



Antioxidant, Hypolipidemic and Angiotensin Converting Enzyme Inhibitory Effects of Flavonoid-rich Fraction of *Hyphaene thebaica* (Doum Palm) Fruits on Fat-fed Obese Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Author MAA designed the study and wrote the protocol. Authors AB, BSA, AZM and AYU carried out the laboratory analysis. Author BK performed the statistical analysis and wrote the first draft of the manuscript. Author RKB managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study investigated the antioxidant, hypolipidemic and angiotensin converting enzyme (ACE) inhibitory effects of flavonoid-rich fraction of *H. thebaica* on fat-fed obese wistar rats.

Study Design: Twenty-five rats were divided into 5 groups of 5 rats each: Control (standard diet, untreated), Obese control (Fat-fed, untreated), Standard control (Fat-fed, treated with 70 mg/kg Atorvastatin), Groups 4 and 5 (Fat-fed, treated with 100 and 250 mg kg⁻¹ flavonoid-rich fraction, respectively). The rats were given high fat diet to induce obesity, after which treatment was administered for fourteen (14) days, and on the 15th day, rats were sacrificed and blood samples collected.

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Results: From the results, induction of obesity significantly ($P<0.05$) increased body weight, some lipoproteins, ACE activity, superoxide dismutase, catalase and glutathione peroxidase levels, while HDL cholesterol and malondialdehyde levels decreased. Treatment of obese rats with the standard drug, atorvastatin and flavonoid-rich fraction of *H. thebaica* significantly ($P<0.05$) decreased ACE activity, total cholesterol, triglyceride and LDL cholesterol, while HDL cholesterol and malondialdehyde increased.

Conclusion: This study has demonstrated that the flavonoid-rich fraction of *H. thebaica* is hypolipidemic, possesses antioxidant activities, and may contain potent ACE inhibitors.

Keywords: Angiotensin converting enzyme; antioxidant; hypolipidemic; *H. thebaica*; obesity.

1. INTRODUCTION

Life style changes due to industrialization have made a significant impact on the health of people, with obesity becoming a fast growing epidemic worldwide. According to the World Health Organization, the prevalence of overweight in adults (≥ 18) rose from 35.7% to 38.9% and obesity rose from 11.2% to 13.1% from 2010 to 2016. Overall, there were 2.01 billion overweight adults of which 678 million were obese [1]. Obesity, a consequence of overnutrition, results in an imbalance between energy intake and expenditure with contributions from genetic, metabolic and behavioral elements. It is one of the major causes of chronic diseases, such as diabetes, cardiovascular diseases, as well as all forms of cancer [2,3,4]. Obesity results in excessive fat accumulation, which alters cholesterol, triglyceride and other lipid levels in plasma and tissues. Most obese individuals are known to be hyperlipidemic, that is they have elevated levels of triglycerides, cholesterol, cholesterol esters, and low-density lipoprotein, with low levels of high-density lipoprotein [5].

The elevation of plasma lipids is associated with chronic inflammation of the adipose tissue due to increased production of reactive oxygen species (ROS) as a result of uncontrolled supply and metabolism of substrates [6] resulting in oxidative stress [7]. Oxidative stress is defined as the excess formation of highly reactive molecules (oxidants) relative to antioxidants [8] leading to the development of obesity-related complications. It results in cellular and molecular tissue damage increasing the risk of several chronic diseases. Highly reactive molecules include reactive oxygen species (ROS) and reactive nitrogen species (RNS) such as hydrogen peroxide, superoxide, hydroxyl radical, and peroxynitrite, produced normally in the cytochrome P450 system during oxidative metabolism of mitochondria [8], oxidative bursts within white blood cells and fatty acid

degradation [6]. They are important signaling molecules, but overproduction causes interference with regular cell function by reacting with biomolecules such as lipids, proteins, carbohydrates, nucleic acids, etc [9]. Superoxide dismutase (SOD), catalase, glutathione peroxidase, glutathione reductase and heme oxygenase (HO) are antioxidant enzymes that alleviate tissue damage caused by free radicals [8].

Obesity is also associated with the activation of the renin-angiotensin system (RAS) in humans [10], most diet-induced obese rodents and genetic models [11]. It increases plasma levels of angiotensinogen as a result of adipocyte hypertrophy. Angiotensinogen produced by the action of renin, is converted to angiotensin II (Ang II) by angiotensin converting enzyme (ACE) [12]. Elevated level of Ang II constricts the vascular system and increases blood pressure. ACE also catalyses the breakdown of a vasodilator, bradykinin [13], ultimately contributing to increased blood pressure. There are also reports suggesting a relationship between body mass Index (BMI), angiotensin II levels, renin and ACE due to increase in adipocytes, an important source of these hormones [14,15].

Inhibition of RAS is an important strategy for the treatment of cardiovascular disorders, all of which are risk factors of obesity. These drugs are not only useful in blood pressure control, but also provide protection against risks associated with cardiovascular diseases by preventing the generation of angiotensin II [16,17]. Other drugs such as statins, ezetimibe, fibrates and niacin are common lipid-lowering drugs known to ameliorate and reduce the incidence of deleterious cardiovascular events. Statins inhibit cholesterol biosynthesis by inhibiting 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which prevents the formation of mevalonate and further reduce the formation of

intermediates such as the 15-carbon isoprenoid farnesol and 20-carbon isoprenoid geranylgeraniol which finally results in decrease in protein prenylation, Coenzyme Q10 and dolichol synthesis [8]. However, most of these drugs have side effects such as creatine kinase level elevation, muscular problems etc., hence, the search for natural alternatives either as drugs or nutraceuticals that are cheap, safe and effective. These natural alternatives include medicinal plants, whose bioactive constituents like alkaloids, tannins, flavonoids, saponins and phenolics [18], produce physiological actions of great importance to the health of individuals and communities [19].

Doum palm (*Hyphaene thebaica*) is an African palm tree, found in the Sahel. It grows in riverine areas and along the coast of West, Central, and East Africa. It is sweet and tastes like ginger bread hence the name "doum fruit". It contains high amount of phenols and flavonoids, and possesses antioxidant and antimicrobial activities [20,21]. Traditionally, the fruit is used for the treatment of diabetes [22], hypertension, dyslipidemia and as a hematinic agent [23]. Components of the fruit have been shown to reduce the risk of cardiovascular diseases [24], but very little has been documented on the effect of flavonoids present in the seeds. The present study aims to investigate the effect of flavonoid-rich fraction of *H. thebaica* on the lipid profile, activity of angiotensin converting enzyme (ACE) and some antioxidant parameters in fat-fed obese wistar rats.

2. MATERIALS AND METHODS

2.1 Collection, Identification and Preparation of Plant Material

Doum palm fruit was collected in February, 2018 from Rimi Market, Kano Municipal, Kano State, Nigeria. The fruit was identified and authenticated by a Botanist at the herbarium of the Department of Plant Biology, Faculty of Life Sciences, Bayero University, Kano, Nigeria. A voucher specimen was deposited at the herbarium for future use.

The doum fruit was cracked to separate the epicarp from the seed, ground into powder using mortar and pestle and then sieved with a mesh (2 mm) before use.

2.2 Experimental Animals

Twenty-five (25) wistar rats (150-240 g) were obtained from the National Veterinary Research

Institute, Vom, Jos, Plateau State, Nigeria and kept in well-ventilated cages under standard conditions. The animals were allowed to acclimatize for 2 weeks, fed normal rat feed (Vital Feed, UAC, Kano) and water *ad libitum* before commencement of experiment.

2.3 Acute Toxicity Test

The lethal dose (LD₅₀) of the flavonoid-rich fraction of *H. thebaica* was determined as described by Lorke [25]. Briefly, nine (9) rats divided into 3 groups of 3 rats each were orally administered 10, 100 and 1000 mg/kg of the extract, respectively. The rats were monitored for any signs of distress or death for 24 hours. In the absence of death or distress, the doses were increased to 1600, 2900 and 5000 mg/kg.

2.4 Experimental Design

High fat diet (HFD) was prepared as reported by Mickelsen et al. [26]. To compose the feed, 60 g crisco, 25 g casein, 7 g sucrose, 1 g starch, 4 g mineral salt, 3 g palm oil, 2g vitamins A and D, and 1 g vitamin E was added to every 100 g of standard rat feed. The feed was compounded daily.

The rats were divided into five (5) groups of five (5) rats each:

- Group 1: Control group (Fed standard rat diet, not treated)
- Group 2: Obese control (Fed HFD, not treated)
- Group 3: Standard (Fed HFD, treated with 70 mgkg⁻¹ standard drug, atorvastatin)
- Group 4: 100 mgkg⁻¹ *H. thebaica* (Fed HFD, treated with 100 mgkg⁻¹ flavonoid-rich fraction of *H. thebaica*)
- Group 5: 250 mgkg⁻¹ *H. thebaica* (Fed HFD, treated with 250 mgkg⁻¹ flavonoid-rich fraction of *H. thebaica*)

Animals were allowed free access to water and food freshly prepared daily. Rats in all groups except group 1 (control) were given HFD for 4 weeks and weighed weekly until they became obese. Obesity was defined as a BMI ≥ 0.35 kg/m², which was determined by dividing the weight of the rat (kg) by the square of the length (distance from head to tail end) in centimeter [27]. The obese rats were treated orally with the flavonoid-rich fraction of doum palm and standard drug (atorvastatin) for 2 weeks.

All experimental protocols were approved and conducted with strict adherence to guidelines and procedures of the Institutional Animal Care and Use Committee of Bayero University, Kano.

2.5 Preparation of Flavonoid-rich Fraction of *H. thebaica*

Flavonoid-rich fraction of *H. thebaica* was obtained as described by Hetta and Yassin [24]. Briefly about 500 g of powdered *Hypaene thebaica* fruit epicarp was extracted using acetone. The crude acetone extract obtained was then fractionated with methanol, followed by ethyl acetate leaving a residual water-soluble flavonoid-rich fraction.

2.6 Serum Lipid Analysis

Lipid profile was determined using enzymatic colorimetric methods. High density lipoprotein (HDL), low density lipoprotein (LDL), total cholesterol (TC) and triglyceride (TG) were determined using commercially available kit, and analyzed according to the manufacturer's instruction (Chemelex, S.A., Barcelona).

2.7 Determination of ACE Activity

Kidneys and lungs were homogenized in cold Trizma-HCl buffer (pH = 7.8), centrifuged at 4°C for 15 min and 5000 xg to obtain the supernatants used for the assay. The activity of ACE was determined as described by Cushman and Cheung [28]. Briefly, 50 µl deionized water and 0.2 ml of 5 mmol L⁻¹ hippuric-histidyl-leucine (HHL) was added to 50 µl of either serum, kidney or lung sample, and allowed to stand for 15 minutes at 37°C. This was followed by adding 0.25 ml of 1.0 N hydrochloric acid to terminate the reaction. To extract hippuric acid, 2 ml ethyl acetate was added, vortexed and centrifuged for 2 mins at 3600 x g, and 1 ml of the supernatant dried in a water bath at 100°C. Hippuric acid was dissolved in 3 ml distilled water and absorbance taken at 228 nm.

2.8 Determination of Antioxidant Parameters

2.8.1 Determination of malondialdehyde concentration

Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation [29]. Briefly, 200 µl of each sample was de-proteinized using 0.5 ml trichloroacetic acid (TCA) and

centrifuged at 3000 rpm for 10 minutes. To 0.1 ml of supernatant obtained, 1 ml of 0.75% TBA was added, boiled in water bath for 20 minutes at 100°C and then cooled on ice. The absorbance was read at 535 nm.

2.8.2 Determination of catalase activity

This was determined as described by Brannan et al. [30] with some modifications. About 50 µl of blood sample was mixed with 2.5 ml Ammonium ferrous sulphate, 100 µl xylenol orange, 250 µl sorbitol and 25 µl hydrogen peroxide. This was incubated at room temperature for 30 minutes, and absorbance read at 560 nm. The concentration of hydrogen peroxide generated was extrapolated from the standard curve.

2.8.3 Determination of Superoxide Dismutase (SOD) activity

To determine SOD activity, 1 ml of blood sample was added to 9 ml of distilled water; then 0.2 ml of the diluted sample added to 2.5 ml of 0.05 carbonate buffer (pH 10.2). The reaction was initiated by adding 0.3 ml to freshly prepared 0.3 mM adrenaline and quickly mixed by inversion. The increase in absorbance was measured at 480 nm every 30 seconds for 150 seconds, using a reference cuvette containing 2.5 ml buffer, 0.3 ml adrenaline and 0.2 ml water [31].

2.8.4 Determination of reduced glutathione concentration

Reduced glutathione was determined according to Ellman's method [32]. Briefly, to 200 µl of blood sample, 9 ml of distilled water was added. This was followed by addition of 1 ml of the phosphate buffer (pH 7.4). An aliquot of 3 ml of the mixture was transferred into two different cuvettes, and 200 µl Ellman's reagent added to one, while the other was used as blank when absorbance was read at 412 nm.

2.9 Statistical Analysis

All experiments were conducted in triplicates and results expressed as Mean±SD. Data were analysed by one-way ANOVA using Graphical Instat3 software (2000) version 3.05 by Graphical Inc. Values of *P* < 0.05 were considered significant.

3. RESULTS

3.1 Acute Toxicity Test

Treatment of rats with the different concentrations of the flavonoid-rich fraction

showed no signs of toxicity, distress or death. Hence, 100 and 250 mg kg⁻¹ doses were chosen in the present study.

3.2 Effects of Flavonoid-rich Fraction of *H. thebaica* on Body Mass Index

In Fig. 1 shows that induction of obesity using HFD significantly increased BMI of rats, and made them obese. However, treatment with atorvastatin and flavonoid-rich fraction of *H. thebaica* reduced BMI compared to obese control rats, in a dose-dependent manner (Fig. 1).

3.3 Effect of Flavonoid-rich Fraction of *H. thebaica* on Lipid Profile

Changes in plasma lipoprotein levels in control and fat-fed obese wistar rats are shown in Table 1. Induction of obesity increased TC, triglyceride and LDL, while HDL cholesterol reduced significantly ($P < 0.05$). Treatment of obese rats with atorvastatin and the flavonoid-rich fraction reduced serum levels of total cholesterol,

triglyceride and LDL cholesterol. A significant increase was observed in HDL cholesterol level in all treated groups. The effects of the drugs were dose-dependent.

3.4 Effect of Flavonoid-rich Fraction of *H. thebaica* on Antioxidant Parameters

From the results of the antioxidant parameters, it was observed that induction of obesity in rats reduced serum catalase, glutathione peroxidase and superoxide dismutase, while MDA increased significantly ($P < 0.05$). Atorvastatin and the flavonoid-rich fraction of *H. thebaica* given to obese rats caused a significant increase in catalase, glutathione peroxidase and superoxide dismutase compared to obese control rats. While the increase in catalase and glutathione peroxidase was dose-dependent, the increase in superoxide dismutase level wasn't. Also, although MDA levels of treated rats decreased compared to obese rats, the level of MDA in control rats was significantly lower (Table 2).

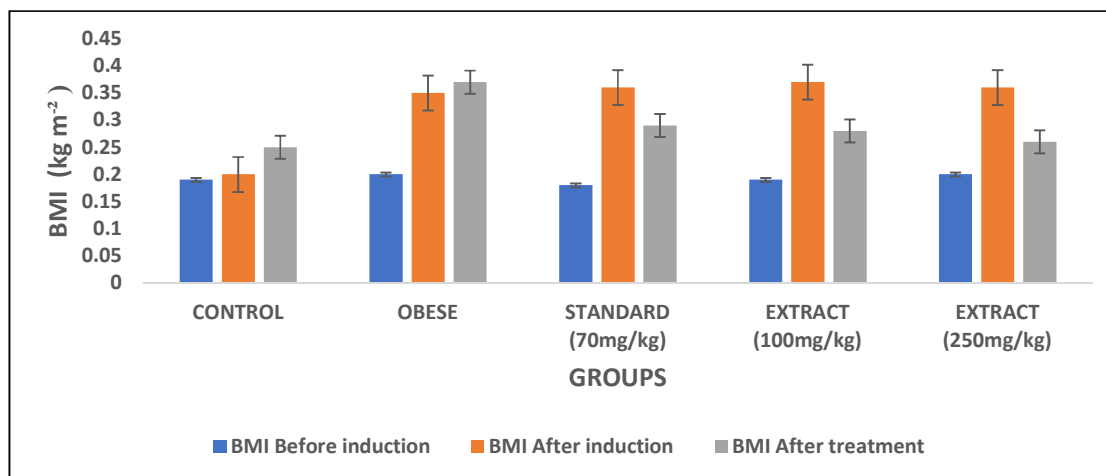


Fig. 1. Effect of flavonoid-rich fraction of *H. thebaica* (Doum palm) on body mass index of fat-fed obese Wistar rat

Table 1. Effect of flavonoid-rich fraction of *H. thebaica* on lipid profile of fat-fed obese Wistar rats

Parameters (mg/dl)	Control	Obese control	Standard control (Atorvastatin 70 mg/kg)	<i>H. thebaica</i> (100 mg/kg)	<i>H. thebaica</i> (250 mg/kg)
Total Cholesterol	85.44 ± 1.11 ^a	113.12 ± 2.23 ^b	61.56 ± 1.31 ^c	80.72 ± 1.69 ^e	74.28 ± 1.85 ^d
HDL Cholesterol	42.12 ± 0.90 ^j	10.48 ± 1.51 ^m	53.96 ± 1.01 ⁿ	14.40 ± 0.76 ^r	31.36 ± 1.31 ^p
Triglycerides	102.4 ± 3.02 ^f	113.4 ± 1.82 ^g	30.51 ± 2.47 ^h	71.44 ± 1.65 ^k	61.56 ± 1.65 ^j
LDL Cholesterol	22.84 ± 0.91 ^z	79.89 ± 1.17 ^p	7.65 ± 0.99 ^w	51.86 ± 1.16 ^y	24.09 ± 0.51 ^z

Values are expressed as mean ± SD (n=5). Values with different superscript across the group are significantly different $P < 0.05$

Table 2. Effect of flavonoid-rich fraction of *H. thebaica* on some antioxidant parameters of fat-fed obese Wistar rats

Groups	Superoxide dismutase (U/ml)	Glutathione peroxidase (U/ml)	Catalase (U/ml)	Malondialdehyde ($\mu\text{mol/ml}$)
Control	96.0 \pm 2.44 ^b	123.0 \pm 9.90 ^c	77.05 \pm 2.50 ^a	33.40 \pm 15.22 ^b
Obese Control	70.9 \pm 5.55 ^c	72.12 \pm 6.50 ⁿ	33.05 \pm 2.50 ^w	91.78 \pm 1.29 ^p
Standard Control (70 mg/kg Atorvastatin)	96.4 \pm 0.27 ^b	93.52 \pm 8.41 ^m	66.37 \pm 5.5 ^e	54.52 \pm 10.52 ^x
<i>H. thebaica</i> (100 mg/kg)	92.19 \pm 1.29 ^b	82.00 \pm 7.43 ^s	45.05 \pm 9.50 ^f	65.00 \pm 9.22 ^x
<i>H. thebaica</i> (250 mg/kg)	91.20 \pm 4.54 ^b	92.00 \pm 6.50 ^m	60.05 \pm 8.20 ^e	67.34 \pm 8.32 ^x

Values are expressed in mean \pm S.D Values with the same superscript shows no significant difference at value of $P < 0.05$

Table 3. Effect of flavonoid-rich fraction of *H. thebaica* on serum, kidney and lung ACE activity of fat-fed obese Wistar rats

Groups	ACE activity ($\mu\text{mol/min/mg}$)		
	Serum	Kidney	Lungs
Control	0.584 \pm 0.058 ^l	0.569 \pm 0.025 ^d	0.565 \pm 0.36 ^a
Obese Control	0.804 \pm 0.050 ^k	0.811 \pm 0.040 ^e	0.909 \pm 0.073 ^b
Standard Control (70mg/kg Atorvastatin)	0.572 \pm 0.022 ^l	0.485 \pm 0.072 ^d	0.513 \pm 0.083 ^a
<i>H. thebaica</i> (100 mg/kg)	0.729 \pm 0.013 ⁱ	0.677 \pm 0.027 ^d	0.679 \pm 0.045 ^a
<i>H. thebaica</i> (250 mg/kg)	0.796 \pm 0.027 ⁱ	0.639 \pm 0.021 ^d	0.728 \pm 0.53 ^a

Values are expressed as Mean \pm SD Values with different superscript down the column are significantly different at ($P < 0.05$)

3.5 Effect of Flavonoid-rich Fraction of *H. thebaica* on Activity of ACE

Table 3 depicts the effect of the flavonoid-rich fraction of *H. thebaica* on ACE activity in serum, kidneys and lungs of fat-fed obese wistar rats. The activity of ACE in obese rats was significantly ($P < 0.05$) higher than rats in control and all treated groups. There was no significant ($P > 0.05$) difference in ACE activity in serum, kidneys and lungs of rats in the control group and those given atorvastatin and the fraction at 100 and 250 mg/kg.

4. DISCUSSION

Obesity is identified as a prominent cause of morbidity and mortality and is associated with various medical disorders [33]. Several factors such as sedentary lifestyle, consumption of HFDs and large amount of modern fast foods have been attributed to the epidemic of obesity [34]. Fat increases the palatability of food and is converted into body fat far more efficiently than carbohydrates [35], resulting in body weight increase that subsequently leads to obesity and related disorders. The first step in research studies underlying disease pathogenesis and

drug development is the use of experimental animal models [36]. High fat-induced obese animal models represent the most common route of obesity in humans [37]. The mechanism of HFD-induced obesity is still unclear, but long-term exposure increases body weight and adiposity in human and animals [38,39]. Also, HFD-induced obese rodents have low sympathetic activity, which in turn results in decreased energy expenditure. However, it is not known whether reduced sympathetic activity is due to a defect in the central nervous system (CNS) during the development of diet-induced obesity [40].

The BMI of rats in each group were determined weekly as a general index of overall health. The significant ($P < 0.05$) increase in BMI after induction of obesity conforms to studies by Rinku et al. [41], who demonstrated that the intake of HFD for 28 days significantly increased the body weight and produced hyperlipidemia in rats. The increased BMI may be attributed to the increased level of leptin or the stimulation of ghrelin and peptide YY (PYY) by the high fat meal [42]. It is evident from our study that the administration of flavonoid-rich fractions of *H. thebaica* at both low and high doses provoked a reduction in BMI

when compared to the fat-fed obese group. It is possible that the fractions increased the catabolism of accumulated lipids in adipose tissue resulting in a decrease in mean body weight and consequently BMI [43]. Also, treatment with atorvastatin decreased BMI significantly compared to obese rats. Atorvastatin inhibits HMG-COA reductase, causing a decrease in cholesterol synthesis, and increasing expression of LDL receptors on hepatocytes [8].

Hyperlipidemia is another major risk factor associated with obesity. The findings from this study agree with Hariri and Thibault [44] who showed that HFD increase triglycerides. The higher TG levels compared to other lipids has been attributed to the inhibition of triglyceride degradation, due to a direct inhibitory effect on lipoprotein lipase bound to capillary endothelium. Lipoprotein lipase is vital in the metabolism of triglycerides and is involved in several pathological disorders, including atherosclerosis and obesity [45,46]. Also, the significantly higher levels of LDL-cholesterol than HDL-cholesterol in obese rats could be due to a shift in the lipoprotein distribution from HDL-cholesterol to predominantly LDL-cholesterol [45,46]. The decreased levels of serum TG, LDL, and TC, and increased HDL observed in rats treated with flavonoid-rich fraction of *H. thebaica* agrees with the findings of Kamis et al. [47] who reported that doum fruit is hypolipidemic. The low TG level in treated animals shows that *H. thebaica* may have an inhibitory effect on lipoprotein lipase activity. The increased levels of plasma HDL-cholesterol concentrations at a higher dose of 250 mg/kg body weight suggests that *H. thebaica* may protect against cardiovascular diseases that result from hyperlipidemia. It is a known fact that HDL-cholesterol carries cholesterol and cholesterol esters from the peripheral tissues and cells to the liver, where cholesterol is metabolized into bile acids. This pathway plays a very important role in reducing cholesterol levels in the blood and peripheral tissues and in inhibiting atherosclerotic plaque formation in the aorta [48].

Obesity is a chronic inflammatory disease associated with an unbalanced production and elimination of reactive oxygen species (ROS). High fat diet causes the formation of toxic intermediates capable of inhibiting the activity of antioxidants, leading to the accumulation of H_2O_2 and O_2 , which in turn forms free hydroxyl radicals [49]. The antioxidant system represents the main pathway for free radicals detoxification. In the

present study, induction of obesity decreased the levels of catalase, glutathione peroxidase and superoxide dismutase and increased MDA in obese rats compared to control. This may be attributed to the increased cellular accumulation of lipid peroxides and depletion of endogenous antioxidants due to the high-fat diet given to the rats, indicating the reduced the ability for free radical scavenging in obese rats and subsequent development of oxidative stress [50]. This signifies that the first line of antioxidant defense diminishes on induction of obesity [49]. On the contrary, the increase in serum catalase, glutathione peroxidase and superoxide dismutase and decrease in MDA levels of rats treated with the flavonoid-rich fraction of *H. thebaica* is evident of its antioxidant activity. Although there is paucity of information on the antioxidant properties of *H. thebaica*, studies have attributed it to its high phenolic contents [51]. This conforms to reports by Zübeyir et al. [52] that plants containing high levels of flavonoids and polyphenol possess very good antioxidant properties. Phenolic compounds are recognized as antioxidants due to their ability to donate hydrogen atoms. Furthermore, flavonoids are a large group of naturally occurring plant phenol compounds that inhibit lipid oxidation by scavenging radicals, singlet oxygen quenching, metal chelation, and lipooxygenase inhibition [53].

The elevated levels of ACE in kidneys and lungs of fat-fed obese rats agrees with reports by Sherifi et al. [54] that ACE activity increases in all diseases that involves proliferation of endothelial cells, including diabetes, obesity and hypertension. Also, Abdulazeez et al. [55] demonstrated that serum and tissue ACE activity were significantly elevated in two-kidney-one-clip hypertensive rats. Obesity increases the risks of cardiovascular diseases, including hypertension, by altering endothelin and RAAS and the increase in ACE activity in obese condition is due to the activation of sympathetic nervous system by adipose tissue derived hormones, resulting in the production of renin that converts angiotensinogen to angiotensin I, which is then converted to angiotensin II by ACE [57]. Thus, from this result it is evident that increase in ACE activity in obese rats may be due in part, to alteration of RAAS. The significant decrease in ACE activity observed when rats were given flavonoid fraction of *H. thebaica* indicates that the fraction contains potent ACE inhibitors. This conforms to studies by Abdulazeez et al. [58], who reported that plants rich in ACE inhibitor(s),

reduce ACE activity. However, atorvastatin, known to inhibit HMG-CoA reductase, prevent the production of cholesterol and retards or even reverse progression of coronary artery disease, might have reduced ACE activity based on its ability to cause weight loss. This is because, it has been demonstrated that ACE activity reduces with weight loss [17]. This is however inconclusive since the effectiveness of atorvastatin is dose-related.

5. CONCLUSION

In conclusion, this study has demonstrated that the flavonoid fraction of *H. thebaica* may possess antioxidant, hypolipidemic and ACE inhibitory properties, and may be a promising candidate for managing the adverse effects of obesity.

ETHICAL APPROVAL

As per international standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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