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Research Article
Evaluation of Hypolipidemic Effects of *Caesalpinia bonduc* in a Murine Model of High Fat Diet Induced Hyperlipidemia

Pankaj G. Jain and Sanjay J. Surana

R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, District Dhule, 425405, Maharashtra, India

Abstract

Background: Hyperlipidemia is a disorder characterized by the increase in blood lipoprotein or cholesterol levels. *Caesalpinia bonduc* (family: Caesalpiniaceae) is medicinal plants with significant pharmacologic activity. **Materials and Methods:** Fruits of *Caesalpinia bonduc* were used to prepare aqueous (AQ-CBF), ethanolic (ET-CBF) and petroleum ether extract (PE-CBF). Hyperlipidemia was induced in male Sprague-Dawley rats (180-220 g) by administering them with High fat Diet (HFD, 58% fat) for 60 days. Rat were either concomitantly administered orally with vehicle or AQ-CBF (100, 200 and 400 mg kg^{-1}) or ET-CBF (100, 200 and 400 mg kg^{-1}) or PE-CBF (100, 200 and 400 mg kg^{-1}) for 60 days. Various biochemical parameters were evaluated in serum. **Results:** Concomitant administration of AQ-CBF (400 mg kg^{-1}), ET-CBF (200 and 400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) showed significant inhibition in HFD induced elevated serum triglyceride (TG), Total Cholesterol (TC), Low Density Lipoproteins (LDL-C), Very Low Density Lipoproteins (VLDL-C), LDL to HDL ratio and atherogenic index as compared to HFD control group. Decreased High Density Lipoproteins (HDL-C) level was significantly increased by AQ-CBF (400 mg kg^{-1}), ET-CBF (200 and 400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) treatment as compared to HFD control group. **Conclusion:** ET-CBF and PE-CBF improved the serum lipid profile in rats by decreasing serum TC, TG, LDL-C and increasing serum HDL-C, thus improving the atherogenic index. This finding provides some biochemical basis for the use of *C. bonduc* as hypolipidemic agent having preventive and curative effect against hyperlipidemia.

Key words: *Caesalpinia bonduc*, hyperlipidemia, high fat diet, hypolipidemic, serum cholesterol level

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Corresponding Author: Pankaj G. Jain, R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, District Dhule, 425405, Maharashtra, India

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The term hyperlipidemia refers to the elevated lipid levels in the body including high cholesterol and high triglyceride levels[1,2]. 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[3,4]. 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 h[5].

Worldwide around 30% of the deaths has been occurred due to cardiovascular diseases (CVD)[6,7] and the risk factor associated with it includes hypertension, hypercholesterolemia, diabetics and obesity[8,9]. It has been projected that the number of hypercholesterolemia individual would be increase two fold by 2025[10].

Research carried out over a decade's showed that not only total serum cholesterol but also different lipoproteins is responsible for lipid associated disorders[11,12]. The major role of Low Density Lipoproteins (LDL) is to transport of cholesterol towards tissue with atherogenic potential whereas, High Density Lipoproteins (HDL) conduct cholesterol towards the liver from peripheral tissues hence HDL consider as protective lipoproteins against many cardiac problems and obesity[13].

Animal models played important role in the development of various therapeutic moieties against various diseases[14-20]. Induction of hyperlipidemia in rats by feeding them with High Fat Diet (HFD) is well established animal model used for evaluation of antihyperlipidemic potential of various drugs[21,22]. The HFD induced hyperlipidemia is associated with elevated HDL-C levels in rodents[23], non human primates[24] and in humans also[25].

Currently available pharmacotherapy for hyperlipidemia includes agents like statin, fibrates, niacin, bile acids, ezitimibe etc. which has an ability to reduce cholesterol level with different condition[26]. However, these therapeutic moieties are associated with a number of side effects including hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function which leads to limit their usage[27].

Therapeutic moieties from plant source have been explored from ancient times. It consider as the major constitute of raw materials source in drugs for treating various ailments of human being[28-33]. Herbal medicine consist of an array of biochemical moieties including saponins, tannins, essential oils, flavonoids, alkaloids, etc. which have curative potential[34-42]. In many traditional and folklore medicine plant have been utilized for the treatment of hyperlipidemia. *Caesalpinia bonduc* (Nata Karanja) (family: Caesalpiniaceae) is one of such medicinal plants with significant pharmacologic activity[43,44]. It is abundantly present in the hotter parts of India, Burma and Sri Lanka[45]. It's having an wide range of pharmacological potential including anti-inflammatory, analgesic, antibacterial, antidiarrhoeal, antipyretic, antidiabetic, anticarcinogenic, antifungal, immunomodulatory, insecticidal, anxiolytic and antioxidant properties. The aim of present study was to investigate hypolipidemic properties of *Caesalpinia bonduc* in a model of hyperlipidemia induced by HFD in Sprague-Dawley rats.

MATERIALS AND METHODS

Drugs and chemicals: The HFD (60 kcal % fat, # D12492, 5.24 kcal g^{-1}) was purchased from Research Diet Inc., New Brunswick, NJ, USA. Cholesterol, triglyceride, HDL-C and LDL-C kits were purchased from Accurex Biomedical Pvt. Ltd., Mumbai, India. Petroleum ether (60:80) and diethyl ether were purchased from Merck, India.

Collection of plant material: The fresh fruits of the plant *Caesalpinia 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.

Preparation of aqueous, ethanolic and petroleum ether extract: Fruits of *Caesalpinia bonduc* 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 (500 g) was passed through 40 mesh and macerated with petroleum ether (60:80) at room temperature and filtered. The filtrate was evaporated. The yield obtained were 2.1% w/w, respectively. The remaining marc was extracted with 70% ethanol using Soxhlet extractor. The extract was concentrated in vacuum evaporator below 40°C. The yield obtained were 12.8% w/w, respectively. Furthermore, the remaining marc was macerated with distilled water at room temperature for 7 days and filtered. The filtrate was dried on tray dryer at 60°C. The yield obtained were 21.5% w/w, respectively. The semi-solid petroleum extract was emulsified with 2% Tween-80 in distilled water and powder of aqueous and ethanolic extract was dissolved in distilled water to prepare the drug solution of concentration of 10 mg mL^{-1} and used for pharmacological studies.

Preliminary phytochemical screening: The preliminary phytochemical analysis for aqueous extract of *Caesalpinia bonduc* fruit (AQ-CBF), ethanolic extract of *Caesalpinia bonduc* fruit (ET-CBF) and petroleum ether extract of *Caesalpinia bonduc* fruit (PE-CBF) was carried out for the alkaloid (Mayer's, Hager's, Dragendorff's and Wagner's test), flavonoids (Shinoda test), steroids (Salkowski, Liberman-Burchard, Libermann's test), carbohydrate, phenolic compounds, glycosides and volatile oils[46-48].

Experimental animals: Male Sprague-Dawley rats (180-220 g) were obtained from college animal house of R.C. Patel Institute of Pharmaceutical Education and Research, Shirpur, India. 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 RCPIPER College, Shirpur approved the study protocol (IAEC/RCPIPER/2012-13/09).

Acute oral toxicity study: Acute toxicity was studied in rats to determine safe oral dose of drugs. 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)[49].

Development of high fat diet fed rats: Rat was fed with two dietary regimes such as Normal Pellet Diet (NPD) and High Fat Diet (HFD). The rat was feeding either NPD or HFD (58% fat, 25% protein and 17% carbohydrate, as a percentage of total kcal) *ad libitum*, respectively, for the initial period of 60 days. The composition and preparation of HFD as were described elsewhere[22].

Experimental design: The studies were conducted in the following groups of animals.

Group 1 : Normal rats: Received vehicle (10 mg kg^{-1} of 2% Tween-80 in distilled water)

Group 2 : HFD control: Received vehicle (10 mg kg^{-1} of 2% Tween-80 in distilled water)

Group 3 : AT (1.2): Received atorvastatin (1.2 mg kg^{-1})

Group 4 : AQ-CBF (100): Received aqueous extract of *Caesalpinia bonduc* fruit (100 mg kg^{-1})

Group 5 : AQ-CBF (200): Received aqueous extract of *Caesalpinia bonduc* fruit (200 mg kg^{-1})

Group 6 : AQ-CBF (400): Received aqueous extract of *Caesalpinia bonduc* fruit (400 mg kg^{-1})

Group 7 : ET-CBF (100): Received ethanolic extract of *Caesalpinia bonduc* fruit (100 mg kg^{-1})

Group 8 : ET-CBF (200): Received ethanolic extract of *Caesalpinia bonduc* fruit (200 mg kg^{-1})

Group 9 : ET-CBF (400): Received ethanolic extract of *Caesalpinia bonduc* fruit (400 mg kg^{-1})

Group 10 : PE-CBF (100): Received petroleum ether extract of *Caesalpinia bonduc* fruit (100 mg kg^{-1})

Group 11 : PE-CBF (200): Received petroleum ether extract of *Caesalpinia bonduc* fruit (200 mg kg^{-1})

Group 12 : PE-CBF (400): Received petroleum ether extract of *Caesalpinia bonduc* fruit (400 mg kg^{-1})

Vehicle or atorvastatin or drugs were administered orally for 60 consecutive days. After end of treatment, rats were fasted overnight and, after 24 h they were sequentially anesthetized with anesthetic ether for about 30-40 sec. The blood was withdrawn by retro orbital puncture. Each blood sample was collected into separate vials for the determination of serum parameters.

Preparation of serum samples and biochemical estimations: The serum was separated by centrifugation using an Eppendorf cryocentrifuge (model No. 5810, Eppendorf, Hamburg, Germany), maintained at 4°C and run at a speed of 7000 rpm for 15 min. The levels of High-Density Lipoprotein (HDL), Low-Density Lipoprotein (LDL), triglyceride (TG) and Total Cholesterol (TC) were measured by a spectrophotometer (UV–visible spectrophotometer, Jasco V-530, Tokyo, Japan) using commercially available reagent kits according to procedure provided by manufacturer (Accurex Biomedical Pvt. Ltd., Mumbai, India)[50-56].

Statistical analysis: All statistical analysis was performed using GraphPad Prism 6.0 (GraphPad Software, Inc, La Jolla, CA, USA). Data of body weight and biochemical measurements was analyzed by separate One-way ANOVA followed by Dunnett's test separately for each parameter. A value of $p<0.05$ was considered to be statistically significant.

RESULTS

Preliminary phytochemical screening: PE-CBF showed the presence of steroids and volatile oils. ET-CBF showed the presence of glycosides, flavonoids, tannins, steroids and phenolic compounds. The AQ-CBF showed the presence of glycosides, flavonoids, tannins and phenolic compounds (Table 1).

Table 1: Phytochemical analysis of AQ-CBF, ET-CBF and PE-CBF

Tests	AQ-CBF	ET-CBF	PE-CBF
Carbohydrate			
Molish test	+	-	-
Reducing sugar			
Fehling's test	+	+	-
Benedict's test	+	+	-
Monosaccharides			
Barfode's test	-	-	-
Proteins			
Biuret's test	-	-	-
Millon's test	-	-	-
Protein containing sulphur	-	-	-
Protein containing tyrosine and tryptophan			
Xanthoprotein test	-	-	-
Amino acid			
Ninhydrine test	-	+	-
Tannins and phenol			
Ferric chloride test	-	+	-
Lead acetate	-	+	-
Potassium permanganate	-	+	-
Glycosides			
Cardiac glycosides			
Legal's test	+	+	-
Keller killani test	+	+	-
Anthraquinone glycosides			
Test	MI-ALC	MI-AQE	SI-ALC
Borntragers's test	-	-	-
Saponins			
Foam test	+	+	-
Flavonoids			
Shinoda test	+	+	-
Alkaloids			
Mayer's test	-	-	-
Wagner's test	-	-	-
Hager's test	-	-	-
Dragendroff's test	-	-	-
Steroids			
Salkowaski test	+	-	+
Liberman-Burchard reaction	+	-	+
Libermann's test	+	-	+

+: Positive test, -: Negative test, AQ-CBF: Aqueous extract of *Caesalpinia bonduc* fruit, ET-CBF: Ethanolic extract of *Caesalpinia bonduc* fruit, PE-CBF: Petroleum ether extract of *Caesalpinia bonduc* fruit

Acute oral toxicity study: AQ-CBF, ET-CBF and PE-CBF were found to be safe up to the dose of 5000 mg kg^{-1} p.o. No mortality or any toxic reactions were observed up to the end of the study period (Table 2).

Effect of AQ-CBF and PE-CBF on HFD-induced alteration in body weight, serum lipid profile and atherogenic index of rats: There was significant increased ($p<0.001$) in the body weight of the HFD control rats as compared to the normal group. However, increased in the body weight was significantly decreased ($p<0.05$) by treatment with AQ-CBF (400 mg kg^{-1}) as well as PE-CBF (400 mg kg^{-1}) treatment as compared to HFD control rats. Administration of atorvastatin (1.2 mg kg^{-1}) also showed significant inhibition ($p<0.001$) in HFD induced increased in body weight as compared to HFD control rats (Table 3).

When compared with normal rats, HFD control rats showed significantly increased ($p<0.001$) triglyceride, cholesterol, LDL-C, VLDL-C, LDL to HDL ratio and atherogenic index whereas HDL-C level was decreased significantly ($p<0.001$). Administration of AQ-CBF (400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) showed significant inhibition ($p<0.05$) in HFD induced alterations in triglyceride, cholesterol, HDL-C, LDL-C and VLDL-C whereas LDL to HDL ratio and atherogenic index were significantly decreased ($p<0.001$) by AQ-CBF

Table 2: Acute toxicity study of AQ-CBF, ET-CBF and PE-CBF

	Dose (mg kg^{-1}, p.o)				
Extracts	175	550	1750	2000	5000
AQ-CBF	0	0	0	0	0
ET-CBF	0	0	0	0	0
PE-CBF	0	0	0	0	0

0: Alive, AQ-CBF: Aqueous extract of *Caesalpinia bonduc* fruit, ET-CBF: Ethanolic extract of *Caesalpinia bonduc* fruit, PE-CBF: Petroleum ether extract of *Caesalpinia bonduc* fruit

Table 3: Effect of AQ-CBF, ET-CBF and PE-CBF on HFD-induced alteration in body weight, serum lipid profile and atherogenic index of rats

Treatments (mg kg^{-1})	Body weight (g)	Triglycerides (mg %)	Cholesterol (mg %)	HDL-C (mg %)	LDL-C (mg %)	VLDL-C (mg %)	LDL:HDL	Atherogenic index
Normal	234.8±4.78	81.90±4.34	71.42±3.65	60.49±2.09	27.34±2.12	16.38±0.86	0.45±0.04	0.18±0.05
HFD control	350.8±6.85$^{###}$	327.1±18.60$^{###}$	168.9±5.29$^{###}$	43.40±2.40$^{###}$	64.99±1.16$^{###}$	65.42±3.72$^{###}$	1.52±0.10$^{###}$	2.94±0.19$^{###}$
AT (1.2)	276.3±11.77***	131.2±8.74***	88.64±4.32***	56.21±2.19***	34.43±3.38***	26.25±1.74***	0.61±0.05***	0.59±0.11***
AQ-CBF (100)	340.8±7.88	319.8±21.21	161.6±8.73	47.09±1.96	60.22±2.75	63.95±4.24	1.28±0.06	2.47±0.25
AQ-CBF (200)	322.3±6.23	249.3±25.48	151.0±4.29	47.48±3.34	55.38±2.07	49.87±5.09	1.21±0.13*	2.25±0.24
AQ-CBF (400)	315.3±9.74*	230.0±23.39*	138.0±4.41*	52.62±1.87*	55.89±2.14	45.99±4.67*	1.06±0.05***	1.63±0.10***
ET-CBF (100)	336.3±6.79	315.2±22.07	156.8±6.62	48.93±1.02	56.56±2.22	63.04±4.41	1.15±0.02**	2.21±0.18
ET-CBF (200)	295.2±6.16***	213.5±19.61**	137.4±5.13**	54.95±0.92**	51.16±2.82**	42.70±3.92**	0.92±0.03***	1.49±0.06***
ET-CBF (400)	256.0±11.29***	170.7±24.36***	123.4±9.17***	56.60±0.99***	39.16±2.14***	34.14±4.87***	0.69±0.04***	1.19±0.18***
PE-CBF (100)	334.0±4.21	302.9±28.94	147.8±6.73	46.50±1.95	62.04±1.73	60.59±5.78	1.34±0.06	2.21±0.22
PE-CBF (200)	326.0±5.60	269.2±20.63	147.9±9.19	50.39±2.74	54.64±1.77*	53.84±4.12	1.10±0.06**	2.01±0.29**
PE-CBF (400)	306.3±7.06*	235.1±16.85*	141.2±6.49*	52.04±2.25*	54.91±2.93*	47.02±3.37*	1.07±0.09***	1.73±0.15***

Data is expressed as Mean±SEM and analyzed by one-way ANOVA followed by Dunnett's post tests, $^{###}p<0.001$ as compared with normal group, *p<0.05, **p<0.01, ***p<0.001 as compared with HFD control group. HFD: High fat diet, AT (1.2): Atorvastatin (1.2 mg kg^{-1}, p.o.) treated, AQ-CBF: Aqueous extract of *Caesalpinia bonduc* fruit, ET-CBF: Ethanolic extract of *Caesalpinia bonduc* fruit, PE-CBF: Petroleum ether extract of *Caesalpinia bonduc* fruit (n = 6)

(400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) treatment as compared to HFD control rats. Atorvastatin (1.2 mg kg^{-1}) treatment also showed significant inhibition ($p<0.001$) in the HDM induced alterations in triglyceride, cholesterol, HDL-C, LDL-C, VLDL-C, LDL to HDL ratio and atherogenic index as compared to HFD control group (Table 3).

Effect of ET-CBF on HFD-induced alteration in body weight, serum lipid profile and atherogenic index of rats: The HDF induced increased in the body weight was significantly decreased ($p<0.001$) by treatment with ET-CBF (200 and 400 mg kg^{-1}) treatment as compared to HFD control rats. Administration of ET-CBF (200 and 400 mg kg^{-1}) showed significant and dose dependant inhibition ($p<0.01$ and $p<0.001$) in HFD induced alterations in triglyceride, cholesterol, HDL-C, LDL-C, LDL to HDL ratio and atherogenic index as compared to HFD control group. The elevated VLDL-C level was significantly decreased by ET-CBF (400 mg kg^{-1}) treatment as compared to HFD control rats (Table 3).

DISCUSSION

Hyperlipidemia is a result of elevated cholesterol that caused increased oxidative stress with serum LDL levels which in turn increased risk for atherosclerosis development[57]. Cholesterol is the main sterol in animal tissues and it is an important precursor for the synthesis of steroid hormones and vitamin D. Due to its rigid planar structure it's played vital role in stabilization of membrane structures. High level of cholesterol is associated with cardiovascular disease particularly Coronary Heart Disease (CHD)[58]. It has an ability to forms the basis for plaque lining the arteries. In the present study administration of HFD caused significant increased in the level of serum cholesterol whereas, administration of AQ-CBF (400 mg kg^{-1}), ET-CBF (200 and 400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) showed significant inhibition in HFD induced elevated serum cholesterol level.

The HMG CoA reductase is an important rate determining enzyme in cholesterol biosynthesis and it has been reported that diet with saturated fatty acids has an ability to increases the activity of HMG CoA reductase. It may be due to higher availability of acetyl CoA, which stimulated the cholesterogenesis rate. Lipoproteins involved in this process include cholesterol carried by Very Low Density Lipoproteins (VLDL), remnant lipoproteins and Low Density Lipoproteins (LDL)[59]. It is also associated with down regulation in LDL receptors leads to elevation of serum LDL-C levels via inducing alterations in hepatic LDL receptor activity as well as the LDL-C production rate[60]. Furthermore, transesterification of cholesterol, the maturation of HDL-C and the flux of cholesterol from cell membranes into HDL has been carried out by lecithin cholesterol O-acyltransferase (LCAT) enzyme and this enzyme activity has been reported to decreased in HFD induced hypercholesterolemia[61]. Decreased level of HDL-C may resulted in inhibition of 'reverse cholesterol transport' i.e. transport of TG or cholesterol from serum to liver thus its catabolised and excreted out of the body has been decreased which in turn elevates the level of TG and LDL-C in serum. The HFD administration is associated with elevated TG and LDL-C along with decreased HDL-C levels[22]. The result of present study is in line with the findings of previous investigators[22]. However, administration of AQ-CBF (400 mg kg^{-1}), ET-CBF (200 and 400 mg kg^{-1}) and PE-CBF (400 mg kg^{-1}) significantly inhibited HFD induced alterations in TG, LDL-C and HDL-C levels.

Atherogenic index is used as hallmark to assess the susceptibility of atherogenesis. In the present investigation atherogenic index was markedly increased in HFD control rats whereas AQ-CBF (400 mg kg^{-1}), ET-CBF (200 and 400 mg kg^{-1}) and PE-CBF (200 and 400 mg kg^{-1}) treatment significantly decreased atherogenic index.

Previous studies exploring the effect of alcoholic extract of *C. bonduc* in antihyperglycemic as well as antihyperlipidemic effects on STZ-induced diabetes mellitus in rats which may be due to it antioxidants property attributes to presence of glycosides, flavonoids and phenolic compounds in it[62].

CONCLUSION

Result of present study revealed that the ethanolic extract and petroleum ether extract of *C. bonduc* improved the serum lipid profile in rats by decreasing serum triglyceride (TG), Total Cholesterol (TC), LDL-C and increasing serum HDL-C, thus improving the atherogenic index. This finding provides some biochemical basis for the use of *C. bonduc* as hypolipidemic agent having preventive and curative effect against hyperlipidemia. However, further investigation on isolation, purification and characterization of the bio-active phytomolecule is needed to explore the exact mechanism of action of the *C. bonduc* extracts.

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