



AMELIORATION OF LIPIDS BY ALCOHOLIC EXTRACT OF *TRIGONELLA FOENUM GRAECUM* LINN. (TFG) SEEDS IN ATHEROGENIC DIET INDUCED HYPERLIPIDEMIA IN ALBINO RABBITS.

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ABSTRACT

Hyperlipidemia is a major risk factor for cardio-vascular diseases. Hyperlipidemia is characterised by an abnormal elevation in one or more of the serum lipids such as total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG). *Trigonella foenum graecum* linn. resembles guar gum in viscosity and is rich in high density fiber content, the property of which are found to be effective in hyperlipidaemia. The present study was taken to evaluate amelioration of lipids by alcoholic extract of *Trigonella foenum graecum* seeds in suitable animal experimental model. Blood samples were collected on day 0 from albino rabbit and basal serum lipid estimation was done. Hyperlipidaemia was induced with high cholesterol diet in different groups of animals. Blood samples were collected on day 31. Serum was analysed for total cholesterol, TG, HDL and LDL-cholesterol by the enzymatic method. $P < 0.05$ was considered statistically significant. Administration of TFG extract at 100, 200 and 400 mg/kg dose significantly lowered the LDL/HDL ratio and AI values comparable to the standard drug showing the beneficial effect of this plant in preventing atherosclerotic incidence. The beneficial effect of this plant may be due to the presence of diosgenin, flavanoid and antioxidant properties.

KEYWORDS: *Trigonella foenum graecum*, hyperlipidaemia, atherogenic index, high cholesterol diet



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INTRODUCTION

Excessive quantities or improper lipid intake may result in hyperlipidemia which is characterised by an abnormal elevation in one or more of the serum lipids such as total cholesterol(TC), low-density lipoprotein cholesterol (LDL-C) and triglycerides(TG). Hyperlipidemia is considered to be a major risk factor for cardio-vascular diseases including atherosclerosis, myocardial infarction, heart attacks etc¹. Atherogenic diet are high cholesterol diet and saturated fats rich. Anti-hyperlipidemic drugs have attracted considerable attention because of their potential to prevent cardio-vascular diseases by retarding the accelerated atherosclerosis. Further, it has been found that the natural carbohydrates rich in fiber content are effective against hyperlipidemia and ischaemic heart disease². *Trigonella foenum graecum* Linn.(TFG) is one such compound which resembles guar gum in viscosity and is rich in high density fiber content³. More-over, the seeds have been shown to possess hypocholesterolemic effect in rats⁴ and dogs⁵. TFG of the family *fabiaceae* is an annual herbaceous plant grown throughout the tropics. TFG(Fenugreek in English, Methi in Hindi) is claimed to possess medicinal properties like hypolipidemic, anti-diabetic, antioxidant etc⁶. No adverse effect, organ toxicity or mortality were detected in albino mice and rats upto 2mg/kg and 5gm/kg body wt. respectively on acute toxicity testing⁷. So this study was conducted in the Dept. of Pharmacology, RIMS, Imphal to evaluate the hypolipidemic effect of ethanol extract of seeds of TFG in albino rabbits fed with high cholesterol diet(HDC).

MATERIALS AND METHODS

The seeds of TFG were procured from local markets and authenticated (Ac. No.-0003627) by Prof. P.Kumar M.U, Centre of advanced study in life science.

Preparation of extract

The seeds were powdered and defatted with petroleum ether (40°C-60°C) for a period of 10 hours. The defatted materials were evaporated and again extracted with 95% ethanol for 24 hours and dried (yield-10%). The extract was used as suspension in 0.5% carboxy methyl cellulose(CMC).

Animals

Albino rabbits (1.5-2 kg) of either sex were housed in standard cages and kept under controlled room temperature (24±2°C), relative humidity(60-70%) in a 12hours light-dark cycle. The rabbits were given standard laboratory diet and water *ad libitum*.

Drugs and Chemicals

The following chemicals and drugs were used: Tab. Atacor(atorvastatin, Dr.Reddy's Company), TFG extract, 0.5% carboxy methyl cellulose(CMC), Serum cholesterol, High density lipoprotein(HDL) and Triglyceride(TG) estimation kits(Human Company,

Germany), Cholesterol A.R.Powder(Himedia Company), coconut oil(KMP company).

Ethical clearance

All the experimental protocols were approved by the Institutional Animal Ethics Committee(IAEC), RIMS, M.U., Imphal(Reg. no. 1596/GO/a/12/CPCSEA).

Phytochemical studies

Studies showed presence of flavanoids, trigonelline, steroidal saponins, amino acids, plenty of fibers etc.^{5,8}.

Study design

The method of Bopanna KN et al(1997)⁹ and Haytham Met al (2014)¹⁰ with slight modification were followed to investigate the hypolipidemic effect. Thirty albino rabbits (n=30) of either sex weighing between 1.5-2kg were divided into five groups of six animals in each group. During the whole period of experiment they were kept in separate cages and maintained with normal diet(100mg/day) and water *ad libitum*. On day 0, blood samples were collected and basal serum lipid estimation was done by the enzymatic method¹¹.

Induction of hyperlipidemia¹²

Hyperlipidemia were induced in all the animals in different groups by daily feeding with high cholesterol diet(HCD) using intragastric feeding tubes from day 1 to day 30. HCD contains cholesterol A.R powder(400mg/kg) dissolved in 5ml of coconut oil. Along with induction of hyperlipidemia all the animals are maintained on normal diet(100mg/kg daily). The extract and standard drug (atorvastatin) were suspended in 0.5% CMC. The dose of atorvastatin were extrapolated from human dose. Along with induction of hyperlipidemia the animals in the different groups were treated with equal volume of 2 ml/kg body wt. of the different drugs for the same duration of one month in the morning as follows:

- Group 1- HCD+ vehicle(0.5% CMC)
- Group 2- HCD + 100mg/kg wt. of extract
- Group 3- HCD + 200mg/kg wt. of extract
- Group 4- HCD + 400mg/kg wt. of extract
- Group 5- HCD + Atorvastatin(1mg/kg)

Collection of blood

Following overnight fasting blood samples were collected on day 31 from marginal ear vein of rabbits. Serum were separated by centrifugation for 10 minutes at 4000 r.p.m and analysed for total cholesterol, TG, HDL and LDL-cholesterol by the enzymatic method using commercial kits from Human Company, Germany¹¹.

Statistical analysis

Data were analyzed by one way ANOVA followed by Dunnett's test. A level of P<0.05 were considered significant.

RESULTS

Table 1
Effect of TFG extract on serum lipids in albino rabbits

Serum lipids	Group(per kg body wt.)	Basal value(mg/dl)Day 0	Mean changes in values(mg/dl) Day 31
Cholesterol	1-Control	73.0 ±2.6	365.0 ±2.2
	2-100mg(TFG)	73.7 ±1.2	268.3 ±1.0
	3-200mg(TFG)	72.7 ±1.7	211.5± 2.3
	4-400mg(TFG)	73.7 ±1.2	145.8± 4.4 [#]
	5-Standard	73.3 ±1.4	155.2 ±1.8
TG	1-Control	51.2±1.3	116.8±2.3
	2-100mg(TFG)	51.3±1.6	79.3±1.4 [#]
	3-200mg(TFG)	52.7±1.9	25.2±1.7 [#]
	4-400mg(TFG)	51.3±1.2	25.2±2.7 [*]
	5-Standard	51.7±2.4	25.8±2.4 [*]
LDL	1-Control	32.6±0.8	273.9±2.3
	2-100mg(TFG)	32.1±1.9	186.4±1.2 [*]
	3-200mg(TFG)	31.3±2.3	136.9±3.5 [*]
	4-400mg(TFG)	32.0±2.8	85.5±3.7 [#]
	5-Standard	31.8±2.4	84.5±3.3 [*]
HDL	1-Control	30.5±1.0	67.3±1.7
	2-100mg(TFG)	31.3±1.2	66.0±1.1
	3-200mg(TFG)	30.8±1.1	66.0±1.9
	4-400mg(TFG)	31.5±1.9	62.7±2.6
	5-Standard	31.2±1.6	63.8±3.3

$AI = (Total\ cholesterol - HDL-C) / HDL-C$. LDL-C/HDL-C ratio was calculated as the ratio of plasma LDL-C to HDL-C level.

Values are mean± SEM, n=6 in each group

^{*}P<0.001, when compared with control

[#]P>0.05, when compared with standard

Cardio vascular risk- ratio was calculated on day 31 with the help of Atherogenic index(AI) and LDL-C/HDL-C ratio^{13,14}. The results are shown in the table 2.

Table 2
Cardio vascular risk- ratio

Group(mg/kg.)	AI values	AI values
1 (Control)	4.42±0.3	4.07±1.35
2 (100mg extract)	3.07±0.01 [#]	2.82±1.10 ^{##}
3 (200mg extract)	2.20±0.02 [#]	2.07±1.84 ^{##}
4 (400mg extract)	1.34±0.07 [*]	1.36±1.42 [*]
5 (Standard)	1.43±0.45 [*]	1.32±1.00 [*]

^{*}P<0.01, compared to control

[#]P<0.01, compared to standard

^{*}P<0.01, compared to control

^{##}P<0.01, compared to standard

Feeding with HCD for 1 month severely increased plasma total cholesterol, TG and LDL-C levels reaching about 5 times, 3 times and 8 times the basal values of control animals respectively. The reduction in total cholesterol, TG and LDL-C levels at different doses of the test drug (100 mg, 200 mg and 400 mg/kg) and also the standard drug (1.4 mg/kg) after 1 month's concurrent feeding with HCD were found statistically significant (P<0.001) when compared with control values. The 400 mg/kg dose of test drug also showed comparable results with that of the standard drug. Whereas, no significant change was detected in the plasma HDL-C levels. The cardiovascular risk ratio calculated with the help of atherogenic index and LDL-C/HDL-C showed significant reduction with the test drug at 100, 200 and 400 mg/kg dose and also the standard drug. The reduction in these values at 400 mg/kg dose of the extract was comparable with that of standard drug, atorvastatin.

DISCUSSION

Hyperlipidemia specially total cholesterol and LDL-C is a major risk factor that can facilitate the development of coronary artery disease and progression of atherosclerotic lesions. It is recently reported that TG play a key role in regulation of lipoprotein interaction to maintain normal lipid metabolism leading to coronary artery disease¹⁵. Moreover, higher plasma TG levels have been attributed mainly to increase population of small dense LDL-deposits which are very atherogenic and enhanced cholesteryl ester mass transfer from apolipoprotein B containing lipoprotein (i.e VLDL and LDL) and HDL remodelling in human plasma¹⁶. So, this study was undertaken to evaluate the hypolipidemic effect of TFG extract in high cholesterol diet fed rabbits. Induction of hypercholesterolemia by feeding experimental animals a high cholesterol diet (HCD) has been suggested by many scientists as a reliable model for atherosclerosis in human^{12,17}. In the animal model used, feeding with HCD for 1 month severely increased plasma total cholesterol, TG and LDL-cholesterol levels reaching about 5 times, 3 times and 8 times the basal values in control animals respectively which is in collaboration with the findings of other

workers^{18,19}. Administration of TFG extract at doses of 100mg, 200mg, 400mg/kg dose is comparable with that of standard drug, atorvastatin. No significant change is detected for HDL level. It is desirable to have higher plasma HDL and lower LDL-C levels to prevent atherosclerosis, since there is a correlation between an increase LDL/HDL ratio and raised atherogenic index(AI) values and development of atherosclerosis. Since the atherogenic index of plasma predicts the presence of small, dense and highly atherogenic LDL and HDL particles, therefore it serves as a reliable tool for screening dyslipidemia²⁰. Administration of TFG extract at 100, 200 and 400 mg/kg dose significantly lowered the LDL/HDL ratio and AI values, showing the beneficial effect of this plant in preventing atherosclerotic incidence was studied. In the present study, it may be suggested that the ethanolic extract of TFG seeds containing steroidal saponins specially diosgenin may interact with bile acids in the digestive

tract to reduce serum lipids¹⁷. Moreover its antioxidant properties may have a role in its hypolipidemic effect²¹. The flavanoid content of TFG may also have a role in raising LDL receptor densities in liver to remove LDL cholesterol from blood.

CONCLUSION

The present study shows that the ethanolic extract of seeds of *Trigonella foenum graecum* Linn. has significant anti-hyperlipidemic action independent of the concentration. However, further study is needed in order to assess the long term benefits of TFG administration.

CONFLICT OF INTEREST

Conflict of interest declared None.

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