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Original article

Morin attenuates high-fat diet induced-obesity related vascular endothelial dysfunction in Wistar albino rats

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ABSTRACT

Vascular endothelial dysfunction is caused by dyslipidemia, hypertension, and deficiency of antioxidant systems. In this study, the protective effect of a flavonol, morin was investigated in high-fat diet (HFD)-induced dyslipidemia and vascular endothelium dysfunction. The dose-dependent attenuating effect of morin was tested at doses of 50 and 100 mg/kg/day in an *in-vivo* model of HFD-induced dyslipidemia using rats whereas vascular endothelial reactivity was assessed in isolated rat aorta using *ex-vivo* organ bath setup. Morin administration in HFD-induced dyslipidemic rats for three weeks, resulted in a significant decrease in the body weight, LW/BW ratio as compared to rats treated with HFD only where the increase in body weight was observed. Significant reduction in the waist, BMI and lee index was also observed after morin treatment in HFD-induced dyslipidemic rats. In the lipid profile studies, HFD group showed a significant increase in the total cholesterol, triglyceride, LDL, and VLDL levels while HDL levels were decreased significantly, whereas morin treatment reversed all these parameters which were comparable to standard diet (SD) group. In the *ex-vivo* isolated aorta studies, HFD-induced endothelium dysfunction was observed, whereas it was reversed in the aorta of animals treated with morin at doses of 50 and 100 mg/kg/day, comparable to SD group. Morin treatment produced dose-dependent improvement in lipid profile and vascular endothelium protection, thus rationalizing its medicinal use in dyslipidemia and cardiovascular-related endothelial disorders.

1. Introduction

Obesity is a chronic disease with steadily increasing prevalence worldwide, including Saudi Arabia. The changes in the lifestyle among Saudis that have resulted from the country's economic development over the last four decades lead to an increase in national obesity rates (Al-Nozha et al., 2005). The wave of urbanization, as well as the increase in affluence and physical inactivity has resulted in these changes in lifestyle, contributing ultimately to obesity (Al-Nozha et al., 2007). Consequently, the frequency of obesity-related ailments is also increasing very fast, among them a risk for type-2 diabetes mellitus and cardiovascular diseases are very common (Bray, 2004). The endothelium plays pivotal roles in human vascular homeostasis via controlling the balance in the production of vasodilator factors, e.g., nitric oxide, endothelial-derived hyperpolarizing factor (EDHF) and vasoconstrictor, e.g., angiotensin-II (Lüscher, 1990; Epstein, et al., 1990; Vallance, 1989). Accordingly, endothelial dysfunction (ED) can be simply defined as a disturbance in the balance between endothelium-dependent vasodilators and vasoconstrictors. Endothelial dysfunction is currently regarded as one of the key elements in the development of cardiovascular diseases such as atherosclerosis (Ross, 1999; Heitzer et al., 2001). Several human and experimental studies have linked ED to obesity and obesity-associated cardiovascular diseases (Steinberg et al., 1996; Vita et al., 2002). Previously, it has been reported that a high-fat diet (HFD) plays an imperative role in the development of vascular lesions by inducing oxidative stress and systemic inflammation (Napoli et al., 2003). Furthermore, HFD reduced the production of nitric oxide (NO) in the vascular endothelium (Yang et al., 2007). Berg and his colleagues indicated that the increase in adipose tissue mass in obese animals induce systemic inflammation via actions of secreted pro-inflammatory factors, including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) (Berg et al., 2005). Obesity disease

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exhibited a correlation with elevated markers of oxidative stress such as Glutathione (GSH), superoxide dismutase (SOD). However, oxidative stress and inflammation associated with obesity represent a crucial early event in the development of obesity-related endothelial dysfunction (Kobayasi et al., 2010). There is firm evidence that HFD induces central obesity, which is the accumulation of fat in visceral and abdominal areas that are closely linked to endothelial dysfunction (Costa et al., 2011). On the other hand, studies have demonstrated that weight loss interventions successfully reversed endothelial function abnormalities in subjects with obesity-associated endothelial dysfunction (Bigornia et al., 2010).

Growing evidence has linked oxidative stress to obesity-related endothelial dysfunction in which the increase in the production of oxidative stress species and the reduction of antioxidant scavengers, e.g., SOD, GPx catalase and vitamin C result in the diminished nitric oxide bioavailability (Ting et al., 1997). Studies also established that hypercholesterolemia in humans is associated with ED as a consequence of the detrimental effects of oxidative stress (Perticone et al., 2001).

Morin (3,5,7,2,4-pentahydroxy flavone, Fig. 1) found in several natural products such as almond (*prunus dutcis*), guava leaves (*psidium guajava*), figs (*chlorophora tinctoria*) and some other Maraceae family plants (Xie et al. 2006; Caselli et al., 2016). Several studies reported a variety of beneficial pharmacological effects of morin and other natural products, including antioxidant, anti-inflammatory, antinociceptive, antihyperglycemic, and antiangiogenic effects (Jung et al., 2009; Kapoor and Kakkar, 2012; Wu et al., 1993, Rehman et al., 2013, Ahmed et al., 2018, Rehman et al., 2018). Previously, morin was reported to have beneficial effect in reducing the blood pressure (Prahalathan et al., 2012), improve endothelial dysfunction in diabetic animals (Taguchi et al., 2015), reduces blood glucose, serum lipid and liver triglycerides (TG) levels (Vanitha et al., 2014; Naowaboot et al., 2016), shows the same effect as of insulin (Paoli et al., 2013) and inhibit enzyme fatty acid synthase (FAS) (Tian, 2006). In earlier studies, the low cytotoxicity of morin has been reported on cellular cultures and animal models. In one of the studies (in vitro), Sergediene et al., (1999) reported weak cytotoxic effects (IC50 = 250 ± 40 μM) of morin on human promyelocytic leukemia cells. Moreover, in vivo studies revealed no toxic effects of Morin on F344 rats. The reported LD50 dose of morin is 555 mg/kg intraperitoneal and treatment with morin orally at high doses (from about 300 to 2400 mg/Kg b.w.) in rats for 13 weeks did not show any toxicity, only a modest alteration of liver functionality or a moderate increase in liver or kidney weight was reported. Moreover, no-observed toxicity level of Morin was reported at 300 mg/Kg of body weight per day (Cho et al., 2006), hence it's safe to use 50 and 100 mg/kg oral doses of morin in our study. It has been reported that neurodegeneration, inflammation in cardiovascular disease, and cancer protected by the process of autophagy, which occurring in all cells to maintain mammalian homeostasis (Mizushima et al., 2002; White et al., 2010). Moreover, autophagy has also been reported to play a crucial role in preventing the risk of atherosclerosis by removing misfolded proteins in endothelial cells. In previous studies, it also has been reported that the presence of morin increases the level of intracellular cAMP (Degenhardt et al., 2006), which is known to activate protein kinases (PKA) in mammalian cells (Summanen et al., 2001; Canalli et al., 2007). Liang et al. (2007) reported that activation of the cAMP/PKA signaling pathway in endothelial cells plays an essential role in the induction of autophagy.

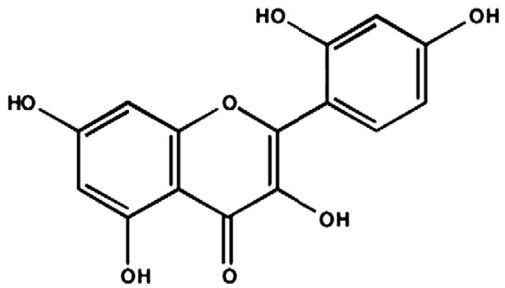

Fig. 1. Morin Chemical Structure.

However, the curative effects of morin on endothelial dysfunction in the obesity model have not investigated yet. Therefore, in the present study, we aimed to examine the possible beneficial effects of morin on endothelial dysfunction in HFD-induced obesity rats.

2. Material and methods

2.1. Reagents and chemicals

Acetylcholine chloride (Ach), phenylephrine (PE), and diagnostic kits for the total serum cholesterol (TC), TG, and high-density lipoproteins (HDL) estimations were purchased from Sigma Aldrich Chemical Co (St Louis, MO, USA). The salts for physiological Krebs solution; calcium chloride, glucose, magnesium chloride, magnesium sulfate, potassium dihydrogen phosphate, sodium bicarbonate, sodium chloride, and sodium dihydrogen phosphate were purchased from E. Merck KGaA (Darmstadt, Germany). Krebs solution was prepared fresh in distilled water on the day of the experiment, whereas all other drugs were prepared as stock, and only dilutions were made on the day of the experiment.

2.2. Animals

Twenty-four adult Wistar albino rats (150–180 g) of either sex were used in the present study. All rats were in healthy condition according to the history and a thorough physical examination performed by the investigators. The study protocol fulfilled requirements and approved by the Institutional Animal Ethics Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University, Kingdom of Saudi Arabia (BERC-001–10-18). Animals were acclimatized for a week before treatment and kept under standard laboratory conditions, in a ventilated room at 25 ± 2 °C, with food and water *ad libitum*, under a 12 h light/12 h dark cycle, during the study.

2.3. Preparation of high-fat diet

HFD was prepared as described by Jakobsdottir and his colleagues (Jakobsdottir et al., 2013) with some modifications. Briefly, normal pellet diet i.e., standard diet (SD, 73%) was ground and mixed thoroughly with cholesterol powder (1% w/w), tallow (10% w/w), egg yolk powder (10% w/w), milk powder [(6% w/w; with 21% carbohydrate, 40% fat, 15% protein and 25.9 kJ/g)]. Tallow was used to induce obesity in HFD, as shown in Table 1. The resultant mixture was mixed with water and made into pellets, which were then oven-baked for proper drying to avoid fungal contamination.

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

Ingredients	Percentage of total content
Powdered Normal Pellet Diet (NPD)	73%
Cholesterol	1%
Tallow	10%
Egg Yolk Powder	10%
Milk Powder	6%

2.4. Experimental design

Animals were randomly divided into four experimental groups (n = 6). Group I: standard diet (SD) fed rats. Group II: high-fat diet (HFD) fed rats. Group III and IV rats administered with HFD plus oral administration of two increasing doses of morin at 50 and 100 mg/kg/day, respectively (Fig. 2).

2.5. Evaluation of morin effect on body and organ weight

To investigate the effect of morin on body weight (BW), and liver weight (LW), two doses of morin (50 and 100 mg/kg/day) were given orally for three weeks to HFD fed animals (9 weeks on HFD prior to morin administration).

2.6. Evaluation of morin effect on waist, Lee index and BMI

Waist, Lee index, and BMI of the rats fed with SD, HFD, and HFD + morin (50 and 100 mg/kg/day) were determined according to the previously reported method (Bernardis, 1970).

2.7. Evaluation of morin effect on food intake

The average daily feed intake of all the animals was determined according to the method followed by Diniz et al (2005). Food intake was measured every day by weighing the amount of food put into the feeders and that remaining in them.

2.8. Evaluation of morin effect on lipid profile

Blood samples, collected in suitable centrifuge tubes from orbital sinus under light ether anesthesia by glass capillary, were centrifuged (3,000 rpm) at 4 °C for 20 min, and separated serum samples were stored at −80 °C for lipid profiling studies. Finally, serum concentrations of TC, TG, and HDL were analyzed using diagnostic kits as described in the instructions of the manufacturer (Sigma–Aldrich Chemical Co., USA). VLDL and LDL were calculated by the given formula (Ansari and Bhandari, 2008):

$$VLDL = TGs/5$$

$$LDL = TC - (VLDL + HDL)$$

2.9. Measurement of vascular endothelial dysfunction

At the end of the experiment, all animals, SD, HFD, and morin (50 and 100 mg/kg/day) were fasted for 16 h and sacrificed by a blow on the head. After opening the abdomen and thoracic cage, their aortae were carefully isolated and kept in physiological Krebs solution with the following composition in mM: NaCl: 118.4, KCl: 4.7, CaCl2: 2.5, KH2PO4: 1.2, MgSO4: 1.2, $NaHCO_3$: 25 and glucose: 11) aerated with oxygen. After cleaning and transverse cutting rings of 2–3 mm length, the tissues were further studied for endothelial reactivity using isolated organ bath filled with Krebs solution (37 °C), bubbled with atmospheric oxygen and connected to a force transducer attached with emkaBath2 data acquisition system and a computer running the Chart software (IOX2) for measuring isometric tension. The experimental protocol was followed as previously described by Furchgott and Zawadski (1980) with slight modifications. Aortic rings were allowed to equilibrate in an organ bath for 45–60 min, at a resting tension of 2 g with the replacement of fresh Krebs solution every 15 min. After the stabilization of the isometric tension, inhibitory concentration–response curves (CRCs) of ACh (1×10^{-9}–1×10^{-5} M) were prepared against induced contractions with a submaximal dose of PE (1×10^{-6} M).

2.10. Aorta histological examination

At the end of the experiment, thoracic aortae were isolated and fixed in 4% formaldehyde and processed routinely for paraffin embedding. Aortae fixed tissue slices of 5 μm thickness were obtained with a rotary microtome. Connective tissue fibers are collagen, elastic, and reticular fibers. The main histological structure of aorta wall is elastic fibers, which are correlated with the function of aorta i.e., elasticity to allow blood flow out of the heart. Thus, the general structure of the aortae of all group of animals were investigated using hematoxylin and eosin (H&E) stain, collagen fibers using van Gieson stain and elastic fibers using Verhöeff's stain. Stained sections were examined under a light microscope (Hund Wetzlar H600/12, Germany, fitted with a digital camera, Canon EOS 550D).

2.11. Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) and considered to be significant at *P*-value of <0.05. To evaluate the variability, data were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test using GraphPad Prism 7.0 (GraphPad Software, Inc., USA). For all comparisons, statistical significance was defined as $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ and $^{****}P \leq 0.0001$.

3. Results

3.1. Effect of morin on body and organ weight

The results were shown in Fig. 3. Animals feed with HFD have significantly increased in body weight, and LW/BW ratio compared with rats fed with a standard diet (SD) ($P < 0.001$). However, body weight, and LW/BW ratio were found to be significantly decreased in HFD-induced obese rats treated with morin doses, in a dose-dependent manner.

3.2. Effect of morin on the waist, Lee index, and BMI

Results of the effect of morin on Waist, Lee index, and BMI of rats were shown in Fig. 4 and found to be significantly increased after nine weeks of HFD treatment compared to SD fed rats, irrespective of sex. However, after morin administration to the

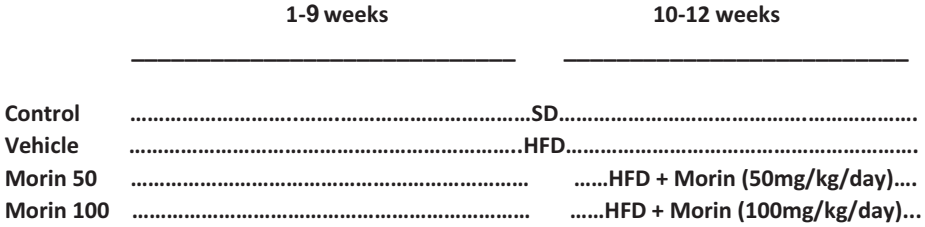

Fig. 2. Experimental protocol of the present study.

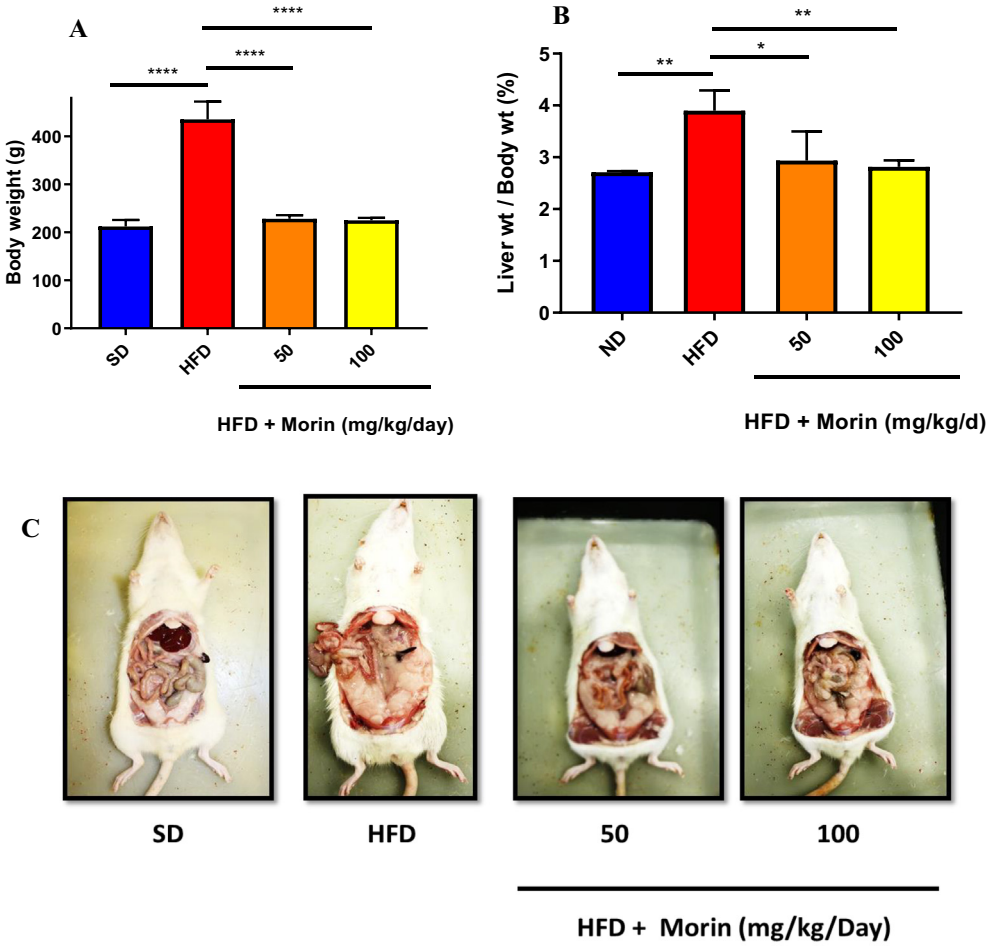

Fig. 3. Effect of morin on (A) Body weight (at the end of study) (B) Liver weight / Body weight ratio (C) Retroperitoneal fat content. Values represented by the mean ± S.E.M (n = 6), $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{****}P \leq 0.0001$ represents comparison of HFD with SD and treated groups (Morin; 50 and 100 mg/kg/day) with HFD group.

HFD-induced obese rats for three weeks at both doses (50 and 100 mg/kg/day), waist, Lee index, and BMI were found to be significantly reduced.

3.3. Effect of morin on food intake

The average daily feed intake of all rats was found to be the same at the beginning of the experiment; however, HFD treatment for nine weeks resulted in a minor increase in food intake (Fig. 5) as compared to SD rats. Both Morin treatments for three weeks in HFD fed rats significantly decreased food intake compared to the HFD fed rats.

3.4. Effect of morin on lipid profile

As depicted in Table 2, rats fed with HFD for nine weeks have shown a significant increase in the levels of TC, TG, LDL-C ($P < 0.001$), VLDL-C, and a significant decreased in levels of HDL-C ($P < 0.001$) when compared to SD feed rats. However, morin treatment for three weeks in HFD-fed rats significantly reversed the hyperlipidemic effect produced by HFD ($P < 0.001$) in a dose-dependent manner.

3.5. Effect of morin on endothelial dysfunction

When the aortae of the morin treated animals were tested for possible endothelium protection, acetylcholine failed to produce relaxation against PE (1 μM)-induced contractions in HFD group with maximum relaxation of 17% ($p > 0.05$), while SD group showed a significant vasodilatation with EC50 values of 76.12 uM (58.26–92.12; n = 4). Similar to SD group, aorta of the animals treated with morin at higher dose (100 mg/kg/day) also showed endothelium-dependent vasodilatation with EC50 values of 88.62 uM (62.34–102.26; n = 4), whereas morin at 50 mg/kg/day dose showed less relaxation compared to higher dose of morin but significantly higher when compared with HFD with EC50 values of 918.32 uM (866.86–1022.42; n = 5) as shown in Fig. 6.

3.6. Effect of morin on histopathological changes

Photomicrographs of the aorta of HFD-induced obese rats showed the presence of fat accumulation in the form of adipocytes, weakness of elastic fibers layers compared to both normal and treated groups. Increased collagen elastic ratio indicated by the increased collagen layer. Morin treatment at the dose of 100 mg/kg/day resulted in better improvement, and photomicrographs showed the almost normal architecture of aorta regarding all aspects of general features, elastic collagen ratio, and elastic fibers status (Fig. 7).

4. Discussion

Obesity is a chronic disease and well known significant risk factor for various associated pathological disorders, morbidity, and

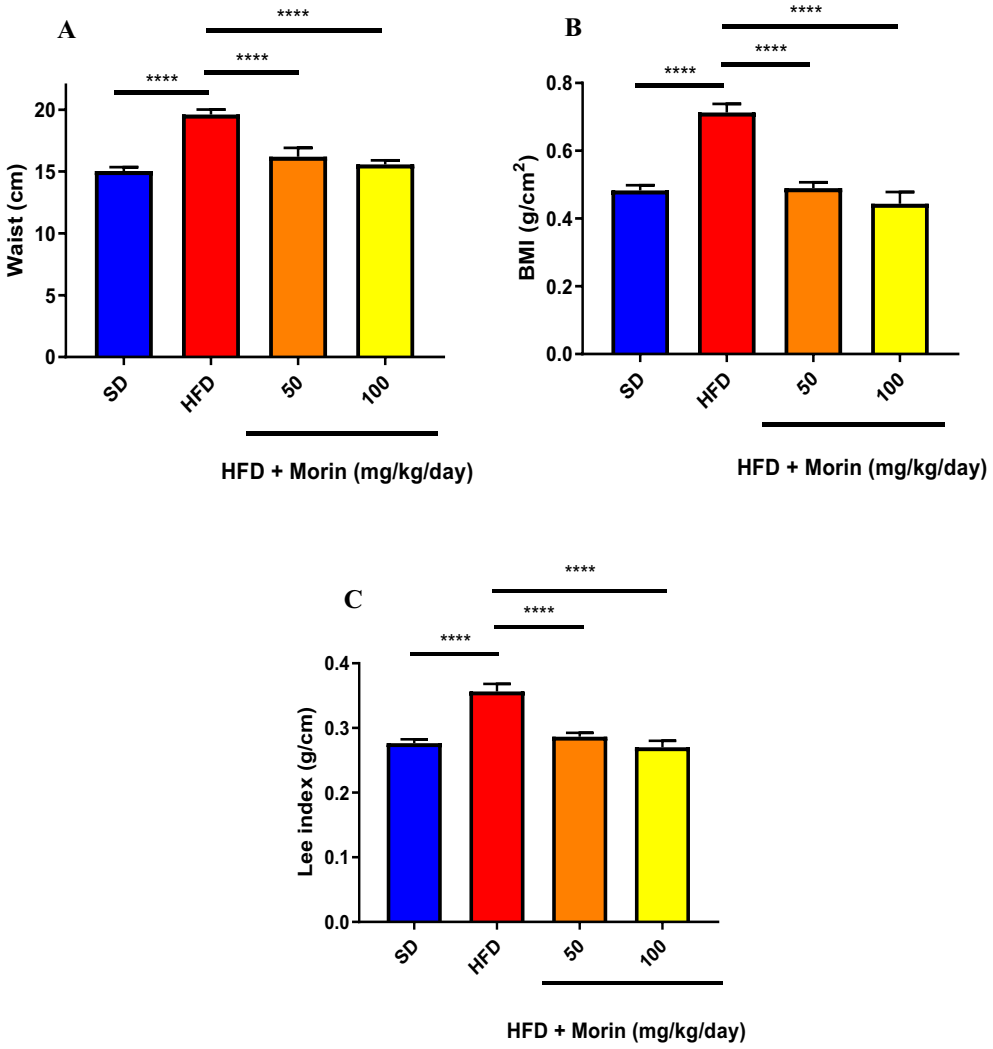

Fig. 4. Effect of Morin on (A) waist, (B) BMI and (C) Lee index. Values represented by the mean ± S.E.M (n = 6), $^{****}P \leq 0.0001$ represents comparison of HFD with SD and treated groups (Morin; 50 and 100 mg/kg/day) with HFD group.

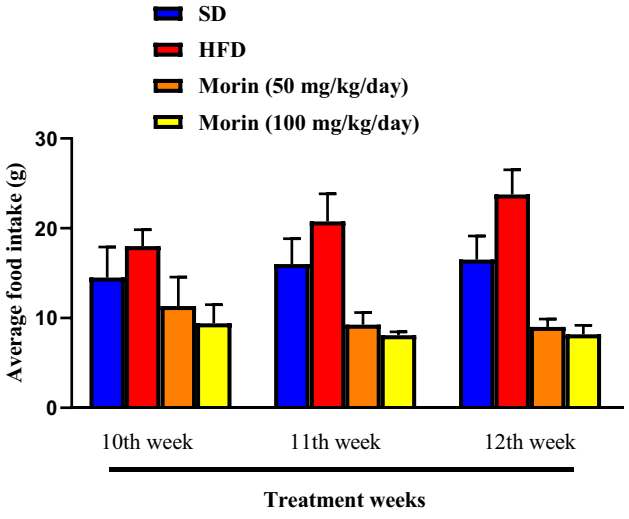

Fig. 5. Effect of morin on feed intake.

mortality. It is confirmed and reported in earlier studies that obesity is associated with cardiovascular disorders, even though there is no effective treatment available for obesity and related vascular diseases. Therefore, the present study was aimed to investigate the medicinal use of morin in obesity-induced dyslipidemia and vascular endothelial dysfunction.

The induction of obesity in animals by feeding with HFD has been reported in several studies and considered as the most popular and reliable model for studying obesity because the usual route of obesity episodes in human are pronounced similar (Buettner et al., 2007).

In this present study, the medicinal use of morin was investigated at two doses of 50 and 100 mg/kg/day for three weeks against HFD-induced dyslipidemia and related disorders such as vascular endothelial dysfunction. HFD used in this study induced significant differences in adiposity as compared with SD groups, confirming our experimental model. Previous studies have shown and confirmed that excess fat intake is associated with increased body weight, which can further lead to a risk of obesity and several other metabolic disorders related complications (Costa et al., 2011). This study corroborated with the previous findings and confirmed that rats fed with HFD for 12 weeks cause a significant increase of body weight, LW/BW, waist, Lee index, BMI, and food intake when compared with SD only fed rats. Whereas, morin treatment at both doses for three weeks in HFD-induced obese rats produced a significant decrease in body weight, LW/BW, waist, Lee

Table 2
Effect of morin 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 + Morin 50	254.84 ± 8.83***	68.52 ± 6.28 ns	53.38 ± 2.80 ns	13.70 ± 1.25 ns	187.75 ± 5.95***
HFD + Morin 100	215.05 ± 10.54***	61.80 ± 4.45*	62.76 ± 0.87 ns	12.36 ± 0.89*	139.93 ± 11.64***

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).

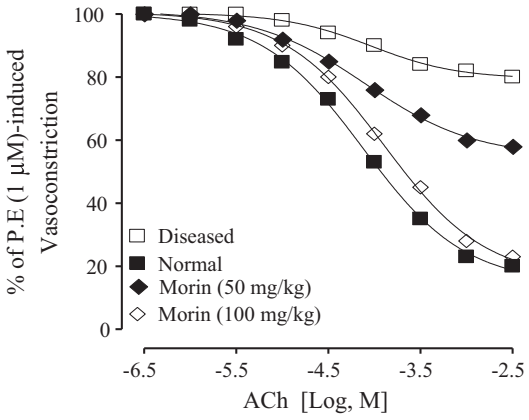

Fig. 6. Effect of Morin on vascular endothelium. ■ Normal (SD), □ Diseased (HFD),◆ HFD + Morin (low dose), ◇ HFD + Morin (high dose). Values are presented as mean ± S.E.M. (n = 6).

index, BMI, and also decreased the amount of food consumed daily by rats when compared with HFD group. Therefore, it evidenced the weight-reducing potential of morin. The administration of morin to the HFD-fed rats at both the doses significantly suppresses the increased body weight accompanied by the significant decreases in average food intake comparative to rats fed with SD.

It has been reported earlier that HFD-induced dyslipidemia resulted in a significant increase in serum TC, TG, LDL, and decreased HDL levels by improving the intestinal absorption and secretion, and decreasing cholesterol metabolism (Mariee et al., 2012). In the present study also, HFD treatment resulted in a significant increase of TC, TG, VLDL, and LDL levels while decreased HDL levels whereas, rats treated with morin at both tested doses significantly decreased the TC, TG, VLDL, and LDL levels while increased HDL levels similar to animals fed with SD. Morin administration significantly improves the lipid profile in HFD treated rats, as earlier Koshy and Vijayalakshmi (2001) reported that flavonoids exerted hypolipidaemic activity in rats. It has been reported earlier that flavonoids lower the LDL levels and increases the HDL concen-

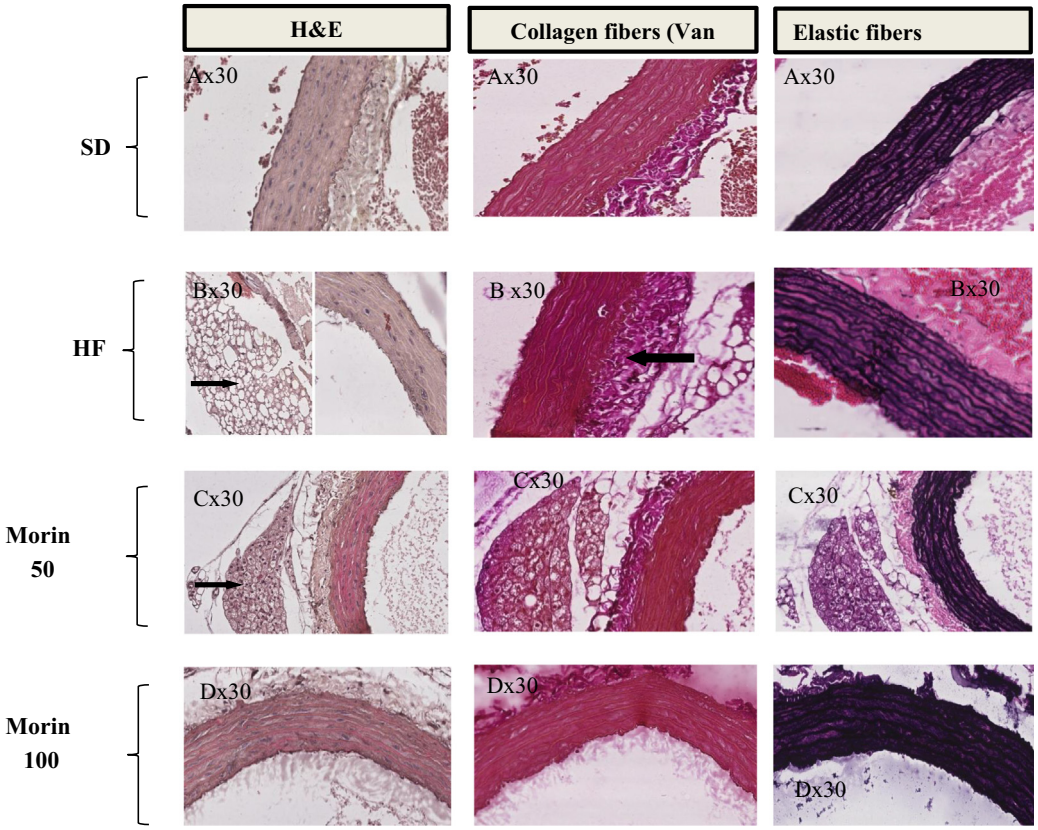

Fig. 7. Effect of Morin on histology of isolated rat aorta. Histological sections of the aortae of all animals were stained with hematoxylin and eosin (H & E) to study their general structures, whereas to investigate collagen and elastic fibers sections, van Gieson and Verhöeff's stains, respectively, were used (x30).

trations in hypercholesteremic animals (Daniel et a., 2003). HFD also resulted in the induction of oxidative stress in rats and caused increased oxidation of LDL, which plays a vital role in atherosclerosis. Therefore, antioxidants are the best option in preventing cellular damage caused by oxidative stress (Vijayakumar et al., 2004). Previous literature reported that flavonoids offer further benefits against oxidative stress caused by hypercholesterolemia (Mariee et al., 2012).

Obesity is also associated with vascular endothelial dysfunction probably because of several metabolic disorders, including atherosclerosis, hypertension, hyperglycemia, and dyslipidemia, which are accompanying vascular oxidative stress (Carvalho et al., 2015). Many scientific reports confirmed the relation of hypercholesterolemia with impaired endothelium-dependent relaxation in atherosclerotic coronary arteries and angiographically smooth coronary arteries (Hayashi et al., 1991; Seiler et al., 1993). Various vasoactive substances are synthesized and released by the endothelium to regulate peripheral vascular resistance. Also, vascular endothelial dysfunction induced by HFD has been suggested to be caused by various factors, such as increased blood pressure, increased serum triglyceride levels, the overproduction of oxidants and insufficiency of antioxidant systems (Vanhoutte and Boulanger, 1995; Akpaffiong and Taylor, 1998; Bartus et al., 2005). In the present study, HFD-induced dyslipidemic rats, treated with morin dose-dependently reversed the endothelial dysfunction (confirmed by increased Acetylcholine-induced vasorelaxation) comparable to SD group, whereas HFD rat's aorta did not show significant vasodilatation. This shows that morin treatment protected vascular endothelium from the harmful effects of HFD. Endothelial dysfunction provides a reasonable explanation of pathophysiologic mechanisms for the various harmful risk factors on coronary artery disease.

Obesity can also lead to blood vessel disorders through the changes in vascular histology. In this study, the aorta of HFD fed rats showed the presence of fat accumulation in the form of adipocytes, weakness of elastic fibers layers, increased elastic collagen ratio indicated by increased collagen layer compared to the aorta of SD fed rats. Previous literature reported that increased body mass index is usually accompanying by hardening and thickening of the arterial wall (Martínez-Martínez et al., 2014). These alterations found in this study corroborates with the previous findings as a significant predictors of increased cardiovascular mortality. However, morin treatment at the dose of 100 mg/kg/day significantly restores the integrity of aorta and resulted in better improvement as showed by the almost normal architecture of aorta regarding all aspects of general features, elastic collagen ratio, and elastic fibers status. These findings suggest that treatment with morin restores vascular structural and functional integrity and histopathological alterations in obesity and other pathological conditions.

5. Conclusion

Antidyslipidemic and endothelial protecting effects of morin were found dose-dependent, as evident by improvement in the lipid profile and vascular endothelial function. Thus, this present study validates the medicinal use of morin in dyslipidemia and related cardiovascular disorders. However, there is still a need for further detailed studies to explore its mechanistic approach that helps in obesity and related disorders management.

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Conflict of interest

Authors declare that they have no conflict of Interest.

References

Ahmad, B., Rehman, M.U., Amin, I., Mir, M.U.R., Ahmad, S.B., Farooq, A., Muzamil, S., Hussain, I., Masoodi, M., Fatima, B., 2018. Zingerone (4-(4-hydroxy-3-methylphenyl) butan-2-one) protects against alloxan-induced diabetes via alleviation of oxidative stress and inflammation: probable role of NF-kB activation. Saudi Pharmaceutical Journal 26, 1137–1145. https://doi.org/10.1016/j.jsps.2018.07.001.

Akpaffiong, M., Taylor, A., 1998. Antihypertensive and vasodilator actions of antioxidants in spontaneously hypertensive rats. Am. J. Hypertens. 11, 1450–1460. https://doi.org/10.1016/s0895-7061(98)00183-6.

Al-Nozha, M.M., Al-Mazrou, Y.Y., Al-Maatouq, M.A., Arafah, M.R., Khalil, M.Z., Khan, N.B., Al-Marzouki, K., Abdullah, M.A., Al-Khadra, A.H., Al-Harthi, S.S., Al-Shahid, M.S., 2005. Obesity in Saudi Arabia. Saudi Med J 26 (5), 824–829.

Al-Nozha, M.M., Al-Hazzaa, H.M., Arafah, M.R., Al-Khadra, A., Al-Mazrou, Y.Y., Al-Maatouq, M.A., Khan, N.B., Al-Marzouki, K., Al-Harthi, S.S., Abdullah, M., Al-Shahid, M.S., 2007. Prevalence of physical activity and inactivity among Saudis aged 30-70 years. A population-based cross-sectional study. Saudi Med. J. 28 (4), 559–568.

Ansari, M.N., Bhandari, U., 2008. Antihyperhomocysteinemic Activity of an Ethanol Extract fromEmbelia ribes. in Albino Rats. Pharm. Biol. 46, 283–287. https://doi.org/10.1080/13880200701741146.

Bartus, M., Lomnicka, M., Lorkowska, B., Franczyk, M., Kostogrys, R.B., Pisulewski, P. M., Chlopicki, S., 2005. Hypertriglyceridemia but not hypercholesterolemia induces endothelial dysfunction in the rat. Pharmacol Rep 57 (Suppl), 127–137.

Berg, A.H., Scherer, P.E., 2005. Adipose tissue, inflammation, and cardiovascular disease. Circ Res 96, 939–949. https://doi.org/10.1161/01.RES.0000163635.62927.34.

Bernardis, L.L., 1970. Prediction of carcass fat, water and lean body mass from Lees 'nutritive ratio' in rats with hypothalamic obesity. Experientia 26, 789–790. https://doi.org/10.1007/bf02232553.

Bigornia, S.J., Mott, M.M., Hess, D.T., Apovian, C.M., Mcdonnell, M.E., Duess, M.-A., Kluge, M.A., Fiscale, A.J., Vita, J.A., Gokce, N., 2010. Long-term Successful Weight Loss Improves Vascular Endothelial Function in Severely Obese Individuals. Obesity 18, 754–759. https://doi.org/10.1038/oby.2009.482.

Bray, G.A., 2004. Medical consequence of obesity. J Clin Endocrinol Metab 89, 2583–2589. https://doi.org/10.1210/jc.2004-0535.

Buettner, R., Schölmerich, J., Bollheimer, L.C., 2007. High-fat Diets: Modeling the Metabolic Disorders of Human Obesity in Rodents*. Obesity 15, 798–808. https://doi.org/10.1038/oby.2007.608.

Canalli, A.A., Franco-Penteado, C.F., Traina, F., Saad, S.T., Costa, F.F., Conran, N., 2007. Role for cAMP-protein kinase A signalling in augmented neutrophil adhesion and chemotaxis in sickle cell disease. Eur. J. Haematol. 79, 330–337. https://doi.org/10.1111/j.1600-0609.2007.00926.x.

Carvalho, K.M., Marinho Filho, J.D., de Melo, T.S., Araújo, A.J., Quetz Jda, S., da Cunha, Mdo P, et al., 2015. The resin from protium heptaphyllum prevents high-fat diet-induced obesity in mice: scientific evidence and potential mechanisms. Evid Based Complement Alternat Med 2015, 106157. https://doi.org/10.1155/2015/106157.

Caselli, A., Cirri, P., Santi, A., Paoli, P., 2016. Morin: A Promising Natural Drug. Curr. Med. Chem. 23, 774–791. https://doi.org/10.2174/0929867323666160106150821.

Cho, Y.-M., Onodera, H., Ueda, M., Imai, T., Hirose, M., 2006. A 13-week subchronic toxicity study of dietary administered morin in F344 rats. Food Chem. Toxicol. 44, 891–897. https://doi.org/10.1016/j.fct.2005.12.002.

Costa, R.R., Villela, N.R., Souza, M.D.G.C., Boa, B.C., Cyrino, F.Z., Silva, S.V., Lisboa, P.C., Moura, E.G., Barja-Fidalgo, T.C., Bouskela, E., 2011. High fat diet induces central obesity, insulin resistance and microvascular dysfunction in hamsters. Microvasc. Res. 82, 416–422. https://doi.org/10.1016/j.mvr.2011.08.007.

Daniel, R.S., Devi, K.S., Augusti, K.T., Sudhakaran Nair, C.R., 2003. Mechanism of action of antiatherogenic and related effects of Ficus bengalensis Linn. flavonoids in experimental animals. Indian J Exp Biol 41 (4), 296–303.

Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., Mukherjee, C., Shi, Y., Gélinas, C., Fan, Y., Nelson, D.A., Jin, S., White, E., 2006. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell 10, 51–64. https://doi.org/10.1016/j.ccr.2006.06.001.

Diniz, Y.S., Faine, L.A., Galhardi, C.M., Rodrigues, H.G., Ebaid, G.X., Burneiko, R.C., Cicogna, A.C., Novelli, E.L., 2005. Monosodium glutamate in standard and high-fiber diets: metabolic syndrome and oxidative stress in rats. Nutrition 21, 749–755. https://doi.org/10.1016/j.nut.2004.10.013.

Epstein, F.H., Vane, J.R., Änggård, E.E., Botting, R.M., 1990. Regulatory Functions of the Vascular Endothelium. N. Engl. J. Med. 323, 27–36. https://doi.org/10.1056/nejm199007053230106.

Furchgott, R.F., Zawadzki, J.V., 1980. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. Nature 288, 373–376. https://doi.org/10.1038/288373a0.

Hayashi, T., Ishikawa, T., Naito, M., Kuzuya, M., Funaki, C., Asai, K., Hidaka, H., Kuzuya, F., 1991. Low level hyperlipidemia impairs endothelium-dependent

relaxation of porcine coronary arteries by two mechanisms. Functional change in endothelium and impairment of endothelium-dependent relaxation by two mediators. Atherosclerosis 87, 23–38. https://doi.org/10.1016/0021-9150(91)90229-v.

Heitzer, T., Schlinzig, T., Krohn, K., Meinertz, T., Thomas, Münzel, 2001. Endothelial Dysfunction, Oxidative Stress, and Risk of Cardiovascular Events in Patients With Coronary Artery Disease. Circulation 104, 2673–2678. https://doi.org/10.1161/hc4601.099485.

Jakobsdottir, G., Xu, J., Molin, G., Ahrné, S., Nyman, M., 2013. High-Fat Diet Reduces the Formation of Butyrate, but Increases Succinate, Inflammation, Liver Fat and Cholesterol in Rats, while Dietary Fibre Counteracts These Effects. PLoS ONE 8. https://doi.org/10.1371/journal.pone.0080476.

Jung, H.-J., Kim, S.-J., Song, Y.-S., Park, E.-H., Lim, C.-J., 2009. Evaluation of the Antiangiogenic, Anti-inflammatory, and Antinociceptive Activities of Morin. Planta Med. 76, 273–275. https://doi.org/10.1055/s-0029-1186079.

Kapoor, R., Kakkar, P., 2012. Protective Role of Morin, a Flavonoid, against High Glucose Induced Oxidative Stress Mediated Apoptosis in Primary Rat Hepatocytes. PLoS ONE 7. https://doi.org/10.1371/journal.pone.0041663.

Kobayasi, R., Akamine, E.H., Davel, A.P., Rodrigues, M.A., Carvalho, C.R., Rossoni, L.V., 2010. Oxidative stress and inflammatory mediators contribute to endothelial dysfunction in high-fat diet-induced obesity in mice. J. Hypertens. 28, 2111–2119. https://doi.org/10.1097/hjh.0b013e32833ca68c.

Koshy, A.S., Vijayalakshmi, N.R., 2001. Impact of certain flavonoids on lipid profiles potential action of Garcinia cambogia flavonoids. Phytother. Res. 15, 395–400. https://doi.org/10.1002/ptr.725.

Liang, Z., Liu, F., Grundke-Iqbal, I., Iqbal, K., Gong, C.-X., 2007. Down-regulation of cAMP-dependent protein kinase by over-activated calpain in Alzheimer disease brain. J. Neurochem. 103, 2462–2470. https://doi.org/10.1111/j.1471-4159.2007.04942.x.

Luscher, T.F., 1990. Imbalance of Endothelium-derived Relaxing and Contracting Factors: A New Concept in Hypertension?. Am. J. Hypertens. 3, 317–330. https://doi.org/10.1093/ajh/3.4.317.

Mariee, A.D., Abd-Allah, G.M., El-Beshbishy, H.A., 2012. Protective effect of dietary flavonoid quercetin against lipemic-oxidative hepatic injury in hypercholesterolemic rats. Pharm. Biol. 50, 1019–1025. https://doi.org/10.3109/13880209.2012.655424.

Martínez-Martínez, E., Miana, M., Jurado-López, R., Bartolomé, M.V., Neto, F.V.S., Salaices, M., López-Andrés, N., Cachofeiro, V., 2014. The potential role of leptin in the vascular remodeling associated with obesity. International Journal of Obesity 38, 1565–1572. https://doi.org/10.1038/ijo.2014.37.

Mizushima, N., Ohsumi, Y., Yoshimori, T., 2002. Autophagosome Formation in Mammalian Cells. Cell Struct. Funct. 27, 421–429. https://doi.org/10.1247/csf.27.421.

Naowaboot, J., Wannasiri, S., Pannangpetch, P., 2016. Morin attenuates hepatic insulin resistance in high-fat-diet-induced obese mice. J. Physiol. Biochem. 72, 269–280. https://doi.org/10.1007/s13105-016-0477-5.

Napoli, C., Martin-Padura, I., Nigris, F.D., Giorgio, M., Mansueto, G., Somma, P., Condorelli, M., Sica, G., Rosa, G.D., Pelicci, P., 2003. Deletion of the p66Shc longevity gene reduces systemic and tissue oxidative stress, vascular cell apoptosis, and early atherogenesis in mice fed a high-fat diet. Proc. Natl. Acad. Sci. 100, 2112–2116. https://doi.org/10.1073/pnas.0336359100.

Paoli, P., Cirri, P., Caselli, A., Ranaldi, F., Bruschi, G., Santi, A., Camici, G., 2013. The insulin-mimetic effect of Morin: A promising molecule in diabetes treatment. Biochimica et. Biophysica Acta (BBA) - General Subjects 1830, 3102–3111. https://doi.org/10.1016/j.bbagen.2013.01.017.

Perticone, F., Ceravolo, R., Candigliota, M., Ventura, G., Iacopino, S., Sinopoli, F., Mattioli, P.L., 2001. Obesity and Body Fat Distribution Induce Endothelial Dysfunction by Oxidative Stress: Protective Effect of Vitamin C. Diabetes 50, 159–165. https://doi.org/10.2337/diabetes.50.1.159.

Prahalathan, P., Kumar, S., Raja, B., 2012. Morin attenuates blood pressure and oxidative stress in deoxycorticosterone acetate-salt hypertensive rats: A biochemical and histopathological evaluation. Metabolism 61, 1087–1099. https://doi.org/10.1016/j.metabol.2011.12.012.

Rehman, M.U., Tahir, M., Khan, A.Q., Khan, R., Lateef, A., Oday-O-Hamiza, Qamar, W., Ali, F., Sultana, S., 2013. Chrysin suppresses renal carcinogenesis via amelioration of hyperproliferation, oxidative stress and inflammation: plausible role of NF-κB. Toxicology Letters 216, 146–158. https://doi.org/10.1016/j.toxlet.2012.11.013.

Rehman, M.U., Rashid, S.M., Rasool, S., Shakeel, S., Ahmad, B., Ahmad, S.B., Madkhali, H., Ganaie, M.A., Majid, S., Bhat, S.A., 2018. Zingerone (4-(4-hydroxy-3-methylphenyl)butan-2-one) ameliorates renal function via controlling oxidative burst and inflammation in experimental diabetic nephropathy. Arch. Physiol. Biochem. 125, 201–209. https://doi.org/10.1080/13813455.2018.1448422.

Ross, R., 1999. Atherosclerosis—an inflammatory disease. N Engl J Med. 340, 115–126. https://doi.org/10.1056/NEJM199901143400207.

Seiler, C., Hess, O.M., Buechi, M., Suter, T.M., Krayenbuehl, H.P., 1993. Influence of serum cholesterol and other coronary risk factors on vasomotion of angiographically normal coronary arteries. Circulation 88, 2139–2148. https://doi.org/10.1161/01.cir.88.5.2139.

Sergedien≐, E., Jönsson, K., Szymusiak, H., Tyrakowska, B., Rietjens, I.M., Č≐Nas, N., 1999. Prooxidant toxicity of polyphenolic antioxidants to HL-60 cells: description of quantitative structure-activity relationships. FEBS Letters 462, 392–396. doi:10.1016/s0014-5793(99)01561-6

Steinberg, H.O., Chaker, H., Leaming, R., Johnson, A., Brechtel, G., Baron, A.D., 1996. Obesity/insulin resistance is associated with endothelial dysfunction. Implications for the syndrome of insulin resistance. Journal of Clinical Investigation 97, 2601–2610. https://doi.org/10.1172/jci118709.

Summanen, J., Vuorela, P., Rauha, J.-P., Tammela, P., Marjamäki, K., Pasternack, M., Törnquist, K., Vuorela, H., 2001. Effects of simple aromatic compounds and flavonoids on Ca2 fluxes in rat pituitary GH4C1 cells. Eur. J. Pharmacol. 414, 125–133. https://doi.org/10.1016/s0014-2999(01)00774-9.

Taguchi, K., Hida, M., Hasegawa, M., Matsumoto, T., Kobayashi, T., 2015. Dietary polyphenol morin rescues endothelial dysfunction in a diabetic mouse model by activating the Akt/eNOS pathway. Mol. Nutr. Food Res. 60, 580–588. https://doi.org/10.1002/mnfr.201500618.

Tian, W.-X., 2006. Inhibition of Fatty Acid Synthase by Polyphenols. Curr. Med. Chem. 13, 967–977. https://doi.org/10.2174/092986706776361012.

Ting, H.H., Timimi, F.K., Haley, E.A., Roddy, M.-A., Ganz, P., Creager, M.A., 1997. Vitamin C Improves Endothelium-Dependent Vasodilation in Forearm Resistance Vessels of Humans With Hypercholesterolemia. Circulation 95, 2617–2622. https://doi.org/10.1161/01.cir.95.12.2617.

Vallance, P., Collier, J., Moncada, S., 1989. Effects Of Endothelium-Derived Nitric Oxide On Peripheral Arteriolar Tone In Man. The Lancet 334, 997–1000. https://doi.org/10.1016/s0140-6736(89)91013-1.

Vanhoutte, P.M., Boulanger, C.M., 1995. Endothelium-Dependent Responses in Hypertension. Hypertens. Res. 18, 87–98. https://doi.org/10.1291/hypres.18.87.

Vanitha, P., Uma, C., Suganya, N., Bhakkiyalakshmi, E., Suriyanarayanan, S., Gunasekaran, P., Sivasubramanian, S., Ramkumar, K., 2014. Modulatory effects of morin on hyperglycemia by attenuating the hepatic key enzymes of carbohydrate metabolism and β-cell function in streptozotocin-induced diabetic rats. Environ. Toxicol. Pharmacol. 37, 326–335. https://doi.org/10.1016/j.etap.2013.11.017.

Vijayakumar, R., Surya, D., Nalini, N., 2004. Antioxidant efficacy of black pepper (Piper nigrumL.) and piperine in rats with high fat diet induced oxidative stress. Redox Rep. 9, 105–110. https://doi.org/10.1179/135100004225004742.

Vita, J.-A., Keaney Jr., J.-F., 2002. Endothelial function: a barometer for cardiovascular risk?. Circulation 6 (106), 640–642. https://doi.org/10.1161/01.cir.0000028581.07992.56.

White, E., Karp, C., Strohecker, A.M., Guo, Y., et al., 2010. Role of autophagy in suppression of inflammation and cancer. Curr. Opin. Cell Biol. 22, 212–217. https://doi.org/10.1016/j.ceb.2009.12.008.

Wu, T.-W., Zeng, L., Wu, J., Fung, K., 1993. Morin hydrate is a plant-derived and antioxidant-based hepatoprotector. Life Sci. 53. https://doi.org/10.1016/0024-3205(93)90266-6.

Xie, M.-X., Long, M., Liu, Y., Qin, C., Wang, Y.-D., 2006. Characterization of the interaction between human serum albumin and morin. Biochimica et Biophysica Acta (BBA) - General Subjects 1760, 1184–1191. https://doi.org/10.1016/j.bbagen.2006.03.026.

Yang, N., Ying, C., Xu, M., Zuo, X., Ye, X., Liu, L., Nara, Y., Sun, X., 2007. High-fat diet up-regulates caveolin-1 expression in aorta of diet-induced obese but not in diet-resistant rats. Cardiovasc. Res. 76, 167–174. https://doi.org/10.1016/j.cardiores.2007.05.028.