Hypolipidemic and antioxidant potential of hydroalcoholic leaf extract of *Talinum Portulacifolium* against Triton WR-1339 induced dyslipidemia in experimental animals

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**ABSTRACT**

*Talinum portulacifolium* which is known as water plant is traditionally used for its various properties and hence in present study hydroalcoholic extract of plant leaves has been screened for its hypolipidemic activity. Hyperlipidemia is a highly prognostic risk factor for atherosclerosis, coronary artery diseases and cerebrovascular diseases. Triton WR-1339, a non ionic surfactant has been widely used to produce acute dyslipidemia in animal models in order to screen hypolipidemic activity of the extract and serum levels of various biochemical parameters such as total cholesterol, triglycerides, LDL, VLDL and HDL cholesterol and atherogenic index were determined. The animals are grouped into normal, Triton treated group, Triton + Standard, Triton + herb extract (200mg/kg) and Triton + herb extract (400mg/kg). The plant extract showed significant hypolipidemic effect by lowering all biochemical parameters and increases HDL cholesterol levels. These biochemical observations are also confirmed by Histopathological examination of liver. The plant extract also showed good antioxidant potential by increasing SOD, CAT, GSH and decreasing lipid peroxidation.

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INTRODUCTION

Experimental and epidemiological studies shown that the plasma hypercholesteremic state could contribute to the development of atherosclerosis and related cardiovascular diseases which are the most common cause of death in both western and eastern societies (Nilsson PM et al., 2014). Triton WR-1339, a non ionic detergent (oxyethylated tertiary octyl phenol formaldehyde polymer) has been generally used to produce acute dyslipidemia in animal models in order to screen natural or chemical drugs (Schurr PE et al., 1972), and to study cholesterol and triglyceride metabolism (Ghatak SB et al., 2012). The accumulation of plasma lipids by this detergent appears to be especially due to inhibition of lipoprotein lipase activity (Scharwey M et al., 2013). Hyperlipidemia is a highly predictive risk factor for atherosclerosis, coronary artery diseases (Elhissi JH et al., 2014). Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular diseases (Saravanan R et al., 2003).

Atherosclerosis of arteries is a generalized disease of arterial network known as progressive and silent killer disease characterized by formation of lesions called atherosclerotic plagues in the walls of large and medium sized coronary arteries and reduces blood flow to myocardium called coronary
artery disease (Mohale DS et al., 2008). Medicinal plants play a major role in hyperlipidemic activity, literature suggests that the lipid lowering action is mediated through, inhibition of hepatic cholesterol biosynthesis and reduction of lipid absorption in the intestines (Gramza A et al., 2005).

The aim of this study was to evaluate the hypolipidemic and antioxidant activities of hydroalcoholic extract of Talinum portulacifolium leaves related to both human health condition and interest in the development of new drugs. Talinum portulacifolium leaves are used to treat polyuria, internal heat, measles, GIT ailments, hepatoprotection and also for hypoglycemic activity. This leaves are frequently used for cooking purpose also.

MATERIALS AND METHODS

Plant material

The plant material was collected at S.V. Government Polytechnic, Tirupati during rainy season and the same was authenticated by Department of Dravya Guna, S. V. Ayurvedic College, Tirupati.

Preparation of plant extract

The fresh leaves of Talinum portulacifolium were shade dried. The coarse power was subjected to cold maceration process using hydro alcohol as solvent (70:30 Water: Ethanol) for 72 hrs. The filtrate is concentrated; residue was reconstituted in distilled water and stored in refrigerator.

Animals

Healthy Sprague Dawley rats, weighing 150-200g were housed at 22±1°C under 12 hrs light-dark cycle. Animals were allowed free access to food and water. The experimental protocol of the present study was approved by Institutional animal Ethical Committee (IAEC No: 1016/A/CPCSEA/ 014/2013) and the experiments were carried out as per CPCSEA guidelines.

Acute toxicity studies

According to OECD-423 guidelines acute oral toxicity was performed. No toxicity was produced in different animals which were fed with doses of 50, 300, 1000 and 2000 mg/kg and observed continuously for 24 hrs to detect behavioral, neurological and autonomic parameters.

Induction of Hyperlipidemia by Triton

A non ionic surfactant triton WR-1339 induced Hyperlipidemia within 24hrs by the dose of 200mg/kg B.W. intraperitoneally diluted in normal saline (Khanna AK et al., 2002).

Experimental design

Experimental schedule was conducted for 8 days. 30 Rats were divided into 5 groups each consisting of 6 animals. The first group received only the vehicle i.e. normal saline. Second group received vehicle up to 7 days +Triton WR-1339 (200mg/kg B.W) dissolved in normal saline on 8th day. Third group was given Rosuvastatin (1mg/kg B.W) for 7 days and Triton WR-1339 (200mg/kg B.W) on 8th day. Fourth group was given HATP (200mg/kg B.W) in normal saline p.o for 7 days + Triton on 8th day. Fifth group was given HATP (400mg/kg B.W) P.O for 7 days and Triton on 8th day. Blood samples were withdrawn from the retro orbital venous plexus of rats for separation of serum at the end of treatment schedule. The serum samples were analyzed for biochemical estimation of lipid parameters.

Instruments used

Analytical UV-Visible Spectrophotometer, Electric balance, Cooling centrifuge, Homogenizer.

Biochemical studies

The lipid profiles such as total serum cholesterol, TG, HDL cholesterol, LDL, VLDL, Atherogenic index, LDL/HDL ratio were studied. Liver homogenate was tested for SOD, CAT, GSH and lipid peroxidation. On 8th day, at the end of treatment schedule, Histopathological study of liver was also carried out.

Statistical analysis

All results were expressed as mean ± S.E.M and statistically analyzed by one way ANOVA and two way ANOVA followed by Bonferroni test for multiple comparison. All the statistics were performed prism graph pad version 5.0 and statistically significance set at p<0.001.

RESULTS

Preliminary phytochemical screening

Preliminary phytochemical screening of the hydroalcoholic extract of leaves of Talinum portulacifolium was shown the presence of alkaloids, flavonoids, glycosides, carbohydrates, saponins and tannins.

DISCUSSION

Hyperlipidemia causes about 17 million deaths worldwide each year; (WHO, 2011) in addition, it is also a key factor for the development of heart, coronary diseases and atherosclerosis.

Many studies have proved that reactive oxygen species and free radicals play a major role in maintaining human health. When the balance between generating and scavenging of ROS and free radicals in-vivo is destroyed, an oxidative stress would hap-
effects of foods have been linked to the presence of dants from exogenous sources. The beneficial e-
ing an inhibiting lipid peroxidation of preventing the deleterious effects of oxidation of fense mechanism (Bonomini has enzymatic and non enzymatic antioxidant de-
2014). In order to control this imbalance, the body has enzymatic and non enzymatic antioxidant defense mechanism (Bonomini et al., 2008) capable of preventing the deleterious effects of oxidation, inhibiting lipid peroxidation, free radical scavenging and maintaining redox balance in cells. In addition to endogenous antioxidants, there are antioxidants from exogenous sources. The beneficial effects of foods have been linked to the presence of bioactive compounds and other nutrients (Zonotti et al., 2015). Thus, plants have been described as an alternative to the development of new drugs (Calixto JB et al., 2005) applied to treatment of many diseases such as hypercholesterolemia, ulcers, depurative blood and cancer (De Souza PM et al., 2012, De Toledo CEM et al., 2011 and Melo F et al., 2009). The importance of new products in the treatment and prevention of dyslipidemia becomes essential to reduce the mortality and morbidity due to cardiovascular complications. In addition, the search for less toxic drugs have increased the interest of the scientific community for natural products. The present extract showed to manage Hyperlipidemia induced by Triton WR-1339 reducing serum levels of total cholesterol and triglycerides without signs of change in hepatic and renal function, suggesting that extract is safe in the evaluated condition. The hypolipidemic action of natural products can be correlated to the pres-

Table 1: Effect of Talinum portulacifolium on serum lipid parameters (Total cholesterol, Triglycerides, VLDL-C)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Total cholesterol</th>
<th>Triglycerides</th>
<th>VLDL-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>99 ±1.1</td>
<td>110 ±0.48</td>
<td>22 ±0.41</td>
</tr>
<tr>
<td>II</td>
<td>Triton</td>
<td>153 ±2.4###</td>
<td>137 ±0.85###</td>
<td>28 ±0.065###</td>
</tr>
<tr>
<td>III</td>
<td>Rosuvastatin + Triton</td>
<td>96 ±2.2***</td>
<td>128 ±0.65***</td>
<td>22 ±0.065***</td>
</tr>
<tr>
<td>IV</td>
<td>Test drug 200mg/kg</td>
<td>78 ±0.48***</td>
<td>91 ±1.06***</td>
<td>18 ±0.25***</td>
</tr>
<tr>
<td>V</td>
<td>Test drug 400mg/kg</td>
<td>99 ±0.29***</td>
<td>111 ±0.65***</td>
<td>22 ±0.048***</td>
</tr>
</tbody>
</table>

All values shown are mean ± SEM and n=6; ### indicates p<0.001 when compared with Normal groups; *** P<0.001. ** P<0.01, * P<0.05.

Table 2: Effect of Talinum portulacifolium on serum lipid parameters (HDL-C, LDL-C, LDL/HDL Ratio)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>HDL-C</th>
<th>LDL-C</th>
<th>LDL/HDL Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>51 ±1.1</td>
<td>26 ±0.25</td>
<td>0.56 ±0.0014</td>
</tr>
<tr>
<td>II</td>
<td>Triton</td>
<td>24 ±0.85#</td>
<td>108 ±0.48###</td>
<td>4.9 ±0.046###</td>
</tr>
<tr>
<td>III</td>
<td>Rosuvastatin + Triton</td>
<td>49 ±1.3***</td>
<td>39.3 ±0.075***</td>
<td>0.18 ±0.0026***</td>
</tr>
<tr>
<td>IV</td>
<td>Test drug 200mg/kg</td>
<td>46 ±1.0***</td>
<td>17 ±0.028***</td>
<td>0.41 ±0.056***</td>
</tr>
<tr>
<td>V</td>
<td>Test drug 400mg/kg</td>
<td>42 ±0.71***</td>
<td>26 ±0.24***</td>
<td>0.92 ±0.0054***</td>
</tr>
</tbody>
</table>

All values shown are mean ± SEM and n=6; ### indicates p<0.001 when compared with Normal groups; *** P<0.001. ** P<0.01, * P<0.05.

Table 3: Effect of Talinum portulacifolium on serum lipid parameters (Atherogenic index, Total proteins)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>Atherogenic index</th>
<th>Total proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal</td>
<td>1.27 ±0.0068</td>
<td>14.42 ±0.38</td>
</tr>
<tr>
<td>II</td>
<td>Triton</td>
<td>6.20 ±0.013###</td>
<td>5.66 ±0.91##</td>
</tr>
<tr>
<td>III</td>
<td>Rosuvastatin + Triton</td>
<td>0.73 ±0.0054###</td>
<td>10.10 ±1.28**</td>
</tr>
<tr>
<td>IV</td>
<td>Test drug 200mg/kg</td>
<td>0.90 ±0.057###</td>
<td>10.97 ±1.07***</td>
</tr>
<tr>
<td>V</td>
<td>Test drug 400mg/kg</td>
<td>1.45 ±0.0023***</td>
<td>12.06 ±1.16***</td>
</tr>
</tbody>
</table>

All values shown are mean ± SEM and n=6; ### indicates p<0.001 when compared with Normal groups; *** P<0.001. ** P<0.01, * P<0.05.
Table 4: Effect of *Talinum portulacifolium* in liver biochemical parameters (SOD, CAT, GSH, LPO Values)

<table>
<thead>
<tr>
<th>S. No</th>
<th>Groups</th>
<th>IN VIVO ANTIOXIDANT LEVELS</th>
<th>PRO-OXIDANT LEVELS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>SOD (U/mg protein)</td>
<td>Catalase (µM H₂O₂ consumed/mg protein)</td>
</tr>
<tr>
<td>I</td>
<td>Normal</td>
<td>1.791±0.43</td>
<td>4.466±0.734</td>
</tr>
<tr>
<td>II</td>
<td>Triton</td>
<td>0.438±0.157**</td>
<td>1.487±0.219**</td>
</tr>
<tr>
<td>III</td>
<td>Rosuvastatin</td>
<td>1.670±0.030**</td>
<td>3.819±0.795###</td>
</tr>
<tr>
<td>IV</td>
<td>Test 200mg/kg</td>
<td>0.970±0.30**</td>
<td>2.086±0.86**</td>
</tr>
<tr>
<td>V</td>
<td>Test 400mg/kg</td>
<td>1.540±0.201***</td>
<td>3.521±0.13***</td>
</tr>
</tbody>
</table>

Values were expressed as means ± S.E.M of 6; ### indicates P<0.001 when compared with Normal groups; *** P<0.001. ** P<0.01, * P<0.05.

Figure 1: Histophathology of Liver a). Normal Group b). Control Group c). Rosuva Statin Group d). Test 200mg/kg Group e). Test 400mg/kg Group

ence of flavonoids due to their properties of inhibiting cholesterol biosynthesis and absorption and modifying the activity of lipogenic and lipolytic enzymes, leading to reduced lipid metabolism (Borradale NM et al., 2003, Whitman SC et al., 2005, Brusq JM et al., 2006), as observed in hyperlipidemic rats, treated with extract, which showed significant reduction in levels of total cholesterol and triglycerides. Other molecules able to decrease the serum level of cholesterol are saponins (Patel S
et al., 2012), also present in extract. It is very interesting that extract is able to decrease both serum levels of cholesterol and triglycerides. The present study also reported that the extract reduces the generation of free radicals.

CONCLUSION

These findings indicated that the extract may contain biologically active phytoconstituents that may lower lipid levels and might be beneficial in treatment of Hyperlipidemia and atherosclerosis, and the extract also increased the levels of in-vivo antioxidants simultaneously reducing lipid peroxidation when compared to Triton control groups.

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