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Research Article

ANTIDYSLIPIDEMIC AND ANTIOXIDANT ACTIVITY OF HYDROETHANOLIC FRUIT EXTRACT OF *FICUS GLOMERATA*

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ABSTRACT

Ficus glomerata Roxb (Family: Moraceae) is an evergreen tree found throughout India and other parts of the world. The lipid lowering action of hydroethanolic fruit extract *Ficus glomerata* has been studied in triton induced hyperlipidemic rats. Serum lipids were found to be lowered by *Ficus glomerata* (200mg/kg b. w.) in triton WR-1339 induced hyperlipidemia *in vivo*. The hypolipidemic activity of the extract is compared with gemfibrozil (100mg/kg) a known lipid lowering drug and *in vitro* antioxidant activity of this extract shown potent inhibition of superoxide anions, hydroxyl redicals and microsomal lipid peroxidation and scavenger of oxygen free redicals.

Keywords: Hypolipidemic, Dyslipidemic, Antioxidants, Triton model, Hyperlipidemia.

INTRODUCTION

Coronary heart disease(CHD) are the leading cause to death in the world[1,2], low density lipoprotein-cholesterol (LDL-C) accounts for approximately 2/3rd of the serum cholesterol pool in a normal subject and is believed to play an important role in a atherosclerosis. LDL-C and high density lipoprotein - cholesterol (HDL-C) respectively are positive and negative cardiovascular (CV) risk factors associated with deaths by myocardial infarction[3]. Oxidative stress via the increased production of free radicals and other reactive oxygen species and decreased antioxidative defenses, is known to occur during cardiovascular diseases (CVD)[4]. Numerous studies have shown that oxidative stress causes structural and functional alterations in molecular, cellular tissues and organ systems[5]. Therefore functional foods which have antioxidant properties may be effective and useful for reducing the risk of CVD associated pathological damage. Epidemiological studies have consistently shown that a high dietary intake of fruits and vegetables as well as whole grains is strongly associated with reduced risk of developing chronic diseases, such as, cancer, CVD, diabetes, hepatotoxicity etc.[6-8].

Several members of the genus Ficus (family:Moraceae) are being used traditionally in a wide variety of ethnomedical remedies. One among them, *Ficus glomerata* syn. *Ficus racemosa* (Gular), is widely distributed in all over India, northern Australia and other parts of Asia. Its stem bark has shown anti-diarrhoeal, antidiuretic, antitussive, anti-pyretic, chemomodulatory effect and hypoglycemic activities[9, 10]. The fruit extracts of it showed antifungal, antimicrobial, antipyretic, antidiabetic activities in previous studies[11, 12]. Since detailed *in vitro* antioxidant activity and anti-dyslipidemic activities of *F. glomerata* fruit (ripened) have not yet been explored. In the present study, we have investigated the antidyslipidemic and antioxidant activity of the hydroethanolic extract from *Ficus glomerata.*

MATERIALS AND METHODS

Collection of plant material

Samples were collected from Raebarely and Faizabad regions of Uttar Pradesh and washed with tap water twice, air dried, powdered and finally stored in polythene bags at 4°C till further processing. The identification of plant was confirmed by Dr. T. Hussain, Department of Taxonomy, National Botanical research Institute (NBRI), Lucknow, India. The voucher specimens were also deposited in institute library.

Extraction and yield: The powdered fruit of *F. glomerata* (50g) was extracted in a rotatory shaker at constant stirring rate for 2 days. The process was carried out thrice with 500 ml of hydroethanolic solvent (1:1). Solids were removed by filtration and the solvent from combined extracts was removed under reduced pressure at 150 PSI and 40°C in a Buchi Rotavapor. The yield of powdered extract was 5g after solvent evaporation. Powdered plant extract was used for their further activities.

Animals used

Rats (Charles foster strain, adult body weight 200-225 g) were kept in room with controlled temperature (25-26°C), humidity (60-80%) and 12/12 hr dark and light cycle (light on from 08:00 AM to 8:00 PM) under hygienic conditions. Animals which were acclimatized for one week before starting the experiment had free access to the normal diet and water *ad-libitum.*

Triton induced hyperlipidemia

The rats were divided in control, triton induced, triton plus *F. glomerata* and triton plus Gemfibrozil (standard drug) treated groups containing six rats in each group. In this acute experiment triton WR-1339 (Sigma chemical Co., St. Louis, USA) was administrated at a dose of (400 mg/kg, b. wt.) by intraperitoneal injection. for 18 hr. *F. glomerata* and gemfibrogil (CIPLA Ltd. Bombay, India) were macerated with aqueous gum acacia (0.2% w/v) suspension fed orally (200 mg/kg and 100 mg/kg b. wt.) simultaneously with triton. At the end of experiments rats were fasted overnight and blood was withdrawn. The animals were sacrificed and the liver was excised immediately[13].

Biochemical analysis of plasma/serum

Plasma post heparin, lipolytic activity (PHLA) was assayed[14]. Serum was analyzed for their total cholesterol activity (TC), phospholipids (PS), Triglycerides (TG) and protein by standard procedure[15-18].

Biochemical analysis of liver

Liver was homogenized (10% w/v) in cold 100mM phosphate buffer pH 7.2 and used for the assay of total lipolytic activity as mentioned above. The lipid extract of each homogenate was used for the estimation of TC, PL, TG and protein as mentioned above.

Antioxidant activity (generation of free radicals)

Super oxide anions were generated enzymatically by xanthine (160 mM), xanthine oxidase (0.04 U) and nitroblue tetrazolium (320 M) in absence or presence of the extract of *F. glomerata* (100 and 200 ug/ml) in 100 mM phosphate buffer (pH 8.2) extract was sonicated well in phosphate buffer before use. The reaction mixtures were incubated at 37°C and after 30 min the reaction was

stopped by adding 0.5 ml glacial acetic acid. The amount of formazone formed was measured spectrophotometrically at 560nm. In another set of experiment effect of extract on the generation of hydroxyl radical was also studied by non-enzymatic reactants[19]. Briefly, ·OH were generated in a non-enzymatic system comprising deoxyribose (2.8 mM), FeSO4.7H2O (2 mM), sodium ascorbate (2.0 mM) and H_2O_2 (2.8 mM) in 50 mM KH_2PO4 buffer (pH 7.4) to a final volume of 2.5 ml. The above reaction mixtures in the absence or presence of test compounds (200 µg /ml) were incubated at 37^{0}C for 90 min. The test compounds were also studied for their inhibitory action against microsomal lipid peroxidation *in vitro* by nonenzymatic inducer. Reference tubes and reagents blanks were also run simultaneously. Malondialdehyde (MDA) contents in both experimental and reference tubes were estimated spectrophotometrically by thiobarbituric acid[20].

Statistical evaluation

Data were analyzed using student t test. The hyperlipidemic groups were compared with control and extract treated groups. Similarly the oxygen free radicals with *F. glomerata* extract was compared with that of their formation without extract. P<0.01 was considered to be significant.

RESULTS

Effects of *F. glomerata* in triton induced hyperlipidemia

The acute administration of triton WR-1339 caused a markeable increase in serum levels of TC (+4.94 F), PL (+3.90 F), TG (+4.43 F) and protein (+ 1.52 F). Treatment with *F. glomerata* caused a significant reversal in their level. The triton induced caused the inhibition of PHLA (- 31%) and treatment with the extract and gemfibrozil partially reactivated these lipolytic activities in plasma of hyperlipidemic rats (Table 1).

Effects of *F. glomerata* on hepatic lipids

There is a marked increase in hepatic lipids i.e,TC (+ 1.64 F), PL (+36%), TG (+32%) and protein (+ 34%) in triton treated hyperlipidemic rats and in triton plus *F. glomerata* extract showed reversal in the level of hepatic lipids i.e, TC (-21%), PL (- 24%), TG (-22%) and protein (-23%) respectively (Table 2).

Effects of *F. glomerata* extract in oxygen free radical generation *in vitro*

The scavenging potential of *F. glomerata* extract at a dose of (100 and 200 ug/ml) against formation of O_2^- and ·OH in non enzymatic system was studied (Table 3).

DISCUSSION

Hyperlipidemia is one of the important risk factors involved in the development of cardiovascular diseases. Atherosclerosis and congestive heart diseases are strongly associated with disorders of lipid metabolism and plasma lipoprotein[21]. In the present study the extract of *F. glomerata* produced potent hypolipidemic and antioxidant activity. This can be attributed to the flavonoids and other phytoconstituents present in the extract.

F. glomerata and Gemfibrozil both caused a significant decrease in the serum levels of lipids in triton induced hyperlipidemic rats and this model has been successfully used for the evaluation of lipid lowering activity of natural products in rats[22]. Triton WR-1339 acts as a surfactant and suppresses the action of lipases to block the uptake of lipoproteins from circulation by extra-hepatic tissues resulting into increased blood lipid concentration[23]. The lipid lowering effect of *F. glomerata* extract might be due to an early clearance of lipids from circulation of lipids from circulation in both hyperlipidemic models and it might be due to reactivation of lipolytic enzymes as evidenced by increased PHLA, which play a key role in lipid catabolism and their utilization in the body leading to decrease in the level of plasma and liver lipids in the above models[24]. It is reported that hypolipidemic action of Guggulsterone, the active principle is mediated through activation of PHLA, LPL and lecithin Cholesterol acyl transferase (LCAT), inhibition of hepatic cholesterol biosynthesis and increased faecal bile acid excretion[25]. The similar mechanism might also interplay with hypolipidemic effect of *F. glomerata* extract.

The potentially reactive derivative of oxygen described as ROS such as superoxide radicals and hydrogen peroxide are continuously generated inside the human body as a consequence of exposure to exogenous chemicals and or a numbers of endogenous metabolic processes involving redox enzymes and bioenergetics electron transfer owing to the ROS overproduction and or inadequate antioxidant defense, there is upsurge of ROS and this ultimate's in oxidative stress. It is quite interesting to note that plants extract have efficient antioxidant ability and are safer to use as a drug in comparison to synthetic antioxidants[26]. The antioxidants activity can be attributed to various mechanisms like prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, reactive capacity and free radical scavenging activity[26]. Furthermore we are also evaluating antimalarial and hepatoprotective activity of the plant to explore its medicinal aspects.

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Table 1: Lipid lowering activity of *F. glomerata* extract in triton treated hyperlipidemic rats

Groups	Total Cholesterol[a]	Phospholipids[a]	Trigycerides[a]	Protein[b]	PHLA[c]
Control	80.32 ± 6.87	76.94 ± 6.23	78.68 ± 5.64	6.12 ± 0.17	17.12 ± 1.10
Triton treated	397.12 ± 28.84*** (+ 4.94 F)	300.33 ± 24.17*** (+ 3.90 F)	348.62 ± 25.81*** (+ 4.43 F)	9.32 ± 0.28*** (+ 1.52 F)	11.84 ± 0.82*** (- 31%)
Triton + *F. glomerata*	300.41 ± 24.77*** (- 24%)	230.44 ± 18.76*** (- 23%)	254.37 ± 20.44*** (- 27%)	7.44 ± 0.48** (- 20%)	15.57 ± 0.78*** (+ 23%)
Riton + Gemfibrozil (Std. Drug)	260.40 ± 20*** (- 34%)	200.12 ± 17.14*** (- 33%)	230.10 ± 20.14*** (- 34%)	6.80 ± 0.27*** (-27%)	16.23 ± 1.00*** (+27%)

Units: a mg/dl, b g/dl, c nmol free fatty acid formed/h/ml plasma, Values are mean ± SD of six rats *** P< 0.001; **p<0.01 triton treated group compared with control and triton plus drug treated group compared with triton only (F=Fold increase).

Groups	Total Cholesterol[a]	Phospholipids[a]	Triglycerides[a]	Protein[b]	PHLA[c]
Control	6.82 ± 5.17	24.14 ± 2.00	11.00 ± 0.72	145.82 ± 12.11	132.64 ± 10.10
Triton treated	11.23 ± 0.72*** (+1.64 F)	38.17 ± 3.11*** (+36%)	16.37 ±1.00*** (+32%)	220.00 ± 16.12*** (+34%)	73.22 ± 5.17*** (-45%)
Triton + *F. glomerata*	8.80 ± 0.32** (- 21%)	28.66 ± 1.62 *** (- 24%)	12.80 ± 0.68*** (- 22%)	170.37 ± 11.82*** (- 23%)	83.37 ± 6.12** (+ 15%)
Riton + Gemfibrozil (Std. Drug)	8.10 ± 0.33*** (- 28%)	25.88 ± 1.12*** (- 32%)	11.38 ± 0.62*** (- 30%)	160.37 ± 12.82*** (-27%)	87.77 ± 8.00** (+16%)

Table 2: lipid lowering activity of hepatic lipids in triton treated hyperlipidemic rats.

Units: a mg/dl; b g/dl; c nmol free fatty acid formed/mg/protein ,Values are mean ± SD of six rats ***P< 0.001; **p<0.01 triton treated group compared with control and triton plus drug treated group compared with triton only, (F=Fold increase)

Table 3: Antioxidant activity of *F. glomerata* extract.

Groups	Dose (µg/ml)	Superoxide anions[a]	Hydroxy radicals[a]	Microsomal lipid peroxidation[b]
Control	-	120.22 ± 9.77	75.50 ± 7.00	85.56 ± 7.39
F. golmerata	100	90.33 ± 6.89*** (- 25%)	57.30 ± 3.80*** (- 24%)	65.48 ± 4.00*** (- 23%)
	200	78.17 ± 6.00*** (- 35%)	42.14 ± 4.00*** (- 44%)	51.20 ± 3.77*** (- 40%)
Standard drugs	200	50.78 ± 3.62*** (- 58%) (Allopurinol)	40.36 ± 2.71*** (- 47%) (Manitol)	38.20 ± 2.51*** (- 55%) (α- tocopherol)

Units: a. nmole formazone formed/ min.
b. nmole MDA formed/hr.
Drug treated groups compared with reference group, Values are mean ± SD of four values *** P<0.001

REFERENCES

1. World Health Report, Report of the Director General WHO, Geneva, 1998.
2. Kannel WB, Dawber TR, Kagan A, Revotski N, Stokes JI Factors of risk in the development of coronary heart disease: six-year follow-up experience. Ann Int Med 1961; 55:33-50.
3. Kastelei JJP The future of best practice. Atherosclerosis 1999; 143 Suppl 1: S17-S21.
4. Bagchi K, Puri S Free radicals and antioxidants in health and disease. Eastern Mediterranean Health Journal 1998; 4 (2): 350-360.
5. Kregel KC, Zhang HJ An integrated view of oxidative stress in aging: basic mechanisms, functional effects, and pathological considerations. Am J Physiol Regul Integr Comp Physiol 2007; 292:R18-R36.
6. WHO Technical Report Series. Diet, nutrition and the prevention of chronic diseases. Report of a Joint WHO/FAO Expert Consultation; Geneva 2003. Retrieved 2011-03-07.
7. Reddy KS, Yusuf S Emerging epidemic of cardiovascular disease in developing countries. Circulation 1998; 97:596-601.
8. Ignarro LJ, Balestrieri ML, Napoli C Nutrition, physical activity, and cardiovascular disease: an update. Cardiovasc Res 2007; 73:326-340.
9. Baskara RR, Murugesan T, Pal M, Saha BP, Mandal SC Antiqussive potential of methanolic extract of stem bark of Ficus racemosa Linn. Phytotherap Res 2003; 17:1117-1118.
10. Khan M, Sultana S Chemomodulatory effect of Ficus racemosa extract against chemically induced renal carcinogenesis and oxidative damage response in wistar rats. Life Sciences. 2005, 77:1194-1210.
11. Mukherjee K, Saha K, Murugesan T, Manal SC, Pal M, Saha BP Screening of antidiarrhoeal profile of some plant extract of a specific region of West Bangal, India. J Ethnopharmacol 1998; 60:85-89.
12. Rao RB, Anupam K, Swaroop KR, Murugesan T, Pal M, Mandal SC Evaluation of anti-pyratic potential of Ficus racemosa bark. Phytomedicine 2002; 9:731-33.
13. Schurr PE, Schultz JR, Parkinson TM. Triton induced hyperlipidemia in rats as an animal model for screening of hypolipidemic drugs. Lipids 1972; 7:68–74.
14. Wing DR, Robinson DS Clearing-factor lipase in adipose tissue. Studies with puromycin and actinomycin. Biochem J 1968; 106(3):667-76.
15. Deeg R, ziegenhorn J Kinetic enzymic method for automated-determination of total cholesterol in serum. Clinical chemistry 1983; 29: 1798-1802.
16. Zilversmith DB, Davis A K Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J Lab Clin Med 1950; 35(1):155-60.
17. Bucolo G, David H Quantitative determination of serum triglycerides by the use of enzymes. Clin Chem 1973; 19(5):476-82.
18. Lowry OH, Rosenbrough NS, Farr AL, Randall RJ Protein measurement with Folin phenol reagent. J Biol Chem 1951; 193: 265 – 75.
19. Halliwell B, Gutteridge JMC, Arouma OI. The deoxyribose method: a simple test tube assay for determination of rate

constants for reaction of OH- radicals. Anal Biochem 1987; 165:215–9.
20. Ohkawa H, Ohishi N, Yagi K Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95(2) 351-8.
21. Gofman JW, Lindgren F. The role of lipids and lipoproteins in atherosclerosis. Science 1950; 111(2877):166–71.
22. Khanna AK, Chander R, Singh C, Srivastava AK, Kapoor NK Hypolipidemic activity of achyranthus aspera Linn in normal and triton induced hyperlipemic rats. Indian J Exp Biol. 1992; 30(2):128-30.
23. Patil UK, Saraf S, Dixit VK Hypolipidemic activity of seeds of *Cassia tora* Linn . J Ethanpharmacol 2004; 90:249-52.
24. Deborah MA, Andrew PG, Pykalisto OJ, Brunzell JD, William RH Effect of estrogen on post-heparin lipolytic activity: Selective decline in hepatic triglyceride lipase. The Journal of Clinical Investigation.1977; 59: 601-8.
25. Fredrickson Ds, Loud Av, Hinkelman Bt, Schneider Hs, Frantz Id J The effect of ligation of the common bile duct on cholesterol synthesis in the rat. J Exp Med 1954; 99(1):43-53.
26. Kaur G, Alam M.S, Jabbar Z, Javed K, Athar M. Evaluation of antioxidant activity of *Cassiasiamea* flowers. J Ethnopharmacol 2006; 108(3): 340-48.