Original Article

Absence of hypolipidemic effect of *Holoptelea integrifolia* leaf extract in tyloxapol-induced hyperlipidemic rats

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Abstract

Objective: The leaves of *Holoptelea integrifolia* are used in the treatment of diabetes and lipid disorders. The present study demonstrates the influence of the aqueous extract of leaves of *Holoptelea integrifolia* (HIAE) on the lipid profile in normal and Tyloxapol-induced hyperlipidemic rats. **Materials and Methods:** The vehicle, the aqueous extract of leaves of *Holoptelea integrifolia* (250 and 500 mg / kg), and fenofibrate, reference standard (65 mg / kg), were administered orally for seven days to different groups of normal and Tyloxapol-induced hyperlipidemic rats. The lipid profile was assessed on the eighth day by estimating the plasma levels of total cholesterol, triglycerides, HDL-c, LDL-c, VLDL-c, and the corresponding atherogenic index and the LDL-c/HDL-c ratio. **Results:** Administration of the HIAE neither caused any significant effect on the lipid profile in normolipidemic rats nor attenuated tyloxapol-induced hyperlipidemia. **Conclusion:** The present investigations revealed that the aqueous extract of the leaves of *Holoptelea integrifolia* did not exhibit a hypolipidemic effect and did not substantiate its traditional use in lipid disorders and obesity.

Key words: Atherosclerosis, *Holoptelea integrifolia*, hypolipidemic activity, triton WR 1339, tyloxapol

INTRODUCTION

Increase in the plasma lipid levels, mainly total cholesterol, triglycerides, and low density lipoprotein, along with a decrease in high-density lipoproteins are known to cause a metabolic disturbance called hyperlipidemia. An elevation of plasma lipids may be the result of a primary genetic defect or it may be secondary to diet, drugs or diseases. Hyperlipidemia is considered as an important risk factor in the initiation and progression of atherosclerotic impasse[1,2] and its cardiovascular complications.[3] Hence, the prime consideration in the therapy of hyperlipidemia and the subsequent atherosclerotic condition is to attenuate the elevated blood levels of the lipids, to reduce the incidence of cardiovascular diseases. The current interest in the natural herbal-based products has stimulated the search for new lipid-lowering agents from plant sources. In India, most of the hyperlipidemic individuals use medicinal plants and folk medicines to treat hyperlipidemia and atherosclerosis. Many herbal medicinal products have been reported to have the potential to reduce lipid and cholesterol levels in the body and to counter check further complications.[4] Therefore, a strong interest has arisen to search for hypolipidemic substances derived from medicinal plants.

Holoptelea integrifolia (Ulticaceae) is a medium-sized to large deciduous tree, having a whitish or yellowish-

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gray bark in irregular flakes when freshly cut; the leaves are simple, alternate, elliptic, entire, glabrous, rounded or cordate; the flowers are greenish-yellow, racemes or fascicles; and the fruits are suborbicular samara. The tree is distributed throughout India in the deciduous forests.[5,6] It is considered an important pollen allergen in India and sensitizes almost 10% of the atopic population in Delhi.[7] The recent investigations on this plant have indicated that the plant demonstrates antiviral,[8] antioxidant, antimicrobial, wound-healing,[9] and antiemetic activities.[10] The leaves of *Holoptelea integrifolia* have also been shown to possess antibacterial,[11] antidiarrheal,[12] anti-inflammatory,[13] and recently antitumor activities.[14] Ethnomedically, the leaves and stem bark of this plant are used by local people for skin disease, obesity, cancer, and wound-healing purposes.[15] The plant has been demonstrated to possess anti-obesity activity in experimentally induced obesity in rats.[16] In the Ayurvedic system of medicine, the plant is given in the case of lipid disorder (*medoroga*) and diabetes (*prameha*), and it is known to have antiatherosclerotic property (*lekhna*).[7] Although the plant possesses an anti-obesity property and it is used in case of a lipid disorder, no scientific studies have been carried out to delineate its influence on hyperlipidemia. Therefore, the present investigations propose to study the effect of the leaves of *Holoptelea integrifolia* on experimentally-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Chemicals and drugs

Triton WR 1339 (Tyloxapol) (TLX) was purchased from Sigma-Aldrich, USA, while Fenofibrate (Lipicard) (FBT) was a gift sample from USV Ltd., Mumbai, India. Biochemical kits for estimations of cholesterol (Siemens Healthcare Pvt. Ltd., Vadodara, India) and triglycerides and HDL-c (Span Diagnostic Ltd., Surat, India) were procured from the local market. All the remaining chemicals used in the experiment were of the highest grade commercially available.

Plant material

The leaves of the plant *Holoptelea integrifolia* Roxb. were collected from the local area of Gwalior, Madhya Pradesh, India, in March 2011. The identification of the plant material was carried out by Dr. N.K. Pandey, Research Officer (Botany), National Research Institute for Ayurveda-Siddha Human Resource Development, Gwalior, India. A voucher specimen (Accession no. 437) of the authenticated *Holoptelea integrifolia* R. plant has been deposited in the herbarium of the Institute.

Processing of the plant material and preparation of the aqueous extract of leaves of *Holoptelea integrifolia*

The leaves were segregated from other extraneous matter and subjected to shade drying. The dried leaves were coarsely powdered by simple hand smashing. The powder (180 g) was packed into Soxhlet apparatus in stages (30 g in each packet) and was defatted with petroleum ether (60 – 80°). The total defatted marc was then cold macerated with distilled water for 48 hours, thrice. The mixture was then filtered through a muslin cloth. The filtrate was then concentrated to dryness in a Buchi-type rotary evaporator under reduced pressure at a temperature of 35°C, to obtain a dark brown aqueous extract (Percentage yield — 14% w / w). The dried extract was stored in a dessicator for use in subsequent experiments.

The aqueous leaf extract was dissolved in deionized water for oral administration to animals.

Preliminary phytochemical screening

Preliminary phytochemical screening[17] was carried out to detect the presence of steroids, alkaloids, saponins, terpenes, tannins, phenolic substances, carbohydrates, volatile oil, and so on.

Animals

Healthy adult albino rats of Wistar strain weighing about 200 – 250 g, of either sex, between two to three months of age, were selected for the experiments. They were housed in groups, in polypropylene cages, maintained under standard conditions (12 : 12 hour light : dark cycle; 25 ± 3°C; 40 – 60% humidity) with free access to the standard rat pellet diet (Ashirwad brand, Chandigarh, India) and water *ad libitum*. Food was withdrawn during fasting, but water remained available *ad libitum*.

The experiments were carried out in accordance with guidelines described by the Institutional Animal Ethics Committee of the National Research Institute for Ayurveda–Siddha Human Resource Development (Ay), Gwalior, India (Proposal No. CRI-GWL/IAEC/2010/06).

Acute toxicity study

Healthy female Wistar rats, starved overnight were subjected to acute toxicity studies to determine the safe dose by acute toxic class method of oral toxicity as per Organization for Economic Cooperation and Development (OECD) 423 guidelines.[18] The rats were administered HIAE in the limit dose of 2000 mg / kg orally and continuously observed for two hours, for behavioral, neurological, and autonomic profiles, and after a period of 24 and 72 hours, and thereafter up

to 14 days for any lethality, moribund state or death. The experiment was repeated in three more animals to determine its Globally Harmonized System (GHS) category.

Induction of acute experimental hyperlipidemia

Several previous studies[19] showed that systemic administration of Tyloxapol (Triton WR 1339, an ionic surfactant) to fasted rats caused elevation in the plasma lipid levels, reaching a peak two-to-three times the control value, by 24 hours, after administration of the triton injection. In the present study, acute hyperlipidemia was induced by a single intraperitoneal injection of tyloxapol in a dose of 200 mg / kg. The lipid profile was assessed in the plasma after 24 hours of tyloxapol administration, to confirm the hyperlipidemia.

Experimental design

The normolipidemic rats were divided into four different groups (n = 5). Group I received deionized water as the vehicle. The extract-treated groups (II and III) were orally administered aqueous extract of *Holoptelea integrifolia* (HIAE) in two different doses (250 and 500 mg / kg). The standard treated group (group IV) was administered fenofibrate (FBT) 65 mg / kg,[20] a standard antihyperlipidemic agent. The drugs were administered orally for seven days.

In another set of experiments, the vehicle or HIAE or fenofibrate were administered orally for seven days as mentioned earlier, and tyloxapol was administered one hour after the last dose of drugs on the seventh day, to induce hyperlipidemia.

The blood was withdrawn 24 hours after the administration of tyloxapol from the retro-orbital plexus via the venipuncture method.[21] The blood was centrifuged at 3000 rpm and the plasma separated. The plasma was then assessed for estimation of cholesterol, triglycerides,[22,23] and HDL-c.[24] LDL-c, VLDL-c, atherogenic index, and the LDL-c / HDL-c ratio were calculated as mentioned herewith.

Calculation of very low density lipid and low density lipid

VLDL (Very low density lipid) and LDL (Low density lipid) were calculated by using the following formula

VLDL-c = TG / 5

LDL-c = Total Cholesterol – (VLDLc - HDLc)

Calculation of atherogenic index (AI) and LDL-c / HDL-c ratio

The AI was calculated by using the following formula:

AI = (total cholesterol - HDL-c) / HDL-c

The LDL-c / HDL-c was calculated as the ratio of plasma LDL-c to HDL-c levels.

Statistical analysis

The data were analyzed with one-way ANOVA followed by the Newman Keul's multiple comparison post hoc test. A statistical difference of $P < 0.05$ was considered significant in all cases.

RESULTS

Preliminary phytochemical screening

The preliminary phytochemical screening of HIAE revealed the presence of carbohydrates, proteins, sterols, glycosides, flavonoids, phenolic compounds, and alkaloids.

Acute toxicity study

Acute toxicity studies revealed that HIAE was safe up to 2000 mg / kg of body weight (limit test) and approximate LD_{50} is more than 2500 mg / kg. No lethality or any toxic reactions or moribund state was observed up to the end of the study period.

Effect of HIAE on lipid profile in normolipidemic rats

One-way ANOVA indicated oral administration of HIAE did not show any significant effect on lipid profile in normal rats [Table 1]. HIAE at 250 and

Table 1: Effect of HIAE on lipid profile in normolipidemic rats

Lipid profile parameters	Treatments			
	Vehicle	HIAE 250	HIAE 500	FBT
Total Cholesterol (mg / dl)	68.40 ± 3.31	66.40 ± 1.36	66.80 ± 5.06	67.60 ± 3.93
Triglycerides (mg / dl)	95.35 ± 6.40	80.27 ± 4.96	77.94 ± 6.50	92.80 ± 8.50
HDL-c (mg / dl)	9.018 ± 0.34	10.32 ± 0.79	10.79 ± 0.95	10.30 ± 0.76
LDL-c (mg / dl)	58.33 ± 2.55	61.60 ± 1.14	61.06 ± 4.83	59.34 ± 5.55
VLDL-c (mg / dl)	19.09 ± 1.28	16.05 ± 0.99	15.59 ± 1.30	18.56 ± 1.70
Atherogenic Index	6.60 ± 0.29	5.59 ± 0.50	5.34 ± 0.64	5.68 ± 0.59
LDL-c / HDL-c ratio	6.49 ± 0.26	6.10 ± 0.43	5.80 ± 0.60	5.80 ± 0.51

Normolipidemic rats were treated with vehicle or HIAE (250 and 500 mg / kg, orally) or FBT (65 mg / kg, orally) for 7 days. The plasma lipid profile was estimated at 24th h on day 8. Values are the mean ± SEM of five rats, HIAE: Aq. extract of *Holoptelea integrifolia* FBT: Fenofibrate

Table 2: Effect of HIAE on lipid profile in hyperlipidemic rats

Lipid profile parameters	Treatments				
	Saline	Vehicle+TLX	HIAE 250+TLX	HIAE 500+TLX	FBT+TLX
Total Cholesterol (mg / dl)	71.20 ± 2.70	1043 ± 25.40*	1119 ± 87.16	930.6 ± 57.19	607.2 ± 89.10@
Triglycerides (mg / dl)	95.60 ± 6.66	636.0 ± 57.49*	593.2 ± 29.16	643.3 ± 22.64	456.9 ± 19.96@
HDL-c (mg / dl)	9.48 ± 0.43	11.00 ± 0.65	10.72 ± 0.53	10.18 ± 1.18	10.24 ± 0.68
LDL-c (mg / dl)	61.56 ± 1.64	926.60 ± 28.86*	1011.00 ± 88.98	812.10 ± 57.44	525.80 ± 86.56@
VLDL-c (mg / dl)	19.12 ± 1.33	127.20 ± 11.50*	118.60 ± 5.83	128.70 ± 4.53	91.63 ± 3.93@
Atherogenic Index	6.58 ± 0.50	95.40 ± 7.22*	104.50 ± 9.42	94.39 ± 10.12	0.92 ± 0.10@
LDL-c / HDL-c ratio	6.56 ± 0.39	85.80 ± 7.10*	95.29 ± 9.06	82.83 ± 8.27	51.94 ± 8.85@

Rats were treated with vehicle or HIAE (250 and 500 mg / kg, orally) or FBT (65 mg / kg, orally) for 7 days and on day 7 tyloxapol was administered to induce hyperlipidemia. The plasma lipid profile was estimated at 24th h on day 8. Values are the mean ± SEM of five rats. *P < 0.001 compared to saline, @P < 0.001 compared to vehicle, HIAE: Aq. extract of *Holoptelea integrifolia* FBT: Fenofibrate, TLX: Triton WR 1339 (Tyloxapol)

500 mg/kg did not exhibit any significant change in the plasma levels of total cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c compared to vehicle treated animals. The HIAE also did not influence the corresponding atherogenic index and LDL-c/HDL-c ratio. The standard antihyperlipidemic agent, FBT (fenofibrate) also showed similar effect and did not affect the lipid profile compared to vehicle treated rats [Table 1].

Effect of HIAE on lipid profile in tyloxapol-induced hyperlipidemic rats

One-way ANOVA showed that tyloxapol has significantly influenced lipid profile while HIAE treatment failed to attenuate the induction of hyperlipidemia by tyloxapol [Table 2]. Post hoc test indicated that tyloxapol significantly raised total cholesterol, triglycerides, LDL-c and VLDL-c and decreased HDL-c. It has also exhibited elevation in atherogenic index and LDL-c/HDL-c ratio. The prior treatment with HIAE, however, did not attenuate the tyloxapol induced hyperlipidemia. Unlike HIAE, the standard drug, FBT (fenofibrate) caused significant reduction tyloxapol induced increase in total cholesterol, triglycerides, LDL-c and VLDL-c and decrease in HDL-c along with corresponding lowering of atherogenic index and LDL-c / HDL-c ratio [Table 2].

DISCUSSION

The main causative factor for atherothrombotic diseases is the disturbance occurring in lipid metabolism. Though there is a large class of hypolipidemic drugs used in the treatment, none of them is fully effective, absolutely safe and free from side effects.[25] Hence, efforts are being continuously made to find out safe and effective agents that may be beneficial in correcting the lipid metabolism and preventing cardiac diseases.

In the present study, *Holoptelea integrifolia*, a medicinal plant that is used traditionally in the Indian system of medicine for treatment of lipid disorders and obesity was studied for its influence on experimentally-induced hyperlipidemia and to substantiate its claim as natural hypolipidemic agent.

Single oral administration of aqueous extract of *Holoptelea integrifolia* (HIAE) in limit test of acute toxicity study in rats at 2000 mg/kg did not produce any changes in the autonomic and behavioral responses and no mortality was observed even after 14 days. This indicated the extract's safety and absence of toxicity in the higher dose. The approximate LD_{50} is above 2500 mg/kg. On this basis, the dose for administration of HIAE was selected in the range of 250 mg / kg on tenth of the LD_{50} dose.

Administration of HIAE for seven days in the dose of 250 and 500 mg/kg did not affect the total cholesterol, triglycerides, HDL-c, LDL-c and VLDL-c and corresponding atherogenic index and LDL-c/HDL-c ratio. This suggests that HIAE *per se* has no influence on the lipid profile of normolipidemic animals. Similar effect was also observed for standard antihyperlipidemic drug, FBT.

In another set of experiment, effect of HIAE treatment for seven days was studied on Triton WR 1339 (Tyloxapol, TLX) induced hyperlipidemia. Single intraperitoneal administration of TLX (200 mg/kg) has caused significant hyperlipidemia as observed by elevations in total cholesterol, triglycerides levels and increase in levels of LDL-c and VLDL-c. However, HDL-c remained unaffected. It also elevated atherogenic index and LDL-c / HDL-c ratio. The effect of TLX is in concordance with the previous studies[20,26] and indicates the validity of the experimental model. As mentioned previously, TLX produces acute hyperlipidemia by increasing hepatic synthesis of cholesterol with peak

levels at 24 h of administration. The large increase in plasma cholesterol and triglycerides due to Triton WR-1339 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism.[27] Administration of HIAE in the dose of 250 and 500 mg / kg for seven days did not prevent TLX induced hyperlipidemia. It did not attenuate the elevations in the levels of total cholesterol, triglycerides, LDL-c and VLDL-c. It also did not attenuate rise in the corresponding atherogenic index and LDL-c / HDL-c ratio. This indicates the failure of HIAE to normalize the TLX induced hyperlipidemia in contrast to the standard drug, FBT, which has demonstrated the reduction in the elevated levels of total cholesterol, triglycerides, LDL-c and VLDL-c and corresponding atherogenic index and LDL-c / HDL-c ratio. FBT has an acute lowering activity only on plasma triglycerides and LDL-cholesterol. This agrees with the mechanism of action of fibrates.[28] The triglycerides decreasing effect of FBT is very spectacular especially by both stimulation of the gene expression of lipoprotein lipase leading to enhanced catabolism of VLDL, synthesis of fatty acids and reduced VLDL secretion. In the present study, FBT treatment also decreased the total cholesterol which is uncommon observation in contrast to clinical conditions. It is possible that FBT might behave differently in triton hyperlipidemia. Some of the previous studies have also shown similar type of effect of FBT and support the findings.[29-31]

The studies indicate that HIAE *per se* has no influence on lipid profile. However, it is not exactly clear as to why HIAE did not normalize the triton induced hyperlipidemia. Some of the plausible explanations are mentioned here. It is evident from the data that the levels of total cholesterol and triglycerides observed are much higher. Hence, it is possible that the present employed doses of HIAE may not be therapeutically sufficient to bring the detectable and significant normalization of the hyperlipidemia. The present study has only determined the lipid profile in plasma. However, the status of liver lipid profile is not clear. Since, liver is playing a crucial role in the synthesis of cholesterol in triton treated animals. It is very much essential to record the status of liver lipid profile for accurate evaluation of the activity.

The leaves of *H. integrifolia* contain large amounts of flavonoids and saponins.[9] The preliminary phytochemical screening of HIAE has shown the presence of flavonoids and saponins. In the previous studies, flavonoids and saponins have been shown to possess a variety of biochemical and pharmacological activities, including hypolipidemic effects.[32,33] However, despite the presence of these phytochemicals, it is not clear as to why HIAE failed to normalize the triton induced hyperlipidemia.

In light of these facts, it is noteworthy to mention that chronic studies with high fat diet induced hyperlipidemia are required to be carried out to comment exactly on effect of *Holoptelea integrifolia* leaves on lipid profile.

In conclusion, the present study showed that the leaves of *Holoptelea integrifolia* have no hypolipidemic effect in experimentally induced hyperlipidemia and the present study does not substantiate its traditional use in lipid disorders.

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