

EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF *FICUS HISPIDA* LINN LEAVES IN TRITON WR-1339 (TYLOXAPOL) INDUCED HYPERLIPIDEMIA IN MICE

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ABSTRACT

Antihyperlipidemic activity of the methanolic plant extract of *Ficus hispida* Linn. f. leaves was evaluated against elevated cholesterol, triglycerides level in Triton – WR 1339 (Tyloxapol) induced hyperlipidemic mice model. Administration of the leaf extract of *Ficus hispida* at doses of 125, 250, and 500 mg/kg respectively, showed a significant reduction in serum lipid parameters such as Total Cholesterol, Triglycerides, VLDL and LDL level as compared to the Tyloxapol treated control group, indicating that the leaf extract of *Ficus hispida* possessed Antihyperlipidemic properties.

Keywords: Antihyperlipidemic, *Ficus hispida*, Triton WR-1339 (Tyloxapol), Serum lipid parameters.

INTRODUCTION

Mortality rates due to cardiovascular diseases have increased several folds in most developed and undeveloped countries. These cardiac ailments are directly related to hyperlipidemia [1]. Evidence from lipid lowering trials has clearly established that reduction of total cholesterol or low density lipoprotein cholesterol (LDLc) is associated with a decreased risk of atherosclerosis and coronary heart disease [2, 3]. Furthermore, since the last two decades a strong correlation has been observed between levels of circulating lipids and mortality rates from coronary atherosclerotic heart disease [4].

A large number of synthetic antihyperlipidemic drugs are currently available in the market but these lag on the desired properties of safety on long term use and cost. These factors affect a patient's compliance [5]. Plants and herbs are mines of a large number of bioactive phytochemicals that might serve as leads for the development of effective, safe, cheap and novel drugs. A number of medicinal plants have shown their beneficial effect on cardiovascular disease by virtue of their lipid lowering, antianginal, antioxidant and cardioprotective effects [6, 7].

Ficus hispida (Moraceae) [commonly known as Dumoor (Bengali), Gobra (Hindi) and Peyatti (Tamil), bhokada (Marathi), Kadaatthi (Kannada), and Jangli anjir (Gujarati)] is a moderate sized tree which grows well in damp localities and shady places. Almost all parts of this plant are used in traditional Indian medicine for the treatment of various ailments by traditional healers. The leaves are of particular interest from a medicinal point of view [8], as an anti-diarrhoeal [9], hepatoprotective [10], anti-inflammatory [11], antitussive, antipyretic, astringent, vulnerary, haemostatic, and an anti-ulcer drug [12, 13].

The phytochemical constituents of *Ficus hispida* Linn. f. include phenanthroindolizidine alkaloids, n-alkanes, coumarins and triterpenoids [14]. Published reports also show that *Ficus hispida* leaves contain hispidin, oleanolic acid, bergapten, β -amyrin and β -sitosterol [15, 16] and the bark comprises lupeol acetate, β -sitosterol and β -amyrin acetate [17]. Ample evidence is available suggesting that these compounds exhibit significant antioxidant, cardioprotective and hypoglycemic properties [18-21].

However, as there are no reports on the antihyperlipidemic activity of this plant species the present study was initiated to investigate the antihyperlipidemic potential of the methanol extract of the leaf of *Ficus hispida*, in Triton WR-1339 (Tyloxapol) induced hyperlipidemic mice model.

MATERIALS AND METHODS

Chemicals

Tyloxapol (Triton WR 1339) was procured from Sigma Aldrich, USA, while Simvastatin was obtained from Cipla LTD. Enzymatic kits were

purchased from Span Diagnostics India Ltd. All other chemicals used were of Analytical grade.

Plant material and extraction

Ficus hispida fresh leaves were collected from Mumbai, Maharashtra in August 2010. The plant species was authenticated by a botanist. The leaves were washed well with water, dried under shade, pulverised in a mechanical grinder and stored in a closed air tight container till further use. Fifty g of the powdered material was extracted with methanol at 60-70°C for 30 hours in a Soxhlet apparatus. The extract was filtered and concentrated to obtain a greenish semi solid mass (8.42% on dry weight basis). Quantities of the individual drug (extract) to be administered orally were calculated and suspended in vehicle (0.5%CMC) so as to yield dose levels of 125 mg/kg, 250mg/kg and 500 mg/kg body weight.

Preliminary phytochemical screening

The concentrated extracts were analysed to identify various constituents using standard methods [22, 23].

Animals

Adult albino mice of either sex, weighing 25-30g were taken for the study. The animals were housed in standard environmental conditions and fed with food and water *ad libitum*. The mice were acclimatised to the laboratory conditions for 10 days before commencement of the experiment. All experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, India and approved by the Institutional Animal Ethical Committee.

Acute toxicity studies

Acute toxicity was studied using acute toxic class limit test dose guideline 425 of Organisation for Economic Co-operation and Development (OECD). Toxicological effects, if any, were assessed on the basis of mortality and behavioural changes observed (OCED, 2001).

Induction of hyperlipidemia

Hyperlipidemia was induced by a single intraperitoneal injection of Triton WR-1339 (300mg/kg body weight) dissolved in 0.9% saline to overnight fasted albino mice. After one hour of tritonization, the animals were given feed *ad libitum*. Hyperlipidemia was confirmed 24 hours after triton injection by determining the blood cholesterol concentration.

Pharmacological Evaluation

The animals were divided into six groups, (n = 6 per group), Group I normal control, Group II administered Tyloxapol (control), Group III. IV and V administered Tyloxapol + methanolic *Ficus hispida* extract

(MFHE) at 125 mg/kg, 250 mg/kg and 500 mg/kg respectively. The sixth Group was administered Tyloxapol + Simvastatin. This served as the positive control.

All mice except the normal control group were injected with triton WR-1339 (Sigma, USA) at a dose of 300mg/kg to induce hyperlipidemia in the mice, while the normal control group was injected with normal saline (NS). Twelve hour following Triton WR-1339 administration, group III, group IV and group V mice were treated with methanolic *Ficus hispida* extract (MFHE) at doses of 125, 250, and 500 mg/kg respectively, orally by gastric intubation. Simultaneously, the positive control group was given the standard drug Simvastatin at a dose of 80 mg/kg by gastric intubation. Carboxy methyl cellulose (CMC) 0.5% was used as the vehicle to administer MFHE and Simvastatin.

At the end of the experimental period, blood was withdrawn after 24 hours by retro-orbital sinus puncture method and centrifuged at 5000 rpm for 10 minutes so as to obtain serum.

Biochemical assay

The amount of serum total cholesterol, HDL-C was estimated by the enzymatic cholesterol oxidase - phenol + aminophenazone (CHOD-PAP) method [24, 25, 26, 27]. Triglycerides were estimated by the enzymatic glycerol-3-phosphate oxidase - phenol + aminophenazone

(GPO-PAP) method [28]. LDL-cholesterol and VLDL-cholesterol were calculated using the Friedwald formula [29].

Statistical Analysis

The results have been expressed as mean \pm SD for 6 animals in each group. One way analysis of variance (ANOVA) was performed by comparison of values for different groups [30] at $P < 0.05$.

RESULTS

Acute toxicity studies

Administration of methanolic extract of leaves of *Ficus hispida* up to 2000 mg/kg body weight did not produce any mortality and gross behavioural changes.

Antihyperlipidemic activity

From the data presented in Table 1 and 2 it is observed that the administration of Tyloxapol induced hyperlipidemia in mice (Group 2). Concurrent administration of *Ficus hispida* at doses of 125mg/kg, 250mg/kg and 500mg/kg body weight (Group IV, Group V and Group VI) respectively showed a significant reduction in the levels of serum total cholesterol, LDL, VLDL as well as triglycerides. There was a decrease in serum HDL cholesterol when compared with the normal control (Table 2).

Table 1: Effect of methanolic leaf extracts of *Ficus hispida* on Total Cholesterol, Triglycerides in Triton WR-1339 (Tyloxapol) induced hyperlipidemic mice

Parameters	Total Cholesterol (mg/dl)	Triglycerides (mg/dl)
Group-I Normal control	84.5 \pm 11.56	52.42 \pm 19.42
Group-II Triton treated	248.98 \pm 18.19*	695.35 \pm 82.81*
Group-III Triton + Simvastatin (80mg/kg)	181.78 \pm 27.76**	448.54 \pm 77.11**
Group-IV Triton+ MFHE (125mg/kg)	171.61 \pm 33.42**	435.06 \pm 42.40*
Group-V Triton + MFHE (250mg/kg)	153.13 \pm 26.40*	357.14 \pm 67.70*
Group-VI Triton + MFHE (500mg/kg)	132.35 \pm 37.63*	392.47 \pm 47.66*

All value are expressed as Mean \pm SD. Triton-treated group was compared with normal control, Triton + MFHE and drug treated groups were compared with the Triton control group * $P < 0.001$, ** $P < 0.01$.

Table 2: Effect of methanolic leaf extracts of *Ficus hispida* on HDL, LDL, and VLDL Cholesterol in Triton WR-1339 (Tyloxapol) induced hyperlipidemic mice

Parameters	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group-I Normal control	62.97 \pm 9.73	11.04 \pm 4.33	10.48 \pm 3.89
Group-II Triton treated	39.83 \pm 2.81	70.08 \pm 26.37**	139.07 \pm 16.56*
Group-VI Triton + Simvastatin (80mg/kg)	27.51 \pm 3.79	64.57 \pm 31.37	89.71 \pm 5.42**
Group-III Triton+ MFHE (125mg/kg)	36.81 \pm 7.32	44.19 \pm 26.15	90.61 \pm 8.48*
Group-IV Triton + MFHE (250mg/kg)	32.04 \pm 11.27	49.65 \pm 22.58	71.43 \pm 13.54*
Group-V Triton + MFHE (500mg/kg)	34.36 \pm 7.17	19.50 \pm 38.64***	78.49 \pm 9.53*

All value are expressed as Mean \pm SD. Triton-treated group was compared with the normal control group, Triton + MFHE and drug treated groups were compared with Triton control group * $P < 0.001$, ** $P < 0.01$ and *** $P < 0.05$

DISCUSSION

Hyperlipidemia has been documented as one of the major risk factors involved in the development of cardiovascular diseases [31]. Atherosclerosis and congestive heart diseases are strongly associated with disorders of lipid metabolism and plasma lipoproteins. Triton WR-1339 (Tyloxapol) has been widely used for screening of natural or chemical hypolipidemic drugs because it is convenient in terms of length of treatment period and handling [32]. Triton WR-1339 (Tyloxapol) acts as a surfactant which blocks the uptake of lipoprotein from the blood circulation by extra hepatic tissues, resulting in an increase in the level of circulatory lipoproteins [33]. Many medicinal plants like *Phyllanthus niruri*, *Vaccinium myrtillus* and *Erica multiflora*; have been investigated for their acute hypolipidaemic activity in Triton WR-1339 (Tyloxapol) induced hyperlipidaemic animals [34, 35, 36].

The present study was designed to investigate the antihyperlipidemic effect of the extract of the leaf of *Ficus hispida* on Triton WR1339 induced hyperlipidemia in mice. The study

demonstrated that 24 hours after administering *Ficus hispida* leaf extract at different doses (125mg/kg, 250mg/kg, and 500mg/kg), there was a remarkable reduction in the total cholesterol, triglycerides, VLDL and LDL levels in the serum of hyperlipidemic mice, suggesting a beneficial modulatory influence on cholesterol metabolism and turnover. However there was no improvement in HDL cholesterol.

Otway and Robinsons (1967) have stated that the large increase in plasma cholesterol and triglycerides due to Triton WR-1339 administration resulted mostly from an increase of VLDL secretion by the liver, accompanied by a strong reduction of VLDL-C and LDL-C catabolism [37]. In the present investigation the reduction of plasma total cholesterol in response to treatment with the crude extract of *Ficus hispida* is apparently associated with the significant decrease of its LDL fraction (Table 1 and 2), which, in fact, is the target of several hypolipidemic drugs. This result suggests that the cholesterol lowering activity of *Ficus hispida*, could possibly be due to a rapid catabolism of LDLc through its hepatic receptors for its final elimination in the form of bile acids [38].

Published reports have shown that *Ficus hispida* leaves contain hispidin, oleanolic acid, bergaten, β -amyirin and β -sitosterol [14]. These compounds are known to exhibit significant antioxidant, cardioprotective and hypoglycemic properties [18-21]. It is likely that some of these phytoconstituents might be responsible for exerting beneficial antihyperlipidemic effects. The cholesterol lowering effect of β -sitosterol has already been well established [39]. Further, it has been shown that the cholesterol is reduced by preventing its absorption from the gut [40]. Preliminary phytochemical investigations of the methanolic extract of *Ficus hispida* revealed the presence of alkaloids, flavonoids, sterols, saponins, phenols and glycosides. The antihyperlipidemic activity of *Ficus hispida* (500 mg/kg) was equipotent when compared to Simvastatin. The present study has shown that administration of the methanol extract at 500 mg/kg of *Ficus hispida* was more effective than the standard drug for managing hyperlipidemia.

CONCLUSION

From the present study it can be concluded that the methanolic extract of the leaf of *Ficus hispida* improves serum lipid profile in triton WR-1339 (Tyloxapol) induced hyperlipidemia in mice. The extract at 500mg/kg is as effective as the standard drug simvastatin. The current study supports, at least partly, the traditional use of this ethnomedicinal plant. Further, studies have been initiated to gain a deeper insight into the possible mechanism of action of this plant drug.

REFERENCES

- KHO JH, Kim JM, Chang UJ and Suh H J. Hypocholesterolemic effect of hot water extract from mycelia of *Cordyceps sinensis*. Biol. Pharmacol. Bull 2002; 26(1): 84-87.
- Brown AS, Bakker - Arkema, RG and Yellen L: Treating patients with documented atherosclerosis to National Cholesterol Education Program - recommended low density lipoprotein cholesterol goals with atorvastatin, fluvastatin, lovastatin and simvastatin. J. Mol. Cell. Cardiol 1998; 32:665-692.
- Grundy SM, Cleeman JI and Merz C N: Implication of recent clinical trials for the National Cholesterol Education Program Adult Panel on Detection, Evaluation and Treatment of high blood cholesterol in adults. JAMA 2004; 285: 2486-2497.
- Anwar M, Hakeem MH, Din T and Khan AB. Clinical efficacy of Safoof-e-Luk (powdered lac) in the management of hyperlipidemia. Hamdard Med 1999; 40(1): 94-97.
- Davidson MH and Tooth PP: Comparative effect of lipid lowering therapies. Prog. Cardiovasc. Dis 2004 47:173-204.
- Wang HX and Ng TB: Natural products with hypoglycaemic, hypotensive, hypocholesterlemic, antiathero-sclerotic and antithrombotic activities. Life Sci 1999; 65:2663-2677.
- Dwivedi S: Atherosclerosis revisited. Indian J. Cardiol. 2004; 7: 6-12.
- Nadkarni AK. Indian Materia Medica. Popular Prakashan, Bombay; 1976. I: 1031-1035
- Mandal SC and Kumar CKA. Studies on anti-diarrhoeal activity of *Ficus hispida*. Leaf extract in rats. Fitoterapia 2002; 73(7-8): 663-667.
- Mandal SC, Saraswathi B, Kumar CKA, Lakshmi SM and Maiti BC. Protective effect of leaf extract of *Ficus hispida* Linn. Against paracetamol- induced hepatotoxicity in rats. Phytother Res 2000; 14 (6): 457-459.
- Vishnoi SP and Jha. Evaluation of anti- inflammatory activity of leaf extract of *Ficus hispida*. Indian J.Nat. Prod 2004; 20 (3): 27-29.
- Nadkarni AK. Indian Materia Medica. Popular Prakashan, Bombay. 1976; I: 1031-1035
- Rastogi and Mehrotra BN. Compendium of Indian Medicinal Plants, Central Drug Research Institute, Lucknow. Publication and Information Directorate, New Delhi. 1993; II: 27-30.
- Peraza- Sanchez SR, Chai HB, Shin, T Santisuk YG, Reutrakul V, Farnsworth NR, Cordell GA, Pezzuto JM and Kinhorn AD. Constituents of the leaves and twigs of *Ficus hispida*. Planta Med 2002; 68 (2): 186-188.
- Huong VN and Trang VM. Hispidin. A strong anticancer agent isolated from the leaves of *Ficus hispida* L., Tap. Chi. Hoa. Hoc 2006; 44 (3): 345-349.
- Khan MSY, Siddiqui AA and Javed K. Chemical investigation of the leaves of *Ficus hispida*. Indian J. Nat. Prod 1991; 6(2): 14-15.
- Acharya BM and Kumar KA. Chemical examination of the bark of *Ficus hispida* Linn. Curr. Sci 1984; 53 (19): 1034-1035.
- Du Y and KM Ko. Oleanolic acid protects against myocardial ischemia-reperfusion injury by enhancing mitochondrial antioxidant mechanism mediated by glutathione and α -tocopherol in rats. Planta Med 2006; 72 (3): 222-227.
- Khushbaktova ZA, Yusupova SM, Badal'yants KL, Syrov VN and Batirov EKh. Isolation of hispidin from a walnut-tree fungus and its antioxidant activity. Chem. Nat. Comp 1996; 32 (1): 27-29.
- Shanmugarajan TS, Arunsundar M, Somasundaram I, Krishnakumar E, Sivaraman D and Ravichandran V. Cardioprotective effect of *Ficus hispida* Linn. on Cyclophosphamide provoked Oxidative Myocardial Injury in a rat model. International Journal of Pharmacology 2008; 4(2):78-87.
- Ghosh R, Sharatchandra Kh, Rita S, Thokchom IS. Hypoglycemic activity of *Ficus hispida* (bark) in normal and diabetic albino rats. Indian J Pharmacol 2004; 36(4):222-225.
- Kokate CK, Purohit AP, Gokhale SB Pharmacognosy, Nirali Prakashan, 21 edn, Pune; 2002 pp 105-106, 111-113.
- Harborne JB and Baxter HH. Phytochemical Dictionary: A hand Book of Bioactive Compound from plants. Taylor and Francis, Washington, D.C., U.S.A, 1993 pp 237.
- Herbert K. Lipids, In Clinical chemistry; Theory, Analysis and Co-relation, Kaplan L. A. and Pesce A.J., Eds. C.V. Mosby, Toronto, 1984; p 1182-1230.
- Nader R, Paul B, John A. Lipids, Lipoprotein and apolipoprotein, In Tietz textbook of Clinical Chemistry, 3rd ed., Burtis C.A and Ashwood E.R., Eds. W.B. Saunders, Philadelphia, 1994. p 809-852.
- Siedel J, Hagele EO, Ziegenhorn J and Wahlefeld AW. Reagent for the enzymatic determination of serum total cholesterol with improved lipolytic efficiency. Clin. Chem 1983; 29(6): p 1075.
- Cres Cenizio Izzo, Franco Grub, and Enzo Murador. Improved method for determination of High-Density-Lipoprotein cholesterol. Clin. Chem., 1981; 27(3): p 371.
- McGowan MW Artiss JD, Strandbergh DR and Zak B. A peroxidase-coupled method for the colorimetric determination of serum triglycerides. Clinical Chemistry 1983; 29: p 538.
- Freidewald WT, Levy RI and Frederickson DS. Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge. Clin. Chem 1972; 18: 499-502.
- Woodson RF. Statistical Methods for the Analysis of Biochemical Data. Chichester: Wiley, U.S, U.K 1987; p 315.
- Rajani GP, Purnima Ashok. In Vitro antioxidant and antihyperlipidemic activities of *Bauhinia variegata* Linn. Indian J Pharmacol 200; 1(5): 227-232.
- Schurr PE, Schultz JR and Parkinson TM. Triton induced hyperlipidaemia in rats as an animal model for screening hypolipidaemic drugs. Lipids 1972; 7:69-74.
- Schurr PE, Schultz JR, Parkinson TM. Triton induced hyperlipidemia in rats as an animal model for screening of hypolipidaemic drugs. Lipids 1972; 7:68-74.
- Khanna AK, Rizvi F and Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats. J. Ethnopharmacol 2002; 82: 19-22.
- Cignarella A, Nastasi M, Cavalli E and Puglisi L. Novel lipid-lowering properties of *Vaccinium myrtillus* L. leaves, a traditional antidiabetic treatment in several models of

- dyslipidaemia: A comparison with ciprofibrate, *Thrombosis Research* 1996; 84: 311-322.
36. Harnafi H, El-Houda-Bouanani N, Aziz M, Caid HS, Ghalim N and Amrani S. The hypolipidaemic activity of aqueous *Erica multiflora* flowers extract in Triton WR-1339 induced hyperlipidaemic rats: A comparison with fenofibrate. *J. Ethnopharmacol* 2007; 109: 156-160.
 37. Otway S and Robinson DS. The effect of nonionic detergent (Triton WR 1339) on the removal of triglyceride fatty acids from the blood of the rats. *J. Physiol* 1967; 19: 309-319.
 38. Khanna AK, Rizvi F and Chander R. Lipid lowering activity of *Phyllanthus niruri* in hyperlipemic rats, *J. Ethnopharmacology* 2002; 82: 19-22.
 39. Lees AM, Mok HYI, Lees RS, Mc Cluskey MA and Grundy S. Plant sterol as cholesterol lowering agent: clinical trials in patients with hypercholesterolemia and studies of sterol balance, *Atherosclerosis* 1997; 28: 325-338.
 40. Mattson FH, Grundy SM and Crouse JR. Optimizing the effect of plant sterols on cholesterol absorption in man. *Am J Clin Nutr* 1982; 35: 697- 700.