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Antihyperlipidemic activity of *Mangifera indica* l. leaf extract on rats fed with high cholesterol diet

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ABSTRACT

Background: *Hyperlipidemia is defined as increase in the lipid content in blood. Mangifera indica L., known as mango (Family; Anacardiaceae), commonly used herb in ayurvedic medicine, traditionally used for their antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties, antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, anti microbial, hepatoprotective, gastroprotective effects.* ***Objective:*** *To investigate effect of aqueous extract of Mangifera indica L.leaf on high cholesterol fed diet rats.* ***Results:*** *High cholesterol fed diet rats exhibited significant increase in total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and significant decrease in high density lipoproteins. Treatment with aqueous extract of Mangifera indica leaves significantly decreased total serum cholesterol, triglycerides, low density lipoprotein, very low density lipoprotein and increased in high density lipoproteins rats.* ***Conclusion:*** *Hypolipidemic activity of M. indica may be attributed due to presence of flavonoids, Saponins, glycosides, tannins, phenolics.*

Key words: *Mangifera indica*, Lipoprotein, Hyperlipidemia, Triglycerides, Mangiferin

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INTRODUCTION

Hyperlipidemia characterized by hypercholesterolemia is the most prevalent indicator for succeptibility to cardiovascular diseases [1]. World health organization reports that high blood cholesterol contributes to approximately 56% of cases of cardiovascular diseases worldwide and

causes about 4.4 million deaths each year [1]. Hyperlipidemia is a metabolic disorder, specially characterized by alterations occurring in serum lipid and lipoprotein profile due to increased concentrations of Total cholesterol (TC), Low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C), and triglycerides (TAG) with a concominant decrease in the concentrations of high density lipoprotein cholesterol (HDL-C) in the blood circulation[2]. Currently, the use of alternative medicines and especially the consumption of phytochemicals have been rapidly increasing worldwide. As herbal medicines are less damaging than synthetic drugs they have better compatibility thus improving patient tolerance even on long-term use [3].

Leaf of *Mangifera indica* L., commonly known as mango (Family; Anacardiaceae) is large evergreen tree of tropical and subtropical region has been used by traditional medicine of a number of peoples for centuries. In Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments like antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-inflammatory properties, various effects like antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, anti microbial, hepatoprotective, gastroprotective. The extract showed a powerful scavenging activity of hydroxy radicals and acted as a chelator of iron. It also showed a significant inhibitory effeect on the peroxidation of rat brain phospholipid and prevented DNA damage caused by bleomycin or copper-phenenthroline systems [4]. The leaves of *M. indica* for antidiabetic properties using normoglycaemic, glucose-induced hyperglycaemia and streptozotocin (STZ) induced diabetic mice. The aqueous extract of the leaves of *M. indica* possess hypoglycaemic activity [5]. The natural C-glucoside xanthone mangiferin [2-C-β-Dgluco-pyranosyl-1,3,6,7-tetrahydroxyxanthone;$C_{19}H_{18}O_{11}$; Mw, 422.35; melting point, anhydrous 271°C[6] has been reported in various parts of *M. indica* leaves.

Mangiferin has been reported to be present in various parts of *M. indica* viz. leaves, fruits, stem bark, and roots. A number of active principles from this plant have been identified which include polyphenolics, flavonoids, triterpenoids. From literature survey it was found out that there is no study reported for treating hyperlipidemia with aqueous extract of leaf of *M. indica*. So, the present research work was undertaken to investigate the antihyperlipidemic activity of aqueous extract of leaves of *M. indica* by studying *in vivo* effects on cholesterol induced hyperlipidemia in rats using standard lipid lowering drug atorvastatin.

MATERIALS AND METHODS

Chemicals: Cholesterol, sodium cholate and coconut oil were purchased from SD-fine chemicals; atorvastatin was procured from Ranbaxy labs. Ltd., Gurgaon, India. All other reagents used were of analytical grade.

Instrument: UV spectrophotometer (Shimadzu –UV-1601)**,** Centrifuge Machine (Eltek-research centrifuge-TC-4100D), Sonicator (Enertech Lab).

Collection and authentification of plant material: The leaves of *M. indica* L. were collected locally from college campus of Modasa, Gujarat, in December-January (2008). The plants were first identified by comparing them morphologically and microscopically with description given

in different standard texts and floras. The plants were identified and authenticated by Dr. M. S. Jangid, Sir P.T. Science College, Modasa, Gujarat. Herbarium was prepared and submitted at Institute's herbarium department.

Extraction of plant material: The dried leaves of Mango (300 g) are crushed to powder and extracted with water by microwave. (Intensity 60, time 6min), (Intensity 80, time 5min). The extract was filtered & concentrated to dryness; the extract was defatted. After drying, the extract was weighed and percentage yield was determined. It is then stored in a cool & dry place.

Preliminary phytochemical screening: Preliminary phytochemical screening of aqueous extract of mango leaf was carried out for detection of various plant constituents [7].

Experimental animals: Sprague Dawely female rats weighing 200-250 g were used for the study. Rats were maintained under good hygienic conditions in the departmental animal house. Animals were caged in a group with maximum of three animals per cage. Animals were maintained under standard environmental conditions (22 ± 2°C, 55 ± 5% humidity, 12hr L:D cycle) and fed with a standard feed and water *ad libitum*. The experimental protocol was approved by Institutional Animal Ethical Committee (IAEC) constituted as per CPCSEA guidelines, Shri B. M. Shah College of Pharmaceutical Education and Research, Modasa, Gujarat, India (IAEC/BMCPER/04/2008-09).

Induction of Hyperlipidemia: High cholesterol diet was prepared by mixing cholesterol 2%, sodium cholate 1% and coconut oil 2% or 30 %, with standard powdered standard animal food. The diet was placed in the cage carefully and was aministered for seven days [8].

Dose preparation and administration of standard atorvastatin and mango extract: Standard atorvastatin at a dose of 10 mg kg^{-1} was prepared by suspending bulk atorvastatin in aqueous 0.5 % methyl cellulose [9]. The extract of mango leaf was dissolved in water and a dose of 200 mg kg^{-1} was given to the rats once in a day along with high cholesterol diet orally. Treatment was given daily for seven days.

Experimental protocol for antihyperlipidemic activity: The experimental animals were divided into four groups, six animals in each group:

- Group 1: Normal
- Group 2: High cholesterol diet control
- Group 3: Standard atorvastatin (10 mg kg^{-1} body weight (b.wt.), orally (p.o.)
- Group 4: High cholesterol diet treated with mango extract (200 mg kg^{-1} b. wt., p.o.)

Treatment was given daily for seven days orally.

Collection of blood: On the 8^{th}day, blood was collected by retero orbital sinus puncture, under mild ether anesthesia after 8 hr fasting 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.

Biochemical analysis: The Serum samples were analyzed spectrophotometrically for total serum cholesterol(TC), triglyceride (TG) and high-density lipoprotein cholesterol (HDL-C) was

estimated using diagnostic kits which were procured from lab-Care Diagnostics Pvt. Ltd., Mumbai, India. Very low density lipoprotein (VLDL), High density lipoprotein ratio (HDL-C ratio), Atherogenic Index (AI) and low density lipoprotein cholesterol (LDL-C) were calculated by using formula[10].

Statistical analysis: Results were presented as mean ± SEM (Standard error of mean) 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.

RESULTS

The Phytochemical tests with the aqueous extract of leaf of *M.indica* indicated the presence of flavonoids, saponins, glycosides, tannins, phenolics. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world. The rats fed with high cholesterol diet for seven days exhibited significant increase in TC, TG, LDL-C and VLDL and significant decrease in HDL-C, HDL-C ratio as compared to normal animals. Treatment with atorvastatin (10 mg kg^{-1} body weight, p.o.) showed significant decrease in elevated TC, TG, LDL-C and VLDL, with significant increase in HDL-C ($p<0.05$) as compared to high cholesterol diet control. Whereas treatment with aqueous extarct of mango leaves (200 mg kg^{-1} body weight, p.o) showed significant decrease in elevated TC, TG, LDL-C and VLDL, with significant increase in HDL-C ($p<0.05$) as compared to high cholesterol diet control. (Table-1)

Table: 1 effect of *M. Indica* extract on serum lipid profile in hyperlipidemic rats

	Normal	High cholesterol diet control	High cholesterol diet treated with Atorvastatin	High cholesterol diet treated with Mango extract
TC	64.19±8.07	241.5±16.89*	87.25±14.85**	150.15±16.64**
TG	65.75±1.702	130±2.89*	75.25±0.8539**	89.75±0.459**
HDL-C	42.12±3.20	22.23±1.08*	35.29±2.35**	30.21±2.59**
LDl	8.92±5.07	193.27±14.23*	36.91±11.86**	101.99±14.23**
VLDL	13.15±0.34	26±0.58*	15.05±0.17**	17.95±0.09**
HDL-ratio	190.84±14.51	10.13±5.38*	67.91±15.4	25.18±15.6
AI	1.56±0.10	5.84±0.09	2.13±1.43	2.97±1.29

Values are mean ± SEM (n=6). * Significantly different from normal groups ($p<0.05$).
** Significantly different from high cholesterol diet control groups ($p<0.05$)

DISCUSSION

In Ayurvedic literature of India, different parts of this plant have been recommended as a remedy for various ailments like antidiabetic, anti-oxidant, anti-viral, cardiotonic, hypotensive, anti-

inflammatory properties, various effects like antibacterial, anti fungal, anthelmintic, anti parasitic, anti tumor, anti HIV, antibone resorption, antispasmodic, antipyretic, antidiarrhoeal, antiallergic, immunomodulation, hypolipidemic, anti microbial, hepatoprotective, gastroprotective. The present study has been undertaken to demonstrate the effect of *M. indica* l. leaf on rats fed with high cholesterol diet. Modern lipid lowering agents i.e., all statins (simvastatin, atorvastatin etc.,) are expensive. The most important adverse effects of statins are liver and muscle toxicity. Other risk factors are hypothyroidism, renal insufficiency, hepatic dysfunction, advanced age and serious infections. In the present study, parameters of lipid profile were evaluated for all normal and hyperlipidemic rats. It was found that there was a significant ($p<0.05$) decrease in TC, TG, LDL-C, VLDL and significant increase in HDL-C ($p<0.05$). In the present study, the aqueous extract of leaves of *M.indica* showed a significant antihyperlipidemic activity in cholesterol induced hyperlipidemic model of rats, which was almost comparable to that of the standard atorvastatin drug used in treatment. Their actions may be due to increased inhibition of intestinal absorption of cholesterol, interfernece with lipoprotein production, increased expression of hepatic LDL receptors and their protection etc. Leading to an increased removal of LDL-C from the blood and its increased degradation and catabolism of cholesterol from the body. All these events either individually or in combination lead to decreased serum LDl-C levels which may have also reduced serum cholesterol (TC) levels during the treatment with test extract [11,12,13].

In the hyperlipidemic model, the purpose of inclusion of cholesterol and coconut oil might be attributed to well established findings that addition of dietary cholesterol along with saturated fats results in accumaulation of intracellular cholesterol and its esters in the body tissues as coconut oil contains 92% of saturated fats. Antihyperlipidemic agents which are active in cholesterol induced hyperlipidemic model function by one or more other mecanisms given above and by others [11,12,13,14].

The aqueous extarct of leaves of *M.indica* induced an increase in serum HDL-C levels in the hyperlipidemic models. During blood circulation, HDL-C mediates the transfer of excess cholesterol from the peripheral cells to the liver for its catabolism by a pathway termed as “reverse cholesterol transport” hence increased serum HDL-C levels may prove beneficial in lipid disorders and might also serve as a cardioprotective factor to prevent the gradual initiation of atherosclerotic process.

Apart from wide usage of mango as antidiabetic it can also be used for the treatment of hyperlipidemia. The mango exract at a dose of 200 mg/kg b. wt. orally showed significant antihyperlipidemic acticity which may be attributed due to presence of flavonoids, saponins, glycosides, tannins, phenolics.

CONCLUSION

It is concluded that antihyperlipidemic effect of aqueous extarct of leaves of *M. indica* may be attributed due to presence of flavonoids, saponins, glycosides, tannins, phenolics.

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