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Research article

Chloroform extricate of trianthema portulacastrum, whole plant: an anti-hyperlipidemic in atherogenic diet induced hyperlipidemic rats

Yada Divya 1*, T. Sivakkumar 2, M. Sudhakar1

Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, telangana state, India
Annamalai University, Annamalai Nagar, Chidambaram, telangana state, India

ABSTRACT

Hyperlipidemia is contemplated to be among the considerable risk factor that contributes to the severity as well as incidence of cardiovascular diseases. The Anti hyperlipidemic effect of Chloroform extricate of entire plant Trianthema portulacastrum has been studied in atherogenic diet induced hyperlipidemic rats. Group-I acted as controls (untreated), Group-II acted as atherogenic diet induced controls, standard Hypolipidemic drug (Simvastatin 4mg/kg) used for treating Group-III. The Chloroform Extricate of Trianthema portulacastrum were administered orally at 100mg/kg and 200mg/kg doses for Group IV and V respectively. Serum lipid levels were evaluated after oral administration of standard drug and Chloroform extricate of Trianthema portulacastrum (100mg/kg as well as 200mg/kg) respectively at experimental study's end. With 200mg/kg of Trianthema portulacastrum Chloroform Extricate treatment, significant reduction in serum lipid parameters like VLDL, LDL, triglycerides, total cholesterol levels and increased HDL level were seen hyperlipidemic rats as compared to control statistically. From these results, it is evident that, Chloroform Extricate of Trianthema portulacastrum entire plant treats hyperlipidemia and improves the liver lipid profile.

Keywords: Atherogenic diet, Trianthema portulacastrum, Hypolipidemic activity, simvastatin.

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Correspondence: Divya Yada* yada.divya@gmail.com

Department of Pharmaceutical Chemistry, Malla Reddy College of Pharmacy, Maisammaguda, Dhulapally, Secunderabad, telangana state, India

INTRODUCTION

In the atherosclerotic lesions' commencement and progression, hyperlipidemia is the key factor. Arteries' atherosclerosis is an arterial network's generalized disease that is acknowledged as a silent killer and progressive disease described by the lesions formation which is known as atherosclerosis plaques in the medium or large sized coronary arteries' walls that decreases flow of blood to the myocardium - known as "Coronary Artery Disease (CAD)".^[1]

Hyperlipidemia is associated with the atherosclerosis through numerous evidences. It has indicated by the study evidences in both humans and animals that deceleration of progression is possible when atherogenic triglycerides and lipoprotein's increased serum concentration are decreased that results in reducing CAD mortality and morbidity in CAD established patients as well as also newer CAD occurrences as well as mortality in without CAD established patients are also reduced.^[2] In hyperlipidemia patients' treatment's primary aim is reducing the occurrence of further cerebrovascular diseases or cardiovascular diseases or development risk of ischemic heart diseases.^[3] Presently, there is progressive demand for the medicinal plant treatment even though a huge number of synthetic drugs are available to treat, because synthetic drugs are related to various side effects like abnormal liver function, gastric flushing, dry skin, myositis, irritation, diarrhoea, nausea, and hyperuricemia.^[4] Condiments, medicinal plants, fruits(daily use) have been identified as hyperlipidemic in Ayurveda(Indian traditional system).^[5]

Trianthema portulacastrum (Aizoaceae), discovered nearly throughout India as a weed in wastelands and cultivated lands. In taste, this plant is bitter as well as utilized as laxative, stomachic, and analgesic as well as rural people utilized it for inflammation, blood

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anemia, heart disease, and bronchitis. For treating the night blindness, dimness of sight, itching, and corneal ulcers, the plant root is used to the eye.^[6] Isolation of various bioactive compounds from this weed is performed as well as extensively utilized against various diseases.^[7] The herb decoction is utilized as a vermifuge (an anthelmintic medicine) as well as beneficial to treat rheumatoid arthritis. Against the hepatotoxicity induction, a significant protection is exhibited by the plant.^[8] Earlier, T. portulacastrum antifungal activity and in albino rats, Trianthema protulacastrum hepatoprotective activity^[9] against thioacetamide, and paracetamol intoxication are reported by the researchers. Also, in alloxan-induced diabetic rats, TP's methanolic extricate's hyperlipidemic and hypoglycemic activities were reported.^[10]

MATERIALS AND METHODS

Collection of Plant material and Plant Extrication

The Trianthema portulacastrum plant was accumulated from the forests of Maisammaguda, Secunderabad located in the state of Telangana (India), shade dried and powdered mechanically. The plant specimen was certified by pharamacognosist of Osmania University and authenticated voucher Number 145 of the plant has been preserved in department for future use. The dried plant were then mechanically milled to coarse powder and extracted with dichloromethane (CHCl3) in Soxhlet's apparatus and the extract was evaporated to dryness under vacuum and dried in vacuum desiccators. Later stored in refrigerator.

Animals

This study's an ethical approval was acquired from the "Institutional Animal Ethical Committee" with an Approval no: CPCSEA/IAEC/JLS/11/11/19/14. Albino rats with average body weight from 150 to 250 g were utilized for conducting this study. They were procured from Sanzyme Bio-analytical lab, Plot no. 8 Sys.No.542, Kothur (V), Shameerpet, RR dist. Polypropylene cages were used for housing rats as well as standard conditions (12h dark and light cycles at 35-60 % humidity and $25\pm3^{\circ}$ C) were used for their maintenance. Standard tap water and pellet feed were allowed ad-libitum.

Experimental methodology

The rats will be randomly assigned into 5 different groups (n=6).

Group I	: Normal diet ad libitum
Group II	: Atherogenic diet
Group III:	Atherogenic diet + Simvastatin (4mg/kg)
Group IV:	Atherogenic diet+ CHCl3 Extricate of
	Trianthema portulacastrum (CETP)
	(100mg/kg)
Group V	: Atherogenic diet+ CHCl3 Extricate of
	Trianthema portulacastrum (CETP)
	(200mg/kg)

After the treatment duration, on 30th day the animals will be sacrificed and the following parameters will be evaluated.

Induction of hyperlipidemia

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For inducing hyperlipidemia, Bopanna et al.^[11] reported method is utilized for preparing the Atherogenic diet (Table 1). There were 5 animal groups, with 6 rats each. Group-I rats acts with normal diet while Group-II to Group-V served orally with standard powdered animal food following without or with treatment for 30days. The feed produced as pellets was carefully put in the cage as well as supplied for 30days.

Ingredients	Normal	Atherogenic
Protein (Milk powder)	20g	15g
Sucrose	3g	-
Carbohydrate (Wheat flour)	65 g	57.6 g
Fat (Butter)	5g	15g
Salts	4g	4g
Vitamin mix	1g	1g
Fiber	2g	2g
Coconut oil	-	5 m
Cholesterol	-	0.4 g
Total weight	100g	100g

Table1. Normal	and Athera	ogenic diet	compositions.

Fecal cholesterol excretion

During the treatment's last 3days, fecal matter was gathered. CHCl₃: MeOH (2:1) used for extricating the powered and dried fecal matter. After that, for cholesterol contents, analysis of resultant extricate was performed in a way same as that of serum. Further, the fecal matter excreted cholesterol (mg/g) was estimated.

Biochemical assays for Serum lipid profile

At the experiment's end, the rats were anaesthetized by CO₂ inhalation method, blood was collected via carotid bleeding permitted to clot for 10min at room temperature. Next, for 10min at 1000 rpm it was centrifuged, the serum was kept at 4°C, utilized for assessing the Serum triglycerides (TG), total cholesterol (TC) using CHOD-PAP approach as well as "HDL-c (high-density lipoprotein-cholesterol)" through GPO-PAP approach utilizing standard enzymatic colorimetric kits acquired from "M/s Qualigens Diagnostics, Mumbai, India". Serum LDL-c was measured by utilizing the following formula ^[12]:

LDL-cholesterol = total cholesterol – [HDL-C + (triglycerides /5)]

VLDL-cholesterol ^[13] was either measured directly (after ultracentrifugation) or calculated as total triglyceride concentration/5. The following formula was utilized for calculating atherogenic index ^[14]

Atherogenic index = (total cholesterol – HDL-C)/ HDL-C

Statistical analysis

Results were represented as mean \pm SD. Between the groups the difference significance was evaluated with the help of "ANOVA (one way analysis of variance)" following Dunnet's test. **RESULTS AND DISCUSSION**

The chloroform extricate had been tested for the antihyperlipidemic activity and the results were presented in (Table-2), gives us the data of the serum lipid profile in Atherogenic induced hyperlipidemia model. Mean \pm SEM used for expressing values, n=6. Using t-test, the intergroup variation between various groups was conducted by using "ANOVA (one way analysis of variances)".

Groups	Choleste rol (mg/dL)	Triglyceri des (mg/dL)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Athero genic index
Control	94.4 ± 0.16	90.2±0.78	18.04±1 .35	23.16±1 .09	53.2±1. 50	0.77
Atheroge nic diet.	216.8±1. 01	172.2±0.3 1	34.44±1 .06	141.76± 0.45	40.60±1 .29	4.33
Simvasta tin(mg/kg)	114.20±1 .74***	106.60±0. 54***	21.32±0 .71***	22.68±0 .84***	70.20±0 .45***	0.62
CETP(10 0mg / kg)	198.4±0. 13*	162±0.89*	32.40±0 .58*	107.5±1 .21*	58.5±0. 82*	2.39
CETP(20 0mg/kg)	142.6±0. 92**	149.2±0.9 6**	29.84±0 .80**	48.36±0 .40**	64.40±0 .21**	1.21

Table2: Effect of Chloroform extricate of Trianthema portulacastrum

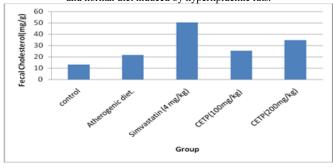
For the Hypocholesterolemic effect evaluation, we successfully used atherogenic diet induced hyperlipidemic model. In this study, it was observe that plasma HDL-cholesterol increases with other associated lipids' reduction. Hyperlipidemia was controlled and normalized with the treatment of chloroform extricate of Trianthema portulacastrum (200 mg/kg) significantly. Chloroform extricate at "100mg/kg (p<0.05), 200mg/kg (p<0.01)" doses as well as in comparison to Atherogenic diet control groups, a considerable rise in cholesterol excretion is shown by standard drug simvastatin 4mg/kg (p<0.001) (Table 3, Figure 1).The chloroform extricate of Trianthema portulacastrum (200 mg/kg) showed better control of the readings and lipid levels in the serum.

Table3: Effect of treatment of Chloroform extract of Trianthema portulacastrum entire plant on fecal cholesterol examination of atherogenic

and normal diet induced by hyperlipidemic rats			
Groups	Cholesterol (mg / 9)		
Control	13.1 ± 0.16		
Atherogenic diet.	21.7±1.01		
Simvastatin (4 mg/kg)	50.20±1.74***		
CETP(100mg/kg)	25.2±0.13 *		
CETP(200mg/kg)	34.6±0.92**		

Mean \pm SEM used for expressing values, n=6. Using t-test, the intergroup variation between various groups was conducted by using "ANOVA (one way analysis of variances)". ***p < 0.001, **p < 0.01, and *p < 0.05.

Figure 1: Treatment effect of Chloroform extricate of Trianthema portulacastrum entire plant on fecal cholesterol examination of atherogenic and normal diet induced by hyperlipidemic rats.



CONCLUSION

The Chloroform extricate of Trianthema portulacastrum entire plant showed a better hypolipidemic activities at 200mg/kg dose in Atherogenic diet induced hyperlipidemic models. Further research has to be done to prove the exact mechanism of action extricate to control the lipid levels in the serum and prevent unnecessary weight gaining of the animals.

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