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Studies on antihyperlipidemic activity of stigmasterol isolated from methanol extract of *Acalypha indica*

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Abstract

The antihyperlipidemic activity of isolated stigmasterol from methanol extract of whole plant of *Acalypha indica* was evaluated using high fat diet induced hyperlipidemia model in rats. Chronic hyperlipidemia was induced by feeding high fat diet for 28 days to rats. Treatment with isolated stigma sterol (20 and 40 mg/kg, p.o) significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol, Triglycerides, Low Density Lipoprotein Cholesterol (LDL-C),Very Low Density Lipoprotein Cholesterol (VLDLC) and increase of serum High Density Lipoprotein Cholesterol when compared to vehicle control and standard drug Atarvastatin. The results demonstrated that isolated stigma sterol from methanol extract of whole plant of *Acalypha indica* possessed significant anti-hyperlipidemic activity.
Keywords: Antihyperlipidemic activity, Atarvostatin, high fat diet method.

1. Introduction

Hyperlipidemia is major risk factor for the atherosclerosis. Other complications are coronary heart disease, ischemic cerebro-vascular disease, hypertension, obesity and diabetes mellitus (Type -II). Although many efficacious lipid-lowering synthetic drugs exist, none is effective and safe for all lipoprotein disorders, and all such agents are associated with some adverse effects. Therefore it is a need of the day to search other materials from natural sources that are less toxic, less expensive and which can provide better safety and efficacy on a long term usage.

Natural products from plants are a rich source used for centuries to cure various ailments. *Acalypha indica* leaf juice or boiled extract is prepared and given in dose of 1-2 tablespoonful to small children and 15-30 ml in case of a adult to induce purgation and expel the intestinal worms and mucus from intestine. The juice of tree is boiled with ginger and applied outwardly on ache areas of body [1].

The leafs are grind with salt and externally above skin infection like scabies [2,3]. The vegetation be ground along with manual and apply outwardly above ulcers, poisonous bites [4-6]. The paste of leaves along with quick lime is externally applied over painful arthritis[7]. The leaf liquid is externally applied over head ache [7,8]. The dry leaf fine particles are bandaged above the bed sore areas and therefore produce anthelmintic action. The whole plant of *Acalypha indica* was subjected to extraction with various solvents and active constituents were isolated from methanolic extract. The present research work evaluates antihyperlipidemic activity of isolated stigmasterol from *Acalypha indica.*

2. Materials and methods

2.1. *Plant Material*

The whole plants of *Acalypha indica Linn.* were collected from Cherlapally Nalgonda rural area Hyderabad road, Telangana in the month of September. Botanical identification was done by Prof Badraiah, Department of Botany, Osmania University, Hyderabad.

The plant material was shed dried and powdered to coarse material and subjected to hot continuous extraction using Soxhlet apparatus method with various solvents. From all the solvent extraction, the yield of methanol extract was good hence the methanol extract was selected for further evaluation and isolation.

2.2. Isolation of compound from extract.

Methanol extract was subjected to TLC and HPTLC method to isolate the phytosterol using optimized solvent ratio is chloroform and ethanol (9.5:0.5). From laboratory studies and other analytical techniques like IR, HPLC, MS, the isolated phytosterol was identified as stigmasterol. Further same solvent ratio is used to isolate the stigmasterol through column chromatography. The isolated compound was used for the animal studies.

2.3. Evaluation of anti-hyperlipidemic activity

The experimental model selected for the present study was high fat diet induced hyperlipidemia in rats. This animal model mimics human hyperlipedemia, inducing radicals showing the signs of oxidative stress. The present study was aimed to evaluate the serum lipid parameters in the high fat diet (HFD) induced hypercholesterolemia rats after administration [9].

2.3.1 Preparation of High Fat Diet (HFD):

Exactly weighed 610 g of powdered NPD, 5 g deoxycholic acid and 5 g cholesterol were added and mixed. After complete mixing the above, 90 g fructose was added and mixed well, later 280 g of coconut oil was added slowly into the above powder with constant mixing, after complete mixing to get dough mass, made in to uniform size balls, stored in refrigerator.

In-house laboratory bred healthy wistar male rats were used for the experiment because, besides preventing environmental variation, inbreeding provides a homogenous population of animals for experiments were acclimatized to the experimental room having temperature 25±2◦C, controlled humidity conditions and 12 h light-dark cycle. The rats were fed with commercially available rat standard pellet diet and water *ad libitum*.

Animals were grouped based on their body weight before feeding the HFD. Group 1 animals are considered as normal control and were fed with (NPD) normal pellet diet. The other animals were fed with HFD for 4 weeks. Cholesterol level in HFD fed animals were estimated and regrouped at the end of 4th week based on Cholesterol levels. Group 1 served as control and received vehicle (SCMC) only. Group 2 received as HFD, then Group 3 received as 20 mg stigmasterol, Group 4 received as 40 stigmasterol, Group 5 received as atarvastatin (4mg/kg), At the end of the treatment schedule on 30th day, animals were fasted overnight and blood samples were collected from all animals by retro orbital puncture under slight anaesthesia and serum were separated by centrifugation and subjected for fasting lipid profiles analysis.

3. Results and discussion

The dried and powdered whole plant material of *Acalypha indica was* subjected to soxhlet extraction with methanol. Later methanol extract was subjected for column chromatography using solvents chloroform and ethanol (9.5:0.5) for isolation of stigmasterol to have anti-hyperlipidemic properties. Treatment with stigmasterol 20 mg and 40mg for prevented the elevation of serum Total Cholesterol, Triglycerides, Low Density Lipoproteins Cholesterol (LDL-C), Very Low Density Lipoproteins Cholesterol (VLDL-C), and decrease of serum High Density Lipoprotein Cholesterol (HDL-C) in Triton model rats respectively. High fat diet method induced hyperlipidemia in rats is a chronic model for the primary screening of antihyperlipidemic agents. High fat diet physically alters very low density lipoprotein cholesterol rendering them refractive to the action of lipolytic enzymes of blood and tissues, preventing or delaying their removal from blood and tissues. Hence the antihyperlipidemic effect of *isolated stigmasterol* administration could be due to an increased catabolism of cholesterol into bile acids. Diet containing saturated fatty acids increases the activity of HMG CoA reductase, the rate determining enzyme in cholesterol biosynthesis; this may be due to higher availability of acetyl CoA, which stimulated the receptors by the cholesterol and saturated fatty acids in the diet, which could also explain the elevation of serum LDL-C levels either by changing hepatic LDLR (LDL receptor activity), the LDL-C production rate or both.

Table 1: Effect of methanol extract of *Acalypha indica* of on cholesterol, triglycerides, HDL, VLDF and LDL level in serum of control and experimental rats

Group	Cholesterol	Tryglycerides	HDL	VLDL	LDL
Control	53.9 ± 1.99	62.27 ± 3.9	36.23 ± 1.66	11.38 ± 0.1	9.66 ±0.1
HFD	243.8 ± 3.1	410.0 ± 4.69	86.28 ± 2.7	43.76 ± 3.2	77.06 ±3.1
Test 20 mg/kg (stigma sterol)	164.8 ± 2.12	221.9 ± 1.38	76.2 ± 3.12	30.86 ± 0.2	55.13 ± 2.1
Test 40 mg/kg (stigma sterol)	110.3 ± 7.2	110.3 ± 6.11	50.51 ± 2.2	22.44 ± 1.2	35.34 ± 0.3
Standard (atarvostatin 25 mg/kg)	62.27 ± 3.2	78.4 ± 6.67	45.21 ± 3.71	15.45 ± 0.2	24.10 ± 0.2

4. Conclusion

The results obtained from the pharmacological screening have led to the conclusions that, isolated stigmasterol from methanolic extract of whole plant of *Acalypha indica* has significant antihyperlipidemic activity. Hence it can be exploited as an anti-hyperlipidemic therapeutic agent or adjuvant in existing therapy for the treatment of hyperlipedemia.

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