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ORIGINAL ARTICLE

Hypolipidimic and Antioxidant Activities of Cold and Hot-pressed linseed oil in High-Cholesterol Fed Rats

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ABSTRACT

Hyperlipidemia is a metabolic disorders of plasma lipids, triglycerides, cholesterol, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C). Hyperlipidemia is among the leading risk factors associated with cardiovascular diseases. This study aimed to compare the efficiency of cold and hot-pressed linseed oil to decrease the levels of total cholesterol and proteins in high cholesterol fed- Wister rats (n =60) weighing (225-252 g). Linseed oil was fed for seven weeks to Wister rats and control. Many biochemical and physiological parameters were measured by automatic analyzer and the results were statistically analyzed by SPSS.12. In comparison to hypercholesterolemic diet-control rats showed highly significant decrease in values of following physiological parameters; glucose, triglycerides, total cholesterol , low density lipoprotein cholesterol, total protein. In contrast, marked increasing in the value of high density lipoprotein cholesterol when compared with hypercholesterolemic diet- control rats. Highly significant decrease in rats fed with hypercholesterolemic diet enriched with(5g/100g cold pressed linseed oil),in blood measurements of triglycerides, total cholesterol, and low density lipoprotein cholesterol. In contrast, marked increasing in the value of high density lipoprotein cholesterol when compared with rats fed with hypercholesterolemic diet enriched with(5g/100g hot pressed linseed oil). These findings indicate that diets containing Linseed oil significantly improved the physiological parameters of rats. We suggest that Linseed oil as part of food might improve blood parameters and increase high density lipoprotein cholesterol in rats. We further suggest that Linseed oil supplementation act as antioxidant agents, and an excellent adjuvant therapy for rats.

Key words: *Linseed oil, on lipid Metabolism,cholesterol, Hyperlipidemia.*

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INTRODUCTION

Flax (common flax or linseed) or *Linumu sitatissimum*, belonged to family Linaceae. The Genus comprised of about 200 species[1]. Linseed contains 35-45 oil, 28% soluble dietary fiber, and 21% protein. Linseed comprises nutritional valuable components such as protein (200-240 g/kg), dietary fiber (250–280 g/kg) and flaxoil (350-450 g/kg). The health benefits are related with the ingestion of polyunsaturated fatty acids (PUFA) and dietary fiber[1-3].

Linseeds among unique oil seed crops because of its exceptionally high content of α-linolenic acid (45 to 52% of its oil) (ALA), each tablespoon of ground linseed contains about 1.8 grams of plant omega- (E1)[4-6]. High amounts of ALA, were derived from Soybean.

Omega-3 fatty acid plays an important role in prevention or treatment of cardiovascular disease, hypertension, arthrosclerosis, cancer neurological disorders and inflammatory disease. More omega-3 fatty acids intake decreases serum cholesterol which beneficially affects blood pressure, skin diseases, thrombosis atherosclerosis and diabetes, arterial compliance and hyperlipidemia response [7-8].Linseed- but not linseed oil - contains soluble fiber. It might cause diarrhea, cramping, wind, and bloating. Large amounts of flaxseed, especially when not taken with enough water, can cause constipation and even bowel obstruction, flatulence, stomach pains, nausea and constipation[9--13].

In the current study aimed to investigate the efficiency of cold and hot-pressed linseed oil to decrease the levels of total cholesterol and proteins in high cholesterol fed- Wister rats model.

MATERIAL AND METHODS

Linseed Oil:

Cold pressed Linseed Oil was obtained by cold pressing method which means the oil is pressed by great pressure of physical machinery under low temperature,

So it is called cold pressing method. Cold pressed Linseed Oil was obtained from Bio Oils, Canterbury, New Zealand, Containing the Essential Fatty Acids Omega 3, 6 and 9; linseed Oil is nature's richest source of Omega 3. Linseed oil was obtained from non-genetically modified seed.

Hot pressed flaxseed oil was commercially purchased from local market. The oil was extracted by hot pressing method. The oil is produced by physical pressing from oil crops after high temperature frying or steaming. It is the traditional pressing technology with high yield efficiency.

Animals

Healthy young adult male Wister rats weighing (225-252 g) were obtained from The Animal physiology Lab of Faculty of Science Hail University.

The rats were housed in well-aerated individual cages and maintained in a temperature-controlled room (24 ± 1 °C) with a 12 h light/12 h dark cycle, 55±10 % humidity. They were fed with normal commercial chow and water *ad libitum*. Throughout the experiments, animals were processed according to the suggested international ethical guidelines for the care of laboratory animals.

Experimental design:

A total of 60 rats were used in the experiment. The rats were divided into 4 groups of 15 animals each as follows:

Group 1- **NC:** Normal control (normal rats) received normal commercial chow diet and were allowed *ad libitum* access to water.

Group 2- **HC:** fed hypercholesterolemia diet (standard diet + 2% cholesterol) and were allowed ad libitum access to water.

Group 3- **HC-HLO:** hot pressed linseed oil Group received (standard diet + 2% cholesterol) enriched with 5g/100g diet of hot pressed linseed oil and were allowed *ad libitum* access to water.

Group 4-**HC-CLO:** cold pressed linseed oil Group received (standard diet + 2% cholesterol) enriched with 5g/100g diet of cold pressed linseed oil and were allowed *ad libitum* access to water.

Blood collection and determination of physiological parameters

At the end of experimental period, blood samples were collected from retro-orbital eye plexus [3].

Each sample was collected into both heparinized tubes to obtain the plasma and into a dry clean centrifuge glass tube without any coagulation to prepare serum.

Blood was left for15 min at room temperature, then the tubes were centrifugation for 15 min at 3000 rpm and the clean supernatant serum was kept frozen at -20 ºC until the time of analysis for different biochemical analyses, prior immediate determination of serum glucose, triglycerides, cholesterol, high density lipoprotein HDL-cholesterol (HDL-C), low density lipoprotein LDL-cholesterol (LDL-C).

All of these parameters were measured using an automatic analyzer (Architect c8000 Clinical Chemistry System, USA).

Statistical analysis

Statistical analyses were performed using SPSS package for Windows version 13.0. Data are expressed as mean ± SE. One-way ANOVA and two-way ANOVA were used to analyze differences among groups. Post-hoc analyses of significance were made using least-significant difference (LSD) test. Differences between groups were considered statistically significant at $p<0.05$.

RESULT AND DISSECTION

Blood glucose

The mean values of blood glucose of both control and experimental groups are presented in figure 1.1.

No significant differences were observed in blood glucose level of normal rats fed on diets containing the oil of linseed when compared with those rats fed on the control diet after 7 weeks of treatment.

Blood triglyceride, cholesterol, LDL-C and HDL-C

The changes in the levels of serum lipids in control and experimental groups are illustrated in Figure 1. The rats were treated with linseed oil resulted in a significant ($p<0.05$) decrease in the levels of triglycerides, cholesterol and LDL- cholesterol compared to hypercholesterolemic diet control group. While HDL-cholesterol level was significantly ($p<0.05$) increased when compared to hypercholesterolemic diet control group.

The rats exposed to the diets containing the linseed oil for 7 weeks had higher blood HDL- cholesterol than those of hypercholesterolemic diet in control group ($p<0.05$).

Highly significant decrease in rats fed with cold pressed linseed oil blood measurements of triglycerides, total cholesterol, and low density lipoprotein cholesterol. In contrast, marked increasing in the value of high density lipoprotein cholesterol when compared with rats fed with hot pressed linseed oil group.

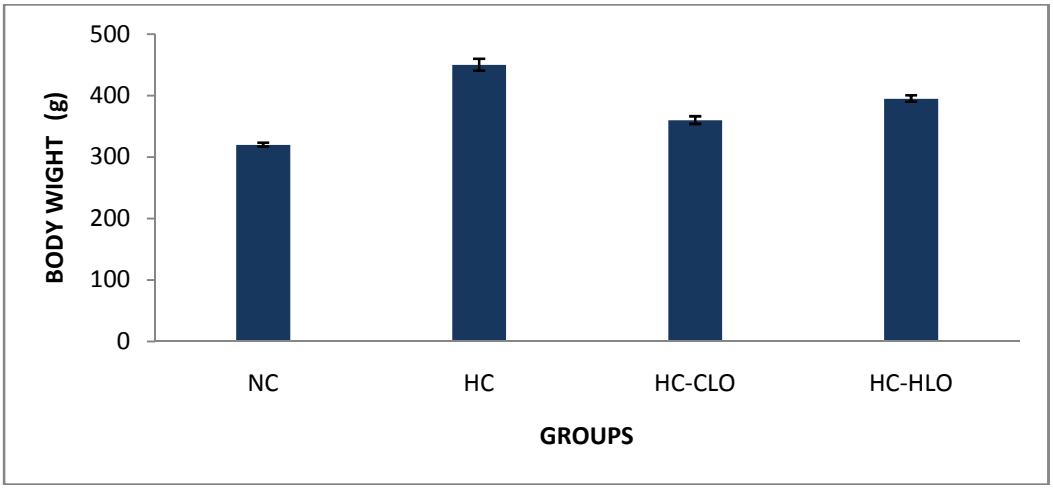

Figure 1.1 Effects of linseed oil supplementation on body weight gain, after 7 weeks of treatment

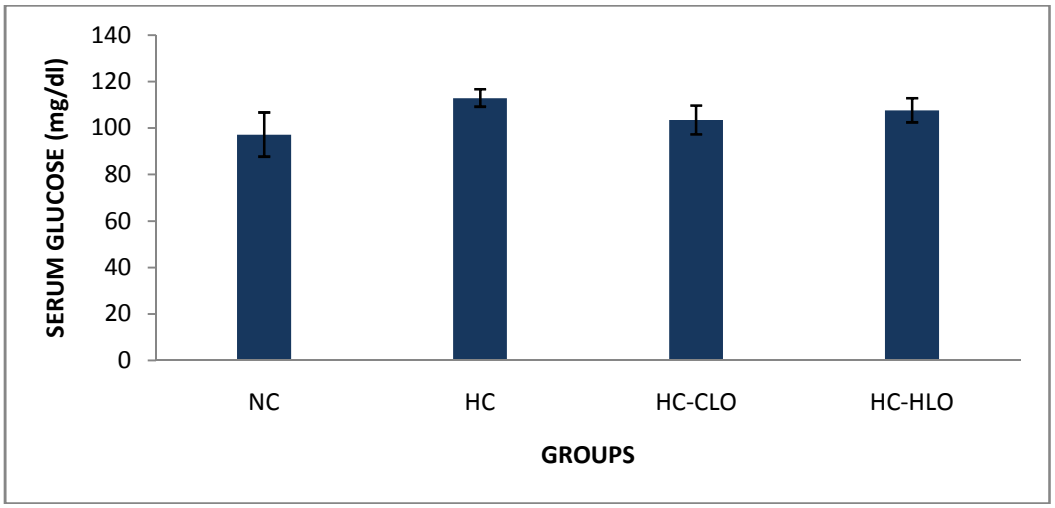

Figure 1.1 Blood serum glucose levels (means ± SE) of different treatments in rats after 7 weeks.

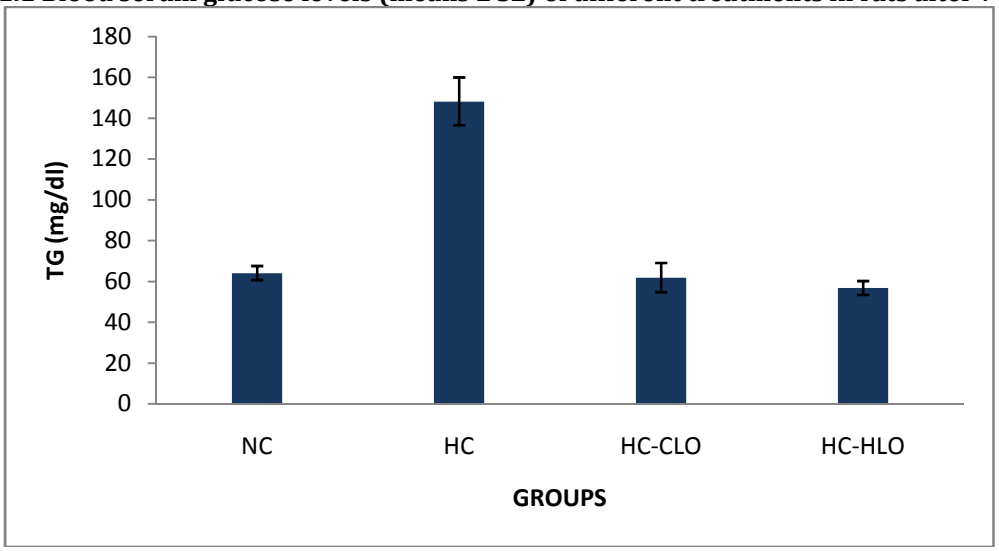

Figure 1.2 Blood triglycerides levels (means ± SE) of different treatments in rats after 7 weeks.

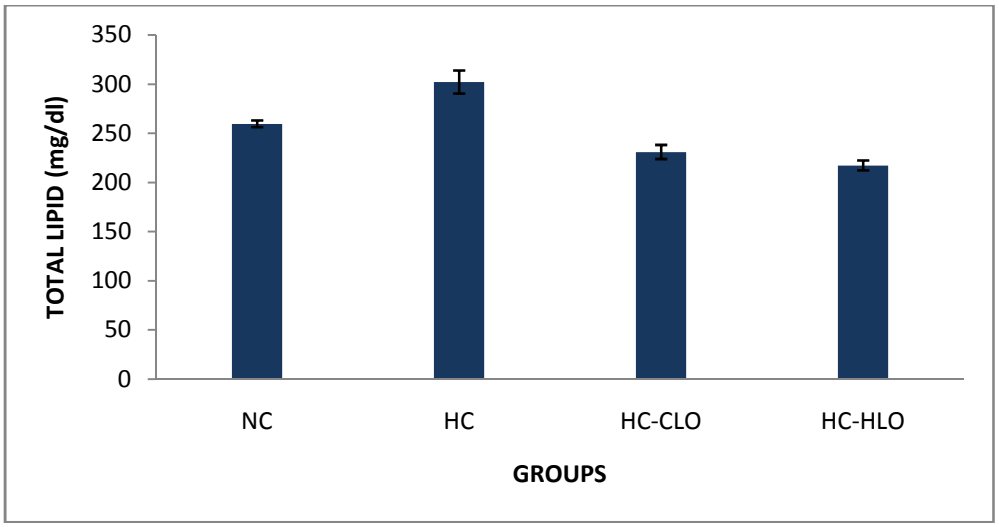

Figure 1.3 Blood total lipid levels (means ± SE) of different treatments in rats after 7 weeks

Table 1.1 Effects of linseed oil supplementation on Cholesterol, HDL-C and LDL-C after 7 weeks of treatment.

Treatments	Cholesterol (mg/dl)	HDL-C (mg/dl)	LDL-C (mg/dl)
NC	88.38±3.31	39.54±2.28	38.52±3.25
HC	253.45±6.49*	21.84±3.92*	90.57±8.19*
HC-HLO	170.64±12.33*#	33.40±3.86*#	69.97±4.56*#
HC-CLO	139.64±7.52*#	37.93±3.41*#	50.43±5.73*#

The number of animals was 10 for each group
NC: Normal control, HC: hypercholesterolemic diet control, HC-HLO: Hot-pressed linseed oil & HC-CLO: cold- pressed linseed oil.
All values are expressed as means ± SE.
Significantly different from normal control (# $p < 0.05$, ## $p < 0.01$ and ## # $p < 0.001$).
Significantly different from: hypercholesterolemic diet control (# $p < 0.05$).

DISCUSSION

Inthis study, it was shown that the levels of high density lipoprotein cholesterol HDL-C in rats fed with HC-CLO hypercholesterolemic diet enriched with(5g/100g cold pressed linseed oil) and with HC-HLO hypercholesterolemic diet enriched with(5g/100g hot pressed linseed oil) groups. These results are in agreement with MORISE et al., 2004 [14].

In the current research illustrated that diets containing Linseed oil significantly improved the physiological parameters of rats and hypolipidimic and antioxidant activities of Cold and hot-pressed linseed oil in high-cholesterol fed rats. Linseed and linseed oil has also been reported to act as anti-arrhythmic, anti-atherogenic and anti-inflammatory agent in addition in its improving of cardiovascular functions [15-17].

The health benefits of all ω-3 fatty acids (ALA, EPA and DHA) have been widely reported for several conditions including cardiovascular disease, hypertension, atherosclerosis, diabetes, cancer, arthritis, osteoporosis, autoimmune and neurological disorders [18].

Linseed oil is the richest source of ALA, which makes about 55–60% of total fatty acids. However, this high content of omega-3 fatty acid makes linseed oil highly sensitive to heat, oxygen and light [18].

The serum cholesterol and triglycerides were significantly decreased in diabetic rats supplemented with of linseed oil. The oil supplementation also result the significant attenuation in the levels of HDL-cholesterol and LDL-cholesterol in serum toward the control level which again strengthen the hypolipidaemic influence of these oils. A variety of derangements in metabolic and regulatory mechanisms, due to insulin deficiency, is responsible for the observed accumulation of lipids [19-20].

Moreover, many minor components of foods, such as secondary plant metabolites, have been shown to alter biological processes which may reduce the risk of chronic diseases in humans *Linumu sitatissimum* popularly known as linseeds an indigenous plant widely available in India and Burma. Different parts

of this plant have been reported to have antiseptic, wound healing and skin disease curing activity [22-24].

We have investigated in this study that Highly significant decrease in rats fed with cold pressed linseed oil blood measurements of triglycerides, total cholesterol, and low density lipoprotein cholesterol. In contrast, marked increasing in the value of high density lipoprotein cholesterol when compared with rats fed with hot pressed linseed oil and These results indicated that the process method has a significant effect on the aroma quality of FSO and may be helpful in evaluating aroma quality as Chang Q. Wei et al reported in 2015 [25].

In conclusion, the present data suggest that using linseed improve blood parameters. The responses in blood parameters in these animals are also demonstrated that oils supplementation may act as antioxidant agents and these oils could be an excellent adjuvant support in the therapy of Hypercholesterolemia and hyperlipidemia.

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