EVALUATION OF HYPOLIPIDEMIC EFFECTS OF THE ETHANOLIC EXTRACT OF CAESALPINIA BONDUC (L.) IN TRITON WR-1339 INDUCED HYPERLIPIDEMIC RATS

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ABSTRACT

Objective: To investigate the hypolipidemic activity of the ethanolic fruit extract of Caesalpinia bonduc in Triton WR 1339 induced hyperlipidemic Sprague Dawley rats. Methods: Triton WR 1339 was used to induce hyperlipidemia in experimental rats. 70 % ethanolic fruit extract of C. bonduc was given orally at three different doses of 200 mg/kg, 400 mg/kg and 600 mg/kg to determine hypolipidemic activity. These hypolipidemic effects were compared with a vehicle control group. Simvastatin was used as standard reference drug. Results: Ethanol fruit extract at its increasing doses significantly reduced plasma cholesterol, triglyceride, VLDL and LDL cholesterol levels as compared to the vehicle control group. Atherogenic index remarkably decreased in extract treated rats compared to vehicle control. HDL cholesterol level was significantly increased in extract treated group. Conclusions: These results suggest that ethanolic fruit extract of C. bonduc possess significant hypolipidemic activity.

KEYWORDS: Hypolipidemic activity, Caesalpinia bonduc, Triton solution.

INTRODUCTION

Hyperlipidemia is a metabolic condition characterized by elevated levels in concentration of blood lipid, especially cholesterol and triglyceride. Abnormal lipid levels accelerate atherosclerosis, a chronic degenerative process resulting in arterial stiffness.[1,2] Hyperlipidemia is a well-established risk factor for development and progression of
cardiovascular disease (CVD)\textsuperscript{[3]}, a leading cause of death and disability worldwide.\textsuperscript{[1]} Mostly hyperlipidemia is genetic in origin.\textsuperscript{[3]} However, it can associate with diabetes, kidney disease, obesity, sedentary lifestyle, and alcohol abuse and smoking.\textsuperscript{[1]} Statins are widely used lipid-lowering drugs for prophylaxis and management of hyperlipidemia.\textsuperscript{[1,3]} But various adverse effects have been noted with use of statin, including myositis and myalgia, cognitive loss, neuropathy, pancreatic and hepatic dysfunction and sexual dysfunction.\textsuperscript{[4]} Other commonly used hypolipidemic agents such as nicotinic acid derivatives, fibrates and bile acid binding resins are also reported to produce mild to moderate side effects.\textsuperscript{[1]} With this aspect, it is worth developing a new therapeutic agent with potent antihyperlipidemic action and fewer side effects. Traditional medicinal system believes on use of natural products derived from plants to control an array of human diseases without producing side effects. Moreover, such products are relatively cheap and easily available to the local public. A number of studies screened plant derived materials for possible phytochemical induced antihyperlipidemic action.\textsuperscript{[5,6,7,8]}

\textit{Caesalpinia bonduc} is one of such medicinal plants well-known for its health benefits in folklore medicine. It is extensively used in Ayurveda, Siddha, Unani and homoeopathy since long time.\textsuperscript{[9]} \textit{C. bonduc}, commonly known as Nata Karanja, is a prickly shrub belongs to the family \textit{Caesalpiniaceae}.\textsuperscript{[9,10]} Plant is widely distributed in the hotter parts of India, Burma and Sri Lanka.\textsuperscript{[10]} Mounting evidence from preclinical studies demonstrated almost all parts of the plant possess remarkable medicinal properties. Recently conducted studies reported different parts of plant possess significant anti-inflammatory, analgesic, antibacterial, antidiarrhoeal and cytotoxic activity.\textsuperscript{[9,11,12]} In addition to this, plant is known to offer multiple pharmacological benefits such as antipyretic, antidiabetic, anticarcinogenic, antifungal, antifilarial, immunomodulatory, insecticidal, anxiolytic and antioxidant effects.\textsuperscript{[10]} \textit{C. bonduc} is known to contain phenolic compounds, alkaloids, glycosides, saponin and isoflavones.\textsuperscript{[9,10,13]} These chemical constituents might be responsible for spectrum of phytochemical and pharmacological activities of \textit{C. bonduc}.

However, little data is available in scientific literature about the role of \textit{C. bonduc} on blood lipid levels. In this regard, it is noteworthy to conduct a study evaluating hypolipidemic potential of \textit{C. bonduc}. The present study was designed to study hypolipidemic effects of 70% ethanolic fruit extract of \textit{C. bonduc} in Triton WR 1339 induced hyperlipidemic rats.
MATERIALS AND METHODS

Drugs and chemicals
Triton WR 1339 (Loba Chemie Ltd.), cholesterol, triglyceride, HDL kits (Beacon Diagnostics Pvt. Ltd.) and 70 % ethanol were used in this study. All the solvents used during experimental study were of analytical reagent (AR) grade and high performance liquid chromatography (HPLC) grade.

Collection of plant material
The fresh fruits of the plant *C. bonduc* were collected from Satpuda region of Maharashtra, India. The plant was identified and authenticated by Professor L. K. Kshirsagar, Taxonomist, Department of botany, S.S.V.P.S’s L. K. Dr. Ghogre Science College, Dhule, North Maharashtra University, Jalgaon. A specimen of same has been submitted to the herbarium of division (Accession No RCIPPER/Pharmacology/27-2013).

Preparation of ethanolic extract
Fruits were cleaned to remove dirt and cut into small pieces and dried in shade. Fruits pieces are crushed in a grinder and pulverized into fine powder. This powdered material was passed through 40 mesh sieve and extracted with 70% ethanol using Soxhlet extractor. The extract was concentrated in vacuum evaporator below 40°C.

Experimental Animals
Total 36 Sprague Dawley rats were obtained from college animal house of R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India. Each animal was in the weight range of 150-200 gm at the beginning of the study. They were housed in well ventilated cages and maintained at controlled temperature of 22±2 °C with a 12 h light/dark cycle and standard lab control. All animals had free access to food during experiments under strict hygienic conditions. Tap water was offered *ad libitum*. Institutional Animal Ethical Committee of RCIPPER College, Shirpur approved the study protocol.

Acute toxicity study
Acute toxicity was studied in rats to determine safe oral dose of 70 % ethanolic fruit extract of *C. bonduc*. For this we followed Organization for Economic Co-operation and Development (OECD) guidelines No. 425 ‘Up and Down’ method of Committee for the Purpose of Control and Prevention of Experiments on Animals (CPCSEA).
Induction of hyperlipidemia
Triton WR 1339 solution at a dose of 200 mg/kg body weight (B.W.) was injected intraperitoneally (i.p) in experimental rats in order to induce hyperlipidemia.

Experimental design
The current study comprises six groups (n = 6). The treatment regimen offered in each group was as follows.
Group I (Normal Control) - Normal saline 1ml
Group II (Vehicle Control) - Triton WR 1339 (200 mg/kg) i.p.
Group III (Standard) - Triton WR 1339 (200 mg/kg) i.p. + Simvastatin 4 mg/kg orally p.o.
Group IV (Test 1) - Triton WR 1339 (200 mg/kg) i.p. + Et-BCF 200 mg/kg p.o.
Group V (Test 2) - Triton WR 1339 (200 mg/kg) i.p. + Et-BCF 400 mg/kg p.o.
Group VI (Test 3) - Triton WR 1339 (200 mg/kg) i.p. + Et-BCF 600 mg/kg p.o.
All the rats except group I were injected with Triton WR 1339 (200 mg/kg i.p.). Group I was a normal control group injected with normal saline (NS). Group II was a vehicle control group administered with vehicle CMC intraperitoneally. Group III received Simvastatin 4 mg/kg orally and acted as standard. In test groups IV, V, VI the extract was given orally at an increasing dose of 200, 400, 600 mg/kg p.o. respectively 30 minutes before the Triton injection.[14]

Sample collection
Blood sample was collected from all the rats before Triton injection (0 h) and after Triton injection (24 h). Blood was withdrawn by making retro-orbital plexus method.

Biochemical parameters
The blood was allowed to clot for 30 min and serum was separated by centrifugation under standard laboratory practices. Serum was analyzed for cholesterol, triglyceride, HDL, LDL and VLDL levels and their levels were estimated using chod pap method.

Statistical analysis
All values were expressed as Mean ± Standard Error of Mean (Mean ± SEM). Data were analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. A \( P \) value < 0.05 was considered as statistically significant.
RESULTS

Acute toxicity study
Oral administration of 70% ethanolic fruit extract of *C. bonduc* did not produce toxicity or cause death at a dose up to 4000 mg/kg of p.o. It’s one tenth i.e. 400 mg/kg was selected as middle evaluation dose, 600 mg/kg as up and 200 mg/kg as down dose.

Hypolipidemic activity
Serum cholesterol and triglyceride levels were significantly increased in triton injected rats as compared to the normal control group. This subsequently raises atherogenic index in triton injected rats. However, these biomarkers values substantially reduced in the Simvastatin and ethanolic fruit extract treated groups as compared to the vehicle control group. Simvastatin significantly declined both cholesterol (*P*<0.001) and triglyceride (*P*<0.001) levels with significant reduction in atherogenic index (*P*<0.01) as compared to the vehicle control group. Ethanolic extract in the lowest dose (200 mg/kg) substantially reduces only triglyceride levels (*P*<0.05). However, at increasing doses (400 mg/kg) there was significant recovery in cholesterol (*P*<0.05) and triglyceride (*P*<0.01) levels with decrease in atherogenic index (*P*<0.05). At the highest test dose (600 mg/kg) there was a considerable reduction in cholesterol (*P*<0.01), triglyceride (*P*<0.01) and atherogenic index (*P*<0.01) suggesting significant hypolipidemic effects. (Table I).

Table I: Effect of Et-CBF on Triton WR 1339 induced hyperlipidemia in rats

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Group</th>
<th>Cholesterol Level (mg/dl)</th>
<th>Triglyceride Level (mg/dl)</th>
<th>Atherogenic Index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I.</td>
<td>Normal</td>
<td>87.54±3.65</td>
<td>78.04±2.31</td>
<td>1.02±0.19</td>
</tr>
<tr>
<td>II.</td>
<td>Triton (200 mg/kg, i.p.)</td>
<td>135.9±4.17***</td>
<td>137.2±3.05***</td>
<td>2.12±0.18##</td>
</tr>
<tr>
<td>III.</td>
<td>Simvastatin (4 mg/kg, p.o.)</td>
<td>101.3±5.01***</td>
<td>95.96±2.94***</td>
<td>1.07±0.15**</td>
</tr>
<tr>
<td>IV.</td>
<td>Et-CBF (200 mg/kg, p.o.)</td>
<td>123.6±1.79</td>
<td>124.9±3.22</td>
<td>1.91±0.16</td>
</tr>
<tr>
<td>V.</td>
<td>Et-CBF (400 mg/kg, p.o.)</td>
<td>113.9±2.52</td>
<td>117.9±2.99</td>
<td>1.30±0.08</td>
</tr>
<tr>
<td>VI.</td>
<td>Et-CBF (600 mg/kg, p.o.)</td>
<td>109.9±4.79**</td>
<td>118.3±1.91**</td>
<td>1.07±0.17**</td>
</tr>
</tbody>
</table>

Data is expressed as mean ± SEM (*n* = 6) # denotes compared to normal control group. * denote the significance levels with *p*<0.05, **p*<0.01; ***p*<0.001, Et-BCF: Ethanolic extract of *Caesalpinia bonduc* fruit

Both serum VLDL and LDL were considerably high in triton induced hyperlipidemic rats in comparison with the normal control group. But treatment with Simvastatin (*P*<0.001) and ethanolic fruit extract at a dose level 200 mg/kg (*P*<0.05), 400 mg/kg (*P*<0.01) and 600 mg/kg (*P*<0.01) produced significant reduction in VLDL. Similarly, LDL value significantly
returned towards normal on treatment with Simvastatin ($P < 0.01$) and ethanolic fruit extract at a dose level 400 mg/kg ($P<0.05$) and 600 mg/kg ($P<0.01$) (Figure 1 & 2).

**Figure 1:** Effects of Et-CBF on VLDL-cholesterol level against triton WR-1339 induced hyperlipidemia in rats.

Data is expressed as mean ± SEM ($n = 6$) # denotes compared to normal control group, * denote the significance levels with *$p<0.05$; **$p<0.01$; ***$p<0.001$, Et-BCF: Ethanolic extract of *Caesalpinia bonduc* fruit. The figures in the parenthesis indicates dose in mg/kg p.o.

**Figure 2:** Effects of Et-CBF on LDL-cholesterol level against triton WR-1339 induced hyperlipidemia in rats.
Data is expressed as mean ± SEM (n = 6) # denotes compared to normal control group, * denote the significance levels with *p<0.05; **p<0.01, Et-BCF: Ethanolic extract of Caesalpinia bonduc fruit. The figures in the parenthesis indicates dose in mg/kg p.o.

Serum HDL cholesterol was low in triton induced hyperlipidemic rats in comparison with the normal control group. On treatment with Simvastatin (P<0.05) and ethanolic fruit extract at the dose level 400 mg/kg (P<0.05) and 600 mg/kg (P<0.05) this level significantly increased as compared to the vehicle control group (Figure 3).

![HDL-cholesterol Level](image)

**Figure 3:** Effects of Et-CBF on VLDL-cholesterol level against triton WR-1339 induced hyperlipidemia in rats.

Data is expressed as mean ± SEM (n = 6) # denotes compared to normal control group, * denote the significance levels with *p<0.05, Et-BCF: Ethanolic extract of Caesalpinia bonduc fruit. The figures in the parenthesis indicates dose in mg/kg p.o.

**DISCUSSION**

Blood lipids are usually divided into two types ‘cholesterol’ and ‘triglycerides’. Cholesterol is of various types, including total cholesterol (TC), high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol and very low-density lipoprotein (VLDL) cholesterol. Body needs both cholesterol and triglyceride in a normal range to carry out vital functions. However, an abnormal lipid levels result in serious life-threatening disorders like hyperlipidemia, atherosclerosis, coronary heart disease and cerebrovascular
accidents. Hyperlipidemia induced CVD risk and lipid lowering agents associated side effects, both warrants the need for development of new therapeutic agents to manage this clinical entity. In this regard, testing hypolipidemic properties of plant-derived material is particularly beneficial.

Plants belonging to *Caesalpiniaceae* family have already confirmed substantial hypolipidemic activity in different studies. A study by Sharma SR K *et al* (1997) reported significant antihypercholesterolemic and antihypertriglyceridemic effects of aqueous extracts of *C. bonduc*ella seeds in diabetic rats.[15] Similarly, a study by Jana K *et al* (2010) successfully demonstrated antihyperlipidemic effects of hydro-methanolic seed extract of *C. bonduc* in streptozotocin induced diabetic rats.[16]

Current study demonstrated that all triton injected rats developed remarkable hyperlipidemia as exhibited by an increase in serum cholesterol, triglyceride, and VLDL and LDL levels. Triton WR 1339, also known as Tyloxapol, is a non-ionic detergent and has been extensively used to induce hyperlipidemia in several animal studies.[6,17] Hyperlipidemic action of triton is attributive to reduction in rate of triglyceride hydrolysis and interference with TG uptake from circulation by the extra-hepatic tissues elevating TG level and clearing of lipoprotein lipase rising VLDL levels.[18]

However administration of Simvastatin and ethanolic fruit extract of *C. bonduc* at increasing doses significantly suppresses levels of serum cholesterol, triglyceride, VLDL and LDL. Simvastatin is a statin commonly used for management of hyperlipidemia due to its antihypercholesterolemic effects. Statins are known to inhibit cholesterol formation by inhibiting HMG-CoA reductase (3-hydroxy-3-methyl-glutaryl-CoA), an important enzyme in cholesterol synthesis. [1] Moreover, statins are proved to be more effective in lowering of LDL cholesterol.[19]

In current study, 70 % ethanolic fruit extract of *C. bonduc* dose-dependently improved the blood lipid profile and normalize atherogenic index in triton induced hyperlipidemic rats. Compared with Simvastatin, hypolipidemic acticity of ethanolic fruit extract is marginally low but significant. However, regarding rising protective HDL levels hypolipidemic action of extract at higher doses (400, 600 mg/kg) is comparable to Simvastatin. With reduction in triglyceride levels there was a subsequent decrease in atherogenic index too. Atherogenic index is the logarithm of the ratio of plasma concentration of triglycerides to HDL.
cholesterol. It is widely accepted as a marker of plasma atherogenicity. In present study, a remarkable reduction in atherogenic index may be due to extract related decrease in triglyceride levels and increase in HDL levels. A decrease in an atherogenic index indicates ethanolic extract has a cardioprotective effect due to potential anti-atherogenic activity.

*C. bonduc* is reported to contain phenolic constituents and saponins. Shukla S *et al* (2009) estimated 62.50 mg/g of total phenolic content only in ethanolic extract of *C. bonducella* seeds. Phenol has a good free radical scavenging activity due to presence of hydroxyl groups. Hence high amount of phenolic contents makes *C. bonducella* as a potent antioxidant agent. 

Studies on different plant reported positive relationship between presence of phenolic compounds and reduction in blood cholesterol concentration. This favorable impact on cholesterol level is might be due to improvement in antioxidant potential of hepatic tissues leading to decrease in oxidative stress and lipid peroxidation. Likewise, saponins are another important phytochemical leading to various biological activities. Saponins are surface-active glycosides abundantly present in various plant species. Several studies have shown that saponins from different plant extract decrease serum cholesterol level. Underlying hypolipidemic mechanism of saponins is not yet fully understood. There are different schools of thoughts suggesting effects of saponins on cholesterol metabolism. Possible hypolipidemic action of saponins is may be due to formation of large mixed micelles by its interaction with bile acids that increase cholesterol excretion. This accelerates metabolism of cholesterol in liver resulting in decrease serum levels.

Thus, possible hypolipidemic mechanism of extract might involve synergistic or independent role of phenolic contents and saponins. These findings suggest that ethanolic fruit extract of *C. bonduc* have favorable impact on cholesterol synthesis and metabolism. Also potential to correct lipid abnormalities make *C. bonduc* as a good cardioprotective agent.

**CONCLUSION**

The current study provides some biochemical basis for use of ethanolic fruit extract of *C. bonduc* as a hypolipidemic agent in prevention and control of CVD. This might be a promising and cost-effective therapy. Hence, further phytochemical screening and scientific evaluation is needed in order to fractionate active principles and determine the mechanistic approach of *C. bonduc* as a hypolipidemic agent.
REFERENCES


