EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 5
Issue: 9
278-283
Year: 2018

EVALUATION OF ANTIHYPERLIPIDEMIC ACTIVITY OF SACRED FIG LEAF

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Article Received on 10/07/2018 **Article Revised on 01/08/2018** **Article Accepted on 22/08/2018**

ABSTRACT
The present study was designed to evaluate antihyperlipidemic activity of leaves ethanolic extract of *Ficus religiosa*. The evaluation of antihyperlipidemic activity was done using Triton X 100 and High Fat Diet induced hyperlipidemia models in Wistar albino rats. The crucial literature survey revealed the presence of alkaloids, carbohydrates, glycosides, saponins, tannins, flavonoids, proteins, and amino acids in ethanolic leaves extract and doses up to 2000mg/kg were found to be safe after acute toxicity tests. Cholesterol, triglycerides, HDL, LDL, VLDL, SGOT, SGPT and Total protein were measured. The results suggested that ethanolic leaves extract of F.religiosa possess anti-hyperlipidemic activity.

KEYWORDS: F. Religiosa, Ethanolic Extract, Triton-X, High Fat Diet, Hyperlipidemia.

INTRODUCTION

Hyperlipidemia is a condition in which there is abnormal high levels of lipids, elevated serum levels of one or more of total cholesterol, low-density lipoprotein cholesterol, triglycerides, or both total cholesterol and total triglycerides(combined Hyperlipidemia), very low density lipoprotein.[1] Hyperlipidemia is a lifestyle disorder which seriously affects the human health.[2] It leads to various cardiovascular disorders like angina pectoris, myocardial infarction, hypertension, atherosclerosis, congestive Heart failure.[3] Hypercholesterolemia is a metabolic condition that determines the onset of chronic degenerative diseases such as atherosclerosis. The formation of initial lesions appears to originate, more often, from the focal increase in lipoprotein content within regions of the intima, not only due to changes in the permeability of the overlying endothelium, but also mainly because of binding to constituents of the cellular matrix, increasing the residence time of lipid-rich particles within the arterial wall. In the extracellular space of the intima, lipoproteins may undergo changes and evidence points to a pathogenic role for such modifications.[4] The major side effects of anti-hyperlipidemic agents include muscle toxicity, rhabdomyolysis, psychiatric adverse reactions which include depression, memory loss, confusion and aggressive reactions.[4,5] These effect the lifestyle again. Hence it is the need of the hour to investigate herbal drugs for treatment of hyperlipidemia which are devoid of the above side effects. **Sacred fig** is commonly known as *Ficus religiosa* is extensively used in Ayurveda for variety of conditions. However its antihyperlipidemic activityhas not been investigated scientifically so far. Keeping in view of pathophysiological complications of hyperlipidemia and therapeutic efficacy of herbal medicines, the plant Ficus leaves was evaluated for antihyperlipidemic activity by enhancement of plasma cholesterol using laboratory animals which also provided help in understanding the relationship between changes in lipid metabolism and atherogenesis and possible treatments for their reduction.[6]

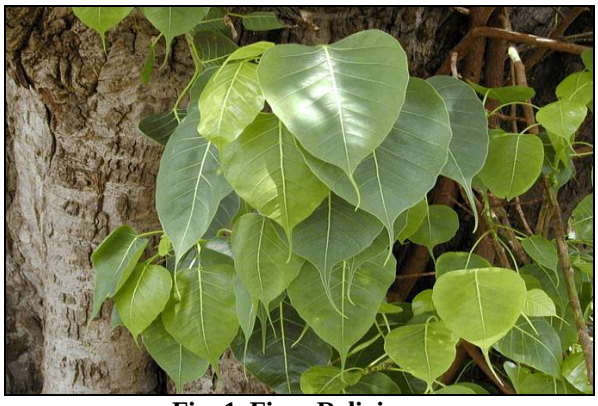


Fig. 1. Ficus Religiosa.

MATERIAL AND METHODS

Collection & Authentification of plant

The plant material was collected from local area of Bhopal in March 2016 and was authenticated.

Preparation of the extract of leaves

The preparation of extract was carried out according to the method of (Oktay, et al, 2003). Briefly, the leaves of *F. religiosa* was shade dried after collection for 5 days

and was powdered. Approximately 0.95 kg of powdered drug material was extracted using 99% pure ethanol in the ratio of 1:2 (w/v) in a air tight container. The extract obtained was dried in a steam bath and the dried mass was weighed and recorded. The percentage of yield was calculated. The weight of dried crude extract obtained was approximately 0.16 g which commemorated with the percentage yield of 17.16%.

Drugs & Chemicals

Atorvastatin(PLEOSTIN-10) was obtained from Mano Pharma Chemical & Pharmaceuticals Ltd. Chennai, Triton was purchased from Neon Laboratories Ltd., Mumbai, ethanol were obtained from Merck Ltd., The other chemicals and solvents used were of analytical grade.

Experimental animals

Wistar rats weighing 130-165g were used in the present study. The experimental animals were maintained under standard laboratory conditions in an animal house approved by the committee for the purpose of control and supervision on experiments on animals (CPCSEA) under 12 h light/dark cycle and controlled temperature (24 ± 2°C) and fed with commercial pellet diet and water *ad libitum*.

Screening Models

Diet-induced hyperlipidemic model

The animals were selected, weighed then marked for individual identification. Rats were made hyperlipidemic by the oral administration of atherogenic diet for 20 days. The rats were then given plant extracts suspended in 2% acacia at the dose of 200mg/kg b.w. once daily in the morning through gastric intubation for 14 consecutive days.

During these days, all the groups also received atherogenic diet in the same dose as given earlier. The control animals received the hyperlipidemic diet and the vehicle. At the end of treatment period, the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.[7]

Experimental Design

Table. 1. Groups & Doses Schedule for Diet-induced Hyperlipidemic Model.

Sl. No.	Group	Treatment	Animal Used
1	I	2% acacia + atherogenic diet for 20 days	6
2	II	positive control received standard drug atorvastatin (10mg/kg/ day p.o.) for 14 days	6
3	III	Aq. Extract (200mg/kg/day) fine suspension of 2% acacia + atherogenic diet for 14 days	6
4	IV	Ethanol Extract (200mg/kg/day) fine suspension of 2% acacia + atherogenic diet for 14 days	6

Triton-induced hyperlipidemic model

Animals kept for fasting for 18 h, will be injected a saline solution of Triton(Triton x-100) at the dose of 100mg/kg b.w. intra-peritoneally. The plant extracts, at the dose of 200mg/kg b.w., was administered orally through gastric intubation. The first dose being given immediately after triton injection and second dose 20 h later and continue the extraction process for 7 days. After 7 days dose the animals were used for various biochemical parameters. Blood was collected by heart puncturing of rat under ether anesthesia and centrifuged by using centrifuge at 2000 rpm for 30 minute to get serum.[8]

Experimental Design

Table. 2. Groups & Doses schedule for Triton-induced hyperlipidemic model.

Sl. No	Group	Treatment	Animal Used
1	I	Control received 2% acacia + triton(100mg/kg) for a days (p.o.).	6
2	II	After 18 hrs. of triton positive control received standard drug atorvastatin (10mg/kg/ day p.o.) for 7 days.	6
3	III	After 18 hrs. of triton Aq. Extract (200mg/kg/day) fine suspension of 2% acacia was given for 7 days.	6
4	IV	After 18 hrs. of triton Ethanol Extract (200mg/kg/day) fine suspension of 2% acacia was given for 7 days.	6

Collection of blood samples

On 8th day of Triton & 21st day of Hyperlipidemic treatment, the blood was collected by Heart puncture, under mild ether anesthesia in tubes. Serum obtained by immediate centrifugation of blood samples using ultra cooling centrifuge(Remi) at 2000 rpm for 30 minutes at room temperature and directly used for estimating serum lipid profiles (TC, TG, LDL,VLDL and HDL).

Estimation of Total-Cholesterol: Autospan Diagnostics kit was used for estimation of Total-Cholesterol, which followed CHOD/POD method.[9]

Reaction Parameters

- Wavelength: 490-510 nm
- Flow cell temperature : 37^0 C
- Incubation: 10 min.
- Sample volume: 10 µl
- Reagent volume: 1ml
- Zero setting with: Reagent blank
- Light path: 1cm

Table. 3. Estimation of Total-Cholesterol.

Pipette into tube marked	Blank	Standard	Test
Serum/plasma	---------	--------	10µl
Reagent 2	---------	10µl	--------
Reagent 1	1000µl	1000µl	1000µl

Mix well; incubate at 37^0 C for 10 minute or at room temperature (15-30^0 C) for 30 minutes.
Programme the analyser as per assay parameters.

1. Blank the analyser with reagent blank.
2. Measure absorbance of standard followed by the test.
3. Calculated the results as per given calculation formula.

Calculation

$$\text{Cholesterol concentration (mg/dl)} = \times 200 \quad \frac{\text{Absorbance of test}}{\text{Absorbance of standard}}$$

Estimation of HDL-Cholesterol

HDL-Cholesterol was estimated using Span Diagnostics kit, which follows the CHOD/POD method.[10]

Reagent composition

The kit contains 4 Reagents

(a) Enzyme Reagents
(b) Diluent buffer
(c) Precipitating reagents PEG-6000
(d) Standard

Preparation of working reagents

Enzyme reagent was dissolved with 25 ml. of diluents buffer and it was kept for at least 10 min. before use. The working reagent was stable for 4 weeks at 2-8^0 C.

Procedure

(a) Taken 200µl of clear serum into tubes added 200 µl of reagent 3 and incubated at temperature for (15-30^0 C) for 10 min.
(b) Kept all tubes in cooling centrifugation chamber at 2000 rpm for 15 minute for HDL Cholesterol estimation.
(c) Added 100 µl of supernatant from step 1 reagent 4. Added 100 µl of reagent 1. Incubate at 37^0 C for 10 min. measured the absorbance of different sample.

Table. 4: Estimation of HDL-Cholesterol Step-I.

Pipette into tube marked	Test
Serum/Plasma	200µl
Reagent 3	200µl

Mixed well and kept at room temperature (15-30^0 C) for 10 minutes. Centrifuge for 15 minutes at 2000 rpm and separated clear supernatant. Use the supernatant for HDL-Cholesterol estimation.

Table. 5: Estimation of HDL –Cholesterol Step-II.

Pipette into tube marked	Blank	Standard	Test
Supernatant from Step-1	--------	--------	100µl
Reagent-4	--------	100 µl	--------
Reagent-1	1000µl	1000µl	1000µl

Mix well incubate at 37 ^{0} C for 10 minutes or at room temperature (15-30^0 C) for 30 minutes.

(a) Programmed the analyzer with reagent blank.
(b) Measured absorbance of standard followed by the test.
(c) Calculated results as per given calculation formula.

Calculation

$$\text{HDL-Cholesterol concentration (mg/dl)} = \frac{\text{Absorbance of test}}{\text{Absorbance of standard}} \times 50 \times 2^*$$

***(2 dilution factor, as sample is diluted 1:1in step 1)**

Estimation of LDL-Cholesterol and VLDL-Cholesterol:

The amount of LDL-Cholesterol and VLDL-Cholesterol were calculated using friedewald's equation**

$$\text{LDL-Cholesterol} = \text{Total Cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL -Cholesterol}$$

VLDL-Cholesterol = Triglycerides/5
LDL-Cholesterol = Total–Cholesterol – (HDL+VLDL)

Estimation of Triglycerides: Triglycerides was estimated using accurate Triglycerides kit of Span Diagnostics Pvt. Ltd. Accurately triglycerides estimation kit is formulated using GPO and peroxide for quantitative estimation of serum triglycerides. This method is more specific due to use of lipase to liberate glycerol which is estimated.[11]

Procedure: Fresh clear serum with no hemolysis was used for estimation.

- Wavelength: 490-550 nm
- Flow cell temperature: 37^0 C
- Incubation: 10 minutes
- Sample volume: 10µl
- Reagent volume: 1000µl
- Zero setting with: Reagent blank

Table. 6: Estimation of Triglycerides.

Pipette into tube marked	Blank	Standard	Test
Serum/Plasma	-------	-------	10µl
Reagent 2	-------	10µl	--------
Reagent 1	1000µl	1000µl	1000µl

Mix well. Incubate at 37^0 C for 10 minutes.

Calculation

$$\text{Triglycerides (mg/dl)} = \frac{\text{Absorbance of Test}}{\text{Absorbance of Standard}} \times 200$$

****For Glycerol free Triglycerides:** Glycerol free Triglycerides = calculated Triglycerides - 10 mg/dl

Statistical analysis

The results were expressed as mean ± SEM. Statistical analysis was carried out using One-way ANOVA followed by Tukey Multiple Comparisons test with the help of Graph pad instant software. Values of P<0.05 were considered statistically significant.

RESULTS AND DISCUSSION

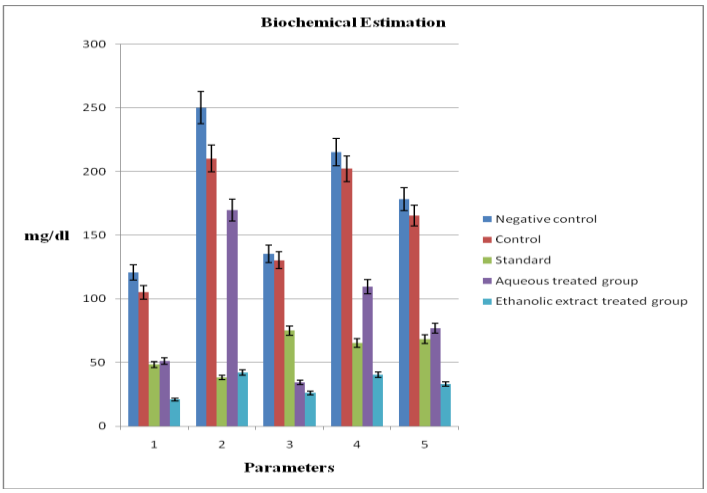
Table 6.1 Effect of *Ficus Religiosa* on serum biochemical parameter in High Fat (Cafeteria diet) diet-induced hyperlipidemia on rat.

GROUP	DOSE	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I (vehicle)	1ml/kg	120.61±1.2	105.13±2.9	48.45±2.6	51.14±0.01	21.02±0.00
Group II (diet)	1.5ml/kg	250.21±1.3 a***	210.35±2.0 a***	38.31±2.5	169.83±0.01 a***	42.07±0.01 a***
Group III (std.+ diet)	10mg/kg	135.44±2.5 a**,b***	130.41±2.3 a***,b***	75.10±1.6 a***,b***	34.26±0.07 a***,b***	26.08±0.02 a***,b***
Group IV (aqs.+ diet)	200mg/kg	215.33±2.5 a***,b***, c***	202.25±1.4 a***,c***	65.33±3.1 a***,b***	109.55±0.08 a***,b***, c***	40.45±0.01 a***,b***, c***
GroupV (etoh.+ diet)	200mg/kg	178.35±3.7 a***,b***, c***,d***	165.33±1.6 a***,b***, c***	68.45±2.4 a***,b***	76.84±0.00 a***,b***, c***,d***	33.06±0.01 a***,b***, c***,d***

[TG-Triglyceride, TC- Total Cholesterol, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL- Very Low Density Low Protein, std.-standard, aqs-aqueous, etoh-ethanol]

The data obtained were analyzed by one way ANOVA followed by Tukey Multiple Comparisons Test. Each values represent the mean ± SEM; n=6. **p< 0.01*p< 0.05, p< 0.001***

a- Significant difference as compare to normal control group
b- Significant difference as compare to negative control group
c- Significant difference as compare to standard group
d- Significant difference as compare to aqueous group



Graph: 6.1- Evaluation of Biochemical Parameters on High Fat (Cafeteria diet) diet induced hyperlipidemia in experimental rats.

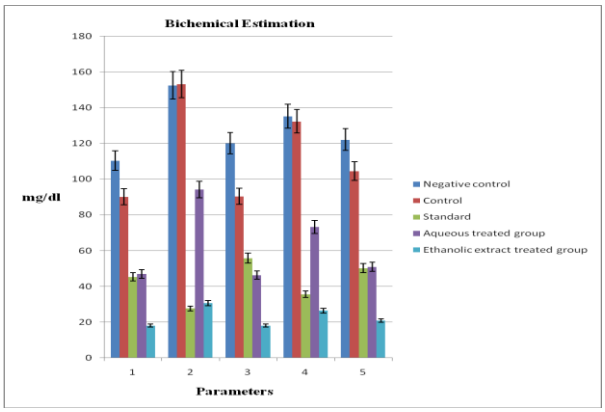
Table 4. Effect of *Ficus Religiosa* on serum Biochemical Parameter in Triton- induced hyperlipidemia in experimental rats.

Group	Dose	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I (vehicle)	1ml/kg	110.34±1.1	90.15±2.7	45.34±2.2	46.97±0.00	18.03±0.01
Group II (triton)	100mg/kg	152.51 + 4.3 a***	153.24 +4.0 a***	27.65+1.6 a**	94.21±0.06 a***	30.62±0.02 a***
Group III (std.+ triton)	10mg/kg	120.15 ± 2.3 b***	90.44 + 7.5 b***	55.84 +1.1 b***	46.26±0.00 a***,b***	18.05±0.00 b***
Group IV (aqs.+ triton)	200mg/kg	135.26 + 2.1 a***,b***, c**	132.37 +5.4 a***,b*, c***	35.56 + 3.2 c***	73.23±0.00 a***,b***, c***	26.47±0.00 a***,b***, c***
Group V (etoh.+ triton)	200mg/kg	122.10 + 1.1 a*,b***, d**	104.55 +3.1 a***,b***, d**	50.22 +5.1 b***,d*	50.96±0.01 a***,b***, c***,d***	20.92±0.00 a***,b***, c***,d***

[TG-Triglyceride, TC- Total Cholesterol, HDL- High Density Lipoprotein, LDL- Low Density Lipoprotein, VLDL- Very Low Density Low Protein, std.-standard, aqs-aqueous, etoh-ethanol]

The data obtained were analyzed by one way ANOVA followed by Tukey Multiple Comparisons Tests. Each values represent the mean ± SEM; n=6. **p< 0.01*p< 0.05, ns p> 0.05. p< 0.001***

a. Significant difference as compare to normal control group
b. Significant difference as compare to negative control group
c. Significant difference as compare to standard group
d. Significant difference as compare to aqueous group



Graph: 6.2- Evaluation of Biochemical Parameters on Triton-induced Hyperlipidemia in Experimental rats.

The physiological effect of flavonoids include possible antioxidant activity, therefore suggestion their role in prevention of coronary heart disease including atherosclerosis. Flavonoids may also work by making liver cells more efficient to remove LDL-C from blood by increasing the LDL-C receptor densities in liver and by binding to apolipoprotein B. It is widely accepted that reduction in plasma HDL is a risk factor for developing atherosclerosis. HDL facilitates the translocation of cholesterol from the peripheral tissue, such as arterial walls to liver for catabolism. The increase in HDL may slow down the atherosclerotic process. Increased levels of HDL (cardio protective lipid) after administration of garlic extracts concluded that the extract is a potent cardio protective agent and this effect may be due to the increase in the activity of Lecithin: cholesterol acyl transferase (LCAT), which play a key role in incorporating the free cholesterol into HDL which is taken back by the liver cells. Several studies show that an increase in HDL-C is associated with a decrease in coronary risk High levels of TC and LDL-C are major coronary risk factors. Administration of *Ficus religiosa* lowered both total and LDL cholesterol in hyperlipidemic rats. This lowering of TC and LDL-cholesterol would reduce the incidence of coronary events .Atherogenic Index was calculated by the equation: (total cholesterol-HDL-cholesterol)/ HDL-cholesterol. The ratio was significantly increased in Triton induced hyperlipidemic rats compared with normal group and these elevated ratios were returned to near normal levels in groups of rats treated with ethanolic extracts of *Ficus religiosa* and atorvastatin. The rise in AI in hyperlipidemic rats enhances the probability of cardiovascular pathogenesis and endothelial dysfunction. A significant decrease in AI value was observed in herbal supplemented animals, suggests the atheroprotective / cardio protective potential of this herb. In conclusion, all the fractions obtained from the ethanolic extracts of *Ficus religiosa* significantly reduced the Triton-X-100 induced hyperlipidemia in rats.

As reported earlier, Injection of Triton X-100 (100 mg/kg) has successfully induced hyperlipidemia in rats by increasing the serum TC, TG and LDL-C levels. The effect of ethanolic extracts of *ficus religiosa* on serum lipid profile levels was showed in Table 1. Treatment with ethanolic extracts of *ficus religiosa* at the doses of 200mg/kg significantly reduced the serum TC, TG and LDLC levels and increased the serum HDL-C levels when compared to the hyperlipidemic control group. The change in lipid levels in groups of II, III and IV were comparable with group of atrovastatin treated rats. Among three fractions, reduced the elevated lipid levels more significantly than the others.

The *ficus religiosa* showed protective action at a dose of 200mg/kg and demonstrated a significant decrease in the raised diet-induced levels of serum TC, LDL-C and triglycerides. At a dose of 200mg/kg, effects were comparable with that of the standard drug atorvastatin.
The present study was designed to investigate the hypolipidemic effect of *ficus religiosa* leaves extracts in high cholesterol diet induced hyperlipidemia. Aqueous and ethanolic extracts of leaves of *ficus religiosa* were administered in doses of 200mg/kg /day p.o. each for 14 days. Simultaneous administration of *ficus religios* leaves extracts significantly ($p<0.001$) prevent the rise in serum levels of total cholesterol, triglyceride, LDL-C,VLDL-C and Atherogenic index whereas significant ($p<0.01$) increases in the level of HDL-C. The present study was designed to investigate the hypolipidemic effect of *Ficus religiosa* leaves extracts in high cholesterol diet induced hyperlipidemia. In this study firstly I have to choose this plant because it having antioxidant activity after that rat were divided in to 5 groups shown in table. Then I will give the vehicle for the 1st group high cholesterol diet for the 2nd atorvastatin for the 3rd group & aqueous ethanolic extract was given for the 4th and 5th group till the end of 20th days. After the end of 20th days or at the end of the experimental period Blood was withdrawn from Heart puncturing of rat under ether anesthesia and centrifuged at 2000 rpm for 30min so at to get serum. Serum total cholesterol, triglyceride HDL was estimated by using span diagnostic kits.

CONCLUSION

Natural remedies have been investigated for centuries for a wide variety of ailments. *Ficus religiosa* has received special attention for its beneficial effects, but until recently there has been little scientific support for its therapeutic and pharmacological properties. In the present study, *Ficus religiosa* was selected to screen for its antihyperlipidemic activity in Triton X-100 (100 mg/kg) induced hyperlipidemic rats & diet induced hyperlipidemic rats.

The present study revealed that the ethanolic extract of *Ficus religiosa* at the dose of 200mg/kg have significant anti-hyperlipidemic activity. Phytochemical screening on *Ficus religiosa* have reported the presence of lignans, alkaloids and flavonoids as main chemicals constituents. As for the phytochemical result the flavonoid may be responsible for the anti-hyperlipidemic activity and further study is required for mechanism of action.

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