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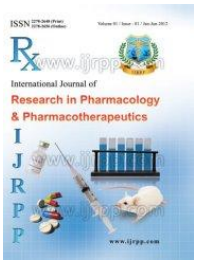

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Research Article ***Open Access***

Evaluation of *Tephrosia purpurea* for anti hyperlipidemic activity in high fat induced rats

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ABSTRACT

The anti-hyperlipidemic effect of methanolic extract of leaves of *Tephrosia purpurea (T.purpurea)* was tested in high fat diet induced hyperlipidemic rat models. Here, chronic hyperlipidemia was induced by feeding fat diet for 21 days. The hyperlipedemic rats are grouped and Treated with fenofibrate (5mg/kg), *T.purpurea* extract, (200 and 400 mg/kg, p.o). The plant extract treated groups are significantly reduced the hyperlipidemia i.e., decreased levels of serum Total Cholesterol, Trigly cerides, Low Density Lipoprotein Cholesterol (LDL-C), and increase of serum High Density Lipoprotein Cholesterol (HDL-C) whencompared to vehicle control and standard drug Fenofibate (5 mg/kg). The results demonstrated that methanolic extract of leaves of *T.purpurea* possessed significant antihyperlipidemic activity.

Keywords: Anti hypelipidemic activity, *Tephrosia purpurea*, Fenofibrate, *Fat* diet

INTRODUCTION

Hyperlipidemia is the term used to denote raised serum levels of one or more of total cholesterol, low density lipoprotein cholesterol, triglycerides or both total cholesterol and triglyceride (combined hyperlipidaemia). Dyslipidaemia is a wider term that also includes low levels of high-density lipoprotein cholesterol[1]. Hyperlipidemia represents a major risk factor for the premature development of atherosclerosis and its cardiovascular complications. Hyperlipidemia is a disorder characterized by the increase in blood lipoprotein or cholesterol levels. Recent studies have shown that lipid associated disorders are not only attributed to the total serum cholesterol, but also to its distribution among different lipoproteins. The low density lipoproteins (LDL) are the major carriers of cholesterol towards tissues having atherogenic potential, while the high density lipoproteins (HDL) carry cholesterol from peripheral tissues to the liver. HDL thus gives protection against many cardiac problems and obesity. Although genetic factors recline behind these lipid disorders[2]. Oxidative stress by oxidation of low density of low density lipoproteins plays a major role in hyperlipidemia. The disorder of hyperlipidemia is considered to be a problem in patients with diabetes and is one of the major risk factor in the development of atherosclerotic heart diseases[3]. Excess triglyceride accumulation and increased fatty acid oxidation in diabetic heart contribute to cardiac dysfunction. Lipid metabolism normally maintains an elegant balance between its synthesis and degradation when this balance is disrupted hyperlipidemia, hypertriglyceridemia, and hypercholesterolemia may develop, this can cause variety of diseases such as hypertension, diabetes, obesity, atherosclerosis[4]. Plants have a significant role in maintaining human health and improving the quality of human life for thousands of years and served humans well as valuable components of

medicine[5]. Ayurveda is an ancient form of Indian medicine which deals with plants products, this indigenous form of medicine uses the active ingredients present in plants for treating disease[6] .Plant products are frequently considered to be less toxic and free from side effects than synthetic ones. The prophylactic and therapeutic effect of plant foods and extracts in reducing cardiovascular disease has been reviewed. A vast number of these plants are to receive attention in this regard and have been shown to lower plasma lipid levels, some examples are *Gacinia cambogia*, *Zingiber officinale* and *Emblica officinalis*[7]. The wide use of these herbal plants had now leads to carry out research in institutions and universities on the potential benefits of herbal drugs. Our present study is also an attempt towards this direction[8].

MATERIALS AND METHODS

Plant collection

Tephrosia purpurea was collected around *Kurnool*, Creative Educational Society, JNTU University, Ananthapur. Leaves and stems were separated, washed in water, chopped into pieces and then shade dried. The coarsely powdered leaves were extracted with methanol using sox let apparatus for 3 hr. The cycle was repeated for three times. The extract was concentrated on rotary flash evaporator to semi solid consistence and then dried over a water bath (yield – 30.6 g/kg).

Animals

Male and Female albino rats (150-200gm) of body weight were obtained from Tirupati, and they were housed under standard husbandry conditions 25±5°C temperature, light/dark cycle with standard rat feed (Pravan agro Ltd. India) and water *ad libitum*. The experimental protocol was approved by the Institutional Animal Ethical Committee of Sri Padmavathi School of Pharmacy (1016/a/06/CPCSEA/008/2009).

Drugs and chemicals

Fenofibate c Epinephrine, DTNB (sigma), Thiobarbituric acid (TBA) and Trichloro acetic acid, hydrogen peroxide (SD fine chemicals Ltd). Sodium dihydrogen phosphate, potassium dihydrogen phosphate, tries buffer and all other reagents used were of analytical grade. Total cholesterol and HDL, Triglycerides and Total protein estimation kits were procured from Kamineni Life Sciences Pvt. Ltd. India.

Instruments

Semi auto analyzer (mixpel)

Acute toxicity studies

Acute oral toxicity study was performed as per OECD – 423 guidelines (acute toxic class method). Wistar albino rats of either sex were selected by random sampling technique and divided into five groups (n = 6). The animals were fasted overnight and methanolic extract in doses of 500, 1000, 2000, 4000 mg/kg body weight, were administered orally to II – V groups. Group I which received vehicle (water) served as control. The animals were observed continuously for 2 hr, then intermittently for 6 hours at the end of 24 hours, the number of were noted deaths and to determine LD_{50} of extract[9].Animals were also observed for behavioral, neurological and autonomic profiles simultaneously[10].

High fat diet (fd) induced hyperlipidemic model

Normal animal food pellets were crushed in mortar and pestle to crush into small pieces and then grinded into fine powder in mixer grinder. The other ingredients i.e. cholesterol 2% , Cholic acid 1% , sucrose 40% , and coconut oil 10% were added in the mixer grinder in an ascending order of their quantity and mixed well. This dried powder was then mixed with same quantity of water every time to make small balls of feed and later this was stored in self sealing plastic covers in refrigerator at 2°C to 8°C. The feed for normal group was prepared similarly by grinding only the normal food pellets and then mixing with water without the other exepients. This preparation of feed was done once in three days for all the animals. Thirty Wister rats were randomly divided into five groups of six each. The chronic experimental hyperlipidemia was produced by feeding the above prepared food for 21 days. The rats are then given test plant extracts i.e., *T.purpurea* (200 and 400 mg/kg, p.o) and fenofibrate (5 mg/kg, p.o) once daily in the morning orally for 28 consecutive days. During these days, all the groups also received fat diet in the same dose as given earlier. The hyperlipidemic control i.e., group II animals received the hyperlipidemic diet and the vehicle. The control group animals received the normal laboratory diet and vehicle.

Experimental design

The experiment was conducted for 28 days, in

which rats n=30 are randomly divided into five groups and fed with modified diet containing 20% ground nut oil, 0.5% cholesterol, 1% cholic acid and followed by treatment with standard, test doses of drugs and periodic blood samplings.

Treatment schedule for Antihyperlipidemic activity

GROUPS	TREATMENT(28DAYS)	PURPOSE
I	Vehicle(Distill water)	To serve as normal animal
II	High fat diet	To serve as control
III	Fenofibate (5mg/kg)	To serve as standard
IV	*Tephrosia purpurea* extract 200 mg/kg + fat diet	To assess the anti hyperlipidemic activity of Tephrosia purpurea
V	*Tephrosia purpurea* extract 400 mg/kg +fat diet	To assess the anti hyperlipidemic activity of Tephrosia purpurea

Collection of blood samples

The blood samples were withdrawn on 28th day from the retrorbital venous plexus of rats without any coagulant for the separation of serum. After collecting the blood in eppendroff tubes kept for 1 hour at room temperature and serum was seperated by centrifugation at 2000 rpm for 15 min and stored analyzed for various bio chemical parameters.

RESULTS

Acute toxicity

Administration of methanolic extract of leaves and stems of Tephrosia purpurea upto 4000 mg/kg body weight did not produce any mortality.

The antihyperlipidemic activity of methanolic extract of *Tephrosia purpurea* was tested at 400mg/kg

Effect on serum total cholesterol

Animals treated with high fat diet (G-II) a significant increase in serum cholesterol was observed on 28th day, when compare to normal animals (G-I). This shows that administration of high fat diet induces hyperlipidemia for the present study. Group-III that received standard drug (Fenofibrate, 5mg/kg) showed a significant decrease of serum cholesterol on 28th day, when compare to high fat diet treated group (G-II).Group IV, V receiving *T.purpurea* extract 400 mg/kg and 200 mg/kg showed a significant reduction in serum cholesterol on 28th day. These observations suggest that *Tephrosia purpurea* had a cholesteol lowering property.

Effect on serum HDL cholesterol

- There was significant decreases in serum HDL cholesterol levels in control group (G-II), on 28th day when compared to normal group (G-I).
- The group-III treated with fenofibrate showed a significant increase in serum HDL cholesterol levels, when compared to control group (G-II). The effect was significant from 28th day onwards.
- The test group (G-IV and V) receiving BMLE in 400 mg/kg and 200 mg/kg also showed a significant increase in serum HDL levels when compared with control group (G-II) on 28th day.

Effect on serum LDL cholesterol

It was observed that there was significant increase in serum LDL cholesterol levels in rats treated with high fat diet group (G-II). The group III treated with standard drug fenofibrate 5mg/kg exhibits a reduction in LDL cholesterol levels on 28th day when compared to control group (G-II). Group VI and V receiving BMLE 400 mg/kg and 200 mg/kg showed significant decrease in serum LDL cholesterol levels on 28th day on comparison with control group (G-II).

Effect on serum triglycerides

Control group receiving high fat diet shows a significant increase in serum triglyceride levels on 28th day, when compare to normal group (G-I). The group III treated with standard drug fenofibrate 5 mg/kg had significantly lowered triglyceride levels on 28th day, when compared with control group (G-III). Decrease in serum triglyceride levels was observed in groups IV and V receiving BMLE 400

mg/kg and 200 mg/kg. This decrease was significant on 28th day.

Effect on total proteins

Administration of high fat diet in control group (G-II), a significant decrease in total proteins was observed on 14th day onwards when compared to normal group (G-I). A significant increase in serum total proteins was found in animals treated with fenofibrate from 28th day onwards. Animals treated with BMLE 400 mg/kg and 200 mg/kg showed a significant increase in serum total proteins on 28th day

Effect on body weight

There was a significant increase in body weight of control group (G-II) administered with high fat diet, compared to normal group (G-I).Also it was found that group (III) receiving 5mg/kg of standard drug there was a significant reduction in body weight. Group (G-IV and V) receiving the extract 400 mg/kg and 200 mg/kg, there was significant decrease in body weight compared to control group.

Effect of Methanolic extracts of *T.purpurea* on total cholesterol, HDL, LDL, triglycerides, total proteins results on 28th day

GROUP	TREATMENT	CHOLESTIROL	HDL	LDL	TRIGLYCERIDES	TOTAL PROTEINS
I	**Normal**	237.3±14.64	61.52± 5.47	139.2±14.11	132.7±13.04	8.195±0.97
II	**High fat diet**	380.0±15.06a	28.54±0.90a	306.±15.9a	290.8±14.23a	3.242.±0.67a
III	**Fenofibrate (5 mg/kg)+fat diet**	269.2±13.87d	49.91±2.56b	189.2±11.32d	190.3±17.56d	6.102±0.84c
IV	**Fat diet +TPE (200 mg/kg)**	280.0±14.83d	48.46±3.23b	198.1±16.31d	198.0±13.36c	5.580±0.96c
V	**Fat diet +TPE (400 mg/kg)**	263.2±14.83d	54.58± 7.17c	192.0±13.90d	185.02±16.81d	6.624±0.93c

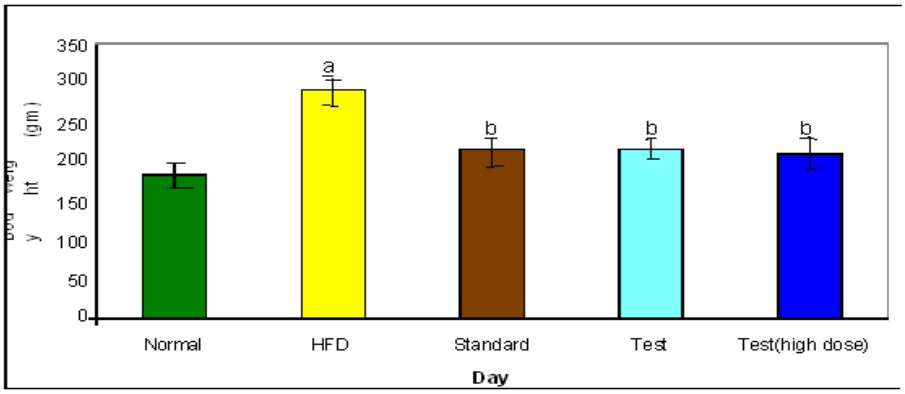
All the values are expressed as Mean±SEM (n=6)
a = p <0.001 When compared to normal (G-I)
b= p <0.01 When compared to control (G-II)
c=p<0.001 When compared to control (G-II)
d=p <0.001 When compared to control (G-II)

Effect of methanolic *T.purpurea* extract on Body weight

GROUPS	TREATMENT	Body Weight	
		0 Day	28th Day
I	**Normal**	180.0±12.71	183.3±14.47
II	**High fat diet**	181.7±16.41	290.0±18.80 a
III	**Fenofibrate (5 mg/kg)+fat diet**	182.0±13.90	213.3±18.51b
IV	**Fat diet +TPE (200Mmg/kg)**	184.8±12.74	216.7±12.81b
V	**TPE (400 mg/kg) +Fat diet**	183.0±14.59	209.2±18.64b

All the values are expressed as Mean ± SEM (n=6)
a= p <0.01 When compared to normal (G-I)
b= p <0.05 When compared to control (G-II)

Effect of methanolic *T.purpurea* extract on Body weight at 28th day



All the values are expressed as Mean ± SEM (n=6)
a= p <0.01 When compared to normal (G-I)
b= p <0.05 When compared to control (G-II)

DISCUSSION

The experimental model selected for the present study is high fat diet induced hyperlipidemia in rats. These animal model of high fat diet in composition of 0.5% cholesterol mimics human hyperlipidemia, inducing fat deposition and damage to the cells of the organs and results in generation of free radicals showing the signs of oxidative stress. In the present study administration of high fat diet to rats causes significant increases in serum cholesterol, LDL, triglycerides on 28th day, with a decrease in protective HDL total proteins. The effect on HDL was significant on 28thday when compared to normal group (G-I). Significant reduction of total cholesterol, LDL, triglycerides levels, with an increase in HDL, total protein levels were found in standard group (G-III) treated with fenofibrate 5 mg/kg. The proposed mechanism of action for the improvement in serum lipid profile is, that fenofibrate is a lipid lowering agent comes under a class of Fibrates. Fibrates act on Peroxidase proliferated activated receptor (PPAR-α).Activation of this receptor leads to lowering of triglycerides, VLDL and increase HDL level which clears the unused cholesterol from blood[12]. It is also found from the present study that administration of *Tephrosia purpurea* extract 200 mg/kg and 400 mg/kg to high fat diet rats of test group (G-IVand V) showed a significant decrease in serum cholesterol. LDL, triglyceride and improvement in HDL, total proteins levels, when compared to control group (G-II). A decrease in body weight of animals treated with 200 mg/kg and 400 mg/kg extract along with fenofibrate 5 mg/kg was found. This reduction in body weight may due to beneficial effects of oleic, linolenic, lenoleic acids on serum lipid profile[13]. The effect could be due to *Tephrosia purpurea* enhancing hypolipidemic action of fibrates. Both polyunsaturated fatty acids and fibrates show similar actions enhancing endothelial nitric oxide synthesis and lower cholesterol levels prevent atherosclerosis, and are benefit in caronary heart diseases. *T.purpurea* extract significantly protects against high fat diet and cholesterol (0.5%) induced oxidative stress in rats. Which was reflected by improved antioxidant parameters like increases in SOD, Catalase, GSH levels and decreased lipid peroxidation (LPO)? The protective effect of *T.purpurea* extract and combination groups may be due to anti-oxidant action of butein of linoleic acid present in *Tephrosia purpurea* leaves[14]. The anti oxidant action depends on its participation in a series of reactions involving radicals[15].

CONCLUSION

In conclusion methanolic extracts of *Tephrosia purpurea* offers significant protection against high fat diet induced hyperlipidemia. The study clearly reveals the antioxidant property of *Tephrosia purpurea* individually and in combination by decreasing lipid peroxidation and enhancing protective antioxidant enzyme levels. *T.purpurea* extract shows synergistic action with fenofibrate.

Histo pathological findings add an additional note for protective effect. Therefore it is a potential therapeutic agent for treating hyperlipidemia and can be further explored for future research studies.

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