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Research Article

Protective Effect of *Dolichos biflorus* Seed Extract on 3T3-L1 Preadipocyte Differentiation and High-Fat Diet-Induced Obesity in Rats

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Obesity is known to be one of the severe health issues worldwide, as its prevalence continues to rise as well as its association with other chronic diseases worsens. Even though various approaches have been underway to prevent or treat obesity, alternative approaches are in need to combat this chronic condition because of the unsatisfactory effectiveness and adverse side effects of the existing approaches. *Dolichos biflorus* L. seeds have been employed as a weight-loss treatment in folk medicine. Considering the necessity to develop a safe alternative remedy to rising obesity, the current investigation has been set up to assess the antiobesity potential and the mode of action of the aqueous seed extract of *D. biflorus* (ASEDB) in a cell line (3T3-L1) and high-fat diet (HFD)-induced rats. For *in-vitro* studies, 3T3-L1 cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) augmented with adipogenic-inducing medium and the influence of the extract (10 μg/mL–500 μg/mL) on 3T3-L1 adipocyte viability, adipogenesis, and lipolysis was assessed. An *in-vitro* study revealed maintenance of cell viability, reduced triglycerides (TG) accumulation, and promoted lipolysis in 3T3-L1 cells by ASEDB. Following *in-vitro* analysis, the HFD-induced obese rats were treated with ASEDB at different concentrations (100 mg/kg, 200 mg/kg, and 300 mg/kg) for 60 days and the effect was evaluated through various anthropometric and biochemical parameters. The findings revealed a significant decrement in total body weight, organ weights, fat pad weights, and restoration of abnormal levels of glucose, leptin, insulin, lipid markers, and antioxidant system to normal by ASEDB treatment. Also, pancreatic lipase inhibition analysis of ASEDB revealed a modest level of inhibition with an IC_{50} value of 213.3 μg/mL. All these findings exposed that ASEDB possesses pronounced antiobesity potential and exhibits its protective effect by suppressing food intake, reducing fat digestion and absorption, limiting adipogenesis, enhancing lipolysis, and alleviating oxidative stress.

1. Introduction

Obesity, the leading public health issue of the 21st century, is a complex and chronic condition of excessive body fat buildup that results from an imbalance between energy and food intake, basal metabolism, and energy outlay. Body mass index (BMI), the metric determined from a person's weight and height, categorizes individuals those who are having BMI from 25 to 29.9 kg/m^2 as overweight and ≥30 kg/m^2 as obese [1]. This additional body fat gets deposited and is predisposed to major health issues like dyslipidemia, coronary artery disease, type 2 diabetes mellitus (T2DM), stroke, and certain malignancies like breast, endometrial, and colon, which shortens life expectancy. In addition, the obese have higher risks of back pain, osteoarthritis, infertility, and deprived psychosocial wellbeing [2]. Even though obesity is multifactorial in etiology, which is influenced by social, physiological, and genetic variables, increased calorie intake from energy-dense meals along with a sedentary lifestyle are thought to be the primary causes of obesity in the majority of instances [3]. According to WHO, the worldwide incidence of obesity was raised threefold between 1975 and 2016. In 2016, it was also reported that more than 340 million children and adults and over 1.9 billion adults were either obese or overweight. Most shockingly, it is noted that around 38.2 million kids under the age of 5 have identified as obese or overweight [4]. As the problem is spreading like an epidemic across the globe and among all age groups over the years, it imposes a promising strategy to treat it.

There are several approaches currently in use to combat overweight and obesity, including dieting, exercise, and medication and in extreme circumstances bariatric surgery. However, all these current approaches have certain limitations. For example, lifestyle modifications were stated to have a let-down rate of higher than 85%, over 5 to 10 years if the modifications were not continued [5]. In the case of pharmacotherapy, the options are very less and orlistat is the only FDA-approved medication which is presently been in the market but which was also reported to have adverse effects upon its prolonged usage, like steatorrhea, flatus, fecal incontinence, and nervousness [6]. Bariatric surgery is generally been suggested for immensely obese people, keeping in mind the associated postoperative complications. Thus, it is of paramount importance to develop an effective and safe alternative strategy to control the obesity epidemic. The scientific community currently believes that revealing the antiobesity capabilities of conventional food/plant healers could lead the path to developing a novel drug for obesity management with fewer side effects.

Dolichos biflorus L., belonging to the *Fabaceae* family, is a leguminous eatable pulse cultivated in subtropics areas. It is usually recognized as horse gram in English, and kulthi in the Indian System of Medicine. It is a suberect, branched herb, indigenous to India and is found up to altitudes of 1000 m. Traditionally, its seeds have been used for various ailments like anthelmintic, expectorant, astringent, diaphoretic, diuretic, ophthalmic, and tonic. The seeds have been reported to possess chemo modulatory [7], antilithiatic [8], antihepatotoxic [9], and antioxidant activity [10]. It is also being used in Ayurvedic medicines for the treatment of medroga (obesity), constipation, piles, hepatomegaly, splenomegaly, and renal calculi. Despite the hot water extract of *D. biflorus* seeds having long historical use for local management of obesity, there is a dearth of published scientific records on its antiobesity potential. From the literature search, it was found that the antiobesity potential of organic solvent extract of *D. biflorus* seeds itself or in combination [11] has been studied extensively. However, no such extensive data are available for hot water (aqueous) extract. Thus, the current investigation has been framed to assess extensively to know the antiobesity efficacy of the plant *D. biflorus* using aqueous of seed (ASEDB) in a cell line, 3T3-L1 adipocytes, and HFD-induced obese rats thereby scientifically validating the plant as a traditional healer.

2. Materials and Methods

2.1. Chemicals. DMEM (Dulbecco's modified Eagles medium), streptomycin, penicillin, dexamethasone, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), DMSO (dimethylsulfoxide), FBS (fetal bovine serum), and 3-isobutyl-1-methylxanthines were purchased from Himedia, Mumbai, India. Oil red O, free glycerol determination kit, PPL (porcine pancreatic lipase), commercial insulin, and leptin ELISA quantification kits were purchased from Sigma-Aldrich, USA. Analytical kits for the determination of blood glucose, serum FFA (free fatty acid), TG (triglycerides), TC (total cholesterol), HDL-C (high-density lipoprotein cholesterol), ALT (alanine transaminase), and AST transaminase (aspartate transaminase) were supplied by Biosystems, Tamil Nadu, India. All other analytical grade reagents used in the current study were obtained from Merck Ltd., India.

2.2. Extract Preparation. Fresh dried seeds of *D. biflorus* were acquired from the natural drug mart in Trichy, India. The clean, dried seeds were then pulverized into tiny powders by using a household electric blender. One part of the powdered sample was soaked with six parts of water and boiled with continuous stirring till the content was reduced to one-third of its original volume. The resultant extract after being filtered through a muslin cloth was then concentrated using a boiling water bath. The yield of the dried extract was found to be nearly 21.4% (w/w) and it was kept in an airtight vessel and stored at 4°C till further usage.

2.3. Inhibition of Pancreatic Lipase Assay. The PPL (porcine pancreatic lipase) inhibition assay was done based on previously described protocols [12–14] with minor modifications. PPL stock (1 mg/mL) was prepared by using potassium phosphate buffer (0.1 mM, pH 6.0) and was kept at −20°C. To assay the inhibitory activity, various dosages (5 μg/mL, 10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL) of 0.2 mL of the extracts were preincubated with

0.1 mL of PPL in 0.1 mM (pH 7.2) potassium phosphate buffer, which contains Tween 80 (0.1%) for 1 hour, at 30°C. To this, 0.1 mL of 25 mM p-nitrophenyl butyrate in acetonitrile (substrate solution) was added and the chemical reaction was initiated by incubating the reaction mixture at 37°C for 5 min. The positive control, orlistat, also underwent the protocol. After the incubation period, the amount of p-nitrophenol freed from p-nitrophenyl butyrate was noted at 405 nm using a spectrophotometer. The negative control was also measured with and without the addition of an inhibitor. By using the following formula, the percentage of lipase inhibition was calculated.

$$\%\text{Inhibition} = 100 - \left[\frac{(B - b)}{(A - a)} \times 100\right], \quad (1)$$

where *B*-activity with an inhibitor; *b*-negative control with an inhibitor; *A*-activity without an inhibitor; and *a*-negative control without an inhibitor.

2.4. Anti-Adipogenic Effects of ASEDB in 3T3-L1 Adipocytes

2.4.1. 3T3-L1 Preadipocytes Culture and Differentiation. Mouse 3T3-L1 preadipocytes were purchased from National Centre for Cell Science, India, and were cultured in DMEM adding with FBS (10%), 100 IU/mL of penicillin and 100 μg/mL of streptomycin were maintained at 37°C with 5% CO_2. For adipocyte differentiation, two days old postconfluent preadipocytes were incubated in an inducer (consisting of 0.25 μM dexamethasone, 0.5 mM 3-isobutyl-1-methylxanthine, and 10 μg/mL insulin) supplemented DMEM for 48 hours (day 2) and then maintained in FBS (10%) and insulin (10 μg/mL) supplemented DMEM for another 6 days (day 8). The culture medium was replaced with a fresh medium every two days.

2.4.2. Cell Viability Test. The influence of the aqueous seed extracts of *D. biflorus* (ASEDB) on 3T3-L1 preadipocyte viability was analyzed through an MTT assay [15]. 3T3-L1 preadipocytes (1×10^5 cells/mL) were taken in a 96-well microtiter plate (in triplicate) and treated with various concentrations of ASEDB (10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL) and incubated in FBS-free culture medium at 37°C for 2 days under 5% CO_2. Instead of extracts, 0.05% of DMSO was added to the control group. After two days, fresh media (FBS-free) with MTT (0.5 mg/mL) was added and incubated at 37°C for an hour. The purple formazan crystals developed were then solubilized with100 μL of DMSO and measured at 560 nm spectrophotometrically. The percentage of the viable cells were determined by (At/Ac) × 100, where At and Ac represent the absorbance of the test group and the control group, respectively.

2.4.3. Oil-Red-O Staining. Efficacy of ASEDB on anti-adipogenic activity was evaluated by measuring the lipid pile-up in adipocytes after the neutral lipids were stained with Oil Red O [14, 16]. For anti-adipogenic studies, 3T3-L1 cells undergoing the first 2 days of adipogenic differentiation were treated with nontoxic doses of ASEDB (10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL). Control 3T3-L1 cells were maintained without extracts. All these cells were subsequently moved to a maintenance medium for the following four days. Following 6 days of incubation, the cells were subjected to PBS (phosphate-buffered saline) wash two times and fixed with formaldehyde (10%) in PBS for 30 minutes at 37°C. The cells were then washed twice with distilled water and finally stained by using Oil Red O (3 mg/mL in 60% isopropyl alcohol) for a period of 1 hour at the temperature of 25°C. After 1 hour, the lipid buildup in the cells was assessed microscopically. In addition, the dye that remained in the cells after being eluted with isopropanol (100%) was quantified spectrophotometrically at 510 nm.

2.4.4. Glycerol Release Assay. Glycerol release was quantified based on the method previously reported [17]. Briefly, the differentiated 3T3-L1 cells were treated with varying dosages of ASEDB (10 μg/mL, 25 μg/mL, 50 μg/mL, 100 μg/mL, 250 μg/mL, and 500 μg/mL) and incubated for 24 hours. Then, the free glycerol reagent was mixed to the tubes and the reaction was allowed to stand for 5 mins, based on the manufacturer's instruction. Distilled water (blank) and glycerol standard were also treated similarly and the absorbance was measured at 540 nm.

2.5. Effect of ASEDB on Antiobesity Properties

2.5.1. Experimental Rats. Wistar male albino rats weighing between 100 and 125 g were procured from Tamil Nadu Veterinary and Animal Sciences University, Chennai, India, for use in this investigation. All of the animals were housed in polypropylene cages under appropriate environmental controls (65 ± 5% humidity, 23 ± 2°C temperature, and 12/12 hours of light-dark cycle) and were given standard commercially available rat chow pellets (manufactured by Sai Durga Feeds and Foods, Bengaluru, India) used as a standard feed along with unlimited access to water. The rats spent roughly a week like this becoming accustomed to the lab environment before being used for the investigation.

All the experiments and protocols carried out in this study complied with OECD guidelines of CPCSEA and were approved by the Institutional Animal Ethics Committee of Srimad Andavan Arts and Science College, Tiruchirappalli, India (Approval No: 790/03/ac/CPCSEA).

2.5.2. Induction of Obesity and Treatment. A total of 40 rats were induced with preobesity before the start of the original experimental period by feeding a calorie-richhigh-fat diet (HFD) for 45 days. The HFD as represented in earlier studies [14, 18] comprised 5% corn oil (45 kcal), 15% corn starch (60 kcal), 20% sucrose (80 kcal), 20% casein (80 kcal), and 35% beef tallow (315 kcal) in addition to 4% mineral and 1% vitamin mixture. After feeding HFD for 45 days, 30 rats

which were considered obese-prone (weighing close to 250 g) were chosen for antiobesity studies. In the same period, another 6 rats were maintained by feeding standard rat chow pellets. The experimental rats were divided randomly into 6 groups ($n = 6$). The rats fed by standard rat chow pellets served to continue as normal control (Group 1), while the HFD-fed preobese rats served as group 2 to group 6. Group 2 was maintained as the obese/HFD control, and kept eating HFD without ASEDB treatment. Groups 3 to 5 continued to feed on HFD and treated with 100 mg/kg, 200 mg/kg, and 300 mg/kg of ASEDB, respectively. Group 6 animals were given standard medication orlistat (30 mg/kg) in addition to HFD. The drugs were injected orally via a feeding tube once a day for 60 days.

2.5.3. Assessment of Antiobesity Activity. Daily food consumption [19] and body weight (at an interval of every 10 days) of experimental animals were documented regularly. The rats were sacrificed by cervical dislocation under diethyl ether anesthesia at the end of study period (60th day) and blood was collected from the heart for various biochemical assays. Necessary organs (kidney, liver, spleen, and heart) as well as fat fads (mesenteric, epididymal, and perirenal) were separated, washed in buffered saline, blotted dry, and weighed immediately to obtain their absolute weights.

The adipocyte area of the visceral fat fad was calculated with the help of a Nikon trinocular light microscope (Eclipse, Ci) furnished with network information system (NIS) digital imaging software. The BMI of the rats, i.e., body weight in gram divided by body length in square cm, was determined and recorded on the initial and final days of the experimentation [20]. Body length (from nose to anus) and waist circumference (on the greatest portion of the stomach) of the experimental rats were measured with a tape by possessing the animals in a ventral posture under mild sodium barbiturate (0.1 ml i.p. of 1%) anesthesia.

Serum levels of TC, TG, HDL-C, FFA, AST, ALT, and blood glucose were determined using commercial kits. The LDL-C and VLDL-C were determined using the formulas: LDL-C = TC−(HDL-C−(TG/5)); VLDL-C = total serum TG/5 [21]. The insulin and leptin were quantified using commercially available ELISA kits. Lipid peroxides (LPO) [22], glutathione reduced (GSH) [23], superoxide dismutase (SOD) [24], and catalase [25] were analyzed using standard protocols.

2.6. Statistical Analysis. All the obtained data were presented as the mean ± SEM (standard error of the mean), and were statistically analyzed by one-way ANOVA, which is followed by Duncan's multiple range test; <0.05 was taken as statistically significant differences among groups.

3. Results

3.1. Inhibitory Effect of Pancreatic Lipase by ASEDB. The porcine pancreatic lipase (PPL) inhibitory action of ASEDB at various concentrations (5 μg/mL to 500 μg/mL) was assessed by evaluating the breakdown of p-nitrophenyl butyrate into p-nitrophenol. Figure 1 demonstrates that ASEDB has a substantial level of inhibition potential on the activity of PPL with IC_{50} of 213.3 μg/mL when matched to the standard drug orlistat, whose IC_{50} value was found to be 9.2 μg/mL.

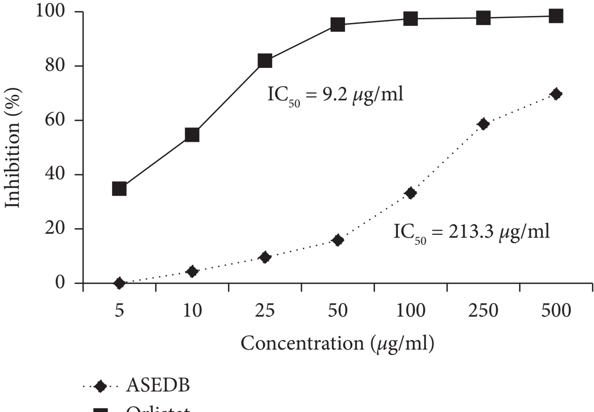

Figure 1: The pancreatic lipase inhibitory effect of ASEDB. Values are the mean of 3 experiments; ASEDB–aqueous seed extract of *Dolichos biflorus* L. Orlistat was used as a positive control.

3.2. Anti-Adipogenic and Lipolytic Effect. First, to assess the possibility of 3T3-L1 preadipocytes upon ASEDB exposure, the cells were treated with a varied dosage of ASEDB (10 μg/mL–500 μg/mL) and the observed findings were expressed as percentage viability, comparing the cell viability of negative control's (Figure 2(a)). These data made it clear that none of the ASEDB doses administered had an impact on the cells' viability. Thus, the selected dosages of ASEDB (10 μg/mL–500 μg/mL) were deemed nontoxic in 3T3-L1 cell lines and considered for further investigation.

Oil Red O staining was used to assess the anti-adipogenic potential of ASEDB. The observed findings (Figure 2(b)), as well as photomicrographs of lipid staining (Figure 2(c)), clearly revealed that ASEDB dose-reliantly decreased lipid accumulation during 3T3-L1 differentiation. At the higher dose of 500 μg/mL, ASEDB exerted around 35.25 ± 1.37% reductions in fat accumulation than control cells.

The lipolytic effect of ASEDB was assessed by estimating the quantity of glycerol liberated from the differentiated 3T3-L1 adipocytes and the obtained data are presented in Figure 2(d). ASEDB had shown a reasonable level of lipolytic activity which was evidenced through a notable increase ($p < 0.05$) in the release of glycerol approximately by 21, 31, and 33% at doses of 100, 250, and 500 μg/mL of ASEDB, respectively.

3.3. Antiobesity Effect of ASEDB on HFD-Induced Obese Rats

3.3.1. Effect of ASEDB on Food Consumption. The food consumption of the experimental groups was determined by subtracting the quantity of remaining chow from the

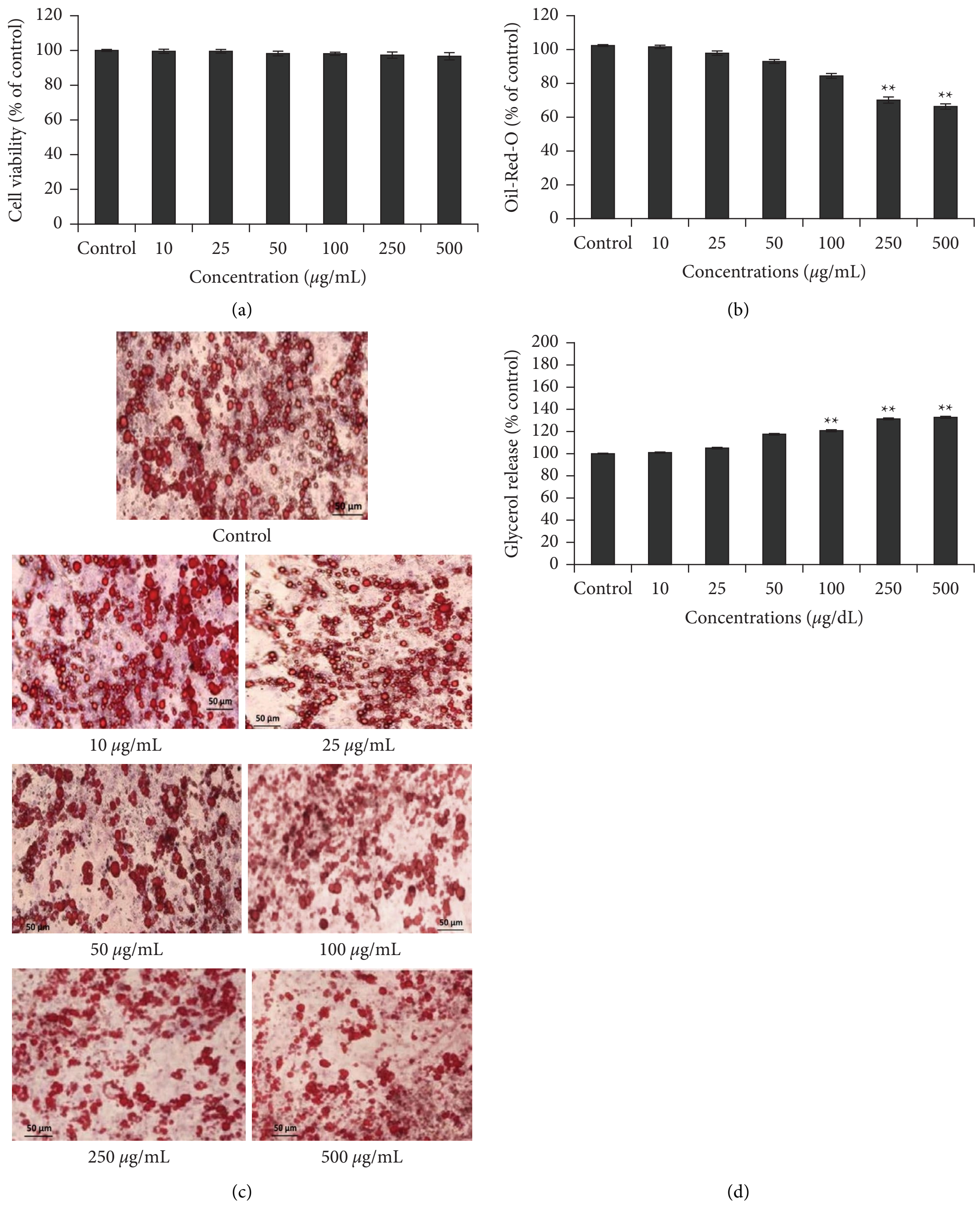

Figure 2: Anti-adipogenic and lipolytic effect of ASEDB on 3T3-L1 adipocytes: (a) cell viability, (b) intracellular lipid content, (c) microscopic photographs of Oil-Red-O staining, and (d) glycerol release. ASEDB–aqueous seed extract of *Dolichos biflorus* L. Values are the mean ± SEM ($n = 3$). $^{**}p < 0.05$, significantly different from the control group.

preweighed chow, every 24 hours per cage basis and the received data are represented in Figure 3 as average food intake. The data have indicated that animals fed with HFD consumed significantly more food on average than animals fed with the ND. Administration of ASEDB along with HFD however showed a slight suppression of food intake, without showing any significant dose-dependent variation. However, orlistat-treated group 6 rats showed significantly lower ($p < 0.05$) food consumption than ASEDB-treated rats.

3.3.2. Influence of ASEDB on Body Weight, Fat Pad Weights, Organ Weights, Adipocyte Area, BMI, and Waist Circumference (WC). An initial body weight of the experimental models were noted on the day one, followed by the changes in those weights brought on by ASEDB supplementation; these changes were documented once every 10 days. Obtained results have shown that there was a significant upsurge in a gain of body weight in HFD control rats (Group 2) when matching to the normal control (Group 1) rats. On the

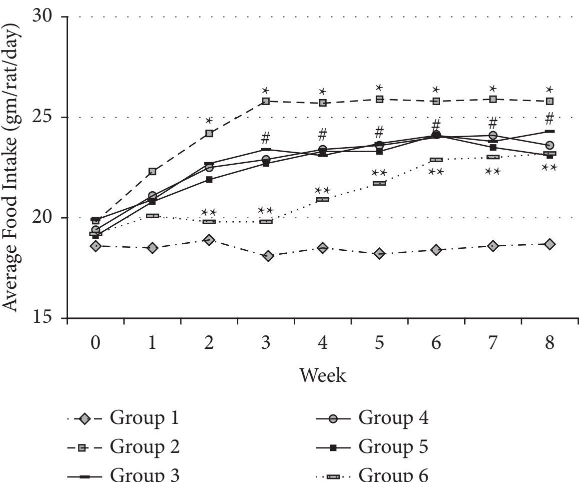

Figure 3: Levels of average food intake in experimental rats. Group 1–fed with ND; group 2–fed with HFD; group 3–HFD + ASEDB (100 mg/kg bw); group 4–HFD + ASEDB (200 mg/kg bw); group 5–HFD + ASEDB (300 mg/kg bw); group 6–HFD + orlistat (30 mg/kg bw); ND–normal diet; HFD–high fat diet; ASEDB–aqueous seed extract of *Dolichos biflorus* L; values are the mean of 6 rats; $^{*}p < 0.05$, significantly different from group 1; $^{**}p < 0.01$, significantly different from group 2; $^{\#}p < 0.05$, group 3, 4, and 5 significantly different from group 2; no significant differences between ASEDB-treated groups (group 3, 4, and 5).

60th day of the experimental period, group 2 animals have shown approximately 15% more weight gain than group 1 animals. However, treatment with various dosages of ASEDB (100 mg/kg, 200 mg/kg, and 300 mg/kg BW) significant reduction ($p < 0.05$) was noted in the percentage body weight gain of the animals are in proportion to the dose when compared to HFD control rats. Adipocyte area, the weights of the liver, heart, kidney, and spleen, as well as the mesenteric, perirenal, and retroperitoneal fat pad weights, were also observed to be increased in HFD fed, untreated rats but dramatically decreased ($p < 0.05$) in ASEDB-treated rats in a dose-reliable manner (Figure 4).

The BMI and WC of the animals were measured on days 1 and 60 and the found data are depicted in Figure 5. When compared to obese rats that were administered HFD without receiving any treatment (Group 2), the administration of ASEDB plus HFD significantly decreased the rats' BMI and WC in proportionate to the concentration.

3.3.3. Effect of ASEDB on Different Biochemical Parameters. The effect of ASEDB on blood glucose, leptin, and insulin levels in the animals is given in Table 1. The increased levels of glucose, leptin, and insulin found in HFD-induced rats were efficiently returned to near-normal levels in ASEDB-treated animals. The data on the effect of ASEDB on hepatic triglycerides and serum lipid profiles are also given in Table 1. HFD feeding to the experimental animals raised the levels of hepatic and serum triglycerides, FFA, TC, LDL-C, and VLDL-C while HDL-C was found to be reduced. However, administrations of ASEDB effectively restored serum lipids and hepatic triglycerides to normalcy in proportion to the dosage. The AST and ALT showed elevated levels in the serum of HFD-fed rats than normal rats. However, on ASEDB treatment, these alterations were reversed to normal, dose-dependently (Table 1).

3.3.4. Effect of ASEDB on Hepatic Antioxidant Status. The data obtained on antioxidant status in liver tissues of HFD-fed obese rats revealed a significant elevation in peroxidation of lipids and depletion in the level of GSH and activities of enzymatic antioxidants such as SOD and CAT. However, the analyses of data from HFD-rats treated with ASEDB have shown improved levels of these alterations in a dose-reliable manner (Table 2).

4. Discussion

Obesity is reaching epidemic proportions worldwide and has led to a rise in the burden of health care, a decline in life expectancy, and many other socioeconomic problems. This epidemic of obesity alerts the public and mainstream researchers to the need to increase the efficacy of the therapies available. In the present study, we have studied the anti-obesity properties of ASEDB in the differentiation of 3T3-L1 preadipocytes and HFD-induced obesity in the experimental rats.

Although numerous factors may be responsible for the pathogenesis of obesity, the availability of energy-rich foods and excessive calorie consumption are the prominent contributors to obesity in maximum cases [26]. Dietary fats are mostly comprised of mixed TGs which get broken down by pancreatic lipase (PL) and serve as the major source of extra calories relative to other nutrients. Therefore, the reduction of the activity of PL and also reduction of the digestion and absorption of dietary fat from the intestine are the cornerstone strategies in the therapeutic attempt to reduce the excessive intake of energy [27]. So far, orlistat, which is derived from *Streptomyces toxytricini*, is the lone drug available in the market to inhibit PL activity. This drug can reduce fat absorption by about 30%. However, its use is limited because its prolonged usage can result in side effects including oily spotting, steatorrhea, fecal urgency, and bloating [28, 29]. Hence, the quest for a safe PL inhibitor is still in pipeline and many researchers in recent days screened hundreds of natural products for possible lipase inhibitory effects. In the current investigation, we analyzed the pancreatic lipase inhibition ability of ASEDB and the found data exposed that ASEDB inhibited PL moderately in different concentrations when compared with the standard drug orlistat. Even though ASEDB was not more effective in inhibiting lipase activity than orlistat, its effect was superior to that of many other dietary plants which were reported previously [30]. Given that the secondary metabolites of ASEDB such as tannins, saponins, polyphenols, or triterpenes have a well-established ability to inhibit pancreatic lipases [31–33], this inhibitory effect of ASEDB might be mediated by these secondary metabolites.

Obesity is highlighted by the accumulation of additional calories in the adipose tissue, which is mostly composed of

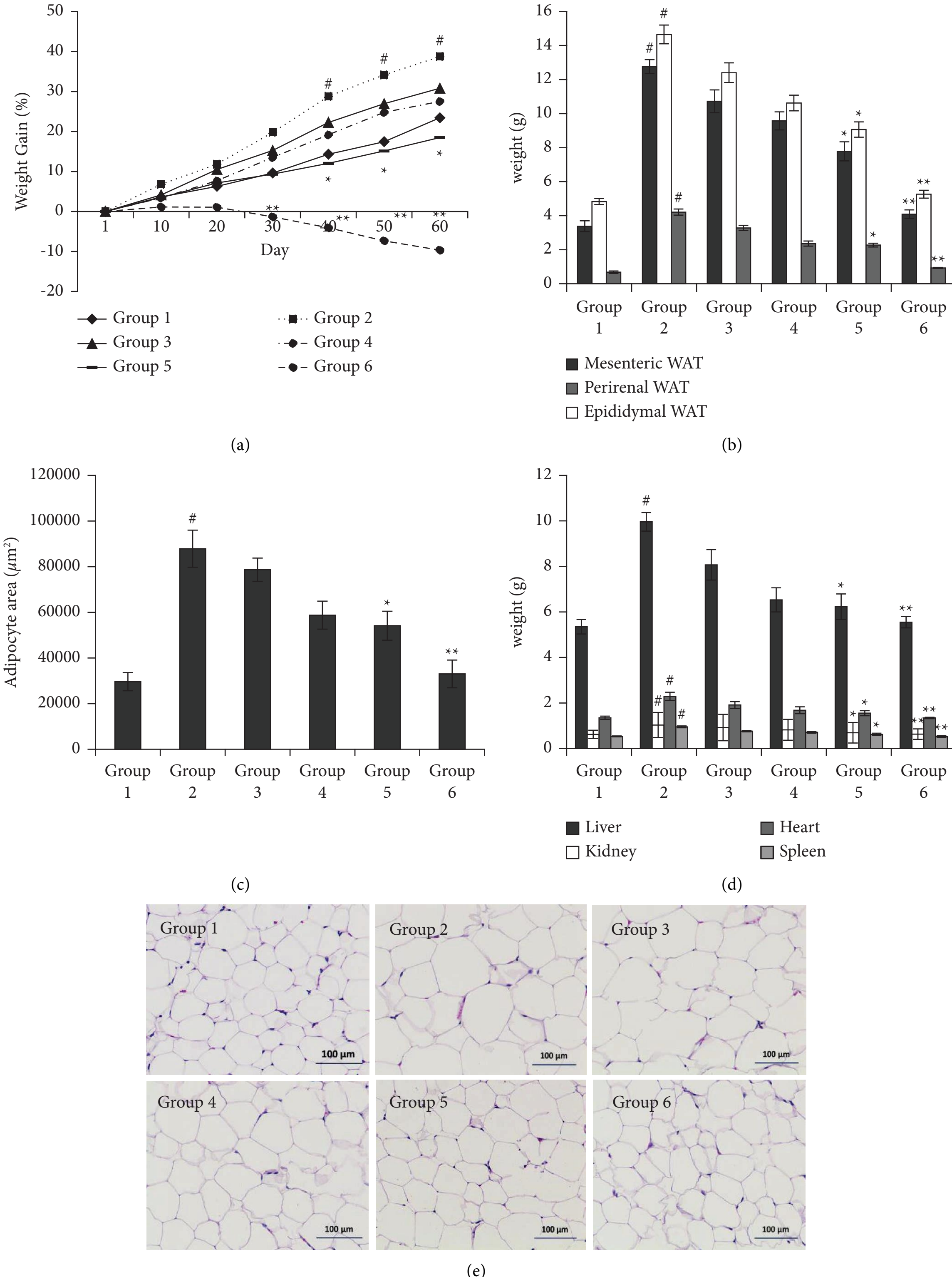

Figure 4: Effect of ASEDB on (a) body weight; (b) fat pad weight; (c) adipocyte area; (d) organ weights; and (e) microscopic images of adipocytes showing variations in adipocyte area among different groups. WAT–white adipose tissue; ASEDB–aqueous seed extract of *Dolichos biflorus* L.; values are the mean ± S.E.M of 6 rats; $^{\#}p < 0.05$, significantly different from group 1; $^{*}p < 0.05$, significantly different from group 2; $^{**}p < 0.01$, significantly different from group 2.

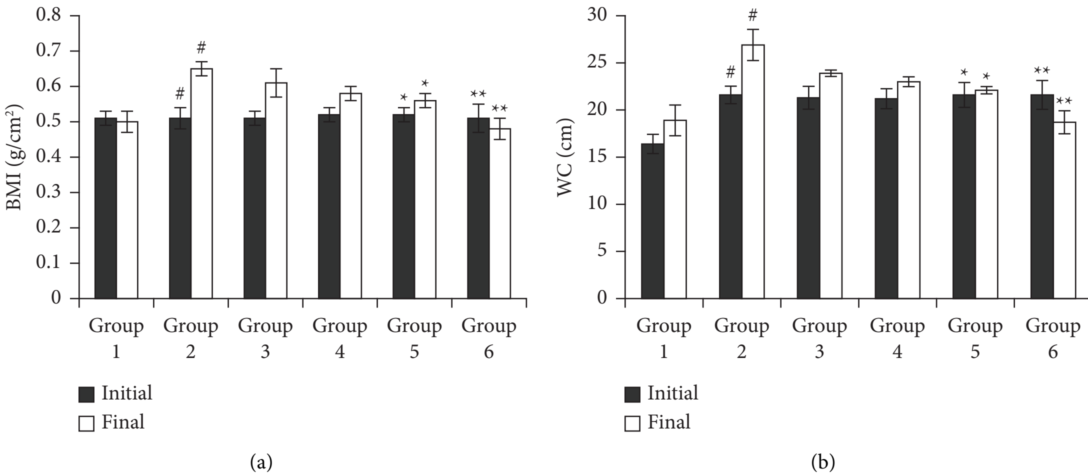

Figure 5: Effect of ASEDB on (a) body mass index; (b) waist circumference of experimental rats. Values are the mean ± S.E.M ($n = 6$); #significant difference from normal control (group 1) at $p < 0.01$; *significant difference from HFD control (group 2) at $p < 0.05$; **Significant difference from HFD control (group 2) at $p < 0.01$.

Table 1: Effect of ASEDB on different biochemical parameters.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Glucose (mg/dL)	71.08 ± 1.65	149.16 ± 2.58#	125.84 ± 1.67	106.43 ± 1.82	93.28 ± 1.87*	76.52 ± 2.12**
Insulin (μIU/mL)	3.42 ± 0.20	7.35 ± 0.22#	6.55 ± 0.16	5.61 ± 0.23	5.02 ± 0.16*	4.04 ± 0.25**
Leptin (ng/mL)	3.12 ± 0.24	7.56 ± 0.32#	6.36 ± 0.38	5.63 ± 0.36	4.95 ± 0.18*	3.83 ± 0.17**
Hepatic TG (mg/g tissue)	9.58 ± 0.48	18.02 ± 0.49#	16.22 ± 0.61	15.33 ± 0.52	14.54 ± 0.13*	11.26 ± 0.23**
Serum TG (mg/dL)	73.85 ± 1.92	169.24 ± 2.54#	146.34 ± 1.77	127.92 ± 2.69	114.47 ± 1.93*	92.19 ± 1.47**
FFA (mg/dL)	20.12 ± 2.08	46.71 ± 1.89#	34.86 ± 1.20	32.80 ± 1.71	29.56 ± 2.32*	21.90 ± 1.03**
TC (mg/dL)	98.02 ± 1.41	174.16 ± 2.48#	158.31 ± 1.96	135.71 ± 1.98	108.65 ± 1.96*	99.54 ± 1.59**
HDL-C (mg/dL)	67.28 ± 0.98	43.05 ± 0.71#	46.93 ± 0.88	49.21 ± 1.32	53.91 ± 0.91**	58.99 ± 1.25**
LDL-C (mg/dL)	42.15 ± 1.66	157.55 ± 1.43#	132.43 ± 1.91	103.48 ± 1.24	73.60 ± 1.58*	57.33 ± 1.34**
VLDL-C (mg/dL)	15.25 ± 0.52	33.16 ± 1.83#	31.28 ± 1.02	25.58 ± 1.14	22.09 ± 0.63*	17.08 ± 0.59**
ALT (U/L)	39.23 ± 1.04	74.19 ± 0.80#	57.18 ± 1.29	51.03 ± 0.88	45.73 ± 0.93*	43.89 ± 1.27**
AST (U/L)	67.83 ± 1.47	150.56 ± 1.32#	135.79 ± 0.62	121.69 ± 1.13	102.06 ± 1.34*	79.63 ± 1.46**

Values are expressed in the mean ± S.E.M ($n = 6$); #significantly differ from normal control at $p < 0.01$; *significantly differ from the HFD-treated group at $p < 0.05$; **significant difference from the HFD-treated group at $p < 0.01$.

fat cells. Because this expansion of adipose mass is associated with both hyperplasia and hypertrophy, the efficiency of ASEDB was studied on the progressions of the proliferation of the adipocyte and differentiation with 3T3-L1 preadipocytes. 3T3-L1 cell line, developed from murine Swiss 3T3 cells, is a well-known model for studying adipogenesis and obesity [14]. These cells display a fibroblast-like morphology and upon suitable conditions undergo differentiation (the process also known as adipogenesis) and develop into mature adipocytes [34, 35]. We first examined the effect of various concentrations of ASEDB on 3T3-L1 preadipocyte viability. As revealed in Figure 2(a), an insignificant reduction in cell viability was noticed as the concentration increased, but still, the viability of the cell was retained at over 95% for all the administered concentrations. Therefore, these concentrations were taken as noncytotoxic doses and were used for further study.

The 3T3-L1 preadipocytes have been exposed to an adipogenic-inducing medium and the same has been treated with various doses of ASEDB. Then, the concentrations of intracellular lipid build-up in maturing 3T3-L1 adipocytes were measured. 3T3-L1 preadipocytes possess the triglyceride accumulation ability upon differentiation. Oil Red O staining results showed that ASEDB decreased the size and number of lipid droplets in a dose-reliant manner and indicates the inhibitory activity on the accumulation of lipids in differentiated 3T3-L1 adipocytes using ASEDB. As the mechanism of antiobesity potential mainly depends upon the balance between lipolysis and lipogenesis [17, 36], we then studied the lipolytic effect of ASEDB on matured 3T3-L1 adipocytes. Adipocytes release glycerol into the medium in proportionate to triglyceride hydrolysis, and this release was found to be significantly elevated after the treatment of matured 3T3-L1 adipocytes with ASEDB, expressing the antiadipogenic effects. The findings of the present study are in agreement with the earlier reported study [11]. Adipocyte differentiation is promoted by a cascade of transcription factors acting sequentially. Likewise, lipolysis was also identified to be regulated by the expression of genes associated with hormone-sensitive lipase and the oxidation of fatty acid. It is presumed that ASEDB inhibits the development as well as the build-up of fat in the cells by obstructing the proliferation and differentiation of adipocytes by promoting lipolysis by regulating the expression of

Table 2: Effect of ASEDB on hepatic enzymatic and nonenzymatic antioxidant parameters.

Parameters	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
LPO (n moles of MDA formed/g tissue)	78.25 ± 1.04	$144.56 \pm 1.72^{\#}$	137.42 ± 1.31	118.78 ± 1.52	$112.12 \pm 1.18^{*}$	$97.42 \pm 1.42^{**}$
GSH (mg/g tissue)	4.47 ± 0.05	$2.33 \pm 0.06^{\#}$	2.51 ± 0.11	3.56 ± 0.10	$3.98 \pm 0.07^{*}$	$4.25 \pm 0.18^{**}$
SOD (μmoles of epinephrine oxidized/mg protein)	5.06 ± 0.19	$1.98 \pm 0.07^{\#}$	2.35 ± 0.15	2.71 ± 0.23	$3.17 \pm 0.24^{*}$	$4.23 \pm 0.26^{**}$
CAT (μmoles of H_2O_2 utilized/min/mg protein)	34.72 ± 1.29	$14.27 \pm 0.84^{\#}$	17.31 ± 0.71	20.50 ± 0.94	$24.41 \pm 1.32^{*}$	$30.07 \pm 1.23^{**}$

Values are expressed in the mean ± S.E.M ($n = 6$); $^{\#}$significantly differ from normal control at $p < 0.01$; *significantly differ from the HFD-treated group at $p < 0.05$; **significant difference from the HFD-treated group at $p < 0.01$.

lipolysis-associated genes. Furthermore, in-depth gene expression studies are needed to confirm this. Many natural compounds, such as epigallocatechin gallate (EGCG), ginkgolide C, capsaicin, resveratrol, and curcumin have also been reported to inhibit adipogenesis and induce the process of lipolysis through regulated gene expression [34].

If a person consumes additional calories, then they use them up or expend; such additional calories get deposited as adipose tissues and result in weight gain. Thus, body weight gain is assessed as one of the early signs of the progression of obesity [37]. In this study, HFD feeding resulted in 15.3% more weight gain in obese control rats (group 2) than in normal control rats (group 1) over 60 days. Increased body weight in group 2 animals might be associated with energy balance and accompanying fat deposition in visceral regions caused by HFD feeding. On the contrary, ASEDB supplementation in addition to HFD significantly ($p < 0.05$) reduced the percent weight gain following the dose to HFD-fed group 2 rats. The decreases in body weight may partially be associated with suppressed food intake as evidenced in this study in ASEDB-treated rats. Also, food intake was not drastically suppressed in these ASEDB-treated rats, and their food intake was still higher than that of normal control rats. This discards the possibility of provocation of ASEDB-induced toxicity. Earlier studies also reported its non-toxicity nature and its safe usage as a food source [38].

Obesity features an upsurge in the number and size of adipocytes at the cellular level because of unnecessary fat deposition [39]. Thus, measurement of adipocyte fat pad weights would help to understand the magnitude of obesity. In this study, we observed the weights were increased in the retroperitoneal, mesenteric, and perirenal fat pads in the obese (HFD-fed) control rats when compared to the normal control rats. However, ASEDB administration, significantly reduced ($p < 0.05$) the weight of such fat pads. Furthermore, the measurement of the size of adipocytes in terms of their area after 9 weeks of treatment with ASEDB had shown a substantial decrease in the area of adipocytes following the dose. Determination of organ weight could be another important index of the severity of obesity, as excess calories slowly deposit in organs like the liver, heart, kidney, and spleen in addition to adipose tissues [40]. It was found that HFD feeding resulted in around 46%, 39%, 41%, and 44% more weight gain in the liver, kidney, heart, and spleen of obese control rats (group 2), respectively, than normal diet-fed rats (group 1). However, ASEDB treatment had shown a significant ($p < 0.05$) decrease in the weight of the organ in a dose-dependent manner. It was reported previously that continuous exposures to HFD decline the resting metabolic rate as well as thermogenesis, thereby promoting the accumulation of triglycerides in abdominal regions [41].

The anthropometric methods such as BMI and WC are standard practical approaches, which are recommended for diagnosis, risk evaluation, and exact tracking of overall body fat distribution. Assessing central obesity (visceral adiposity) through BMI and WC helps to predict metabolic troubles like CVDs, atherosclerosis, hypertension, and dyslipidemia [42]. It was reported that a 1 cm rise in WC raises the threat of the arousal of hypertension by 2.49 times [43]. The rise in WC is also a useful predictor for the subsequent progression of T2DM [44]. In this study, it has been noted that HFD administered for 60 days results in a considerable rise in the BMI and WC; however, ASEDB treatment brought them back significantly ($p < 0.05$) in proportionate to dose. This decline in BMI and WC is well correlated with reduced fat accumulation in fat pads and visceral organs.

The adipose tissue acts as a dynamic endocrine organ, which secretes several biologically active adipokines including leptin, the *ob* gene cytokine product. In this study, it is noted that a significant rise ($p < 0.05$) in leptin levels in the obese rats than in normal rats. The leptin level is found to be positively connected with the proportion of fat stores in white adipocytes [45, 46]. Thus, elevated leptin concentration in serum is a marker of body fat mass increase. At normal conditions, the circulating leptin signals the high energy reserves of the periphery to the hypothalamus thereby regulating energy metabolism by suppressing appetite. However, in obese conditions, elevated leptin levels do not seem to do this role because of leptin resistance. It was proposed that raised leptin levels in obesity can cause leptin receptors desensitization in the hypothalamus, thereby disturbing the downstream regulation of energy consumption and body weight [47]. Elevated leptin levels were found to be significantly reduced with ASEDB intake, in proportionate to the dose. This indicates that intake of ASEDB is effective in refining changes in fat mass and leptin levels, affected by HFD intake.

Being overweight and obese have a strong association with T2DM and this may be because of insulin resistance (IR) [48]. In obese conditions, enlarged adipocytes secrete comparably more proinflammatory cytokines (leptin, TNF-α, and IL-6), which causes IR [32, 49]. The resulting IR in obesity thus affects the liver or muscle cells' insulin-dependent glucose intake and contributes to elevated blood glucose levels. The β-cells of the pancreas continually synthesize and secrete insulin in response to increased blood glucose levels, which in turn contributes to hyperinsulinemia. IR, hyperglycemia, and hyperinsulinemia are frequently connected with obesity [50]. The obtained high levels of both blood glucose and serum insulin in the current study also confirmed hyperglycemia and hyperinsulinemia in obese rats. However, in ASEDB-treated rats, the levels of glucose and insulin seemed to be significantly ($p < 0.05$) reduced at a higher dose. The reduced level of leptin observed in ASEDB intake might have improved insulin sensitivity, which is well correlated with the reduced levels of blood glucose and insulin in ASEDB-treated animals.

Dyslipidemia is one of the major concerns of obesity as it significantly escalates the risk of CVD, T2DM, and certain types of cancer. Mounting evidence suggests that IR is the most probable link between obesity and obesity-associated metabolic dyslipidemia [51]. The main patterns in patients with dyslipidemia are their high concentrations of lipid profiles. In the current study, we also encountered significantly ($p < 0.01$) elevated levels of TC, TG, LDL-C, VLDL-C, FFA, and reduced levels of HDL-C in obese rats than in the control group. Elevated FFAs in an obese state are mainly an outcome of the enhanced release of FFA from adipose fat

mass due to the resistance of adipocytes to the anti-lipolysis deed of insulin and the inability of adipocytes to reutilize FFAs efficiently [52]. Hypertriglyceridemia in obesity is partly due to increased liver FFA fluxes and their accumulation as TG. This causes elevated synthesis of large VLDL by hepatocytes, which interrupts the lipolysis of chylomicrons due to competition for lipoprotein lipase (LPL). Under these circumstances of increased concentration and delayed clearance of VLDL, cholesterol ester transfer protein (CETP) promotes the interchange of cholesterol esters in HDL and LDL with TGs in VLDL. This exchange also results in increased cholesterol ester-enriched VLDL particles and reduced HDL-C concentrations [53]. The administration of ASEDB ameliorated dyslipidemia by substantially reverting the altered levels of these lipid profiles to normal in a dose-based manner. The observed reversion of lipid levels may be due to the actions of phytoconstituents present in the extracts in the enhancement of satiety as well as suppression of digestion and absorption of dietary triglycerides through inhibition of lipase activity.

Hepatic steatosis (fatty liver) is a pathological condition due to the deposition of large fat mass in the liver which frequently occurs in patients with obesity. Although liver steatosis is a multifactorial condition, IR is a prime requirement for hepatocellular fat accumulation. IR in obesity may result in boosted lipolysis of TG in the adipose tissue and it leads to more accessibility of FFAs for an enhanced synthesis and storage of triglycerides in liver cells [54]. Our data showed elevated levels of hepatic TGs in HFD-fed control rats, thereby suggesting the development of a fatty liver. However, the supplementation of ASEDB in addition to HFD bettered the fatty liver by overturning the elevation of liver TG. ALT and AST, the hepatic marker enzymes, were also noted to raise in the HFD control rats and this raise may be attributed to fatty liver and oxidative stress-induced hepatic necro-inflammatory injury upon continuous feeding of HFD. Elevated levels of AST and ALT are the common index of fatty liver and associated hepatotoxicity [55]. Whereas, these raised hepatic enzymes were considerably ($p < 0.05$) restored in the ASEDB-administered rats, suggesting the recovery of necro-inflammatory injury, caused by HFD-induced fatty liver.

Excess intakes of fat-rich diets are capable of generating reactive oxygen species (ROS) due to altered oxygen metabolism. Excessive lipid peroxidation and ROS will utilize the body's antioxidant enzymes and vitamins [56, 57], resulting in the depletion of these protective substances and leading to oxidative stress and ROS-mediated cellular damage. Chronic oxidative stress is an important factor in the pathogenesis of obesity and its related morbidities [58]. In the current study, we witnessed higher content of malondialdehyde (MDA) which is the common biomarker for oxidative stress and end product of lipid peroxidation [59], in liver tissues of obese rats than in standard laboratory diet-fed normal rats, indicating HFD-induced oxidative stress in obese rats. ASEDB administration, however, prevented lipid peroxidation which was evidenced by a substantially diminished quantity of MDA in the liver of the treated rats. The level of glutathione (GSH), an important cytosolic antioxidant, was found to be markedly depleted in obese rats than the control group; however, in ASEDB-treated rats, the concentration of GSH was restored significantly ($p < 0.05$). The depletion of GSH in obese rats could be understandable because whenever the cells experience oxidative stress, GSH could be utilized in large amounts to directly quench the ROS such as lipid peroxides. Cells also have another set of powerful antioxidant enzymes named SOD and CAT to respond to the superoxide anions which are generated during oxidative damage. SODs respond to these superoxide anions ($O_2^{\bullet -}$) by converting them into H_2O_2 while CAT converts this toxic H_2O_2 into O_2 and H_2O [60]. We detected significantly diminished levels of both antioxidant enzymes in obese control rats than in control and the reduction may owe to rapid and more consumption of these enzymes storage, in counteraction to the free radicals produced during the progression of obesity. The levels of SOD and CAT were found to be reversed in ASEDB-treated groups suggesting its free radical scavenging efficacy. Restoration of all these antioxidant systems, both enzymatic and nonenzymatic, might be associated with the effective participation of natural phytoconstituents particularly flavones and polyphenols in the test drug in scavenging the free radicals.

5. Conclusions

Taken together, the study represents that ASEDB possesses formidable antiobesity benefits by *in-vitro* (3T3-L1 adipocytes) and *in-vivo* (HFD-fed obese rats). The potentiality of ASEDB might be associated with the reduction of food intake, blockade of adipocyte differentiation, enhancement of lipolysis, decrease in intestinal fat absorption, and amelioration of dyslipidemia through regulated fat metabolism and improvement of antioxidant grade arbitrated by the defensive phytoconstituents present in it. In addition, exploring the mRNA expression of adipogenic proteins, and fatty acid oxidations, as well as in-depth analysis of adipocytokines and ghrelin levels are warranted to understand the detailed mechanism of action. It is also indispensable to isolate and characterize the lead molecules responsible for antiobesity action to develop a promising pure drug to combat obesity.

Data Availability

The datasets used to support the findings of this study are available from the corresponding author upon reasonable request.

Disclosure

Arumugam Vijaya Anand equally contributed as a first author.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Kumaraswamy Athesh, Nayagam Agnel Arul John, Gurunagarajan Sridharan, Pemiah Brindha, and Arumugam

Vijaya Anand conceptualized the study, prepared the original draft, validated the study, and performed methodology; Balasubramanian Balamuralikrishnan, Wen-Chao Liu, and Arumugam Vijaya Anand reviewed and edited the study and performed formal analysis; Amer M. Alanazi and Kannan RR Rengasamy checked for plagiarism and performed final review.

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References

[1] Who, "Physical status: the use and interpretation of anthropometry," WHO Technical Report Series, No. 854, World Health Organization, Geneva, Switzerland, 1995.

[2] K. Athesh, R. Sivasubramanian, G. Jothi, and P. Brindha, "Evaluation of anti-obesity potential of aqueous extract of Achyranthes aspera Linn. in high fat diet induced obese rats," *Clinical Phytoscience*, vol. 6, no. 1, p. 69, 2020.

[3] B. E. Wisse, F. Kim, and M. W. Schwartz, "An integrative view of obesity," *Science*, vol. 318, no. 5852, pp. 928-929, 2007.

[4] Who, "Obesity and overweight," 2020, https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.

[5] C. Ayyad and T. Andersen, "Long-term efficacy of dietary treatment of obesity: a systematic review of studies published between 1931 and 1999," *Obesity Reviews*, vol. 1, no. 2, pp. 113–119, 2000.

[6] K. A. Hwang, Y. J. Hwang, P. R. Im, H. J. Hwang, J. Song, and Y. J. Kim, "Platycodon grandiflorum extract reduces high-fat diet-induced obesity through regulation of adipogenesis and lipogenesis pathways in mice," *Journal of Medicinal Food*, vol. 22, no. 10, pp. 993–999, 2019.

[7] R. Nanta and R. K. Kale, "Chemomodulatory effect of Dolichos biflorus Linn. on skin and forestomach papillomagenesis in Swiss albino mice," *Indian Journal of Experimental Biology*, vol. 49, no. 7, pp. 483–490, 2011.

[8] T. S. Garimella, C. I. Jolly, and S. Narayanan, "In vitro studies on antilithiatic activity of seeds of *Dolichos biflorus* Linn. and rhizomes of *Bergenia ligulata* Wall," *Phytotherapy Research*, vol. 15, no. 4, pp. 351–355, 2001.

[9] S. Laskar, U. K. Bhattarcharyya, A. Sinhababu, and B. K. Basak, "Antihepatotoxic activity of kulthi (*Dolichos biflorus*) seed in rats," *Fitoterapia*, vol. 69, no. 5, pp. 401-402, 1998.

[10] S. Saha and R. J. Verma, "Antinephrolithiatic and antioxidative efficacy of Dolichos biflorus seeds in a lithiasic rat model," *Pharmaceutical Biology*, vol. 53, no. 1, pp. 16–30, 2015.

[11] K. Sengupta, A. T. Mishra, M. K. Rao, K. V. Sarma, A. V. Krishnaraju, and G. Trimurtulu, "Efficacy of an herbal formulation LI10903F containing Dolichos biflorus and Piper betle extracts on weight management," *Lipids in Health and Disease*, vol. 11, no. 1, p. 176, 2012.

[12] C. Roh and U. Jung, "Screening of crude plant extracts with anti-obesity activity," *International Journal of Molecular Sciences*, vol. 13, no. 2, pp. 1710–1719, 2012.

[13] C. D. Zheng, Y. Q. Duan, J. M. Gao, and Z. G. Ruan, "Screening for anti-lipase properties of 37 traditional Chinese medicinal herbs," *Journal of the Chinese Medical Association*, vol. 73, no. 6, pp. 319–324, 2010.

[14] K. Athesh and P. Brindha, "Anti-obesity potential of Capparis spinosa flower bud extracts in 3T3-L1 adipocytes and in high fat diet induced obese rats," *Pharmaceutical Sciences Asia*, vol. 2022, no. 5, pp. 486–497, 2022.

[15] M. C. Kang, N. Kang, S. C. Ko, Y. B. Kim, and Y. J. Jeon, "Anti-obesity effects of seaweeds of Jeju island on the differentiation of 3T3-L1 preadipocytes and obese mice fed a high-fat diet," *Food and Chemical Toxicology*, vol. 90, pp. 36–44, 2016.

[16] J. L. Ramirez-Zacarias, F. Castro-Munozledo, and W. Kuri-Harcuch, "Quantitation of adipose conversion and triglycerides by staining intracytoplasmic lipids with Oil red O," *Histochemistry*, vol. 97, no. 6, pp. 493–497, 1992.

[17] J. H. Sung, J. W. Chon, M. A. Lee, J. K. Park, J. T. Woo, and Y. K. Park, "The anti-obesity effect of Lethariella cladonioides in 3T3-L1 cells and obese mice," *Nutrition Research and Practice*, vol. 5, no. 6, pp. 503–510, 2011.

[18] R. Buettner, J. Scholmerich, and L. C. Bollheimer, "High-fat diets: modeling the metabolic disorders of human obesity in rodents," *Obesity*, vol. 15, no. 4, pp. 798–808, 2007.

[19] C. L. Hsu and G. C. Yen, "Effect of gallic acid on high fat diet-induced dyslipidaemia, hepatosteatosis and oxidative stress in rats," *British Journal of Nutrition*, vol. 98, no. 4, pp. 727–735, 2007.

[20] E. L. B. Novelli, Y. S. Diniz, C. M. Galhardi et al., "Anthropometrical parameters and markers of obesity in rats," *Laboratory Animals*, vol. 41, no. 1, pp. 111–119, 2007.

[21] W. T. Friedewald, R. I. Levy, and D. S. Fredrickson, "Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge," *Clinical Chemistry*, vol. 18, no. 6, pp. 499–502, 1972.

[22] H. Ohkawa, N. Ohishi, and K. Yagi, "Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction," *Analytical Biochemistry*, vol. 95, no. 2, pp. 351–358, 1979.

[23] M. S. Moron, J. W. Depierre, and B. Mannervik, "Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 582, no. 1, pp. 67–78, 1979.

[24] H. P. Misra and I. Fridovich, "The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase," *Journal of Biological Chemistry*, vol. 247, no. 10, pp. 3170–3175, 1972.

[25] A. C. Maehly and B. Chance, "The assay of catalases and peroxidases," *Methods of Biochemical Analysis*, vol. 1, pp. 357–424, 1954.

[26] C. Y. Wang Cy and J. K. Liao, "A mouse model of diet-induced obesity and insulin resistance," *Methods in Molecular Biology*, vol. 821, pp. 421–433, 2012.

[27] K. Athesh, G. Sridharan, and P. Brindha, "Scientific evaluation of anti-obesity potential of aqueous seed kernel extract of

Mangifera indica Linn," *High Fat Diet Induced Obese Rats Obesity Medicine*, vol. 19, Article ID 100264, 2020.

[28] R. M. Viner, Y. Hsia, T. Tomsic, and I. C. K. Wong, "Efficacy and safety of anti-obesity drugs in children and adolescents: systematic review and meta-analysis," *Obesity Reviews*, vol. 11, no. 8, pp. 593–602, 2009.

[29] T. D. Filippatos, C. S. Derdemezis, I. F. Gazi, E. S. Nakou, D. P. Mikhailidis, and M. S. Elisaf, "Orlistat-associated adverse effects and drug interactions: a critical review," *Drug Safety*, vol. 31, no. 1, pp. 53–65, 2008.

[30] F. Conforti, V. Perri, F. Menichini et al., "Wild Mediterranean dietary plants as inhibitors of pancreatic lipase," *Phytotherapy Research*, vol. 26, no. 4, pp. 600–604, 2012.

[31] R. F. Oliveira, G. A. Goncalves, F. D. Inacio et al., "Inhibition of pancreatic lipase and triacylglycerol intestinal absorption by a pinhao coat (Araucaria angustifolia) extract rich in condensed tannin," *Nutrients*, vol. 7, no. 7, pp. 5601–5614, 2015.

[32] A. L. de la Garza, F. I. Milagro, N. Boque, J. Campion, and J. A. Martinez, "Natural inhibitors of pancreatic lipase as new players in obesity treatment," *Planta Medica*, vol. 77, no. 08, pp. 773–785, 2011.

[33] J. W. Yun, "Possible anti-obesity therapeutics from nature – a review," *Phytochemistry*, vol. 71, no. 14-15, pp. 1625–1641, 2010.

[34] Y. C. Tung, P. H. Hsieh, M. H. Pan, and C. T. Ho, "Cellular models for the evaluation of the antiobesity effect of selected phytochemicals from food and herbs," *Journal of Food and Drug Analysis*, vol. 25, no. 1, pp. 100–110, 2017.

[35] F. J. Ruiz-Ojeda, A. I. Ruperez, C. Gomez-Llorente, A. Gil, and C. M. Aguilera, "Cell models and their application for studying adipogenic differentiation in relation to obesity: a review," *International Journal of Molecular Sciences*, vol. 17, no. 7, p. 1040, 2016.

[36] S. Rayalam, M. A. Della-Fera, and C. A. Baile, "Phytochemicals and regulation of the adipocyte life cycle," *The Journal of Nutritional Biochemistry*, vol. 19, no. 11, pp. 717–726, 2008.

[37] Y. J. Jeong, E. H. Sohn, Y. H. Jung et al., "Anti-obesity effect of Crinum asiaticum var. japonicum Baker extract in high-fatdiet-induced and monogenic obese mice," *Biomedicine & Pharmacotherapy*, vol. 82, pp. 35–43, 2016.

[38] S. U. Upadhyay, V. C. Jain, and U. M. Upadhyay, "Glossary of*Dolichos biflorous*-A Legume with miraculous activities," *Research Journal of Pharmacology and Pharmacodynamics*, vol. 7, no. 2, pp. 103–116, 2015.

[39] T. Furuyashiki, H. Nagayasu, Y. Aoki et al., "Tea catechin suppresses adipocyte differentiation accompanied by down-regulation of PPARγ2 and C/EBPα in 3T3-L1 cells," *Bioscience Biotechnology & Biochemistry*, vol. 68, no. 11, pp. 2353–2359, 2004.

[40] M. Kang, J. W. Oh, H. K. Lee et al., "Anti-obesity effect of PM-F2-OB, an anti-obesity herbal formulation, on rats fed a high-fat diet," *Biological and Pharmaceutical Bulletin*, vol. 27, no. 8, pp. 1251–1256, 2004.

[41] M. Gerbaix, L. Metz, E. Ringot, and D. Courteix, "Visceral fat mass determination in rodent: validation of dual-energyX-ray absorptiometry and anthropometric techniques in fat and lean rats," *Lipids in Health and Disease*, vol. 9, no. 1, p. 140, 2010.

[42] S. C. Savva, D. Lamnisos, and A. G. Kafatos, "Predicting cardiometabolic risk: waist-to height ratio or BMI - a Meta-analysis," *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, vol. 6, pp. 403–419, 2013.

[43] T. Michishita, S. Kobayashi, T. Katsuya, T. Ogihara, and K. Kawabuchi, "Evaluation of the antiobesity effects of an amino acid mixture and conjugated linoleic acid on exercising healthy overweight humans: a randomized, double-blind, placebo-controlled trial," *Journal of International Medical Research*, vol. 38, no. 3, pp. 844–859, 2010.

[44] S. A. Saboor Aftab, N. Reddy, E. Smith, and T. M. Barber, "Obesity and type 2 diabetes mellitus," *Internal Medicine*, vol. S6, p. 002, 2014.

[45] R. A. El-Shiekh, D. A. Al-Mahdy, S. M. Mouneir, M. S. Hifnawy, and E. A. Abdel-Sattar, "Anti-obesity effect of argel (Solenostemma argel) on obese rats fed a high fat diet," *Journal of Ethnopharmacology*, vol. 238, Article ID 111893, 2019.

[46] T. A. Dardeno, S. H. Chou, H. S. Moon, J. P. Chamberland, C. G. Fiorenza, and C. S. Mantzoros, "Leptin in human physiology and therapeutics," *Frontiers in Neuroendocrinology*, vol. 31, no. 3, pp. 377–393, 2010.

[47] S. Lin, T. C. Thomas, L. H. Storlien, and X. F. Huang, "Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice," *International Journal of Obesity*, vol. 24, no. 5, pp. 639–646, 2000.

[48] W. M. Arika, C. M. Kibiti, J. M. Njagi, and M. P. Ngugi, "Anti-obesity effects of dichloromethane leaf extract of Gnidia glauca in high fat diet-induced obese rats," *Heliyon*, vol. 5, no. 11, Article ID e02800, 2019.

[49] M. F. Gregor and G. S. Hotamisligil, "Inflammatory mechanisms in obesity," *Annual Review of Immunology*, vol. 29, no. 1, pp. 415–445, 2011.

[50] B. B. Kahn and J. S. Flier, "Obesity and insulin resistance," *Journal of Clinical Investigation*, vol. 106, no. 4, pp. 473–481, 2000.

[51] J. Vekic, A. Zeljkovic, A. Stefanovic, Z. Jelic-Ivanovic, and V. Spasojevic- Kalimanovska, "Obesity and dyslipidemia," *Metabolism*, vol. 92, pp. 71–81, 2019.

[52] B. I. Frohnert, D. R. Jacobs, J. Steinberger, A. Moran, L. M. Steffen, and A. R. Sinaiko, "Relation between serum free fatty acids and adiposity, insulin resistance, and cardiovascular risk factors from adolescence to adulthood," *Diabetes*, vol. 62, no. 9, pp. 3163–3169, 2013.

[53] B. Klop, J. W. Elte, and M. C. Cabezas, "Dyslipidemia in obesity: mechanisms and potential targets," *Nutrients*, vol. 5, no. 4, pp. 1218–1240, 2013.

[54] S. Petta, A. Gastaldelli, E. Rebelos et al., "Pathophysiology of non-alcoholic fatty liver disease," *International Journal of Molecular Sciences*, vol. 17, no. 12, p. 2082, 2016.

[55] S. I. Choi, I. H. Cho, S. H. Han et al., "Antiobesity effects of Salvia plebeia R. Br. extract in high-fatdiet-induced obese mice," *Journal of Medicinal Food*, vol. 19, no. 11, pp. 1048–1056, 2016.

[56] N. Matsuzawa-Nagata, T. Takamura, H. Ando et al., "Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity," *Metabolism*, vol. 57, no. 8, pp. 1071–1077, 2008.

[57] D. Pessayre, A. Mansouri, and B. Fromenty, "V. Mitochondrial dysfunction in steatohepatitis," *American Journal of Physiology - Gastrointestinal and Liver Physiology*, vol. 282, no. 2, pp. G193–G199, 2002.

[58] S. Furukawa, T. Fujita, M. Shimabukuro et al., "Increased oxidative stress in obesity and its impact on metabolic

syndrome," *Journal of Clinical Investigation*, vol. 114, no. 12, pp. 1752–1761, 2004.

[59] S. A. Noeman, H. E. Hamooda, and A. A. Baalash, "Biochemical study of oxidativestress markers in the liver, kidney and heart of high fat diet induced obesity in rats," *Diabetology & Metabolic Syndrome*, vol. 3, no. 1, p. 17, 2011.

[60] O. Erdeve, Z. Siklar, P. A. Kocaturk, Y. Dallar, and G. O. Kavas, "Antioxidant superoxide dismutase activity in obese children," *Biological Trace Element Research*, vol. 98, no. 3, pp. 219–228, 2004.