Research Article

Anti-obesity potential of *Capparis spinosa* flower bud extracts in 3T3-L1 adipocytes and in high fat diet induced obese rats

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ABSTRACT

Obesity is a raising pandemic and it needs alternative approaches to prevent or treat, as the existing approaches are not highly effective. In this context, the present study has been designed with the aim to investigate the anti-obesity potential of aqueous flower buds extract of *Capparis spinosa* L. (AFBECS) in 3T3-L1 adipocytes and in high fat diet (HFD) induced obesity thereby giving scientific validation to its traditional use. The 3T3-L1 preadi-pocytes were cultured and differentiated in DMEM in the absence and presence of various concentrations of AFBECS (25, 50, 100, 250 and 500 µg/mL) and the influence of the extracts on 3T3-L1 adipocyte viability and lipid accumulation were determined. The results showed that AFBECS maintained the viability of the 3T3-L1 adipocytes and also reduced the lipid accumulation in 3T3-L1 cells, dose dependently. *In-vitro* pancreatic lipase inhibition assay of AFBECS had shown moderate level of inhibition when compared with Orlistat. For *in-vivo* studies, HFD induced obese rats were treated with 100, 200 and 300 mg/kg of extracts for a period of 60 days using orlistat as standard drug. Anti-obesity potential was assessed using food intake, body weight, organ weights, adipocyte area, lipid profiles and many other blood biochemical parameters. Data of *in-vivo* studies revealed, significant reduction in body weight, fat-pad and organ weights of AFBECS treated animals. Altered levels of glucose, insulin, leptin, lipid profiles and antioxidant status were also normalized upon AFBECS treatment. These findings suggested that AFBECS was found to have prominent anti-obesity potential and exhibited its therapeutic efficacy by inhibiting adipogenesis, promoting lipolysis and ameliorating oxidative stress.

Keywords:
Obesity, *Capparis spinosa* L., 3T3-L1 adipocytes, High Fat Diet, Dyslipidemia

1. INTRODUCTION

Obesity, defined as body mass index (BMI) of more than 30 kg/m^2 is a chronic and complex worldwide epidemic health problem of excessive body fat accumulation occurs due to imbalance between energy intake and expenditure. An imbalance of excessive energy consumption and less expenditure results in adipose tissue enlargement due to excessive lipogenesis and scanty lipolysis[1]. This obesity is viewed as a multifactorial disease as it evolves from the interaction between the genotype and the environment and involves social, behavioural, physiological and genetic factors. However, consumption of excessive calorie in the form of energy-dense meals is believed to be the major supplier to obesity, in most of the affected cases[2]. The excess body fat in obesity presents a serious of health problems including dyslipidemia, coronary artery disease, type II diabetes mellitus, osteoarthritis, cognitive impairments, infertility, gallbladder illness, and certain cancers such as of the breast, endometrium and colon, resulting in declined life span[3]. In addition to the medical consequences, obesity also adversely affects an individual's capability to live a fully active life. It affects psychologically their self-esteem, exacerbates depression and poor employment prospects which substantially impacts a person's functional capacity

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and quality of life[4].

Once reflected as a high-income country burden, obesity is now dramatically on the rise in low and middle income countries, notably in urban settings. World Health Organization's recent report states that the worldwide occurrence of obesity has almost tripled between 1975 and 2016. More than 1.9 billion adults, and over 340 million children and adolescents were found to be overweight or obese in 2016. In 2019, 38.2 million children under the age of 5 years were assessed as overweight or obese. On a global scale, obesity has now been ranked as the fifth leading risk factor for global deaths and is projected to be the third leading cause of death by 2030[5]. Currently, obesity represents the most significant health promotion and disease prevention priorities worldwide as the issue is rising in epidemic proportions globally, irrespective of ages and ethnicity and also its increased risks of associated morbidity and mortality.

There are various strategies currently in use to prevent or control obesity, which includes lifestyle modification such as diet regimes, exercise, behavioral therapy etc., to expel additional calories, medications to reduce appetite or inhibit lipid absorption, and bariatric surgery to earlier satiation and reduce nutrient absorption from food[6]. But all these interventions have very modest efficacy in bringing weight loss. Considering pharmacotherapy, orlistat, a pancreatic lipase inhibitor is the only medication currently approved for long-term use by the FDA. But, prolonged intake of orlistat is reported to have many side effects like steatorrhea, fecal incontinence, oily spotting, nervousness, constipation etc[7]. Due to the limitations of the existing therapies, there has been a renewed interest in medicinal plants in recent decades as they are known to be safe, efficacious, affordable and biocompatible. There are many plants that have been mentioned in Ayurvedic texts for treating obesity and one amongst them is *Capparis spinosa* Linn.

Capparis spinosa L. is a common member of the genus Capparis and belongs to the family Capparidaceae. It is a thorny, 0.3-1 m tall perennial shrub, seen abundantly in wild arid regions of Asia, Europe and Africa, especially in the Mediterranean basin. Capparis species are grown for their medicinal properties and as food sources. Its young flower buds commonly known as capers are consumed for their flavour and digestive properties in fresh salads, pizza, and after processing as pickle[8]. Traditionally it has been used for the treatment of toothache, rheumatism, convulsions, gout, skin disease, kidney disease, liver disease, diabetes, haemorrhoids, ulcers, sciatica etc. It is reported to have different pharmacological effects including antioxidant, antimicrobial, anticancer, immuno-modulatory, anti-inflammatory, hepatoprotective, anti-hypertensive, cholesterol-lowering and anti-diabetic[9]. But its anti-obesity potential was not yet fully exposed scientifically. Thus, the current study has been set up, to investigate the effect of aqueous flower buds extract of *Capparis spinosa* L. (AFBECS) on adipogenesis in 3T3-L1 adipocytes as well as to assess the anti-obesity potential in rats fed with HFD thereby providing scientific evidence to this traditional healer.

2. MATERIALS AND METHODS

2.1. Collection and authentication of plant material

The young flower buds of *Capparis Spinosa* L. were purchased from local herbal market in Tiruchirappalli, Tamil Nadu and were identified with the help of 'Flora of Presidency of Madras'[10]. Authentication was done at the Pharmacognosy Department of Drug Testing Laboratory of CARISM, SASTRA University, Thanjavur, India, through pharmacognostic studies.

2.2. Preparation of the aqueous flower bud extract

The clean, shade dried buds of *C. spinosa* were pulverized into coarse powder using domestic blender. 50 g of the powdered sample was then macerated with 500 ml of distilled water and boiled under reflux till the volume reduced to one-third. The resulting extract was then filtered using muslin cloth and concentrated under reduced pressure using a rotary evaporator at 40°C. The weight of the dried extract remained was 9.2 g, giving a yield of 18.4% (w/w), and it was stored in air tight container at 4°C until use.

2.3. Anti-adipogenic studies in 3T3-L1 adipocytes

2.3.1. Culture and differentiation of 3T3-L1 preadipocytes

Mouse 3T3-L1 preadipocytes (NCCS, Pune, India) were grown in Dulbecco's Modified Eagles's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), penicillin (100 IU/mL) and streptomycin (100 µg/mL) at 37°C under 5% CO_2. For adipocyte differentiation, 2-day post-confluent 3T3-L1 preadipocytes (day 0) were incubated for 48 h (day 2) in DMEM with an inducer (10 µg/mL insulin, 0.25 µM dexamethasone, and 0.5 mM 3-isobutyl-1-methylxanthine) and then maintained for 6 days (day 8) in DMEM supplemented with 10% FBS and 10 µg/mL insulin, changing the culture medium every 2 days.

2.3.2. Cell viability test

The impact of AFBECS on 3T3-L1 preadipocyte viability was studied by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[11]. 3T3-L1 preadipocytes were taken in a 96-well plate (1×10^5 cells/mL) in triplicate and treated with different concentrations of AFBECS (25, 50, 100, 250 and 500 µg/mL) in FBS-free medium and incubated for 2 days at 37°C

under 5% CO_2. In control group 0.05% of dimethylsulfoxide (DMSO) was added instead of extracts. Two days later, the medium was replaced with fresh FBS-free medium which containing 0.5 mg/mL of MTT and incubated for an hour at 37°C. The purple formazan crystals formed in proportional to live cells were then solubilized by DMSO (100 μL) and the absorbance was read at 560 nm. Percentage cell viability was calculated using the formula: (At/Ac)×100. Where, At denotes the absorbance of the test and Ac denotes the absorbance of the control.

2.3.3. Oil-Red-O staining

Anti-adipogenic potential of AFBECS was screened through the determination of lipid accumulation in adipocytes by staining the neutral fats using Oil red O[12]. 3T3-L1 cells undergoing 2 days of adipogenic differentiation was treated with 25, 50, 100, 250 and 500 μg/mL concentrations of AFBECS. Control cells were maintained without extract. Cells were then transferred to maintenance medium for the next 4 days without adding extract. Tests were conducted in triplicate. After 6 days, the cells were washed twice with phosphate buffered saline (PBS) and fixed with 10% formaldehyde in PBS for 30 min at 37°C. After washing two times with distilled water, the cells were stained with Oil red O solution (3 mg/mL in 60% isopropyl alcohol) for 1 h at 25°C. The cells were examined microscopically to assess lipid accumulation. Also, the retained dye in the cells was eluted using 100% isopropanol and the eluent was quantified spectrophotometrically at 510 nm.

2.4. *In-vitro* pancreatic lipase inhibition assay

The pancreatic lipase inhibitory assay was carried out as per previously described protocols[13] with minor modifications. 0.2 mL of various concentrations of the extracts (5, 10, 25, 50, 100, 250 and 500 μg/mL) were pre-incubated with 0.1 mL of porcine pancreatic lipase (PPL) (1 mg/mL) in a potassium phosphate buffer (0.1 mM, pH 7.2) containing 0.1% Tween 80 at 30°C for 1 h. The reaction was then initiated by adding 0.1 mL of substrate solution [25 mM p-nitrophenyl butyrate (PNPB) in acetonitrile] and incubated at 37°C for 5 min. The same procedure was followed for reference drug, orlistat. After incubation, the quantity of *p*-nitrophenol released from PNPB was measured spectrophotometrically at 405 nm. The absorbance for the negative control was also noted with and without an inhibitor. Lipase inhibition (%) was calculated using the formula:

$$\text{Inhibition (\%)} = 100 - [(B - b) / (A - a) \times 100]$$

where, *A* is the activity without inhibitor, *a* is the negative control without inhibitor, *B* is the activity with inhibitor, and *b* is the negative control with inhibitor.

2.5. *In-vivo* anti-obesity studies in experimental animals

2.5.1. Experimental animals

Wistar strain of male albino rats (100-125g), used in this study were purchased from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India. The animals were maintained in polypropylene cages under suitable conditions (23±2°C temperature, 65±5% humidity, 12/12 hours light-dark cycle) and fed with standard rat chow pellet (supplied by Sai Durga Feeds and Foods, Bengaluru, India) as basal diet and tap water *ad libitum*. The rats were adjusted to the lab conditions for about 10 days prior to the experimental study.

2.5.2. Induction of obesity

Pre-obesity was induced to a group of 50 animals by feeding high fat diet (HFD) for a period of 45 days. The HFD consisted of beef tallow-35% (315 kcal), casein-20% (80 kcal), corn starch-15% (60 kcal), sucrose-20% (80 kcal), corn oil-5% (45 kcal), mineral mixture-4% and vitamin mixture-1%. After 45 days of HFD feeding, 30 animals which were gained weight rapidly (weighing near 250 g) than others were considered obese prone and chosen for anti-obesity studies.

2.5.3. Experimental design

The obese-prone/pre-obese rats were divided randomly into 5 groups (Group 2 to Group 6), each comprising of 6 rats. Another set of 6 rats which were maintained separately by feeding normal rat chow pellet were continued to serve as Group 1 (normal control). Group 2 rats were served as 'obese control' and continued to feed on HFD, while Group 3 to Group 5 rats were treated with 100, 200 and 300 mg/kg of AFBECS along with HFD. Group 6 (Positive control) rats were treated with 30 mg/kg of orlistat (standard drug) along with HFD. All the drugs were administered orally once a day for 60 days.

Daily food intake and body weight on a regular interval of 10 days were recorded. At the end of the experimental period the rats were sacrificed by cervical dislocation; blood, necessary organs (liver, kidney, heart and spleen) and fat fads (mesenteric, epididymal, and perirenal) were collected for further analysis.

2.5.4. Parameters analysed

The adipocyte size in the form of adipocyte area was measured using Nikon (Eclipse, Ci) trinocular light microscope with NIS digital imaging software. The BMI was calculated on the first and final day of the experiment by the formula: BMI=body weight (g)/body length (cm^2). Body lengths from nose-to-anus and WC

on the largest sector of the rat abdomen were measured using a measuring tape under mild anaesthesia (0.1 ml i.p. of 1% sodium barbiturate) by keeping the rats in ventral position.

Serum triglycerides (TG), free fatty acids (FFA), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C) and blood glucose were measured using commercial kits (Biosystems, TN, India). The low-density lipoprotein cholesterol (LDL-C) content was calculated using the formula: LDL-C = TC - {HDL-C - (TG/5)}[14]. Serum levels of insulin and leptin were measured using commercial enzyme-linked immunosorbent assay kit (Sigma, USA). Lipid peroxides (LPO)[15], reduced glutathione (GSH)[16], glutathione peroxidase (GPx)[17], superoxide dismutase (SOD)[18] and catalase (CAT)[19] were analysed using standard protocols.

2.6. Statistical analysis

All the obtained data were presented as mean± S.E.M. The data were analysed using the SPSS statistics programme by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Inter group comparisons were carried out and a p value of <0.05 was set to indicate statistically significant differences between various groups.

3. RESULTS

3.1. Anti-adipogenic effect of AFBECS in 3T3-L1 adipocytes

The viability of 3T3-L1 preadipocytes on AFBECS treatment was studied by treating the cells with various concentrations (25-500 µg/mL) of extracts and the obtained result was presented as % viability, compared with the viability of the negative control. From the obtained data it was noted that none of the applied AFBECS doses were seem to affect the viability of the cells (Figure 1A). Thus AFBECS dosages of 25-500 µg/mL were considered non-toxic and used for further anti-adipogenic assay in 3T3-L1 adipocytes. The anti-adipogenic effect of AFBECS in the form of lipid accumulation was examined by Oil red O staining. The obtained data (Figure 1B and Figure 1C) of lipid staining have clearly shown that AFBECS reduced the accumulation of lipid droplets during 3T3-L1 differentiation in a dose-dependent manner.
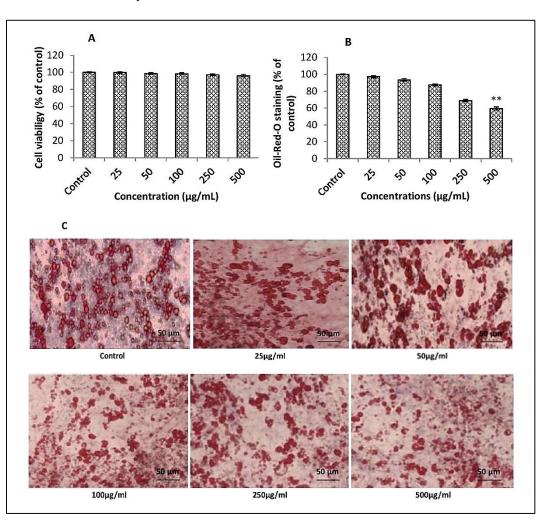

Figure 1. Anti-adipogenic effect of AFBECS on 3T3-L1 adipocytes: cell viability **(A)**, intracellular lipid content **(B)** and microscopic images of Oil-Red-O staining **(C)**. Values are mean±SEM (n=3). **$p<0.05$, significantly different from control group.
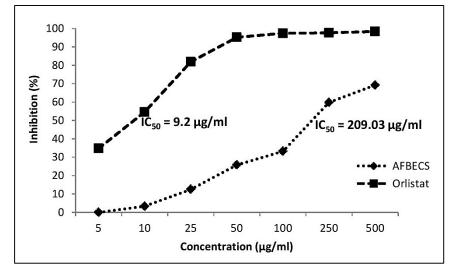
Figure 2. The inhibitory effect of AFBECS on pancreatic lipase activity. Values are mean of 3 experiments; AFBECS-aqueous flower bud extract of *Capparis spinosa* L.

3.2. *In-vitro* Pancreatic lipase inhibitory effect of AFBECS

The inhibitory effect of AFBECS (5-500 µg/mL) on porcine pancreatic lipase was determined by measuring the hydrolysis of PNPB to *p*-nitrophenol. The result (Figure 2) had shown that AFBECS possesses moderate level of pancreatic lipase inhibitory activity with IC_{50} of 209.03 µg/mL compared to the reference drug Orlistat (IC_{50}-9.2 µg/mL).

3.3. Effect of AFBECS on daily food intake and body weight

The food intake of experimental rats was measured on every 24 hours per cage basis (each cage consists of group of 6 rats) by subtracting the residual chow left from the pre-weighed chow added in the cage, and the obtained results were presented as average food intake in Figure 3A. The data revealed a significant rise in average food intake of HFD fed animals than normal diet fed animals. But this raise was slightly suppressed upon AFBECS administration along with HFD dose dependently. However, this suppression in food intake was found to be high in Group 6 rats which were given orlistat along with HFD. The body weights of the rats were recorded on day 1 and subsequently changes in their body weight on treatment with AFBECS were recorded once in every 10 days. We noticed a significant increase in body weight in HFD rats than normal diet fed control rats. However, different doses of AFBECS (100, 200 and 300 mg/kg bw) treated rats had shown significant reduction in body weight in proportion to the dose than HFD fed untreated obese rats (Figure 3B).
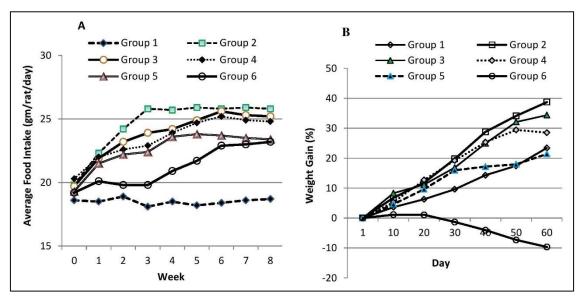

Figure 3. Levels of daily food intake **(A)** and percentage weight gain **(B)** in experimental rats. Group 1 - fed with normal diet; Group 2 - fed with high fat diet (HFD); Group 3 - HFD+AFBECS (100 mg/kg bw); Group 4 - HFD+AFBECS (200 mg/kg bw); Group 5 - HFD+AFBECS (300 mg/kg bw); Group 6 - HFD+orlistat (30 mg/kg bw); AFBECS - aqueous flower bud extract of *Capparis spinosa* L.
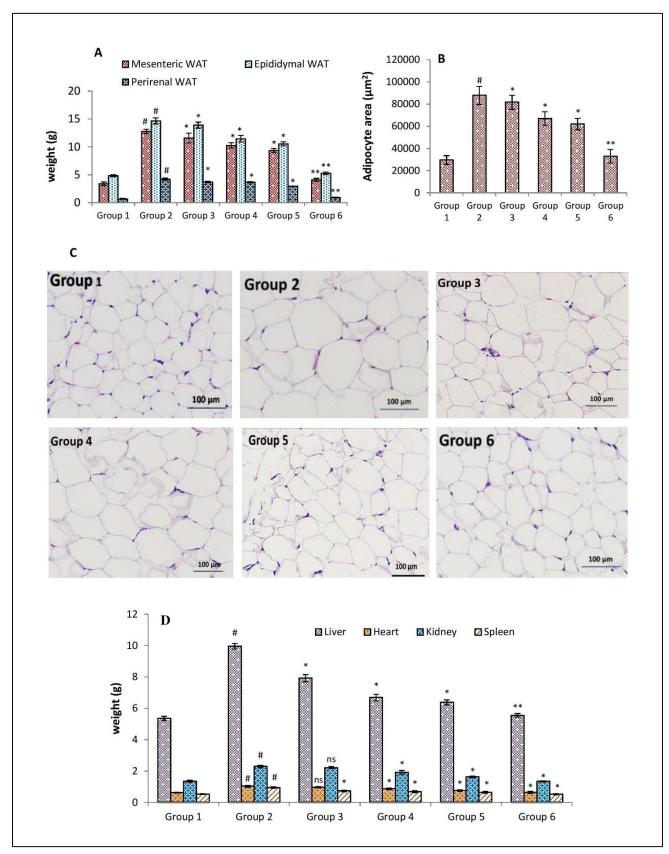
Figure 4. Effect of AFBECS on changes in fat pad weight (**A**); changes in adipocyte area (**B** and **C**); changes in organ weights (**D**); Group 1 - fed with normal diet; Group 2 - fed with high fat diet (HFD); Group 3 - HFD+AFBECS (100 mg/kg bw); Group 4 - HFD+AFBECS (200 mg/kg bw); Group 5 - HFD+AFBECS (300 mg/kg bw); Group 6 - HFD+orlistat (30 mg/kg bw); AFBECS - aqueous flower bud extract of *Capparis spinosa* L.; Values are mean±S.E.M of 6 rats; #$p<0.01$, significantly different from group 1; *$p<0.05$, **$p<0.01$, significantly different from group 2; ns - non significant from group 2.

3.4. Effect of AFBECS on adipocyte size, fat pad weight and organ weight

The major fat pads such as mesenteric, retroperitoneal and perirenal fats were dissected-out, blotted dry and weighed. The weight of the fat pads was found to be raised in HFD fed rats, but the raise was greatly suppressed upon AFBECS treatment in proportionate to dose (Figure 4A). Adipocyte size/area was also found to be significantly reduced in extract treated rats than HFD fed obese rats (Figure 4B and Figure 4C). Moreover, the increased weights of vital organs such as liver, kidney, heart and spleen found in HFD fed obese rats were also reduced

significantly in AFBECS treated animals in a dose dependent manner (Figure 4D).

3.5. Effect of AFBECS on body mass index (BMI) and waist circumference (WC)

The BMI and WC of the experimental rats were calculated on day 1 and final day (60th day) of the experiments and the data obtained were presented in Figure 5A and Figure 5B. The AFBECS administration along with HFD reduced the BMI and WC of the obese rats in a significant level when compared to HFD alone fed obese rats in a dose dependent manner.
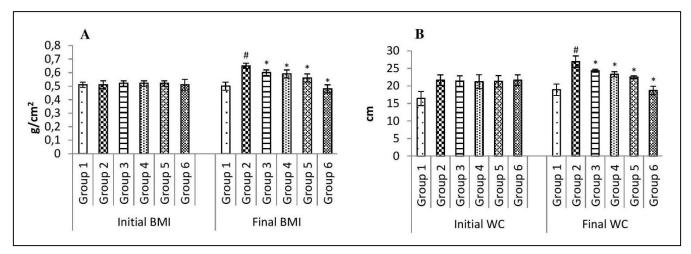

Figure 5. Effect of AFBECS on body mass index (**A**) and waist circumference (**B**) of experimental rats. Group 1 - fed with normal diet; Group 2 - fed with high fat diet (HFD); Group 3 - HFD+AFBECS (100 mg/kg bw); Group 4 - HFD+AFBECS (200 mg/kg bw); Group 5 - HFD+AFBECS (300 mg/kg bw); Group 6 - HFD+orlistat (30 mg/kg bw); AFBECS - aqueous flower bud extract of *Capparis spinosa* L.; Values are mean±S.E.M of 6 rats; $^{#}p<0.05$, significantly different from group 1; $^{*}p<0.05$, significantly different from group 2.

3.6. Effect of AFBECS on biochemical parameters

The effect of AFBECS on various obesity related biochemical parameters such as blood glucose, serum insulin, leptin and lipid profile of experimental rats were presented in Table 1. The altered levels of glucose, insulin and leptin in HFD induced rats were efficiently brought back to normalcy on AFBECS treated rats. Feeding of HFD raised the levels of triglycerides, total cholesterol (TC) and LDL-cholesterol (LDL-C) while HDL-cholesterol (HDL-C) was found to be decreased in group 2 rats. However, administrations of AFBECS along with HFD proficiently brought back these altered serum lipid profiles to normalcy in proportionate to the dose.

3.7. Effect of AFBECS on hepatic anti-oxidant status

The data obtained on hepatic enzymatic and non-enzymatic anti-oxidant status of experimental rats was given in Table 2. The analysis of obtained data had shown, elevated lipid peroxidation, depleted Glutathione (GSH) level, and diminished activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and

catalase (CAT). However, the data obtained for AFBECS treated rats indicated that all these variations observed in HFD induced obese rats were reverted back to near normal values in a dose dependent manner.

4. DISCUSSION

Obesity is rising in epidemic proportions worldwide contributed mainly by reduced physical activity, excess intake of energy dense food, food obsession, depression, genetic predisposition and lifestyle modifications. This rising prevalence has become a real health threat, as obesity itself is a risk factor for many other conditions such as insulin resistance, type 2 diabetes mellitus, hypertension, cardiovascular diseases and different cancers. The swift rises of obesity cases as well as the limitations of the current management therapies warrant the scientific community to divert their focus on safe alternate therapy. This led us to scientifically evaluate the anti-obesity potential of aqueous flower bud extract of *Capparis spinosa* Linn. in 3T3-L1 pre-adipocyte differentiation and high fat diet induced obesity in rats.

Table 1. Effect of AFBECS on levels of glucose, insulin, leptin and lipid profile in experimental rats.

Experimental Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Glucose (mg/dl)	73.63 ± 1.90	147.56 ± 2.55[#]	129.86 ± 1.27	112.83 ± 2.48	100.39 ± 1.95*	73.59 ± 2.06**
Insulin (μIU/ml)	3.55 ± 0.20	7.20 ± 0.27[#]	6.94 ± 0.35	6.03 ± 0.32	5.79 ± 0.36*	3.87 ± 0.27**
Leptin (ng/ml)	3.08 ± 0.24	7.18 ± 0.32[#]	6.93 ± 0.27	6.15 ± 0.16	5.63 ± 0.29*	3.15 ± 0.14**
TG (mg/dl)	67.75 ± 1.99	163.83 ± 2.40[#]	159.91 ± 2.18	142.38 ± 0.71	118.48 ± 1.85*	72.90 ± 0.97**
FFA (mg/dl)	20.12 ± 2.08	41.13 ± 2.91[#]	37.55 ± 1.15	34.52 ± 1.21	31.82 ± 1.35*	23.05 ± 1.07**
TC (mg/dl)	98.02 ± 1.41	194.16 ± 2.48[#]	180.03 ± 2.08	168.39 ± 1.02	137.40 ± 1.05*	99.54 ± 1.59**
HDL-C (mg/dl)	61.28 ± 0.98	38.45 ± 0.71[#]	37.08 ± 0.93	44.82 ± 1.03	48.54 ± 0.91**	58.99 ± 1.25**
LDL-C (mg/dl)	36.98 ± 0.65	107.55 ± 1.43[#]	94.71 ± 1.55	83.14 ± 1.34	62.76 ± 1.34*	37.71 ± 1.34**

Values are mean±S.E.M (n=6); [#]$p<0.01$ vs normal control (group 1); *$p<0.05$ vs obese control (group 2); **$p<0.01$ vs obese control (group 2)

Table 2. Effect of AFBECS on antioxidant status in experimental rats.

Experimental Groups	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LPO (n Moles of MDA formed/g tissue)	82.05 ± 0.94	158.04 ± 1.52[#]	138.12 ± 1.52	125.11 ± 1.38	114.88 ± 2.08*	97.32 ± 1.45**
GSH (mg/g tissue)	5.32 ± 0.08	2.45 ± 0.06[#]	2.63 ± 0.04	3.26 ± 0.07	3.92 ± 0.04*	4.25 ± 0.08**
GPx (μMoles of GSH utilized/min/mg protein)	3.92 ± 0.14	1.23 ± 0.04[#]	1.72 ± 0.07	2.25 ± 0.08	2.61 ± 0.08*	3.38 ± 0.10**
SOD (μMoles of Epinephrine oxidized/mg protein)	4.16 ± 0.14	2.04 ± 0.05[#]	2.42 ± 0.09	2.78 ± 0.10	3.22 ± 0.12*	3.94 ± 0.16**
CAT (μMoles of H_2O_2 utilized/min/mg protein)	34.10 ± 1.43	15.29 ± 0.93[#]	16.34 ± 0.68	18.87 ± 0.58	23.18 ± 0.97*	30.19 ± 0.90**

Values are mean±S.E.M (n=6); [#]$p<0.01$ vs normal control (group 1); *$p<0.05$ vs obese control (group 2); **$p<0.01$ vs obese control (group 2)

Obesity is highlighted by excessive adipose mass, occurs as a result of increased triglyceride accumulation and adipocyte differentiation in a process called adipogenesis. Thus agents which inhibit adipocyte differentiation could be a useful target to reduce adipose mass. In the present study the effect of AFBECS was screened on the processes of adipocyte proliferation and differentiation using 3T3-L1 pre-adipocytes, a well-established cell culture model for adipogenesis and obesity-related studies. Viability studies of AFBECS on 3T3-L1 cells had shown that the cell viability was maintained over 95% for all the selected doses. From the viability studies, the concentrations between the ranges of 25-500 μg/mL were found to be safe doses, and were used for further adipocyte differentiation experiments on 3T3-L1 cells. For the anti-adipogenic study, the 3T3-L1 preadipocytes were added to adipogenic medium and treated with 25, 50, 100, 250 and 500 μg/mL of AFBECS and the intracellular lipid accumulation in maturing 3T3-L1 cells were observed using Oil red O staining. The obtained results had shown that the extract decreased the lipid droplets in 3T3-L1 cells in a dose-dependent manner. The differentiation of preadipocytes into mature adipocytes is regulated by cascade of transcription factors viz. peroxisome proliferator-activated receptor-γ, CCAAT/ enhancer-binding proteins, and sterol regulatory element-binding transcription factor 1, by acting in a sequential fashion[20]. Previously, many natural compounds, such as epigallocatechin gallate, capsaisin, resveratrol, curcumin, rutin and quercetin have been reported their anti-adipo-genesis through regulated gene expression[21-23]. Thus, it is assumed that AFBECS which is reported to contain rutin and quercetin may also exhibit its anti-adipogenic effect through regulated expression of adipogenic genes.

Excessive calorie consumption in the form of dietary lipid is one of the major contributors to obesity among others. This dietary lipids comprised mostly of mixed triglycerides get digested by pancreatic lipase (PL) in the GI tract before being absorbed into the circulation. Thus, inhibition of PL activity and thereby reducing/ delaying dietary fat digestion and absorption from intestine is one of the modern day therapeutic approaches in dropping excessive calorie intake. Orlistat (Xenical), obtained from *Streptomyces toxytricini*, is the only FDA approved PL inhibitor currently available in the market for treatment of obesity; however, its use is been limited because of reported adverse effects such as bloating, faecal urgency, oily spotting, steatorrhea etc upon prolonged usage[24]. In recent days many plant drugs have been reported for their lipase inhibitory activity and in the present study we also tested the PL inhibition ability of AFBECS. The obtained data showed a moderate level of inhibition of PL activity by AFBECS when compared to the standard drug Orlistat. This inhibitory effect of AFBECS may be mediated through the interaction of secondary metabolites such as saponins, tannins, polyphenols or triterpenes of AFBECS with the PL enzyme, as these metabolites are well documented for their inhibition potential of PL[25]. Rutin, the active constituent of *C. spinosa* is reported for its pancreatic lipase inhibitory

potential[26].

An increase in body weight is one of the common indexes for obesity development upon prolonged intake of HFD. Although several factors contribute to the etiology of obesity, consumption of energy-dense meals is the typical contributor to obesity in most cases[27] accompanying with visceral adiposity, hyperglycemia, hyperinsulinemia, dyslipidaemia and oxidative stress. If a person consume more calories than his expenditure these extra calories get dumped in various sites of the body result in weight gain. In the current study we observed approximately 15% more weight gain in the obese control animals upon HFD feeding for a period of 60 days than normal diet fed animals. This weight gain indicates the excess fat deposition in visceral regions associated with positive energy balance on HFD feeding. It was noticed that the percent weight gain in AFBECS supplemented rats were greatly reduced in proportionate to the dose than obese control rats. These reductions in body weight may be partially accomplished through enhanced lipolysis and or reduced fat absorption from the GI tract by inhibiting pancreatic lipase as observed in the present study (Figure 2). The standard drug orlistat, a pancreatic lipase inhibitor, has shown higher percent of weight reduction than the extract as it has the potential to limit fat absorption in intestine. It is also believed that food intake might not play much role in body weight reduction as there was not much decrement in food intake between the obese control and AFBECS treated rats.

The magnitude of obesity can also be determined by the amount of lipid stored in adipocytes as well as other vital organs, as excess energy gets deposited as fat pads mainly in the visceral regions followed by their deposition in organs. Along with fat pads obesity can lead to increase in adipocytes number (hyperplasia) and their size (hypertrophy) as well[28]. In the present study we found approximately a 2 to 3 fold increase in the weights of mesenteric, retroperitoneal and perirenal fat pads, adipocyte area and organ weights (liver, kidney, heart and spleen) in HFD-induced untreated obese rats than extract-treated and normal control rats. These observations indicate that prolonged exposures of rats to energy dense diets facilitated fat build-up in the visceral regions due to the more effective energy content of high fat diets. The observed decrease in body weight, organ weights and fat pad weights were also reflected in the BMI and WC of the AFBECS treated rats as we observed decrement in both of this CVD predictor in the extract treated rats when compared to untreated rats. Reduction in fat pads weights, organ weights, BMI and WC could be in correlation with the observed decreases in body weights of the extract treated rats.

Obesity is one of the well-known risk factor for type 2 diabetes mellitus (T2DM). It was reported that around 60-90% of all patients with T2DM are obese (BMI$\geq$30kg/m^2)[29]. The major link between obesity and

T2DM is the development of insulin resistance (IR) during excess fat storage. Findings of the present study also revealed increased levels of glucose and insulin in HFD-induced untreated rats relative to normal diet fed rats. A number of mechanisms have been proposed to link obesity and IR which predispose to diabetes and one among them is altered profile of 'adipocytokines' (leptin, adiponectin, TNF-α, IL-6 etc.,) secreted by enlarged adipocytes[30]. In obesity adipose tissue secretes proportionally more leptin, TNF-α, IL-6 etc., which cause IR and fewer adiponectin that promote insulin sensitivity. The resulting IR affects the muscle or liver cells' insulin-mediated glucose uptake and creates hyperglycemia. Also, the β-cells of pancreas continually secrete insulin in response to hyperglycemia, results in hyperinsulinemia. However, we observed significant ($p<0.05$) decrease in the levels of serum glucose and insulin in AFBECS treated rats in a dose dependent manner than untreated obese rats. The active phytoconstituents present in the test drug may mediated this hypoglycemic and hypoinsulinemic effect through decreasing fat mass and retrieving the altered adipocytokines to normal thereby promoting insulin sensitivity.

Leptin is a peptide hormone, produced primarily by white adipose tissue and their concentrations are directly dependent upon *Ob* gene transcription, which correlates with adipocyte size and lipid content. In the present study we observed significantly ($p<0.05$) elevated levels of leptin in untreated obese rats than normal control rats. Under normal conditions the circulating leptin plays crucial role in energy balance by communicating the peripheral energy reserves to the hypothalamus thereby regulating appetite and energy expenditure. However, hyperleptinemia the condition associated with obesity do not appear to play this role due to leptin resistance[31]. Although the complete understanding for leptin resistance remains unclear, it has been previously reported that elevated levels of leptin can cause desensitization of hypothalamic leptin receptors and thus affects the downstream neural networks mediating leptin effects on energy intake and body weight[32]. In AFBECS treated rats, the leptin level was found to be reduced in proportionate to the dose and this decrease correlates with reduced fat accumulation that can be mediated by the lipid lowering phytoconstituents present in the aqueous plant extract.

Dyslipidemia, the abnormal level of lipids characterized by high concentrations of triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and the low levels of high-density lipoprotein cholesterol (HDL-C) contributed by obesity is a serious concern as it significantly increases the risk of cardiovascular disease[33]. In the present study also we witnessed significant ($p<0.01$) abnormality in the levels of serum lipids in HFD induced obese rats characterized by increased levels of free fatty acid (FFA), TG, TC, LDL-C,

and decreased levels of HDL-C. The rise in circulating FFAs in obesity is correlated with enhanced lipolysis of fat mass, as obese adipose tissue is resistant to the anti-lipolytic action of insulin as well as reduced ability of obese adipocytes to effectively recycle FFAs via re-esterification[34]. This elevated circulatory FFAs results in increased liver FFA fluxes, which in turn causes increased hepatic synthesis and secretion of large VLDL, thereby raises circulatory TG. The increased concentration of VLDL, causes an increased interchange of cholesterol esters in LDL and HDL with triglycerides in VLDL mediated by cholesterol ester transfer protein (CETP). This CETP mediated exchange produces TG enriched LDL particles that are rapidly lipolysed by hepatic lipase, whose activity was known to be increased in obesity, leaving smaller, denser LDL and HDL particles. The affinity of apoprotein called Apo A-I for small HDL particles is reduced leading to its disassociation and clearance by the kidneys, resulting in reduced levels of HDL-C in patients who are obese[35]. Administration of AFBECS along with HFD significantly improved lipid profiles in rats, as evidenced by lowered FFA, TG, TC, LDL-C and elevated HDL-C. Reductions in TC in serum may partly be the effect of saponin present in the extract (0.83 mg/g) as saponin has the capability to bind with bile acids and cholesterol secreted through bile, thereby averting mixed micelles formation and following reabsorption of cholesterol[36]. Restoration of TG and other lipoproteins might be the result of reduced adipose mass mediated by limited fat digestion and absorption as well as enhanced fatty acid metabolism.

Along with insulin resistance, chronic oxidative stress is another key mechanism underlying obesity-related morbidities[37]. Raised levels of circulatory glucose and lipids increase the energy substrates delivered to cellular metabolic pathways, thereby enhancing the production of reactive oxygen species. Excessive adipose mass is a source of pro-inflammatory cytokines which in turn increase ROS production and lipid peroxidation, leading to oxidative stress[38]. Significantly increased levels of malondialdehyde (MDA), the stable end product of lipid peroxidation, observed in the liver tissues of HFD-induced untreated obese rats than normal diet fed rats is the marker for generation of HFD induced oxidative stress in the present study. The biological system contains many enzymatic and non-enzymatic antioxidant defence molecules to counteract the oxidants produced thereby reducing oxidative stress. Glutathione (GSH) is one such potent cytosolic non-enzymatic antioxidant, which neutralizes ROS such as lipid peroxides directly and plays an important role against oxidative stress. The significantly lowered GSH content observed in liver of HFD fed animals than normal diet fed animals in the present study indicates its large utilization to quench the HFD induced ROS. The cells also contain several enzyme systems like glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalases (CAT) which form the body's enzymatic endogenous defence mechanisms to help protect against free radicals-induced cell damage by metabolizing oxidative toxic intermediates. GPx, major peroxide scavenging enzyme, converts oxygen radicals like H_2O_2 into water and organic peroxides into stable alcohols using GSH as substrate while, SODs act against superoxide anions and convert them into oxygen and H_2O_2. CAT, a tetrameric protein predominantly found in peroxisomes converts toxic H_2O_2 to water and oxygen[39]. In HFD fed obese rats, a fall in the activity of GPx was observed and this might be due to the decreased availability of GSH to act as substrate for GPx under oxidative stress. Also, we observed decreased levels of both SOD and CAT in HFD-induced obese rats than normal rats and this decrease might be attributable to severe oxidative stress generated in obese rats. AFBECS administration, however substantially reduced the MDA content and effectively restored the levels of GSH, GPx, SOD and CAT suggesting its free radical scavenging efficacy. *C. spinosa* is rich in flavonoid compounds such as rutin and quercertin which are well attributed for their positive effects on health because of their anti-oxidative property[40].

5. CONCLUSION

From the obtained data it is evident that the tested plant extract, AFBECS possess potent anti-obesity potential in 3T3-L1 cell lines and in HFD induced obese rats. The anti-obesity potentials of extract could be connected with inhibition of adipocyte differentiation, inhibition of pancreatic lipase activity, betterment of dyslipidemia and enhancement of antioxidant status mediated by the protective phytonutrients present in the extract. The current findings thus provide satisfactory scientific confirmation to the anti-obesity claim of *Capparis spinosa* L. Further in-depth mRNA expression analysis of proteins of adipogenesis, fatty acid oxidation and adipocytokines is warranted to demonstrate the underlying molecular mechanism and substantiate the value of AFBECS.

6. ACKNOWLEDGEMENT

Conflict of interest
None to declare.

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