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Amelioration of high fat diet induced hyperlipidemia by methanol extract of *Tephrosia calophylla* in wistar rats

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Abstarct

Objectives: The present study was performed to determine hypolipidemic potentials of methanol extract of *Tephrosia calophylla* against high fat induced hyperlipidemia in wistar rats.

Methods: Defatted powdered drug of ariel parts of *Tephrosia calophylla* was subjected to methanol extraction using soxhlet extractor. The high fat diet induced hyperlipidemia in rat model was used for the present investigation in which all experimental rats were administered with single dose of triton (250mg/k.g., i.p) on 1st day were given with methanol extract for 21 days. Blood samples were collected from all the animals on day 21st after one hour of administration of the drugs and serum lipid profile (total cholesterol, triglycerides, HDL, LDL and IDL) was estimated. All animals were sacrificed after the blood sampling and liver samples were collected. The collected liver samples were homogenized and estimated for protein and m-RNA expression ranges by western-blotting.

Results: The rats of therapeutic groups ingested with extract of *Tephrosia calophylla* and standard drug atorvastatin have shown significant reduction in serum cholesterol, serum triglycerides, LDL and increase in HDL indicating its ability to attenuates effect of administration of high fat diet. Administration of methanol extract could also normalize levels of protein and m-RNA expression in therapeutic rats.

Conclusion: The methanol extract of *Tephrosia calophylla* shown significant hypolipidemic effects against high fat diet induced hyperlipidemia wistar rats by inhibiting cholesterol synthesis in liver.

Key words: Hyperlipidemia, High Fat Diet, *Tephrosia calophylla*, Lipid profile, HMG CoA reductase, HMG CoA synthetase.

INTRODUCTION

A major risk factor for the onset and progression of cardiovascular disorders is hyperlipidemia which is characterized by elevated levels cholesterol, triglycerides, cholesterol esters VLDL and LDL cholesterol and also reduced concentrations HDL in blood[1,2] [1,2]. Various factors can impact the occurrence of hyperlipidemia among different populations which includes dietary factors, uncontrolled diabetes mellitus, high alcohol intake and stress[3,4] [3,4]. Hyperlipidemia can lead to health impediments such as atherosclerosis, Myocardial infarction(MI), coronary artery disease (CAD), angina pectoris and cerebral ischemic stroke[4] [3]. Important classes of drugs presently used for the management of hyperlipidemia include are cholesterol synthesis inhibitors (statins), inhibitors of lipolysis (nicotinic acid and fibrates) bile acid sequestrants (cholestipol) possess significant serious adverse reactions such as liver damage, rhabdomyolysis and renal failure and hence pharmacological management of hyperlipidemia remains still unsatisfactory[5]. Hence studies on medicinal plants are increasing worldwide and screened for various complications such diabetes, CVS disorders, cancers and etc.

Tephrosia calophylla commonly known as Adavivempalli a traditional plant widely distributed in Karnataka and Andhra Pradesh and extensively used in Ayurveda and Folklore medicine. The ariel parts of this plant had been used in Ayurvedic medicine for the management of various ailments. The plant extracts have been used in treatment of different disease conditions such as liver disease, diabetes mellitus bacterial, fungal, viral infections and hyperlipidemia[6]. The aerial parts of the plant rich in alkaloids, flavonoids, tannins and phenols and scientifically proved for its antidiabetic, antiulcer, anticancer, larvicidal and antimicrobial activities[7]. The various pharmacologically active compounds from the plant such as Betulinic acid, Tephcalostan, Tephcalostan A, Tephcalostan C, Calophione -A, B and C have been isolated and characterized[8]. The *Tephrosia calophylla* belongs to the same genus was essential component of traditional anf folklore medicine and recently reported for anti-ulcer, antidiabetic and anticancer potentials[9,10,11]. Though the plant was extensively used in traditional medicine to reduce serum lipids, there is a lack of scientific evidence for the same. Hence the objective of the present study is to evaluate and provide scientific data for the hepatoprotective potentials of *Tephrosia calophylla* against thioacetamide induced liver damage in experimental rats.

MATERIALS AND METHODS

Collection and authentication of plant material

The ariel parts of *Tephrosia calophylla* were collected and authenticated by Dr. Madhavachetty, HOD, Department of Botany, Sri Venkateswara University, Tirupati.

Preparation of ethanol extract

The ariel parts of *Tephrosia calophylla* were dried under room temperature immediately after collection and subjected to milling to collect the coarse powder. About 250gm of coarse powder of *Tephrosia calophylla* was first defatted with petroleum ether (40°-70°C) and defatted coarse powder was again subjected for extraction with methanol for 72 hours using soxhlet apparatus[12].

Preliminary phytochemical investigation

The methanol extract of *Tephrosia calophylla* (TCME) was investigated for the preliminary phytochemical compounds according to standard protocol described by Khandelwal[13].

Animals

Healthy albino Wistar rats 180-200 of weight range were procured from Sri-Venkateswara Enterprises, Bengaluru. All animals were housed in animal house facility of East West College of Pharmacy provided with well ventilation and standard temperature condition between 28±2^{0}C. The animals were provided to access feed (standard laboratory plellets) and drinking freely. The research protocol was approved by IAEC, IJAHSM (Ref.no.IJAHSM/IAEC/2014/03) with the permission from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

Assessment of methanol extract of *Tephrosia calophylla* hypolipidemic activity

The hypolipidemic activity of methanol extract of *Tephrosia calophylla* was evaluated against high fat diet induced hyperlipidemia in rat's models[14,15,16].

Hypolipidemic activity of TCME against high fat diet (HFD) induced hyperlipidemia

Preparation of (HFD): The High Fat Diet was prepared according to procedures described in previous studies. The composition of HFD consisting of Powdered Normal protein diet (365 g), Lard (310 g), Casein (210 g), Cholesterol (10 g), Vitamin and Minerals (60 g), D1-Methionine (3 g), Yeast powder (1 g) and Sodium chloride (1 g). All the ingredients of High Fat Diet were mixed properly using mixer and made in the form pellets using required amount of distilled water and allowed for drying under shade.

Group design: This study was also consisting of six group of albino rats containing 6 animals in each group and details of treatment are as follows.

- Group I-Normal control: Animals were administered with normal saline 2ml/kg., i.p.
- Group II-Lipid control: Animals were given with 30g of HFD every day and 2% tween 80 for 21 days.
- Group III-Standard control: Animals were given with 30g of HFD every day and standard drug atorvastatin (mg/kg.,p.o) orally for 21 days.
- Group IV- TCME 100: Animals were given with 30g of HFD every day and low dose of methanol extract of *Tephrosia calophylla* orally for 21 days.
- Group V- TCME 200: Animals were given with 30g of HFD every day and medium dose of methanol extract of *Tephrosia calophylla* orally for 21 days.
- Group VI- TCME 400: Animals were given with 30g of HFD every day and high dose of methanol extract of *Tephrosia calophylla* orally for 21 days.

Evaluation parameters

Biochemical parameters: On the 21st day of study, blood samples from all the animals were collected estimated for Total cholesterol, Triglycerides, LDL, HDL, VLDL, Creatinine, urea and BUN. The weight gain of animals during study period was also calculated[14,15,16].

RESULTS

Preparation of extract

The percentage yield of methanol extract of *Tephrosia calophylla* was 8.19 % w/w.

Preliminary phytochemical study

The preliminary phyto-chemical investigation for the methanol extract of *Tephrosia villosa* reveals the presence of poly phenols, flavonoids, tannins, steroids, alkaloids and carbohydrates.

Evaluation of anti-hyperlipidemic activity of methanol extracts

In the present study conducted to determine antihyperlipidemic activity of methanol extract of *Tephrosia calophylla* ingestion of high fat diet caused significant weight gain and also significant increase in the Total cholserol, Triglycerides, LDL and VLDL concentrations in the vehicle control group compare to normal animals. But co-administration of Atorvastatin and TCME significantly reduced above mentioned parameters in therapeutic animals compare to vehicle control rats. The concentration of HDL was significantly declined vehicle control group compare to normal while its range was significantly increased in therapeutic group of animals treated with medium and high doses of TCME and standard drug compare to lipid control animals. But there was no significant change in the biochemical parameters of rats treated with low dose of methanol extracts [Table 1].

Table 1: Effect of methanol extract of *Tephrosia calophylla* on lipid profile against HFD induced hyperlipidemia

Treatment	Serum parameters					
	Total Cholesterol	Triglycerides	LDL	VLDL	HDL	Weight gain
Normal Control	83.61±1.715	101.9±2.187	29.87±0.7571	5.974±0.1514	34.05±1.549	1.978± 0.09163
Lipid Control	156.8^{+++}±3.447	150.7^{+++}±1.790	55.85^{+++}±1.691	11.17^{+++}±0.3382	21.82^{+++}±0.92	18.44^{+++}± 1.084
Standard(Atorvastatin)	86.11***±2.608	102.8***±1.411	32.90***±0.7196	6.58***±0.1439	34.51***±1.469	3.390± 0.4882
TCME 100 mg/kg	147.7±2.807	146.9±3.463	51.17±0.6194	10.234±0.1239	24.53±1.782	15.67±0.8665
TCME 200 mg/kg	109.3**±2.640	125.0*±2.028	46.02*±1.630	9.204**±0.326	29.33**±1.789	13.20**± 1.140
TCME 400 mg/kg	83.64***±2.335	102.1***±3.256	33.01***±0.6840	6.602***±0.137	32.49***±1.358	3.135***±0.7837

Values are mean ± S.E.M, n=6 symbols represent statistical significance.,

^{ns}p>0.05, * p<0.05, ** p<0.01, ***p<0.001 Normal control vs Lipid control.

^{ns}p>0.05, $^{+}$ p<0.05, $^{++}$ p<0.01, $^{+++}$p<0.001 Lipid control vs Therapeutic groups

DISCUSSION

Atherosclerosis and its accompanying diseases, such as peripheral vascular disease, ischemic cerebrovascular disease, and coronary heart disease (CHD), are largely brought on by hyperlipidemia. Among these, ischemic heart disease is intimately associated with hypercholesterolemia and hypertriglyceridemia. CHD risk is decreased by lower serum cholesterol levels. Reducing the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease is the major goal of treatment for people with hyperlipidemia[17]. Several negative effects of the hypolipidemic medications now on the market have been reported. Hyperuricemia, diarrhoea, nausea, myositis, stomach irritation, flushing, dry skin, and altered liver function are all caused by the usage of synthetic medicines. In this regard, herbal medicines are proven to be effective drugs to reduce hyperlipidemia with minimum side effects and hence there is a scope to develop herbal remedy for the hyperlipidemia. High fat diet induced hyperlipidemia, triton induced hyperlipidemia in rats model had earlier been reported as ideal *in vivo* models for testing antihyperlipidemic drugs. Several studies reported that enriched fatty diets cause elevation of plasma TC and LDL cholesterol. High levels of TC and most importantly LDL cholesterol are predictors of atherosclerosis. Another research showed that triglycerides are directly or indirectly related to coronary heart diseases[18,19]. In the present study, high fat diet induced hyperlipidemia in rats model was used for the evaluation of hypolipidemic activity of methanol extract of *Tephrosia calophylla*. The administration of standard drug atorvastatin, medium and high doses of TPME, TVME and TCME could significantly reduce total cholesterol, triglycerides, LDL, VLDL and weight gain while in control group there was increase in these parameters were observed due to hyperlipidemia. There was also significant increase in the concentration of HDL in therapeutic animals was found compare to control animals ultimately suggesting the possible anti-hyperlipidemic activity of methanol extracts.

CONCLUSION

The results obtained from the present investigation suggesting that, methanol extract of *Tephrosia calophylla* possess significant antihyperlipidemic potentials against high fat induced hyperlipidemia in wistar rats. Further studies are require to determine its mechanism of action and also to isolate and test specific constituent present in the methanol extract responsible for the benefits.

Conflict of interest

All authors are hereby declaring that there is no conflict of interest with respect to manuscript.

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Authors contributions

The present research work was performed by Ms. Sushma Bai B under the guidance of Dr. Ramesh C, Professor and HOD, Department of Pharmacology, East West College of Pharmacy, Bangalore. Mrs. Sowmya B.A, Mrs. Pinkey Rawal and Mrs. Sushmitha D.H were also part of research work guided her in literature survey, study design and statistical analysis.

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