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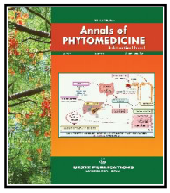

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Evaluation of antiobesity potential of methanolic bark extract of *Alstonia scholaris* (L.) R.Br. in high fat diet induced obese rats: *In vitro* and *in vivo* studies

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Abstract

Global epidemic levels of obesity are frequently accompanied by life-threatening comorbidities. Since the traditional medicinal practices by local healers are safe and efficacious, the scientific community is forced to look into the indigeneous medicinal plants' potential due to the lack of long-term, safe and effective treatments for obesity in modern pharmacotherapy. The objective of this study was to investigate the ability of *Alstonia scholaris* (L.) R.Br. bark extract to prevent obesity in obese rats. Six experimental groups were included in this *in vivo* investigation. Rats in Group I, known as the normal control, received a normal rat ration only; rats in Group II, known as the obese control, received only a HFD; rats in Group III, known as the positive control, received a HFD with orlistat medication; and rats in Groups IV, V, and VI received 100, 300, and 900 mg/kg bw of ASBE, respectively, in addition to HFD. Diet and medicine were administered orally once daily for a duration of 45 days. After the experiment, several observations were made on physical, biochemical and histological parameters. *In vivo* investigations including the treatment of ASBE plant extract revealed notable effects on physical parameters, including body weight, food consumption and organ weight. A marked decrease in the organ and fat pad weights was observed by the histological investigation. Biochemical parameters such as the lipid profile, atherogenic index, glucose, insulin, leptin, hepatic antioxidant enzymes and inflammatory markers like IL-6 and TNF-α were observed and successfully brought back to normal serum levels. The study's observations conclusively proved that *A. scholaris* bark extract has strong antiobesity potential.

1. Introduction

Obesity has reached global epidemic proportions due to an increase in consumption of calorie-dense diets and affluent lifestyles with low physical activity (Sharma and Sarwat, 2022; Kumar and Choudhary, 2023). In recent years, obesity is acknowledged as a clinical illness that impairs life quality and longevity and raises the risk of diabetes, heart disease, and several types of cancer. It also results in premature deaths and a reduction in life expectancy (Sivamaruthi *et al.,* 2019; Bhavani *et al.,* 2023). While genetic predispositions have a major impact in the development of adiposity or obesity, high-calorie meals and sedentary lifestyles are mostly to blame for the pandemic levels of obesity (El-Sayed Moustafa *et al.,* 2013). Thus, obesity is a preventable disorder that may be avoided by changing one's lifestyle (Martel *et al.,* 2017). There are several approaches to tackle obesity and one of them is to combine a diet low in calories with physical exercise; however, this is a challenging strategy to stick to and does not always work. This is mostly due to the body's adaptation processes, which keep its energy stores intact (Dokken *et al.,* 2007; Atkinson *et al.,* 2014). FDA has approved several pharmaceutical drugs for the treatment of obesity. The primary antiobesogenic medication, orlistat, causes an average 3% decrease in body weight over the course of a year, but consumption of these drugs is associated with GI disorders, renal disorders and liver failure (Filippatos *et al.,* 2008; Yanovski *et al.,* 2014). Some other antiobesogenic medications, including fenfluramine, sibutramine, and rimonabant, were taken off the market since they were associated with serious side effects that include heart issues, hypertension, mental health disorders, and even suicidal thoughts (Dietrich *et al.,* 2012; Divya and Sanjeev, 2021). While some well-known weight-loss surgeries like bariatric surgeries, gastric bypass and gastric band, are found to be more successful than antiobesogenic drugs, it is also more costly, more invasive, and ineffective for most overweight individuals (Bult *et al.,* 2008).

This makes the centuries-old ethnomedical use of these herbs all the more important, given their lengthy history of use and negligible to nonexistent adverse effects (Nampoothiri *et al.,* 2021; Pawaskar and Ranade, 2022). The antiobesogenic medications currently on the market need to be able to cure obesity or weight loss with minimal or no side effects. Consequently, due to their total lack of adverse effects, natural products are becoming more and more important

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(Rodgers *et al.,* 2012; Verma *et al.,* 2022). Herbal remedies have been used medicinally by ancient cultures for their antidiabetic, anti-inflammatory, antiobesogenic, antipyretic, and antihypertensive properties and many other benefits (Mehrotra *et al.,* 2021; Rani *et al.,* 2023). Medicinal plants are considered to have therapeutic properties primarily because they naturally produce secondary metabolites including tannin, alkaloids, flavonoids, ascorbic acid, phenolic acid and terpenoids. These are the basic components of many crucial medications (Sangeetha and Jagannath, 2022; Prathyusha, 2023). Due to their great chemical diversity, products made from medicinal or herbal plants, whether as isolated compounds or as a standard crude extract, offer enormous potential for the discovery of new pharmaceutical drugs to treat several ailments without any side effects (Sasidharan *et al.,* 2011; Duraisami *et al.,* 2021; Sundarrajan, 2023).

According to several researches, the bark of *A. scholaris* is widely used by many indigenous tribes and folklore traditions. Other names for it include devil's tree, millwood, milkwood pines, and blackboard tree (Akbar, 2020). This plant is used in its entirety to treat a variety of illnesses, though its bark is the most widely used part. The pharmacological effects of *A. scholaris* are primarily caused by the high content of multiple bioactive compounds, including triterpenoids, alkaloids, flavonoids, phenolic acid and tannins (Baliga, 2012). Some studies reported that *A. scholaris* leaves have antihyperglycemic or antidiabetic properties and its juice has been used in folklore and traditional medicine for reducing weight and obesity (Arulmozhi *et al.,* 2010; Muhammed, 2017). Studies also reported that extract of bark is utilized for weight loss and metabolic diseases (Bandawane *et al.,* 2011; Chhajed *et al.,* 2023). Some preliminary investigation into the diverse pharmacological characteristics of the extract of *A. scholaris* bark has been conducted. However, no experimental validation has been done on its antiobesogenic properties. Thus, this study was carried out to investigate the ability of *A. scholaris* bark extract to prevent obesity in obese rats.

2. Materials and Methods

2.1 Plant material

The bark of *A. scholaris* was gathered from Bhalabari Village, Udalguri, Assam, and was identified with guidance from a taxonomist from the Department of Agronomy, Assam Agricultural University, Jorhat (Voucher specimen number: AAU WEED HERBARIUM Acc. No. 5468).

2.2 Extraction and processing of the plant material

Following the collection of the plant's bark, the diseased and infected areas were removed and the plant's bark was cleaned. Upon a thorough cleaning under running water, the barks were shade-dried after which they were cleaned further with distilled water. Following the proper grinding of plant barks into a fine powder, the materials were labeled and kept in an airtight container. The grounded plant was kept in a refrigerator at 4°C. These materials in powder form were utilized for further chemical analyses.

Approximately 5 g of samples were taken in the thimble for the Soxhlet extraction. A 250 ml solvent and methanol mixture was used, and the Soxhlet was run for 8 h at 45°C for 7-8 cycles. Subsequently, the solvent-extracted samples were added to a rotary evaporator and the excess solvent was then removed.

2.3 Experimental rats

For the investigation, healthy adult male albino rats were selected from the Department of Pharmacology and Toxicology, College of Veterinary Science, Khanapara, Guwahati, India.

The rats (110 to 130 g weight) were kept in controlled environments in polypropylene cages with regular pellet chow and free access to water. Before experiments, they were provided with a week of acclimatization in the lab environment.

2.4 Composition of high-fat diet (HFD)

A normal diet consisting of the following ingredients- corn starch 40 g, sugar 10 g, casein 20 g, soybean oil 17 g, methionine 3 g, vitamin mixture 1 g, mineral mixture 4 g, cellulose 5 g (400 kcal/100 g) and high-fat diet consisted of corn starch 10 g, sugar 10 g, lard 40 g, vitamin mixture 1 g, mineral mixture 4 g, casein 20 g, cellulose 5 g, soybean oil 7 g, methionine 3 g (500 kcal/100 g). The diets were made each day to avoid auto-oxidation of lipids.

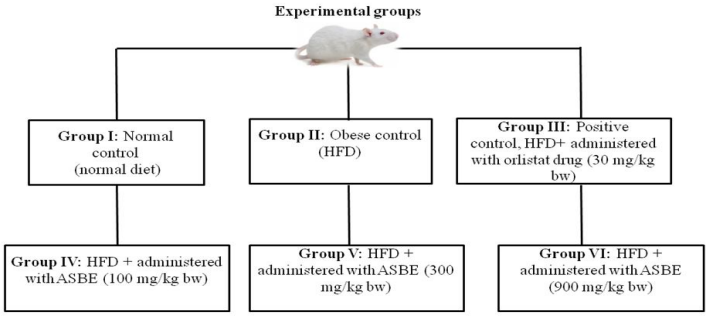
2.5 Obesity induced by HFD

A set of thirty-two albino rats, weighing between one hundred and twenty-five grams, were selected, and they were fed the HFD for thirty days to make them obese. Thirty rats (considered obese prone) weighing approximately 225-250 g were selected for antiobesity studies after the induction period. As a normal control, six more rats were given regular pellet chow.

2.6 Experimental groups

After receiving an oral gavage dose of 2000 mg/kg bw of the methanolic bark extract of the plant, rats were examined for behavioural modifications and mortality. The results showed that a single intake of the plant extract up to 2000 mg/kg bw did not cause any adverse effects.

The obese albino rats on a high-fat diet were categorized as follows:



2.7 Assessment of antiobesogenic potential of the plant extract

2.7.1 Measurement of body weight and daily intake of food

Throughout the experimental period, amount of food consumed was recorded daily. Measurement of the difference in weight of the total and remaining pellet chow at the end of the day was also recorded. The body weights of the rats were recorded once a week.

2.7.2 Estimation of biochemical parameters

Standard ELIZA kits were used to estimate or assay biochemical parameters such as glycemic profile, lipid profile, serum leptin and lipid peroxidation (LPO). Endogenous antioxidant enzymes such as CAT, SOD, GSH and GPx were also estimated.

2.7.3 Histological analysis

For a period of 45 days, the diet and orlistat drug were provided orally once a day. Blood was drawn to perform a biochemical analysis. The white adipose tissue and the organs such as the liver, heart, kidney and spleen were cleaned using ice-cold saline (0.9%). Then, they were dried with a paper towel for measurement of weight. For further histopathological investigations, the liver organ was kept and homogenised in a 0.1 molar phosphate buffer of pH 7.4.

2.7.4 ASBE's impact on energy expenditure

The TSE Lab Master System was utilized to measure the amount of oxygen consumed (Yuan *et al.,* 2017). Before beginning the measurements, all of the rats were given a 24 h period to acclimate. The rats were then put in a metabolic chamber, and for the next 24 h, carbon dioxide and oxygen consumption were monitored. The rats were housed for a 12 h light and dark cycle (25°C) and provided rat ration including water. Next, using the formula from the manufacturer and earlier research, the energy expenditure (EE) and the respiratory exchange ratio (RER) were computed (Chu *et al.,* 2015; Tokubuchi *et al.,* 2017).

$$RER = VCO_2/VO_2$$

$$EE\ (kcal) = [3.815VO_2(l/min) + 1.232\ VCO_2(l/min)]\ 1440\ min.$$

2.8 Ethics statement

The procedures for the study were approved by the Institutional Animal Ethics Committee (Approal No. 770/GO/Re/S/03/CPCSEA/FVSc/AAU/IAEC/21-22/945) and were carried out in compliance with the Guidelines for the Care and Use of Lab Animals (NIH Publication, ISBN-10: 0-309-15400-6, 2010, ISBN-13:978-0-309- 15400-0).

2.9 Statistical analysis

Unpaired t test was used in the analysis of the data using IBM's (USA) SPSS software. p value < 0.05 or < 0.01 indicated statistical significance. The standard deviation (SD) ± mean was used to represent the data.

3. Results

3.1 ASBE's impact on daily intake of food

Figure 1 depicts the daily intake of food of the experimental rats. The average of the six rats in each experimental group was used to determine daily food consumption. Rats in the obese control group showed an increasing trend in food intake, but the treatment groups with varying dosages showed a slight drop in food consumption.

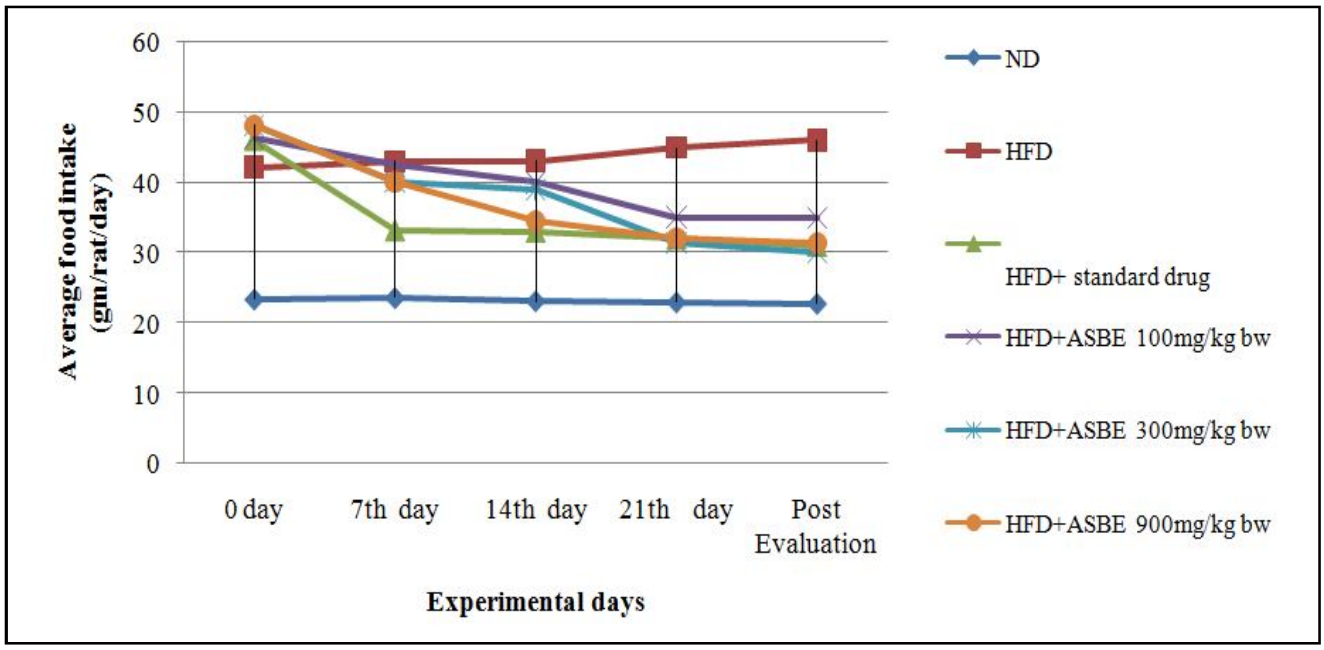


Figure 1: Average food intake of rats, ND-Normal diet, ASBE-*A. scholaris* bark extract, HFD-High fat diet.

3.2 ASBE's impact on body weight

On the first day, the rats' initial body weight was noted for each group and after receiving ASBE treatment, variations in their body weight were noted at regular intervals of seven days as shown in Figure 2. The data collected revealed that obese control group had a slight increase in weight. However, among the ASBE supplementation groups with different doses, a marked reduction was seen throughout the experimental period.

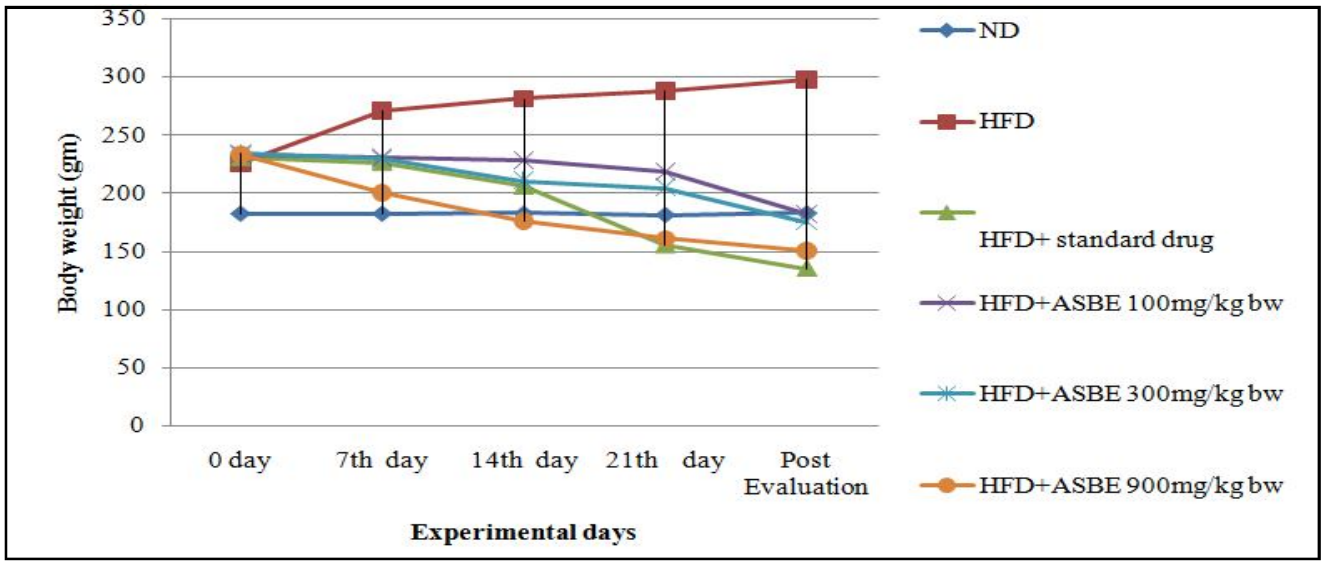


Figure 2: Change in body weight, ND-Normal diet, ASBE-*A. scholaris* bark extract, HFD-High fat diet.

3.3 ASBE's impact on biochemical parameters

Table 1 indicates how ASBE plant extract affects the rats in several experimental groups in terms of their glycemic profile, lipid profile, leptin, and markers of inflammation, including IL-6 and TNF-α. After supplementation of HFD to obese control group rats, a significant increase was seen in most of the parameters including glucose, insulin, atherogenic index, total cholesterol, triglyceride and LDL cholesterol except HDL, where a marked reduction was seen at the end of the experiment. Furthermore, there was a marked increase in inflammatory markers such as TNF-α and IL-6 in Group II. All the biochemical parameters; however, returned to almost normal levels when the obese control group had been given three different dosages of ASBE plant extract.

Table 1: The lipid profile, glycemic profile, leptin, atherogenic index, TNF-α and IL-6 in experimental rats of different groups

Biochemical parameters	Group I	Group II	Group III	Group IV 100 mg/kg bw	Group V 300 mg/kg bw	Group VI 900 mg/kg bw
Glucose (mg/dl)	78.11 ± 1.94	137.91 ± 2.94#	78.66 ± 3.28**	121.50 ± 2.82	110.80 ± 2.06**	91.26 ± 1.11**
Insulin (µIU/ml)	3.15 ± 0.21	7.37 ± 0.34#	4.40 ± 0.34**	6.57 ± 0.73	6.03 ± 1.08**	5.01 ± 1.10**
AI	1.23 ± 0.26	6.21 ± 0.32#	2.38 ± 2.07**	5.07 ± 0.63	3.85 ± 1.33**	2.83 ± 1.77**
TG (mg/dl)	75.66 ± 1.03	168.39 ± 0.77#	75.63 ± 3.29**	141.57 ± 1.74*	125.46 ± 10.72**	104.01 ± 23.01**
TC (mg/dl)	102.15 ± 1.91	198.06 ± 0.66#	119.37 ± 43.1**	162.66 ± 16.40*	160.36 ± 16.33**	147.03 ± 16.55**
HDL-C (mg/dl)	61.31 ± 0.43	41.59 ± 3.72#	56.83 ± 5.65**	46.02 ± 0.88	49.15 ± 2.29**	54.81 ± 5.75**
LDL-C (mg/dl)	50.91 ± 0.74	108.99 ± 4.56#	50.74 ± 22.87**	87.04 ± 8.24	86.17 ± 25.04**	60.02 ± 26.99**
Leptin (ng/ml)	3.42 ± 0.29	8.41 ± 0.20#	4.24 ± 2.26**	7.38 ± 0.80	6.48 ± 0.87**	6.09 ± 1.35**
TNF-α (pg/ml)	156.60 ± 1.09	442.75 ± 24.23#	215.75 ± 120.21**	335.58 ± 77.91	306.11 ± 84.29**	256.08 ± 93.28**
IL-6 (pg/ml)	87.21 ± 3.16	310.28 ± 22.06#	178.05 ± 50.72**	297.74 ± 45.01	229.01 ± 43.65**	219.53 ± 48.28**

All values are mean ± SD of 6 rats

*Significant (p<0.05) and **Significant (p<0.01) in comparison with Group II (Obese control).

Significant (p<0.01) in comparison with Group I (Normal control).

3.4 ASBE's impact on visceral body fat weight, adipocyte size and organ weight

A mark reduction was found in different organ weights such as liver, kidney and heart after dose-dependent administration of ASBE plant extract to obese rats. In fact, the various white adipose tissue or fat pad weights (mesenteric, epididymal and retroperitoneal) and the size of adipocytes were significantly (p<0.05) lowered across the three treatment groups (Table 2).

3.5 ASBE's impact on antioxidant levels of obese rats

It was found that the obese control group exhibited relatively low blood levels of many endogenous antioxidants, including CAT, SOD,

GPx, and GSH. However, a significant (p<0.05) rise was seen after dose-dependent administration of ASBE plant extract to obese rats. In contrary, the obese control group exhibited elevated levels of lipid peroxidation (LPO). But after administration of ASBE plant extract, significant (p<0.05) reduction was seen in lipid peroxidation (Groups V and VI) from 186.43 ± 0.81 to 122.42 ± 0.56 and 186.43 ± 0.81 to 120.43 ± 0.75, respectively (Table 3).

Table 2: ASBE's impact on organ weight, adipocyte size and visceral body fat

Organs and different adipose tissue	Group I	Group II	Group III	Group IV 100 mg/kg bw	Group V 300 mg/kg bw	Group VI 900 mg/kg bw
Liver	5.02 ± 0.41	10.63 ± 0.52#	6.32 ± 0.45**	11.42 ± 1.06	8.01 ± 0.23**	7.01 ± 2.02**
Kidney	1.02 ± 0.06	2.05 ± 0.64#	1.44 ± 0.63**	2.12 ± 0.91	1.87 ± 0.07**	1.73 ± 0.34**
Heart	0.58 ± 0.07	1.43 ± 0.03#	0.51 ± 0.10**	1.39 ± 0.13	0.92 ± 0.02**	0.78 ± 0.06**
Adiopcyte size (Area-µm^2)	27,378.12 ± 4031	91,512.02 ± 9178#	34,308.81 ± 5906**	85,813.09 ± 5089	50,482.72 ± 6084**	48,043 ± 6062**
Mesenteric WAT (g)	3.02 ± 0.35	13.72 ± 0.41#	4.13 ± 0.52**	12.09 ± 2.61	11.66 ± 1.21**	8.15 ± 0.83**
Retro-peritoneal WAT (g)	5.03 ± 1.02	14.43 ± 1.91#	7.03 ± 1.82**	13.51 ± 1.73	10.04 ± 1.62**	9.81 ± 1.97**
Epididymal WAT (g)	1.59 ± 0.13	4.03 ± 0.39#	1.63 ± 0.73**	3.11 ± 0.72	2.65 ± 0.67**	2.11 ± 0.33**

All values are mean ± SD of 6 rats

*Significant (p<0.05) and **Significant (p<0.01) in comparison with Group II (Obese control).

Significant (p<0.01) in comparison with Group I (Normal control).

Table 3: ASBE's impact on antioxidant levels of HFD induced obese rats

Antioxidant levels	Group I	Group II	Group III	Group IV 100 mg/kg bw	Group V 300 mg/kg bw	Group VI 900 mg/kg bw
LPO (n moles of MDA formed/g tissue)	76.23 ± 0.67	186.43 ± 0.81#	80.31 ± 0.93**	178.92 ± 0.72	122.42 ± 0.56**	120.43 ± 0.75**
GSH (mg/g tissue)	16.55 ± 0.43	7.11 ± 0.62#	20.13 ± 0.82**	9.23 ± 0.12	12.31 ± 0.59**	13.63 ± 0.14**
GPx (µmoles/ mg protein)	5.33 ± 0.16	1.23 ± 0.34#	4.91 ± 0.34**	2.39 ± 0.15	2.21 ± 0.32*	2.78 ± 0.43*
SOD (µmoles /mg protein)	6.22 ± 0.52	2.03 ± 0.86#	5.21 ± 0.82**	1.45 ± 0.22	3.05 ± 0.14*	3.71 ± 0.23*
CAT(µmoles/ min/mg protein)	41.72 ± 0.43	12.83 ± 0.42#	36.03 ± 0.31**	14.08 ± 0.75	22.36 ± 1.09**	28.05 ± 0.47**

All values are mean ± SD of 6 rats

*Significant (p<0.05) and **Significant (p<0.01) in comparison with Group II (Obese control).

Significant (p<0.01) in comparison with Group I (Normal control).

3.6 Histopathological studies

The histopathological analysis revealed that rats in the Group II had large fat droplets which were prominent in the liver lobules. However, the test drug and ASBE plant extract (900 mg/kg bw) treatment caused the fat droplets to disappear in the obese rats. The fat droplets in the histopathologic image of adipose tissue were found larger in comparison with rats of the group obese control. On the contrary, when treated with ASBE (900 mg/kg bw) and orlistat drug, a smaller size of adipocytes appeared in adipose tissue (Figures 4, 5).

3.7 ASBE's impact on energy expenditure of high fat diet induced obese rats

Figure 3 demonstrates how the application of bark extracts from *A. scholaris* markedly raised energy expenditure. Furthermore, when the rats were given bark extracts of *A. scholaris* showed noticeably greater O_2 consumption and CO_2 production during the day and at night. ASBE treatment also decreased the value of respiratory exchange ratio (RER). RER is a measure of fat and carbohydrate oxidation duirng the both day and nighttime cycle. After computing energy expenditure, we found that the HFD+ ASBE extracts group showed significantly higher energy expenditure during the day ($p<0.05$) than the other groups.

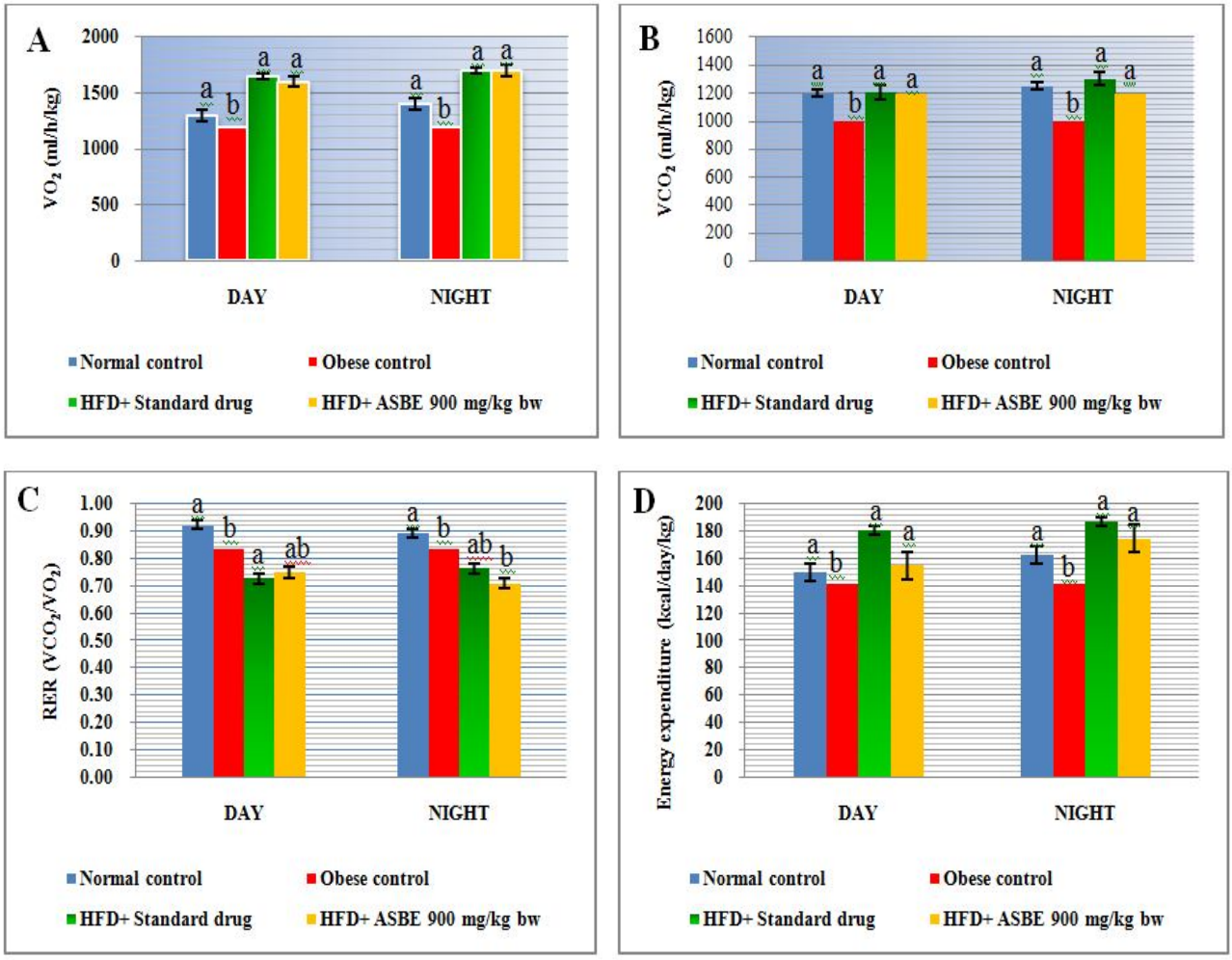
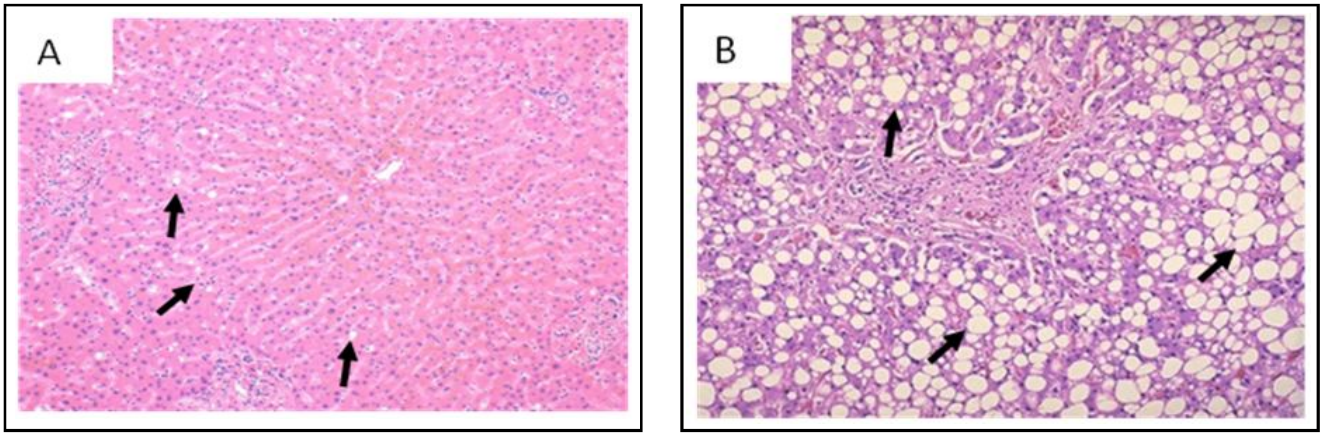


Figure 3: (A) VO_2 consumption of rats, (B) and VCO_2 consumption of rats, (C) Respiratory exchange ratio, (D) Energy expenditure (kcal/day/kg), Mean values with distinct letter combinations between the groups show a significant difference ($p<0.05$).



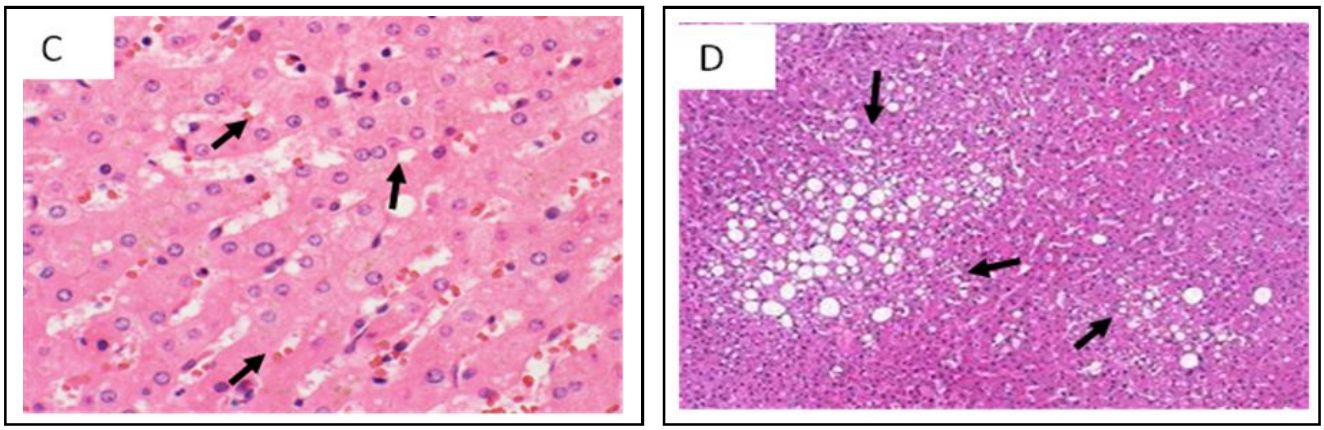


Figure 4: Histopathological examination of liver tissue of different experimental groups of rats; A: Liver tissue of normal control group rats with no fatty droplets; B: Liver tissue of obese control group rats with larger fatty droplets; C: Liver tissue of obese rats administered with orlistat (Standard drug) with reduced fatty droplets, D: Liver tissue of obese rats administered with ASBE plant extract (900 mg/kg bw) showing few fatty droplets.

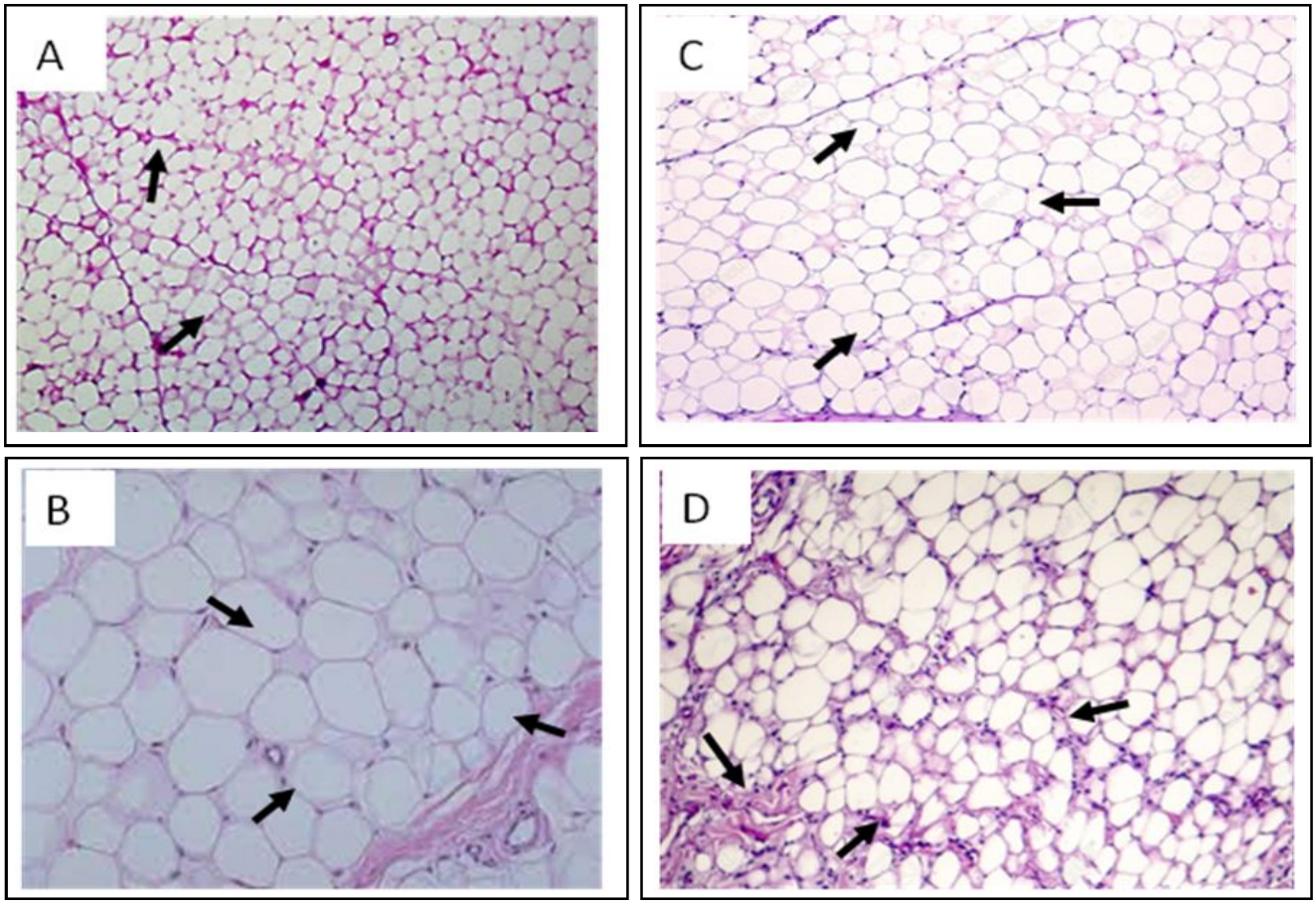


Figure 5: Histopathological examination of adipose tissue of different experimental groups of rats; A: Adipose tissue of normal control group rats with normal size adipocyte; B: Adipose tissue of obese control group rats with large size adipocyte; C: Adipose tissue of obese rats administered with orlistat (Standard drug) with reduced and smaller size adipocyte; D: Adipose tissue of obese rats administered with ASBE plant extract (900 mg/kg bw) with smaller size.

4. Discussion

Since obesity and its associated illnesses are becoming more widespread globally like an epidemic in the twenty-first century, they now pose a serious risk to public health. Increasing awareness of the obesity pandemic within the scientific community and the general public is essential to improve the effectiveness of treatment. Additionally, several pharmaceutical medications are licensed and permitted to treat obesity. Due to several side effects like heart

diseases, hypertension, mood disorders, and even suicidal thoughts, several other antiobesogenic drugs were withdrawn from the market (Dietrich *et al.,* 2012). Although, antiobesogenic drugs are less effective than weight-loss surgery, such as bariatric surgeries, gastric bypass and gastric band, the surgery procedure is also more costly, physically invasive, and unsuccessful for a huge percentage of overweight individuals (Bult *et al.,* 2008). Consequently, due to their total lack of adverse effects, natural products are becoming more and more important. Products created from herbal plants, whether as standardized crude extract or as separated pure chemicals, provide significant prospects for the discovery of novel pharmaceutical medications to treat obesity and related illnesses due to their high chemical variety (Sasidharan *et al.,* 2011).

According to recent research, *A. scholaris* bark extract has been extensively utilized in the field of ethnomedicine as a medicinal plant to cure and prevent hyperglycemia as well as to reduce weight loss (Bandawane *et al.,* 2011; Chhajed *et al.,* 2023). To investigate the diverse pharmacological characteristics of the plant some preliminary research has been conducted on its bark extract. Its antiobesogenic qualities, however, have not been confirmed by experimentation. In order to assess *A. scholaris* bark extract's efficacy to prevent obesity in obese rats, this study was carried out.

A common indicator of the onset of obesity is weight gain (Van der Ploeg *et al.,* 2000). In this study, we discovered that during the experimental days, HFD feeding caused the obese control rats to gain weight at a faster rate. The reason behind the rapid gain in weight could be due to the deposition of visceral fat in response to a positive energy balance created by the intake of a high-fat diet. In contrast, a marked drop in body weight was seen among the rats of ASBE treated groups in dose dose-dependent manner. In the state of obesity, adipose tissue secretes some adipocytokines like IL-6, IL-1and TNF-α*, etc*., which causes insulin resistance. This condition leading to insulin resistance causes hyperglycemia since glucose cannot be carried to muscle or liver for its utilization. In response to hyperglycemia, the beta cells of the pancreas keep synthesizing insulin which further leads to a condition called hyperinsulinemia.

Our study also revealed that the obese rats (Group II) had elevated levels of blood glucose than the rats that received orlistat medication (Group III). Furthermore, obese rats treated with ASBE in addition to HFD demonstrated a dose-dependent, marked (p<0.01) drop in blood glucose levels after a comparison was made with obese control group rats. As per the results of earlier studies, the hypoglycemic effect could be regulated by the bioactive compounds found in the plant extract by improving insulin resistance *via* reduced inflammatory markers or adipocytokines (Francini-Pesenti *et al.,* 2019). The results of our investigation coincide with those of Kim *et al.* (2019), who stated that the only group exhibiting a significant drop in blood glucose levels (p<0.05) was the one administered 400 mg/kg bw of *Diospyros lotus* leaf extract.

The results of the study also revealed that the blood insulin levels of the obese control group increased significantly (p<0.01) in comparison to the rats in the normal control group (Table 1).

The blood insulin levels in the ASBE treatment group, which was administered dosages of 100, 300, and 900 mg/kg bw, markedly decreased in a dose-dependent manner. Insulin levels were lower among the rats of Group IV, but not significantly. In Groups V and VI, insulin level was significantly (p<0.01) lower than in the obese control group. These results align with those of Rahman *et al.* (2017), who observed a significant (p<0.05) drop in blood insulin in HFD rats receiving low (175 mg/kg bw) and high dosages (350 mg/kg bw) of *Cosmos caudatus* Kunth leaf extract.

Triglycerides (TG) and HDL-C cholesterol constitute the novel index known as the atherogenic index. Since it is a reliable indicator of dyslipidemia and conditions related to it, such as cardiovascular disease, stroke, hypertension, *etc*., it is frequently used to estimate serum lipid levels (Zhu *et al.,* 2018). In this study, administering ASBE plant extract to various groups resulted in a reduction of AI, significantly. Comparing the experimental groups, Groups V and VI to the obese control (Group II), our study discovered that they had a significantly (p<0.01) reduced atherogenic index value. After ASBE was administered to the obese rats in addition to HFD, a dose-dependent reduction of triglycerides and total cholesterol levels was observed. Triglyceride levels and total cholesterol were seen to be significantly (p<0.01) lower across all the groups receiving treatment (Groups IV, V, and VI). Similarly, rats in Group II had higher LDL value, while all the groups experienced a significant drop in LDL values after receiving the ASBE plant extract. The findings align with the study of Kongchian *et al.* (2020), which observed a significant drop in triglyceride, LDL, and cholesterol levels in obese mice with administration of extract containing different dose ranging from 250-500 mg/kg bw of *Garcinia Atroviridis.*

The HDL levels were seen to be reduced in rats treated with the ASBE plant extract. Groups V and VI resulted in a significant drop (p<0.01) in HDL value in comparison with the rats of group obese control. The findings align with Oluyemi *et al.* (2007), who discovered that using a dose from 200 mg/kg bw to maximum 400 mg/kg bw of plant extract of *Garcinia cambogia*, resulted in higher HDL level, significantly (p<0.01) in the serum of obese wistar rats.

Leptin is primarily produced and released by the cells of adipose tissue in approximately proportion to the fat that is stored in the human body. It appears to be a highly crucial hormone for controlling blood sugar levels in our bodies. By delivering messages to the hypothalamus, circulating leptin helps reduce appetite and allow for the use of energy. However, because of leptin resistance, increased levels of endogenous leptin do not seem to have the same effect in obesity. Even though, the mechanism is unclear for insensitivity to leptin or leptin resistance, it was asserted that in a state of adiposity or obesity, excess leptin levels might induce desensitization of leptin receptors in the hypothalamic region which further can affect the downstream neural network mediating leptin effect on body weight or energy balance (Considine *et al.,*1997). In our study, leptin was seen elevated significantly (p<0.05) in Group II in contrast to the rats of Group I fed with normal diet. However, the serum leptin was reduced to normal level after receiving ASBE plant extract with different doses of 100 to 900 mg/kg bw. Similar outcomes were observed by Pichiah *et al.* (2012), who reported a dose, from 500-1000 mg/kg bw of *Hippophae rhamnoides* (L.), leaf extract markedly lowered the serum leptin levels in obese mice.

Adipocytokines, or cytokines that promote inflammation like TNF-α and IL-6, are connected to diseases like hyperglycemia and obesity and are playing a crucial part in the pathophysiology of insulin resistance. These are mostly secreted from adipocytes under conditions such as obesity or adiposity. The buildup of excess body

fat results in the proliferation of adipose tissue, which consequently causes the release of cytokines that cause inflammation. These cytokines can be transported by blood capillaries to various locations, where they activate inflammatory signaling pathways and cause long-term inflammation in the affected organ or tissues. Abnormal productions of these cytokines are associated with many metabolic disorders like dyslipidemia, stroke, obesity, coronary heart diseases and diabetes, *etc*. In contrast to the rats (Group I) fed with normal diet, a significant (p<0.01) rise was seen in the levels of TNF-α and IL-6 in the experimental Group II (obese control). A significant (p<0.01) reduction in blood levels of IL-6 and TNF-α was seen in ASBE treatment groups receiving 300 and 900 mg/kg bw in comparison to the obese control group's rats. Similar findings were made by Sripradha *et al.* (2015) when they examined the ability of *Garcinia cambogia* fruit extract to reduce body weight in obese wistar rats. They discovered that when administered with 400 mg/kg bw, it successfully reduced body weight and TNF-α level significantly (p<0.05).

In conditions like obesity, reactive oxygen species (ROS) are produced at higher rate and it is also well correlated with increased metabolism. During normal state, there will be an equilibrium maintained in the production of antioxidants and reactive oxygen species in the body (Gunalan *et al.*, 2023). A deviation from this balance or equilibrium can lead to oxidative stress, which is the underlying factor of metabolic diseases (Gãman *et al.,* 2020). One of the main mechanisms underlying morbidities associated with obesity is chronic oxidative stress. Natural antioxidant-rich foods and herbs are essential for preventing illness and promoting overall health (Husain *et al.,* 2021). Large levels of ROS are created in liver cells as a result of increased metabolism and fat buildup in obesity, which increases the cells' susceptibility to oxidative stress and lipid peroxidation. The end product that forms as a result of lipid peroxidation, MDA, serves as a significant indicator for abnormal levels of free radicals found in the body (Vincent and Taylor, 2006; Athesh *et al.,* 2017). Lipid peroxidation was seen higher in the obese control group, but after the administration of ASBE plant extract, a decreasing trend was seen across all experimental groups. LPO lowered significantly (p<0.01) in ASBE treatment groups receiving 300 and 900 mg/kg bw, going from 151.54 ± 1.32 to 119.34 ± 1.55 and 151.54 ± 1.32 to 93.42 ± 1.20, respectively. These results support the findings of Athesh *et al.* (2017), who observed a mark drop in LPO in the experimental group.

Strong endogenous antioxidants may scavenge free radicals like lipid peroxides and are important in lowering oxidative stress. All of the antioxidant enzymes were found to be reduced in the obese rats (Group II) as compared to the rats (Group I) fed with normal diet. Endogenous antioxidant enzymes, including CAT, GSH, SOD, and GPx, exhibited a significant (p<0.01) drop from 41.72 ± 0.43 to 12.83 ± 0.42, 16.55 ± 0.43 to 7.11 ± 0.62, 6.22 ± 0.52 to 2.03 ± 0.86 and 5.33 ± 0.16 to 1.23 ± 0.34, respectively.

Groups V and VI, on the other hand, revealed a significant rise (p<0.05) in antioxidant value after administration of the ASBE plant extract. For glutathione (GSH), in both the group, Group V and VI it rose significantly (p<0.01) from 7.11 ± 0.62 to 12.31 ± 0.59 and 7.11 ± 0.62 to 13.63 ± 0.14, respectively. Glutathione peroxidase (GPx), also showed similar results, where a significant increase was seen in ASBE treatment groups receiving 300 and 900 mg/kg bw from 1.23 ± 0.34 to 2.21 ± 0.32 and 1.23 ± 0.34 to 2.78 ± 0.43, respectively.

It also turns out that in ASBE treatment groups receiving 300 and 900 mg/kg bw, the levels of superoxide dismutase (SOD) were significantly greater (p<0.01) than those in the obese control group; they rose from 2.03 ± 0.86 to 3.05 ± 0.14 and from 2.03 ± 0.86 to 3.71 ± 0.23, respectively. The serum level of catalase was also found higher in ASBE treatment groups receiving 300 and 900 mg/kg bw. Group V had a significant rise from 12.83 ± 0.42 to 22.36 ± 1.09, while Group VI showed a significant increase from 12.83 ± 0.42 to 28.05 ± 0.47, respectively, in comparison with obese control group.

The obese control group had significantly (p<0.01) greater amounts of all the different forms of adipose tissue than the normal control group. This may be due to high intake of food and HFD which led to increased deposition of fats in visceral organs. Mesenteric, peritoneal and epididymal white adipose tissue (WAT) were increased from 3.02 ± 0.35 to 13.72 ± 0.41 g, 5.03 ± 1.02 to 14.43 ± 1.91 g, 1.59 ± 0.13 to 4.03 ± 0.39 g, respectively. On the other hand, ASBE treatment groups receiving 300 and 900 mg/kg bw demonstrated a significant (p<0.01) drop in all forms of WAT as compared to the obese control group. For mesenteric WAT, in both the group, Groups V and VI, it has drop significantly (p<0.05) from 13.72 ± 0.41 to 11.66 ± 1.21 and 13.72 ± 0.41 to 8.15 ± 0.83, respectively. Similar significant (p<0.05) results were observed for peritoneal and epididymal WAT, Group V: 14.43 ± 1.91 to 10.04 ± 1.62, Group VI: 14.43 ± 1.91 to 9.81 ± 1.97 and Group V: 3.11 ± 0.72 to 2.65 ± 0.67 to Group VI: 3.11 ± 0.72 to 2.11 ± 0.33, respectively.

Various organ weights such as liver, kidney and heart were also found to be elevated in the obese control group; however, a decreasing trend was seen when administered with ABSE plant extract. In the obese control group, the weights of the liver, kidney, and heart rose significantly (p<0.01) from 5.02 ± 0.41 to 10.63 ± 0.52, 1.02 ± 0.06 to 2.05 ± 0.64, and 0.58 ± 0.07 to 0.43 ± 0.03, respectively. After comparison with the obese control group, Groups V and VI demonstrated a significant drop (p<0.01) in all types of organ weights. For the liver, in both groups V and VI, it has attenuated significantly (p<0.05) from 10.63 ± 0.52 to 8.01 ± 0.23 and 10.63 ± 0.52 to 7.01 ± 2.02 respectively. Similar significant (p<0.05) findings were seen for kidney and heart, Group V: 2.05 ± 0.64 to 1.87 ± 0.07, Group VI: 2.05 ± 0.64 to 1.73 ± 0.34, and Group V: 1.43 ± 0.03 to 0.92 ± 0.02 to Group VI: 1.43 ± 0.03 to 0.78 ± 0.06, respectively.

When comparing the obese rats (Group II) to the rats of (Group I), a substantial increase in adipocyte size was observed. However, in ASBE treatment groups receiving 300 and 900 mg/kg bw, a significant drop in adipocyte size was seen. Adipocyte size was significantly (p<0.01) reduced in ASBE treatment groups receiving 300 and 900 mg/kg bw, going from 91,512.02 ± 9178 to 50,482.72 ± 6084 and 91,512.02 ± 9178 to 48,043 ± 6062, respectively.

The rats fed with *A. scholaris* bark extracts showed markedly higher VO_2 and VCO_2 consumption levels in the twelve hours of light and dark cycle than in the rats in Group II treated only with a high-fat diet. The HFD+ *A. scholaris* bark extracts group had a significant rise (p<0.01) in energy expenditure in both the day and night period. These results indicated that the rats in the HFD+ *A. scholaris* bark extracts group consumed more energy than the obese control rats. The obese rats receiving treatment with the highest dose of ASBE did not show any noticeable increase in the RER, indicating that fat

remains the primary energy source. These findings demonstrated that rather than altering food intake or physical activity, ASBE treatment reversed HFD-induced weight gain by increasing energy expenditure. ASBE plant extract may be responsible for this, as they have been shown to improve brown adipose tissue's expression of thermogenesis markers and raise thermogenesis, or energy metabolism, to combat obesity. These outcomes aligned with those of Gao *et al.,* (2022), who showed that medium-chain fatty acids and coconut oil may successfully raise mice's energy expenditure by significant amounts. Furthermore, Chu *et al.* (2015) shown that giving obese rats a 0.5% ursolic acid (UA) supplement for six weeks causes the rats to burn more energy, which in turn causes the rats to lose weight.

5. Conclusion

The data obtained clearly demonstrate that, when applied to HFD-induced obese rats, the standard medication orlistat and the plant extract ASBE have significant antiobesity potential. The plant extract from ASBE was found to be more effective at the highest dose levels (300 and 900 mg/kg bw). Our study's results imply that ASBE's antiobesity potentials may be regulated through several mechanisms such as regulation of fat metabolism, enhanced hepatic antioxidant enzymes, delayed intestinal absorption of dietary fat, and decreased production of inflammatory cytokines through the use of bioactive compounds like carotenoids, phenols, flavones, and alkaloids. These research findings thus support the scientific data supporting the *A. scholaris* plant potential to prevent obesity, which has been used in traditional medicine.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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