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Preventive effects of *Trigonella foenum-graecum* and *Allium sativum* against Triton induced hyperlipidaemia in rabbits

Hina Imran[1,A,D], Tehmina Sohail[1,B-C], Rashid Ali Khan[1,C], Shahla Basit[1,E], Shazia Syed[2,F]

[1] *PCSIR LABS COMPLEX, Karachi, Pakistan*
[2] *Department of Chemistry, Karachi University, Pakistan*
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Abstract

Introduction and Objective. Hyperlipidaemia is the greatest risk factor of coronary heart disease and currently available hypolipidemic drugs have been associated with number of side effects. Several studies have demonstrated the role of *Trigonella foenum-graecum* and *Allium sativum* in improvement of lipid profile and suggest their use as alternative and natural source for prevention / long term management of hyperlipidaemia.

Materials and method. The study investigates synergetic antihyperlipidemic effects of polyherbal formulation containing *T. foenum-graecum* seeds and *A. sativum* buds (ratio 7:3) in Triton-induced acute hyperlipidaemia in rabbits, and compared with standard Atorvastatin. All animals were fed their respective samples orally without any base material for the period of one week. On day 7, blood samples were collected directly from the marginal ear vein of all rabbits before dosing. Immediately after the last dose, Triton WR-1339 (200 mg/kg i.p.) (Tyloxapol) (Sigma-aldrich Corp., USA) in normal saline was injected into all groups. After 24 h of Triton injection, blood samples were again collected from marginal ear vein of all animals for analysis of total cholesterol (TC), triglycerides (TG), HDL cholesterol and LDL cholesterol.

Results. Seven days pretreatment of polyherbal formulation clearly demonstrated significant anti-hyperlipidaemic effects by showing TC 40.4 (p <0.05*), TG 36.6 (p <0.01**), HDL 25.8 and LDL 10.6 mg/dl (p <0.01**) in comparison with control group that exhibited TC 94.6, TG 812.6, HDL 20 and LDL 91.6 mg/dl lipid values. The standard group also exhibited potent results by showing TC 46, TG 198.6 (p <0.01**), HDL 37.6 (p <0.05*) and LDL 31.6 mg/dl (p <0.05*) values.

Conclusion. The results obtained suggest that the regular use of a moderate amount of *T. foenum-graecum* and *A. sativum* in the diet may offer protection against hyperlipidaemia.

Key words

hyperlipidaemia, Triton WR-1339, Rabbits, *T. foenum-graecum, A. sativum*

INTRODUCTION

Hyperlipidaemia has been considered as one of the utmost risk factors contributing to the occurrence and severity of coronary heart diseases [1]. It is characterized by increased serum total cholesterol (TC), low-density Lipoprotein (LDL), very low density lipoprotein (VLDL) cholesterol and decreased high-density lipoprotein (HDL) [2]. Prevention or treatment of such disorders can be achieved by targeting the hyperlipidaemia and hypercholesterolemia through diet and/or drug administration. In order to keep these negative situations under control, it is necessary to reduce the lipid level. Although a range of synthetic drugs are available having anti-hyperlipidaemic effects, they may produce unwanted as well as therapeutic effects [2, 3]. For this reason, herbal products gain more attention as alternative treatment due to the absence of undesirable side-effects, and their economic and easy accessibility. Scientists have reported the role of medicinal plants in controlling, as well as alleviating, elevated serum lipid levels, which result in the reduction of associated morbidity and mortality [4].

✉ Address for correspondence: Hina Imran, PCSIR LABS COMPLEX Karachi, PCSIR Labs Complex Off University Road, 75280 Karachi, Pakistan
E-mail: dr_hinaimran@yahoo.com

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Historically, in the drug discovery system natural products have played a vital role. To treat such diseases as hyperlipidaemia, diabetes and cardiovascular diseases, the use of alternative treatments – especially medicinal plants and their complements – has increased in recent decades in the majority of countries worldwide [2, 5]. Fenugreek (*Trigonella foenum-graecum* L., Fabaceae family) is a plant with high medicinal value. Apart from the traditional medicinal uses, fenugreek is found to have many pharmacological properties, such as anti-diabetic, anti-nociceptive, anti-carcinogenic, anti-oxidant and anti-inflammatory [6, 7]. The anti-cholesterol activity of fenugreek extracts has been well studied by researchers all over the world, and *T. Fenograsicum* leaves, seeds and extracted in different solvents are well reported as anti-hyperlipidaemic agent [8–13].

Garlic (*Allium sativum*), another herbal material, has attracted special attention in modern medicine because of the widespread belief in its effects in maintaining good health. Throughout worldwide history, garlic has been used to treat bronchitis, hypertension, tuberculosis, liver disorders, dysentery, flatulence, intestinal worms, rheumatism, diabetes, and fever. Its health-promoting properties are attributed to its chemical composition: it contains such bioactive compounds as organic sulfur compounds, alkaloids, tannins, flavonoids, saponins and phenolic compounds, which exhibit various

biological activities [14]. Garlic has shown evidence of a significant role in improvement of the lipid profile. It is reported to have similar effects as atorvastatin on protection against lipemic-oxidative disorder in hypercholesterolaemic rats [15]. *A. sativum* extracted oil, its raw buds, dried powder and aquous extracts are reported for a variety of applications [16, 17]. It is documented that a combination of plant extracts or herbs is superior to single plant extracts, and show increased therapeutic efficacy rather than the individual plant extract or herb [18, 19].

Therefore, bearing the above in mind, *T. fenograsium* and *A. sativum* were selected based on the back-up evidence for their widespread effects on maintaining good health. The aim of the study was to investigate *T. fenograsium* and *A. sativum* in a ratio of 7:3 for any possible synergistic effects against Triton-induced acute hyperlipidaemia in a rabbit model.

MATERIALS AND METHOD

Trigonella foenum-graecum seeds and *Allium satvum* bulbs was purchased from a local market. The plant material was authenticated by the department of Pharmacology. A polyherbal formulation was prepared by mixing *Trigonella foenum-graecum* seeds and peeled *Allium satvum* bulb *in a 7:3 ratio for the experimental work.*

Animal selection. Rabbits were obtained from the animal house of PCSIR and were kept under standard environmental conditions. Green vegetables and standard pelleted feed was given throughout the experimental period, and filtered water available in a bowl *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Anti-hyperlipidemic activity. Animals were randomly arranged into 3 groups containing 5 rabbits each. The groups were as follows:

Group-I: Control – distilled water (5 ml/animal p.o.) once daily for 1 week.
Group-II: Test – polyherbal formulation (1g/animal p.o.) once daily for 1 week.
Group-III: Standard atorvastatin (0.5 mg/kg body weight p.o.) once daily for 1 week.

All animals fed their respective doses for the period of one week. On day 7, blood samples were collected directly from the marginal ear vein of all rabbits before dosing. Immediately after the last dose, Triton WR-1339 (200 mg/kg i.p.) (Tyloxapol) (Sigma-Aldrich Co. USA) in normal saline was injected to all groups. After 24 h of Triton injection, blood samples were again collected from the marginal ear vein of all animals for analysis of total cholesterol (TC), triglycerides (TG), HDL cholesterol and LDL cholesterol (Tab. 1, Fig. 1 & 2) [20, 21].

Statistical analysis. All numerical data were expressed as the mean ± SD. All data were statistically analyzed by student t test. The p values at <0.05 were considered significant (*) and highly significant (**) at p <0.01 when compare with respective controls.

RESULTS

The results showed that Triton WR-1339 successfully induced hyperlipidaemia in all 3 groups. In comparison to the control group, the test and standard groups showed a reduction in total blood cholesterol, LDL, and triglycerides, and an increase in HDL cholesterol level after consumption of the prepared formula. Results showed that high, significant reduction in total cholesterol (TC) 40.4, triglyceride (TG) 36.6, HDL 25.8 and LDL 10.6 mg/dl was observed in the test group. The standard group also exhibited potent results by showing TC 46, TG 198.6, HDL 37.6 and LDL 31.6 mg/dl values (Tab. 1).

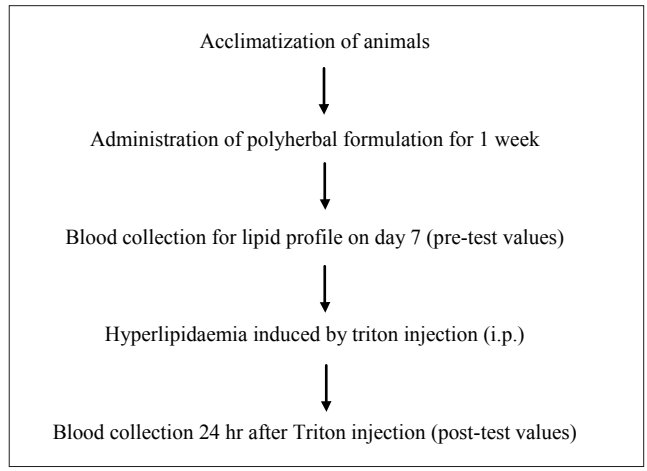

Figure 1. Study flow chart

DISCUSSION

Triton WR-1339 has been widely used to produce acute hyperlipidaemia in animal models in order to screen natural and chemical drugs. It is a non-ionic detergent that suppresses the action of lipases and blocks the uptake of lipoprotein from the circulation, resulting in an increase in the levels of circulating lipid – a primary risk factor for vascular diseases [22, 23]. Nowadays, herbal medicines are the most popular

Table 1. Effect of polyherbal formulation on lipid profile (TC, TG, HDL, LDL)

Parameters (mg/dL)	Control group		Test group		Standard group	
	Pre	Post	Pre	Post	Pre	Post
TC	22±7.21	94.6±47.9	26.6±6.26	40.4±4.15*	27.6±17.5	46±18.27
TG	62.6±13.5	812.6±176.9	66.4±14.9	36.6±10.26**	71.8±8.22	198.6±161.12**
HDL	24±5.47	20±6.04	25.8±6.97	35±2.91	27.6±5.77	37.6±8.35*
LDL	11.6±1.14	91.6±35.5	14±2.91	10.6±1.81**	17.6±8.79	91.6±26.26*

All values are expressed as mean ± STDEV; n=5; value ** p< 0.01; *p< 0.05

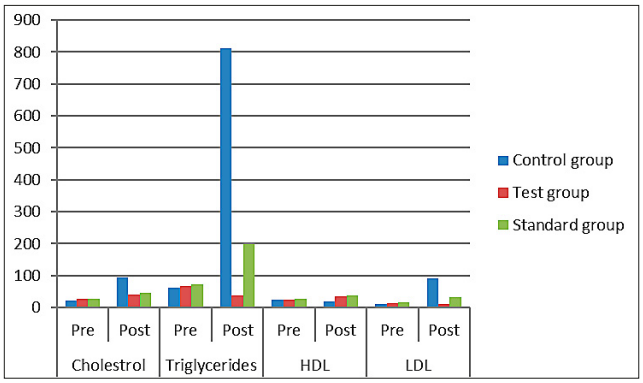

Figure 2. Effect of polyherbal formulation on lipid profile in Triton-induced hyperlipidaemia

forms of alternative medicine. They have multiple effects in that some plants or plant products not only having anti-diabetic effects, but have also shown a reduction in blood fat [24, 25].

In the present study, acute hyperlipidaemia was produced by Triton i.p. injection in rabbits. The pre-feeding of a polyherbal formula for 1 week produced a lowered value of serum TC, TG and LDL levels in the test group, compared to the levels in the control group and standard group treated with atorvastatin (0.5 mg/kg b.w.). Test group results indicated a significant reduction in TC 40.4 (p <0.05*), TG 36.6 (p <0.01**) and LDL 10.6 mg/dl (p <0.01**), while slightly raised HDL 25.8 mg/dl in contrast to control that showed TC 94.6, TG 812.6, HDL 20 and LDL 91.6 mg/dl. The standard group exhibited TC 46, TG 198.6 (p <0.01**), HDL 37.6 (p <0.05*) and LDL 31.6 mg/dl (p <0.05*) values in the 7 day study period (Tab. 1, Fig. 2).

It is believed that if levels of lipids, especially LDL-cholesterol and triglycerides, are controlled, it can markedly prevent many chronic inflammatory diseases that result from obesity-related low-grade inflammation [26]. The results of the present study suggest that a pohyherbal mixture supplementation significantly lowered serum TC, TG and LDL cholesterol concentrations in the test group when compared with the hyperlipidaemic control group. These findings are generally in accordance with the results from previous studies that showed improvements in TC and LDL cholesterol concentration in relation to consumption of these herbs [26–29]. The results are supported by previous studies. An article [9] reported that the aqueous solution of fenugreek leaves possesses significant activity against serum triglyceride and total cholesterol. Two more studies [30, 31] on fenugreek seed powder and freeze-dried garlic cloves used as individual and in 10% and 2% combination, respectively, in a high cholesterol diet of rats for 8 weeks in a myocardial infarction model, reported that both herbal materials had significant effect on TC, TG, HDL, LDL on heart muscle and liver. They also reported more beneficial effects associated with combination of fenugreek and garlic. The current 7-day study on pre-feeding a polyherbal formula in Triton-induced acute hypercholestremia also produced similar effects. It can therefore be concluded that either of these plant materials utilized by mixing them in a high fat diet, or pre-feeding them with normal diet prior to inducing hyperlipidaemia, produced almost similar effects. Similarly reported documentation on *A. sativum* alone and in combination with other synthetic and herbal materials also supports the findings of the current study.

A study conducted [32] on lipid profile assessment by the intake of garlic alone and in combination with atorvastatin reported a significant reduction in TC, TG, LDL and VLDL, and an increase HDL after 12 weeks of drug treatment in animal models. On the basis of this statement, it can be assume that the combined administration of atorvastatin with a moderate quantity of this polyherbal formulation may improve lipid profile by producing synergistic effects. Few more studies on the use of raw garlic in a 2% cholesterol diet in rat model also reported the improved level of lipid profile [33–34]. They also reported that water extract inhibited cholesterol synthesis more than methanol and petroleum extractable fraction. These statements support the current findings in which the polyherbal formulation was also based on an aqueous homogenous mixture of *Trigonella foenum-graecum* and *Allium sativum*. All the above-mentioned studies support the current findings, and it can be stated that a daily intake of polyherbal formulation definitely improves the lipid profile.

There is another reason for the anti-hyperlipidaemic effects of the polyherbal formula used in the current study – their phytochemical constituents, which inhibit cholesterol biosynthesis, their absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism. A study [35] reported that phytochemical constituents (steroids, flavonoids, saponins) possess lipid lowering activity. The steroids reduce the absorption of cholesterol and thus increase its faecal excretion, while saponins cause a reduction in blood cholesterol by binding with the cholesterol in the intestinal lumen, so that cholesterol is less readily absorbed [14]. Another study [13] reported that dietary fibre plays a key role in reducing the cholesterol levels through increased faecal excretion of bile acids and salts, as well as inhibitors of hepatic cholesterol biosynthesis. The presence of important phytochemicals renders fenugreek as one of the important medicinal plants. The major constituents that are present in fenugreek seeds are carbohydrates, proteins, lipids, alkaloids, flavonoids, fibres, saponins, steroidal saponins, vitamins, minerals and nitrogen compounds [6, 7, 36]. A scientific document [37] reported a compound GII purified from the water extract seeds of fenugreek was able to reduce the total cholesterol level and increase HDL cholesterol which is an indicator of good cholesterol.

A. sativum is also reported for disease preventive phytochemicals. Research studies [38, 17] have reported that the anti-hyperlipidaemic effects of *A. sativum* are due to its water-soluble sulfur compounds that are converted to the active ingredient 'allicin'. This compound has an inhibitory effect upon the key enzymes involved in cholesterol biosynthesis, such as HMG-CoA reductase. *A. sativum* also scientifically validates the presence of saponin, alkaloids, tannin, steroids, flavonoids, glycosides, cardiac glycosides, phenolic compounds, terpenoids, steroids, ketones and phlobutanin [39, 40, 14, 41].

A study conducted on fenugreek seeds and garlic reported improvement in serum cholesterol level (induced by high cholesterol diet). It was suggested that this is a synergistic effect that not only provides dietary fibre, but also act as hypocholesterolemic agents [42]. All the above-cited studies support the current findings, as the improvement in lipid profile in Triton-induced acute hyperlipidemic model may be due to the presence of these phytochemicals act as ant-

ihyperlipidaemic agents. It can be assumed that the currently presented test formula may act by inhibiting cholesterol synthesis and increasing its excretion, or due to presence of photochemical constituents responsible for this effect.

In this short duration study, the rabbits had normal eating and drinking habits, without incurring any mortality which demands a long-term toxicity study. Hence, it can be concluded that the combination has a possible synergistic effect in controlling hyperlipidaemia. A study [22] reported that proloned use of atorvastatin at a higher dose has some adverse effects. Thus, the possible synergistic effect can help in reducing the dose of atorvastatin without compromising the therapeutic effect. To overcome hyperlipidaemia problems and its side-effects, the proposed polyherbal formulation might provide a useful source for a new, oral hypolipidaemic formula for the development of pharmaceuticals entities, or as a dietary adjunct to existing therapies.

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