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Ethanolic extract of cashew apple inhibits lipid metabolism and ameliorates obesity in atherogenic diet−induced obese rats

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ABSTRACT

Objective: To evaluate the anti-obesity activity of ethanolic extract of cashew apple using various *in vitro* and *in vivo* models.

Methods: Phytochemical screening was carried out in ethanolic extract of cashew apple, followed by quantification of phenol and flavonoid. Antioxidant potential was evaluated using 2,2-diphenyl-1-picrylhydrazyl and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) scavenging assays. The inhibitory effect of ethanolic extract of cashew apple on α-amylase and pancreatic lipase was also studied. In addition, anti-obesity activity was determined in two *in vivo* models, lipid emulsion model and atherogenic diet-induced obese rat model. Levels of postprandial plasma triglycerides were assessed in lipid emulsion model, whereas serum lipid profile, *in vivo* antioxidants and histopathological studies of the carotid artery and liver were performed in an atherogenic diet-induced obese model.

Results: Phytochemical screening revealed the presence of carbohydrates, alkaloids, polyphenols, terpenoids, and steroids. The *in vitro* assays showed inhibition of α-amylase and pancreatic lipase and strong antioxidant potential. Ethanolic extract of cashew apple showed significant and time-dependent inhibitory activity on postprandial triglycerides after administration of lipid emulsion for 5 h. Ethanolic extract of cashew apple at 200 and 400 mg/kg on day 60 showed a significant reduction in body weight, body mass index and atherogenic index, whereas lipid profile and liver function marker levels in the serum were decreased in a dose-dependent manner at time intervals (day 0, 20, 40, and 60) compared to the atherogenic diet-induced obese rats. Histological observations showed reduced non-alcoholic fatty liver deposits and decreased atherosclerotic fatty streak plaques (carotid artery) after treatment with ethanolic extract of cashew apple.

Conclusions: Ethanolic extract of cashew apple ameliorates obesity, which may be partly mediated by its delayed absorption of cholesterol and carbohydrates.

1. Introduction

Obesity is often defined as a variation between intake of energy and its expenditure[1]. Since decades, studies have been reporting that excessive intake of calories develops many chronic ailments, including obesity, type 2 diabetes (also known as adult-onset diabetes), hyperlipidemia, and cardiovascular diseases[2]. An earlier

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study revealed that an atherogenic high-fat diet containing 1% cholesterol caused various health issues like obesity and coronary heart diseases[3]. Moreover, it was evidenced that persistence of hypercholesterolemia led to obesity through enhanced oxidative stress[4]. Therefore, the diet containing cholesterol was employed in the study to assess the anti-obeisty activities of selected nutraceutical. In addition, research focused on the development of lipid absorption inhibitors to reduce energy intake and to prevent obesity.

The manipulation of essential enzymes involved in lipid digestion and absorption has paved a new way to reduce energy intake through gastrointestinal mechanisms and this strategy has received much attention in the obesity therapy[5]. Though drugs like orlistat, acarbose, miglitol, and voglibose are known enzyme inhibitors to reduce lipid absorption and have played an important role in the obesity treatment, they are compromised with gastrointestinal adverse effects like oily stools, flatulence, and diarrhea[6]. Hence, in the current situation, the inhibition of the breakdown of complex carbohydrates and fats occupied a major research area, which further helps to develop potent nutraceuticals, supplements, and pharmaceuticals.

Cashew apple (*Anacardium occidentale* L.), belonging to the family Anacardiaceae, is a small tree grown in many tropical countries, with great economic and medicinal value including antitumor, antimicrobial, urease inhibitory and lipoxygenase enzyme activity[7]. Studies reported the antioxidant potential of the juice made from cashew apple by proving their ability to scavenge free peroxyl radicals[8]. Crushed cashew apple pulp (Cashewin™) was found to reduce body-weight gain, fat storage, hyperglycemia, hyperinsulinemia, and insulin resistance in the diet-induced obese mouse model. Also, Cashewin™ was reported to contain 5% total polyphenols which include myricetin and quercetin derivatives[9]. According to another study, cashew apple juice was found to improve the exercise performance in athletes by increasing the fat oxidation[10]. We hypothesized that extract prepared from the whole cashew apple without removing the juice may contain the higher amount of bioactive polyphenols which might be useful in obesity treatment. Thus, the study was aimed to verify the anti-obesity potential of cashew apple employing standard *in vitro* and *in vivo* models.

2. Materials and methods

2.1. Chemicals and reagents

Chemicals like 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) were procured from Sigma Chemical Co. (St. Louis, MO, USA). Porcine pancreatic lipase-type Ⅱ (PPL), α-amylase and all other chemicals of analytical grade were obtained from Hi-media laboratories, India. The kits for biochemical estimation were procured from Erba Mannheim, India.

2.2. Plant material

Fresh cashew apples were collected from the local market of Nellore district, Andhra Pradesh, India during March 2016. Cashew apples were authenticated by Dr. K. Madavachetty from Department of Botany, Sri Venkateswara University, Tirupati and the voucher specimen was No. 0976.

2.3. Extract preparation

The freshly procured cashew apples were cut into pieces and shade dried at room temperature. Cashew apple powder (250 g) was extracted with 90% ethanol (1:9 w/v) using Soxhlet apparatus in 3 h cycles until the solvent became pale. Rotary evaporator at 30-40 ℃ was used for concentrating the collected solvent to semi-solid mass which was designated as an ethanolic extract of cashew apple. The extract was dried and preserved in storage vials at 4 ℃ until further investigation.

2.4. Phytochemical screening

The ethanolic extract of cashew apple was screened for phytochemical constituents such as alkaloids, terpenoids, flavonoids, saponins, steroids and phenolic compounds using qualitative phytochemical methods available in the literature[11].

2.5. Determination of total phenolic content

Folin-Ciocalteu method was used to estimate the total phenolic content[12]. A total of 0.5 mL of Folin–Ciocalteu reagent and 2 mL of 20% of sodium carbonate were added to 200 μL of the extract at 100, 200, 400, 800 and 1 600 μg/mL and mixed thoroughly. The resulting solution was then placed in the dark at room temperature for 30 min and the absorbance was measured at 765 nm. A similar procedure was carried out for the standard gallic acid and the calibration curve was constructed by plotting the absorbance against concentration. The total content was calculated from the standard graph, and results were expressed as milligrams of gallic acid equivalent (GAE)/g of extract.

2.6. Determination of total flavonoid content

Total flavonoid content was determined according to an earlier method[13] at 100, 200, 400, 800 and 1 600 μg/mL with quercetin as a standard. Ethanolic extract of cashew apple (0.5 mL) was added to the 2% of $AlCl_3$ and kept at normal regular temperature, and all the concentrations were determined in triplicates. After 1 h, the absorbance was measured at 420 nm. The total flavonoid content was calculated from the reference compound curve. The results were expressed as mg quercetin equivalent (QE)/g of extract.

2.7. In vitro assays

2.7.1. DPPH assay

Stock solution (1 mg/mL) of the crude extract in distilled water was used. Ethanolic extract of cashew apple at different concentrations was assessed for free radical scavenging ability using DPPH assay[14]. A total of 3 mL of freshly prepared DPPH in methanol (0.002%) was added with 1 mL of ethanolic extract of cashew apple (100, 200, 400, 800 and 1 600 μg/mL) and then was kept in darkness for 15 min. All the samples were determined in triplicates and the absorbance was recorded at 515 nm. Linear regression analysis was used to calculate the inhibitory concentration (IC_{50}). The percentage of inhibition of DPPH was calculated using the following formula:

(%) Inhibition of DPPH=[Abs (control)-Abs (sample)]/[Abs (control)] ×100

2.7.2. ABTS radical scavenging activity

Stock solution (1 mg/mL) of the crude extract in distilled water was used. The ethanolic extract of cashew apple was evaluated for antioxidant activity using $ABTS^{\bullet+}$ radical cation decolorization assay[15]. Breifly, 7 mM ABTS solution in the H_2O was mixed with 2.45 mM potassium persulphate mixture in H_2O at the ratio of 1:1 (v/v). This working solution was kept in darkness at room temperature for 16 h, then diluted with ethanol until the absorbance was (0.706 ± 0.010) units at 734 nm. A total of 200 μL of ethanolic extract of cashew apple (100, 200, 400, 800 and 1 600 μg/mL) was added with 2.9 mL of ABTS working solution. An equal amount of ethanol (200 μL) was taken as a blank (control). All the concentrations were prepared in triplicates and vortexed for 2 min. After 6 min, the absorbance of the sample was taken against ethanol as blank at 734 nm and the results were reported as IC_{50} values.

(%) Inhibition of ABTS= [Abs (control)-Abs (sample)]/[Abs (control)]×100

2.7.3. α –amylase inhibition assay

Inhibition of α-amylase was determined as per the earlier described method[16]. Ethanolic extract of cashew apple (0.5 mL) at 100, 200, 400, 800 and 1 600 μg/mL was mixed with 0.5 mL of sodium phosphate buffer (0.02 M) containing α-amylase solution (0.5 mg/mL) and incubated for 10 min at 25 ℃. To the pre-incubated solution, 0.5 mL of 1% starch prepared in 0.02 M phosphate buffer was added to each tube. The reaction mixture was allowed to stand for 10 min at 25 ℃ and the reaction was terminated by adding 1 mL of dinitrosalicylic acid color reagent. The test tubes were then kept in a boiling water bath for 5 min and cooled to room temperature. The same procedure was followed for control samples without the enzyme. The reaction mixture was diluted with 10 mL of distilled water and the absorbance was recorded using spectrophotometer. Acarbose was used as positive control at the same concentrations as the test sample. Inhibition percentage of α-amylase was calculated using the following formula:

(%) α-amylase inhibition=[Abs (control)-Abs (sample)]/[Abs (control)]×100

2.7.4. Pancreatic lipase inhibition assay

Inhibition of pancreatic lipase was estimated using *p*-nitrophenyl butyrate as substrate[17]. The PPL (type Ⅱ) stock solution at 1 mg/mL was prepared in 0.1 mM phosphate buffer (pH 6.0). To measure lipase inhibitory activity, 200 μL of ethanolic extract of cashew apple at 1, 2, 4, 8 and 16 mg/mL were pre-incubated with 200 μL of PPL for 1 h in potassium phosphate buffer at 30 ℃. The reaction was initiated by adding 150 μL of nitrophenyl butyrate, and then incubated for 5 min. The *p*-nitrophenol released in the reaction mixture was measured at 405 nm using UV-spectrophotometer. Orlistat was taken as a standard drug (positive control) and the above procedure was repeated at the same concentrations as the test sample. Dimethylsulfoxide was used as negative control and control was kept with and without inhibitor.

% Inhibitory activity=100-[(B-b)/(A-a)×100]

Where A is the activity without inhibitor; a is the negative control without inhibitor; B is the activity with inhibitor; and b is negative control with inhibitor.

2.8. In vivo studies

2.8.1. Experimental animals

Adult male Wistar rats weighing 180-200 g were used. Rats were kept under standard air conditioning maintaining the normal room temperature (25 ± 1) ℃ with 45% to 55% of relative humidity and 12:12 h light and dark cycle for one week with food and water provided *ad libitum*. All procedures were done according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee with No. 1677/PO/Re/S/2012/ CPCSEA/01, dated 6th May 2016.

2.8.2. Dose fixation

Based on the previous study on cashew apple, the dose of ethanolic extract of cashew apple was fixed at 200 and 400 mg/kg, *p.o.* to determine its effect in the lipid emulsion model and anti-obesity activity[18].

2.8.3. Estimation of postprandial plasma triglyceride (TG) levels using lipid emulsion model

In order to assess the ability of ethanolic extract of cashew apple to inhibit lipid digestion and absorption, postprandial TG levels were assessed after administration of lipid emulsion[19]. Rats were divided into five groups (n=6) and fasted overnight. Animals of group one received vehicle 1% carboxymethyl cellulose; Group two received lipid emulsion (*p.o.*); Group three received lipid emulsion and standard drug (atorvastatin, 10 mg/kg *p.o.*); Group four and five received lipid emulsion along with ethanolic extract of cashew apple at 200 and 400 mg/kg, *p.o.* Deionized water was added to 7 mL of olive oil, 93 mg of cholic acid for the preparation of lipid emulsion. Food was withdrawn throughout the experimental procedure. At 0, 1, 2, 3, 4 and 5 h, blood samples were collected from retro-orbital plexus using the heparinized capillary tube and centrifuged at 6 300 rpm for 10 min. Commercially available triglycerides assay kit was

used to measure TG levels in the plasma (Erba diagnostics).

2.8.4. Atherogenic diet–induced obesity in male Wistar rats

2.8.4.1. Preparation of diet

Atherogenic diet was prepared by mixing 1% cholesterol, 0.5% cholic acid and 5% olive oil with 100 g of normal rat diet[20]. Atherogenic diet was freshly prepared daily, dried and fed along with water *ad libitum* for 60 d for the induction of obesity in rats.

2.8.4.2. Experimental design

Male Wistar rats weighing 180-220 g were randomly divided into five groups of six rats each and kept on following the treatment schedule for 60 d. Animals of group one were given the vehicle 1 % carboxymethyl cellulose and taken as a normal control. Group two received atherogenic diet and served as an obese control. Group three was fed with an atherogenic diet and a standard drug (atorvastatin, 10 mg/kg, *p.o.*). Group four and five received atherogenic diet with ethanolic extract of cashew apple at 200 mg/kg and 400 mg/kg, *p.o.*, respectively which served as a low dose treatment group and a high dose treatment group.

On day 0 and 60, percentage increase in body weight was calculated as following formula and body mass index (BMI) was calculated as per literature[21].

% increase in body weight = (Final body weight-Initial body weight)/(Initial body weight) ×100

BMI= Body weight/ length^2

Blood was withdrawn from retro-orbital plexus on day 0, 20, 40, and 60. Serum was separated by centrifuging blood samples for 5 min at 2 500 rpm to estimate biochemical parameters including lipid profile [total cholesterol (TC), trigylerides (TG), high-density lipoproteins (HDL), very-low-density lipoproteins (VLDL), and low-density lipoproteins (LDL)] and liver function markers [aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP)] using commercially available autoanalyzer kits in an autoanalyzer (Erba Mannheim, EM- 200). Moreover, atherogenic index (AI) and % protection were calculated according to the following formula[22,23].

AI=(TC-HDL)/HDL

% Protection = [AI (control) – AI (treated)]/[AI (control)]×100

2.8.4.3. Isolation of carotid artery and liver

All animals were anesthetized on day 60 by administering ketamine (80 mg/kg, *i.p.*) and xylazine (5 mg/kg, *i.p.*). A sterile scalpel was used to make a midline incision and the left carotid artery was wrapped with cotton dipped in 1% $FeCl_3$ solution. After 30 min, the artery was ligated on both the ends with nylon structure and excised carefully[24]. Animals were sacrificed immediately employing cervical dislocation and two liver lobes were collected for the estimation of *in vivo* antioxidant status. Furthermore, the excised carotid artery and remaining liver lobe were fixed in the 10% formalin solution. After fixation, the tissues were dehydrated through ascending grades of ethanol. Thereafter, it was cleared in xylene and finally embedded in paraffin wax. Using a rotary microtome, specimens were sectioned at 5 μm and sections were mounted on clean slides and stained with hematoxylin-eosin. The histopathological examinations of liver sections and carotid artery were carried out using Olympus microscope (Model BX43) at 40 × magnification.

2.8.4.4. Measurement of endogenous antioxidants in liver homogenate

Teflon coated homogenizer was used for the homogenization of the excised liver in phosphate buffer and centrifuged at 3 000 rpm for 15 min at 4 ℃ to separate the nuclear debris. The supernatant of the liver homogenate was used to estimate the endogenous antioxidants including catalase (CAT), superoxide dismutase (SOD), and reduced glutathione (GSH)[25]. The oxidative stress levels were measured in terms of malondialdehyde (MDA) content[26].

2.9. Statistical analysis

All results were indicated as mean ± SEM and IC_{50} values were estimated using linear regression analysis. The statistical significance was interpreted using unpaired student *t*-test for *in vitro* assays. Statistical significance was analyzed by one way/two–way analysis of variance (ANOVA) choosing Bonferroni post-test using Graph Pad Prism version 5 software and $P < 0.05$ was considered significant.

3. Results

3.1. Preliminary phytochemical analysis and yield

The preliminary phytochemical screening of the ethanolic extract of cashew apple showed the presence of carbohydrates, alkaloids, polyphenols, terpenoids, and steroids. The yield of the extract obtained was 5.24 g/100 g of dry material.

3.2. Estimation of total phenolic and flavonoid content

Total phenolic content in ethanolic extract of cashew apple was (209.58 ± 1.23) mg GAE/g and the total flavonoid content was (249.60 ± 1.40) mg QE/g of extract. Total phenolic content was calculated from standard curve of gallic acid (standard curve equation: y= 0.002 8x+0.062 5, R^2=0.992 7) and total flavonoid content was calculated by using the quercetin as a standard (equation of standard curve: y= 0.002 5x+0.366, R^2= 0.998 1).

3.3. In vitro assays

DPPH assay showed that the IC_{50} value of ethanolic extract of cashew apple was (636.50 ± 21.41) μg/mL while the IC_{50} value of rutin was (773.60 ± 49.04) μg/mL ($P < 0.05$). Furthermore, the percentage inhibition of ethanolic extract of cashew apple against

DPPH radical was 68.07% at 1 600 μg/mL, comparable to standard rutin (73.40%) (Supplementary Figure 1).

ABTS assay showed that IC_{50} value of ethanolic extract of cashew apple was (595.70 ± 36.42) μg/mL, while the IC_{50} of rutin was (844.80 ± 13.07) μg/mL ($P < 0.05$). The percentage inhibition of ethanolic extract of cashew apple and rutin was 70.67% and 68.6%, respectively at 1 600 μg/mL (Supplementary Figure 2).

The IC_{50} values of ethanolic extract of cashew apple and acarbose for the inhibition of α-amylase were found to be (716.56 ± 3.19) and (896.99 ± 4.96) μg/mL, respectively. Similarly, IC_{50} values of ethanolic extract of cashew apple and orlistat for the inhibition of pancreatic lipase were (7.16 ± 0.47) and (6.28 ± 0.68) mg/mL, respectively ($P < 0.05$) (Supplementary Figure 3).

3.4. Effect of ethanolic extract of cashew apple on postprandial plasma TG levels after oral administration of lipid emulsion

Both ethanolic extract of cashew apple (at 200 and 400 mg/kg) and atorvastatin (10 mg/kg) significantly reduced the plasma TG from 1 h onwards, when compared to the disease control rats which were given lipid emulsion alone ($P < 0.05$) (Figure 1).

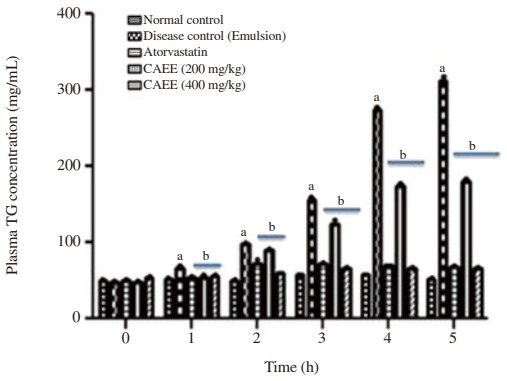

Figure 1. Effect of ethanolic extract of cashew apple on postprandial plasma triglyceride (TG) levels after oral administration of lipid emulsion. Values were expressed as mean ± SEM (n=6). [a]$P < 0.05$ when compared with the normal group; [b]$P < 0.05$ when compared with the control group. CAEE: ethanolic extract of cashew apple.

Table 1. Effect of CAEE on BMI, body weight, % increase in body weight, atherogenic index and % protection.

Group	BMI (g/cm^2)		Body weights		% increase in body weight	Atherogenic index (TC-HDL)/HDL	% Protection
	Day 0	Day 60	Day 0	Day 60			
Normal	0.72±0.02	0.77±0.01	206.66±6.66	223.33±3.33	7.44±2.78	2.85±0.07	-
AD	0.80±0.07	2.23±0.09[a]	196.66±6.14[a]	405.00±6.70[a]	51.41±1.36[a]	32.16±1.74[a]	-
AD + atorvastatin (10 mg/kg)	0.68±0.07	0.86±0.05[b]	193.33±6.66[b]	236.66±12.01[b]	14.74±3.61[b]	4.73±0.24[b]	85.18±1.29
AD + CAEE (200 mg/kg)	0.68±0.02	1.65±0.12[b]	196.66±6.14[b]	338.33±13.27[b]	41.65±1.79[b]	17.26±0.56[b]	46.40±3.20
AD + CAEE (400 mg/kg)	0.77±0.03	0.78±0.02[b]	210.00±4.47[b]	226.66±6.66[b]	8.58±2.15[b]	3.40±0.15[b]	89.36±0.38

AD: atherogenic diet; BMI: body mass index; TC: total cholesterol; HDL: high density lipoproteins; CAEE: ethanolic extract of cashew apple. Values were expressed as mean ± SEM of 6 rats per group; [a]$P < 0.05$ when compared with the normal group; [b]$P < 0.05$ when compared with AD obese group.

Table 2. Effect of ethanolic extract of cashew apple on lipid profile (mg/dL).

Group	Day	TC	TG	HDL	VLDL	LDL
Normal	0	49.88±2.51	63.05±1.54	16.65±0.23	17.80±0.18	16.50±0.21
	20	53.46±2.13	63.53±1.17	16.40±0.27	17.35±0.20	18.63±0.23
	40	52.91±1.76	63.85±1.48	15.65±0.22	17.68±0.25	18.12±0.37
	60	64.21±1.11	61.00±1.20	16.68±0.16	18.43±0.22	18.26±0.29
AD	0	49.13±2.58	56.73±2.21	16.26±0.20	17.76±0.33	17.45±0.32
	20	133.11±2.64[a]	96.91±0.92[a]	14.23±0.29[a]	29.58±1.13[a]	29.20±0.81[a]
	40	226.5±8.82[a]	137.20±1.43[a]	11.98±0.29[a]	52.06±1.30[a]	45.38±1.50[a]
	60	306.10±12.08[a]	190.85±2.51[a]	9.26±0.19[a]	79.53±2.18[a]	76.63±0.78[a]
AD + atorvastatin (10 mg/kg)	0	49.70±1.79	58.68±2.12	16.56±0.30	18.50±0.67	17.36± 0.30
	20	71.13±1.41[b]	71.16±0.82[b]	15.60±0.14[b]	17.53±0.53[b]	17.41±0.31[b]
	40	72.80±1.31[b]	82.63±1.45[b]	15.05±0.22[b]	20.90±0.63[b]	21.76±0.89[b]
	60	86.73±2.68[b]	95.65±1.23[b]	15.50±0.26[b]	26.40±0.87[b]	19.60±0.52[b]
AD + CAEE (200 mg/kg)	0	49.33±2.02	57.46±1.32	17.13±0.18	17.75±0.45	17.96±0.46
	20	112.93±5.27[b]	85.50±2.13[b]	14.48±0.12[b]	20.01±0.52[b]	19.46±0.36[b]
	40	172.05±4.46[b]	95.65±1.23[b]	14.20±0.20[b]	30.53±0.79[b]	25.40±1.12[b]
	60	203.98±5.63[b]	127.05±2.60[b]	11.20±0.32[b]	53.88±1.65[b]	27.66±0.87[b]
AD + CAEE (400 mg/kg)	0	50.81±1.82	55.84±2.50	16.83±0.29	18.06±0.12	19.45±0.65
	20	65.03±1.69[b]	70.18±0.40[b]	16.55±0.27[b]	17.71±0.23[b]	18.50±0.23[b]
	40	70.23±0.93[b]	73.80±0.94[b]	16.23±0.19[b]	19.00±0.57[b]	15.28±1.40[b]
	60	74.66±1.61[b]	70.50±1.65[b]	17.00±0.36[b]	20.88±0.83[b]	18.91±0.33[b]

CAEE: ethanolic extract of cashew apple; AD: atherogenic diet; TC: total cholesterol; TG: triglycerides; LDL: low density lipoprotein; VLDL: very low density lipoprotein; HDL: high density lipoproteins. Values were expressed as mean ± SEM. [a]$P < 0.05$ when compared to the normal group; [b]$P < 0.05$ when compared to AD group.

3.5. Effect of ethanolic extract of cashew apple in atherogenic diet–induced obese rat model

3.5.1. Effect of ethanolic extract of cashew apple on BMI, AI and % protection

BMI, body weight and AI were significantly increased in the atherogenic diet-induced obese group on day 60 compared to the normal group. Treatment with ethanolic extract of cashew apple significantly ($P < 0.05$) ameliorated the increased BMI and body weight compared to atherogenic diet–induced obese group. AI was reduced in the rats treated with atorvastatin and ethanolic extract of cashew apple at both low and high doses. Besides, % protection of ethanolic extract of cashew apple at 400 mg/kg was similar to that of atorvastatin (Table 1).

3.5.2. Effect of ethanolic extract of cashew apple on the serum lipid profile

Administering atherogenic diet significantly ($P < 0.05$) increased serum TC, TG, LDL and VLDL phospholipids and reduced HDL levels compared with the normal group. Treatment of atorvastatin (10 mg/kg) and ethanolic extract of cashew apple (400 mg/kg) maintained the lipid profile at the normal level compared to the atherogenic diet–induced obese group on day 20, 40 and 60. At 200 mg/kg, ethanolic extract of cashew apple reduced TC, TG, LDL, VLDL and increased HDL levels when compared to the disease control (Table 2). The effect of ethanolic extract of cashew apple was dose dependent and comparable to that of atorvastatin.

3.5.3. Effect of ethanolic extract of cashew apple on liver biomarkers

The levels of AST, ALT and ALP were significantly ($P < 0.05$) increased in atherogenic diet-induced obese group compared to the normal group. Treatment with atorvastatin and ethanolic extract of cashew apple at 200 and 400 mg/kg decreased the levels of AST, ALT, and ALP in a dose-dependent manner (Table 3).

3.5.4. Effect of ethanolic extract of cashew apple on carotid artery histology

Histopathological observations of the carotid artery of the atherogenic diet-induced obese group showed degenerative changes in the endothelium membrane (Figure 2B). Atorvastatin (10 mg/kg) and ethanolic extract of cashew apple (200 and 400 mg/kg) revealed a remarkable decrease in morphological alterations of endothelium (Figure 2C-2E).

3.5.5. Effect of ethanolic extract of cashew apple on liver histology

The histopathological examination of the liver in normal group showed normal cytoarchitecture of the liver (Figure 3A) whereas in animals with atherogenic diet–induced obesity, the liver showed lipid droplets near central vein along with necrotic changes, indicating degenerative changes (Figure 3B). However, the administration of atorvastatin and ethanolic extract of cashew apple (200 and 400 mg/kg) offered protection from the necrotic changes by maintaining the normal cytoarchitecture of the liver as in the normal group (Figure 3C-3E).

3.5.6. Effect of ethanolic extract of cashew apple on liver lipid peroxidation and antioxidant enzymes

In atherogenic diet–induced obese group, CAT, SOD and GSH levels were significantly ($P < 0.05$) decreased and MDA was increased compared to the normal rats (Table 4). However, atorvastatin (10 mg/kg) and ethanolic extract of cashew apple (200 and 400 mg/kg) significantly increased CAT, SOD, GSH levels and decreased MDA level (Table 4).

Table 3. Effect of ethanolic extract of cashew apple on liver enzymes (IU/L).

Groups	Day	AST	ALT	ALP
Normal	0	2.66±0.26	2.28±0.11	2.31±0.17
	20	2.62±0.10	2.16±0.03	2.25±0.17
	40	2.63±0.13	2.23±0.08	2.42±0.12
	60	2.57±0.07	2.65±0.16	2.23±0.12
AD	0	2.65±0.18	2.50±0.13	2.13±0.15
	20	4.49±0.14[a]	3.44±0.14[a]	4.42±0.11[a]
	40	8.00±0.13[a]	7.63±0.15[a]	8.22±0.13[a]
	60	9.89±0.15[a]	9.88±0.15[a]	12.13±0.19[a]
AD + atorvastatin (10 mg/kg)	0	2.86±0.26	2.36±0.09	2.25±0.19
	20	2.37±0.11[b]	2.34±0.13[b]	2.86±0.16[b]
	40	3.40±0.12[b]	3.61±0.09[b]	3.49±0.12[b]
	60	3.36±0.05[b]	3.65±0.11[b]	3.08±0.03[b]
AD + CAEE (200 mg/kg)	0	2.58±0.38	2.13±0.04	1.96±0.21
	20	3.45±0.12[b]	3.07±0.04[b]	3.87±0.03[b]
	40	4.86±0.22[b]	6.24±0.12[b]	6.35±0.07[b]
	60	5.46±0.14[b]	6.80±0.17[b]	7.52±0.18[b]
AD + CAEE (400 mg/kg)	0	2.78±0.20	2.52±0.11	2.23±0.20
	20	2.59±0.12[b]	2.44±0.11[b]	2.50±0.10[b]
	40	3.47±0.23[b]	3.06±0.14[b]	2.80±0.24[b]
	60	2.89±0.06[b]	3.45±0.06[b]	2.81±0.14[b]

CAEE: ethanolic extract of cashew apple; AD: atherogenic diet; AST: aspartate aminotransferase; ALT: alanine aminotransferase; ALP: alkaline phosphatase. Values were expressed as mean ± SEM of 6 observations; [a]$P<0.05$ when compared to the normal group; [b]$P<0.05$ when compared to AD induced obese group.

Table 4. Effect of ethanolic extract of cashew apple on antioxidant enzymes and lipid peroxidation in atherogenic diet-induced oxidative stress in rat liver homogenate.

Group	GSH (mg/g tissue)	CAT (μmol H_2O_2/ min/mg protein)	SOD (U/mg protein)	MDA (nmoles/ g of protein)
Normal	22.56±0.67	61.55±2.13	38.28±1.96	18.23±0.59
AD	7.86±0.20[a]	17.18±1.07[a]	11.87±1.71[a]	58.77±1.21[a]
AD+atorvastatin (10 mg/kg)	16.88±0.20[b]	54.18±1.11[b]	28.73±1.34[b]	17.81±0.23[b]
AD + CAEE (200 mg/kg)	12.92±0.16[b]	23.83±0.62[b]	18.16±0.35[b]	30.73±0.95[b]
AD + CAEE (400 mg/kg)	21.83±0.53[b]	56.50±1.17[b]	31.21±0.95[b]	16.99±0.44[b]

CAEE: ethanolic extract of cashew apple; AD: atherogenic diet; GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase; MDA: malondialdehyde. Values were expressed as mean ± SEM (n=6); [a]$P<0.05$ when compared with the normal group; [b]$P<0.05$ when compared with atherogenic diet–induced obese group.

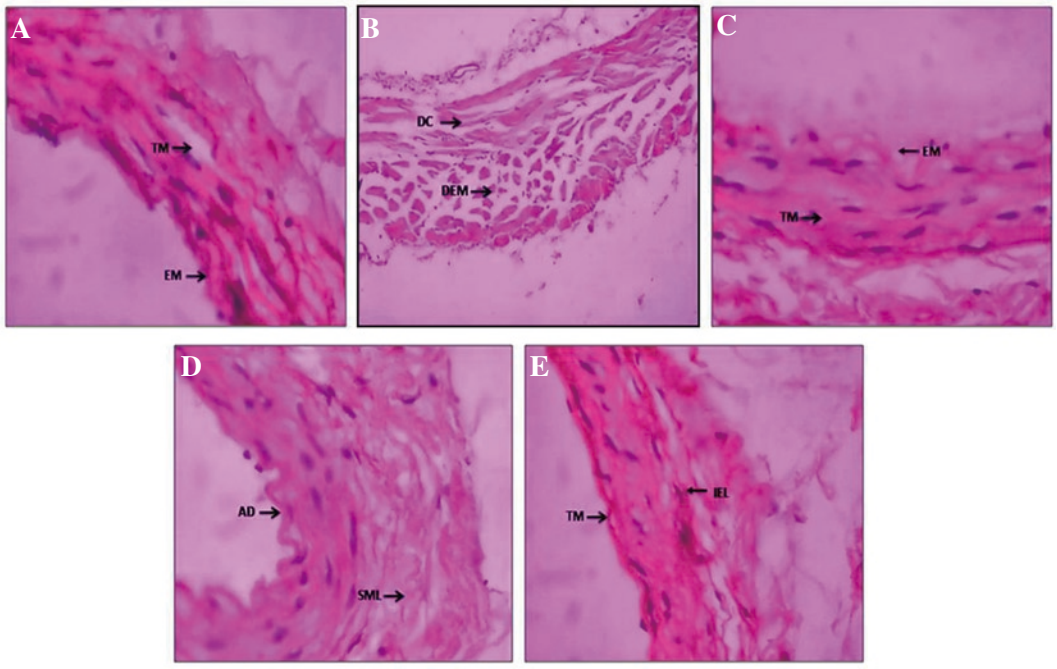

Figure 2. Effect of ethanolic extract of cashew apple on carotid artery histopathology (40× magnification). The tissue sections were stained with hematoxylin-eosin (H&E). (A) normal group; (B) atherogenic diet–induced obese group; (C) atherogenic diet-induced obese group treated with atorvastatin (10 mg/kg); (D) atherogenic diet-induced obese group treated with ethanolic extract of cashew apple (200 mg/kg); (E) atherogenic diet–induced obese group treated with ethanolic extract of cashew apple (400 mg/kg). IEL = Internal elastic lamina; EM = Endothelium; AD = Adventitia; SML = Smooth muscle layer; DC = Degenerative changes; TM=Tunica media; DEM=Degenerative changes in endothelium.

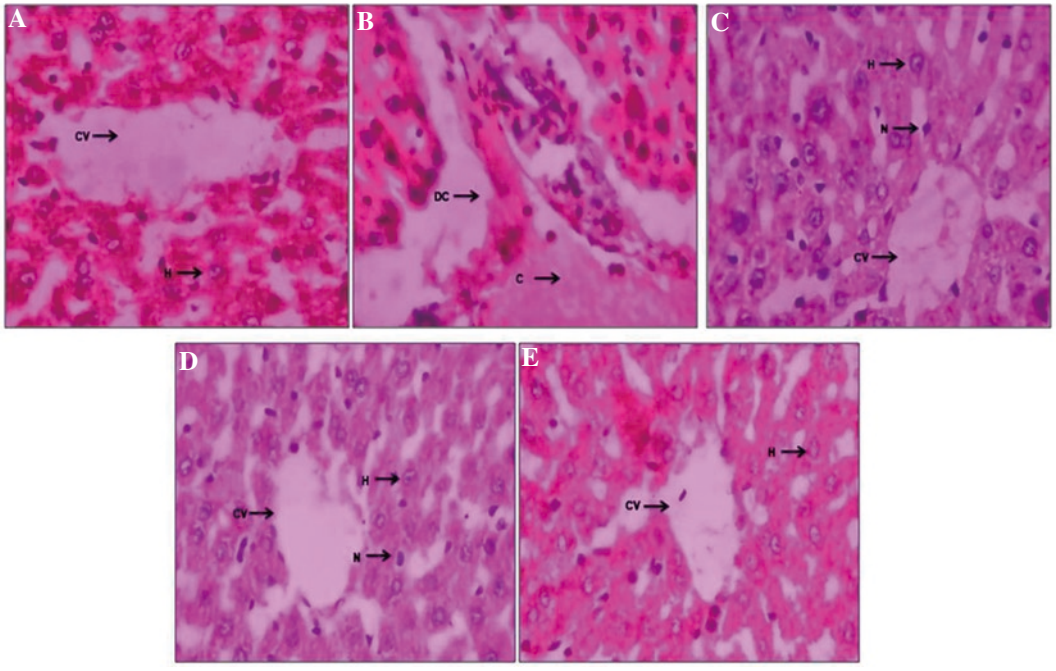

Figure 3. Effect of ethanolic extract of cashew apple on liver histopathology (40× magnification). The tissue sections were stained with hematoxylin-eosin (H&E). (A) normal group; (B) atherogenic diet-induced obese group; (C) atherogenic diet–induced obese group treated with atorvastatin (10 mg/kg); (D) atherogenic diet-induced obese group treated with ethanolic extract of cashew apple (200 mg/kg); (E) atherogenic diet-induced obese group treated with ethanolic extract of cashew apple (400 mg/kg). DC= Degenerative changes; N= Nucleus; C = Congestion; CV = Central vein; H = Hepatocytes.

4. Discussion

Though currently varied pharmacological interventions and surgeries were available, undesirable side effects of these strategies had attracted interested in nutraceutical field. Currently, a lot of medical plants are being investigated as a probable treatment option for obesity and weight loss management, which stimulates the research to evaluate and report the anti-obesity properties of nutraceuticals such as conjugated linoleic acid (CLA), capsaicin, *Momordica charantia* and Psyllium fiber[27].

The cashew apple, which is known to be discarded as a byproduct after separating cashew, is one such dietary source on which research is going on to unlock its potential. Multiple techniques have been industrialized to convert the cashew apple to commercialized products like juice, jam, syrup, and chutney. Studies also supported the cashew apple as a fine source of vitamins and bioactive compounds contributing to its antioxidant[28]. Fermented cashew apple juice demonstrated probiotic nature which could improve intestinal microbial balance. Convincing evidence was available, suggesting the anti-obesity potential of probiotics from bacteria in high fat/high cholesterol-fed mice[29]. These observations revealed that seasonally available fruit cashew apple (*Anacardium occidentale*, family-Anacardiaceae) might be considered as a new strategy for ameliorating obesity and its associated metabolic disorder.

As humans and rats have a similar tendency of weight gain by diet containing cholesterol and fatty acids, it is an appropriate model to predict weight loss in human. Moreover, obesity was also reported to be a high-risk factor for higher amounts of cholesterol and also evidenced through many cholesterol diet models[30]. Thus, the study was designed to determine the anti-obesity potential of cashew apple using an atherogenic diet. Body weight and BMI were found to be the major indices used for categorizing the obesity. Administration of atherogenic diet has resulted in marked elevation in body weight whereas, treatment with ethanolic extract of cashew apple at both doses and a standard drug (atorvastatin) have effectively reduced body weight gain and BMI, which might be mediated through the inhibition of dietary fat absorption. This is consistent with the earlier study[31]. Moreover, we did not assess food intake, which may attribute to lower body weight, so we cannot confirm that ethanolic extract of cashew apple has caused hypophagia.

Obesity is integrated with lipid metabolism abnormality. The typical dyslipidemia associated with obesity includes elevated levels of TG, VLDL, TC, LDL and reduced HDL levels[32]. Cholesterol diet for 60 d has also shown a similar pattern of altered lipid profile, indicating the induction of hyperlipidemia. In this study, supplementation with ethanolic extract of cashew apple at two doses (200 & 400 mg/kg, *p.o.*) notably reduced the plasma TG, LDL, VLDL, TC, and increased HDL, similar to the effect shown by standard drug.

Long-term sustained dyslipidemia has been characterized as a major factor for cardiovascular threats like atherosclerosis[33]. The atherogenic index is considered as a marker towards cardiovascular diseases. Feeding with a cholesterol diet for 60 d resulted in increased atherogenic index in the atherogenic diet group while treatment with ethanolic extract of cashew apple exhibited a remarkable reduction in the atherogenic index, thus providing cardio-protection.

In animal models with the diet–induced obesity, the liver of obese rats was characterized by hepatic steatosis such as fat accumulation in hepatocytes[34]. Earlier studies also reported that atherogenic diet-induced accumulation of hepatic TG caused leakage of transaminases like ALT, ALP, and AST[35]. One of the potential mechanisms for liver damage by high–fat diet includes endoplasmic reticular, stress-mediated apoptosis, a central feature of liver injury[36]. Though our studies did not include exact mechanisms of liver damage, similar alterations were noted in terms of altered liver function markers and histological changes. Treatment with ethanolic extract of cashew apple noticeably improved the liver function by decreasing enzyme markers like AST, ALT, and ALP levels which proved an important role of cashew apple in preventing the liver damage.

Dietary high fat has been reported to increase oxidative stress. Similarly, atherogenic diet in the current study results in high lipid peroxidation and lower levels of CAT and GSH and SOD, indicating the induction of oxidative stress in the atherogenic diet-induced obese group. However, treatment with ethanolic extract of cashew apple increased the levels of CAT, GSH, SOD and reduced MDA by inhibiting the lipid accumulation in the liver.

LDL oxidation and the generation of free radicals within the endothelium are an early pro-atherogenic event in disease progression[37]. In order to gain insight into the mechanisms underlying the association between dietary factors and the extent of atherosclerosis in the carotid artery, histopathological examinations were carried out. Treatment with ethanolic extract of cashew apple demonstrated a reduction of atherosclerotic narrowing of the arterial lumen which contributes to a decrease in MDA as noticed from the histological observations of the carotid artery. This positive correlation between atherosclerosis and reduced MDA was consistent with earlier studies[38].

Dietary cholesterol has been strongly associated with liver TG metabolism which further leads to complications like cirrhosis and NAFLD in obese people[39]. Histological observations of liver showed no fat depositions in treatment groups, indicating the protection offered by the extract. This protection could be credited to its ability to minimize the absorption of postprandial TG. Hence, the ethanolic extract of cashew apple may be a potent dietary supplement or adjunct therapy for the management of anti-obesity.

In this study, we showed that the ethanolic extract of cashew apple has prominent *in vitro* scavenging effect in DPPH and ABTS scavenging assays. The effect was found to be dose based and similar to the standard. Though earlier work reported on cashew apple juice for its polyphenolic and flavonoid contents, it may vary due to genetic factors, variety, the degree of ripeness and environmental conditions[40]. The current study also revealed the presence of rich

flavonoids and polyphenols which explains the *in vitro* antioxidant effect of the extract and these findings are consistent with previous studies[41].

Pancreatic lipase inhibitors of both natural and synthetic origin are worthwhile in obesity prevention through inhibition of intestinal lipid absorption[42]. The dietary habits of specific geographical locations like Asian countries include much more carbohydrates than those of western countries; the mechanism of inhibiting the absorption of carbohydrates should be combined with the mechanism of inhibiting fat absorption to improve obesity[43]. Majority of dietary substances like garlic, artichoke, green tea and soy *etc* were reported to be dietary lipid-lowering nutraceuticals in clinical practice[44]. Thus, we evaluated the effect of ethanolic extract of cashew apple on inhibition of α-amylase, pancreatic lipase at *in vitro* condition and *in vivo* postprandial TG absorption using lipid emulsion model. Our results revealed the ability of ethanolic extract of cashew apple to inhibit both α-amylase and pancreatic lipase. Similarly, reduced postprandial TG absorption was observed in the lipid emulsion model which confirms that the extract can inhibit the absorption of dietary lipids.

Studies reported many bioactive compounds in chloroform extract of cashew apple in GC-MS analysis, such as 9,12-octadecadienoic acid (Z, Z)-(50.16%), 9,12-octadecadienoyl chloride (Z, Z)-(21.35%) and 15-hydroxy-pentadecanoic acid (16.32%). These compounds belong to the polyunsaturated fatty acids and have been reported to regulate activities like lipid metabolism, cardiovascular function, and various immune mediated functions[45]. Hence, the study indicates that the cashew fruit has the potential to be used as a nutritional supplement or adjunct therapy against obesity, atherosclerosis and other probable metabolic disorders.

In conclusion, cashew apple has a potent anti-obesity effect. The benefit could be attributed to its ability to inhibit the intestinal absorption of fat and carbohydrates present in the diet. Antioxidant property of ethanolic extract of cashew apple may be helpful in reducing the necrotic changes in the liver and carotid artery caused by the atherogenic diet. However, further studies are required to determine and identify the active compounds in whole cashew fruit and to unveil the mechanism mediating the lipid metabolism in obesity.

Conflict of interest statement

The authors declare that there is no conflict of interest.

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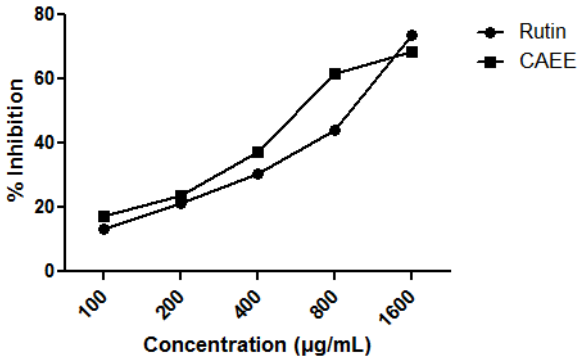


Figure 1. DPPH radical scavenging activities of the ethanolic extract of cashew apple and standard rutin. Results were expressed as mean ± SEM of three replicate determinations (*n*=3). CAEE: ethanolic extract of cashew apple.

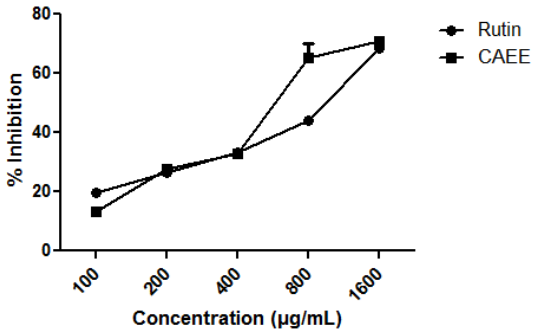
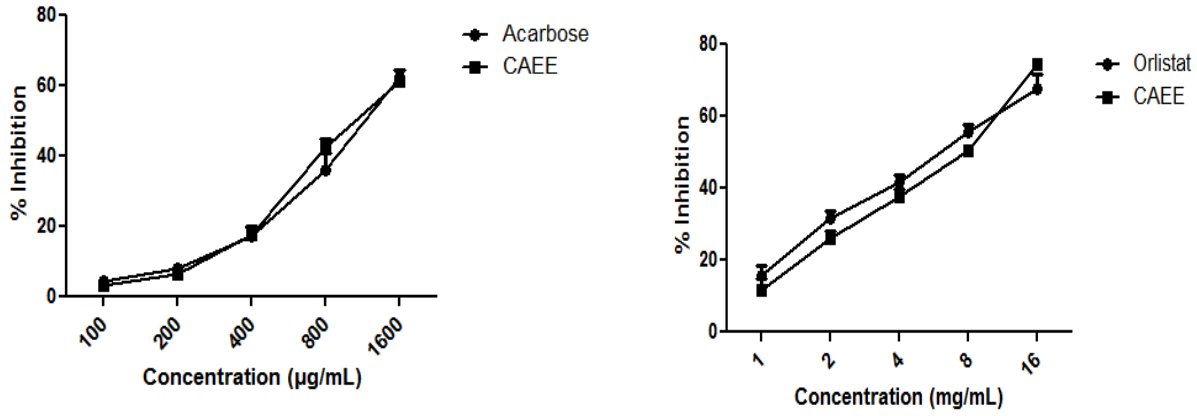


Figure 2. ABTS radical scavenging activities of the ethanolic extract of cashew apple and standard rutin. Results were expressed as mean ± SEM of three replicate determinations (*n*=3). CAEE: ethanolic extract of cashew apple.



(a) **(b)**

Figure 3. Inhibitory effects of ethanolic extract of cashew apple on (a) α-amylase and (b) pancreatic lipase. Results were expressed as mean ± SEM (*n*=3). CAEE: ethanolic extract of cashew apple.