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HYPOCHOLESTEROLEMIC AND HEPATOPROTECTIVE EFFECTS OF FLAXSEED CHUTNEY : EVIDENCE FROM ANIMAL STUDIES

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ABSTRACT

Rats fed with hypercholesterolemic diet showed a significant increase in serum total – cholesterol, liver homogenate total –cholesterol, HDL-cholesterol and changed LDL-cholesterol, and HDL/LDL ratio in comparison to control. Flaxseed chutney *(FC) supplemented diet (15%, w/w) was found to be more effective in restoring lipid profile changes in rats fed with cholesterol (1.0%). The activities of serum marker enzymes glutamate oxaloacetate transminase (GOT), glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) were elevated significantly in carbon tetrachloride induced rats. Administration of flaxseed* chutney *(15%, w/w) resulted in depletion of serum marker enzymes and exhibited recoupment thus showing significant hepatoprotective effect. It was observed that flaxseed* chutney *supplemented diet could lower the serum cholesterol and as a potential source of antioxidants it could exert protection against hepatotoxic damage induced by carbon tetrachloride (CCl_4) in rats.*

KEY WORDS

Flaxseed chutney*, Carbon tetrachloride, Hypocholesterolemic effect, Hepatoprotective effect*

INTRODUCTION

Flaxseed or linseed (*Linum usitatissimum* L.) is a common name for Linaceae family of plants and for plants of a genus Linum within that family. One species (*Linum usitatissimum*) is a shrub grown extensively for its fibre and seeds. The major nutritional components of flaxseed include oil, viscous lignan-rich fibres (mucilage), protein and minerals, which are analyzed by American Oil Chemists Society (AOCS). Flaxseed is the richest source of a –linolenic acid (18:3n-3), soluble and insoluble fibre, and mammalian lignan precursor secoisolariciresinol diglucoside (SDG) (1, 2). Flaxseed has long history of use in India. Most of us do not know that flaxseed was of native of India and once a staple food crop. Even now, in southern India, flaxseed is partly being consumed at lower levels as flaxseed chutney (FC) and as a raw material for medicines. Flaxseed chutney could be stored for months as a food reserve and valued as a source of nutritional compounds, energy and food ingredients on long journeys. In Northern America and Europe flaxseed has been accepted at low levels as a component in some brands of cereals in specialty breads, as a seed dressing on buns and various other bakery products (3). An expert panel of food safety and nutrition recently highlighted flaxseed as one of the ten promising plant sources of functional food (4). There are numbers of studies indicating the hypolipidemic, hypoglycemic and hypocholesterolemic effect of raw flaxseed and its baked products (1, 5, 6). Current research in nutritional biochemistry continues to identify various therapeutic substances in flaxseed. However, studies on either clinical or nutritional properties of FC have not been undertaken which deserves special attention. The present study was aimed to examine the possible hypocholesterolemic and hepatoprotective effects of FC supplemented diet in rats.

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MATERIALS AND METHODS

Flaxseeds (*Linum usitatissimum*), brown variety, were purchased from the local market of Davanagere, Karnataka, India. University of Agricultural Sciences, Hebbal, Bangalore,

India identified these cultivars as LVF-01. A specimen sample of seeds was preserved for reference. The traditional method of preparation of FC practiced in Southern India is given in Fig.1.

Hypocholesterolemic effect of FC : A total of 18 female mature Wister rats (8 – 10 weeks of age) weighing between 70 and 80 ± 8 gm were provided by the Department of Small

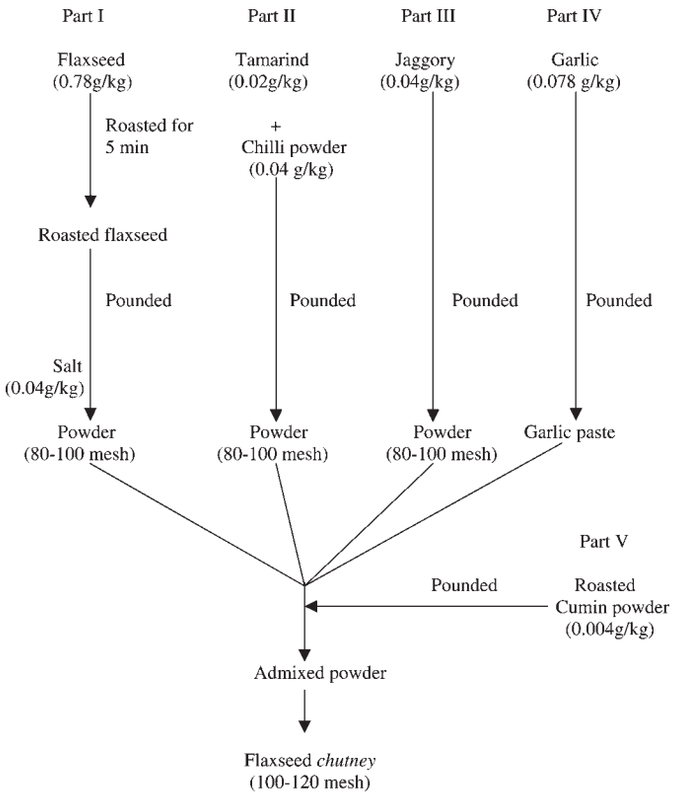

Fig. 1. Diagram showing traditional processing method for flaxseed *chutney*

Animals, Veterinary College, Hebbal, Bangalore, India. Rats were divided into three groups of six animals each and the animals were kept in individual cages under standard conditions (22 - 25 °C; 60-70% relative humidity; 12 h dark/ day cycle). All the animals were fed control diet, test flaxseed chutney diet and water *ad* libitum. The animals were sacrificed according to the guidelines of the current laws of CPCSEA (Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals), India. Groups A-animals were given the basal diet (BD) as control which consisted of % (g/ kg) casein: 240.0; dl-methionine: 2.0; groundnut vegetable oil: 1.0; mineral mix: 40.0; vitamin mix: 80.0; and cornstarch: 658.0. Group B-animals were fed hypercholesterolemic diets (HCD) consisting of BD with cholesterol: 1.0% for three weeks. Group C- animals were supplemented with 15 FC (w/w) and HCD for three weeks. After three weeks of dietary regimen the animals were kept for overnight fasting, sacrificed, blood was drawn by direct cardiac puncture. The liver was immediately removed and stored below 0°C until further use.

Serum and liver homogenate lipids : The lipid peroxidation was measured by the liver homogenate of TBARS by Ohkawa *et al* (7). The malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. Liver homogenate total- lipids were isolated using chloroform: methanol: water (8:4:3 v/v) according to Folch *et al* (8). Serum and liver homogenate total-cholesterol and serum levels of HDL-cholesterol and LDL-cholesterol were determined according to the method described by Zeltkis and Zak (9).

Hepatoprotective effect of FC : In another set of experiments, female mature Wister rats (8 – 10 weeks of age) were randomized into three groups of six animals each. Group A-served as BD control. Group B- animals were fed BD and administered 0.25ml/kg body weight CCl_4 added with liquid paraffin (1:1) orally thrice a week for three weeks. Group C-animals were fed BD supplemented with 15% FC (w/w) for three weeks prior to CCl_4 treatment and continued for another three weeks during CCl_4 treatment. Four hours after the last dose of CCl_4, the animals were sacrificed. The blood was collected by direct heart puncture and serum was separated by centrifugation (3000 rpm at 4 °C for 10 min). Liver homogenate enzymes such as and serum glutamate oxaloacetate transminase (GOT) glutamate pyruvate transaminase (GPT) and alkaline phosphatase (ALP) activities were determined according to Reitmann and Frankel (10). Serum bilirubin was estimated as described by Malloy and Evelyn (11) and total protein was determined by Lowery method (12). Both serum total –cholesterol and liver homogenate total –cholesterol were determined according to Zeltkis and Zak method (9).

Statistical analysis : The results were analysed using Student's *'t'*- test and p values ≤ 0.05 were considered as statistically significant.

RESULTS AND DISCUSSION

Coronary heart disease (CHD) is the leading cause to death in the world (13). LDL—cholesterol (LDL-C) accounts for approximately two thirds of the serum cholesterol pool in a normal subject and is believed to play an important role in arteriosclerosis. Therefore, monitoring of LDL-C in the serum

Table 1 : Effect of flaxseed *chutney* on serum lipids profile of hypercholesterolemic female mature Wistar rats (8 – 10 weeks of age).

Treatment	Serum				
	Liver total Cholesterol (mg/100 ml)	Serum total Cholesterol (mg/100 ml)	HDL - cholesterol (mg/100 ml)	LDL – cholesterol (mg/100 ml)	HDL/LDL ratio
Group A	1.226 ± 0.077	57.2 ± 2.86	18.0 ± 1.62	40 ± 2.56	0.45 ± 0.032
Group B (HCD)	2.025 ± 0.1	68.0 ± 3.65	18.0 ± 1.36	50.0 ± 2.57	0.36 ± 0.036
Group C (HCD + 15% FC)	1.66 ± 0.05	46.4 ± 3.52	18.0 ± 1.76	28.0 ± 3.31	0.643 ± 0.029

Values are mean ± SD (n=6); FC= flaxseed *chutney*, P< 0.05; student *t*-test; cholesterol 1% was administered to group B and C for three weeks.

via diet also provides a basis for the prevention of possible threat of hyperlipidemia (14). LDL-C and HDL-cholesterol (HDL-C) respectively, are positive and negative cardiovascular (CV) risk factors associated with deaths by myocardial infarction (15, 16). The study on group B rats fed with HCD showed 18.88% increase in serum cholesterol over Group A (control) rats. Administration of 15% FC to hypercholesterolemic rats, diet prevented progression of hypercholesterolemia and produced significant reductions in serum total-cholesterol (13.76%), LDL-cholesterol (44.0%) and liver homogenate total – cholesterol (18.02%) without changing HDL-cholesterol levels. A remarkable increase (78.65%) in HDL-cholesterol/LDL-cholesterol ratio was observed (Table 1).

Weekly body weight profile of CCl_4 intoxicated rats showed marginal decrease in body weight gain, which was not significant when compared to control. Generally, CCl_4 is used as model toxicant for the screening of hepatoprotective drugs and nutraceuticals (17). Significantly increased activity of GOT, GPT and ALP from serum and liver homogenate of CCl_4 intoxicated rats. This may be due to cellular damage occurred

Table 2 : Effect of flaxseed *chutney* on serum enzymes profile, serum lipids and liver lipids in CCl_4 intoxicated female mature Wistar rats (8 – 10 weeks of age).

Treatment	Group A (BD)	Group B BD + CCl_4)	Group C (BD + CCl_4 + 15% FC)
Serum Parameters			
SGOT (U/L)	70.45 ± 2.89	96.79 ± 5.90	72.56 ± 3.53*
SGPT (U/L)	50.82 ± 2.89	94.28 ± 8.56	75.42 ± 4.77**
Alkaline phosphatase (U/L)	490. 45 ± 9.31	585.5 ± 9.92	501.33 ± 9.45*
Bilirubin (mg/100ml)	0.28 ± 0.11	0.40 ± 0.019	0.32 ± 0.01*
Total-cholesterol (mg/100ml)	53.87 ± 3.15	64.00 ± 3.18	50.20 ± 3.10
Liver parameters			
SGOT (μ mol/g tissue)	23.25 ± 1.43	68.06 ± 0.16	31.06 ± 1.10
SGPT (μ mol/g tissue)	25.81 ± 0.86	72.11 ± 1.13	36.80 ± 2.60
ALP (μ mol/g tissue)	1.86 ± 0.58	4.83 ± 1.36	2.81 ± 1.05
Total proteins (mg/100ml)	0.88 ± 0.06	1.10 ± 0.04	0.67 ± 0.09
Lipid peroxidation (m^{-1} C^{-1} 10^{-5} units)	13.001 ± 1.08	51.26 ± 8.49	39.914 ± 4.73
Total cholesterol (mg/100ml)	0.32 ± 0.01	1.356 ± 0.027	0.293 ± 0.016*

Values are mean ± SD (n=6); FC= flaxseed *chutney*, **p< 0.05; p<0.005*; student *t*-test; paraffin mixed CCl_4 (1:1) administered orally (1.25ml /kg body weight) to all groups except control group A; thrice a week for three weeks.

in the liver cells (Table 2). There was significant increase in the concentration of serum bilirubin, serum total-cholesterol and liver homogenate TBARS after CCl_4 administration in HCD fed group B rats in comparison to control group. There was no significant variation in the activities of serum GOT, GPT, ALP and hepatic lipid peroxidation in 15% FC *per se* administered group. This indicates that administration of FC into the rats diet prevents leakage of these enzymes and restores the activity of emzymatic variables. These findings are also substantiated by studies with the treatment of Ginkgo biloba (18).

Significant increase in hepatic lipid peroxidation was observed after CCl_4 intoxication. Its toxicity requires cleavage of the bond between carbon and chlorine. Cleavage takes place after binding of CCl_4 to cytochrome p-450 apoprotein in the mixed function system located in the hepatocellular endoplasmic reticulum (19). CCl_4 cleaved into trichloromethyl radical (CCl_3^{o}), which further reacts with molecular oxygen to form trichlormethyl peroxy radicals ($CCl_3\ O_2^{o}$) (20, 21).

These two radical (CCl_3^{o} and $CCl_3\ O_2^{o}$) combine with cellular lipid and proteins to induce lipid peroxidation by abstracting hydrogen (22). In lipid peroxidation, due to oxidative deterioration of polyunsaturated lipids in the presence of reactive oxygen species and transition metal ions, several peroxide radicals are generated. These highly reactive species will get decomposed to yield a wide range of cytotoxic products. Such as aldehydes (including malondialdehyde, MDA), 4-hydroxynonenal and others. Since flaxseed contains a wide variety of phenolic compounds mainly lignans, it may neutralize the free radicals including CCl_3^{o}, $CCl_3\ O_2^{o}$, and peroxides formed due to CCl_4 intoxication thus showing its hepatoprotective effectiveness. Thus flaxseed *chutney* possesses antioxidant and significant hepatoprotective properties. This makes us to extrapolate the benefits to human beings and consider flaxseed *chutney* (~15%) as an affordable ingredient for formulating functional foods of human diet. Further studies are warranted to assess the active nutraceutical principles present in the FC.

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