

# Evaluation of Hypolipidemic and Hepatoprotective Effects of *Linum Usitatissimum* L. Seeds on Hypercholesterolemic Rats

Chaitali Pattanayak <sup>a≡</sup>, Jyotirmayee Bahinipati <sup>b∞</sup>, Vartika Srivastava <sup>a#</sup>  
and Sougata Sarkar <sup>ct</sup>

<sup>a</sup> Department of Pharmacology, Kalinga Institute of Medical Sciences (KIMS), KIIT deemed to be University, Bhubaneswar, India.

<sup>b</sup> Department of Biochemistry, Kalinga Institute of Medical Sciences (KIMS), KIIT deemed to be University, Bhubaneswar, India.

<sup>c</sup> DM Clinical Pharmacology, Department of Clinical and Experimental Pharmacology, School of tropical medicine, Kolkata, India.

Original Research Article

## ABSTRACT

**Background:** Hyperlipidaemia is a chronic progressive disease, which encompasses various genetic and acquired conditions resulting in inappropriately elevated lipid levels in humans. Several preclinical and clinical studies have demonstrated that dietary supplementation with *Linum Usitatissimum* have beneficial cardiovascular effects like antihypertensive action, antiatherogenic effects, lowering of cholesterol, anti-inflammatory action and inhibition of arrhythmia. There are few studies on hepatoprotective effects of flax seeds in hyperlipidemic animal models. Aim and objectives: Hence, this study was undertaken to evaluate the hypolipidemic and hepatoprotective effect of *Linum* seeds.

**Methods:** The study was conducted for the period of two months in Department of Pharmacology KIMS, Bhubaneswar. Total 36 Albino wistar rats were taken and divided into 6 groups containing 6 rats each. Group II was hypercholesterolemic (HC) control, Group III was treatment control and Group IV to VI rats were given different doses of extract. Blood samples were taken at the end of two months.

**Results:** All the values are expressed as Mean±SD (standard deviation). Significant decrease in lipid parameters and liver enzymes is seen at the end of two months in all 3 groups receiving the extract, where the highest dose of extract used, is near to the standard drug.

**Conclusion:** From this study, it can be concluded that the addition of the flaxseeds to the diet may alleviate the rise in circulating cholesterol levels induced by the high cholesterol diet through its content of alfa-linolenic acid (ALA) and lignins. The hepatoprotective role of flaxseed in hypercholesterolaemia has also been demonstrated in this study.

**Keywords:** *Linum Usitatissimum* L. seeds; hyperlipidemia; hepatoprotective effect; high fat diet.

## 1. INTRODUCTION

Hyperlipidaemia is a chronic progressive disease, which encompasses various genetic and acquired conditions resulting in inappropriately elevated lipid levels in humans. This condition is common throughout the world and the numbers continue to rise at an alarming rate. Lipids traditionally include cholesterol, lipoproteins, chylomicrons, very low-density lipoprotein (VLDL), low-density lipoprotein (LDL), apolipoproteins, and high-density lipoprotein (HDL) [1].

Hyperlipidaemia, particularly elevated LDL cholesterol, is one of the most prevalent risk factors contributing to the development of atherosclerosis, leading to subsequent vascular disease. In contrast, HDL cholesterol helps to regulate cholesterol levels by preventing imbalances that would increase the risk of atherosclerotic vascular disease. Recent studies have reported that high cholesterol is present in 25-30% of urban and 15-20% rural population in India [2]. The most common dyslipidaemia in India are borderline high LDL cholesterol, low HDL cholesterol and high triglycerides [2]. Complications from undertreated or untreated hyperlipidaemia include coronary artery disease, peripheral artery disease, cerebrovascular accidents, aneurysms, type II diabetes, high blood pressure and even death [3]. Hyperlipidaemia is also a known risk factor for fatty infiltration of the liver, which can progress to cirrhosis and liver failure. The hepatic involvement is detected early through elevated liver enzyme tests [4]. The initial treatment modalities of hyperlipidaemia are diet, lifestyle modification and one of the lipid-lowering agents like statins, fibrates, nicotinic acid may be added if required. Statin therapy is very beneficial for a majority of the patients but there are associated complications like myopathy, renal injury, arthralgia, extremity pains, nausea, myalgia, elevated liver enzymes/hepatotoxicity, diarrhoea, and rhabdomyolysis. Up to 5 to 20% of patients taking a statin have reported a muscle-related intolerance [5].

It is well known that essential fatty acids like omega-3 and omega-6 are important for control of cholesterol homeostasis. Long-chain omega-6 fatty acids include linoleic, gamma-linolenic, and arachidonic acids. Omega-3 fatty acids comprise of long-chain alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA). Omega-3 fatty acids have anti-inflammatory, antiarrhythmic and anti-thrombotic properties whereas omega-6 fatty

acids, though proposed to be lipid lowering agents, are proinflammatory and prothrombotic [6]. The typical Western diet includes corn, cotton seed, sunflower and safflower oils which is rich in omega-6 fatty acids due to the abundance of linoleic acid. Conversely, omega-3 fatty acids account for only a small percentage of the daily dietary fat intake walnuts, flaxseed, and canola [7]. This has drastically shifted the dietary ratio of omega-6 to omega-3 fatty acids from an estimated 1:1 in the early human diet to approximately 10:1 in the typical modern American diet. Indian diets are principally vegetarian and relatively low in fat. Also, the main sources of fat are of plant origin rather than animal origin resulting in a diet that is relatively low in saturated FA, high in omega -6 polyunsaturated fatty acids (PUFA), and very low in omega-3 PUFA. The dietary recommendations by the Indian Council of Medical Research (ICMR) have always emphasized on increasing omega-3 PUFA intake in Indian diets; but, it is not quite clear how this can be achieved [8].

*Linum Usitatissimum* L. Seeds is one of the richest plant sources of ALA (alpha linolenic acid), an omega-3 polyunsaturated fatty acid (PUFA). Several preclinical and clinical studies have demonstrated that dietary supplementation with flaxseed have beneficial cardiovascular effects like antihypertensive action, antiatherogenic effects, lowering of cholesterol, anti-inflammatory action and inhibition of arrhythmias [9]. However, studies of hepatoprotective effect of *Linum Usitatissimum* L. Seeds extract on hypercholesterolaemic models are sparse.

### 1.1 Aim of the Study

Hence, this study was undertaken to evaluate the effect of flaxseeds extract on hyperlipidaemia and liver function in albino rats fed on high fat diet and to compare the activity with standard drug Rosuvastatin in animal models.

## 2. MATERIALS AND METHODS

### 2.1 Experimental Animals

The study was conducted on healthy adult albino wistar rats in the Department of Pharmacology in collaboration with Department of Biochemistry at Kalinga Institute of Medical Sciences, Bhubaneswar. The experiments were done from January 2021 to March 2021. Healthy male albino wistar rats weighing between 150-200g were used in this study for assessment of hypolipidemic and hepatoprotective effects. They were housed in standard polypropylene cages

and kept under controlled room temperature ( $24 \pm 2^{\circ}$  C) and humidity ( $55 \pm 5\%$ ) with a 12hr light/dark cycle. The animals were fed with standard laboratory diet and water ad libitum. The animals were acclimatized to the surroundings for one week before being grouped for the experiment. In addition, all the precautions were taken to minimize pain and discomfort to the animals.

## 2.2 Preparation Of Flax Seeds Extracts

*Linum Usitatissimum* L. Seeds were purchased from the local market and shade dried for 2-3 days. The seeds were then powdered by mechanical grinder and stored properly in air tight containers. The powdered form of flax seeds was used for the preparation of aqueous extract. About 100 g of the powdered seed material was soaked in 2 litres of distilled water and shaken thrice daily. The extract obtained after this was concentrated under reduced pressure in rotary evaporator at 50-60 °C leaving a brown residue. The aqueous extracts of the seeds obtained was transferred to a petri dish and kept over water bath (50 °C) until the solvent got completely evaporated. It was stored in air tight glass containers in refrigerator at 2-8 °C for further use in experiments. Toxicity studies done previously in rats at 2000mg/kg dose showed no mortality of the animals [10]. Hence, 100mg/kg, 200mg/kg and 400mg/kg doses of *Linum Usitatissimum* Seed Extract (LUSE) were selected and prepared by reconstituting the extract in distilled water [10].

## 2.3 Hyperlipidemic Diet

High fat diet was prepared by mixing Indian vanaspati ghee and coconut oil in the ratio of 3:1 (v/v). It was administered to the experimental rats at a dose of 3 ml/kg body weight per day by mixing it with the standard laboratory rat diet [11]. This comprised of the high fat diet (HFD) animal model based on the traditional fatty diet consumed by the Indian population.

## 2.4 Study Design

About 36 albino wistar rats of either sex and those having the body weights, biochemical parameters within normal limits were selected for the study. The selected animals were divided randomly into 6 equal groups (Group I – VI). The study was conducted for a period of 2 months during which the animals were administered the drugs along with continuous feeding.

Group I – served as Vehicle control, received standard laboratory rat diet and distilled water orally daily Groups II - VI were fed with high fat diet (HFD) for 2 months along with the drugs, out of which.

Group II – served as Hypercholesterolemic (HC) control, received distilled water (5ml/kg orally) daily Group III – served as Treatment control, received standard drug Rosuvastatin (10 mg/kg, orally) daily Group IV, V, VI – served as Test drug groups, received graded doses of flax seed extract (LUSE) 100mg/kg, 200mg/kg and 400mg/kg orally daily, respectively for a period of 2 months.

The body weights of the experimental animals were measured at the end of 2 months. Blood samples for biochemical analysis were taken from retro bulbar venous plexus of each rat at the end of the experiment [12].

## 2.5 Biochemical Analysis

3 ml of retro-orbital blood sample was collected after overnight fasting. Serum was then separated after centrifuging it at 3000rpm for 20 minutes. Serum was then analyzed for the parameters of lipid profile and liver function tests. Total cholesterol (TC) was done by Cholesterol oxidase peroxidase, end point method [13]. Serum Triglyceride (TG) was done by Glycerol phosphate oxidase method based upon the methodology of Mc Gowen and Fossati et al [14,15]. Calculation of VLDL, LDL and HDL was done by the formula developed by Friedwald et al [16].

Serum SGOT (Serum glutamate Oxaloacetate transaminase), SGPT (Serum glutamate pyruvate Transaminase) and ALP (Alkaline phosphatase) was done by IFCC, kinetic method [17]. All the biochemical analysis was done by semi-automated analyser, Photometer 5010 by Diasys India and Reagents used were from Erba Diagnostics (Mannheim).

## 2.6 Statistical Analysis

Data obtained were expressed as mean  $\pm$  SD (standard deviation) values. The values were analysed by using one-way ANOVA followed by *post-hoc* tests using the software sociostatistics.com. The chosen level of significance was  $p < 0.05$  and interpretation was done accordingly.

## 3. RESULTS

This experiment was undertaken to evaluate the hypolipidemic and hepatoprotective effect of flax seed extract in albino rats whose basal body weights and biochemical parameters were within normal limits. The rats were divided into six groups (I-VI). Group I was the vehicle treated group, Groups II-VI were the hypercholesterolaemic (HC) rats which were administered the standard drug Rosuvastatin (Group III) and the Groups IV-VI received graded doses of *Linum Usitatissimum* Seed Extract (LUSE), group II did not receive any drug. There was an increase in bodyweights of the HC rats as well as the LUSE treated rats who were also on high fat diet.

### 3.1 Effect of LUSE Administration (100 mg/kg, 200mg/kg and 400mg/kg) on Serum Lipid Profile (mg/dl) in Normal and Hypercholesterolaemic Rats

The values (mg/dl) of total cholesterol (TC), triglycerides (TG), very low density lipoproteins (VLDL), low density lipoproteins (LDL) and high density lipoproteins (HDL) obtained from the serum of the experimental rats at the end of the experiment were expressed as Mean  $\pm$  SD and tabulated as shown in Table 1. The vehicle

treated rats (Group I) showed no significant changes in their TC, TG, VLDL, LDL and HDL levels at the end of the experiment. There was a significant increase in the levels of TC, TG, VLDL, LDL and a significant decrease in HDL of the HC rats (Group II) at the end of 2 months and this change is also significant ( $p < 0.001$ ) when compared to the vehicle control rats. It is observed that in the treatment control Group III which received the standard lipid lowering drug - Rosuvastatin, the lipid profile parameters TC, TG, VLDL, LDL were significantly less than the HC Group II and HDL levels improved significantly as compared to Group II. In the LUSE treated rats i.e, Groups IV, V and VI, there was a significant decrease in the levels of TC, VLDL, LDL seen with increase in dose of the LUSE from 100 mg/kg to 400 mg/kg and a highly significant decrease at 400 mg/kg, demonstrating a graded dose response. The effect seen with 400 mg/kg dose of LUSE was almost near to the effect with Rosuvastatin treatment in rats. Whereas, significant decrease in TG levels was observed at the highest dose of 400mg/kg LUSE in Group VI only and not with the lower doses of LUSE in groups IV and V. The HDL levels of the test groups did not show any significant changes as compared to the HC group II.

**Table 1. Effect of LUSE administration (100 mg/kg, 200mg/kg and 400mg/kg) on serum lipid profile (mg/dl) in normal and hypercholesterolaemic rats**

Group	Treatment	TC (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
I	Vehicle control	91.16 $\pm$ 2.11	66.59 $\pm$ 1.77	14.08 $\pm$ 0.43	42 $\pm$ 3.09	58.5 $\pm$ 2.87
II	Hypercholesterolemic control	147.5 $\pm$ 3.30 <sup>a</sup>	101.84 $\pm$ 2.55 <sup>a</sup>	21.09 $\pm$ 0.52 <sup>a</sup>	68.83 $\pm$ 2.43 <sup>a</sup>	25 $\pm$ 3.31 <sup>a</sup>
III	Treatment control (Rosuvastatin 10mg/kg)	93.67 $\pm$ 1.58 <sup>***</sup>	69.48 $\pm$ 1.55 <sup>***</sup>	15.28 $\pm$ 1.43 <sup>***</sup>	37.17 $\pm$ 2.60 <sup>***</sup>	59.81 $\pm$ 2.40 <sup>***</sup>
IV	LUSE-100mg/kg	128.17 $\pm$ 3.22 <sup>*</sup>	99.69 $\pm$ 2.32	18.16 $\pm$ 2.13 <sup>*</sup>	59.83 $\pm$ 2.68 <sup>*</sup>	32.17 $\pm$ 3.41
V	LUSE-200mg/kg	111.83 $\pm$ 2.75 <sup>**</sup>	92.67 $\pm$ 1.45	17.88 $\pm$ 0.23 <sup>**</sup>	49.87 $\pm$ 2.26 <sup>**</sup>	36 $\pm$ 3.33
VI	LUSE-400mg/kg	107.67 $\pm$ 4.52 <sup>***</sup>	86.29 $\pm$ 2.54 <sup>*</sup>	16.32 $\pm$ 0.82 <sup>***</sup>	48.83 $\pm$ 4.80 <sup>***</sup>	39.5 $\pm$ 1.72

Total Cholesterol (TC), Triglycerides (TG), Very low density lipoproteins (VLDL), Low density lipoproteins (LDL) and High density lipoproteins (HDL), *Linum Usitatissimum* Seed Extract (LUSE), All the values are mean  $\pm$  SD, n=6, data analyzed by ANOVA and post hoc test. <sup>a</sup>P < 0.001 when compared to group I (vehicle control), \*P value < 0.05- significant, \*\*P value < 0.01- highly significant, \*\*\*P value < 0.001- very highly significant when compared to Group II (Hypercholesterolemic control)

**Table 2. Effect of LUSE administration (mg/kg) on serum liver enzymes in normal and high cholesterol fed male rats (mg/dl)**

Group	Treatment	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)
I	Vehicle control	102.67 $\pm$ 6.28	27.17 $\pm$ 3.66	83.83 $\pm$ 7.58

II	Hypercholesterolemic control	170.50 ± 5.38 <sup>a</sup>	92.67 ± 7.39 <sup>a</sup>	151 ± 5.10 <sup>a</sup>
III	Treatment control (Rosuvastatin 10mg/kg)	111.66 ± 3.98 <sup>***</sup>	41.17 ± 2.32 <sup>***</sup>	99 ± 3.04 <sup>***</sup>
IV	LUSE-100mg/kg	136.67 ± 3.21 <sup>*</sup>	67.67 ± 2.66 <sup>*</sup>	136.67 ± 3.5 <sup>*</sup>
V	LUSE-200mg/kg	122.16 ± 3.18 <sup>**</sup>	55.1 ± 4.74 <sup>**</sup>	121.17 ± 3.19 <sup>**</sup>
VI	LUSE-400mg/kg	113 ± 3.74 <sup>***</sup>	48.33 ± 3.77 <sup>***</sup>	103 ± 3.89 <sup>***</sup>

*Serum glutamate Oxaloacetate transaminase (SGOT), Serum glutamate pyruvate Transaminase (SGPT), alkaline phosphatase (ALP), Linum Usitatissimum Seed Extract (LUSE), All the values are mean ± SD, n=6, data analyzed by ANOVA and post hoc test. <sup>a</sup>P <0.001 when compared to group I (vehicle control), \*P value <0.05–significant, \*\*P value <0.01- highly significant, \*\*\*P value <0.001- very highly significant when compared to Group II (Hypercholesterolemic control)*

### 3.2 Effect of LUSE Administration (100 mg/kg, 200mg/kg and 400mg/kg) on Serum Liver Enzymes (mg/dl) in Normal And High Cholesterol Fed Male Rats

The level of clinical biochemistry such as Serum glutamate Oxaloacetate transaminase (SGOT), Serum glutamate pyruvate Transaminase (SGPT), alkaline phosphatase (ALP) was evaluated to determine the enzymatic activities of the livers, of the control groups and the experimental groups. The values obtained were expressed as Mean ± SD and tabulated as shown in Table 2. The levels of SGOT, SGPT and ALP of the vehicle treated rats did not show any significant changes from the normal baseline levels at the end of the experiment, however these levels showed a significant rise in the HC Group II. It is seen that in the treatment control Group III which received the standard lipid lowering drug - Rosuvastatin, the SGOT, SGPT and ALP levels were significantly less than the HC Group II. In the LUSE treated rats i.e, Groups IV, V and VI, there was a significant decrease in the levels of SGOT, SGPT and ALP seen with increase in dose of the LUSE from 100 mg/kg to 400 mg/kg and a highly significant decrease at 400 mg/kg, demonstrating a graded dose response.

## 4. DISCUSSION

Coronary heart disease is one of the leading causes of death in the world, this is attributable to genetic factors, unhealthy diet and lifestyle. The principal metabolic causes of atherosclerosis include hyperlipidaemia, hypertension and diabetes mellitus. Most of the population in India consumes food which is rich in oil and/or ghee, which increases the susceptibility to atherosclerotic vascular disease. Coconut oil and vanaspati ghee is also consumed by majority of population leading to development of

hyperlipidaemia. The inclusion of plant-based foods in the diet are gaining importance as lifestyle changes in the fight against coronary heart disease. Several preclinical and clinical studies have shown the beneficial cardiovascular effects of dietary supplementation with flaxseed [9]. This study was undertaken to further evaluate the hypolipidemic and hepatoprotective effects of flax seed extract on a rat experimental model using high fat diet prepared by a combination of vanaspati ghee and coconut oil. The advantage of this model was that it simulates the common man's diet in India and could be easily developed within a shorter period of time [11].

The results obtained from the study showed a significant increase in the serum levels of TC, TG, VLDL, LDL, while the HDL levels decreased significantly in the high fat diet group, similar to the study by Munshi, Renuka P et al. [11]. A major risk factor for the development of cardiovascular disease is increased lipid parameters and decreased plasma concentrations of HDL-C [18]. Supplementation of cholesterol in diet results in a marked increase in the production of cholesteryl ester rich-VLDL by the liver and intestine. The high levels of LDL found in hypercholesterolemic rats may be due to down regulation in LDL receptors by cholesterol and saturated fatty acids present in the high fat diet [19]. It is well documented that elevated total cholesterol and LDL levels promote atherosclerosis and cardiovascular complications. Elevated serum triglycerides are considered as independent risk factor for cardiovascular disease [20]. The high levels of TG observed in the experimental rats may be due to inhibition of 7 $\alpha$ -hydroxylase activity [21]. The results of our study demonstrate that flaxseed extract administration in high fat diet fed rats caused a significant decrease of TC, VLDL, LDL at all doses and a significant decrease in TG levels at only the highest dose. Many animal and

human studies as well have demonstrated no changes in serum HDL-cholesterol and triglyceride (TG) levels. A meta-analysis of 28 studies have concluded that flaxseed produces no significant changes in HDL and TG levels. The same absence of flaxseed effect on HDL and TG levels was observed in an interventional clinical trial [9]. It is observed that whole flaxseed and flaxseed powder can moderately reduce LDL by 5-10% without affecting HDL or TG. Conversely, flaxseed oil does not reliably lower LDL-C, but can reduce TG at high doses [17]. In our study, flaxseed extract did not produce any significant changes in the HDL levels, which is similar to a study where defatted flaxseed (2-3% ALA, but similar in lignan content to traditional flaxseed) reduced TC and LDL-C in rabbits fed an atherogenic diet for eight weeks when compared to controls, but had no effect on HDL-C [22].

Flaxseed, *Linum usitatissimum* also known as linseed, is one of the richest plant sources of ALA (alpha linolenic acid) an omega-3 polyunsaturated fatty acid (PUFA). It also contains phytoestrogen "lignans," specially secoisolariciresinol diglucoside (SGD) and both insoluble, soluble fibers. Alpha-linolenic acid (ALA) is responsible for higher cholesterol secretion into bile leading to a depletion of the intrahepatic pool of cholesterol and thus to an increase in cholesterol synthesis and turnover, this being the major cholesterol-lowering mechanisms of flaxseed. ALA rich diet also decreases hepatic lipid accumulation both by stimulating  $\beta$ -oxidation and by inhibiting fatty acid synthesis [19]. ALA is further metabolized to the longer chain fatty acids eicosapentaenoic acid (EPA), docosapentaenoic acid (DPA), and docosahexaenoic acid (DHA). Eicosapentaenoic acid replaces arachidonic acid in membrane phospholipids, this may reduce the oxidative stress by scavenging reactive oxygen species and subsequently decreasing the activation of proinflammatory transcription factors. DPA is known to inhibit cyclooxygenase-I activity and cellular TNF- $\alpha$  activity, whereas EPA and DHA are known to be involved in various anti-inflammatory and antiatherogenic activities through activation of various peroxisome proliferator-activated receptors [9]. Animal studies indicate lignans and associated substances are responsible for reduced LDL and total cholesterol with flaxseed consumption rather than ALA. Dietary flaxseed lignans are known to possess lipid-lowering and antioxidant properties [17]. It is suggested that the lignans

precursor SGD may directly lower serum cholesterol as well as through modulation of enzymes involved in cholesterol metabolism, including 7 $\alpha$ -hydroxylase and acyl CoA cholesterol transferase [22]. Flaxseed fibres may exert its lipid-lowering effect by producing satiety and thereby reducing caloric intake. They may act by enhancing gastric emptying, decreasing transit time, increasing the rate of bile acid excretion and by reducing bile acid reabsorption through increased faecal excretion of cholesterol. Fibers may also get converted to small-chain fatty acids like acetate and propionate. These small-chain fatty acids can affect multiple metabolic pathways like fatty acid synthesis, oxidation, lipolysis, and cholesterol synthesis. Increased levels of propionate also inhibit the conversion of acetate to lipids and 3-hydroxy-3-methylglutaryl-CoA reductase, thus decreasing hepatic cholesterol synthesis [9]. However, nutrient composition and bioavailability of flaxseed depends on the form used. Three distinct forms of flaxseed are available: oil extract, whole, and milled (ground) seed. Flaxseed oil is composed of 73% PUFA (ALA and linoleic acid), 18% monounsaturated fatty acids, and 9% saturated fatty acids. Soluble fiber and lignans are absent in the oil; however, oil has the highest ALA content and bioavailability. Nonetheless, flaxseed oil alone does not appear to improve the lipid profile and associated CVD risk. Other functional components associated with the lignin complex include cinnamic acid glucoside, an antioxidant, and hydroxymethylglutaric acid, which has hypolipidemic effects. Grinding flaxseed does not alter the composition, but it does improve ALA bioavailability, in part because whole seeds may pass undigested [23], this justifies the use of ground flaxseed for the preparation of the LUSE extract.

Serum SGOT, SGPT, and ALP are the enzyme biomarkers used to monitor the structural integrity of the liver, kidneys, heart and specifically help in the clinical diagnosis of liver damage [24]. In case of injury to the organs due to any reason, these enzymes leak into the blood stream. Therefore, AST, ALT and ALP levels in rat serum were examined to detect the liver damage due to HF diet and to analyse the hepatoprotective effect of flax seed extract on the induced liver damage. The reference ranges of AST, ALT and ALP are 50 to 150 IU/L, 10 to 40 IU/L and 30 to 130IU/L respectively [25]. In the high fat diet fed rats, liver which is the primary organ to metabolise the excess cholesterol, is

affected by oxidative stress. This occurs due to imbalance between the production of free radicals and decreased effectiveness of antioxidant defence system. Increased oxidative stress plays an important role in the chronic inflammatory responses to hypercholesterolemia and atherosclerosis. Thus oxidative stress leads to cell damage related to free radicals and lipid peroxidation, including the destruction of the cell membrane structure and elevation of the liver enzymes in the hypercholesterolemic rats [26].

Our study showed that the flaxseed extract prevented elevation of liver enzymes in rats on HF diet, demonstrating a hepatoprotective effect on the proposed oxidative liver damage. This might be attributed to the fact that ALA needs specific *in vivo* conditions to produce its metabolite, namely dihydrolipoic acid, which has been also reported to be hepatoprotective [26].

Our observation is consistent with other studies on flax and pumpkin seed mixture which have suggested anti-atherogenic and hepatoprotective effects, probably mediated by unsaturated fatty acids present in seed mixture [27]. Another study reported that flaxseed *chutney* possesses antioxidant and significant hepatoprotective properties [28].

Soluble flaxseed gum, also called mucilage, is a series of water-soluble polysaccharides from the husk of flaxseed. *In vitro* assays have demonstrated the powerful antioxidant activity of soluble flaxseed gum. In addition to polysaccharides, oligosaccharides from flaxseed exhibit biological activity as well. These oligosaccharides have several physiological functions, such as regulation of gastrointestinal function, liver protection, and antitumor, reducing the risk of cardiovascular disease. The monosaccharide composition of flaxseed oligosaccharide includes rhamnose, fructose, xylose, mannose, arabinose, glucose, and galactose. The flaxseed oligosaccharide exhibited a strong potential to scavenge hydroxyl radicals. The proposed antioxidant mechanism for the flaxseed oligosaccharide involves hydroxyl radical inducing abstraction of the hydrogen atom bonded to a carbon on the carbohydrate. The resultant carbon centred radical then reacts with hydroxyl radical to form carbohydrate hydroxyl radical complexes [29,30]. It may be valuable to conduct further studies to assess the antioxidant activity of flax gum in cardiovascular diseases that may have a component of oxidation-like atherosclerosis.

Through this study, we observed that raised TG, HDL levels, which are important predictors of cardiovascular disease are not reduced significantly by the flaxseed extract. Presently used hypolipidemic drugs are associated with various complications. Plant derived products like flaxseed are relatively devoid of such side effects, also flaxseed consumption did not interfere and was additive to the cholesterol-lowering effects of cholesterol-lowering medications like statins [9]. Hence, these can be combined with other drugs like statins whose dose can thus be reduced to achieve the desired therapeutic effect, especially in patients who are not able to tolerate the higher doses of statins due to the adverse effects. Flaxseed supplementation can be given in hypertensive, diabetic and obese individuals at risk of hyperlipidaemia. But, more studies are needed in larger populations to determine the dose of flaxseed as adjuvant therapy in patients and as supplements in high risk population.

## 5. CONCLUSION

From this study, it can be concluded that the addition of the flaxseeds to the diet may alleviate the rise in circulating cholesterol levels induced by the high cholesterol diet through its content of ALA and lignins. The hepatoprotective role of flaxseed in hypercholesterolaemia has been demonstrated in this study. Hence, flaxseed or flaxseed powder can be a useful part of a cholesterol-lowering diet, such as the Therapeutic Lifestyle Changes (TLC) diet, which was recently endorsed by the National Cholesterol Education Program.

## DISCLAIMER

The products used for this research is commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

## CONSENT

It is not applicable.

## ETHICAL APPROVAL

All experimental protocols were approved by Institutional Animal Ethics Committee vide No. 1730/PO/Re/13/CPCSEA/13/19.

## ACKNOWLEDGEMENT

We are thankful to the staff and technicians of department of Pharmacology and Biochemistry, who were constantly involved with us in this project.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

## REFERENCES

1. Ballantyne CM, Grundy SM, Oberman A, Kreisberg RA, Havel RJ, Frost PH, et al. Hyperlipidemia: diagnostic and therapeutic perspectives. *J Clin Endocrinol. Metab.* 2000;85(6):2089-112.
2. Gupta R, Rao RS, Misra A, Sharma SK. Recent trends in epidemiology of dyslipidemias in India. *Indian Heart J.* 2017;69(3):382-392.
3. Ford I, Murray H, McCowan C, Packard CJ. Long-Term Safety and Efficacy of Lowering Low-Density Lipoprotein Cholesterol with Statin Therapy: 20-Year Follow-Up of West of Scotland Coronary Prevention Study. *Circulation.* 2016;133(11):1073-80.
4. Assy N, Kaita K, Mymin D, Levy C, Rosser B, Minuk G. Fatty infiltration of liver in hyperlipidemic patients. *Dig Dis Sci.* 2000;45(10):1929-1934.
5. Nissen SE, Stroes E, Dent-Acosta RE, Rosenson RS, Lehman SJ, Sattar N, et al. GAUSS-3 Investigators. Efficacy and Tolerability of Evolocumab vs Ezetimibe in Patients with Muscle-Related Statin Intolerance: The GAUSS-3 Randomized Clinical Trial. *JAMA.* 2016;315(15):1580-90.
6. Covington MB. Omega-3 fatty acids. *Am Fam Physician.* 2004;70(1):133-40.
7. Bradberry JC, Hilleman DE. Overview of omega-3 Fatty Acid therapies. *P T.* 2013;38(11):681-91.
8. Mani I, Kurpad AV. Fats & fatty acids in Indian diets: Time for serious introspection. *Indian J Med Res.* 2016;144(4):507-514.
9. Parikh M, Netticadan T, Pierce GN. Flaxseed: its bioactive components and their cardiovascular benefits. *Am J Physiol Heart Circ Physiol.* 2018;314:H146-H159.
10. Kapuriya PB, Sadariya KA, Bhavsar SK, Thaker AM. Antidiabetic activity of aqueous extracts of *Linum usitatissimum* in streptozotocin induced diabetic rats. *The Pharma Innovation journal* 2018;7(7):149-154
11. Munshi RP, Joshi SG, Rane BN. Development of an experimental diet model in rats to study hyperlipidemia and insulin resistance, markers for coronary heart disease. *Indian J Pharmacol.* 2014;46(3):270-276.
12. Schermer S. *The Blood Morphology of Laboratory Animals.* Green and Co., Ltd., Longmans; 1967:350.
13. Roeschlau P, Bernt E, Gruber W. Enzymatic determination of total cholesterol in serum. *Z Klin Chem Klin Biochem.* 1974;12(5):226.
14. McGowan MW, Artiss JD, Strandbergh DR, Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. *Clin Chem.* 1983;29(3):538-42.
15. Fossati P, Prencipe L. Serum triglycerides determined colorimetrically with an enzyme that produces hydrogen peroxide. *Clin Chem.* 1982;28(10):2077-80.
16. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem.* 1972;18(6):499-502.
17. Tietz N. *Fundamentals of clinical chemistry.* W.B. Saunders Co. Philadelphia PA; 1986.
18. Barakat LAA, Mahmoud. RH The antiatherogenic, renal protective and immunomodulatory effects of purslane, pumpkin and flax seeds on hypercholesterolemic rats. *North Am J Med Sci.* 2011;3:351-357.
19. Hussein SA, Senosi YAE, Ragab MR, Hammad MMF. Beneficial effect of flaxseed oil on lipid metabolism in high cholesterol diet fed rats. *Benha Veterinary Medical Journal* 2014; 27(2):290-301.
20. Parameshwari S, Nazni P. Fatty Acid Composition and Hypolipidemic Effect of Roasted Flaxseed Powder. *Int. J. Pharm. Med. & Bio. Sc.* 2012;1(2): 150-158

21. Prasad K, et al. Reduction of hypercholesterolemic atherosclerosis by CDC-flaxseed with very low alpha-linolenic acid. *Atherosclerosis*. 1998;136: 367-375.
22. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA*. 2001;285: 2486-2497.
23. Korayem GB, Rollins CJ. How useful is flaxseed in improving lipid profiles? *Pharmacy Today*. 2017:16
24. Simon-Giavarotti KA, Giavarotti L, Gomes LF, Lima AF, Verid-iano AM, Garcia EA, Mora OA, Fernandez V, Videla LA, Junqueira VB. Enhancement of lindane-induced liver oxidative stress and hepatotoxicity by thyroid hormone is reduced by gadolinium chloride, *Free Radic. Res*. 2002;36:1033–1039.
25. Sharp PE, La Regina MC, Suckow MA. *The Laboratory Rat*, CRC Press; 1998. Available:<https://www.crcpress.com/The-Laboratory-Rat/Sharp-La-Regina/p/book/9780849325656>.
26. Elshazly SM, El-Moselhy MA, Barakat W. Insights in the mechanism underlying the protective effect of  $\alpha$ -lipoic acid against acetaminophen-hepatotoxicity. *Eur. J. Pharm*. 2014;726:116–123.
27. Faseehuddin Shakir KA, Madhusudhan,B. Hypocholesterolemic and Hepatoprotective Effects of Flaxseed Chutney: Evidence from animal studies. *Indian Journal of Clinical Biochemistry* 2007; 22(1):117-121
28. Makni M, Fetoui H, Gargouri NK, Garoui El M, Jaber H, Makni J, Boudawara T, Zeghal N. Hypolipidemic and hepatoprotective effects of flax and pumpkin seed mixture rich in omega-3 and omega-6 fatty acids in hypercholesterolemic rats. *Food Chem Toxicol*. 2008;46(12):3714-20.
29. Bouaziz F, Koubaa M, Barba FJ, Roohinejad S, Chaabouni SE. Antioxidant properties of water-soluble gum from flaxseed hulls. *Antioxidants* 2016;5:26.
30. Liang S, Liao W, Ma X, Li X, Wang Y.  $H_2O_2$  oxidative preparation, characterization and antiradical activity of a novel oligosaccharide derived from flaxseed gum. *Food Chem* 2017;230:135–144.