JMBFS

Journal of Microbiology, Biotechnology and Food Sciences

International peer-reviewed scientific online journal

Published by Faculty of Biotechnology and Food Sciences

ANTIHYPERLIPIDEMIC EFFECT OF *TEPHROSIA VILLOSA* IN ACUTE AND CHRONIC HYPERLIPIDEMIA RAT MODELS

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https://doi.org/10.15414/jmbfs.4222

ARTICLE INFO

Received 16. 1. 2021
Revised 10. 4. 2021
Accepted 13. 4. 2021
Published 1. 10. 2021

Regular article

OPEN ACCESS

ABSTRACT

Atherosclerosis plays a pivotal role in various cardiovascular disorders. Most of the antiatherogenic currently available drugs are associated with many side effects than plant derived formulations. *Tephrosia villosa* (Fabaceae) plant is being used traditionally, in the treatment of hyperlipidemia, diabetes, jaundice and used as antioxidant and antimicrobial. The objective of the current experiment is to evaluate the antihyperlipidemic potency of chloroform (CETV) and ethanolic extract of *Tephrosia villosa* (EETV) in acute (Triton WR-1339 induced) and chronic (cholesterol diet-induced) hyperlipidemia models. Simvastatin, standard drug used to compare the effect of the both extracts at 125mg/kg, 250mg/kg and 500mg/kg b.w). The serum lipid parameters were analysed using enzymatic kits. The preliminary phytochemical screening of extracts of *Tephrosia villosa* showed the presence of phenols, flavonoids, saponins, coumarins, glycosides and alkaloids. In acute model, the pretreatment with both extracts for 7 days showed significant reduction ($p<0.001$) in serum triglycerides (TG), total cholesterol (TC), very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL-C) levels, and subsequent rise ($p<0.01$) in high-density lipoprotein cholesterol (HDL-C) levels after 24h tritonisation in hyperlipidemic rats. Similarly, in chronic hyperlipidemia model, all the lipid (TG, TC, LDL-C & VLDL-C) levels were reduced significantly ($p<0.001$) with successive rise in HDL-C level after 30 days of study with 15 days of extract treatment. The current study indicates the antihyperlipidemic activity of *Tephrosia villosa* is probably due to the phenolic constituents in extracts, which may inhibit HMG CoA reductase or activate lipoprotein lipase and lecithin acyl transferase (LCAT). The results would provide a larger insight in design and development of newer therapeutics for a wide arena of lifestyle disorders.

Keywords: *Tephrosia villosa*, Triton WR-1339, Cholesterol, Simvastatin, LDL-C, HDL-C, Atherogenic index

INTRODUCTION

Hyperlipidemia is defined as alterations in lipid metabolism characterised by collateral increase in plasma lipids such as phospholipids, total cholesterol, triglycerides, LDL-C and VLDL-C and HDL-C **(Nelson *et al.*, 2013; Kreisberg *et al.*, 2005; Sikarwar *et al.*, 2014).** It is generally classified as primary (Familial) which could be due to genetics, and secondary caused by underlying disorder. Hyperlipidemia provides the well-established associations in causing cardiovascular diseases (CVD), as a result of lipid deposition, calcification and migration of muscle cells to intima layer in arteries **(E Souza *et al.*, 2017)**. It is also believed to increase the death rate by 50% **(Karr *et al.*, 2017)**. Thus, diagnosing and management of hyperlipidemia is necessary.
Statins, fibric acid derivatives, selective cholesterol absorption inhibitors and PSCK 9 (Proprotein Convertase Subtilsin-Kexin type 9) inhibitor are few lipid lowering drugs available in the market for the treatment **(Zodda *et al.*, 2018)**. Although there are many pharmaceutical products available for therapeutic management of hyperlipidemia, many people prefer the nutraceuticals due to their lesser side effects, less expensive and its natural **(Santini *et al.*, 2017)**. This favour the discovery of new lead molecule in treating hyperlipidemia of various aetiology through different regulating mechanism **(Gupta *et al.*, 2018)**. Hence, the current researchers were focusing on new lead components from natural sources.
The *Tephrosia* genus is belongs to the family Leguminosae, a large group of plant consists of more than 400 species worldwide where most of them grown as shrub and weeds. They primarily distributed in Africa, Australia, America and Asia. Moreover, in India 24 species were identified in which *Tephrosia villosa* is one among them. This genus is rich in prenylated flavonoids and traditionally used as antihyperlipidemic, antidiabetic, antimicrobial and antioxidant **(Samuel *et al.*, 2019; Mani *et al.*, 2017; Krishnasamy *et al.*, 2019)**. Several previous studies showed the effect on lipid parameters are due to the presence of polyphenols like flavonoids, triterpenoid, sterol, retinoid and triterpene in various plants **(Hmidani *et al.*, 2020)**. According to Namdeo, most of the marketed products are directly or indirectly derived from the plant derived products called Green medicine, is believed to be safe and trustworthy. Hence the curiosity about the plant derived medicine has been special interest for researcher and for many pharmaceutical companies who are concentrating on substantial research on plants products for their medicinal value **(Kumar *et al.*, 2016)**.

MATERIALS AND METHODS

Collection of plant material

Tephrosia villosa, a perennial under shrub found largely in Kolar district of Karnataka, India, was collected as whole plant. The collected plant material was authenticated by Dr. V. Rama Rao, Research Officer (Scientist-2) Botany, Regional Ayurveda Research Institute for Metabolic Disorders, Bengaluru. The herbarium specimen of the collected plant was conserved for future reference in the department.

Extraction

The entire plant of *Tephrosia villosa* was sliced down into small pieces, shade dried for 45 days and grounded to get moderately coarse powder, which is sieved under mesh. About 1000 g of coarse powder of plant was subjected for extraction process using with chloroform and ethanol at 50° - 60°C for 72 h by using soxhlet apparatus. The chloroform and ethanolic extracts are obtained successively and subsequently concentrated to obtain residue by using vacuum distillation. The extracts obtained was subjected for phytochemical screening.

Preliminary phytochemical studies

Chloroform (CETV) and ethanolic (EETV) extract of *Tephrosia villosa* are subjected for preliminary phytochemical investigation for qualitative

identification to discover phytochemical constituents present in the extract **(Gokhale *et al.*, 2016)**.

Experimental Animals

The male Wistar albino rats (180 - 220 g) were procured from Sri Raghavendra Enterprises, Bangalore. As per standard laboratory conditions, rats were housed in polyacrylic cages for 12 h light/dark cycle at the temperature of 26 ± 2 ℃ and relative humidity 44 –56 %, and standard food and water *ad libitum* throughout the study. About 7 days of acclimatization of animals to laboratory conditions was subjected before the test. Institutional Animal Ethics Committee (IAEC) approval (DSU/Ph.D/IAEC/19/2018-19) was obtained prior to the experiment and studies were performed as per CPCSEA guidelines.

Acute oral toxicity studies

Organization for economic cooperation and development (OECD) guidelines, class method 423 was used to perform acute oral toxicity study for CETV and EETV. The study was carried out to determine the LD_{50} of the extracts in female albino Wistar rats weighing between 180 and 220 g. About 300 mg/kg b.w.*p.o* was taken as initial dose and increased up to 5000 mg/kg b.w. *p.o.* to observe signs of toxicity and mortality **(Guideline, O. E. C. D., 2002)**.

Induction of Hyperlipidemia

Triton WR-1339 (200 mg/kg b.w), used intraperitoneally to induce acute hyperlipidemia on the 7th day of the study **(Ibrahim *et al.*, 2016)**. On the other hand, oral administration of cholesterol diet (cholesterol 400mg/kg + coconut oil as a vehicle, *p.o*) for 30 days was followed to induce chronic hyperlipidemia **(Vijaya *et al.*, 2009)**.

Pharmacological activities

Acute hyperlipidemia model

To observe the short-term effect of CETV and EETV on Triton-induced hyperlipidemia, rats were allotted into nine groups comprising six rats in each group (n=6). Group I served as normal control (NC), which received 1% CMC (vehicle) throughout the study. Group II received Triton WR-1339 (200 mg/kg b.w *i.p*) which served as hyperlipidemic control (HC). Group III (STD) served as standard, received Simvastatin at 10 mg/kg b.w *p.o* dose. **(Hmidani *et al.*, 2020).** Group IV to VI received CETV 125 mg/kg, 250 mg/kg and 500 mg/kg b.w *p.o* respectively. Similarly, group VII to IX received EETV 125 mg/kg, 250 mg/kg and 500 mg/kg b.w *p.o* respectively. All rats were treated with respective drug treatments from day 0 to day 7. On 7th day animals of group II to IX were treated with Triton WR-1339 (200 mg/kg; *i.p*), immediately after drug administration. After 6 and 24 h tritonisation, blood samples were collected from all the rats by retro-orbital puncture which was further processed into serum and used for estimation of lipid parameters **(Dubey *et al.*, 2005)**.

Chronic hyperlipidemia model

Induction of hyperlipidemia in rats was achieved using cholesterol rich diet to analyse the antihyperlipidemic effect of CETV and EETV. The rats were grouped similar to Triton-induced hyperlipidemic model where all animals were fed with cholesterol diet (400mg/kg, *p.o*) except normal control (NC) group (Group I), received normal diet throughout 30 days of the study. Group II served as hyperlipidemic control (HC), which received only cholesterol diet. Group III (STD) rats received Simvastatin (10 mg/kg b.w *p.o*) whereas Group IV to VI received CETV 125 mg/kg, 250 mg/kg and 500 mg/kg b.w *p.o* respectively. Similarly, group VII to IX received EETV 125 mg/kg, 250 mg/kg and 500 mg/kg b.w *p.o* respectively. Animals were gavaged with respective drug treatments from day 16 to day 30. Blood samples were collected on 0th, 15th and 30th from the retro-orbital vein of the rats **(Vijaya *et al.*, 2009; Sampathkumar *et al.*, 2011)**.

Estimation of serum lipid profile

The overnight fasted animals were anesthetized with pentobarbitone (50 mg/kg b.w *i.p*) on last day of the study. The blood samples were collected in the eppendorfs tube, kept in upright position for approximately 10-15 min to facilitate blood clotting. The sample was centrifuged at 3000rpm for 15 min. The separated serum was used for the estimations of TG, TC, HDL-C and LDL-C levels by using enzymatic kits (Erba diagnostics, Germany). The VLDL-C level and Atherogenic index (AI) were determined by equations given below:
VLDL = TG/5, AI = TC/ HDL-C (**Khazaal, 2013)**

Statistical analysis

All the values were specified in Mean ± SEM (Standard Error of Mean). GraphPad instat (GraphPad Software Inc.) was used to find the statistical differences ($p< 0.05$) between the groups using one-way ANOVA and followed by Tukey multiple comparison test.

RESULTS

Preliminary phytochemical studies of *Tephrosia villosa* extracts

The phytoconstituents present in the CETV and EETV was obtained after phytochemical evaluation. On phytochemical screening the presence of phenols, flavonoids, saponins, glycosides, alkaloids, fixed oils and fats were revealed.

Table 1. Phytochemical investigation of extracts of *Tephrosia villosa*

S.No	Chemical constituents	Chloroform (CETV)	Ethanol (EETV)
1.	Alkaloids	+	+
2.	Carbohydrates	-	-
3.	Glycosides	+	+
4.	Saponins	+	+
5.	Phytosterols	-	-
6.	Fats and oils	+	+
7.	Resins	-	-
8.	Flavonoids	+	+
9.	Proteins	-	-
10.	Steroids	-	-
11.	Phenolic compounds	+	+
12.	Tannins	-	-

+ Present, - Absent, CETV: Chloroform extract of *T.villosa*, EETV: Ethanolic extract of *T.villosa*

Acute oral toxicity studies of *Tephrosia villosa* extracts

During the period of observation (14 days) no mortality was exhibited up to 5000 mg/kg b.w. dose and also there was no gross distinct effect on general motor activity, feeding behaviour, muscular weakness, faecal output etc seen by both CETV and EETV extracts. Thus, the dose for further pharmacological study was selected as 125 mg/kg, 250 mg/kg and 500 mg/kg b.w *p.o* for both the extracts.

Effect of *Tephrosia villosa* extracts on lipid profile
Acute hyperlipidemia model

As anticipated, after 6 h and 24 h of administration (*i.p*) of Triton in hyperlipidemic control (HC) group resulted in significant ($p< 0.001$) increase in TC, TG, VLDL-C and LDL-C levels, and marked decrease in HDL-C level, related to normal control (NC) group. In case of pretreatment with CETV and EETV at 3 different doses (125 mg/kg, 250 mg/kg and 500 mg/kg b.w) exhibited dose-dependent effect on serum lipid parameters. Both CETV and EETV had showed highly significant ($p< 0.001$) suppression of LDL-C, VLDL-C, TC and TG levels and marked increase ($p< 0.01$) in HDL-C level after 24 h tritonisation at high dose (500 mg/kg b.w), whereas moderated changes in serum lipid parameters at 250 mg/kg b.w dose and no significant effect at 125 mg/kg b.w dose. But the results obtained after 6 h tritonisation for both extracts showed moderate changes in lipid parameters only at 500 mg/kg b.w dose, and no significant changes with other two doses. On the other hand, these findings were compared with the standard drug Simvastatin which showed a remarkable reduction ($p< 0.001$) in serum lipid parameter expect HDL-C in group III (STD). All the above said results were shown in Table 2 and Figure 1.

Table 2 Effect of chloroform and ethanolic extract of *T. villosa* on lipid profile in hyperlipidemic rats of acute model

Groups	TG mg/dL		TC mg/dL		LDL-C mg/dL		HDL-C mg/dL	
	6 h	24 h	6 h	24 h	6 h	24 h	6 h	24 h
NC	81.16±4.82	82.23±3.09	60.32±2.21	61.18±2.92	20.15± 1.47	21.49± 1.95	34.48± 2.26	36.21± 3.86
HC	188.36±5.17$^{***(a)}$	308.16±6.06$^{***(a)}$	170.96±4.16$^{***(a)}$	281.64±4.37$^{***(a)}$	94.06±3.49$^{***(a)}$	165.15±4.32$^{***(a)}$	24.16±1.22$^{**(a)}$	22.16± 1.26$^{***(a)}$
STD	151.19±4.25$^{***(b)}$	201.47±3.27$^{***(b)}$	142.96±3.46 $^{***(b)}$	198.78±5.86 $^{***(b)}$	59.12±3.41$^{***(b)}$	127.03±5.02$^{***(b)}$	33.18± 2.33$^{*(b)}$	38.48± 1.31$^{***(b)}$
CETV (125 mg/kg)	174.19±4.17^{ns}	280.62± 5.16$^{*(b)}$	154.25±5.61 ^{ns}	263.29±5.14^{ns}	89.41± 4.39 ^{ns}	154.61± 4.18 ^{ns}	26.85± 2.17^{ns}	21.98± 2.36 ^{ns}
CETV (250 mg/kg)	178.41±2.47^{ns}	271.62± 5.42$^{**(b)}$	166.17±4.17^{ns}	234.15±6.93$^{***(b)}$	96.64± 4.03 ^{ns}	142.46± 3.67$^{*(b)}$	25.05± 1.09^{ns}	29.19± 0.84$^{*(b)}$
CETV (500 mg/kg)	156.74±2.41$^{***(b)}$	216.81±1.96$^{***(b)}$	151.25±3.28$^{**(b)}$	209.54±3.06$^{***(b)}$	80.26± 2.03$^{*(b)}$	108.43±4.12$^{***(b)}$	29.14± 1.39^{ns}	32.12± 1.21$^{**(b)}$
EETV (125 mg/kg)	180.11±5.35^{ns}	289.22±5.43 ^{ns}	158.57±6.15 ^{ns}	267.91±4.48^{ns}	92.18± 4.19 ^{ns}	160.28± 4.87 ^{ns}	30.11± 2.03^{ns}	24.38± 2.06 ^{ns}
EETV (250 mg/kg)	172.83±3.24$^{*(b)}$	279.32±4.57$^{**(b)}$	169.26±5.08^{ns}	251.27±4.11$^{***(b)}$	93.58± 3.08 ^{ns}	154.46± 2.67^{ns}	24.05± 1.09^{ns}	28.24± 1.41$^{*(b)}$
EETV (500 mg/kg)	169.74±2.97$^{**(b)}$	238.97±4.43$^{***(b)}$	160.33±4.18^{ns}	221.16±6.46$^{***(b)}$	95.62± 3.75 ^{ns}	120.54±4.56$^{***(b)}$	28.65± 1.03^{ns}	30.57± 2.11$^{**(b)}$

Values are expressed as Mean ± SEM;
[a] Values were against normal control (NC).
[b] Values were against hyperlipidemic control (HC).
Values are statistically significant at ***p< 0.001, **p< 0.01, * p< 0.05 and ^{ns} nonsignificant.
NC: Normal control, HC: Hyperlipidemic control, STD: Standard control (Simvastatin), CETV: Chloroform extract of *T.villosa*, EETV: Ethanolic extract of *T.villosa*, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

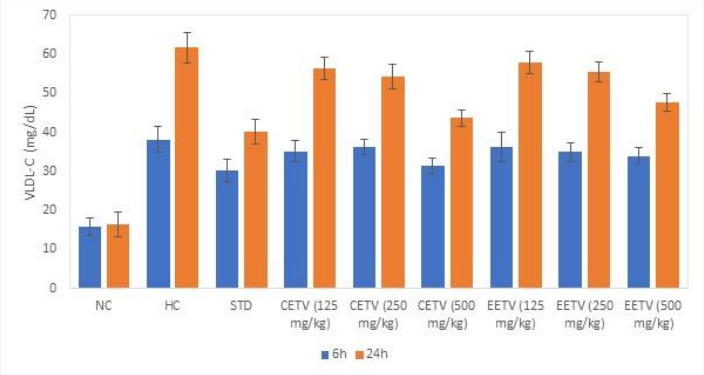

Figure 1 Effect of chloroform and ethanolic extract of *T. villosa* on VLDL-C level in hyperlipidemic rats of acute model.

Furthermore, atherogenic index (AI) was evaluated and showed that there is an increase in AI of hyperlipidemic control (HC) group in contrast to normal control (NC) group. But there was a dose dependent reduction (125 mg/kg, 250 mg/kg and 500 mg/kg b.w) in AI of both CETV and EETV treated groups. The changes in AI both in all the groups were expressed in Figure 2.

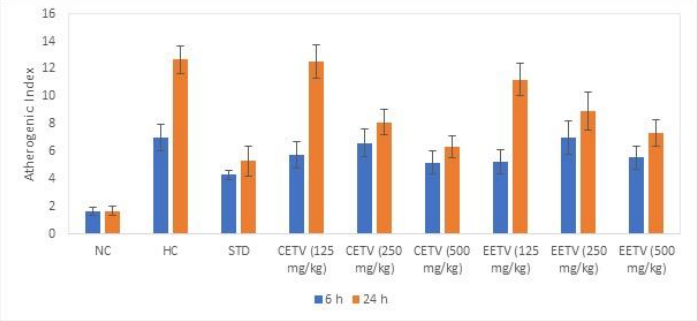

Figure 2 Effect of chloroform and ethanolic extract of *T. villosa* on Atherogenic index (AI) in hyperlipidemic rats of acute model.

Chronic hyperlipidemia model

In this model, all rats from group II to IX were fed with diet rich in cholesterol for 30 days. The serum lipid parameters analysed in all group of rats on day 0, 15 and 30 were showed in Table 3 and Figure 3. The results show that, on 15th day of serum analysis there is a highly significant (p< 0.001) rise in TC, VLDL-C, LDL-C TG levels and alleviation in HDL-C levels, in all the groups compared to NC group. After treating the rats with CETV and EETV at 3 different doses (125 mg/kg, 250 mg/kg and 500 mg/kg b.w) exhibited remarkable effect on lipid parameters in dose dependent manner. On 30th day, treatment groups at the dose of 250mg/kg and 500mg/kg b.w of both extracts exhibited significant decrease (p< 0.001) in the serum TG, TC, LDL-C and VLDL-C along with slightly elevation (p< 0.01 in CETV and p< 0.05 in EETV) in HDL levels. The results obtained where compared with Simvastatin (Standard) which showed significant modifications (p< 0.001) in the serum lipid parameters in treated group (STD).

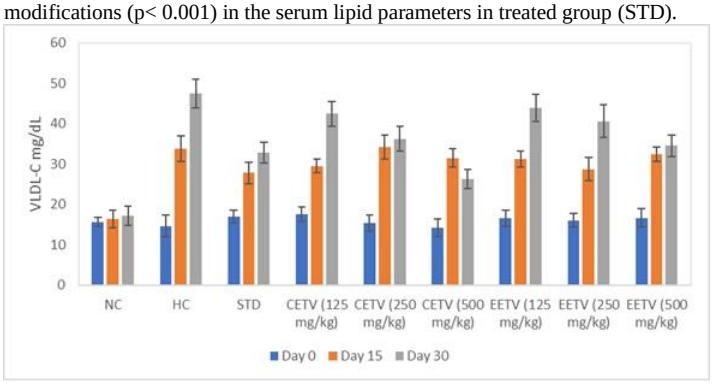

Figure 3 Effect of chloroform and ethanolic extract of *T. villosa* on VLDL-C level in hyperlipidemic rats of chronic model.

Similar to Triton model, Atherogenic index (AI) value were significantly increased in HC group in contrast to NC group. The group which received the standard drug observed highly significant dip in AI value and the treatment group (Group IV to IX) which received both CETV and EETV extracts (125mg/kg, 250mg/kg and 500mg/kg) respectively exhibited dose dependent decrease in the atherogenic index, is depicted in Figure 4.

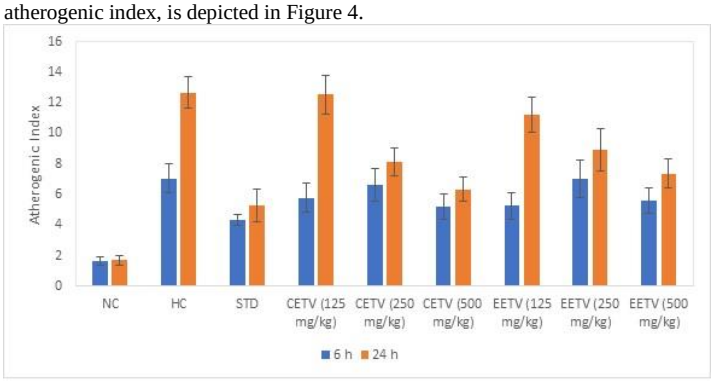

Figure 4 Effect of chloroform and ethanolic extract of *T. villosa* on Atherogenic index (AI) in hyperlipidemic rats of chronic model.

Table 3 Effect of chloroform and ethanolic extract of *T. villosa* on lipid profile in hyperlipidemic rats of chronic model

Groups	TG mg/dL			TC mg/dL			LDL mg/dL			HDL mg/dL		
	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30	Day 0	Day 15	Day 30
NC	78.21 ± 2.87	82.18 ± 3.14	85.51 ± 4.07	95.25 ± 2.96	97.15 ± 3.09	92.48 ± 3.84	44.13 ± 2.24	38.1 ± 1.98	47.34 ± 3.02	40.46 ±2.43	42.46 ± 2.09	41.29 ± 3.27
HC	72.65 ± 3.54	164.35 ± $3.62^{***(a)}$	236.13 ± $6.42^{***(a)}$	86.31 ± 3.67^{ns}	151.21 ± $3.89^{***(a)}$	210.28 ± $5.27^{***(a)}$	40.78 ± 2.23	79.23 ± $2.45^{***(a)}$	139.21 ± $4.35^{***(a)}$	38.12 ±2.86	28.91 ± $2.98^{***(a)}$	22.95 ± $3.23^{***(a)}$
STD	84.86 ± 3.11	139.34 ± $3.21^{***(a)}$	163.91 ± $5.61^{***(b)}$	92.98 ± 2.56^{ns}	147.67 ± $4.15^{***(a)}$	101.74 ± $3.04^{***(b)}$	36.16 ± 2.46	76.09 ± $2.34^{***(a)}$	68.46 ± $3.16^{***(b)}$	41.35 ±1.75	37.32 ± 1.21^{ns}	43.41 ± $2.96^{***(b)}$
CETV (125 mg/kg)	87.68 ± 2.34	149.41 ± $2.19^{***(a)}$	212.22 ± 7.51^{ns}	79.81 ± 2.29^{ns}	155.65 ± $3.98^{***(a)}$	182.58 ± $4.12^{**(b)}$	35.81 ± 2.08	84.73 ± $3.67^{***(a)}$	126.51 ± 4.25^{ns}	35.19 ±2.98	29.12 ± $2.23^{***(a)}$	28.76 ± 3.08^{ns}
CETV (250 mg/kg)	76.52 ± 3.19^{ns}	174.34 ± $4.61^{***(a)}$	180.06 ± $6.25^{***(b)}$	89.48 ± 2.36^{ns}	149.23 ± $2.43^{***(a)}$	156.35 ± $3.58^{***(b)}$	38.18 ± 3.24	79.92 ± $3.26^{***(a)}$	110.71 ± $4.64^{***(b)}$	42.46 ± 2.32	36.75 ± $1.30^{*(a)}$	38.54 ± $2.89^{**(b)}$
CETV (500 mg/kg)	72.86 ± 2.24^{ns}	159.01 ± $3.69^{***(a)}$	132.25 ± $4.68^{***(b)}$	84.21 ± 1.91^{ns}	160.31 ± $3.14^{***(a)}$	137.52 ± $2.96^{***(b)}$	42.12 ± 1.84	82.12 ± $4.34^{***(a)}$	89.69 ± $3.51^{***(b)}$	44.54 ±3.11	31.45 ± $1.55^{***(a)}$	40.25 ± $3.13^{**(b)}$
EETV (125 mg/kg)	82.82 ± 3.14^{ns}	156.32 ± $3.45^{***(a)}$	219.46 ± 5.28^{ns}	94.28 ± 3.14^{ns}	140.83 ± $2.34^{***(a)}$	190.84 ± 4.12^{ns}	45.88 ± 3.12	90.08 ± $3.32^{***(a)}$	130.56 ± 4.12^{ns}	39.55 ± 1.30	25.19 ± $2.37^{***(a)}$	32.26 ± 2.79^{ns}
EETV (250 mg/kg)	80.63 ± 3.28^{ns}	145.18 ± $3.18^{***(a)}$	203.21 ± $6.41^{**(b)}$	95.21 ± 3.71^{ns}	165.28 ± $4.04^{***(a)}$	182.64 ± $4.90^{**(b)}$	39.16 ± 2.28	87.86 ± $3.14^{***(a)}$	122.41 ± $3.95^{*(b)}$	43.06 ±2.33	33.64 ± $1.19^{**(a)}$	35.41 ± $2.43^{*(b)}$
EETV (500 mg/kg)	83.14 ± 2.68	162.54 ± $2.76^{***(a)}$	170.55 ± $4.97^{***(b)}$	90.29 ± 2.82^{ns}	159.48 ± $2.34^{***(a)}$	165.38 ± $5.74^{***(b)}$	38.85 ± 2.24^{ns}	72.92 ± $2.87^{***(a)}$	106.54 ± $3.23^{***(b)}$	37.46 ±1.48	25.28 ± $1.64^{***(a)}$	34.32 ± $2.05^{*(b)}$

Values are expressed as Mean ± SEM;
[a] Values were against normal control (NC).
[b] Values were against hyperlipidemic control (HC)
Values are statistically significant at ***p< 0.001, **p< 0.01 * p< 0.05 and [ns] nonsignificant.
NC: Normal control, HC: Hyperlipidemic control, STD: Standard control (Simvastatin), CETV: Chloroform extract of *T.villosa*, EETV: Ethanolic extract of *T.villosa*, TC: Total cholesterol, TG: Triglycerides, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol

DISCUSSION

Hyperlipidemia is termed as disorder of lipid metabolism characterised by abnormal rise of serum lipid parameter such as TG, TC, LDL-C and VLDL-C, which are accompanied by atherosclerosis and many other CVD **(Sikarwar *et al.*, 2014; Khazaal, 2013)**. Increase in the mortality due to vascular disease and atherosclerosis is a major concern. Thus, prevention and control of hyperlipidemia is prerequisite to reduce complications **(Touiss *et al.*, 2019)**. However, there are many treatment options readily available to treat hyperlipidemia but herbal medicines are extensively used, due to their lesser side effects.

The present study was aimed to demonstrate the antihyperlipidemic effect of *Tephrosia villosa* using triton WR-1339 and high cholesterol diet model. Triton WR-1339**,** an anionic surfactant increases TC, VLDL-C, LDL-C and TG levels. The underlying mechanism would be by inhibiting lipoprotein lipase enzyme (LPL) and by blocking the triacyl glycerol rich lipoproteins from peripheral tissues **(Gupta *et al.*, 2018)**. Inhibition of LPL enzyme leads to hydrolysis of TGs present in plasma as in the form of chylomicron and VLDL-C lipoproteins. On top of it, 3-hydroxy-3-methyl-glutaryl CoA reductase (HMG-CoA reductase) and hepatic synthesis of cholesterol were induced by triton **(E Souza *et al.*, 2017)**. In this study, it was observed that there was significant increase in TC, VLDL-C, LDL-C and TG and declined HDL-C levels after triton WR-1339 administration, whereas CETV and EETV treated rats reversed the triton effect significantly when compared to hyperlipidemic control (HC) rats**.**

Administrations of high fat-diet (HFD)/cholesterol diet causes increase in all serum lipid parameters in rats similar to the diseased condition like in humans. Thus, it is one of the models used to study the hypolipidemic effect of the plant. Upon HFD in rats, it increases the availability of acetyl coenzyme A, a prime substrate for synthesis of cholesterol by the oxidation of fatty acid **(Irudayaraj *et al.*, 2013)**. It is also believed that it modifies the circulation of arginine rich apoprotein which is usually responsible for migration of all lipoproteins in plasma **(Murugaiyah *et al.*, 2018)**. The above proposed mechanism explains the induction of hyperlipidemia in rats. In our study both extracts were able to deplete the TC, VLDL-C, LDL-C and TG levels, critically when hypercholesteremia was induced by cholesterol diet.

The decline of serum cholesterol was accompanied with reduction in the LDL-C fraction. Through hepatic LDL receptors, LDL-C undergo catabolism leading to formation of bile salts and secreted into the bile**.** HDL-C is a "cardio protective lipid" and is believed that increase in HDL-C levels reduced the risk of atherosclerosis through reverse cholesterol transport (RCT). Many studies suggested that that HDL-C mediated RCT occurs by three process: it translocates the cholesterol from peripheral cell to circulating lipoprotein in to the liver for catabolism and excretion, mobilization of cellular cholesterol results in formation of HDL-C particles and to the liver it transports the HDL-C derived cholesterol **(Millar *et al.*, 2017)**. Presence of flavonoids and polyphenols in *Tephrosia villosa* leads to increase the HDL-C levels due to potentiation of lecithin acyl transferase (LCAT) activity and constraining the HMG CoA reductase activity**.** LCAT plays a crucial part in the incorporation of free cholesterol into HDL-C (this may elevate HDL-C) and moving it back later in liver cells, while inhibition of HMG-CoA reductase hinders the cholesterol synthesis **(Zeka *et al.*, 2017)**. The above two mechanism plays a synergistic activity in increasing the HDL-C level, lowering of which is associated with ischemic heart disease.

In addition to the lowering of serum lipid profile, CETV and EETV showed effect on atherogenic index (AI). AI is complex made up of TG and HDL-C gives correlation between atherogenic and protective lipids and a strong predictor of the CAD and atherosclerosis. Reductions in the AI is a positive physiological effect because it is believed that high plasma HDL-C levels and low LDL-C levels is desired to prevent the atherogenesis **(Oršolić *et al.*, 2019; Niroumand *et al.*, 2015)**. During the study both extracts showed significant decrease in the AI thus maintained the LDL-C/HDL-C ratio which explains the action of lipid lowering activity of extracts.

In the present study both extracts CETV and EETV elucidated the antihyperlipidemic activity by reducing the serum cholesterol. It significantly suppressed TG, TC, LDL-C and VLDL-C levels and induce high serum concentration of HDL-C. Thus, suggest the partial restoration of catabolism of TGs, underlying mechanism is not explained by this study. The preliminary phytochemical study report provides the additional support for *Tephrosia villosa* being used traditionally for treating hyperlipidemia. Earlier studies on *Tephrosia villosa* established the presence of various flavonoids, triterpenoid (Lupenone), triterpene (Lupeol) and sterol (Stigmasterol). Hence, the above mentioned phytoconstituents were proven for its anti-hypercholesterolemic property by earlier studies **(Sudhahar *et al.*, 2006; Chandler *et al.*, 1979; Madhusudhana *et al.*, 2010)**. Nevertheless, hypothesized by other studies that it may be due to stimulation of lipoprotein lipase (LPL) and presence of phenolic compounds **(Gopichandchinta *et al.*,2009)**.

Based on various studies, the extraction of polyphenols and supplementary phytoconstituents will be influenced by their diverse structures especially in phenolic compounds. The Polyphenols have better solubility in more polar solvents in nature **(Aires *et al.*, 2017)**. Hence, in this study both the extracts showed good antihyperlipidemic activity but chloroform extract showed slightly better than ethanolic extract.

Study limitation

The future perspective of the study would be isolation and characterisation of the compounds from *Tephrosia villosa* extracts to explain the mechanism of hypolipidemic activity and using the extracts of different parts of plants can be done to isolate more precise compounds for their potent activity.

CONCLUSION

In conclusion to the results obtained both chloroform and ethanolic extract of *Tephrosia villosa* was able to mitigate the levels of TG, TC, VLDC-C and LDC-C, and contribute to rise the HDL-C levels in Triton WR-1339 induced and Cholesterol diet induced hyperlipidemic rats. This effect may be due to their influence in some mechanisms like inhibition of HMG CoA reductase, activation of lipoprotein lipase and LCAT. Thus, it can be considered as antihyperlipidemic agent in the treating hyperlipidemia.

Acknowledgement: The authors urge to convey their gratitude to the Management of Dayananda Sagar University, Bengaluru for providing facilities and necessary support for pursuing the research work.

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