ejpmr, 2014, 1(1), 183-191

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF HYPOLIPIDEMIC ACTIVITY OF METHANOLIC EXTRACT OF FICUS DALHOUSIAEMIQ. ON TRITON-X AND HIGH FAT DIET INDUCED HYPERLIPIDEMIA IN ALBINORATS

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Article Received on 02/10/2014 **Article Revised on 25/10/2014** **Article Accepted on 17/11/2014**

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects compare with allopathic drugs, *Ficus dalhousiae* (FD) was selected and the present study on anti-hyperlipidemic activity of Methanolic extract of FD against triton and high fat diet induced hyperlipidemia in rats. FD administered at a dose of 200mg/kg (p.o) to the triton and high fat diet induced hyperlipidemic rats. FD has shown a significant decrease in the levels of serum cholesterol, triglyceride, LDL, VLDL and significant increase in the level of serum HDL. Methanolic extract fraction decreased serum level of total cholesterol, LDL and increased the serum HDL cholesterol level.

KEYWORDS: *Ficus dalhousiae*, Hyperlipidemia, Cholesterol, Triglyceride, LDL, VLDL, HDL.

INTRODUCTION

Hyperlipidemia is an abnormally high levels of lipids i.e. the fatty substances are found in the blood and it also called hypercholesterolemia or hyperlipoproteinemia. Virchow in 19th century who identified cholesterol crystals in atherosclerotic lesion and stated that endothelial cell injury initiates atherogenesis. [1] But now it is clear that higher lipids level, especially cholesterol and triglycerides leads to hyperlipidemia which ultimate speeds up the process of atherosclerosis and disease associated with it. [2] The major treatment available for the hyperlipidemia includes statins, fibrates, bile acid sequestrents. [3,4]

Consumption of much fat may lead to the production of VLDL levels increases, resulting in the formation of large amounts of LDL which may stick to the walls of the blood vessels if the quantity of HDL is insufficient, it induce hyperlipidemia and atherosclerosis. Hyperlipidemia always associated with diabetes mellitus.[5] Dietary fibres is highly recommended for disease prevention. The medicinal plants play a major role in hypolipidemic activity.[6,7] *Ficus dalhousiae* Miq. (Moraceae) is a small spreading tree; young branches softly pubescent. And it ultivated throughout the India.[8] Phytochemical Screening shows presence of carbohydrate, fats, oils, Steroids, Glycosides, flavonoids, Saponins and alkaloids are present strongly in methanol extract of ficusdalhousiae. [9] Fruits-used for constipation during fevers. Leaf-juice-antidysenteric. Root bark- mixed with water, given internally in coryza, asthma and bronchial diseases. Root-antispasmodic. [10]

MATERIALS AND METHODS

Collection of Medicinal plant

Plant material: The plant of *Ficusdalhousiae* plants were collected from the certified ayurvedic wholesaler. The plant was identified and authenticated by Asst Prof. K. Dr. K. Madhava chatty, MSc, Med, Department of Botany, S.V. University, Tirupati.

Preparation of Plant Extract

The collected fresh plantmaterials were dried and made in to coarse powder. The powder of *Ficusdalhousiae* transferred to a round bottomed flask and extracted with soxhlet apparatus using 70% methanol for 24 hours. Then the extract of ethanol was concentrated. Extract obtained was dried at 30ºC for 2 hour. The percentage yield of the extract was 7.5%. [9, 11]

Animals

Wister albino of male sex (150–220 g) was obtained from the central animal house of Sigma Institute of Clinical Research and administration Pvt Ltd Hyderabad. The animals were housed at room temperature (22-28 ºC) for 12 hr dark and 12 hr light cycle and given standard laboratory feed and water *ad-libitum*. The study was approved and conducted as per the norms of the Institutional Animal Ethics Committee (769/2010/CPCSEA).

Chemicals and Drugs

Triton x 100(Hi media, Mumbai), Cholesterol (SRL Mumbai), Atorvastatin (Micro labs Pvt Ltd, B, lore) Cholic acid (SRL, Mumbai) Anaesthetic ether-SD Finechem Ltd., Mumbai,Chloroform-SD Finechem Ltd .Mumbai.Formaline-SD Finechem Ltd., Mumbai and

all other chemicals and reagents were of analytical grade and Diagnostic kits from Robonik Diagnostic Ltd India**.**

Preliminary Phytochemical Screening

In order to detect the various constituents present in the methonolic extract of *Ficusdalhousiae* was subjected to the tests as per standard method. [12]

Determination of acute toxicity

Acute toxicity studies were performed according to OECD-423guidelines category IV substance (acute toxic class method). Swiss albino mice (n=3) ofeither sex selected by random sampling technique were employed in this study. The animals were fasted for 4 hrs with free access to water only. The plant extracts of *Ficusdalhousiae* were administered orally with maximum dose of 2000 mg/kg body weight. The mortality was observed for 3days. If mortality was observed in2/3 or 3/3 of animals, then the dose administered was considered as a toxic dose. [13]

Experimental Design for Hypolipidemic Activity

1. Triton x 100 induced hyperlipidemia [14]

Five groups of rats, six in each received the following treatment schedule. The first group served as normal control.

Group I: Normal saline 0.9% w/v or Distilled water

Group II: Control Triton-X-100 (100 mg/kg)i.p

Group III: Triton-X-100 (100 mg/kg)+ Atorvastatin (10mg/ kg) p.o

Group IV: Triton-X-100 (100 mg/kg)+ Methanolic extract of *Ficus dalhousiae* (200mg/kg) p.o

Group V: Triton-X-100 (100 mg/kg)+ Methanolic extract of *Ficus dalhousiae* (400mg/kg) p.o.

2. Hyperlipidemia induced by High Fat Died

The rats were randomly assigned to five groups of six rats each and Then chronic hyperlipidemia produced by feeding cholesterol 1%, cholic acid 0.5% suspended in 25% coccunut oil once a day for 28 days.. The feeding and treatment schedule was as follows:

Group I: Normal diet (for 28 days)

Group II: High-fat diet (for 28 days)

Group III: High-fat diet (for 28 days) + atorvostatin 10 mg/kg/, p.o.

Group IV: High-fat diet (for 28 days) +*Ficus dalhousiae* (200 mg/kg/) p.o

Group V: High-fat diet (for 28 days) +*Ficus dalhousiae* (400 mg/kg/) p.o

Collection of Blood

The experiment was continued for 30 days (7days only for Triton x 100 induced hyperlipidemia) and the animals were sacrificed on the 31st day by cervical decapitation. The blood was collected and samples were centrifuged for 15 minutes at 2500rpm. Then serum samples were collected and analysed for serum Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein Cholesterol (HDL-C), Low Density Lipoprotein Cholesterol (LDL-C) and Very Density Lipoprotein Cholesterol (VLDL-C) serum blood glucose and atherogenic index (AI) [71] Histopathological analysis of liver has done. Triglyceride, cholesterol and HDL-C were measured with enzymatic kits. LDL and VLDL concentration were calculated from the Friedewald's equation.

Treated groups	Serum Lipid profile and Glucose mg/dl					
	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Glucose mg/dl
Sham operated Normal	92.48±0.40	85.89±0.28	71.15±0.77	4.167±0.84	17.15±0.04	66.44±2.15
Control (Triton X 100)	132.2±2.76a	109.3±1.76a	52.19±4.96b	18.40±4.79a	21.88±0.36a	146.0±12.59a
Standard (ATS 10mg/kg)	88.49±0.33***	74.33±0.59***	67.95±3.57*	5.683±3.69**	14.87±0.12**	99.99±8.87**
MFD 200mg/kg	98.15±8.32***	81.00±0.48***	57.57±4.19ns	24.38±8.58ns	16.20±0.10**	104.0±9.03*
MFD 400mg/kg	86.50±6.83***	77.23±0.53***	66.09±2.46*	15.97±2.93ns	15.47±0.09**	101.6±9.61**

VLDL-C= TG/5, **LDL-C**= TC- HDL- (TG/5)

AI was calculated by using the formula of Schulpis:

Atherogenic Index (AI): $\frac{\text{TC- Total serum HDL-C}}{\text{Total serum HDL-C}}$

Liver lipid extraction

The liver was homogenized in cold 0.15M KCl and extracted with CHCl3: CH3OH (2% *v/v*). This lipid extract was used for the estimation of lipid parameters.

Statistical analysis

All the data expressed as mean SEM will evaluated by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparisons test using Prism Graphpad version 5.0 and values of $P<0.05$ will considered as statistically significant.

Biochemical analysis

The serum and liver were assayed for total cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), very low-density lipoprotein (VLDL). The serum cholesterol levels were determined by Zak's method The triglyceride, phospholipids, serum HDL, LDL and VLDL was calculated by using standard method. [15-18]

Histopathological studies

At the end of the study period, animals from all the five groups were sacrificed and liver was dissected out, washed, 5µm thick section slides were prepared and stain with heamatoxyline-eosin and examined by light microscopy.

RESULTS AND DISCUSSION

Phytochemicals are secondary metabolites is one more parts of the medicinal plants. These have the ability to produce a definite physiological action on the human body. The most important of these bioactive constituents of plants are alkaloids, flavonoids, tannins, steroids, saponins, glycosides, carbohydrate, protein and aminoacids.

Lipid profile in serum indicates the increased TC, TG and Serum glucose levels were significantly reduced by treatment of 200 mg /kg. LDL and VLDL levels were significantly increased in triton injected animals to control rats (table-1)

Table 1: Effect of MFD on serum lipid profile and serum glucose in triton induced method.

The values are Mean±SEM, n=6, ns= not significant, One way ANOVA followed by multiple comparision of Dunnett's test,*$p<0.05$,**$p<0.01$, ***$p<0.001$ as compared to control and $^{a}p<0.001$,$^{b}p<0.01$ as when compared to normal.

The increased AI by Triton x100 injection was decreased by administration of 200mg or 400mg/kg of MFD significantly (shown in table2).

2. High Fat Diet Induced Hyperlipidemia

Lipid profile in serum indicates the increased TC, TG and Serum glucose levels were significantly reduced by treatment of 200 mg /kg. LDL and VLDL levels were significantly increased in high fat diet animals to control rats (shown in table-3).

Table.2: Effect of MFD on atherogenic index in triton x induced hyperlipidemic rats.

Treated Group	Atherogenic Index
Sham operated Normal	0.31±0.01
Control (Triton X 100)	0.85±0.19
Standard (ATS 10mg/kg)	0.31±0.09**
MFD 200mg/kg	0.81±0.24ns
MFD 400mg/kg	0.53±0.21ns

Table.3: Effect of MFD on serum lipid profile and serum glucose level in HFD induced hyperlipidemia in rats.

All the values are Mean±SEM, n=6, ns= not significant, One way ANOVA followed by multiple comparision of Dunnett's test, **p<0.01 as compared to control.

Treated Groups	Serum Lipid profile and Glucose mg/dl					
	TC mg/dl	TG mg/dl	HDL mg/dl	LDL mg/dl	VLDL mg/dl	Glucose mg/dl
Sham operated Normal	81.69±1.58	79.93±2.16	28.49±1.23	40.35±1.00	14.93±1.23	54.24±3.95
Control (HFD)	132.1±0.56a	125.5±2.44a	20.61±1.00b	64.71±1.60a	24.99±1.13a	83.19±2.90a
Standard (ATS 10mg/kg)	74.39±1.26***	70.78±1.46***	31.97±0.90***	34.41±1.53***	14.37±1.57***	52.18±2.34***
MFD 200mg/kg	103.7±2.58***	105.8±3.35***	28.62±1.54**	54.67±1.33***	19.80±0.57*	73.71±3.84*
MFD 400mg/kg	87.37±2.82***	89.41±2.73***	27.63±2.29**	41.38±0.27***	18.81±0.79**	68.47±1.96**

The values are Mean±SEM, n=6, ns= not significant, One way ANOVA followed by multiple comparision of Dunnett's test,*p<0.05,**p<0.01, ***p<0.001 as compared to control and ^{a}p<0.001,bp<0.01 as when compared to normal.

The increased AI by high fat diet treatment,it was decreased by administration of 200mg or 400mg/kg of MFD significantly(shown in table-4)

Table.4: Effect of methanolic extract of *Ficus dalhousiae*on atherogenic index in HFD induced hyperlipidemicrats.

Treated Gruop	Atherogenic Index
Sham operated Normal	1.61±0.08
Control (Triton X 100)	5.73±0.56a
Standard (ATS 10mg/kg)	1.83±0.16***
MFD 200mg/kg	3.61±0.59**
MFD 400mg/kg	2.75±0.29***

All the values are Mean±SEM, n=6, One way ANOVA followed by multiple comparision of Dunnett's test,**p<0.01, ***p<0.001 as compared to control and [a]p<0.001as when compared to normal.

Histopathology of Liver Tissue

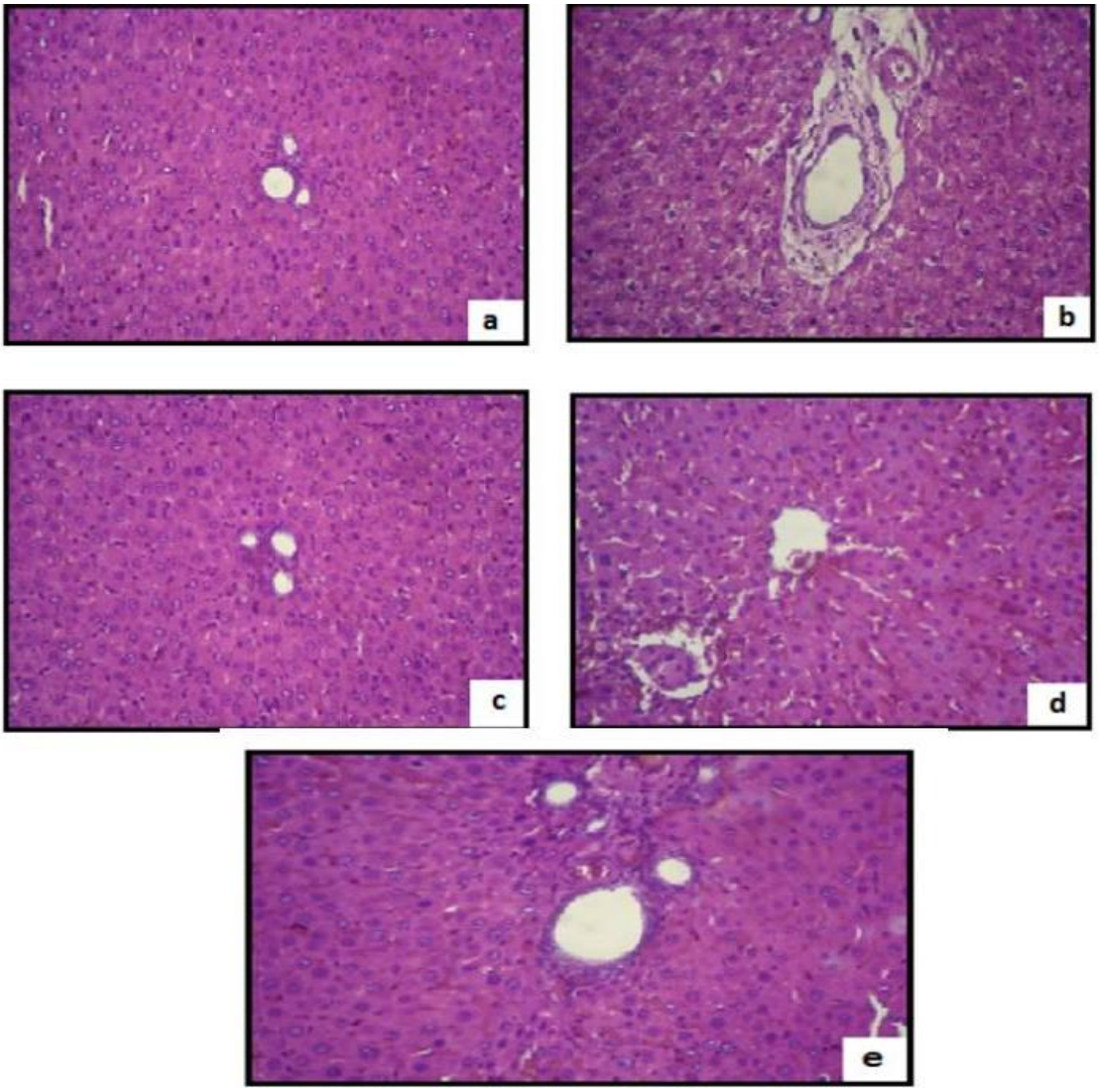

Fig.9: Histopathology of Liver.

(a) normal group showing normal architecture; (b) Control (Triton x 100) showing fatty infiltration and granular degeneration; (c) Standard (Atorvastatin 10mg/kg) treated group showing negligible cytoplasmic fatty infiltration and granular degeneration; (d) MFD 200mg/kg treated group showing mild to moderate cytoplasmic fatty infiltration and granular degeneration; (e) MFD 400mg/kg treated group showing mild cytoplasmic fatty infiltration and mild granular degeneration.

CONCLUSION

In conclusion the findings of the study suggest that MFD is a potent antihypercholesterolemic, antihyperglycerolemic drug lowering LDL, VLDL and increasing HDL levels in all the two models such as Triton x 100 and HFD. The mechanism has point towards cholesterol and triglyceride synthesis.

The drugs has also seems have potent antihyperglycemic activity which has been seen in all the two models. The mechanism for antihyperglycemic activity may be by improving insulin sensitivity and by decreasing TG level.

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