

EFFECT OF METHANOLIC EXTRACTS OF *TYLOPHORA INDICA* L. LEAVES ON LIPID PROFILE IN RATS

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ABSTRACT

Tylophora indica L is important medicinal plant of Ayurvedic system of medicine and used as home remedy as cardioprotective agent without any scientific background. In this investigation an attempt was made to study the effect of methanolic extract of *Tylophora indica* L (HETI) on serum lipid profile. Albino rats were administered HETI (100 mg/kg & 500 mg/kg) once daily orally for 30 days. Accordingly, serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were among the parameters investigated. We also evaluated the activities of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels as a measure of hepatic cell damages. It was found in our study that the administration of both doses of HETI to animals fed with high fat diet (HFD) lowered their serum TC, TG and LDL-C significantly, while raised the serum HDL-C. Treatment with HETI reduced serum ALT and AST levels compared to HFD groups. Further, it was also observed that the high dose of HETI significantly decreases serum TC, TG and LDL-C and increases HDL-C in animals fed with normal fat diet (NFD). Based on these data, it is concluded that the chronic administration of HETI in high dose up to 500 mg/kg has definite cardioprotective potential and these results constitute a valid scientific basis for consuming *Tylophora indica* L for medicinal application.

Key words: *Methanolic extract of Tylophora indica L; high fat diet; lipid profile; normal fat diet.*

INTRODUCTION

Hyperlipidaemia is considered a predisposing factor involved in the development of cardiovascular disease.^{1,2} The search for new drugs capable of reducing and regulating serum cholesterol and triglyceride levels has gained momentum over the years, resulting in numerous reports on significant activities of natural agents.³ Plant products are frequently considered to be less toxic and free from side effects than synthetic agents. These properties have led to the discovery of new therapeutic agents including antioxidants, hypoglycemics, and hypolipidemics. It is well established that diet rich in vegetables and fruits can reduce cardiovascular diseases.^{4,6} In view of the increasing use of herbal remedies by the general public and subsequent interest by the physicians, it is imperative to promote credible research for validation of their claimed activities based on modern scientific techniques.

Tylophora indica (Asclepiadaceae) leaves of this plant is traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma and bronchitis.⁷ It was reported to cause

myocardial depression and fall of blood pressure in relatively large doses.^{8,9} The extract of *tylophora* is known to have anti-oxidant and potent NO scavenging activity.¹⁰ An alcoholic extract from the leaves of the plant *Tylophora indica* were studied for their pharmacological effects on various intact and isolated biological preparations and reported for its protective effect during events of injury such as myocardial stress.⁷

Thus aim of the present study was to evaluate the influence of the HETI on serum lipid levels in rats fed high-fat diets and normal fat diet. The lipid profile was taken as the major marker of hypercholesterolaemia. Accordingly, serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C) and high-density lipoprotein cholesterol (HDL-C) were among the parameters investigated. In addition, to have a measure of the effects of the plant extract on liver function of the treated rats, the serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated relative to plant-untreated control rats.

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EXPERIMENTAL

Plant extract

The leaves of *Tylophora indica* were purchased from medicinal garden Danvantri vana, Bangalore University, in the month of June 2007. The plant material (Voucher Specimen No.-RRCBI 0691) was authenticated by Regional Research Institute (Ay.), Bangalore. The leaves were shade dried and powdered (moderately coarse). The extraction was carried out with 70% of methanol in soxhlet for about 72 hrs. The obtained syrupy mass was concentrated and dried in hot air oven at 70°C. The dried syrupy mass was dissolved in water before administration.

Phytochemical estimations of the extracts

The methanolic extracts of *Tylophora Indica* L. were subjected to qualitative analysis for various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids.¹¹

Experimental animals

Laboratory bred female Wistar albino rats (200-250 g) were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Acute toxicity studies

The acute toxicity study was carried out according to the limit test described by the OPPTS guidelines (<http://www.epa.gov/oppts/home/guideline.htm>). The oral doses of HETI were selected on the basis of acute toxicity study, which was carried out as per limit tests of OPPTS guidelines.¹² Test dose of 2 g/kg and 5 g/kg were given to mice. Both doses were found to be safe. Hence, 1/10th and 1/50th of the maximum safe dose corresponding to 100 mg/kg and 500 mg/kg orally were selected as low and high dose respectively.

Experimental Protocol

The animals were either fed normal fat diet (NFD) or high fat diet (HFD). The NFD comprised of standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) containing protein 22.10%, oil 4.13%, fibre 3.15%, ash 5.15%, sand (silica) 1.12% w/w), whereas, HFD was composed of standard rat chow - 68%, Dalda (saturated fat) - 30% and cholesterol - 2%.¹³

Measurement of body weight and diet intake

Diet intake was measured daily for individual animal by taking mean among the number of animals in cage and the body weights were recorded before starting the plant treatments as well as after each week of the plant treatments.

Treatment

The rats were divided into six groups, each containing six animals: the first three group of rats were fed NFD (Group I-III); first group was considered as NFD control, whereas, the second and third groups were treated with low and high dose of HETI (HETI-100 & HETI-500 mg/kg) respectively. The IV, V and VI groups were fed with HFD; IV group served as HFD control, while, fifth and sixth groups were administered HETI-100 and HETI-500 mg/kg respectively. The administration of HETI was done orally at same time once daily for 30 days. On 14th, 21st and 30th days, the overnight fasted rats were anesthetized with diethyl ether and their blood samples were collected by retro orbital vein and centrifuged at 3000 rpm, 4°C for 15 min. Serums were separated from the blood samples and were stored at -70°C pending biochemical analyses. The triglycerides (TG), total cholesterol (TC) and HDL-cholesterol (HDL) were estimated using autoanalyzer.¹⁴⁻¹⁶

The LDL-cholesterol was calculated by using the formula:

$LDL \text{ in mg/dl} = \text{Total cholesterol} - (\text{HDL-Cholesterol} + \frac{1}{5} \text{Triglycerides})$.¹⁷

AST and ALT were assayed using the corresponding commercial kits (Crest Biosystems Goa, India).

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey multiple comparison tests. p<0.05 was considered significant.

RESULTS

Phytochemical analysis of the extracts

The methanolic extracts of *Tylophora Indica* L. were found to contain various phytoconstituents such as alkaloids, phytosterols, saponins, amino acids and flavonoids.

Effects of HETI on diet intake and body weights

At the end of 7th and 14th days, there was no significant change in diet intake in all groups when

compared to NFD control. The daily diet intake of the rats decreased, in groups II, III, IV, V and VI, from the 21st day of the experiment (Fig. 1). Moreover, the fall in diet intake reaches an extremely significant level on 30th day of the experiment in HFD groups when compared to NFD control.

There was no significant change in body weight in all groups when compared to NFD control. High-fat diet apparently caused an increase in the body weight of rats despite their lower food intake by 21st day. However, in Group V and VI the body weights of the HETI treated rats decreased after day 21 that becomes highly significant by day 30, as shown in Fig. 2.

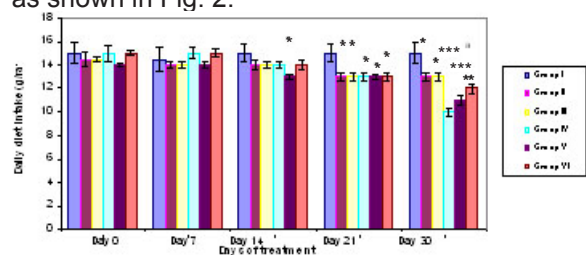


Fig.1. Effect of HETI on daily diet intake

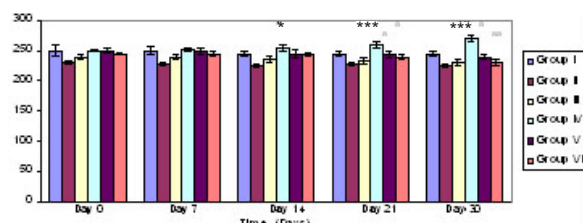


Fig.2. Effect of HETI on Body weight

Values are expressed as mean \pm SEM for six rats in each group; ***Significantly different from NFD control; ^{###}Significantly different from HFD control in HFD-HETI; Group I, NFD; Group II, NFD+HETI-100; Group III, NFD+HETI-500; Group IV, HFD; Group V, HFD+HETI-100; Group VI, HFD+HETI-500; * $p < 0.05$; ** $p < 0.01$; ^{###} $p < 0.001$.

Effect of HETI on serum lipid profile

The general effect of HETI on serum lipid levels was examined in rats fed NFD and HFD. In NFD groups, there was no significant difference in serum lipid levels of rats. Feeding the animals a high-fat diet resulted in a significant increase in the serum total cholesterol (TC), triglycerides (TG), and low-density lipoprotein-cholesterol (LDL-C) levels. However, the level of serum high-density lipoprotein-cholesterol (HDL-C) decreased (Fig. 3-6) compared to NFD control group. Treatment of HFD rats with HETI at a dose of 100 and 500-mg/kg body weight for 21 days shows lowering of lipid level. After 30 days of continuous treatment with plant extract, almost the same observations were made demonstrating the hypolipidaemic effect of high dose of HETI (serum TC, TG, LDL-C and HDL-C were same like NFD control). There was significant reduction in lipid levels of TC, TG and LDL-C in HETI groups when

compared to HFD control group. On the other hand, there was significant elevation in HDL-C level in HETI treated groups fed HFD.

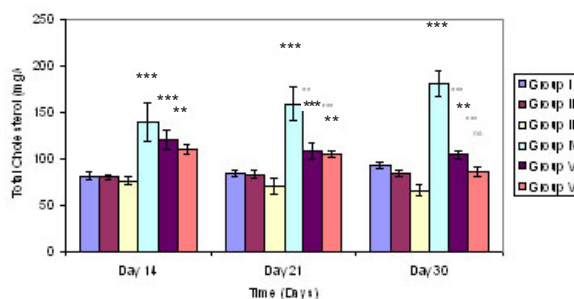


Fig.3. Effect of HETI on serum total Cholesterol

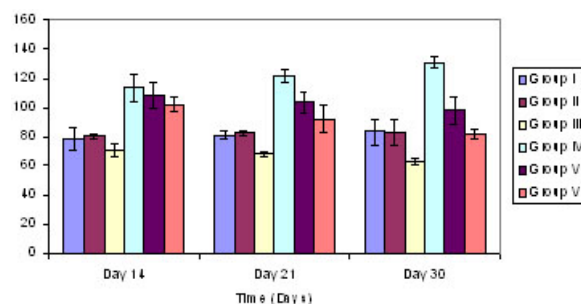


Fig.4. Effect of HETI on serum triglycerides

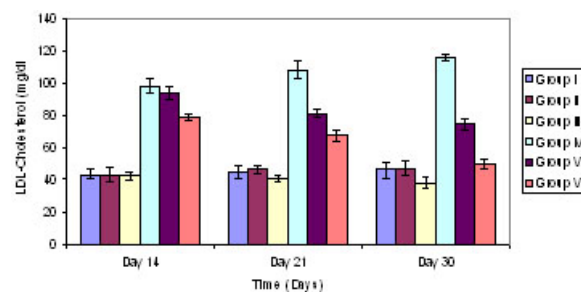


Fig.5. Effect of HETI on serum LDL-Cholesterol

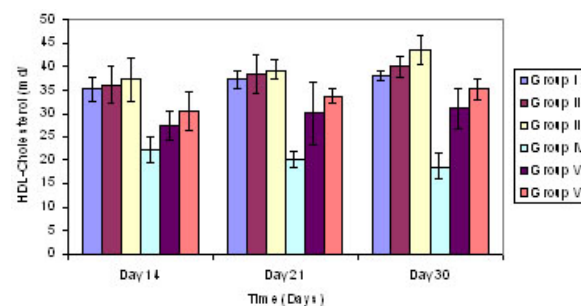


Fig.6. Effect of HETI on serum HDL-Cholesterol

Values are expressed as mean \pm SEM for six rats in each group; ***Significantly different from NFD control; ^{###}Significantly different from HFD control in HFD-HETI; Group I, NFD; Group II, NFD+HETI-100; Group III, NFD+HETI-500; Group IV, HFD; Group V, HFD+HETI-100; Group VI, HFD+HETI-500; * $p < 0.05$; ** $p < 0.01$; ^{###} $p < 0.001$; ns, not significant when compared to NFD control.

Effect of high-fat diet on hepatic enzymes in serum

The effects of HETI on serum aminotransferases (AST, ALT) are shown in Table 1. Animals fed high-fat diet exhibited an elevation in serum AST (GOT) and ALT (GPT) compared to NFD control group. Treatment with plant extract reduced activities of AST and ALT compared to animals of NFD control, after 21 days. After 30 days of continuous treatment with plant extract, almost the same observations were made.

Table 1. Effect of HETI on hepatic enzymes in serum

Treatment	ALT (IU/L)		AST (IU/L)	
	Day 21	Day 30	Day 21	Day 30
Group I	14.70±1.0	16.93±2.1	23.90±3.0	23.30±2.5
Group II	16.01±2.3	17.80±3.3	22.70±2.1	23.20±1.9
Group III	15.10±1.1	16.10±2.2	21.20±1.7	20.32±1.6
Group IV	34.20±2.6 ^{***}	40.21±1.2 ^{***}	40.20±3.9 ^{***}	43.21±1.4 ^{***}
Group V	32.70±2.3 ^{***}	25.90±1.3 ^{***}	31.30±1.9 ^{***}	29.91±1.8 ^{***}
Group VI	21.30±2.27 ^{***}	17.90±2.9 ^{***}	27.20±1.3 ^{***}	24.83±2.0 ^{***}

Values are expressed as mean ± SEM for six rats in each group; ^{***}Significantly different from NFD control; ^{aaa}Significantly different from HFD control in HFD-HETI groups $P < 0.001$; Group I, NFD; Group II, NFD+HETI-100; Group III, NFD+HETI-500; Group IV, HFD; Group V, HFD+HETI-100; Group VI, HFD+HETI-500; ^a * $p < 0.05$; ^{aa} ** $p < 0.01$; ^{aaa} *** $p < 0.001$; ns, not significant when compared to NFD control.

Discussion

The main causative factor for atherothrombotic diseases is the disturbances occurring in lipid metabolism. Despite the presence of different hypolipidaemic drugs in the market, their therapeutic application is usually associated with severe side effects.¹⁸ Hence, efforts are being made to find safer and more efficient anti-hyperlipidaemic drugs. In that respect, medicinal plants have been considered as promising resources for the discovery of new drugs. In the present study, the hyperlipidaemic activity of HETI was evaluated among the hypercholesterolaemic rats. Based on our data, the body weight of rats of Group IV increased relative to NFD control group. This is probably due to supplementation with cholesterol and cholic acid. Treatment with HETI effectively prevented the increase in body weight to a significant extent.

Cholesterol is an essential structural element of the biological membranes. In addition, it is the precursor of many compounds such as the starting materials for the synthesis of bile acids, steroid hormones, and vitamin D among others. Despite this knowledge, high concentration of serum cholesterol increases the risk of developing CHD.¹⁹ The present study demonstrated that rats fed a HFD showed a higher concentration of serum TC compared to rats fed a NFD, while oral administration of HETI reduced the high level of TC. It has been reported that low plasma TG levels are associated with lower risks of CHD.²⁰ In our study, the administration of HETI to hyperlipidaemic rats significantly lowered their serum TG levels. This reduction might be related to decreased triglyceride absorption as well as increased excretion of TG via

feces.²¹ It is widely accepted that elevation of plasma LDL levels are major risk factors for CHD.²² Direct correlation between LDL and atherosclerosis and also the reversibility of the related pathological events by lowering the serum level of LDL has already been reported by many research groups.^{22,23} Our data indicated that, the high concentration of LDL-C in hypercholesterolaemic rats was significantly reduced by oral administration of HETI. Therefore, HETI might constitute a good candidate for the treatment of atherosclerosis by lowering serum LDL-C level. Another risk factor for developing atherosclerosis is the reduced serum level of HDL-C. This effect of HDL is largely attributed to its central function in the reverse cholesterol transport, a process whereby excess cell cholesterol is taken up and processed by HDL particles for further delivery to the liver for metabolism.²⁴ Therefore, it is logical that an increase in HDL level can contribute to lower risk of atherosclerosis.²⁵ Our results clearly showed that HETI is capable of increasing the serum level of good cholesterol (i.e. HDL-C) in the treated rats relative to HFD rats. Our data clearly indicated that HETI is capable of potentially decreasing this risk factor after 21 days of drug treatment. Phytochemicals, especially the phenolics found in fruits and vegetables, have been proposed as the major bioactive compounds providing the health benefits associated with diets rich in plant-foods. Flavonoids are a class of secondary plant phenolics found ubiquitously in fruits and vegetables as well as food products which act as pharmacological active compounds in many medicinal plants.^{26,27} Many of the biological action of flavonoids have been attributed to their powerful hypolipidaemic properties.^{28,29} It is reported that flavonoids intake decreased LDL-C and increased HDL-C in hypercholesterolaemic individuals that may hasten removal of cholesterol from peripheral tissue to liver for catabolism and excretion.³⁰ Independent studies have confirmed the presence of antioxidant phenolic compounds, mainly flavonoids HETI.³¹ Regarding the high total phenolics and flavonoids of HETI, the results strongly suggest that the hypolipidaemic activity of this medicinal plant could be attributed to the presence of the valuable polyphenolic compounds. It might be possible that these active constituents reverse the adverse effects associated with hyperlipidaemia.²⁹ The decrease in cholesterol balance, which indicates the total change in body pools of cholesterol, may be due to the compensatory mechanisms, such as a decrease in resorption of endogenous cholesterol or an increase in the rate of secretion into intestinal tract or both. The effect(s) of HETI on liver function was evaluated by measuring the serum transaminase activities of ALT and AST. These enzymes leak into the circulation when hepatocytes are damaged.³² It is believed that high serum cholesterol level can cause liver damages.³³ Our results show that high-fat diet has caused significant increase in serum ALT and AST levels. However, rats

treated with HETI had lower serum ALT and AST levels compared to rats of Group NFD control.

Thus on the basis of the result of the present research, it is concluded that the high dose of HETI is a powerful agent for combating elevated levels of lipids during CHD and other cardiac manifestation. However, further studies should be carried out to understand the influence of HETI during myocardial infarction.

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