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Research Article

**EVALAUTION OF ANTIHYPERLIPIDEMIC ACTIVITY OF
TEPHROSIA PURPUREA PLANT EXTRACTS IN MICE**

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Abstract:

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool. Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of Tephrosia purpurea while only Phenol were present in Phenolic Extract of Tephrosia purpurea. The results of the study clearly indicate that METP Extract and PETP Extract at a dose of 500 mg/kg & 400 mg/kg significantly lowered serum lipid levels ($P < 0.01$). PETP Extract at a dose of 500 mg/kg significantly lowered serum lipid levels, ($P < 0.001$) i.e. antihyperlipidemic activity which was found to be more effective in higher dose of PETP as compared to METP and lower dose of PETP when administered orally in triton induced hyperlipidemic models. The results concluded that PETP (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model and which is equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

Keywords: Tephrosia purpurea, Antihyperlipidemic activity

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INTRODUCTION:

Lipid is the scientific term for fats in the blood. At Normal levels, lipids perform important functions in your body, but can cause health problems if they are present in excess. The term hyperlipidemia means high lipid levels. Hyperlipidemia includes several conditions, but it usually means that you have high cholesterol and high triglyceride levels[1-5].

The pathophysiology of hyperlipidemia can be studied under two headings, i.e., primary hyperlipidemia and secondary hyperlipidemia. The pathophysiology of primary hyperlipidemia involve that the idiopathic hyperchylomicronemia defect in lipid metabolism leads to hypertriglyceridemia and hyperchylomicronemia which is caused by a defect in lipoprotein lipase activity or the absence of the surface apoprotein CII. Moreover, hyperchylomicronemia in cats with autosomal recessive defect in lipoprotein lipase (LPL) activity showed the occurrence of primary hyperlipidemia[6-9].

In secondary hyperlipidemia, the postprandial absorption of chylomicrons from the gastrointestinal tract occurs 30- 60 min after ingestion of a meal containing fat that may increase serum triglycerides for 3-10 hours. The diabetes mellitus patients have been noted to possess low LPL activity which further caused high synthesis of VLDL cholesterol by the liver ultimately leading to hyperlipidemia. Moreover, hypothyroidism-induced low LPL activity and lipolytic activity has been noted to reduce hepatic degradation of cholesterol to bile acids. Furthermore, hyperadrenocorticism increased the synthesis of VLDL by the liver causing both hypercholesterolemia and hypertriglyceridemia.[10-15] Liverdisease hypercholesterolemia has been noted to be caused by reduced excretion of cholesterol in the bile. Furthermore, in nephrotic syndrome, the common synthetic pathway for albumin and cholesterol causes low oncotic pressure ultimately leading to enhanced cholesterol synthesis[15-17].

The mainstay of treatment for hyperlipidemia is dietary and lifestyle modification, followed by drug therapy, as necessary. Hyperlipidemia should not be considered refractory to dietary treatment if the therapeutic regimen included animal products or more than minimal amounts of vegetable oils. Such diets do not lower LDL cholesterol concentrations as effectively as high-fiber, low-fat diets that exclude animal products[18-20].

Regular exercise can improve lipid concentrations. Low to moderate amounts of physical activity such as walking lower triglyceride concentrations by an average of 10 mg/dL, while raising HDL by 5 mg/dL (these numbers are means drawn from large groups). More strenuous activity may have greater effects. Patients with familial hypercholesterolemia typically require medications starting in early childhood. Major complications of hyperlipidemia are atherosclerotic heart disease, heart attack and heart stroke, but atherosclerosis is primary cause of death. Developing countries are reliant on medicinal plants as their main source of treatment for diseases. As *Tephrosia purpurea* have the native habitat the production is more so it is locally available cost effective with no side effects. As *Tephrosia purpurea* is cost effective and beneficiary in metabolism of cholesterol, so it has been taken in to consideration in order "To evaluate Anti-hyperlipidemic activity of Methanolic Extract and Phenolic Extracts of *Tephrosia purpurea* in triton X -100 induced hyperlipidemic mice".

MATERIALS AND METHODS:**Materials**

Atorvastatin, Normal saline, Chloroform, Diethyl ether, Triton X-100, Shimadzu Electronic Balance, U.V spectrophotometer, Ultra homogenizer, Centrifuge RM-12C, Rotary Evaporator, Total cholesterol kits, Triglycerides Kits, HDL-Cholesterol Kits.

Methods**Cold Extraction (Methanol Extraction)**

In this work the cold extraction process was done with the help of methanol. About 200gms of powdered material was taken in a clean, flat bottomed glass container and soaked in 750 ml of methanol. The container with its contents were sealed and kept for period of 7 days accompanied by continuous shaking with the shaker. The whole mixture then went under a coarse filtration by a piece of a clean, white cotton wool.

Evaporation of Solvent

The filtrates (methanol extract) obtained were evaporated using Rotary evaporator in a porcelain dish. They rendered a gummy concentrate of greenish black. The extract was kept in vacuum desiccator for 7 days.

Preliminary Phytochemical Screening

Preliminary phytochemical screening of the *Tephrosia purpurea* extract was carried out for the analysis of Alkaloids, Carbohydrates, Tannins, Saponins, Steroids, Phenols, Flavonoids. as per the standard methods.

Acute toxicity studies

The Acute Toxicity Studies was performed using female rats as per OECD Guideline No.423 (Short term toxicity). Male mice were selected of weight around 50 ± 10 gm for main test. Single animals are dosed in sequence usually at 48 h intervals. A Dose Progression Factor of 3.2 is used. Using the default dose progression factor, doses would be selected from the sequence (1.75, 5.5, 17.5, 55, 175, 550, 1750, and 5000). However, the time intervals between dosing are determined by the onset, duration, and severity of toxic signs. Treatment of an animal at the next dose should be delayed until one is confident of survival of the previously dosed animal. If the animal survives, the second animal receives a higher dose. If the first animal dies or appears moribund, the second animal receives a lower dose. The toxicological effects were observed in terms of mortality expressed as LD50. The number of animals dying or surviving during a period was noted⁴¹.

Method of Induction

The systemic administration of the surfactant Triton X-100 to mice results in a biphasic elevation of plasma cholesterol and triglycerides. Hyperlipidemia was induced in Wistar albino mice by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg/kg) in physiological saline solution after overnight fasting for 18 h.

Experimental Animal Protocol

Experimental mice, starved for 18 hr, were provided water *ad libitum*. The rats were divided in to six groups containing four animals in each group.

Group – I : Normal Control.(Normal saline 10ml/kg orally for 7 days)

Group – II : Hyperlipidemic control, (Triton x 100.)

Group – III : Hyperlipidemic mice treated with METP at dose of 500mg/kg. for 7days

Group – IV : Hyperlipidemic mice treated with PETP at dose of 400mg/kg for 7days.

Group – V : Hyperlipidemic mice treated with PETP at dose of 500mg/kg. for 7days.

Group – VI : Hyperlipidemic mice treated with Atorvastatin at 10 mg/kg for 7days.

All the groups receives single i.p. injection of Triton X-100 at dose of 100mg/kg, simultaneously with Group- II, Group – III, Group – IV, Group – V, Group – VI , except Group – I (Normal control). After 72 hours of Triton X-100 injection. The Group – VI receives Atorvastatin at dose of 10 mg/kg, was prepared by suspending bulk Atorvastatin in aqueous 0.5% methyl cellulose⁴² for 7 days. The Group– III, receive METP, at daily dose of 500mg/kg orally for 7 days and Group – IV, Group – V receives PETP at daily dose of 400mg/kg and 500mg/kg orally for 7 days .

RESULTS AND DISCUSSION:**Preliminary Phytochemical Screening**

Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of *Tephrosia purpurea* while only Phenol were present in Phenolic Extract of *Tephrosia purpurea*

Table 1: Preliminary Phytochemical Screening

Phytochemical	Results
Steroid	+
Alkaloid	+
Tannin	+
Carbohydrate	-
Phenol	+
Flavonoid	+
Saponin	-

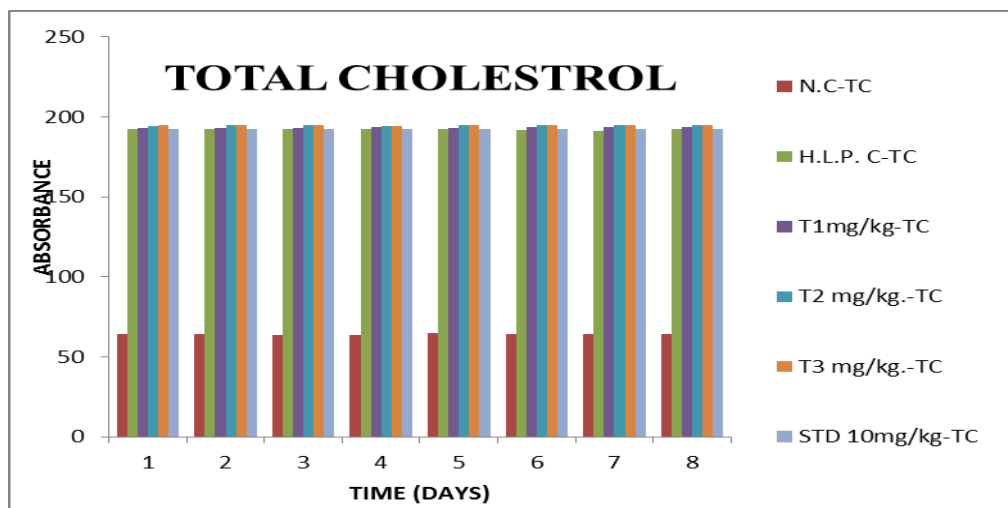
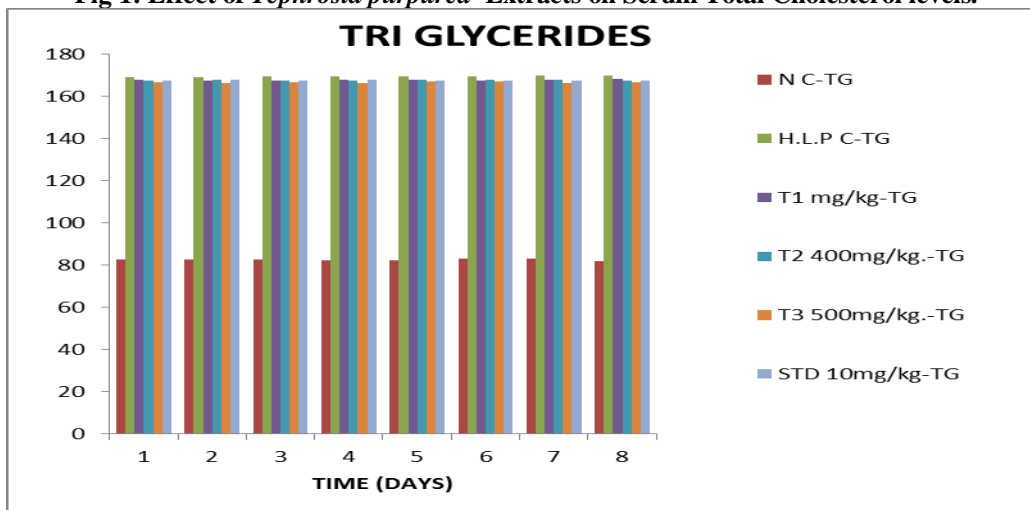
(+) Present.

(-) Absent

Table 2: Lipids Levels Obtained on 8th Day (After Treatment)

SL.NO	GROUPS	TC	TG	HDL	LDL	VLDL
I	Normal Control	64.03 ± 1.45	82.66 ± 2.46	38.91 ± 2.33	8.45 ± 3.43	16.53 ± 0.49
II	Hyperlipidemic Control	192.47 ± 5.05	168.9±5.28	21.86±2.74	136.82±7.00	33.79±1.05
III	METP 500mg/kg	124.19 ± 9.5*	127.7 ± 10.5*	29.1 ± 2.9***	86.8 ± 6.6*	25.5 ± 2.05***
IV	PETP400mg/kg.	120.4 ± 9.4*	109.3 ± 6.6*	30.0 ± 3.3**	70.1 ± 10.5***	22.5 ± 2.3***
V	PETP 500mg/kg.	132.7 ±9.25*	102.5 ±9.2*	33.1 ± 3.1**	56.1 ± 5.9*	28.1 ± 1.4***
VI	Standard Atorvastatin10mg/kg	92.29 ± 5.63*	102.26 ± 7.68*	39.18 ± 3.14**	32.91 ± 7.61*	20.44 ± 1.53**

All the data are expressed as MEAN ± S.D (n=4), *P = < 0.001, **P = < 0.01, ***P = < 0.05. vs GROUP .II TC: Total Cholesterol ; TG: Triglycerides ; HDL-C : High Density Lipoprotein cholesterol ; LDL-C : Low Density Lipoprotein- cholesterol ; VLDL-C : Very Low Density Lipoprotein ; METP: Methanolic Extract of *Tephrosia purpurea* .; PETP: Phenolic Extract of *Tephrosia purpurea*

Fig 1: Effect of *Tephrosia purpurea* Extracts on Serum Total Cholesterol levels.Fig 2: Effect of *Tephrosia purpurea* Extracts on Serum Triglycerides levels.

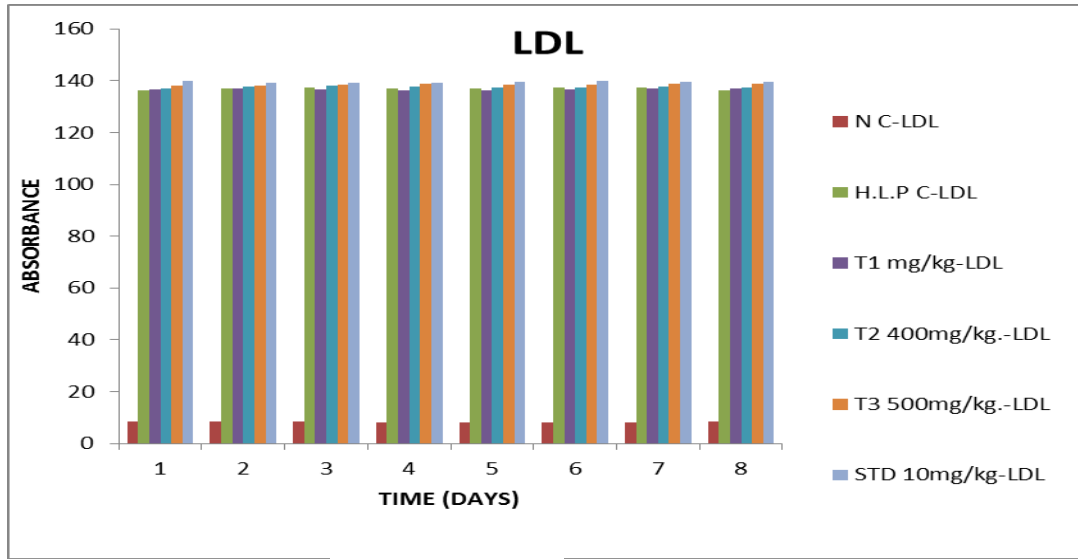


Fig 3: Effect of *Tephrosia purpurea* Extracts on Serum LDL-C levels.

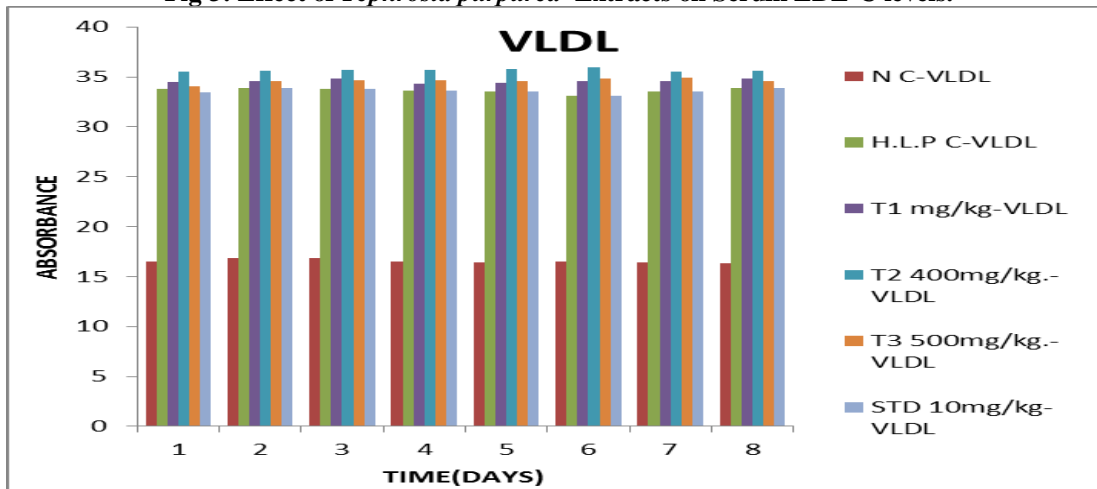


Fig 4: Effect of *Tephrosia purpurea* Extracts on Serum VLDL-C levels.

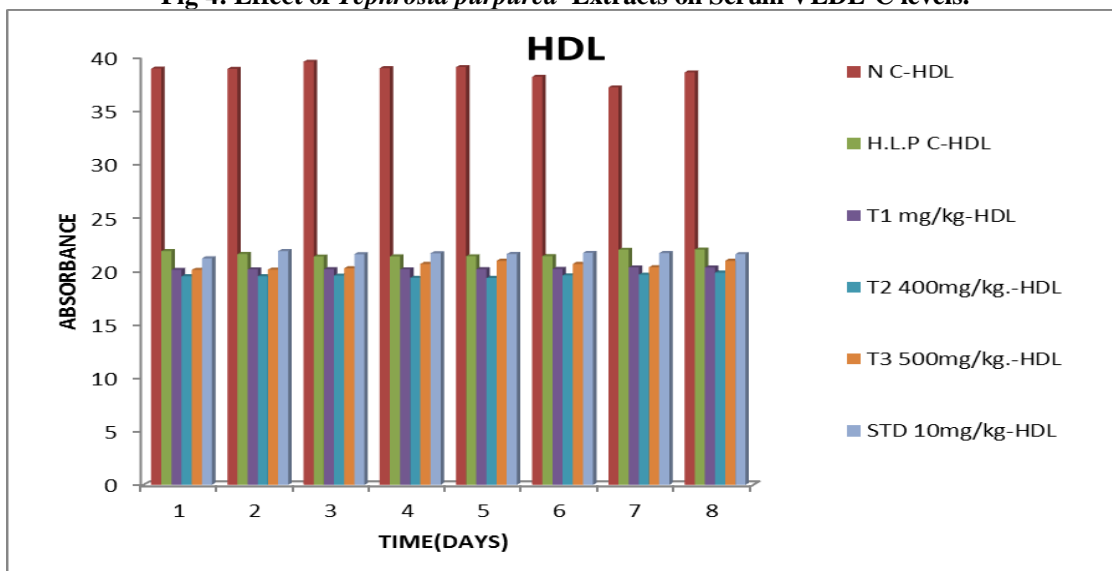


Fig 5: Effect *Tephrosia purpurea* on Serum HDL-C levels.

DISCUSSION:

The present study was designed to investigate the antihyperlipidemic activity of *Tephrosia purpurea* extract in Triton X-100 induced hyperlipidemic mice.

Phytochemical Investigation revealed the presence of Alkaloid, Tannin, Saponin, Phenol in Methanolic Extract of *Tephrosia purpurea* while only Phenol were present in Phenolic Extract of *Tephrosia purpurea* % Yield value of Methanolic Extract from Aerial Parts of *Tephrosia purpurea* was found to be 25.7 % Yield value of Phenolic Extract from Aerial Parts of *Tephrosia purpurea* was found to be 12.6 %.

Administration of Triton-X-100 (100mg/kg) to all the fasted rats caused an elevation of TC, TG, VLDL and LDL and reduction in HDL levels. After 72 hrs of induction of Triton X-100 results in hyperlipidemia which is compared with normal control group .which results in significantly increased serum lipid levels in hyperlipidemic group.

The change in lipid levels in group number III to VI, were comparable with group of Hyperlipidemic control (i.e Triton X-100 ,Group- II) . The Standard group (i.e Atorvastatin group) significantly lowers the serum lipid level (P<0.001).

The results of the study clearly indicate that METP Extract and PETP Extract at a dose of 500 mg/kg & 400 mg/kg significantly lowered serum lipid levels (P<0.01). PETP Extract at a dose of 500 mg/kg significantly lowered serum lipid levels, (P<0.001) i.e. antihyperlipidemic activity which was found to be more effective in higher dose of PETP as compared to METP and lower dose of PETP when administered orally in triton induced hyperlipidemic models.

METP Extract having very low hypolipidemic activity. PETP Extracts showed a dose dependant decrease in the levels of cholesterol, Triglyceride, LDL-C and VLDL-C level. Among three groups (i.e. group number III-V), Group number- V reduced the elevated lipid levels more significantly than the other Groups.(P<0.001)

Flavonoids have exhibited a variety of pharmacological activities, including the antiatherogenesis and antioxidant effect. Thus the present result strongly suggests that the hypolipidemic activity of this medicinal plant could be attributed to the presence of Tannis, Phenols, Flavonoids. in the Extracts.

CONCLUSION:

The results concluded that PETP (500 mg/kg) have definite antihyperlipidemic activity in Triton X-100 induced hyperlipidemic model and which is

equipotent activity when compared with Atorvastatin treated groups. Further studies on this extract may lead to identify the possible mechanism of action and isolation of active principle from the same.

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