ORIGINAL ARTICLE

Hepatoprotective Effect of Flaxseed Oil on Hypercholesterolemia Induced Hepatotoxicity

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ABSTRACT

Aim: To prove the effects of flaxseed oil on hypercholesterolemia induced hepatotoxicity
Methods: Thirty two male rabbits, each fifteen hundred grams weight, mean age of 4 months, were classified into 3 categories. Group 1 administered standard diet in addition half % of lipid dehydrated egg origin, in a course of 64 days. Category two received similar food during initial 32 days, eight milligram per kilogram of baseline flaxseed was also given during upcoming days, Category three was given last category enhanced food in full duration of study. Hypercholesterolemia induced hepatotoxicity was evaluated, serum parameters of total cholesterol, low density lipoprotien, cholesterol, high density lipoprotien, lipids, whole pectrum was evaluated.
Results: Elevated amount of full lipid profile was determined in either category least changes were observed in group three, (p=0.002). Identical changes found present as amount of low density lipoprotien, cholesterol were assessed (p=0.001). The lowering of triglyceride levels at termination of research work in G3 (p=0.008). Variation was observed amongst hypercholesterolemia induced hepatotoxicity and serum parameters of total cholesterol, low density lipoprotien, cholesterol, high density lipoprotien, cholesterol, triglycerides, body weight, groups, Induced reduction was not found to be statistically significant.
Conclusion: Flaxseed has beneficial effects in hypercholesterolemia-induced hepatotoxicity as well as in diseases that have risk factors for the development of the disease.
Keywords: Hypercholesterolemia, Hepatotoxicity, Flaxseed, Experimental study, Functional food

INTRODUCTION

Flaxoil, an ancient medicine and modern functional food, is emerging as an important functional food ingredient because of its rich contents of α-linoleic acid (ALA, omega-3 fatty acid), lignans, and fiber. The flaxseed oil played a worthy role on liver diseases (nonalcoholic) and on the lipid contents of rabbits who are high on cholesterol.[1] Wide range of better outcomes related to the health have been found associated with flaxseed oil, fibers, lignans of flax. Specifically mentionable clinical settings in this regards is in lowering angina pectoris and other heart related disorders like narrowing and hardening of blood vessels, syndrome of elevated blood glucose level, growth, joint pains/achs, resorption and softening of bones, diseases of immune system, diseases of nervous systems. Flaxseed oil has proved to be of immense assistance in protecting plus management of cardiovascular disorders precipitating better outcomes in immune working of body. As a functional food ingredient, flax or Flaxseed oil plays a vital role in bakery items diets, fruit mixtures, milk and milk items, muffins, dehyltingdrated pasta items, macaroni/meat items. Current research results play a part to prove possible anticipated better health outcomes resulting from flaxseed[2].

A large number of acquired and inherited liver diseases are related to metabolism dysfunction. The hypercholesterolemia induced hepatotoxicity which is lipid deposition hepatic problem due to any factor but not due to sedatives. This is frequently observed problem with the intracellular catabolism and anabolism and is featured by the liver steatosis in people who are either scanty users of alcohol or does not use it at all. There has been high index of suspicion about the incidence of hypercholesterolemia induced hepatotoxicity not it is frequently encountered long standing hepatic issue in the European community's cases have been reported in our part of the globe as well. Hypercholesterolemia induced hepatotoxicity includes isolated liver steatosis associated with trivial or aggressive infection plus 'steatohepatitis'. 'Bland steatosis' stays asymptomatic for years and months and does not turn into chronicity[3].

Comparatively hypercholesterolemia induced steatohepatitis remains an entity characterized by liver cell insult resulting into fibrosis of the liver in

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15% of patients. Characteristic presentation of Hypercholesterolemia induced steatohepatitis is swelling of liver cells small areas of liver parenchymal infection plus 'steatosis'. As the sickness advances, cirrhosis appears. Hypercholesterolemia induced steatohepatitis influences both genders similarly, the clinical picture has link with weight of the patient and with "metabolic syndrome" further featured by disturbed levels of cholesterol, deranged levels of insulin in blood, and insulin intolerance[4].

Hypercholesterolemia induced steatohepatitis has been observed predominantly in overweight people and this link has been strongly documented. The "Metabolic syndrome" is another a compliment with hypercholesterolemia induced steatohepatitis where it has been observed that the CVS disease leads to catastrophe in hypercholesterolemia induced steatohepatitis[5]. The reversal of the steatohepatitis leading to cirrhosis is the ultimate target of the regime. Reduction of weight, lowering increased cholesterol in the blood and management of the insulin intolerance lead to the better clinical outcomes[6]. It has been scientifically proved based o observatory finding that inclusion of flaxseed in routine daily diet leads to favorable clinical settings oil. Hypercholesterolemia[7].

Many brands of eatables containing "n-three fat products, sterol esters of botny origen, plus vegetable stuff" many brands have been tried to hault narrowing of the blood vessels due to deposition of cholesterol in tunica media of the arteries[8]. In this context world health organization ranks the flaxseed quite high to lower down the risk of atherosclerosis. Mixture of linolenic acid, lignans, and soluble fibers is the main factor in the food for better outcomes principally for the myocardium[9].

MATERIALS AND METHODS

Thirty two white adult male rabbits, mean age of 4 months were selected for current research work. The study was performed in Zoology Department of University of the Punjab, having ideal environment living conditions for rabbits. Rabbits were classified into three groups, spanning to duration of 8 weeks, Group I (Control group) eleven rabbits; Group II, 11 animals, in them flaxseed was given diet initial half of duration. In Category 3, thirteen animals were administered full duration of research work with flaxseed. During the 57-day research work, rabbits belonging to GI were given unique food "Nuvilab", with one percent lipid of origin dried egg. That food "Nuvilab" did not change cholesterol anabolism and catabolism of rabbits. Category 2 were administered, from 5th week onwards, in addition to standard food, eight milligram per kilogram flaxseed. Category 3 were given Category 2, second food of full duration. Hepatic surgery of animals were done on 57th day. Ketamine 30mg/kg plus intramuscular xylazine 6mg/kg. After study, animals were sacrificed as barbiturate was given.

Laboratory investigations: Serum was taken by putting needle directly into heart on day one of research and 2nd sample just prior to sacrifice. The blood chemistry involved full lipid profile, HDL, LDL and triglycerides were tested in Laboratory. Recommended methods were used to take measurements.

Miccroscopic survey: Hepatic slides were formed by fixing with ten percent formaldehyde buffered phosphate pH=7.6, later covalently bounded with paraffin. Total of 3 pieces and 2 microscopic sections were made, First section was taken from left medial hepatic lobe sample, 2nd from under surface hepatic lobe. In first piece E/H staining was used in 2 pieces "periodic acid Schiff/Gomori's trichrome" stain were used. Microscopic examination was performed for steatosis, hepatic lobe infection, liver cell ballooning, fibrosis status.

Statistical analysis: Percentages represented categorical variables, Average±Standard deviation represented continuous variables Shapiro-Wilks test was put into use for assessing sample normality. Student t-test for quantitative parameters, Mann-Whitney nonparametric test was put into use for comparative study amongst different categories. P value<0.05 was taken as statistical significance. Fisher's exact test instituted for comparisons amongst more than two categories. Bonferroni was used to understand parameters of significance. p<0.05 was considered significance.

RESULTS

Rabbits weight gain was documented to be similar among groups between baseline and euthanasia (Table 1), but isolated assessed, p<0,001 was adjusted value of statistical significance amongst basal weight verses euthanasia's weight. With reference to lipid profile, significant difference amongst different categories was depicted as measurements were taken of full ranges lipids, low density lipoprotein-lidids, plus high density lipoprotein lipids (Tables 2-3).

Table 1: weight changes amongst different categories

Weight	Category	*No.*	Mean ± Standard deviation	*P*
Basal (B)	Group I Group II Group III	11 10 11	1882±267 1876±209 2053±345	0.268
Euthanasia (E)	Group I Group II Group III	11 10 11	2975±165 3154±268 3080±380	0.367
Difference (E-B)	Group I Group II Group III	11 10 11	1120±277 1280±204 1015±244	0.071

Table 2: Lipid profile at baseline (T0) and euthanasia (T8)

Variable	G1 – T0 Mean±SD	G1-T8 Mean±SD	G2 – T0 Mean ± SD	G2 – T8 Mean ± SD	G3 – T0 Mean ± SD	G3–T8 Mean±SD	*p* value G1xG2xG3
Total cholesterol	74.7±35.6	651.2±369.2 **Variation G1** 570.4±368.6	72.9±26.2	733.8±260.8 **Variation G2** 659.2±252.6	63.9±16.8	319.6±213.4 **Variation G3** 258.2±206.5	T0: 0.439 **T8: 0.08** **0.08**
LDL-cholesterol	33.7±32.5	602.3±369.2 **Variation G1** 569.5±367.3	32.4±23.4	693.9±256.2 **Variation G2** 662.8 ±248.4	13.9±7.8	292.6±209.3 **Variation G3** 279.2±204.7	T0: 0.086 **T8: 0.03** **0.02**
HDL-cholesterol	22.2±10.2	22.6±9.6 **Variation G1** 3.4±10.9	19.9±5.6	18.9±5.7 **Variation G2** -2.3±6.9	23.5±4.7	14.0±2.9 **Variation G3** -8.3±5.5	T0: 0.0379 **T8:0.04** **0.023**
Triglycerides	97.7±41.5	64.8±24.9 **Variation G1** -35.6±50.9	115.3±34.4	86.9 ± 35.2 **Variation G2** -21.9 ± 46.5	159±86.2	62.7±29.8 **Variation G3** -96.3±97.6	T0:0.049 T8:0.172 0.155

Table 3: Results of histological analysis

Item	Definition	Score	G1	G2	G3
Steatosis disorder	< 2% 5 to 32% 33 to 66% > 66%	0 1 2 3	28.28% 64.65% 8.11% -	10% 70% 20% -	36.36% 63.64% - -
Fibrosis grade	nill Parallel to sinusoid, parallel to portal	- 1	81.82% 18.18%	100% -	81.82% 18.18%
Lobular inflammation	No foci <2 foci per 200x field 2-4 foci per 200x field >4 foci per 200x field	- 1 2 3	90.91% 9.09% - -	70% 30% - -	72.73% 27.27% - -
Hepatocellular ballooning	None Few balloon cells Many cells/ prominent ballooning	- 1 2	- 72.73% 27.27%	- 60% 40%	- 36.36% 63.64%

DISCUSSION

Hypercholesterolemia induced steatohepatitis has been found to be frequently occurring hepatic problem universally epidemiology of Hypercholesterolemia induced steatohepatitis affected cases is on the increase.[10] This is speculated to be hepatic manifestation of metabolic syndrome. Hypercholesterolemia induced hepatotoxicity has normal links to insulin resistance and metabolic syndrome be its chief part. Hypercholesterolemia induced steatohepatitis, presently has been documented to be an established clinical setting.[11] In progression of disease presence of large amount of fat in cytoplasm of liver cells have been documented, and oxidative pressure precipitating fat peroxidation, that stimulates pro-inflammatory cytokines[12]. No drugs are yet available to manage hypercholesterolemia induced hepatotoxicity or hypercholesterolemia induced steatohepatitis. Basis of treatment is symptomatic relief, reduce weight, manage insulin resistance, lower plod pressure, dyslipidemias-particularly hypertriglyceridemia.[13] Alpha-linolenic fatty acids, is maximally present in larger quantities in flaxseed which makes it storehouse of animal lignan precursors, e.g. secoisolariciresinol diglucoside, pinoresinol, matairesinol, lariciresinol. Flaxseed has properties to act against oxidation, anti-growth, antimitotic, to act against female hahmones like estrogen, to act against aromatase, then having activity against formation of blood vessels, so very effective in hypercholesterolemia induced hepatotoxicity[14]. It secoisolariciresinol diglucoside

lowers elevated cholesterol parameters in animals, that was managed via diet which contained elevated levels of lipids. Another research work done by Carter[15] stated that in comparison to a placebo, giving 100mg of secoisolariciresinol diglucoside consequence as remarkable lowering of LDL/HLD cholesterol ratio, and of the levels in AAT and GGTP, but those levels were elevated in hypercholesterolemia induced hepatotoxicity.

In current research work we found significant elevation of lipid profile during whole period in all categories, plus category three **(**food I association flaxseed addition from very beginning), that change remained lesser verses various categories**.** Identical findings were noted in association with low density lipoprotien[16,17]. It was found that 12-week addition with flaxseed, plus a schedule of altering manners of life, markedly lowered presentation of metabolic syndrome, like lowering weight, measurement of abdominal diameter, lipid profile, low density lipoprotein, apolipoproteins B&E, vascular pressure[18]. About high density lipoprotein findings were that nill marked variation amongst different categories, it was proven in our research work, documented high density lipoprotein lowered in category three in conclusion of work.[19] Meta-analysis by Bueno-de-mesquita et al[20] documents that role of flaxseed in triglyceride levels found G84.

It is observed that in human studies better outcomes in steatosis remained linked to correction of cholesterol abnormalities plus various diseases in line to metabolic syndrome and diseases causing oxidative stress larger samples More research required for longer studies and larger samples.[21] Bjrok et al[22] put forwards the comparison to placebo, giving 100mg in SDG consequence as a marked lowering in low density lipoprotien/high density lipoprotien cholesterol ratio, amount of alanine aminotransferase/ and gamma-glutamyl-transpeptidade, all of them are classically elevated in microscopic range in hypercholesterolemia induced hepatotoxicity. Significant variation between groups of hypercholesterolemia induced hepatotoxicity activity parameter plus isolated segments of hypercholesterolemia induced hepatotoxicity activity score, that lowering was not significant statistically, indicating that flaxseed did not lower severity of steatosis in animals given food containing high levels of lipids as half percent liquid squeezed eggs[23]. All depends upon ample size and duration of research work. We prove better outcome of flaxseed in managing lipid problems linked with hypercholesterolemia induced hepatotoxicity, but remained not effective in lowering liver steatosis.[24] Agular et al[25] concluded that measurement hepatic function indicated an enhanced activity of gamma-glutamyl transpeptidase, but aminotransferase es remained unaffected. So it is stated that flaxseed brings about better outcome to protect and then halts progression of hepatic insult. On the basis of biochemical, hematological and histopathological assessments received, we postulate equally beneficial outcomes of flaxseed in mankind, as pathogenesis and dynamics inflammatory insult in man and rabbits are identical. Yet another research work conducted by Aldercreutz[26] stated that flaxseed reduced low density lipoprotien-cholesterol and total cholesterol. This is stated that ongoing atherosclerotic process was minimally affective in category that got seed verses class that did not.

CONCLUSION

Our results are Identical and comparable to international research, It is documented that administration of flaxseed brings about better outcomes of fat and fat metabolism products in blood plus low density lipoprotien-lipid, flaxseed protects also halts advancement of disease process of hypercholesterolemia induced hepatotoxicity.

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