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Phytochemical Investigation and Evaluation of Antihyperlipidemic Activity of Ficus Carica Linn Fruit Extracts

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ABSTRACT

Ficus carica Linn the common fig belongs to family Moraceae is a source of energy and nutrition. The ethanolic and hexane extracts of were screened for the antihyperlipidemic activity using; High fat diet induced Hyperlipidemia and Triton X -100 model in Wistar Albino rats. Biochemical parameters such as Total Cholesterol, Triglycerides, HDL, LDL, VLDL, Liver Cholesterol, SGOT & SGPT levels were estimated. It was clearly observed that after feeding the animals with High fat diet for one month Hyperlipidemia was induced and the animals gained weight. In both the models the Hexane and ethanolic extract were found to lower the elevated Cholesterol as well as triglyceride levels. The Hexane extract was significant in increasing the HDL ratio as well. The extracts were able to control the elevated Liver lipid levels too. The results clearly indicate the potential capability of Ficus carica Linn fruit extracts to lower elevated Lipid levels.

KEYWORDS : Antihyperlipdemic, Cholesterol, Ficus carica Linn, High Fat diet, Triglycerides

Introduction

The plant has been a source of medicine since the origin of mankind. The natural sources have been used in various forms to cure ailments. It has been a permutation combination and continuous experimentation which has lead to the development of drugs with plant origin. The medicinal properties of plants can be attributed to the different secondary metabolites present in the plants. Plants have also been a major source of nutrition and energy. *Ficus carica Linn* Linn (Family-Moraceae) is one such plant in the Indian system of medicine with the reference of medicinal properties.

It is commonly found in India in various states like Maharashtra, Karnataka, Punjab, Uttar Pradesh. In Ayurvedic literature it is known as Vatt kul, in Sanskrit named as Falgu and known as Anjeer in Marathi.

Figs are an excellent source of minerals, vitamins [1] and dietary fibre; they are fat and cholesterol-free and contain a high number of amino acids . The milk contains resin, sugar and protein. F.carica Linn has been reported to have numerous bioactive compounds [2] such as arabinose, β-amyrins, β- carotenes, glycosides, β-sitosterol and xanthotoxol. Nematicidal coumarin, Campesterol, Stigmasterol [3], Fucosterol, fatty acids , 6-(2- methoxy-Z-vinyl)-7-methyl-pyranocoumarin and an anticancer agent [4],19-cycloarlane triterpenoid have been reported [5]. The Hypoglyceamic and antioxidant activities of fig & leaves are been reported [6]. The figs have proven to possess laxative effect [7]. The adaptogenic activity of figs has also been reported [8].

Hyperlipidemia is an elevation of lipids in the bloodstream including fats, fatty acids, cholesterol, cholesterol esters, phospholipids, and triglycerides. Hyperlipidemia leads to complications like CVD [9], Type 2 diabetes, etc. It mainly results due to various lifestyle related diseases as well as due to the lack of activity & exercise and in certain cases it is associated with the genetic makeup. There are various agents proven to lower the elevated lipid levels. In recent era huge number of herbal extracts and formulations are being explored for its Antihyperlipidemic action.

Materials & Methods

Collection and Authentication

Plant material was collected from the surroundings of Pune and authentication was done by Dr. J. Jayanathi Scientist 'C' from the Botanical survey of India, Pune. The herbarium was preserved with the Voucher specimen No: DAYFIC3.

Extraction

The plant material was size reduced and was further extracted by maceration with 95% Ethanol and Hexane separately and further concentrated using Rota evaporator and dried under vacuum. The Ethanolic extract was reddish brown syrupy semisolid mass (32.2% w/w). The Hexane extract was yellowish waxy mass (2.8%w/w). The extract was formulated into an emulsion using CMC (1%). Atorvastatin 10 mg/kg was used as a standard Antihyperlipidemic drug.

Phytochemical analysis

Qualitative Tests

The preliminary phytochemical investigation of the alcoholic extract was done and the tests for the presence of various phyto-constituents were carried out as per standard procedure [10].

HPTLC Fingerprint analysis

HPTLC fingerprint analysis of the ethanolic extract was carried, as well as the class of compound analysis was carried out using various standard solvent systems for different classes of phytoconstituents. The extract was dissolved in HPLC grade Methanol and solution was prepared. 0.1 & 0.2 µl of extract was spotted on pre-coated silica gel G60 F254 HPTLC plates (Merck) (20 cm x 10 cm) with the help of CAMAG Linomat V applicator and Syringe, 100 ml (Hamilton). The plate was developed in glass twin trough chamber (20 cm x 10 cm) pre-saturated with mobile phase (Toluene : Chloroform : Ethanol 4:4:1) and developed to a distance of 80 mm The plate was derivatized using methanolic Anisaldehyde-H_2SO_4 reagent, was observed under UV light at 200 nm, 254 nm & 540 nm and scanned densitometrically using CAMAG TLC Scanner 3 linked to WINCATS software. The Rf values and color of the resolved bands were noted [11].

HPLC Analysis

The Ethanolic extract (5 mg) was dissolved in of the HPLC grade methanol (5 ml). A part this solutions (10 µl) was analyzed by high performance liquid chromatography (HPLC) using a Zorbax ODS C18 4.6 mm x 250 mm, 5 µm reverse phase column attached to Agilent 1100 high performance pump and Agilent 1100 variable wavelength UV detector (254 nm) using optimized solvent systems at a flow rate of 1 ml/min.

Triton X - 100 Model

Healthy Wistar albino rats of either sex weighing between 150-200 gm were used for the evaluation of antihyperlipidemic activity. The animals were fed ad-libitum with commercial pellet diet (Lipton India Ltd., Mumbai) and had free access to water. The *Ficus carica Linn* ethanolic extract - FCE and hexane extracts - FCH were used in this model at a double dose of 250 & 500 mg/kg bw. The animals were divided into 7 groups and were treated with respective extracts.

Group I: Normal
Group II: Negative Control (1 % CMC)
Group III: Standard (Atrovastatin 10mg/kg p.o.)

Group IV: FCEA (250 mg/kg b.w. p.o.)
Group V: FCEB (500 mg/kg b.w. p.o.)
Group VI: FCHA (250 mg/kg b.w. p.o.)
Group VII: FCHB (500 mg/kg b.w. p.o.)

The 1st Group served as normal, in the groups from 2nd to 7th Hyperlipidemia was induced by injecting freshly prepared Triton-X 100 at a dose of 100 mg/kg b.w. in normal saline solution by the intraperitoneal route after fasting the animals for 18 hrs. After 72hrs of Triton injection the animals were treated for 8 days. On the 8th day blood was withdrawn by retro-orbital puncture. Various biochemical parameters such as Total Cholesterol, Triglycerides, HDL, LDL levels were estimated using different biochemical kits (Biolabs/ Span Kits) [12].

High Fat Diet-Induced Hypercholesterolemia

Healthy Wistar albino rats of either sex weighing between 150-200 gm were used for the evaluation of antihyperlipidemic activity. The *Ficus carica Linn* ethanolic extract - FCE and hexane extracts - FCH were used in this model at a single dose of 500 mg/kg b.w. The animals were divided into 5 groups and were treated with respective extracts.

Group I: Normal
Group II: Negative Control (1 % CMC)
Group III: Standard (Atrovastatin 10mg/kg p.o.)
Group IV: FCE (500 mg/kg b.w. p.o.)
Group V: FCH (500 mg/kg b.w. p.o.)

The 1st Group served as normal, the animals were fed ad-libitum with commercial pellet diet (Lipton India Ltd., Mumbai). In the groups from 2nd to 5th Hyperlipidemia was induced by allowing the animals to feed on a High Fat Diet with a formula devised by us (Table 1). All the animals and had free access to water [13].

Table 1. High Fat Diet Formula

Sr. No	Ingredients	% Qty
1	Wheat Flour	35
2	Besan	15
3	Sucrose	20
4	Vanaspati Ghee	20
5	Milk Powder	5
6	Groundnut oil	5
7.	1 Multivitamin Tablet (Supradyn)/100gm	

The ingredients were mixed and dough was rolled into roundies, this was allowed to dry in oven. The animals were fed on this diet ad-libitum for 30 days. On the 30th day blood was withdrawn by retro orbital puncture and various biochemical parameters such as Total cholesterol, Triglycerides, HDL, LDL levels were estimated using different biochemical kits (Biolabs/ Span Kits). For the next 30 days the 2, 3, 4 & 5 groups were treated with the respective drugs along with high fat diet. On the 60th day the animals were sacrificed by decapitation under mild anesthesia, blood was collected by the heart puncture method and again all the biochemical parameters were analyzed. The liver was separated and liver homogenate was analyzed for hepatic lipid levels.

Results & Discussion

Preliminary phytochemical tests

Table 2. Phytochemical evaluation of F. carica

Sr No.	Phytoconstituents	FCE	FCH
1	Sterols & Terpenoids	+VE	+VE
2	Alkaloids	+VE	-VE
3	Vitamins	+VE	+VE
4	Glycosides	-VE	-VE
5	Tannins	+VE	-VE
6	Flavonoids	+ VE	+VE
7	Proteins & Amino acids +VE		-VE
8	Carbohydrates	+VE	-VE
9	Free sugars	+VE	-VE

Note: The +VE indicate presence and –VE indicates absence.

HPLC analysis of Ficus carica Linn fruit extract

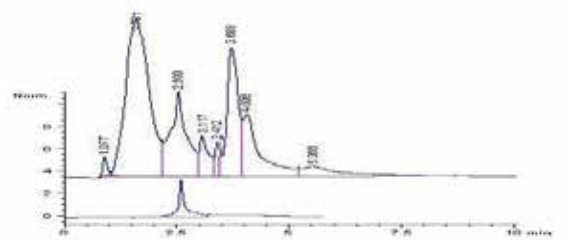


Fig.1. Chromatogram of F.carica Linn ethanolic extract with Std. Quercetin.

The HPLC analysis of Ficus carica Linn ethanolic extract was carried out using Acetonitrile: Methanol: Water (12: 8: 20), which exhibited 07 prominent peaks as well as the presence of Quercetin was also confirmed as shown in Figure 1.

HPTLC studies of Ficus carica Linn fruit extract.

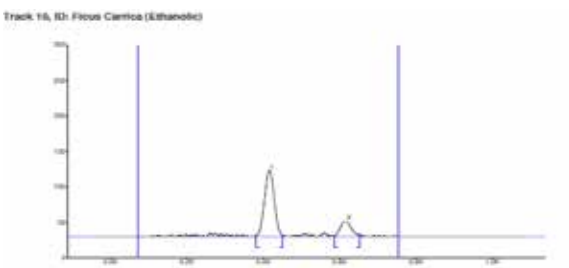


Fig 2. Chromatogram of Ficus carica Linn ethanolic extract @ 254 nm (Toluene: Chloroform: Ethanol 4:4:1)

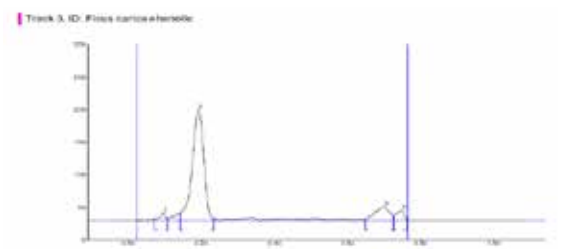


Fig 3. Chromatogram of Terpenoids in Ficus carica Linn ethanolic extract @254nm (n-hexane : ethyl acetate (1:1) Derivatization:- Anisaldehyde Sulphuric acid)

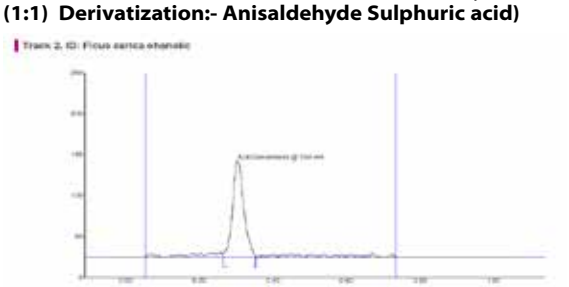


Fig 4. Chromatogram of alkaloids in Ficus carica Linn ethanolic extract @254 (Toluene : Ethyl acetate : Diethylamine (7:2:1) Derivatization :- Dragendroff reagent)

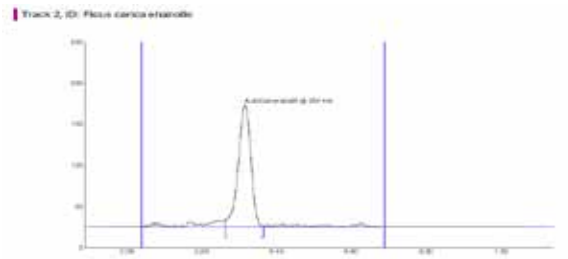


Fig 5. Chromatogram of Sterols in Ficus carica Linn ethanolic extract @254 (n-butanol : methanol : water(3:1:1) Derivatization:- Anisaldehyde Sulphuric acid)

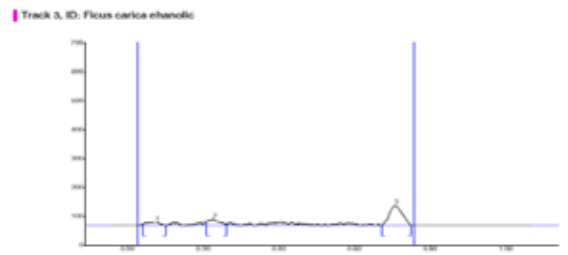


Fig 6. Chromatogram of Flavonoids in Ficus carica Linn ethanolic extract @254 (Ethyl acetate : Formic acid : Glacial acetic acid : Water (10 : 0.5 : 0.5 :1.3) Derivatization:- Anisaldehyde Sulphuric acid

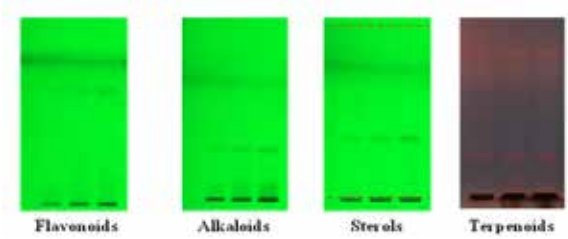


Fig 7. Chromatograms of Ficus carica Linn ethanolic extract.

The HPTLC analysis of the extract exhibited the presence of alkaloids, terpenoids & sterols & flavonoids indicating the complex chemical nature of the extract as observed in figure 2 to 6.

Antihyperlipidemic Activity
Triton X - 100 Model

In the Triton X -100 model, it was observed that severe hyperlipidemia was induced in the animals (Table 5). In the untreated animals the Triglyceride as well as the Cholesterol levels were significantly elevated in comparison to the normal animals. The LDL too was significantly increased and the HDL to Total Cholesterol ratio was decreased thus indicating severe hyperlipidemia.

Table 2. Lipid profile of Ficus carica Linn treated groups in Triton X 100 model induced hyperlipidemia

	TGL mg %	TC mg %	HDL mg %	LDL mg %	VLDL mg %	HDL ratio
Normal (CMC 1%)	69.67 ± 9.00	78.33 ±8.45	40.33 ±3.88	24.07 ±6.26	13.93 ±1.80	51.49
Negative control	136.50 ±13.02	144.33 ±12.29	48.33 ±3.50	68.70 ±11.84	27.30 ±2.60	33.49
Standard (Atorvastatin)	76.17 ±10.38***	89.17 ±13.08***	38.33 ±5.35**	35.60 ±14.03***	15.23 ±2.08***	42.99***
FCEA 250mg	111.67 ±15.34**	98.33 ±6.62**	35.83 ±4.45**	40.17 ±8.93**	22.33 ±3.07NS	36.44 NS
FCEB 500 mg	96.50 ±14.02***	87.33 ±9.20***	37.33 ±5.89***	30.70 ±8.86***	19.30 ±2.80**	42.75***
FCHA 250 mg	100.83 ±14.37**	107.50 ±19.47**	43.17 ±6.31**	44.17 ±15.36**	20.17 ±2.87 NS	40.16***
FCHB 500mg	88.67 ±11.41***	80.50 ±16.34***	39.67 ±5.09***	23.10 ±14.39***	17.73 ±2.28***	49.28***

Data represented as Mean ± S.E.M. (n =6). All Values are expressed as mg/dl. *** P < 0.0001,
**** P < 0.05, NS – Not Significant when compared to Negative control.**

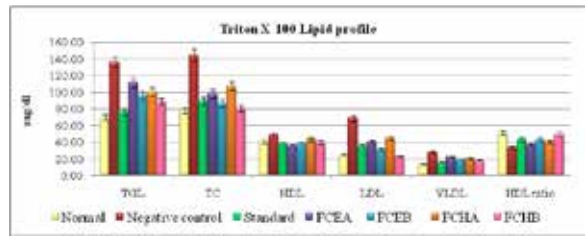


Fig.8. Biochemical analysis of F.carica fruit extracts in Triton X 100 model

The extract treated animals exhibited cholesterol as well as triglyceride lowering effect. The results obtained show that due to Triton X 100 there was significant rise in the normal lipid levels leading to induction of Hyperlipidemia. The above figure indicates the ability of the fractions to lower the elevated lipid levels as well as improve the HDL ratio (Table. 2).

High Fat Diet-Induced Hypercholesterolemia

The animals were allowed to feed on the HFD for 30 days ad libitum and on 30th day blood was withdrawn by retro-orbital puncture. Various biochemical parameters were estimated to confirm the induction of hyperlipidemia (Table. 3).

Table 3. Lipid Profile on 30th day in High fat diet fed animals

	TG mg %	TC mg %	HDL mg %	LDL mg %	VLDL mg %	HDL ratio
I -Normal	83.67 ±6.89	76.17 ±4.31	34.17 ±3.06	25.27 ±4.81	16.73 ±1.38	44.86
II	125.00 ±11.83	98.33 ±6.12	40.50 ±8.78	32.83 ±12.97	25.00 ±2.37	41.19
III	131.67 ±14.53	94.58 ±4.82	35.33 ±5.09	32.92 ±6.03	26.33 ±2.91	37.36
IV	135.50 ±10.93	94.33 ±4.23	39.83 ±5.91	27.40 ±4.96	27.10 ±2.19	42.23
V	128.00 ±13.52	93.83 ±8.73	39.33 ±2.42	28.90 ±9.68	25.60 ±2.70	41.92

Hyperlipidemia with Extract treatment for 30 days

Table 4. Lipid Profile on 60th day in Ficus carica Linn treated animals in High fat diet fed animals after 30 days treatment

FC	TGL mg %	TC mg%	HDL mg%	LDL mg%	VLDL mg%	HDL ratio
Normal 1%CMC	79.05 ±7.76	75.17 ±6.59	37.50 ± 3.27	21.86 ±7.83	15.81 ±1.55	49.89
Negative control	148.79 ±10.54	119.50 ±10.70	38.55 ±5.99	51.19 ±7.88	29.76 ±2.11	32.26
STD Atorvastatin	85.13 ±9.39***	85.34 ±10.90**	41.22 ±7.06***	27.10 ±6.45***	17.03 ±1.88***	48.30***
FCE 500 mg/kg	98.17 ±12.35**	83.67 ±7.08**	31.67 ±7.20	32.37 ±9.58NS	19.63 ±2.47**	37.85NS
FCH 500 mg/kg	95.83 ±14.25**	82.35 ±8.78**	35.48 ±4.80**	27.70 ±11.60**	19.17 ±2.85**	43.09**

Data represented as Mean ± S.E.M. (n =6). All Values are expressed as mg/dl. *** P < 0.0001,
**** P < 0.05, NS – Not Significant when compared to Negative control.**

The Ficus carica Linn extracts were moderately significant in lowering the elevated lipid levels; the hexane extract was more effective in lowering the triglycerides as well as Total cholesterol in comparison to the ethanolic extract. The HDL ratio too was elevated in the extract

treated groups (Table.4).

Table 5. SGOT, SGPT & Liver Lipid Levels on 60th day post treatment by F.carica Linn

	L –TC mg%	L-TGL mg%	SGPT mg%	SGOT mg%
Normal 1%CMC	89.67 ± 4.49	152.67 ±11.62	53.50 ±6.36	193.50 ±10.67
Negative control	141.83 ±9.33	263.67 ±13.20	76.33 ±5.53	225.33 ±9.67
STD Atorvastatin	72.17 ± 15.22***	186.67 ± 31.55***	58.17 ± 1.36***	169.00 ± 7.75***
FCE 500 mg/kg	96.33 ±6.64**	204.33 ±17.40**	54.33 ±1.83***	188.17 ±11.47***
FCH 500 mg/kg	93.48 ±4.58**	195.44 ±9.73**	57.83 ±1.84***	196.50 ±5.03NS

Data represented as Mean ± S.E.M. (n =6). All Values are expressed as mg/dl. *** P < 0.0001, ** P < 0.05, NS – Not Significant when compared to Negative control.

The SGOT and the SGPT levels and the liver lipid levels were highly raised in the disease control group, indicating fatty liver condition (Table.5). The extract treated groups were able to reduce both SGOT & SGPT levels. The Liver Cholesterol as well as Liver Triglycerides too was lowered by all the extracts. The results distinctly show the ability of the plant extracts to protect/revert the fatty liver condition.

The Ficus carica Linn fruit extracts exhibit significant antihyperlipidemic action. The Hexane extract at a dose of 500 mg/kg b.w. is found to be effective in controlling the elevated lipid levels as well as the fatty liver conditions. The ethanolic extract too exhibits cholesterol as well as triglyceride lowering effect. The ethanolic extract was found to be more effective in controlling the SGOT & SGPT levels, exhibioting hepatoprotective action.

The chromatographic analysis indicates the presence of multi component system. From the HPLC analysis Quercetin is found to be present in the ethanolic extract. The overall activity observed could be attributed to the presence of sterols and terpenoids, which are known to poses the antihyperlipidemic action[14].

Conclusion: Thus from these studies it can be concluded that the Ficus carica Linn fruit extract posses antihyperlipidemic activity.

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