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Effect of pumpkin seed oil on lipid metabolism in experimental hyperlipidemic rats

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A B S T R A C T

The present study was designed to investigate the hypolipidemic effect of pumpkin seed oil in experimental hyperlipidemic rats. Fifty male rats were divided into five equal groups. Group 1: normal rats fed on normal diet. Group 2: (hyperlipidemic group) rats administered standard diet+ 20%coconut oil and 1% cholesterol daily for eight weeks. Group 3: (hyperlipidemic +pumpkin seed oil treated group) rats administered standard diet+ 20%coconut oil and 1% cholesterol daily for four weeks followed by atherogenic diet+ 50 mg/Kg b. wt. pumpkin seed oil daily for other four weeks. Group 4: (hyperlipidemic +pumpkin seed oil protected group) rats administered standard diet+ 50 mg/Kg b. wt. pumpkin seed oil daily for two weeks followed by standard diet+ 20%coconut oil and 1% cholesterol+ 50 mg/Kg b. wt. pumpkin seed oil daily for eight weeks. Group 5 : (pumpkin seed oil group) rats administered standard diet+ 50 mg/Kg b. wt. pumpkin seed oil daily for eight weeks. Blood samples collected for serum separation and used for determination of lipids profile (total cholesterol, triacylglycerol, LDL-C, HDL-C, VLDL-C, total lipids and phospholipids), liver marker enzymes (ALT and AST), albumin, kidney function markers (urea and creatinine) and glucose concentration. The results revealed that in group 2 there were significant increases in serum total cholesterol, triglycerides, LDL-C, VLDL-C, phospholipids, total lipids, ALT, AST, urea, creatinine and glucose while HDL-C and albumin were significantly decreased. Meanwhile, administration of pumpkin seed oil resulted in significant decrease in all elevated mentioned parameters and increase in HDL-C and albumin levels. Therefore, it could be concluded that pumpkin seed oil has hypolipidemic and hypoglycemic effect in rats fed high fat diet.

Key words: Hyperlipidemia, Lipids profile, Pumpkin seed oil-

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1. INTRODUCTION

Hyperlipidemia refers to elevated levels of lipids in the blood to describe the manifestations of different disorders of lipoprotein metabolism. LDL stills the best indicator of atherosclerosis risks (Jacobson, 1998). Chemical lipid lowering agents include HMG COA reductase (3-hydroxy-3-methylglutaryl-coenzyme A) as statins, Nicotinic acid, bile acid sequestrants and Fibric acids (Safeer & Lacivita, 2000). The increasing needs for medicinal plants associated with some serious side effects of chemical lipid lowering agents such as rhabdomyolysis, myopathy and an increased

risk of gallstones (Chattopadhyaya & Jindal, 1996). The importance of medicinal plants is for pharmacological research and drug development . Regulation of exploitation and exportation is therefore essential, together with international cooperation and coordination for their conservation so as to ensure their availability for the future (Jayasuriya , 1996).Examples of lipid lowering plants are: leaves of Amaranthus species, Cynara scolymus, Erythrina Indica, etc., seeds of Coriandrum sativum, Celastrus paniculatus, and others (Saghir et al., 2014). The antiatherogenic effect of pumpkin seed might be due to presence of polyunsaturated fatty acids, phytosterols, tocopherols and B-carotene (ElAdawy and Taha, 2001). The majority of total fatty acids in the seeds are isoflavones which act by making the liver better in removal of bad cholesterol from the body by increasing LDL-receptor densities in the liver. The cholesterol lowering effect of flavones might be due to the up-regulation of LDL receptors (Lecumberri et al., 2007). The aim of this study was to evaluate the hypolipidemic effect of pumpkin seed oil in rats through the investigation of serum lipids profile, serum albumin, liver functions (ALT &AST), kidney function markers (urea & creatinine) and glucose.

2. MATERIALS AND METHODS

2.1. Animals

A total number of 50 adult male rats weighting 150-200g about 5 weeks old obtained from the United Company for chemicals, Elsalam city, Egypt. The animals were fed with standard diet and water ad libitum and housed for 7 days for acclimatization before the beginning of the experiment.

2.2. Chemicals' :

Cholesterol powder was obtained from Elbadr Company, Giza. Coconut oil obtained from Salsabil Company, Egypt. Pumpkin seed oil (Pepon) obtained from Arab Company for Pharmaceutical and Medicinal Plants (MEPACO) as box of thirty capsules each contain 300mg. Atherogenic diet was standard diet + 20% coconut oil (Mohamed et al., 2010)+ 1% cholesterol (Lamiaa and Barakat, 2011). Pumpkin seed oil was used in a concentration of 50 mg/kg b. wt. (Friederich et al., 2000).

2.3. Experimental design:

Fifty male adult white albino rats (150-200) gm body weight were used. The animals were divided into 5 equal groups as following: Group 1 (Control normal group): rats fed on normal standard ration. Group 2 (Hyperlipidemic group): rats fed on high fat diet containing 20% coconut oil + 1% cholesterol (20 g + 1 g /100 gm diet respectively) daily for 8 weeks. Group 3 (Hyperlipidemia + pumpkin seed oil treated group): rats fed on high fat diet for 4 weeks followed by atherogenic diet + 50 mg/kg b. wt. pumpkin seed oil daily for 4 weeks. Group 4 (Hyperlipidemia + pumpkin seed oil protected group): rats fed on standard diet + 50 mg/kg b. wt. pumpkin seed oil daily for 2 weeks followed by high fat diet +50 mg/kg b. wt. pumpkin seed oil daily for 8 weeks. Group 5 (Pumpkin seed oil treated group): contained 10 rats fed with standard diet + 50 mg/kg body weight pumpkin seed oil daily for 8 weeks.

2.4. Sampling:

Blood samples were collected after overnight fasting by retro-orbital method for serum separation from all groups at the end of 8th week of high fat diet.

Serum samples: one ml blood was collected in gel tubes then separated by centrifugation at 2500r.p.m. for 15 minutes for serum separation which preserved in refrigerator at -20 °C until used for subsequent biochemical analysis.

2.5. Biochemical analysis:

Hematological studies were determined according to Feldman et al. (2000). Total cholesterol and high-density lipoprotein were determined according to Roeschlau et al. (1974). Triglycerides were determined according to Fossati and Prencipe, (1975). Low density lipoprotein and very low-density lipoprotein were determined according to

Young (1995). Phospholipids and glucose were determined according to Young and Friedman (2001). Total lipids were determined according to Chabrol (1961). Serum Albumin and urea were determined according to Tietz (1995). AST and ALT estimated according to Schumann and Klauke (2003). Creatinine was determined according to Husdan and Rapoport (1968).

2.6. Statistical analysis:

Statistical analysis was performed using the statistical software package SPSS for windows (Version 16.0: SPSS Inc., Chicago, III.). The significance of differences between groups was evaluated by one-way analysis of variance (ANOVA). If one-way ANOVA indicated a significant difference then differences between individual groups were estimated using Fisher s least significant difference (LSD) test, results were expressed as the mean (+) standard error of mean (SEM). A p-value of less than 0.05 was considered significant (Gray and Kinnear, 2012).

3. RESULTS

Results of lipid profile (table 1) after 8 weeks of high fat diet revealed that hyperlipidemic rats (group 2) showed significant increase in total cholesterol, triacylglycerol , LDL-C, VLDL-C, total lipids and phospholipids levels when compared with normal rats (group 1). In contrast, HDL-cholesterol showed significant decrease. Moreover, rats in pumpkin oil treated (group3) and protected group (group 4) showed significant decrease in serum total cholesterol, triacylglycerol, LDL-C, VLDL-C, total lipids and phospholipids concentrations with significant increase in HDL-cholesterol level when compared with hyperlipidemic rats (group 2).

The values of hepato-renal function tests and glucose were changes after 8 weeks of high fat diet presented in table (2). Liver enzymes (AST&ALT) and glucose level showed significant increase in hyperlipidemic rats (group 2) when compared to normal rats (group 1) while serum albumin level showed significant decrease. Serum creatinine and urea concentrations were significantly increased. When compared with pumpkin oil treated (group3) & protected group (group 4) to hyperlipidemic rats (group 2). Liver marker enzymes (AST&ALT) and serum glucose values showed a significant decrease. In contrast, serum albumin level showed significant increase. Urea values showed significant decrease in protected group (group 4) only while for creatinine values, showed significant decrease only in rats in pumpkin oil treated (group3).

Table (1) Effect of pumpkin seed oil on serum lipids profile in rats fed with high fat diet

Parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
T. Cholesterol (mg/dl)	$82.60^{c} \pm 4.85$	$159.00^{a} \pm 9.30$	$131.60^{b} \pm 7.74$	$127.60^{b} \pm 6.88$	$62.20^{d} \pm 2.71$
Triacylglycerol (mg/dl)	$84.20^{c} \pm 2.60$	$155.80^{a} \pm 3.28$	$129.40^{b} \pm 2.79$	$124.60^{b} \pm 2.73$	$66.20^{d} \pm 5.07$
HDL-C (mg/dl)	$28.24^{a} \pm 3.71$	$15.55^{c} \pm 0.85$	$22.78^{b} \pm 1.36$	$21.48^{b} \pm 0.84$	$27.16^{a} \pm 1.67$
LDL-C (mg/dl)	$33.50^{c} \pm 3.26$	$113.00^{a} \pm 9.82$	$95.60^{b} \pm 8.79$	$90.20^{b} \pm 6.73$	$21.80^{c} \pm 2.54$
VLDL-C (mg/dl)	$16.84^{c} \pm 0.52$	$31.16^{a} \pm 0.66$	$25.88^{b} \pm 0.56$	$24.92^{b} \pm 0.55$	$13.24^{d} \pm 1.01$
Total lipids (mg/dl)	$303.40^{d} \pm 8.10$	$569.20^{a} \pm 23.71$	$494.80^{b} \pm 18.11$	$467.80^{c} \pm 15.94$	$222.80^{e} \pm 8.11$
Phospholipids (mg/dl)	$45.00^{d} \pm 1.52$	$85.00^{a} \pm 3.54$	$74.00^{b} \pm 2.72$	$70.00^{c} \pm 2.39$	$34.40^{e} \pm 0.93$

Data are presented as means ± S.E. Different superscripts (a, b, c, d) in the same row indicate significant differences at $P<0.05$. Group 1: normal rats. Group 2: hyperlipidemic group. Group 3: hyperlipidemic +pumpkin seed oil treated. Group 4: hyperlipidemic +pumpkin seed oil protected. Group 5: pumpkin seed oil treated

Table (2) Effect of pumpkin seed oil on hepato-renal functions and serum glucose in rats fed with high fat diet

Parameters	Groups				
	Group 1	Group 2	Group 3	Group 4	Group 5
AST (IU)	$119.20^{c} \pm 2.20$	$226.80^{a} \pm 4.40$	$181.20^{b} \pm 7.68$	$174.60^{b} \pm 7.30$	$88.00^{d} \pm 3.21$
ALT (IU)	$69.80^{c} \pm 2.80$	$132.80^{a} \pm 5.19$	$110.60^{b} \pm 4.18$	$106.60^{b} \pm 3.97$	$63.60^{c} \pm 5.81$
Albumin (gm/dl)	$4.08^{a} \pm 0.14$	$2.52^{d} \pm 0.06$	$2.94^{c} \pm 0.07$	$3.16^{b} \pm 0.07$	$4.08^{a} \pm 0.10$
Urea (mg/dl)	$23.60^{c} \pm 1.75$	$44.20^{a} \pm 3.48$	$48.20^{a} \pm 9.44$	$35.40^{b} \pm 2.86$	$17.60^{c} \pm 0.93$
Creatinine (mg/dl)	$0.51^{b} \pm 0.03$	$0.68^{a} \pm 0.03$	$0.60^{b} \pm 0.05$	$0.68^{a} \pm 0.03$	$0.44^{c} \pm 0.02$
Glucose (mg/dl)	$74.60^{c} \pm 4.18$	$148.00^{a} \pm 4.55$	$123^{b} \pm 3.73$	$122.60^{b} \pm 4.45$	$68.80^{c} \pm 4.18$

Data are presented as means ± S.E. Different superscripts (a, b, c, d) in the same row indicate significant differences at $P<0.05$.
Group 1: normal rats. Group 2: hyperlipidemic group. Group 3: hyperlipidemic +pumpkin seed oil treated. Group 4: hyperlipidemic +pumpkin seed oil protected. Group 5: pumpkin seed oil treated

4. DISCUSION

In this study; the result of serum lipids profile; hyperlipidemia group showed significant increase in total cholesterol, triacylglycerol and total lipids concentrations when compared to control group. These results agree with Lamiaa and Barakat (2011), Sivakumar and Sivakumar (2004) and Shajiselvin and Muthu (2013). Diets supplemented with varying amounts of cholesterol can induce hypercholesterolemia in mice and rats by interfering with the hepatobiliary excretion of cholesterol (Kamesh and Sumathi., 2012). Treated and protected groups caused significant decrease in total cholesterol, TAG and total lipids concentrations when compared to hyperlipidemia group. Such results agree with Gossell-Williams et al. (2008) and FadlAlla et al. (2014). The antiatherogenic effect of pumpkin seed might be due to presence of polyunsaturated fatty acids, phytosterols, tocopherols and B-carotene (ElAdawy and Taha, 2001). The majority of total fatty acids in the seeds are isoflavones which act by making the liver better in removal of bad cholesterol from the body by increasing LDL-receptor densities in the liver. The cholesterol lowering effect of isoflavones might be due to the up-regulation of LDL receptors (Lecumberri et al., 2007). Regarding to serum LDL-C, VLDL-C, HDL-C and phospholipids in hyperlipidemia, rats gave a significant increase in LDL-C, VLDL-C and phospholipids with a significant decrease in HDL were obtained when compared to control group. These results agree with that of Lamiaa and Barakat (2011) and Shajiselvin and Muthu (2013). High-fat diet changes the lipoprotein profile to a more atherogenic one by increasing levels of LDL-C and lowering HDL-C (Srivastava et al., 2000). When comparing treated and protected groups with hyperlipidemia, they showed significant decrease in LDL-C, VLDL-C and phospholipids with a significant increase in HDL-C. Such results agree with Shobana and Jayachitra (2015) and Onuegbu et al. (2015). LDL-C is likely to decrease following a reduction in cholesterol levels. Through regulation of LDL receptor gene, flavonoids increase the number of LDL receptors on the surface of liver cells and LDL is driven into the hepatocyte and removed from the blood stream (Pal et al., 2003).

Concerning to liver markers; hyperlipidemia associated with significant increase in both ALT and AST activities when compared with control group. These results agree with Wang et al.(2013) & De Miranda et al. (2014). Cholesterol-enriched diet increase LDL-C and the oxidative stress (Bhosale et al., 2012). The oxidative stress increased both AST and ALT due to damage to the integrity of liver and consequently, leakage of the enzymes into the serum (Yun et al., 2009). Both treated and protected groups showed significant decrease in liver marker enzymes compared to hyperlipidemia group. Such results agree with Al-Okbi et al. (2014).

pumpkin polysaccharides could increase the activity of superoxide dismutase and glutathione peroxidase and reduce the malonaldehyde in mice serum which shows an increase in antioxidant capacity (Xu, 2000). Hyperlipidemia group showed significant decrease in albumin levels comparing to control group. These results agree with Olorunnisola et al. (2012). The reduction in concentration of serum albumin may be due to the liver damage induced by hypercholesterolemia together with its effect on liver enzymes (Al Hamedan, 2010). Treated and protected groups showed significant increase in albumin levels comparing to hyperlipidemia group. Higher concentrations of albumin emphasize adequate energy status which associated with pumpkin (Wathes et al., 2009).

Regarding to kidney functions; hyperlipidemia group showed significant increase in both serum urea and creatinine concentrations compared to control group. These results agree with FadlAlla et al. (2014). High cholesterol diet was found to increase blood pressure and causing renal injury (Zou et al., 2003). Treated group showed a significant decrease in creatinine level compared to hyperlipidemic group while protected group gave significant decrease in urea parameters. Such results agree with FadlAlla et al. (2014). Antiradical/antioxidant activities of pumpkin seeds might be the cause of improving the renal histological alterations (Makni et al., 2010).

A significant increase in serum glucose level was obtained in hyperlipidemia group compared to control group. These results agree with Ajayi et al. (2004). High fat diet associated with dyslipidemia, insulin resistance and impaired glucose tolerance (Naidu et al. 2016). Compared to hyperlipidemia group, both treated & protected groups caused significant decrease in serum glucose values. Such results agree with Abuelgassim and Al-showayman (2012). Globulins present in pumpkin seeds have a significant anti-hyperglycemic activity (Teugwa et al., 2013).

6. CONCULOSIONS

It could be concluded that, pumpkin seed oil is an effective hypolipidemic and hypoglycemic natural agent and has hepato-renal protective effects against high fat diet induced lipid metabolic disorders in rats.

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