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Research Article

ANTI-ATHEROGENIC ACTIVITY OF ETHYL ACETATE AND ETHANOLIC EXTRACTS OF *CAESALPINIA BONDUCELLA* LINN.F. ON HIGH-FAT DIET-INDUCED ATHEROSCLEROSIS IN RATS

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ABSTRACT

Objective: The aim is to investigate the anti-atherogenic effect of ethyl acetate (EACB) and ethanolic extracts of *Caesalpinia bonducella* (ETCB) kernels against high-fat diet (HFD) induced atherosclerosis (AS) in male Wistar rats.

Methods: Rats were divided into seven groups of six each. Group I served as normal control, group II received HFD (20.5% of wheat flour, 52.6% of roasted bengal gram, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% starch with salt mixture, 9% of coconut oil, 0.5% of choline mixture and vitamin and 0.4% of cholesterol), group III received HFD+Standard drug atorvastatin (1.2 mg/kg b.w.) and the remaining four groups IV, V, VI, and VII received HFD along with EACB (100 and 200 mg/kg b.wt) and HFD along with ETCB (100 and 200 mg/kg b.wt) respectively for 90 days. Anti-atherogenic activity was assessed by quantifying the concentration of serum total cholesterol (TC), triglyceride (TG), low-density lipoprotein (LDL) cholesterol, high-density lipoprotein cholesterol, very LDL cholesterol, and phospholipids (PL). Tissue levels of TC, TG, and PL were also determined. Lipid peroxidation and enzymatic anti-oxidants were determined in the liver, heart, and aorta tissue. The histopathological analysis of the liver and aorta tissue supported the dose-dependent anti-atherogenic effects of EACB and ETCB.

Results: EACB and ETCB significantly ($p \leq 0.05$) protected the above-mentioned parameters to fall from the normal levels.

Conclusion: These observations suggest that the EACB and ETCB have a dose-dependent anti-atherogenic effect against HFD-induced AS.

Keywords: Atherosclerosis, Antioxidants, Atorvastatin, Cholesterol, *Caesalpinia bonducella*, High fat diet, Hperlipidemic effect.

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INTRODUCTION

Atherosclerosis (AS) is one of the leading risk factors for cardiovascular diseases. With the improvement in living standards and the changes in people's dietary habits, the cardiovascular diseases caused by AS are increasing. AS has become the principal cause of morbidity and mortality worldwide. Increasing evidence supporting diet plays a crucial role in the prevention and treatment of AS [1,2]. *Caesalpinia bonducella* Fleming, also known as Fever nut or Bonduc nut, is a prickly herb distributed all over India, especially in the Andaman and Nicobar Islands. All parts of the plant have been in use in traditional medicine. The plant has been reported to possess antidiabetic and antihyperlipidemic, abortifacient, anti-oxidant, analgesic, anti-inflammatory, antifilarial, anticonvulsive, antibacterial, antidiarrheal, antimalarial, antipyretic, antifungal, antitumor, antiulcer, antipsoriatic, immunomodulatory, anticataract, anthelmintic, and anticancer activities. Literature reviews have indicated that although the antihyperlipidemic potential of the seed extract of this plant on diabetes-induced hyperlipidemia has been documented, no studies of the seed kernel extract of the plant on high-fat diet (HFD) -induced anti-atherogenic effect has been conducted so far. As such, the present study was aimed at evaluating the action of ethyl acetate and ethanolic extract of seed kernel of *C. bonducella* Fleming on HFD -induced anti-atherogenic effect [3].

METHODS

Collection of plant material and extraction

The seeds of *C. bonducella* Fleming were collected from the local market in Krishnankoil in June to August. Authentication of the plant material was done by Dr. D. Stephen, Professor, Department of Botany, The American College, Madurai. Manual separation of the seed kernels of *C. bonducella* Fleming from the outer seed shell was first carried out. The seed kernels were then air dried, powdered, and ethyl acetate and ethanolic extracts were prepared using the soxhlet extraction method. The extract was dried and the final yields were obtained.

Preliminary phytochemical analysis

The ethyl acetate (EACB) and ethanolic extract of the seed kernel of *C. bonducella* Fleming (ETCB) were subjected to phytochemical analysis as per the standard methods [4].

Animals

The study was carried out in healthy adult male Wister rats. They were taken care of as per the guidelines of the committee for the purpose of control and supervision of experiments on animals. Permission from the Institutional Animal Ethical Committee for laboratory use of animals (Registration no: AKCP/IAEC/01/19-20) was duly obtained.

Method of preparation of HFD

HFD was prepared by mixing 20.5% of wheat flour, 52.6% of roasted bengal gram, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% starch with salt mixture, 9% of coconut oil, 0.5% of choline mixture and vitamin and 0.4% of cholesterol [5].

Experimental protocol

The animals were divided into seven groups (Six rats/group).

- Group I - Standard chow diet (Control)
- Group II - HFD
- Group III - HFD+Standard drug atorvastatin (1.2 mg/kg b.wt.)
- Group IV- HFD+Ethyl acetate extract of *C. bonducella* (100 mg/kg b.wt.)
- Group V- HFD+Ethyl acetate extract of *C. bonducella* (200 mg/kg b.wt)
- Group VI- HFD+Ethanolic extract of *C. bonducella* (100 mg/kg b.wt)
- Group VII- HFD+Ethanolic extract of *C. bonducella* (200 mg/kg b.wt).

Table 1: Effect of EACB and ETCB on serum lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C) and PL in control and experimental animals

Groups	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)	PL (mg/dL)
Group I	122.61±1.45^{b*}	56.72±0.58^{b*}	59.25±0.38^{b*}	25.12±0.38^{b*}	11.78±0.15^{b*}	97.58±0.18^{b*}
Group II	182.80±1.18^{a*}	75.26±0.62^{a*}	36.86±0.40^{a*}	43.86±0.26^{a*}	15.12±0.18^{a*}	140.26±0.26^{a*}
Group III	109.90±1.63$^{a*, b*}$	59.64±0.40$^{a*, b*}$	59.05±0.34$^{a*, b*}$	20.10±0.32$^{a*, b*}$	10.44±0.10$^{a*, b*}$	100.24±0.26$^{a*, b*}$
Group IV	161.10±2.02 $^{a**, b*}$	70.23±0.40$^{a*, b*}$	40.24±0.28$^{a*, b*}$	38.28±0.24$^{a**, b*}$	14.12±0.12$^{a*, b*}$	134.62±0.24$^{a*, b*}$
Group V	149.28±1.28 $^{a**, b*}$	69.42±0.43$^{a*, b*}$	42.30±0.32$^{a*, b*}$	34.76±0.18$^{a**, b*}$	13.58±0.08$^{a*, b*}$	132.60±0.25$^{a*, b*}$
Group VI	142.03±1.70$^{a*, b*}$	66.52±0.42$^{a*, b*}$	46.42±0.30$^{a*, b*}$	32.41±0.28$^{a*, b*}$	12.96±0.10$^{a*, b*}$	131.48±0.22$^{a*, b**}$
Group II	135.74±1.46$^{a*, b*}$	66.92±0.52$^{a*, b*}$	48.12±0.40$^{a*, b*}$	28.48±0.26$^{a*, b*}$	12.86±0.14$^{a*, b*}$	128.72±0.22$^{a*, b**}$

*p<0.001, **p<0.05, [a]Group I compared with groups II, III, IV, V, VI and VII, [b]Group II compared with groups I, III, IV, V, VI and VII. TG: Triglyceride, PL: Phospholipids, HDL-C: High density lipoprotein cholesterol, LDL-C: Low density lipoprotein cholesterol, TC: Total cholesterol

Table 2: Effect of EACB and ETCB on tissue lipid contents in control and experimental animals

Groups	FC	EC	TG	PL	FFA
Group I - liver	0.82±0.02^{b*}	1.70±0.04^{b*}	8.38±0.12^{b*}	18.94±0.18^{b*}	14.16±0.12^{b*}
Heart	0.71±0.02^{b*}	2.88±0.03^{b*}	11.12±0.04^{b*}	24.35±0.10^{b*}	15.82±0.09^{b*}
Aorta	0.46±0.02^{b*}	2.10±0.38^{b*}	11.20±0.08^{b*}	9.88±0.09^{b*}	13.08±0.07^{b*}
Group II - liver	1.32±0.04^{a**}	3.62±0.06^{a*}	28.78±0.20^{a*}	30.38±0.15^{a*}	33.92±0.20^{a*}
Heart	1.05±0.03^{a*}	7.06±0.08^{a*}	49.25±0.20^{a*}	38.16±0.14^{a*}	44.23±0.18^{a*}
Aorta	2.42±0.05^{a*}	6.92±0.32^{a*}	22.32±0.32^{a*}	17.23±0.10^{a*}	29.35±0.08^{a*}
Group III - liver	0.88±0.05$^{a*, b*}$	1.80±0.02$^{a*, b*}$	10.38±0.07$^{a*, b*}$	19.22±0.24$^{a**, b*}$	16.12±0.14$^{a*, b*}$
Heart	0.66±0.06$^{a*, b*}$	2.96±0.04$^{a*, b*}$	18.90±0.14$^{a*, b*}$	27.54±0.23$^{a*, b*}$	17.16±0.25$^{a*, b*}$
Aorta	0.65±0.03$^{a*, b*}$	2.78±0.10$^{a*, b*}$	13.22±0.08$^{a*, b*}$	11.12±0.12$^{a*, b*}$	15.86±0.22$^{a*, b*}$
Group IV - liver	1.06±0.03$^{a*, b**}$	3.32±0.06$^{a*, b**}$	23.94±0.20$^{a*, b**}$	26.58±0.18$^{a*, b**}$	28.24±0.20$^{a**, b*}$
Heart	0.98±0.02$^{a**, b*}$	6.58±0.18$^{a**, b*}$	42.21±0.18$^{a**, b*}$	35.28±0.24$^{a*, b*}$	36.75±0.18$^{a*, b**}$
Aorta	2.18±0.02$^{a*, b*}$	6.48±0.18$^{a*, b**}$	20.14±0.14$^{a*, b*}$	14.28±0.10$^{a**, b*}$	26.30±0.09$^{a**, b*}$
Group V - liver	1.14±0.01$^{a*, b**}$	3.15±0.05$^{a*, b**}$	21.48±0.18$^{a*, b**}$	26.32±0.10$^{a*, b**}$	27.45±0.23$^{a**, b*}$
Heart	0.90±0.02$^{a**, b*}$	6.21±0.08$^{a**, b*}$	39.55±0.24$^{a**, b*}$	34.82±0.22$^{a*, b*}$	35.18±0.16$^{a*, b**}$
Aorta	2.10±0.02$^{a*, b*}$	6.12±0.30$^{a*, b**}$	18.26±0.18$^{a*, b*}$	13.25±0.18$^{a**, b*}$	24.82±0.08$^{a, b**}$
Group VI - liver	1.07±0.01$^{a*, b*}$	3.06±0.05$^{a*, b*}$	20.58±0.23$^{a*, b*}$	24.12±0.16$^{a*, b*}$	25.10±0.18$^{a**, b*}$
Heart	0.92±0.03$^{a*, b*}$	6.12±0.10$^{a*, b*}$	37.76±0.20$^{a*, b*}$	32.46±0.20$^{a*, b*}$	32.44±0.14$^{a*, b**}$
Aorta	2.02±0.05$^{a*, b*}$	6.20±0.30$^{a*, b*}$	18.26±0.18$^{a*, b*}$	13.12±0.14$^{a*, b*}$	22.82±0.07$^{a, b*}$
Group VII - liver	1.10±0.04$^{a*, b*}$	2.96±0.04$^{a*, b*}$	18.92±0.14$^{a*, b*}$	23.72±0.14$^{a*, b*}$	22.56±0.18$^{a*, b*}$
Heart	0.84±0.01$^{a*, b*}$	5.52±0.04$^{a*, b*}$	34.84±0.21$^{a*, b*}$	30.70±0.26$^{a*, b*}$	30.84±0.20$^{a*, b*}$
Aorta	1.88±0.04$^{a*, b*}$	5.68±0.10$^{a*, b*}$	16.32±0.20$^{a*, b*}$	12.54±0.12$^{a*, b*}$	20.68±0.14$^{a*, b*}$

*p<0.001, **p<0.05, [a]Group I compared with groups II, III, IV, V, VI and VII, [b]Group II compared with groups I, III, IV, V, VI and VII. FC: Free cholesterol, EC: Ester cholesterol, TG: Triglyceride, PL: Phospholipids, FFA: Free fatty acid

Table 3: Effect of EACB and ETCB on tissue lipid peroxidation in control and experimental animals

Groups	TBARS	CD
Group I - liver	24.16±0.22^{b*}	162.80±0.35^{b*}
Heart	39.82±0.18^{b*}	141.12±0.33^{b*}
Aorta	17.23±0.18^{b*}	166.24±0.14^{b*}
Group II - liver	72.38±0.18^{a*}	228.65±0.10^{a*}
Heart	81.26±0.20^{a*}	218.44±0.25^{a*}
Aorta	70.52±0.12^{a*}	658.75±0.56^{a*}
Group III - liver	27.58±0.34$^{a*, b*}$	172.38±0.20$^{a*, b*}$
Heart	41.22±0.18^{b*}	150.58±0.12$^{a*, b*}$
Aorta	18.76±0.18$^{a*, b*}$	318.54±0.23$^{a*, b*}$
Group IV - liver	60.92±0.30$^{a**, b*}$	211.38±0.28$^{a*, b*}$
Heart	75.85±0.20$^{a**, b**}$	202.26±0.20$^{a*, b*}$
Aorta	61.65±0.25$^{a**, b*}$	603.42±0.30$^{a*, b*}$
Group V - liver	59.24±0.38$^{a*, b*}$	208.85±0.28$^{a*, b**}$
Heart	71.45±0.24$^{a**, b**}$	198.78±0.20$^{a**, b*}$
Aorta	59.32±0.38$^{a*, b*}$	596.84±0.33$^{**.b*}$
Group VI - liver	54.54±0.24$^{a*, b*}$	202.64±0.24$^{a*, b*}$
Heart	70.28±0.22$^{a**, b**}$	195.82±0.14$^{a*, b*}$
Aorta	55.18±0.36$^{a*, b**}$	548.06±0.35$^{a*, b*}$
Group VII - liver	47.38±0.28$^{a**, b*}$	199.45±0.26$^{a*, b*}$
Heart	64.34±0.36$^{a*, b**}$	191.54±0.14$^{a*, b*}$
Aorta	51.62±0.14$^{a**, b*}$	528.33±0.18$^{a*, b*}$

TBARS: Thiobarbituric acid reactive substances, CD: Conjugated diene

The animals were fed for 90 days. At the end of the experiment, the animals were sacrificed under mild ether anesthesia and the blood was collected from the neck blood vessels and the serum was separated by centrifugation [6-9].

Biochemical analysis

Serum analysis

AS was assessed by quantifying the serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), phospholipid (PL), AST, ALT, LDH, and CPK by using the kit of Agappe Diagnostic Ltd., India. Activities of these serum parameters were measured using semi autoanalyzer (RMS, India). Low-density lipoprotein cholesterol (LDL-C) and very LDL-C (VLDL-C) were calculated as per the standard methods [10-12].

Tissue analysis

The heart was excised, washed thoroughly in ice-cold saline to remove the blood. Ten percent of homogenate was prepared in 0.1M Tris HCl buffer (pH – 7.4). The homogenate was centrifuged at 3000 rpm for 20 min at 4°C and the supernatant was used for the estimation of catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR), glutathione-S-transferase (GST), reduced glutathione (GSH), lipid peroxidation product (thiobarbituric acid reactive substances – [TBARS]), and total protein. Tissue CAT activity was determined from the rate of decomposition of H_2O_2. GPx activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H_2O_2 and NaN_3. GR activity was assayed at 37°C and 340 nm by following the oxidation of NADPH by GSSG. GST activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB. GSH was determined based on the formation of a yellow-colored complex with DTNB. The level of lipid peroxidation was measured as malondialdehyde, a TBARS, using 1'1'3'3' tetramethoxypropane as standard. Protein content in the tissue was determined using bovine serum albumin as the standard. The

Table 4: Effect of EACB and ETCB on tissue enzymatic anti-oxidants in control and experimental animals

Groups	SOD	CAT	GPx	GR	GST	Glutathione
Group I – liver	3.68±0.01^{b*}	29.54±0.25^{b*}	9.14±0.06^{b*}	1.52±0.02^{b*}	29.18±0.14^{b*}	4.938±0.04^{b*}
Heart	1.76±0.04^{b*}	50.18±0.20^{b*}	12.72±0.14^{b*}	2.76±0.01^{b*}	21.10±0.25^{b*}	7.42±0.10^{b*}
Aorta	2.89±0.03^{b*}	31.22±0.30^{b*}	15.72±0.12^{b*}	1.74±0.02^{b*}	16.94±0.14^{b*}	6.72±0.02^{b*}
Group II – liver	1.82±0.03^{a*}	17.68±0.22^{a*}	5.65±0.09^{a*}	0.69±0.01^{a*}	11.25±0.15^{a*}	1.35±0.05^{a*}
Heart	0.92±0.02^{a*}	31.55±0.18^{a*}	8.35±0.08^{a*}	1.66±0.01^{a*}	7.22±0.12^{a*}	3.58±0.12^{a*}
Aorta	1.40±0.08^{a*}	21.58±0.22^{a*}	8.40±0.08^{a*}	0.81±0.03^{a*}	8.24±0.09^{a*}	3.40±0.04^{a*}
Group III – liver	3.65±0.02$^{a*, b*}$	28.38±0.10$^{a*, b*}$	8.90±0.09$^{a*, b*}$	1.42±0.02$^{a**, b*}$	27.16±0.10$^{a*, b*}$	4.76±0.02$^{a*, b*}$
Heart	1.70±0.03$^{a*, b*}$	30.58±0.22$^{a*, b*}$	11.38±0.09$^{a*, b*}$	2.70±0.02$^{a*, b*}$	17.35±0.18$^{a*, b*}$	7.14±0.05$^{a*, b*}$
Aorta	2.83±0.02$^{a*, b*}$	30.72±0.14$^{a*, b*}$	12.10±0.09$^{a*, b*}$	1.62±0.01$^{a*, b*}$	13.72±0.10$^{a*, b*}$	6.10±0.04$^{a*, b*}$
Group IV – liver	2.25±0.01$^{a*, b*}$	19.66±0.09$^{a*, b*}$	6.22±0.06$^{a*, b*}$	0.98±0.02$^{a**, b*}$	14.26±0.12$^{a**, b**}$	2.98±0.20$^{a**, b**}$
Heart	1.16±0.02$^{a*, b*}$	23.14±0.23$^{a*, b*}$	9.38±0.14$^{a**, b*}$	1.86±0.01$^{a*, b*}$	10.68±0.12$^{a*, b**}$	4.38±0.10$^{a*, b**}$
Aorta	1.96±0.01$^{a*, b*}$	23.68±0.20$^{a*, b*}$	9.30±0.12$^{a*, b*}$	1.05±0.01$^{a*, b*}$	9.52±0.08$^{a*, b**}$	4.26±0.03$^{a**, b*}$
Group V – liver	2.38±0.02$^{a*, b**}$	20.84±0.18$^{a*, b*}$	6.54±0.08$^{a**, b**}$	1.04±0.01$^{a*, b**}$	14.66±0.16$^{a*, b*}$	3.12±0.18$^{a**, b**}$
Heart	1.20±0.02$^{a*, b*}$	24.48±0.22$^{a*, b**}$	9.58±0.14$^{a*, b**}$	2.28±0.02$^{a*, b**}$	11.56±0.14$^{a**, b*}$	4.66±0.09$^{a*, b**}$
Aorta	2.03±0.02$^{a*, b*}$	24.76±0.15$^{a**, b*}$	9.68±0.06$^{a**, b**}$	1.12±0.01$^{a**, b*}$	9.78±0.06$^{a*, b**}$	4.52±0.03$^{a**, b*}$
Group VI – liver	2.56±0.01$^{a*, b*}$	21.58±0.20$^{a*, b*}$	6.78±0.09$^{a*, b*}$	1.06±0.01$^{a**, b*}$	18.78±0.14$^{a*, b*}$	3.28±0.12$^{a*, b*}$
Heart	1.32±0.01$^{a*, b*}$	25.02±0.20$^{a*, b*}$	9.76±0.10$^{a*, b*}$	2.10±0.01$^{a*, b*}$	13.32±0.16$^{a**, b*}$	4.74±0.07$^{a*, b*}$
Aorta	2.32±0.02$^{a*, b*}$	24.94±0.28$^{a*, b*}$	9.84±0.08$^{a*, b*}$	1.20±0.01$^{a*, b*}$	10.36±0.14$^{a*, b**}$	4.90±0.04$^{a*, b*}$
Group VII – liver	2.65±0.02$^{a*, b*}$	22.92±0.14$^{a*, b*}$	7.12±0.10$^{a*, b*}$	1.10±0.01$^{a**, b*}$	20.38±0.12$^{a*, b*}$	3.62±0.14$^{a*, b*}$
Heart	1.35±0.01$^{a*, b*}$	26.52±0.23 $^{a*, b*}$	10.20±0.12$^{a*, b*}$	2.28±0.02$^{a*, b*}$	14.20±0.09$^{a*, b*}$	5.08±0.05$^{a*, b*}$
Aorta	2.36±0.03$^{a*, b*}$	26.58±0.12 $^{a*, b*}$	10.02±0.05$^{a*, b*}$	1.28±0.02$^{a*, b*}$	10.90±0.14$^{a*, b*}$	5.02±0.04$^{a*, b*}$

SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, GR: Glutathione reductase, GST: Glutathione-S-transferase

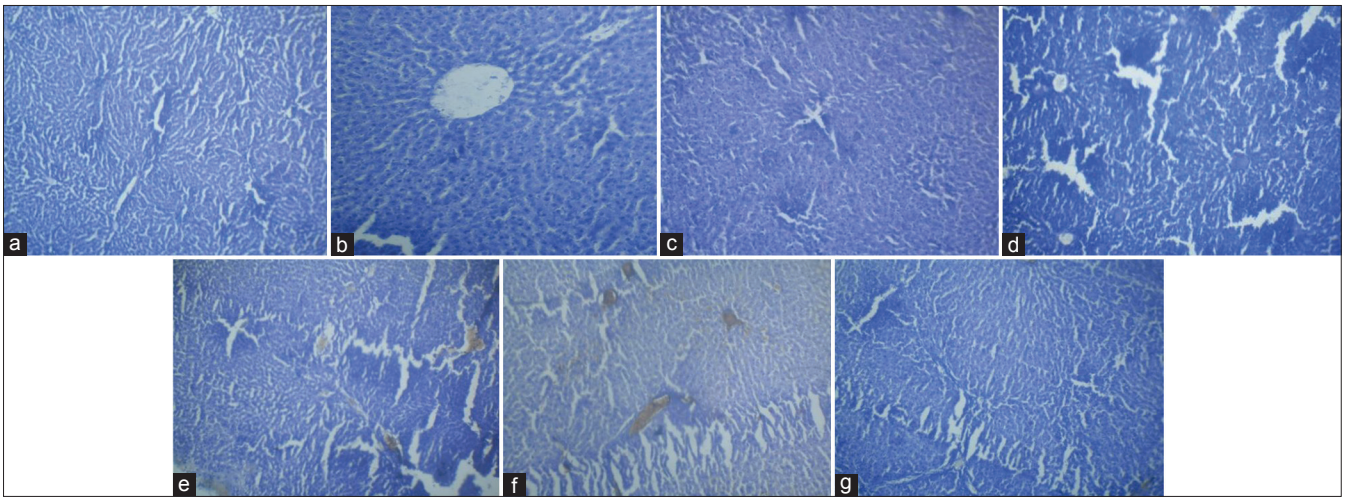

Fig. 1: Histopathological studies for liver (a-g: group I – group VII)

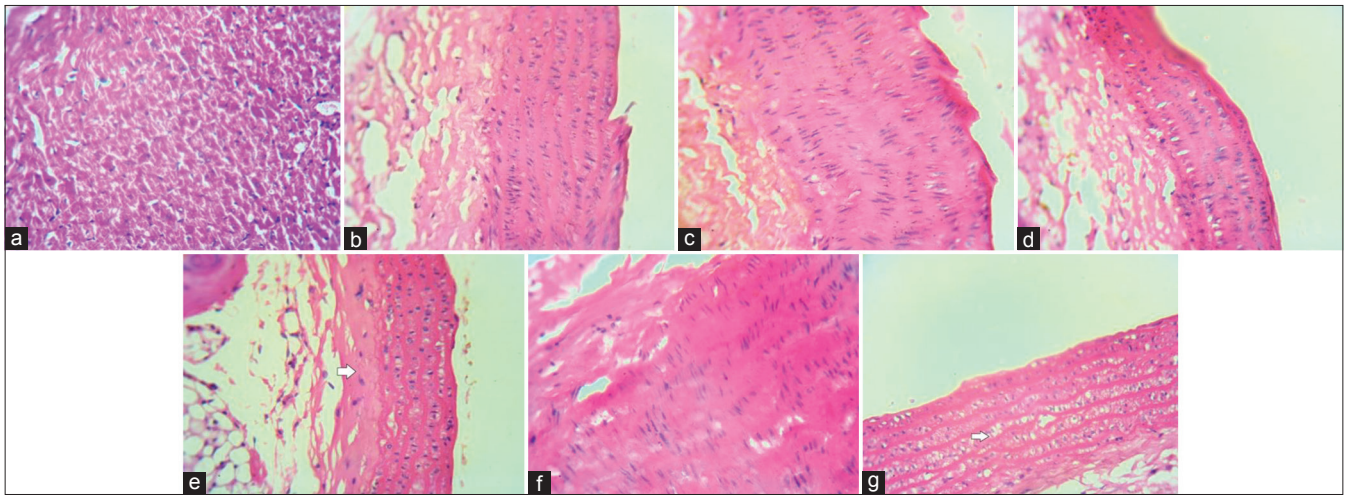

Fig. 2: Histopathological studies for aorta (a-g: Group I – Group VII)

tissue lipids were extracted. The lipid parameters, including TC, TGs, and phospholipids (PL) were estimated.

Histopathological studies

Sections were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope. The cell architecture in the liver and aorta was observed under a high-power objective in a microscope.

Statistical analysis

Results were expressed as mean ± SE of 6 rats in each group. One-way analysis of variance with Scheffe's multiple comparisons test was used to determine the statistical significance. The significance level was fixed at 0.05 [13-22].

RESULTS

Phytochemical analysis

Phytochemical analysis of EACB and ETCB revealed the presence of phytochemical constituents such as alkaloids, triterpenes, steroids, glycosides, phenols, flavonoids, saponins, and carbohydrates.

Serum analysis

Effect of EACB and ETCB on serum lipid profile and phospholipid in HFD-fed rats

HFD-fed rats produced a significant increase ($p \leq 0.05$) in serum cholesterol, TG, LDL-C, VLDL-C, and PL levels, while HDL-C decreased significantly ($p \leq 0.05$) as compared to normal animals (Table 1). Treatment with ETCB and EACB at a dose of 100 and 200 mg/kg showed a significant decrease ($p \leq 0.05$) of TC, TG, LDL-C, VLDL-C, and PL. HDL-C was increased significantly ($p \leq 0.05$) in ETCB- and EACB-treated groups (100 and 200 mg/kg). Treatment with atorvastatin (1.2 mg/kg) also prevented the elevation of TC, TG, LDL-C, VLDL-C, and PL levels. HDL-C is increased significantly in atorvastatin treatment. The protective effect of the extract was in a dose-dependent manner.

Effect of EACB and ETCB on tissue lipid contents in HFD-fed rats

Effect of ETCB and EACB on tissues (aorta, heart, and liver), free cholesterol, ester cholesterol, TG, PL, and free fatty acids levels were elevated in high-fat diet-fed rats (group II) as compared to control rats (group I) (Table 2). The tissue lipid content levels were significantly reduced in EACB and ETCB and standard drug atorvastatin along with HFD-fed rats when compared with rats fed with HFD (group II).

Effect of EACB and ETCB on tissues lipid peroxidation in HFD-fed rats

The elevated levels of TBARS and conjugated dienes (CD) were observed in the aorta, heart, and liver of high-fat diet-fed rats (group II) are a clear manifestation of excessive formation of free radicals and activation of lipid peroxidation reaction. This results in increased lipid peroxidation leading to elevated concentrations of TBARS and CD (Table 3).

Effect of EACB and ETCB on tissue enzymatic anti-oxidants in HFD-fed rats

The results indicated that the concentration of tissue enzymatic anti-oxidants such as Superoxide dismutase, CAT, GPX, GR, GST, and Glutathione decreased in tissues (aorta, heart, and liver) of HFD-fed rats as compared to the control rats. HFD-fed rats decreased the ratio of anti-oxidant enzymes in tissues. Administration of EACB and ETCB along with the HFD-fed rats increased the activities of EACB and ETCB in all the tissues compared to HFD-fed rats. A standard drug atorvastatin administered to rats also showed elevated levels of enzymatic anti-oxidants.

Effect of ETCB and EACB treatment on histopathological changes of rat in liver and aorta

Congestion of the blood vessels and steatosis of microvesicular type were observed in the liver of HFD-fed rats. Rats who received HFD+Atorvastatin showed a significant reduction in congestion of blood vessels vesicular and steatosis of microvesicular type. On the other hand, rats who received HFD+EACB (100 mg/kg) and HFD+EACB (200 mg/kg) showed a moderate reduction in congestion of blood vessels vesicular and steatosis of microvesicular type. Rats received HFD+ETCB (100 mg/kg) and HFD+ETCB (200 mg/kg) showed significant reduction in congestion of blood vessels vesicular and steatosis of micro vesicular type in the liver (Fig. 1).

The Aorta of HFD-fed rats showed deposition of fat. Rats who received HFD+Atorvastatin showed significant disappearance of fat. Rats received HFD+EACB (100 mg/kg) and HFD+EACB (200 mg/kg) showed moderate disappearance of fat deposition. Rats that received HFD+ETCB (100 mg/kg) and HFD+ETCB (200 mg/kg) showed significant disappearance of fat deposition in the aorta (Fig. 2).

CONCLUSION

The result of serum and tissue biochemical parameters, level of lipid peroxides, tissue anti-oxidants, and histopathological studies together support the highly potent anti-atherogenic and anti-oxidant activity of EACB and ETCB. These were also found to confer protection against HFD-induced AS. The lipid-lowering action of the extract may be due to the presence of triterpenoids, polyphenols, flavonoids, and saponins in it. Flavonoids inhibit the oxidation of LDL cholesterol to form foam cells and thus exert protective effects. It has also been reported that triterpenoids and flavonoids have lipoprotein lipase-releasing activity. Sitosterol, a constituent of the seed kernel of *C. bonducella*, has been reported to possess hypocholesterolemic action by inhibiting the intestinal absorption of cholesterol and accelerating the catabolism of cholesterol to bile acid. The tissue enzymatic anti-oxidants assays and the tissue lipid peroxidation assays conducted in the present study showed that the extract has anti-oxidant properties, and this might be because of the presence of phytochemicals such as flavonoids and triterpenoids in it. Flavonoids directly scavenge free radicals by acting as chain-breaking anti-oxidants or they help in recycling other chain-breaking anti-oxidants as well. They also prevent the formation of free radicals by inhibiting several pro-oxidant enzymes like lipooxygenase. Triterpenoids cause an increase in the content of transcription factor, nuclear factor- erythroid-2related factor 2 (Nrf2) and thereby increases the expression of its target gene products like CAT. Triterpenoids also decrease the expression of nuclear factor kappaβ and thus contributes to the attenuation of oxidative stress.

In this study, the administration of HFD to the rats for 90 days resulted in an increase in their body weights. An increased consumption of HFD causes a change in the composition of the intestinal microbial community, which promotes hepatic lipogenesis and enhances lipoprotein lipase-directed incorporation of TGs into adipocytes; this, in turn, increases body weight and obesity. The extract inhibited the gain in body weight probably by restoring the composition of the gut microflora and thereby inhibiting hepatic lipogenesis and lipoprotein lipase activity.

Thus, although the exact mechanism for the anti-atherogenic action of the seed kernel of *C. bonducella* remains unknown, it can be presumed that the presence of medicinally important phytochemicals and anti-oxidants in the seed kernel of *C. bonducella*, is responsible for its anti-atherogenic activity. It can be concluded that ETCB may be a beneficial anti-atherosclerotic agent and is more potent than EACB. However, further studies to find out the exact mechanism of anti-atherogenic action and to elucidate the active principle responsible for the anti atherogenic action can be carried out.

CONFLICTS OF INTERESTS

The authors claim to have no conflicting interests.

REFERENCES

1. Lusis AJ. Atherosclerosis. Nature 2000;407:233-41. doi: 10.1038/35025203, PMID 11001066

2. Ikewuchi CJ, Ikewuchi CC. Alteration of plasma lipid profile and atherogenic indices of cholesterol loaded rats by *Tridax procumbens* Linn: Implications for the management of obesity and cardiovascular diseases. Biokemistri 2009;21:95-9. doi: 10.4314/biokem.v21i2.56477
3. Sarma G, Das S. The hypolipidemic activity of ethanolic extract of seed kernel of *Caesalpinia bonducella* Fleming on serum lipids and atherogenesis in albino rats fed with high fat diet. Int J Basic Clin Pharmacol 2018;7:266-72. doi: 10.18203/2319-2003.ijbcp20180096
4. Wagner H, Bladt S, Zgainski EM. Plant Drug Analysis. Berlin, Heidelberg: Springer Verlag; 1984.
5. Kumar SD, Muthu KA. Evaluation of hypolipidemic activity of various extracts from whole plant of *Ionidium suffruticosum* (Ging.) (Family: *Violaceae*) in rat fed with high fat diet. Int J Pharm Pharm Sci 2012;4:69-73.
6. Ray SK, Rege NN. Atorvastatin in the management of hyperlipidemia. J Postgrad Med 2000;46:242-3. PMID 11298482
7. Choudhary R. Beneficial effect of *Allium sativum* and *Allium tuberosum* on experimental hyperlipidemia and atherosclerosis. Pak J Physiol 2008;4:7-9.
8. Saikrishna N, Ranganayakulu D, Gopisudheerkumar J, Vijaykumar G, Anusha M. Antiatherosclerotic activity of the ethanolic extract of the *Butea monosperma* leaves as alone and in combination with the atorvastatin. J Adv Drug Res 2010;1:20-6.
9. Muddapur UM, More S, Karekar T, Mungi H, Thakkannavar S, Savadatti N. Evaluation of acute and subacute toxicity effect of ethanol extract from *Caesalpinia bonducella* seed kernel. J Pharmacogn Phytochem 2014;3:1204.
10. Prabha SP, Ansil PN, Nitha A, Wills PJ, Latha MS. Anti-atherogenic activity of methanolic extract of *Gardenia gummifera* Linn.f. on high fat diet induced atherosclerosis in rats. Int J Pharm Pharm Sci 2013;5:388-93.
11. Beutler E. Red Cell Metabolism: A Manual of Biochemical Methods. 3rd ed. New York: Grune and Startton; 1984.
12. Folch J, Lees N, Solane S. A simple method for the isolation and purification of total lipids from animal tissue. J Biol Chem 1957;226:497-509.
13. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974;249:7130-9. doi: 10.1016/S0021-9258(19)42083-8, PMID 4436300
14. Shyamala MP, Venukumar MR, Latha MS. Antioxidant potential of the *Syzygium aromaticum* (Gaertn.) Linn. (cloves) in rats fed with high fat diet. Indian J Pharmacol 2003;35:99-103.
15. Beers RF Jr., Sizer IW. A spectrophotographic method for measuring the breakdown of hydrogen peroxide by catalase. J Biol Chem 1952;195:133-40. doi: 10.1016/S0021-9258(19)50881-X, PMID 14938361
16. Allain CC, Poon LS, Chan CS, Richmond W, Fu PC. Enzymatic determination of total serum cholesterol. Clin Chem 1974;20:470-5. doi: 10.1093/clinchem/20.4.470, PMID 4818200
17. Izzo C, Grillo F, Murador E. Improved method for determination of high-density-lipoprotein cholesterol I. Isolation of high-density lipoproteins by use of polyethylene glycol 6000. Clin Chem 1981;27:371-4. doi: 10.1093/clinchem/27.3.371, PMID 7471383
18. Jain RC. Onion and garlic in experimental cholesterol induced atherosclerosis. Indian J Med Res 1976;64:1508-15.
19. Allouche Y, Beltrán G, Gaforio JJ, Uceda M, Mesa MD. Antioxidant and anti atherogenic activities of pentacyclic triterpenic diols and acids. Food Chem Toxicol 2010;48:2885-90. doi: 10.1016/j.fct.2010.07.022, PMID 20650302
20. Carlberg I, Mannervik B. Glutathione reductase. Methods Enzymol 1985;113:484-90. doi: 10.1016/s0076-6879(85)13062-4, PMID 3003504
21. Aminzadeh MA, Reisman SA, Vaziri ND, Khazaeli M, Yuan J, Meyer CJ. The synthetic triterpenoid RTA dh404 (CDDOdhTFEA) restores Nrf2 activity and attenuates oxidative stress, inflammation, and fibrosis in rats with chronic kidney disease. Xenobiotica 2014;44:570-8. doi: 10.3109/00498254.2013.852705, PMID 24195589
22. Theriault A, Wang Q, Van Iderstine SC, Chen B, Franke AA, Adeli K. Modulation of hepatic lipoprotein synthesis and secretion by taxifolin, a plant flavonoid. J Lipid Res 2000;41:1969-79. doi: 10.1016/S0022-2275(20)32358-0, PMID 11108730