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ORIGINAL RESEARCH ARTICLE

Hypolipidemic activity of *Ipomoea aquatica* Forsk. Leaf extracts on lipid profile in hyperlipidemic rats.

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ABSTRACT

The study aimed at evaluating the hypolipidemic effects of *Ipomoea aquatica Forsk* (MEIA) and investigating the potential mechanisms by which MEIA modulated lipid profiles in hyperlipidemic rats. Hypolipidemic effects of the single, daily oral dosing of 200 and 400 mg/kg of methanol leaf extract of *Ipomoea aquatica* Forsk (MEIA) in Swiss albino rats for 30 days. On day 30, blood samples from the rats were collected for the estimation of total cholesterol, total lipid, free fatty acid, phospholipids, and triglycerides. The concentrations of plasma total cholesterol, total lipid, free fatty acid, phospholipid, and triglycerides in rats treated with MEIA at 200 and 400mg /kg were significantly decreased ($P < 0.05$), accompanied with significantly decreased concentrations of liver, kidney and heart total cholesterol and triglyceride ($P < 0.05$). These results indicated that MEIA largely improved the lipid profiles in the hyperlipidemic rats

Key Words: *Ipomoea aquatica* Forsk; Total Cholesterol; Total Lipid; Free Fatty Acid; Phospholipids.

INTRODUCTION

Heart disease remains the leading cause of death in most parts of the world. Epidemiological studies have established a direct relationship with serum cholesterol, and coronary artery disease. The advantages of lowering lipid levels to satisfactory levels have been confirmed by several experimental and interventional studies indicating lower morbidity and mortality in coronary heart disease which commensurate with reduction of serum cholesterol.

Herbal medicine is the oldest form of healthcare known to mankind. Herbs have been used by all cultures throughout history. It was an integral part of the development of modern civilization. The World Health Organization (WHO) estimates that 4 billion people, 80 percent of the world population, presently use herbal medicine for some aspect of primary health care .Substances derived from the plants remain the basis for a large proportion of the commercial medications used today for the treatment of heart disease, high blood pressure, pain, asthma, and other problems.

Ipomoea aquatica Forsk (IA) belongs to the family Convolvulaceae grows wild and is cultivated throughout Southeast Asia and is a widely consumed vegetable in the region. Many of the waters where MEIA grows serve as recipients for domestic and other types of waste water. Water spinach is also supposed to possess an insulin-like activity according to indigenous medicine in Sri Lanka [1]. Only a very few scientific studies have been conducted on its medicinal aspects. These include the inhibition of effects on liver diseases [2], constipation[2].IA is considered a tonic the species contains several vitamins, including A, B, C, E, and "U" (S-methyl-methionine), and is used to treat gastric and intestinal disorders.[3]The species also contains aliphatic pyrrolidine amides, carotenoids, hentriacontane, β-sitosterol and its glycosides, prostaglandin, leukotrine, N-trans- and N-cis feruloyltyramines .[4-7] It is runner type plant with numerous small flowers. [8 - 9] The current study was undertaken to evaluate the hypolipidemic activity of methanolic leaf extract of MEIA by, till now no pharmacological evaluation has been done on IA especially in leaf for its hypolipidemic activity. This prompted us to pursue the activity and was examined for their efficacy and for determination of their possible mechanism of action.

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MATERIALS AND METHODS.

Plant material.

The fresh leaf's of *Ipomoea aquatica* Forsk (IA) were collected from (Changlepet, Tamilnadu, India) western Ghats of South India during March 2008. The plant was identified and authenticated by Dr. Sasikala Ethirajulu.Captain srinivasan research Foundation, Chennai, Tamil nadu, India. The specimen voucher was deposited in the Department of Pharmacology and toxicology, C.L.Baid Metha College of Pharmacy, Chennai, Tamil nadu, India.

Preparation of the Methanolic Extract of IA.

The fresh leaf of IA was collected and washed with running water. It was shade dried at room temperature and 1 kg of the dried leaf was made in to coarse powder. The powder was passed through a 60 No mesh sieve. Air dried Powdered drug was macerated with ethanol (90 % v/v) in glass percolator and allowed to stand at room temperature for about 24 hours. Then the extract obtained was filtered, concentrated by rotary vacuum pump to get the solid mass. The weight of extract obtained was 20.5 %.

Phytochemical screening:

The freshly prepared leaf extract of IA was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Mayer's, Hager's, and Dragendorffs reagent; Flavonoids with the use of sodium acetate, ferric chloride, amyl alcohol; Phenolic compounds and tannins with lead acetate and gelatin; carbohydrate with Molish's,Fehling's and Benedict's reagent; proteins and amino acids with Millon's,Biuret, and xanthoprotein test.Saponins was tested using hemolysis method; Gum was tested using Molishs reagent and Ruthenium red;Coumarin by 10% sodium hydroxide and Quinones by Concentrated Sulphuric acid. These were identified by characteristic color changes using standard procedures. [10] These were identified by characteristic color changes using standard procedures.

The screening results were as follows: Alkaloids + ve; Carbohydrates + ve; Proteins and amino acids +ve; Steroids - ve; Sterols + ve; Phenols + ve; Flavonoids + ve; Gums and mucilage + ve; Glycosides + ve; Saponins + ve; Terpenes + ve and Tannins – ve,

Where + ve and – ve indicates the presence and absence of compounds.

Animals

Swiss albino rats of either sex, weighing 180–200 g were obtained from animal house of C.L.Baid Metha College of pharmacy, Chennai, Tamil nadu, India. Animals were kept in raised mesh bottom cages to prevent coprophagy .The animals were maintained in colony cages at 25±2 ◦C, relative humidity 50–55% maintained under 12:12 h light and dark cycle. The animals were fed with Standard animal feed (Hindustan Lever Ltd.) and water ad libitum. All the animals were acclimatized to the laboratory conditions prior to experimentation. All the experiments were conducted between 10-00 and 17.00 h and were in accordance with the ethical guidelines of the International association for Study of Pain. **[11]**All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The study was approved by the Institutional Animal Ethical Committee.

Acute toxicity studies

Acute toxicity study was performed for the extracts to ascertain safe dose by acute oral toxic class method of Organization of Economic Co-operation and Development, as per 423 guidelines (OECD). [12]

Antihyperlipidemic activity

Rats were divided into five groups each consisting of six animals. The first group received only the vehicle, viz. hydrogenated groundnut oil (HGNO) while the second group was administered vehicle and cholesterol (500 mg/kg, p.o.). Groups III and IV were given standard drugs, clofibrate (10 mg/kg) and guggul (50 mg/kg, p.o.) respectively in addition to HGNO and cholesterol. MEIA was given orally using gavage to groups IV and V in the doses of 200 and 400 mg/kg along with HGNO and cholesterol. The experiment was continued for 30 days and the body weight changes were recorded every 5 days from day 0. On the 31st day, the animals were sacrificed and the blood was withdrawn by retro-orbital method.

Blood and tissues collection.

Blood was collected in heparinized tubes, and the serum was separated by centrifugation and

refrigerated. The vital organs liver, kidney, and cardiac tissues were quickly removed, washed in ice cold, isotonic saline and blotted individually on ash-free filter paper and organ weights were measured. The tissues were then homogenized in 0.1MTris–HCl buffer, pH7.4. The homogenate was used for the estimations of lipid profile and other parameters.

Biochemical studies

The lipid profiles such as total lipids, total cholesterol, triglycerides, phospholipids and free fatty acids of serum, liver,heart and kidney were studied by standard methods.[13 - 17]

Statistical analysis

All values are expressed as mean ±SEM. Data were analyzed by non-parametric ANOVA followed by Dunnett's multiple comparison tests, evaluated using Graph Pad PRISM software. A p-value <0.05 was considered significantly different.

RESULTS

Effect of MEIA on body and liver weights

After 30 days of study, the body weight of the cholesterol treated animals in group II increased significantly when compared to normal group ($P < 0.05$). Treatment with standard drugs (clofibrate) as well as MEIA (200 and 400 mg/kg) appreciably decreased the gain in the body weight ($P < 0.05$). The liver weight of the animals in group's I–VI was 3.5±0.11, 4.5±0.31, 3.6±0.10, 3.9± 0.5, 4.2±0.9, and 3.8±0.6 g, respectively.

Antihyperlipidemic activity

Administration of exogenous cholesterol resulted in significant increase of various parameters of lipid profile. Treatment with standard drugs (clofibrate) or MEIA altered this elevation to different degrees. In animals that were treated with cholesterol (group II), the total lipid level was increased in serum, liver, heart and kidney while the increase was prevented in animals that received standard drugs (**Table 1**). The lipid lowering activity was dose-dependent in the case of MEIA treatment and significant at 200 and 400 mg/kg dose (**Table 2, 3, and 4**). The levels of total cholesterol were increased in cholesterol administered group whereas decreased in clofibrate and guggul (Standard) treated animals. The same trend was noticed in a dose-dependent manner with MEIA treated animals, group VI 400mg/kg shows significant decrease in the cholesterol level when compare to group V 200mg/kgdose. Compared to control group of animals, the amount of triglyceride was found to be more in cholesterol-fed group. In standard drugs and higher doses of MEIA (400mg/kg) treated animals, the triglyceride levels remained unaltered. This proved to be the maximum effective dose. In the case of phospholipids and free fatty acids too, their levels were increased in cholesterol-treated animals while there was a dose-dependent decrease in them with MEIA administration especially in higher dosages of the extract.

Table 1: Effect of MEIA on serum lipid profile in control and experimental animals.

Treatment Group (mg/kg/p.o.)	TL (mg/dl)	FFA (mg/dl	PL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I Control (HGNO)	253.6 ± 1.2	13.12 ± 0.9	96.5±3.5	74.11± 4.6	83.33± 8.5
II Cholesterol	486.3 ± 10.9	24.66± 1.1	163.0± 6.3	125.5± 4.9	198.21± 3.2
III Clofibrate (10mg/kg)	225.3 ± 9.1	9.6± 0.9	89.0± 0.5	72.22± 2.9	83.6± 4.7
IV Guggul (50 mg/kg,)	227.4 ± 0.6	10.2 ± 1.2	90.6 ± 2.6	74.51 ± 3.3	85.1± 1.9
V MEIA (200 mg/kg)	320.7± 7.9	15.6± 10.9	130.8± 6.1	116.5± 3.3	165.5± 5.1
VI MEIA (400 mg/kg)	236.0± 4.0	11.0± 8.1	90.6± 0.5	78.15± 6.6	80.8 ± 0.9

Table.2: Effect of MEIA on liver lipid profile in control and experimental animals.

Treatment Group (mg/kg/p.o.)	TL (mg/dl)	FFA (mg/dl	PL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I Control (HGNO)	40.21 ±5.2	0.96 ± 0.1	28.10 ±4.2	5.01± 0.6	9.9±0.1
II Cholesterol	81.1 ± 3.3	2.56± 0.3	50.0± 1.25	11.6± 1.7	40.1± 3.3
III Clofibrate (10mg/kg)	39.35 ± 6.2	1.1± 0.02	26.3± 6.6	4.80± 3.1	8.6 ± 7.2
IV Guggul (50 mg/kg,)	39.95 ± 1.1	1.9 ± 0.3	28.8 ± 0.1	5.22 ± 2.9	9.1 ± 4.1
V MEIA (200 mg/kg)	52.1± 2.5	2.1± 0.4	30.18± 7.1	6.0± 0.09	14.61± 3.5
VI MEIA (400 mg/kg)	40.0± 0.9	0.9± 0.05	21.2.± 5.3	4.90+0.5	9.16 ± 0.1

Table No.3: Effect of MEIA on heart lipid profile in control and experimental animals.

Treatment Group (mg/kg/p.o.)	TL (mg/dl)	FFA (mg/dl	PL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I Control (HGNO)	23.9 ± 9.1	0.71± 0.11	18.41 ±7.0	3.31± 0.09	3.61± 0.2
II Cholesterol	35.0 ± 6.4	2.6±0.13	25.60± 0.1	6.56± 0.06	7.58± 4.6
III Clofibrate (10mg/kg)	22.41±1.1	0.70±0.9	15.18± 4.9	3.30± 0.14	3.0 ± 2.11
IV Guggul (50 mg/kg,)	23.66±2.6	0.95±0.5	16.22± 1.6	3.91± 0.92	3.16± 0.8
V MEIA (200 mg/kg)	29.11± 4.2	1.51±0.60	22.33± 0.5	4.3± 0.60	5.95± 1.44
VI MEIA (400 mg/kg)	27.0± 0.5	0.86 ±0.33	20.19.± 2.6	3.80+0.05	3.83 ± 1.28

Table. 4: Effect of MEIA on kidney lipid profile in control and experimental animals.

Treatment Group (mg/kg/p.o.)	TL (mg/dl)	FFA (mg/dl	PL (mg/dl)	TG (mg/dl)	TC (mg/dl)
I Control (HGNO)	22.63±0.46	2.11±0.11	20.82±4.0	4.75±0.92	5.46±0.6
II Cholesterol	35.0 ± 0.55	2.6± 0.13	22.75± 0.7	5.22± 0.71	10.67±1.8
III Clofibrate (10mg/kg)	14.97 ± 2.75	1.01± 0.45	16.33± 1.5	2.93± 1.0	4.45 ± 0.28
IV Guggul (50 mg/kg,)	13.39 ± 1.7	1.31± 0.33	17.52± 0.7	2.86±0.5	4.82±0.91
V MEIA (200 mg/kg)	16.7± 0.62	1.7± 0.80	18.75± 2.9	3.6± 0.78	5.40± 0.71
VI MEIA (400 mg/kg)	15.4 ± 0.58	1.53 ± 0.74	14.79.± 4.4	3.10+1.13	5.81 ± 0.45

DISCUSSION AND CONCLUSION

Continuous administration of cholesterol to group II animals for 30 days resulted in the elevation of various parameters of lipid profile. A significant increase in the body weight was also noticed in these animals during the study period. Treatment with the standard drugs, clofibrate and guggul effectively prevented the increase in body weight to a large extent ($P < 0.05$). Guggul has also been included in the study in addition to clofibrate in order to understand how far MEIA's activity is comparable to that of a standard drug, [18] from the natural source. Biochemical assay of various lipid profiles in serum, liver and heart of the animals revealed that EPE reduced the elevated levels of total lipids, total cholesterol, triglycerides, phospholipids and free fatty acids in a dose-dependent manner and a significant activity was observed with 400 mg/kg dose. The extract of *Ipomoea aquatica* Forsk might have acted at the liver level in metabolising and hastening the process of excretion of excess lipids thereby producing hypolipidemic condition, as liver is the key organ in the synthesis and metabolism of lipids shown in **Fig .1**

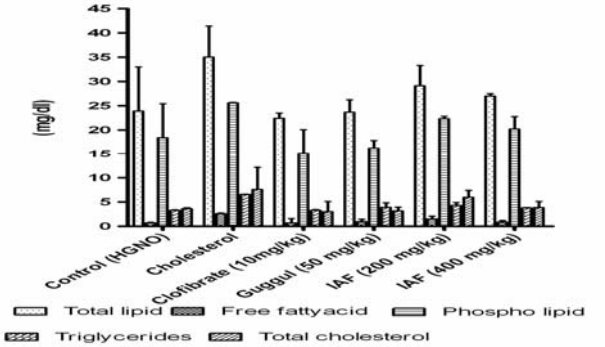

Fig 1: Effect of MEIA on Serum lipid profile in control and experimental animals

In conclusion, our results indicate that MEIA has potential hypolipidemic activity, which provide pharmacological evidence for folk uses of *Ipomoea aquatica* Forsk. In the treatment of liver diseases. Further molecular and cellular experiments are warranted to explore its action mechanisms. Identification of its active components is also warranted.

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