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INTERACTION OF *TRIGONELLA FOENUM-GRAECUM* (FENUGREEK) AND *ACACIA CATECHU* (BLACK CATECHU) FOR ANTIHYPERLIPIDEMIC ACTIVITY

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ABSTRACT

Combined herbal preparations may contain ingredients which interact with each other and an example like Liquorice which states that it is used as a common ingredient in many traditional chinese medicines prescriptions because it reduces the toxicity of other herbal ingredients. High cholesterol diet in rats exhibited significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C. Effect of one week treatment with Fenugreek ethanol extract (FE) at a dose of 250 mg/kg and Catechu ethyl acetate extract (CE) at a dose 500 mg/kg and its low dose combination (150 mg/kg of FE and 250 mg/kg of CE) and in high dose combination (350 mg/kg FE and 750 mg/kg of CE) along with high cholesterol diet in rats. Antihyperlipidemic effects in lipid profile were exhibited in FE, CE and its low and high dose combination. Combine therapy of FE and CE showed higher anti hyperlipidemic effect than the individual FE and CE. Hypolipidemic activity of FE may be attributed due to presence of steroidal saponins, alkaloids and free amino acids. Hypolipidemic activity of CE may be attributed due to presence of carbohydrates, tannins, flavonoids and fixed oils.

Key words: Anti hyperlipidemic, Black catechu, Fenugreek, Cholesterol, Triglyceride

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INTRODUCTION

Hyperlipidemia is a major cause of atherosclerosis and atherosclerosis associated conditions such as coronary heart disease, ischemic cerebrovascular disease and peripheral vascular disease[1,2]. Animal and Human studies have established the role of cholesterol in the development and progression of atherosclerosis. LDL-cholesterol (LDL-C) constitutes approximately 60-70% of total serum cholesterol. Epidemiological studies directly implicated LDL-C to the development of atherosclerosis and coronary heart disease (CHD)[3]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease. Currently available hypolipidemic drugs have been associated with a

number of side effects like diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin etc[4].

Drug interaction between different herbs is an upcoming and important aspect to be studied these days. If these interactions between different herbs are studied, best utilization of the available herbal therapies can be done which can give a valuable treatment and cure with low number of side effects to the society[5]. Hence, for present study two known antidiabetic and antihyperlipidemic plants *Trigonella foenum graecum* Linn. and *Acacia catechu* (L.f.) Willd. are taken to study the interaction between the two herbs for its antihyperlipidemic activity. Fenugreek (*Trigonella foenum graecum* Linn.) has been used as a cooking spice and flavouring agent for centuries and it is a member of Leguminosae family[6]. In Ayurveda and Siddha, Methi has been mentioned as *Tikta rasam, Ushanveeryam, Vata-Kalphaharam*, in fevers, dysentery. In Unani, it is described as resolvant, aphrodiasic, diuretic, emenagogue, expectorant in bronchitis, piles, externally in inflammatory conditions[7]. *Acacia catechu* (L.f.) Willd (Leguminosae), is a moderate size tree. The tree is widely distributed in India from the Indus eastward to Assam and throughout the peninsula. It is also common in the dry plains and lower hill forests of upper and lower Burma. It is used in asthma, cough, bronchitis, colic, diarrhea, dysentery, boils, in skin afflictions and sores and for stomatitis[8]. The concentrated aqueous extract of heartwood of acacia catechu is known as khayer gum used in leprosy[9].

MATERIAL AND METHODS

Collection and Authentification of the Seeds and Heartwood

The seeds of *Trigonella foenum graecum* Linn and Heart wood of *Acacia catechu* (L.f.) Willd. were collected in January, 2008, from Modasa, and authentified by Dr. M.S. Jangid, Botany Department, Sir P. T. Science College, Modasa, Gujarat, India.

Extraction of Plant material

Fenugreek: 500 g of dried and coursely powdered seeds of fenugreek was extracted with petroleum ether by maceration for two days. Filter and mark is extracted with 95% ethanol in soxhlet apparatus for 6 hr. Filter the filtrate. The filtrate was concentrated on water bath using petridish. The temperature was maintained at 55 ^{0}c. The Yield is 25 g[10].

Black Catechu: The powdered catechu was defatted with non polar organic solvent such as petroleum ether (40-60^{0}c) to remove the non polar substances from the dried material. After defatting, catechu was extracted with 95% ethanol. Then the dried ethanol extract was again extracted with ethyl acetate. The filtrate was concentrated on water bath up to drying. The yield at end of extraction was 27%[11].

Pharmacological Evaluation for Anti Hyperlipidemic Activity

Animals: Sprague Dawely female rats weighing 200-250 gm were acclimatized to the experimental room having temperature 23 ± 2 ^{0}C, controlled humidity conditions, and 12:12 hour light and dark cycle. Animals were caged in polypropylene cages in a group with maximum of three animals per cage. The rats were fed with standard food pellets and water *ad libitum*. The study was approved by Institutional Animal Ethical Committee, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa, Gujarat, India (IAEC/BMCPER/07/2008-09).

Induction of Hyperlipdemea: High Cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2% , with standard powdered standard animal food. The diet was placed in the cage carefully and was aministered for seven days.

Preparation of the Extracts: The extract of Fenugreek seed was dissolved in water and different doses of 150 mg/Kg, 250 mg/Kg and 350 mg/Kg were prepared. In the same manner the extract of Black Catechu heart wood was dissolved in water and different doses of 250mg/Kg, 500 mg/Kg and 750 mg/Kg were prepared.

Treatment Protocol: The experimental animals were divided into six groups, six animals in each group.
Group-R1:-Normal,
Group-R2:-High cholesterol diet control,
Group-R3:-High cholesterol diet treated with alcoholic extract of Fenugreek (250mg/kg, p.o.),
Group-R4:-High cholesterol diet treated with Ethyl actate extract of Catechu. (500 mg/Kg, p.o.),
Group-R5:-High cholesterol diet treated with mixture of alcoholic extract of Fenugreek (150mg/kg, p.o.) & Ethyl actate extract of Catechu (250 mg/Kg, p.o.),
Group-R6:-High cholesterol diet treated with mixture of alcoholic extract of Fenugreek (350mg/kg, p.o.) & Ethyl actate extract of Catechu. (750 mg/Kg, p.o.)
Treatment was given daily for seven days orally.

Blood Sample Collection and Analysis: After seven days, blood samples were collected from the tail vein after 8 h fast and allowed to clot for 30 minutes at room temperature. Blood samples were centrifuged at 3000 rpm for 20 minutes. Serum was separated and stored at -20°C until biochemical estimations were carried out. serum samples were analyzed spectrophotometrically for cholesterol, triglyceride and HDL-C was estimated using diagnostic kits. VLDL, LDL, HDL-ratio and atherogenic index were calculated[12, 13].

Statistical Analysis: Results are presented as mean ± SEM of 6 animals. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey test. Data were considered statistically significant at P value ≤ 0.05.

RESULT AND DISCUSSION

High cholesterol diet in rats exhibited significant elevation of plasma total cholesterol, triglycerides, LDL-C, atherogenic index and reduction of HDL-C. Earlier studies have shown a significant elevation in the lipid parameters in response to high cholesterol diet.[5,6]. Effect of one week treatment with Fenugreek ethanol extract at a dose of 250 mg/kg and Catechu ethyl acetate extract at a dose 500 mg/kg and its low dose combination (150 mg/kg of FE and 250 mg/kg of CE) and in high dose combination (350 mg/kg FE and 750 mg/kg of CE) along with high cholesterol diet in rats. Results were summarized in following Table 1 &2.

Serum Cholesterol (SC): Result (Figure 1) showed that High cholesterol diet rats exhibited higher serum cholesterol levels as compared to untreated rats. The FE showed reduction in cholesterol level equal to CE in hyperlipidemic rats. The decrease in cholesterol level in low dose combination was less than high dose combination. In low dose combination, the decrease in cholesterol levels in hyperlipidemic rats was higher as compared to the individual FE and CE. Result showed that the high dose combination gave maximum reduction in cholesterol level as compared to the FE and CE doses as well as the low dose combination.

Serum Triglyceride: Results stated that (Figure 2) FE showed higher reduction in triglyceride level than CE in hyperlipidemic rats. While low and high dose combination showed significant decrease in triglyceride level. The decrease in triglyceride level in high dose combination was more than low dose combination. Low dose combination of drugs lowered the serum triglycerides up to normal.

Serum HDL-Cholesterol: The CE showed (Figure 3) increase in HDL-C level as compared to FE. The increase in HDL-C level in low dose combination was less than the high dose combination. Result showed that the high dose combination of FE and CE shows more increase in HDL-C level than FE and CE doses as well as the low dose combination. High dose combination of drugs increased the serum HDL-C up to normal.

Serum LDL: High cholesterol diet rats exhibited significantly higher LDL levels than untreated rats. Treatment with FE and CE and its combination (low dose and high dose) significantly lowered levels of LDL in rats. The decrease in LDL levels in combination doses was very high than individual FE and CE. High dose combination showed maximum decrease in LDL level than FE and CE doses as well as the low dose combination. (Figure 4)

Serum VLDL: High cholesterol diet rats exhibited significantly higher VLDL levels than untreated rats. Treatment with FE and CE and its combination (low dose and high dose) significantly lowered levels of VLDL as compared to high cholesterol diet rats. The decrease in VLDL levels in combination doses was very high than individual FE and CE. High dose combination of FE and CE showed Maximum decrease in VLDL level than FE and CE alone as well as the low dose combination. Low dose combination of drugs lowered the serum VLDL up to normal. (Figure 5)

Atherogenic index: Serum atherogenic index of high cholesterol diet rats was significantly decreased when treated with FE and CE and its low and high dose combination. The FE and CE showed equal reduction in atherogenic index. The decrease in atherogenic index in high dose combination was maximum. In low dose combination the decrease in atherogenic index in hyperlipidemic rats was higher than the individual FE & CE. (Figure 6)

HDL-Ratio: HDL-Ratio of high cholesterol diet rats was significantly increased in serum when treated with single standard dose of FE and CE and its low and high dose combination. The increased HDL-ratio in combination doses was very high as compared to single standard dose of FE and CE. High dose combination of FE and CE showed maximum increased in serum HDL-Ratio. (Figure 7)

CONCLUSION

The above results summarized that Hypolipidemic effect were exhibited in FE, CE and in combination. Combination therapy of FE and CE showed higher anti-hyperlipidemic effect than the individual FE

and CE. The High dose combination showed the maximum anti hyperlipidemic effect compared to individual dose as well as low dose combination. Hypolipidemic activity of FE may be attributed due to presence of steroidal saponins (diosgenin, yamogenin, tigogenin and neotigogenin), alkaloids (mainly trigonelline) and free amino acids and activity of CE may be attributed due to presence of carbohydrates, tannins, flavonoids and fixed oils.

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TABLES AND FIGURES

Table: 1 Serum Lipid Profile in Rat (n=6)

Sr. no.	Group	LDL-C	VLDL	HDL-Ratio	Atherogenic index
1	Normal	6.21±6.57	17.49±0.49	215.72±70.51	2.08±0.10
2	Control	326.14±21.65	36.36±1.29	4.47±0.35	11.2±0.09
3	FE	262.26±2.63	18.35±0.73	11.15±5.80	2.93±0.18
4	CE	256.83±5.53	19.74±2.73	12.50±2.43	2.85±0.67
5	FE + CE (Low dose)	204.95±1.86	16.05±3.55	17.30±4.47	2.09±0.73
6	FE + CE (High dose)	150.91±7.95	12.61±2.17	24.48±3.95	1.57±0.27

Table: 2 Serum Lipid Profile in Rat (n=6)

Sr. no.	Group	Serum Cholestrol	Triglyceride	HDL-C
1	Normal	65.82±1.90	77.44±2.45	42.12±1.20
2	Control	378.73±14.25	181.80±6.47	16.23±1.90
3	FE	311.89±5.32	91.79±3.69	31.27±1.95
4	CE	311.13±10.28	98.71±13.65	34.56±2.01
5	FE + CE (Low dose)	259.24±7.84	80.25±17.79	38.28±2.41
6	FE + CE (High dose)	203.54±4.55	63.07±10.24	40.02±0.60

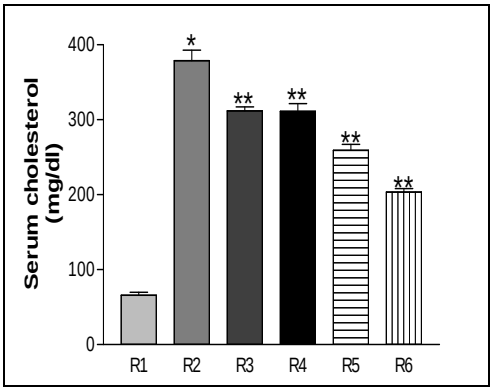

Figure 1: Serum Cholesterol Levels in Rats.

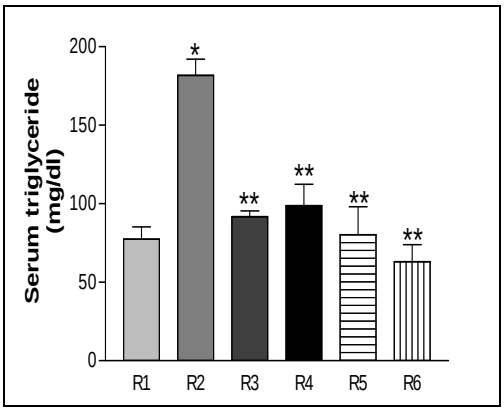

Figure 2: Serum Triglycerides Levels in Rats.

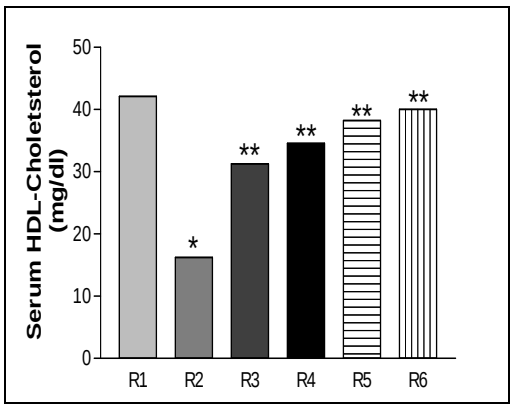

Figure 3: Serum HDL-C Levels in Rats.

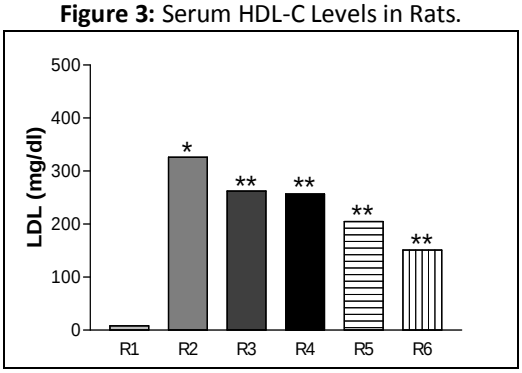

Figure 4: Serum LDL Levels in Rats.

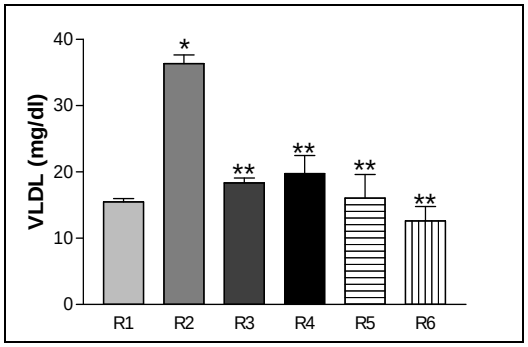

Figure 5: Serum VLDL Levels in Rats.

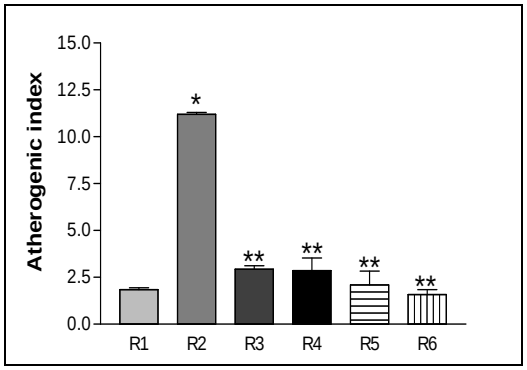

Figure 6: Atherogenic Index Levels in Rats.

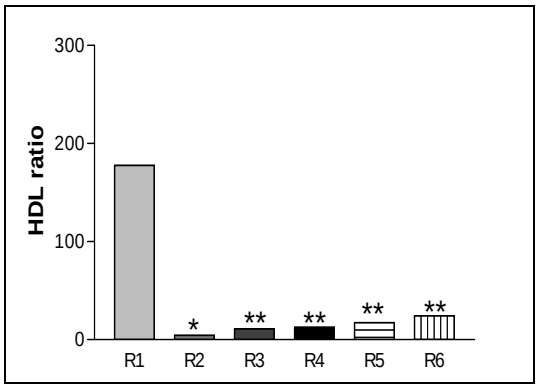

Figure 7: Serum HDL-Ratio in Rats.