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Original Article

Rutin treatment alleviates obesity-related aortic endothelium dysfunction in albino rats fed a high-fat diet

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Abstract

Flavonoids have recently been shown to be useful to people suffering from vascular disorders caused by a high-fat diet (HFD). The flavonoid rutin (RT) exhibits numerous pharmacological effects, including antioxidant, cytoprotective, vasoprotective, and cardioprotective activities. The primary objective of this research was to assess the efficacy of RT against obesity-related vascular endothelial dysfunction (VED) in rats fed HFD. A total of 24 mature Wistar rats were blindly categorized into 4 treatment and control groups: normal control, obese control, and obese which were given RT at 50 and 100 mg/kg for the final 3 weeks of the experimental period. Animals' body mass and food consumption have been estimated periodically. In addition, liver mass and retroperitoneal fat mass per body mass, abdominal circumference (AC), LEE index, and body mass index (BMI) were estimated. Moreover, lipid profile parameters were assessed in serum. The effect on vascular endothelium reactivity was investigated in an isolated rat aorta. A histopathological investigation of the aorta was performed. The obese control group exhibited higher body, liver, and retroperitoneal fat weights. Significantly, RT intake reverses all these alterations. Furthermore, RT decreased food intake, AC, Lee index, and BMI in HFD-fed rats. The lipid profile of HFD-fed rats was also improved after RT treatment, with lower triglycerides, total cholesterol, LDL-C, and VLDL-C levels and higher HDL-C levels in the serum of HFD-fed rats. Through the ex-vivo investigation, RT groups showed improved vascular endothelium function in HFD-fed animals compared to the obese control group. Taking together, RT could be a promising option for preventing obesity-associated VED.

Keywords: High-fat diet, Dyslipidemia, Rutin, Vascular endothelial dysfunction.

1. Introduction

Nowadays, obesity is recognized as a great challenge in global health as well as in Saudi Arabia (KSA) and has reached epidemic proportions with approximately 12% of the world's population [1]. Since the last few decades, KSA has become progressively more westernized and is considered one of the countries with the highest obesity and overweight prevalence rates [2].

Obesity and dyslipidemia have become pivotal risk factors for the development of heart diseases [3]. One of the features of heart disease is vascular endothelial dysfunction (VED) [4]. VED plays a crucial role in the progression of obesity-related cardiovascular events. VED can be identified by two major hallmarks, including the decrease in plasma levels of the endothelial nitric oxide synthase (eNOS) enzyme activity as well as the bioavailability reduction of potent endothelial vasodilator and anti-aggregatory mediator nitric oxide (NO) [5]. Even though the processes that connect obesity to VED are still not grasped, numerous aspects have been suggested to mediate this approach.

Hyperlipidemia is an important independent cause of VED and can even induce cardiovascular disorders [6]. Hyperlipidemia-induced VED is mediated through diverse mechanisms that include oxidant/antioxidant imbalance [7], elevated generation or action of known vasoconstric-

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tor substances, which include the two potent bioactive agents Endothelin-1 (ET-1) and angiotensin II (Ang II) [8], and alterations in the vascular panel of prostanoid liberation [9].

Although there is a wide variety of lipid-lowering agents, including statin (HMG-CoA reductase inhibitors), ezetimibe (cholesterol absorption inhibitor), and niacin (an inhibitor of lipolysis), their effectiveness is restricted, and they have been linked to a range of adverse effects [10]. Hence, the treatment of conditions that are related to obesity has piqued the interest of researchers in the field to develop plant-derived bioactive compounds. Dietary flavonoids are phenolic molecules that are found in different plants, fruits, seeds, and vegetables. Hence, they are extensively dispersed in nature. They exhibit a variety of pharmacological actions, including anti-inflammatory, antiviral, hepatoprotective, antithrombotic, anticancerogenic, and other biological effects [11]. Additionally, flavonoids are strong antioxidants that can suppress lipid peroxidation and preserve tissue from damage caused by free radicals. The antioxidant activity of flavonoids appears either directly by reactive oxygen and nitrogen species (ROS/RNS) scavenging or by activating the enzymes known to pass antioxidant activity [12].

RT, also known as 3,3',4',5,7-pentahydrohyflavone-3-rhamnoglucoside, is a flavonoid that belongs to the flavonol class (Fig. 1). It is present in a wide variety of common plants, including buckwheat and apples, amongst others. Moreover, it is an essential nutrient component of a variety of meals and beverages made from plants [13]. RT has various pharmacological advantages, including anti-tumor, anti-mutagenic, anti-diarrheal, anti-inflammatory, cytoprotective, vasoprotective, and cardioprotective actions. It is also a powerful antioxidant [12, 14-18]. Additionally, it was discovered that RT acts as a neuroprotective agent and can alleviate tissue damage caused by ischemic/reperfusion injury (aka reperfusion injury) in skeletal muscle, the heart, and the brain [19,20].

Overall, the main purpose of conducting the current research was to explore the effects of RT on obesity-associated VED in rats rendered hyperlipidemic by an HFD.

2. Materials and methods

2.1. Drugs and chemicals

Acetylcholine chloride, phenylephrine (PE), and diagnostic kits for the estimation of TG, TC, and HDL-C were bought from Sigma Aldrich Chemical Co. (St. Louis, MO, USA). The salts for the physiological Krebs solution were procured from E. Merck KGaA (Darmstadt, Germany).

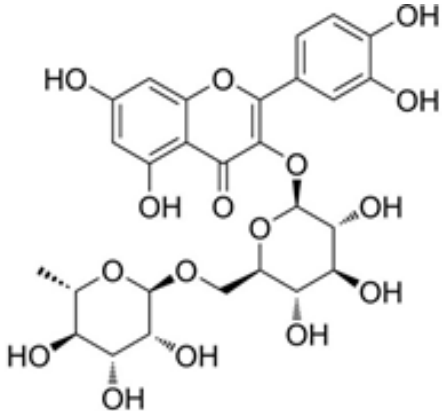

Fig. 1. Rutin Chemical Structure [21].

2.2. Experimental animals

A total of 24 adult healthy male Wistar albino rats (150-180 g) were procured from the animal care unit of the College of Pharmacy, Prince Sattam Bin Abdulaziz University (PSAU), Kingdom of Saudi Arabia (BERC-001–10-18). Animals were acclimatized for one week and housed in transparent cages under standard laboratory conditions in a ventilated room at 25 ± 2 °C with a 12 h light/12 h dark cycle and free access to food and water. No mortalities were observed during the study period. In addition, no animal showed signs of pain or distress throughout the experimental period.

2.3. Preparation of a high-fat diet

The composition of the HFD was done according to the formula described by Xu et al. [22], with some modifications as shown in Table 1. Briefly, the pellets of standard diet (SD) were powdered and mixed thoroughly with cholesterol powder, tallow, egg yolk powder, and milk powder. The resultant mixture was mixed with a sufficient volume of water to make pellets, followed by baking in an oven to avoid fungal contamination.

2.4. Induction of obesity

After one week of adaptation, the first group of animals was given an SD, while other groups were given a HFD for 9 weeks. Water was freely accessible to all animals. After 9 weeks of HFD feeding, blood samples were collected from the induced rats for estimation of triglyceride (TG) and total cholesterol (TC) levels to confirm hyperlipidemia. Rats with blood TG and TC levels >160 and 200 mg/dL, respectively, were considered hyperlipidemic [23].

2.5. Experimental design

A total of 24 rats were assigned to four equal groups:

Standard diet (SD): Rats were fed with SD for the duration of the experiment (12 weeks) plus the vehicle.

High-fat diet (HFD): Rats were fed with HFD for 12 weeks plus the vehicle.

HFD + RT-50: Rats were fed with HFD for 12 weeks plus RT at 50 mg/kg (10–12 weeks).

HFD + RT-100: Rats were fed with HFD for 12 weeks

Table 1. Composition of high-fat diet (HFD).

Ingredient	% of HFD
Powdered normal pellet diet (NPD)	73
Cholesterol	1
Tallow	10
Egg yolk powder	10
Milk powder	6

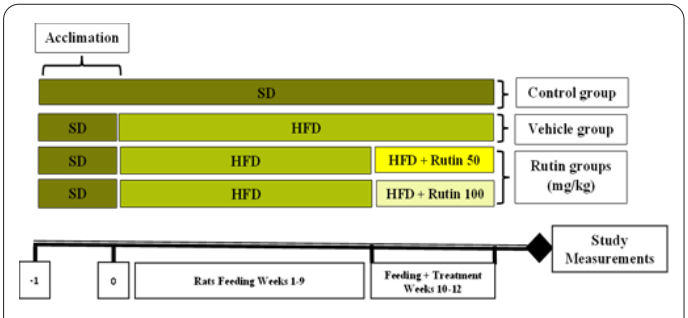

Fig. 2. Experimental Design of HFD-induced dyslipidemia and vascular endothelial dysfunction.

plus RT at 100 mg/kg (10–12 weeks).

1. The vehicle and RT were administered daily by oral gavage during weeks 10-12 (3 weeks) (Fig.2).

2.5.1. Effect on food intake

Food consumption was measured once every week (from the end of the 9th week until the end of the study). Average food consumption per rat was recorded as the difference between the food weight at the beginning of each week and its weight at the end of the week divided by 6 (the number of rats in the group) multiplied by 7 (the number of days) [24]. The percentage of body weight gain was calculated using the following equation: (final body weight minus initial body weight)/final body weight×100 [25,26].

2.5.2. Determination of body weights

The animals were observed daily, and their body weights were recorded before starting the treatment (0-time) and at the ends of the 10th, 11th, and 12th weeks at 10:00 am using an electronic balance (Shimadzu Electronic Balances, Kyoto, Japan).

2.5.3. Anthropometrical determinations [27]

At the end of the experimental period, the abdominal circumference (AC), body mass index (BMI), and Lee index were calculated as indicators of obesity. The AC (immediately anterior to the forefoot) was determined in all anesthetized rats using a plastic non-extensible measuring tape with an accuracy of 0.1 cm [28]. The body weight and body length (naso-anal length) were used to calculate both anthropometrical parameters in anesthetized rats by using the formulas:

$$Body\ Mass\ Index\ (BMI) = \frac{Body\ weight\ (g)}{Body\ length^2\ (cm^2)}$$

$$Lee\ Index = \frac{Cube\ root\ of\ Body\ weight\ (g)}{Body\ length\ (cm)}$$

2.5.4. Blood sampling

At the end of the experiment, the rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (50 mg/kg) to collect blood samples via cardiac puncture. The blood samples were allowed to coagulate at room temperature, followed by centrifugation (3500 rpm, 15 minutes). The clear, non-haemolyzed supernatant serum was separated and stored at -80°C for the assessment of biochemical parameters.

2.5.5. Determination of relative weights of the liver and retroperitoneal fat pad

After successful serum collection, rats were sacrificed by decapitation followed by abdominal incision. The liver and retroperitoneal fat pad of each rat were immediately excised and weighed (g).

2.5.6. Effect on serum lipid profile

The serum samples were assayed for triglycerides (TG), total cholesterol (TC), and high-density lipoprotein cholesterol (HDL-C) using commercially available diagnostic kits. The low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were calculated according to the method of Madkhali et al. [29]:

$$LDL = TC - (\mathrm{HDL} - \frac{\mathrm{TG}}{5})$$

$$VLDL = \frac{\mathrm{TG}}{5}$$

The atherogenic index (AI) and the cardiac risk factor (CRF) were calculated according to Jeong et al. [30] using the

$$AI = \frac{\mathrm{TC} - \mathrm{HDL}}{\mathrm{HDL}}$$s:

$$CRF = \frac{\mathrm{TC}}{\mathrm{HDL}}$$

2.5.7. Estimation of the aortic endothelium dysfunction

Following the cervical dislocation of rats, their thoracic aorta was isolated and immediately transferred to the physiological Krebs solution aerated with carbogen gas. The millimolar (mM) composition of Krebs solution was as follows; Sodium chloride: 118.4, potassium chloride: 4.7, calcium chloride: 2.5, potassium dihydrogen phosphate: 1.2, magnesium sulphate: 1.2, sodium bicarbonate: 25 and glucose: 11. The aorta after cleared off from surrounding tissues, was cut into 7 to 8 isolated tissues having 2–3 mm length of each aortic ring. Each tissue was then suspended in a tissue bath containing 10 mL Krebs having a pH of 7.4, and a constant temperature of 37 °C was maintained by a controlled heating system attached to the organ bath. To stabilize the tissue and to proceed with the experiment, the earlier described protocol by Furchgott and Zawadski [31] was followed with slight modifications. An equilibration period of 50 min was given to each tissue with the replacement of the Krebs solution every 15 min without the addition of any drug, and the tissue tension was maintained around 1 g. The aortic ring isometric contractile and relaxant responses were monitored by the attached transducer with emkaBath2 data acquisition with IOX2 software. Following the successful tissue stabilization, the contraction was induced with a submaximal concentration of alpha-1 agonist drug, phenylephrine (PE; 1×10^{-6} M), and the concentration-dependent relaxation response curves (CRCs) of muscarinic drug; acetylcholine (Ach; 1×10^{-9}–1×10^{-5} M) were recorded.

2.5.8. Histology of the isolated aortic rings

After complete relaxation was achieved with inhibitory CRCs of acetylcholine, each aortic ring was removed from the organ bath. Their fixation was done in 10% buffered formalin and processed routinely for paraffin embedding. These fixed paraffin-embedded aortic rings resulted in a tissue having 5 µm thickness after cutting with a rotary microtome. The primary histological layer of the aorta wall is elastic fibers, which are correlated to the elastic nature of the aorta and are considered important to allow blood flow out of the heart during systolic. Moreover, the general structure of the aortic tissues of all representative animal groups was studied using hematoxylin and eosin (H&E) stain and collagen fibers using van Gieson stain. In contrast, Verhöeff's stain was used to study the nature of elastic fibers. All the microscopic observations of these stained sections were conducted under a light microscope

(Hund Wetzlar H600/12, Germany, fitted with a digital camera, Canon EOS 550D).

2.6. Statistical analysis

All findings are expressed as mean ± standard error (SE) of the mean. The significance of the results was considered where the *P* value was less than 0.05 ($p < 0.05$). The obtained data was analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. GraphPad Prism 9.0 (GraphPad Software, Inc., USA) was used to draw graphs and to analyze all the results.

3. Results

3.1. Effect of rutin treatment on body weight and relative weights of the liver and retroperitoneal fat pad

The average body weight of rats fed with HFD was markedly increased following 9 weeks of feeding, comparable to those fed with SD (data not shown). The difference between the body weight of the animals of the HFD and the SD groups was more apparent at the end of the experimental period. In contrast, the increase in the body weight of HFD-fed rats was dose-dependently suppressed with RT treatments. Importantly, the body weight of the obese rats treated with RT at 100 mg/kg attained a more significant decrease in comparison to HFD rats. RT at 100 mg/kg brought back the body weight gain toward normal value.

In addition to the change in body weight, the relative weights of liver and retroperitoneal fat pad were increased in the HFD group compared to the SD animals. The ability of RT-100 to restore the increase in relative liver and retroperitoneal fat pad weights of obese rats to their normal values confirms its efficacy (Fig. 3).

3.2. Effect on food intake

No significant variations in food intake were noted throughout the first 9 weeks of the study (obesity induction period) in normal and HFD groups (data not shown). The food intake was the highest during the treatment period (10^{th} – 12^{th} weeks) in HFD rats as compared to the SD group. RT treatment of HFD-fed animals during the last 3 weeks of the experiment significantly produced a decrease in the mean food intake compared to HFD rats (Fig. 4).

3.3. Anthropometry measurement

The anthropometry, including AC, BMI index, and Lee index, are shown in Fig. 5. Significantly higher AC, BMI index, and Lee index were observed in the HFD group compared with the SD rats. In contrast, the HFD-induced increase in AC was dose-dependently suppressed with RT treatments (50 and 100 mg/ kg). In addition, RT treatments produced a dose-dependent decrease in BMI and Lee indices as compared with the respective HFD groups.

3.4. Effect on lipid profile

Serum levels of TG, TC, HDL-C, LDL-C, and VLDL-C were estimated, and the results were recorded in Table 2. Compared with the SD, the HFD for 9 weeks produced a marked elevation in serum TG, TC, LDL-C, and VLDL-C (data not shown). The oral administration of RT (50 mg/kg) to the obese rats for 3 weeks induced a marked decline in serum levels of TG (52.89±3.32 mg/dL), TC (236.02±11.69 mg/dL), LDL-C (189.83±11.74 mg/dL) and VLDL-C (10.58±0.66 mg/dL), compared to SD rats (85.41±5.99, 375.26±16.30, 344.43±17.46 and 17.08±1.19 mg/dL, respectively). RT in a dose of 100 mg/kg reduced significantly, better than its low dose, the level of these parameters in obese rats. These declines were accompanied by a significant elevation of serum HDL-C in both RT-treated groups.

On the other hand, the values of the atherogenic index

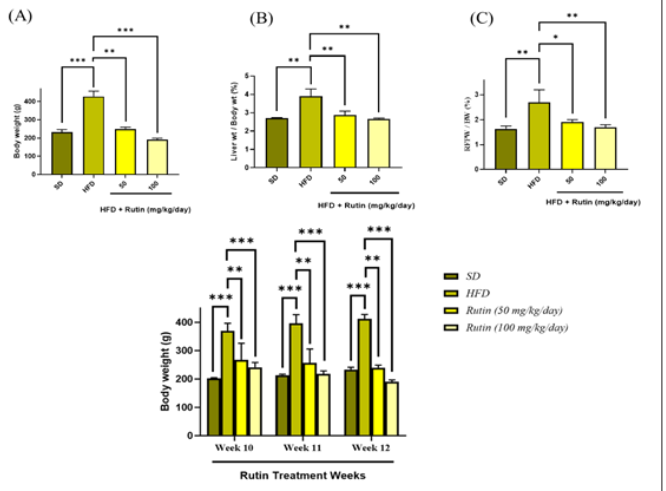

Fig. 3. Effect of Rutin on body weight and relative weights of the liver and retroperitoneal fat pad. **(A)** Body weight at the end of the study; **(B)** LW/BW; **(C)** RFPW/BW; **(D)** body weight at treatment weeks. Values represented are mean ± S.E.M. (n=6). Significantly different from the values *P < 0.05, **P < 0.01, ***P < 0.001.

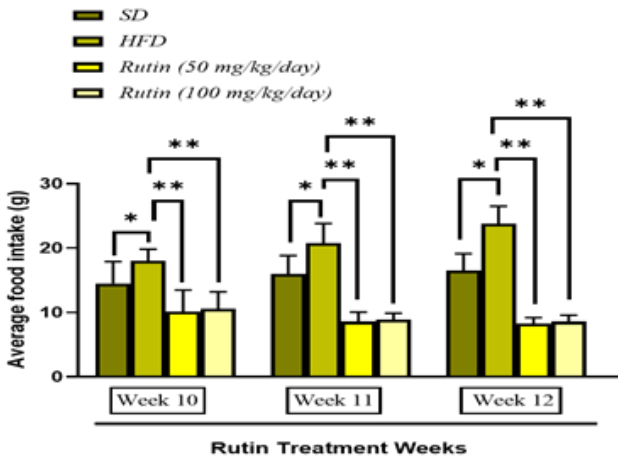

Fig. 4. Effect of Rutin on food intake. Values represented are mean ± S.E.M. (n=6). Significantly different from the values *P < 0.05, **P < 0.01.

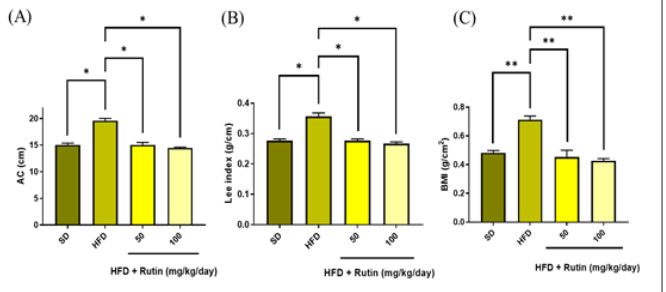

Fig. 5. Effect of Rutin on anthropometric measures. (A) waist; **(B)** Lee index; **(C)** BMI. Values represented are mean ± S.E.M. (n=6). Significantly different from the values *P < 0.05, **P < 0.01.

Table 2. Effect of Rutin on lipid profile.

Groups	Cholesterol (mg/dL)	Triglycerides (mg/dL)	HDL-C (mg/dL)	VLDL-C (mg/dL)	LDL-C (mg/dL)
SD	152.95±10.45	22.91±2.72	83.98±3.84	4.76±0.41	64.20±9.55
HFD	375.26±16.30$^{###}$	85.41±5.99$^{###}$	47.91±1.28$^{###}$	17.08±1.19$^{###}$	310.27±17.46$^{###}$
HFD + RT-50 mg/kg/day	236.02±11.69***	52.89±3.32***	56.77±3.36^{ns}	10.57±0.66***	168.67±11.74***
HFD + RT-100 mg/kg/day	207.52±8.92***	43.75±1.52***	64.84±1.71*	8.75±0.30***	133.93±9.87***

$^{###}p < 0.001$, compared with SD (Student t test), $^{ns}p > 0.05$, *p < 0.05, ***p < 0.001 compared with HFD (One-way ANOVA followed by post-Tukey's test).

and the cardiac risk factor were significantly higher in the HFD group than those in SD rats. A significant reduction in these two parameters was observed in HFD-fed animals treated with RT in a dose-dependent fashion (Fig. 6).

3.5. Protective effect of RT on endothelial dysfunction

In the aortic rings of the diseased group (HFD), acetylcholine failed to show inhibition of the pre-contracted PE (1 µM) rings where non-significant ($p > 0.05$) relaxation of 17% was recorded. On the other hand, in the normal control group, ACh produced significant vasodilation with resultant EC_{50} values of 64.32 µM (44.14-76.58; n=4). The treatment group aortic rings of rats with higher dose (100 mg/kg) showed aortic relaxation with Ach in concentration mediated manner where the EC_{50} values were recorded as 68.82 µM (52.44-79.68; n=4), whereas the lower dose of RT (50 mg/kg) administered rat aortic rings showed comparatively less relaxation to that observed with higher dose, but the relaxation was found significantly higher than HFD group with EC_{50} values of 540.78 µM (492.32-586.28; n=4) as shown in Fig.7.

3.6. Effect of RT on histopathological changes

In HFD-induced obese rats, photomicrographs of the aorta showed the presence of fat accumulation in the form of adipocytes (Arrow at photo A2 x30) with weakened elastic fiber layers in comparison to both normal and RT-treated groups. The collagen elastic ratio is found to increase as indicated by the increased collagen layer (arrow points at red color in photo B3x30). In general, RT treatment at 100 mg/kg resulted in improved architecture of the aorta regarding all aspects of general features, collagen elastic ratio, and elastic fibers status, which were found close to the normal group, as shown by photomicrographs (Fig. 8).

4. Discussion

Obesity is a serious public health issue that affects individuals across the globe. It is caused by a confluence of factors, including factors related to genetics, insufficient nutrition, and an absence of regular exercise. Since 1980, there has been a remarkable rise in the prevalence of obesity, which has more than doubled during that period [32]. Recently, approximately one-third of the global population has fallen within the obesity range, and it is even predicted that this number will rise to nearly half by the year 2030 [33]. Given that obesity is linked to a wide variety of negative health impacts, such as the higher risk of heart disease, inflammatory bowel disease, and various types of cancer, it has now become a serious threat to the general population's health. It is regarded as a major public health concern [34]. The HFD is a critical contributor to the pathogenesis of obesity and the health issues that come along

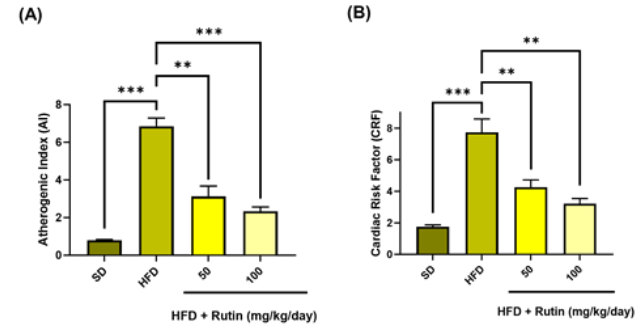

Fig. 6. Effect of Rutin on the values of atherogenic index (AI) and cardiac risk factor (CRF) in high-fat diet (HFD)-fed rats at the end of the experimental period. Values of AI and CRF are expressed as mean ± S.E.M. Significantly different from the values **P < 0.01, ***P < 0.001.

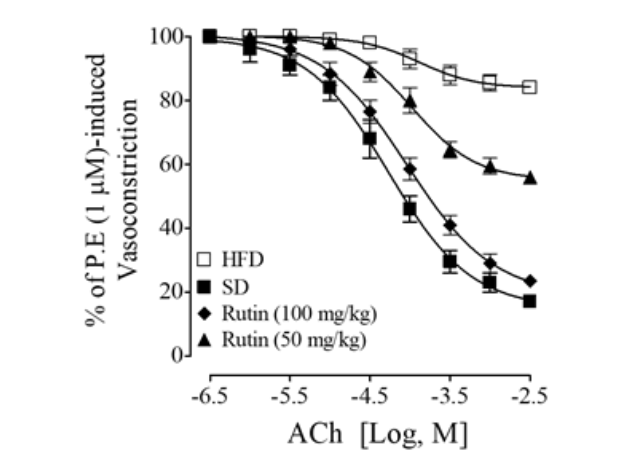

Fig. 7. Effect of Rutin on vascular endothelium. Values are presented as mean ± S.E.M. (n = 6).

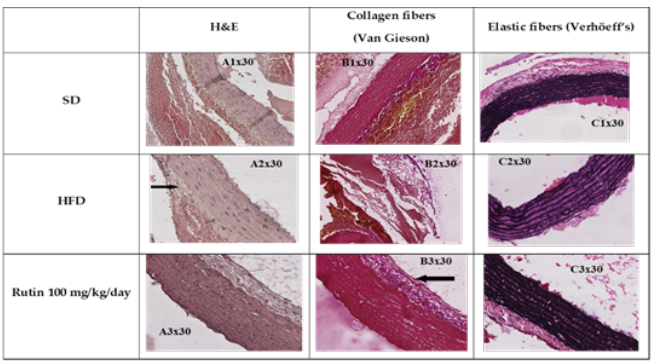

Fig. 8. Effect of Rutin on histology of isolated rat aorta. Photomicrographs of H&E, Van Gieson and Verhöeff's-stained sections of the thoracic aorta of HFD-fed rats. Aortas are from: (a) SD; (b) HFD; and (c) RT-100 (Rutin 100 mg/kg/day) groups.

with it. The obesity that HFD causes in animals is considered a useful model because it closely mimics the obesity that occurs in humans [35].

According to the findings of this study, rats given an HFD had a discernible rise in their overall food intake, as well as an increment in their body weight and retroperitoneal fat. When compared to animals fed a normal diet, the rats in the control group that were fed with HFD had a relatively large liver in terms of weight. The consumption of food with many calories is the primary factor in the development of obesity. This is because excess energy is saved in adipocytes, which thereby expand in either their size or number or both, particularly visceral adipocytes, resulting in a rise in the rate at which fat is broken down [36]. The higher rate of lipolysis drives the generation of cytokines by the infiltration of leukocytes and macrophages, which generates inflammation in the adipocytes and ultimately leads to a pro-inflammatory response as well as endothelial dysfunction. Therefore, malfunctioning in adipose tissue is the etiopathogenic pathway in the progression of cardiovascular disease forced by abdominal obesity [33].

Based on the findings of this study, rats receiving RT treatment were found to have positive impacts on overcoming obesity. It was observed that RT-treated obese rats had significantly lower food intake, body weight, and the weight of retroperitoneal fat, which led to a decrease in the Lee index and BMI. In this context, a person is considered to have a normal BMI between 18.5 and 24.9 kg/m3, to have an overweight BMI between 25 and 29.9, and to have an obese BMI of 30 or more [37]. The capability of RT to protect against obesity results from its strong capacity to reduce the amount of food intake. Various animal studies have demonstrated that RT has beneficial impacts on the animals' health. In mice fed with HFD, it is reported that RT was able to inhibit the adipocyte differentiation of 3T3-L1 cells, as well as its capability to lower gains in body weight and liver weight [38].

There is a proven connection between obesity and elevated serum lipid levels. Individuals suffering from obesity, in comparison to normal-weight people, have higher serum levels of total cholesterol, LDL-cholesterol, triglycerides, and total lipids [39]. These lipid panel results can thus be utilized as indicators of obesity. In the current research work, the lipid profile index was abnormal in the obesity control group, as demonstrated by elevated blood concentrations of TG, TC, VLDL-C, and LDL-C and lowered HDL-C concentration. In HFD-treated rats, RT dose-dependently reversed these alterations in lipid profile. Previously published research indicates that flavonoids exhibited strong anti-obesity properties. RT exerts anti-obesity actions by blocking glycerol-3-phosphate dehydrogenase (GPDH) enzyme, which is known to play a key role in deriving triglyceride from glycerol, either in 3T3–L1 preadipocytes as well as in the fat and liver tissues of obese animals that fed HFD [40].

The reduction in the activities of the liver enzyme 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is known to catalyze the rate-limiting step in the biosynthesis of cholesterol, could be one possible mechanism for the significant lowering of lipid elements in the serum of RT-treated HFD-fed group [41]. Furthermore, the decline in blood lipid levels might be caused by a blockage of lipid absorption in the gastrointestinal system.

Amongst the most reliable and substantial markers for predicting the risk of cardiovascular disorders are the atherogenic index (also called atherosclerosis index, AI) and Castelli's risk indexes (also known as cardiac risk indexes, CRF). The greater the value of these two markers, the greater the risk of getting cardiovascular events; inversely, the smaller the reads, the less possible the risk [42, 43]. Our findings indicated that RT-treated HFD-fed rats have significant reductions in the values of AI and CRF, proving the protective effects of RT against cardiovascular events.

According to the findings of Li et al. [44], vessels isolated from HFD-fed animals demonstrate noticeably irregular endothelium-driven vascular relaxation in response to stimulants such as acetylcholine and thrombin. On the other hand, vasodilation induced by substances directly working on the vascular smooth muscle (such as nitroglycerin, sodium nitroprusside, or SNAP) stays unaltered. Although the pathways and aspects that contribute to obesity-related cardiovascular events, particularly in the vascular endothelium, remain unclear, various indicators in animal models, including insulin resistance and hyperlipidemia, have been suggested as potential predictors for cardiovascular dysfunction associated with obesity [45].

The findings of the present study show that HFD-induced obesity led to endothelial dysfunction, as indicated by a diminished Endothelium-dependent vasorelaxation in response to ACh. Thus, aortic vasorelaxation was significantly impaired in rats fed with HFD. Additionally, previous studies have reported a defective acetylcholine response in the aortas of obese rats fed with HFD, as well as endothelial dysfunction has been linked to impaired aortic vasorelaxation in obese Zucker rats [46, 47].

Endothelial function in rats treated with RT was improved. The vasorelaxant response to acetylcholine was significantly restored with RT in both doses of 50 and 100 mg/kg. Endothelial dysfunction has indeed been linked to oxidative stress, which ultimately resulted in a reduction of NO bioavailability []. Thereby, the antioxidant effect of RT and its ability to reduce oxidative stress in plasma is responsible for its protective effect against VED [49].

Moreover, obesity can cause vascular pathogenesis, including effects on the aorta, which can lead to abnormalities in the histology of the vascular system. In our investigations, the aorta of animals fed HFD exhibited fat accumulation in the form of adipocytes, impairment of elastic fibers layers, and enhanced collagen elastic ratio, as seen by an increase in the collagen content compared to the aorta of obesity control rats. According to the findings of previous research, a higher BMI is typically associated with hardening and enhanced arterial wall thickening [50].

This study confirms prior findings that these changes are a major predictor of higher cardiovascular mortality. Nonetheless, treatment with RT at the dose of 100 mg/kg/day significantly repairs the integrity of the aorta, as evidenced by the practically normal architecture of the aorta in terms of all general features, collagen elastic ratio, and elastic fibers status. These results imply that RT treatment recovered the structural and functional integrity of the vascular system as well as histological changes in obesity and other medical illnesses.

In conclusion, the rats that were fed with HFD developed obesity, dyslipidemia, and endothelial dysfunction. These alterations caused by HFD in rats were either prevented or reversed by RT, a flavonoid found in plants. The mechanisms by which RT alleviates changes induced by

HFD could be either through improving lipid profile or by its known antioxidant activity. Consequently, RT could be a potential therapeutic option for the management of obesity-related VED.

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Conflict of interest
No conflict of interest.

Data availability
All data are included in the manuscript.

Ethics approval and consent to participate
The study has obtained approval from the Bio-Ethical Research Committee (BERC) at PSAU, with reference number BERC-001-10-18.

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Authors' contributions
HAM, MAG, and MNA designed the research study. HAM, MAG, MNA, NUR, MMA, AMH, and KFA performed the research. HAM, MAG, NUR, MNA, and GAS analyzed the data. HAM, GAS, MNA, NUR, AYH, and NMA wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors have read and approved the final manuscript.

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