuChE may play a role in lipid metabolism whether directly or through a synergistic action with cholesterol esterase (Van Lith and Beynen, 1993). Lipid profile analysis of HFD rats recorded a significant increase in the concentration of all the parameters and AI except in HDL-C level which was decreased by (17%) from the standard diet fed group, Table, (4). Moreover, liver and heart MDA levels of this group were significantly elevated with a significant reduction in the antioxidant capacity compared to the control group, Table (5), indicating dysfunctional lipid homeostasis, which induces oxidative stress by reducing the antioxidant defense potential of tissues and generating free radicals. The oxidation of LDL is a free radical driven lipid peroxidation process with the production of aldehydes that modify LDL apoprotein. The modified apoprotein has an altered affinity to LDL receptor and is scavenged by macrophages in an uncontrolled manner with the development of foam cells and initiation of atherosclerosis (Supriya Simon and Vijayakumar, 2013).

Serum uric acid is considered the major antioxidant in the body that protects against oxidative stress.
It loses its antioxidant ability in the hydrophobic environment and becomes a pro-oxidant. A significant elevation in serum uric acid level was observed in group II compared to normal control group. The pathophysiological link between elevated serum uric acid and atherosclerosis are endothelial dysfunction and inflammation. Superoxide radical produced by xanthine oxidoreductase (XO) [an enzyme involved in the production of uric acid] has been identified as the most probable ROS contributing to cardiac dysfunction. It can bind nitric oxide (NO) to form peroxynitrite (OONO−), a potent non-radical oxidant species inducing endothelial dysfunction by reducing bioavailability of nitric oxide. Also, Gersch *et al.,* (2008) have demonstrated the direct irreversible reaction of uric acid with NO and the production of 6-aminouracil with the consequent reduction of NO. In addition, Gersch *et al.*, (2009) have described the complexity of the reaction of uric acid with peroxinitrite and its pro-oxidative effect through the production of active intermediates capable of alkylating biomolecules containing OH, labile hydrogen or reactive functional groups. In 2006, Ruggiero *et al* reported the significant associations between serum uric levels and inflammatory markers, such as white blood cells, C-reactive protein, interleukin-1 (IL-1), IL-6, IL-6 receptor, IL-10, IL-18, and tumor necrosis factor-alpha suggesting its pro-inflammatory effect. Moreover, uric acid can act as an endogenous danger signal capable of stimulating the innate immune response (Liu *et al.,* 2007).

The molecular analysis carried out in this study correlate with the above findings as it revealed an increase in the expression level of the inflammatory markers CRP, SAP and IL-6 in both liver and heart tissues of HFD fed rats compared to standard diet fed group which showed no expression of the investigated genes in both tissues (Fig. 1A, B & C) and this agree with (Mahmoudi *et al.*, 2007) that inflammation plays a key role in the progression of atherosclerosis. Paffen and De Maat, (2006) found that the acute phase reactant CRP facilitates atherosclerosis through influences on vascular cell activation, monocyte recruitment, lipid accumulation, and blood clot formation. Moreover, Hirano *et al*., (1990) indicated that SAP exists in human atherosclerotic aortic intima and the plasma SAP levels are associated with cardiovascular disease. Lin *et al.,* (1990) noticed that mouse SAP gene can be induced by the direct action of either IL-1 or IL-6. Thus, both CRP and SAP are usually considered to be class 1 acute- phase gene products. Riess *et al,* (2017) concluded that IL-6 elevation in atherosclerosis results in effects on multiple cells involved in lipid processing and plaque formation. It is also the primary determinant of acute phase protein production and helps in the development of cardiovascular disease through the activation of endothelial cells, its pro-thrombotic effects on platelets and promotion of smooth muscle proliferation and macrophage lipid accumulation.

Liver and heart functions analyses were performed in the current work to detect the extent of hepatic and cardiac injury. As shown in Table (6), induced hyperlipidemia causes a significant elevation in the

activity of serum GGT, LDH and CKMB with a marked reduction in serum proteins concentration compared to control rats, indicating functional and structural disturbance in hepatic and cardiac cells.

It is worthy to identify the role of GGT in atherosclerosis. The hypothetical mechanism between GGT and CVD is its prooxidant effect (Onat *et al.*, 2012). GGT catalyses the hydrolysis of reduced glutathione with the production of cysteinyl glycine that triggers iron dependant production of ROS. This reaction leads to the oxidation of LDL and contributing to oxidative events influencing plaque evolution and rupture (Dominici *et al.*, 1999). Also thus far in literature, a linear positive correlation was found between GGT and TC, TG, LDL, uric acid and hs-CRP levels (Jiang *et al.*, 2013).

The aforementioned biochemical and molecular observations were confirmed by the histological results of HFD group (Fig 3. Ab & Bb) which demonstrate the deleterious fatty change in liver and heart cells with the presence of edema and infiltration of the inflammatory cells in subpericardium and myocardium.

The enhanced hyperlipidemia, BuChE activity, hyperuracemia, lipid peroxidation and the reduction in TAC observed in HFD group were ameliorated by supplementing 20% of CO or WGO in HFD. These results are in agreement with (Mutalib *et al.,* 1999) who found that hydrogenated rapeseed oil decreased TC and LDL and increased HDL levels compared to palm oil, also, agree with those of (Hamed *et al,* 2013) who observed the improvement of lipid profile and MDA levels in hypercholesterolemic rat after oral administration of 500mg/kg b w of WGO. In addition, administration of 20% CO or WGO preserves liver and heart tissues against structural and functional disruption as indicated by the marked decrease in serum GGT, LDH and CK-MB activity and the normalization of serum proteins. Of interest, the effect of WGO is found to be superior to that of CO. On the molecular level, a significant reduction in the expression of CRP, SAP and IL-6 genes was recorded in hepatic cells after adding 20% of CO or WGO to HFD, the relative density of the expressed genes was lower in the WGO group compared to CO group (Figs 2.A, B & C). On the other hand, no expression was found for any of the three genes in the cardiac cells of both groups. These data are in harmony with the histopathological results of Gr III that reveal the attenuation of fatty change in hepatocytes and in the reduction of inflammatory cells infiltration in the cardiac tissue (Fig 3. Ac & Bc) respectively. Also, they coincide with those of Gr IV which show the nearly complete amelioration of hepatic and cardiac cells on mixing WGO with HFD (Fig 3. Ad & Bd).

The protective effect of CO is attributed to its constituents as it contains low saturated fatty acids (<7% of total fatty acids), has a sustainable balance betweenω-3 andω-6 fatty acids, It contains a relatively high level of oleic acid (61%) and an intermediate level of PUFA (32%) of which α-linolenic acid makes up approximately one-third of total fatty acids in addition to phytosterols and tocopherols. Hypocholesterolemic effect of OA and LA has been documented which suggested that cholesterol improving components of CO might be due to either reducing synthesis or increasing removal of lipoprotein particles rather than alteration in cholesterol content of LDL fraction (Ranjbar-Zahedani *et al.,* 2015). Albert *et al.* (2002) and Calder (2006) attributed the antithrombotic effect of ω-3 polyunsaturated fatty acids to their incorporation into the platelets where they compete with arachidonic acid for the 2-acyl position of the phospholipid membrane and as substrate for the cyclooxygenase enzyme complex that modulates the production of prothrombotic ecosanoids which decreases the production of the classic inflammatory cytokines TNF, IL-1, and IL-6 and the expression of adhesion molecules involved in inflammatory interactions between leukocytes and endothelial cells.

Moreover, ω-3 polyunsaturated fatty acids reduce the adhesion and the migration of monocytes which are important in atherosclerosis, also, they enhance the production of endothelial derived relaxing factor which is reduced in atherosclerotic vessels (Editorial, 1995).

Oleic acid has also been reported as anti-apoptotic and anti-inflammatory agent via down regulation of cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS) through the activation of nuclear factor-kappa B (NF-κB) resulting in the activation of downstream inflammatory mediators (Kim *et al*, 2015). Wheat germ oil is a valuable source of essential fatty acids including linoleic (56%) and alpha linonelic acids.

It contains also phytosterols which have a role in reducing blood cholesterol level and have an antioxidant effect (Richard *et al*., 2003; Hassanein and Abdel-Razek, 2009). It was reported that policosanole in WGO inhibited the absorption of bile acids (Ng *et al*., 2005), decreased cholesterol synthesis (singh *et al*., 2006**)** and reduced LDL-C levels.

Furthermore, the phenolic compounds found in WGO may also have free radical scavenging activity (Zhu *et al*. 2011), similar to vitamins, and thus preventing lipid peroxidation of cell. WGO is also known as the richest natural dietary source of tocopherols (vitamin E) of plant origin. Vitamin E is a strong antioxidant due to its radical scavenging activity preventing lipid peroxidation of the cell membranes (Liu *et al*., 2008). Furthermore, ceramides in WGO are good anti-inflammatory agents that inhibit neutrophil elastase which plays a role in inflammatory process and alteration of connective tissue constituents (Bizotfoulon, 1995). The anti-inflammatory activities of ceramides are augmented by the presence of vit E in WGO. Carreras, (2000) related the anti-inflammatory effect of vit E to its activity against lipoperoxidation thus preventing the synthesis of inflammation mediator prostaglandins from arachidonic acid.

Interestingly, it was observed throughout the study that combination of CO with WGO in the administrated diet has a lower protective efficiency than the individual oils. This was indicated in the biochemical and histological results of Gr V shown in (Tables 4, 5 & 6) and (Fig 3. Ae & 3.Be) respectively. Although, the expression levels of the inflammatory markers in the liver and heart tissues of the same group are significantly lower than that of HFD group, they are higher than those of GrIII and Gr IV, (Fig. 1. a, b & c and Fig. 2. a, b & c).

In our suggestion, this behavior is attributed to the difference in ALA and LA contents in both oils. Since, CO contains 9% ALA and 14% LA while, WGO contains 7% ALA and 55%LA so mixing both oils may result in an unbalanced omega-6/omega-3 ratio in favor of omega-6 PUFAs which is highly prothrombotic and proinflammatory (Simopoulos, 2016). However, this finding needs further investigation.

Conclusion

The current study demonstrated that dietary supplementation with CO or WGO reduced hyperlipidemia and atherosclerosis by virtue of their lipid lowering effect, antioxidant and anti-inflammatory effects. Also, it demonstrated that WGO has more protective effect than CO.

Thus, the study rationalizes the use of either CO or WGO as a strategy of healthy diet against atherosclerosis.

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Conflict of interest: None

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