INTRODUCTION

Hyperlipidemia is defined as alterations in lipid metabolism characterised by a 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, diagnosis and management of hyperlipidemia is necessary.

Statins, fibrice acid derivatives, selective cholesterol absorption inhibitors and PSC9K (Proprotein Convertase Subtilisin-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 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, saponins, coumarins, glycosides and alkaloids (Suresh and Kumar, 2019; Suresh 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 °C 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 LD₅₀ 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. (Himadi 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 (Erla 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 phytocomponents 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>
<thead>
<tr>
<th>Chemical constituents</th>
<th>Chloroform (CETV)</th>
<th>Ethanol (EETV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Alkaloids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2. Carbohydrates</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3. Glycosides</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4. Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5. Phytosterols</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6. Fats and oils</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7. Resins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8. Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9. Proteins</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>10. Steroids</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>11. Phenolic compounds</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>12. Tannins</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Chronic 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. The results were obtained 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

<table>
<thead>
<tr>
<th>Groups</th>
<th>TG mg/dL</th>
<th>TC mg/dL</th>
<th>LDL-C mg/dL</th>
<th>HDL-C mg/dL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6 h</td>
<td>24 h</td>
<td>6 h</td>
<td>24 h</td>
</tr>
<tr>
<td>NC</td>
<td>81.16±5.82</td>
<td>82.23±3.09</td>
<td>60.32±2.21</td>
<td>61.18±2.92</td>
</tr>
<tr>
<td>HC</td>
<td>188.36±5.17</td>
<td>308.16±6.06</td>
<td>170.96±4.16</td>
<td>281.64±4.37</td>
</tr>
<tr>
<td>STD</td>
<td>151.94±2.25</td>
<td>201.47±3.27</td>
<td>142.96±3.46</td>
<td>198.78±5.86</td>
</tr>
<tr>
<td>CETV (125 mg/kg)</td>
<td>174.19±4.17</td>
<td>280.62±5.16</td>
<td>154.25±5.61</td>
<td>263.29±5.14</td>
</tr>
<tr>
<td>CETV (250 mg/kg)</td>
<td>178.41±2.47</td>
<td>271.62±5.42</td>
<td>166.17±4.17</td>
<td>234.15±6.93</td>
</tr>
<tr>
<td>CETV (500 mg/kg)</td>
<td>156.74±2.41</td>
<td>216.81±1.96</td>
<td>151.25±3.28</td>
<td>209.54±3.06</td>
</tr>
<tr>
<td>EETV (125 mg/kg)</td>
<td>180.11±5.35</td>
<td>289.22±5.43</td>
<td>158.57±6.15</td>
<td>267.91±4.48</td>
</tr>
<tr>
<td>EETV (250 mg/kg)</td>
<td>172.83±3.24</td>
<td>279.32±4.57</td>
<td>169.26±5.08</td>
<td>251.27±4.11</td>
</tr>
<tr>
<td>EETV (500 mg/kg)</td>
<td>169.74±2.97</td>
<td>238.97±4.43</td>
<td>160.33±4.18</td>
<td>221.16±6.46</td>
</tr>
</tbody>
</table>

Values are expressed as Mean ± SEM; *Values were against normal control (NC). **Values were against hyperlipidemic control (HC). Values are statistically significant at ***p< 0.001, **p< 0.01, * p< 0.05 and "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-LC 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< 0.01) 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.
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 (Toissu et al., 2019). However, there are many treatment options readily available to treat hyperlipidemia but traditional 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 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. 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 (Orsoli 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 lipoprotein 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 the TC, TG, 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 (Lupene) and steroid (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, hypothesised 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, VLDLC-C and LDL-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 HMGC-CoA reductase, activation of lipoprotein lipase and LCAT. Thus, it can be considered as antihyperlipidemic agent in the treating hyperlipidemia.
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REFERENCES


