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ORIGINAL ARTICLE

Hypocholesterolemic Effects of Cold and Hot-pressed linseed oil in A Wistar Rat Model

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ABSTRACT
Hypercholesterolemia is metabolic deregulation of cholesterol levels in the blood and the major risk factor that precipitate coronary heart disease and atherosclerosis. This study aimed to compare the efficiency of cold and hot-pressed linseed oil to decrease the levels of total cholesterol and proteins in 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 control rats showed highly significant decrease in values of following physiological parameters; triglycerides, total cholesterol ,low density lipoprotein cholesterol, total protein. In contrast, marked increasing in the value of high density lipoprotein cholesterol. 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. 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.*

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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 linseedoil - 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].

This study aimed to compare the efficiency of cold pressed Linseed oil and hot pressed linseed oil to decrease the levels of total cholesterol, hypocholesterimic Effects and total lipids in a Wister rats model.

MATERIALS 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 by 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: Normal control (normal rats) received normal commercial chow and water *ad libitum*.
Group 2: fed hypercholesterolemic diet (standard diet + 2% cholesterol) and water *ad libitum*.
Group 3: hot pressed linseed oil Group received diet was enriched with 5g/100g diet of hot pressed linseed oil.
Group 4: cold pressed linseed oil Group received diet was enriched with 5g/100g diet of cold pressed linseed oil.

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 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 DISCUSSION

Blood glucose

The mean values of blood glucose of both control and experimental groups are presented in Table 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 Table 1.
The rats were treated with linseed oil resulted in a significant ($p<0.01$) decrease in the levels of triglycerides, cholesterol and LDL- cholesterol compared to untreated rats. While HDL- cholesterol level was significantly ($p<0.01$) increased.
The rats exposed to the diets containing the linseed oil for 7 weeks had higher blood HDL- cholesterol than those of the 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.

Fig: 1.a Effects of linseed oil supplementation on body weight gain, After 7 weeks of treatment.

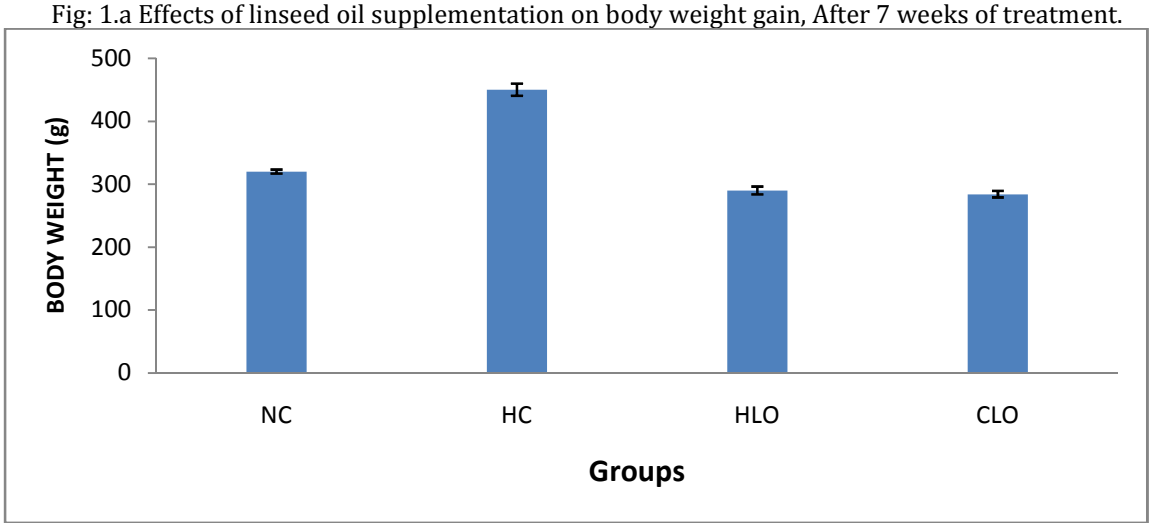

Table 1.a Effects of linseed oil supplementation on blood glucose, triglyceride and total lipid After 7 weeks of treatment.

Treatments	Glucose (mg/dl)	Plasma TG (mg/dl)	Total lipid (mg/dl)
NC	97.18±9.50	64.10±3.46	259.63±2.51
HC	108.50±3.74*	148.21±11.62*	302.11±3.80*
HLO	95.13±2.45#	61.90±2.15*#	230.96±7.01*#
CLO	90.77±3.92#	56.81±3.47*#	217.28±5.97*

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

Table 1.b 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*
HLO	62.21±1.25*#	43.62±2.75*#	31.51±2.67*#
CLO	51.01±9.77*#	50.62±5.53*#	29.44±2.37*#

The number of animals was 10 for each group
NC: Normal control, HC: hypercholesterolemic diet control, HLO: Hot-pressed linseed oil & CFO: 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

Several studies demonstrated that a variety of herbal extracts effectively lowered the glucose level in STZ- induced diabetes mellitus rats).In the present study, normal rats fed on diets containing the oil of fish when compared with those rats fed on the control diet after 7 weeks of treatment[3,6,8]. On the other hand, several researchers also concluded that *Azadirachta indica* alcoholic Leaf Extract significantly lowered the blood sugar level in glucose-fed and adrenaline induced hyperglycemic rats. [8,14].
The effect of diabetes mellitus on lipid metabolismis well established. The association of hyperglycaemia with an alteration of lipid parameters presents a major risk for cardiovascular complications in

diabetes. Many secondary plant metabolites have been reported to possess lipid-lowering properties [15-18].

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].

The impairment of insulin secretion results in enhanced metabolism of lipids from the adipose tissue to the plasma. Further, it has been reported that diabetic rats treated with insulin show normalized lipid levels [3,9.21].

We suggest that the present effects of these oils-treated diabetic rats may be due to its role in normalization of insulin secretion, lowering activity of lipid biosynthesis enzymes, especially cholesterol and or lowering level of lipolysis.

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. *Azadirachta indica* 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].

Several studies demonstrated that water soluble portion of alcoholic extract of leaves of *Azadirachta indica* possesses significant anti-inflammatory, antiserotonin, antifertility and hepatoprotective activity [10-13]. Significant hypolipidemic activity in rats fed on atherogenic diet and antihyperglycemic as well as hypotensive activity have also been reported by us [8].

Significant blood sugar lowering effect of *A. indica* in alloxan and streptozotocin induced diabetic rats have also been reported by several workers [6]. It is well documented that cardiovascular disease induced by hyperglycemia is associated with alterations in serum lipid profiles [9].

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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