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ORIGINAL ARTICLE

Embelin from *Embelia ribes* Ameliorates Oxidative Stress and Inflammation in High-Fat Diet-Fed Obese C57BL/6 Mice

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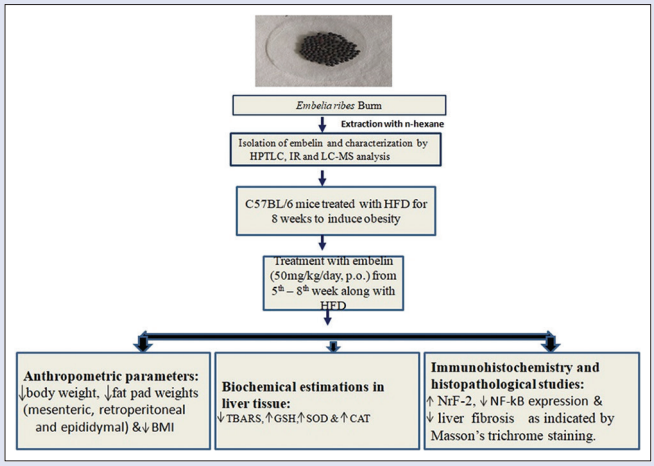
ABSTRACT

Background: A safe, efficacious, and economical drug for the treatment of obesity is the need of the time. Literature published in previous years referred to embelin as a potential investigational therapeutic agent to manage obesity. **Objectives:** This study was designed to isolate and characterize embelin from *Embelia ribes* and further to assess its role in alleviating oxidative stress and chronic inflammation in the high-fat diet (HFD) fed obese C57BL/6 mice. **Materials and Methods:** Embelin extracted from berries of *E. ribes* with n-hexane by soxhlet extraction was characterized using high-performance thin-layer chromatography, infrared and liquid chromatography-mass spectrometry analyses. The obesity was induced by feeding of HFD for 8 weeks. Embelin (50 mg/kg/day, p.o.) was administered from 5th to 8th weeks along with HFD. After 8 weeks, the body weight gain and body mass index were calculated. Then, animals were sacrificed; serum and tissues were collected to further assess the various biomarkers of oxidative stress and inflammation. **Results:** The presence of embelin was confirmed using the above-mentioned analytical techniques. Treatment with Embelin showed amelioration of obesity biomarkers along with the substantial decline in levels of protein expression of nuclear factor erythroid 2-related factor and nuclear factor kappa-B in liver tissue. Treatment with embelin also normalizes the liver tissue levels of thiobarbituric acid reactive substances, glutathione, superoxide dismutase, and catalase. The histopathological analysis of liver tissue showed significant prevention of necrotic and inflammatory changes in embelin treated HFD fed mice. **Conclusion:** The results of the study clearly indicated the potential of embelin in ameliorating obesity by alleviating oxidative stress and inflammation induced by HFD in C57BL/6 mice.

Key words: Embelin, high-fat diet, nuclear factor erythroid 2-related factor, nuclear factor kappa-B, obesity, oxidative stress

SUMMARY

- *Embelia ribes* Burm. (family: *Myrsinaceae*) which conventionally used to treat inflammatory diseases such as rheumatism and fever. Vidangadya churna (powder of vidanga), an ayurvedic formulation, contains vidanga as the main ingredient. It is frequently taken with honey to alleviate obesity, but the mechanism of action is still unexplored. Embelin, a major component of *E. ribes* Burm. reportedly has a wide spectrum of pharmacological activities such as anti-inflammatory, antibacterial, antihyperlipidemic and antioxidant. In the present study, high-fat diet-induced obesity through elevation in oxidative stress, and the inflammatory pathway was investigated in C57BL/6 mice. Embelin proved to be effective in reducing obesity through a decrease in oxidative stress and inflammation.



Abbreviations used: CAT: Catalase; GSH: Glutathione; HFD: High-fat diet; HPTLC: High performance thin layer chromatography; IHC: Immunohistochemistry; IR: Infrared; NF-κB: Nuclear factor kappa-B; KBR: Potassium bromide; NrF-2: Nuclear factor erythroid 2-related factor; LC-MS: Liquid chromatography-mass spectrometry; ROS: Reactive oxygen species; SOD: Superoxide dismutase; TBARS: Thiobarbituric acid reactive substances.

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INTRODUCTION

Obesity causes 2.8 million adult deaths each year which made obesity as the fifth major cause of mortality globally.[1] It contributes to the various secondary manifestations such as type 2 diabetes mellitus, nonalcoholic liver disease, kidney, cardiovascular diseases, and cancers.[2,3] It usually started with the imbalance between energy expenditure and storage, which later initiate the excessive or abnormal fat accumulation and generation of adipogenesis.[1] Adipogenesis is a process of conversion of preadipocyte into adipocyte, which plays a key role in fat mass formation and the pathogenesis of obesity.[4,5] Literature search showed that inflammation and oxidative stress are interlinked, and they may contribute to the metabolic syndrome such as obesity and diabetes.[6,7] Moreover, oxidative stress has a major role in obesity-related problems like increasing the production of cellular reactive oxygen species (ROS) and increased lipid peroxidation.[8] The liver is a key site of ROS damage and majorly involved in the oxidation

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of fatty acids, which induces inflammation.[7,9,10] In addition, nuclear factor erythroid 2-related factor (NrF-2) has a role in the regulation of both inflammation and oxidative stress.[11] In the liver, constitutive activation of NrF-2 causes restoration of the expression of genes involved in various obesity biomarkers, thereby alleviating obesity and other metabolic diseases.[12,13] Earlier reports suggested a cross-link between NrF-2 and nuclear factor kappa-B (NF-kB) pathways. The absence of NrF-2 causes an increase in the oxidative stress and further leads to the progression in inflammation via an increase in the NF-kB activation.[14] NF-kB signaling controls the release of proinflammatory cytokines and subsequent oxidative stress, which are involved in the pathogenesis of obesity.[15] Hence, the modulation in the cross-link between NrF-2 and NF-kB might be an efficacious therapeutic approach for the management of obesity.

Numerous molecules are marketed as weight loss agents, but the majority of drugs have been withdrawn due to safety issues. Currently, orlistat is the only remaining drug for long-term management of obesity, but orlistat also has some undesirable side effects such as diarrhea and abdominal pain.[16] Natural compounds from the traditional system of medicines are the potential candidate for the research as they supposedly have minimal side effects as compared to the commercially available drugs.[17] Embelin (a benzoquinone derivative), a bioactive constituent of *Embelia ribes* Burm have a wide spectrum of pharmacological activity including anti-inflammatory, antioxidant, and anti-obesity, but the molecular mechanism remains unclear.[18] Recently, Abo El-Magd *et al*. 2017 reported that NrF-2 modulation by glycyrrhizin improves the high-fat diet (HFD)-induced obesity.[19] Most of the foods which we are consuming are rich source of saturated fatty acids, and it plays a crucial role in the development of obesity. Many studies providing evidence that chronic consumption of fatty acids rich food caused inflammation, dyslipidemia, and obesity.[20,21] Thus, the HFD-induced obesity model provides a better understanding to find out a novel therapeutic target to treat obesity. In our knowledge, there is no study investigating whether embelin regulates NrF-2 and NF-kB pathways in HFD-induced obesity. Hence, the present research work was designed to investigate the effective modulatory potential of embelin on NrF-2 and NF-kB intervention in HFD-induced obesity in C57BL/6 mice.

METHODS

Plant material and isolation of embelin

E. ribes Burm was procured from the local market of Delhi, India and authenticated by Dr. Sayeed Ahmad, Associate Professor, Department of Pharmacognosy and Phytochemistry, School of Pharmaceutical Education and Research, Jamia Hamdard, India, where a Voucher Specimen has been deposited (#. BNPL/JH/Ph.D/05/2018/01). Extraction and isolation of embelin were done using the modified methodology of Suthar *et al*. 2009.[22] The *E. ribes* berries were dried in the air, coarsely powdered and stored in airtight container. Dried powder (50 gm) was extracted with n-Hexane using the Soxhlet apparatus for 6 h, and the solvent was removed by the distillation process. Further, the collected residue was washed with cold pet ether. After that, the residue was dissolved in a mixture of dichloromethane (DCM) and methanol (50 ml × 50 ml) and the resulting solution was kept for crystallization for 24 h. After crystallization, the crystals were filtered and washed first with n-hexane and followed by DCM. The embelin was obtained in golden colored crystal crystal form characterized by appearance, color, consistency and using analytical techniques like high-performance thin-layer chromatography (HPTLC), infrared (IR), and liquid chromatography–mass spectrometry (LC-MS).

High-performance thin layer chromatography

HPTLC analysis of isolated embelin and the standard compound was done according to the previously published methodology.[23,24] Chloroform: ethyl acetate: formic acid in the proportion of 5:4:1 v/v/v was used as a mobile phase. Samples of test and standard compounds were prepared in the concentration of 20 mg/ml and 1 mg/ml, respectively. Samples of test 2 µl and standard (0.2–2 µl) were applied to 6 mm wide bands on 10 × 10 cm precoated silica G 60 F_{254} TLC plate (E. Merck, 0.20 mm thickness) using Linomat-V automated applicator (CAMAG, Switzerland) with the nitrogen flow with the delivery speed of 150 nL/s. Further, the plate was developed in a presaturated glass tank at room temperature. The scanning was done at 254 nm with a Camag TLC scanner III by the winCATS 1.2.3 software (Camag, Switzerland).

Infrared (analysis

IR spectra were obtained using an IR spectrophotometer (Bruker Advance, Alpha Model). The potassium bromide (KBr) disk method was applied, little amount of drugs mix with spectroscopic KBr and compressed using a vacuum press to obtain a disk. Infrared spectra were recorded by scanning between the wavelengths of 400–4000 cm^{-1}. The obtained peaks and spectra were compared with the previous spectra of the standard and isolated embelin.[23]

Liquid chromatography-mass spectrometry analysis

Embelin (25 mg) was dissolved in 5.0 mL of LC-MS grade methanol in a volumetric flask to get 5.0 mg/mL solution. This solution was filtered and used for UPLC-MS analysis. Chromatography was performed on the monolithic capillary silica-based C_{18} column (ACQUITY UPLC (R) BEH C_{18}, 1.7 µm, 2.1 mm × 100 mm). Acetonitrile (A) and water (B) were used as the solvent system. The chromatographic separation was achieved by an isocratic elution solvent system (initially, A - 50% ACN, B - 50% water) and total run time was 16 min with the flow rate 10 µL/min at a temperature at 100°C. The capillary and cone voltages were set to 3.0 and 40 KV, respectively. Argon was employed with the pressure of 5.3 × 10-5 Torr for collision, and voltages were set to capillary (3.0) and cone (40 KV). The peak of isolated embelin was matched with PubChem CID: 3218. Data obtained from UPLC-MS were processed using Mass Lynx V 4.1 software (Waters Corporation, USA).

Chemicals and materials

Standard embelin (≥98% high-performance thin-layer chromatography) was purchased from Chembio Lifesciences, India. HFD (45% kcal fat, 20% kcal protein, and 35% kcal carbohydrates), was procured as a gift sample from Ashirwad Industries, Chandigarh, Panjab, India.

Animals

C57BL/6 mice for the experiment were procured from Central Animal House Facility of Jamia Hamdard, New Delhi, India after the approval of Institutional Animal Ethics Committee as per Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines (Registration no-173/GO/Re/s/2000/CPCSEA). Animals were kept in a controlled room temperature (22°C ± 2°C) and humidity (55% ± 5%) with a regularly followed 12-h day/12-h night cycle with food and water *ad libitum*.

Experimental protocol

Animals were randomly divided into four groups, with each group containing six animals. Animals had free access to a standard chow

diet (normal control) or HFD (toxic control and treatment groups, i.e., embelin and orlistat) for 8 weeks.

- Group I: Mice received normal chow diet for 8 weeks (normal control group)
- Group II: Mice received HFD for 8 weeks (toxic control group)
- Group III: Mice received HFD for 8 weeks + embelin (50 mg/kg/day, p.o.) from 5th week to 8th week (test drug group)
- Group IV: Mice received HFD for 8 weeks + orlistat (10 mg/kg/day, p.o.) from 5th week to 8th week (standard drug group).

The dose of embelin was based on the previous effect of embelin on body weight gain by Bhandari *et al*.[25] and Chaudhary *et al*.[26] and toxicological study of embelin by Debebe *et al*.[27]

On the 57th day, mice were sacrificed under anesthetic condition and the fat of different types were collected and weighed, and liver tissue also removed and homogenized using phosphate-buffered saline in ice water and further, centrifuged to extract the supernatant to perform the antioxidant parameters.

Biochemical estimation in liver tissue

The levels of malondialdedhyde, endogenous glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) were estimated by the previously published methods, respectively.[28-30]

Histopathological study

Isolated liver tissue was transferred into 10% formalin. It was cut into 5μm thickness and stained with Masson's trichrome dye on glass slide after then viewed under a light microscope (Meiji microscope) to confirm the fibrosis.

Immunohistochemical analysis

Immunohistochemical staining was used to observe changes in liver NrF-2 and NF-kB levels. Percentage of NrF-2 and NF-kB expression levels was quantified by measuring the brown staining of liver tissue using ImageJ software.

Statistical analysis

The statistical analysis was performed using GraphPad Prism 5.0 software (San Diego, California, USA). The body weight and body mass index (BMI) were statistically assessed using two-way analysis of variance (ANOVA) followed by Bonferroni's *post hoc* test. The significant differences among groups for all other parameters were analyzed using one-way ANOVA followed by Tukey's multiple comparisons test. Statistical significance differences are set at $P < 0.05$.

RESULTS

Isolation and characterization of embelin

The pure embelin was obtained after crystallization of n-Hexane fraction with DMSO and methanol, and % yield was found to be 0.096% w/w. The collected embelin was in yellow crystalline powder form and further identified with HPTLC, IR, and LC-MS analysis. HPTLC spectrum of isolated embelin and standard embelin showed similar Rf values – 0.60 [Figure 1] and % assay was found to be 95.22%. The IR spectrum of isolated embelin confirmed the presence of hydroxyl (3615.14 cm^{-1}) and carbonyl (1697.92 cm^{-1}) groups, which was comparable to the IR spectra shown by standard embelin.[23] The LC-MS spectrum of isolated embelin showed *m/z* ratio of 282.44 which was in accordance with the *m/z* value of standard embelin (PubChem CID: 3218).

Changes on body weight, body fat weights, body mass index, and food intake

As shown in Figure 2a that at the end of 2nd week, the gain in body weight of the mice in HFD treated group was significantly higher ($P < 0.01$) in comparison of the mice in normal control group. At the end of the study, a continuous significant rise ($P < 0.001$) in body weight was observed in HFD treated mice. Treatment of embelin (50 mg/kg/day, p.o.) for 28 days (5th–8th week) along with HFD causeda significant decrease ($P < 0.05$ on 6th week and $P < 0.001$ from 7th to 8th weeks) in body weight gain as compared to HFD treated mice. However, treatment with standard drug orlistat (10 mg/kg/day, p.o.) for 28 days (5th–8th week) showed a significant decline ($P < 0.001$) in body weight from 7th to 8th week. The body fat pad weights (mesenteric, retroperitoneal and epididymal) of HFD treated mice were significantly increased in comparison to the normal control mice. On the contrary, treatment with embelin significantly decreased the body fat pad weights in comparison to the HFD treated mice [Figure 2b]. Administration of embelin significantly decreased the BMI in HFD fed mice [Figure 2c]. There is no significant change in food intake among all groups [Figure 2d].

Effect of embelin on oxidative stress

The status of antioxidant parameters is shown in Table 1. HFD administered mice showed significant rise ($P < 0.001$) in liver thiobarbituric acid reactive substances (TBARS) and decline ($P < 0.001$) in GSH, SOD and CAT levels as compared with normal control mice. Administration of embelin (10 mg/kg/day, p.o.) and orlistat (10 mg/kg/day, p.o.) showed a similar significant decrease ($P < 0.001$) in liver TBARS and rise in GSH, SOD, and CAT levels when compared with HFD treated mice.

Effect of embelin on liver histopathology

As shown in Figure 3, Masson's trichrome staining of liver tissue of normal control mice showed normal structure with no development of fibrosis. However, HFD administration for a period of 8 weeks resulted in the development of fibrosis around the sinusoids and hepatic cells. In the treatment groups, HFD + embelin (50 mg/kg/day, p.o.) and HFD + orlistat (50 mg/kg/day, p.o.), the level of fibrosis was lower as compared with only HFD treated mice; liver structure in the mice treated HFD + embelin (50 mg/kg/day, p.o.) showed a lower reduction in the development of fibrosis as compared with orlistat. The percentage area of liver fibrosis is depicted in Figure 3.

Effect of embelin on the expression of nuclear factor erythroid 2-related factor

Results of immunohistochemical staining showed NrF-2 expression in liver cells. More positive staining of NrF-2 was observed in normal control mice, whereas the mice in the toxic group treated with HFD fed showed less NrF-2-positive staining. Treatment with embelin and orlistat in HFD treated mice showed an increase in positive staining when compared with the HFD treated group. However, there was no marked difference observed within treatment groups. The percentage change of NrF-2 positive area is depicted in Figure 4.

Effect of embelin on the expression of nuclear factor kappa-B

Results of immunohistochemical staining showed NF-kB activation in liver cells. No positive staining of NF-kB was observed in normal control

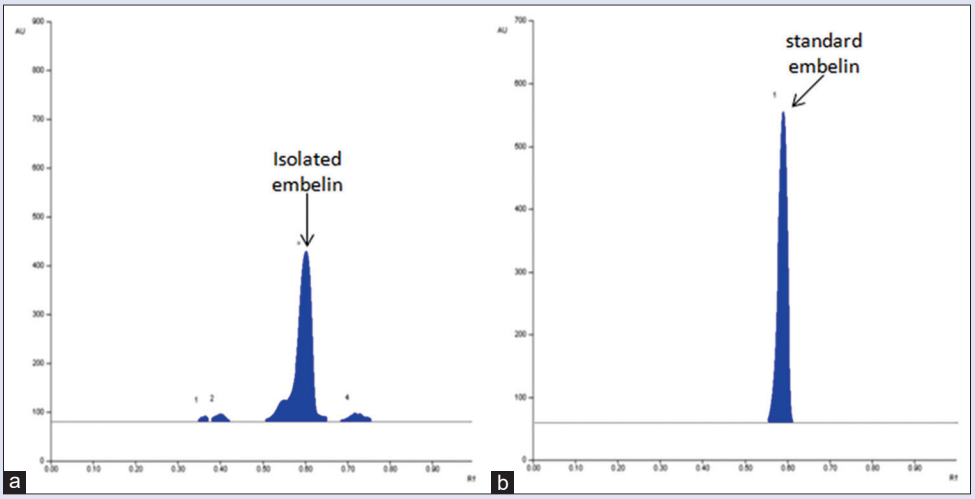

Figure 1: High performance thin layer chromatography chromatograms of isolated embelin (a) and standard embelin (b)

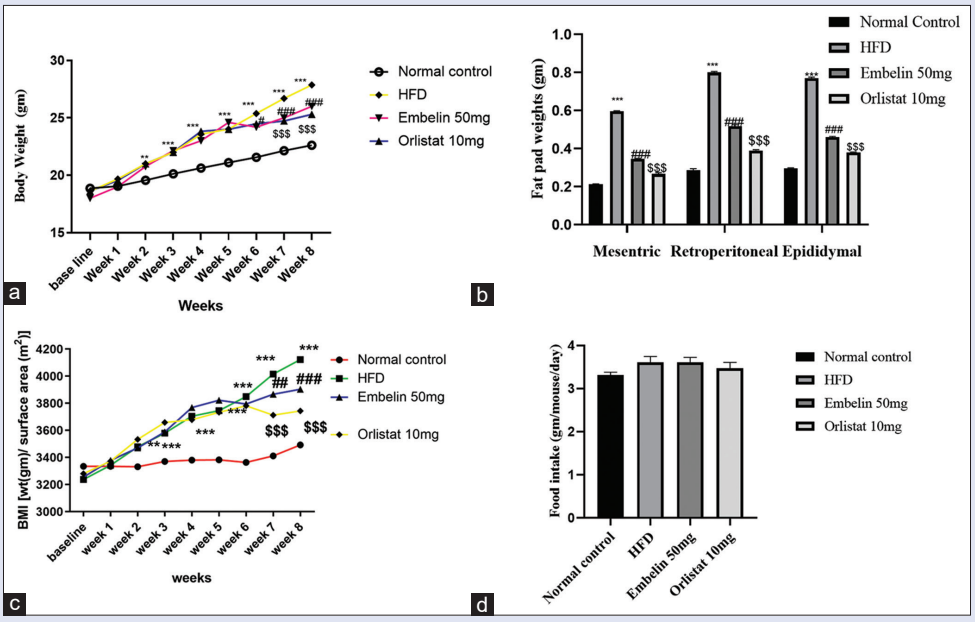

Figure 2: Effect of embelin on body weight gain, body fat weights and body mass index. (a) Body weight gain. (b) Body fat weights. (c) Body mass index. (d) Daily food intake. Data are expressed as mean ± standard error of mean ($n = 6$). ***$P < 0.001$ Normal control versus high fat diet; ###$P < 0.001$ high fat diet versus embelin; #$P < 0.05$ high fat diet versus embelin; $$$$P < 0.001$ high fat diet versus orlistat

Table 1: Effect of embelin on thiobarbituric acid reactive substance, glutathione, superoxide dismutase and catalase in liver tissue of high fat diet fed C57BL/6 mice

Groups	TBARS (nmol MDA/mg protein)	GSH (µmol of GSH/mg protein)	SOD (U/mg protein)	CAT (nmoles of H_2O_2/min/mg protein)
Normal control	0.04±0.0027	20.38±0.12	4.06±1.65	40.3±0.303
HFD	0.25±0.0162***	8.75±0.12***	2.76±1.12***	21.9±0.327***
Embelin 50 mg	0.10±0.0051###	16.22±0.05###	3.53±1.44###	32.2±0.059###
Orlistat 10 mg	0.06±0.0019†††	18.61±0.16†††	3.83±1.56†††	34.6±0.209†††

Data are expressed as mean±SEM (n=6 animals per group). Significance difference was determined by one-way ANOVA followed by Tukey's multiple comparison test. ***P<0.001 HFD versus normal control; ###P<0.001 embelin 50 mg versus HFD; †††P<0.001 orlistat 10 mg versus HFD. TBARS: Thiobarbituric acid reactive substance; GSH: Glutathione; SOD: Superoxide dismutase; CAT: Catalase; HFD: High fat diet, SEM: Standard error of mean

mice, whereas the mice in the toxic group treated with HFD fed showed more NF-kB positive staining. Treatment with embelin and orlistat in HFD treated mice showed less positive staining as compared with HFD treated group. The percentage of NF-kB positive area is depicted in Figure 5.

DISCUSSION

Obesity is a major health problem worldwide, causing many adverse metabolic disorders such as type 2 diabetes mellitus, dyslipidemia, and cancers.[31] It attributes to the imbalance between energy intake,

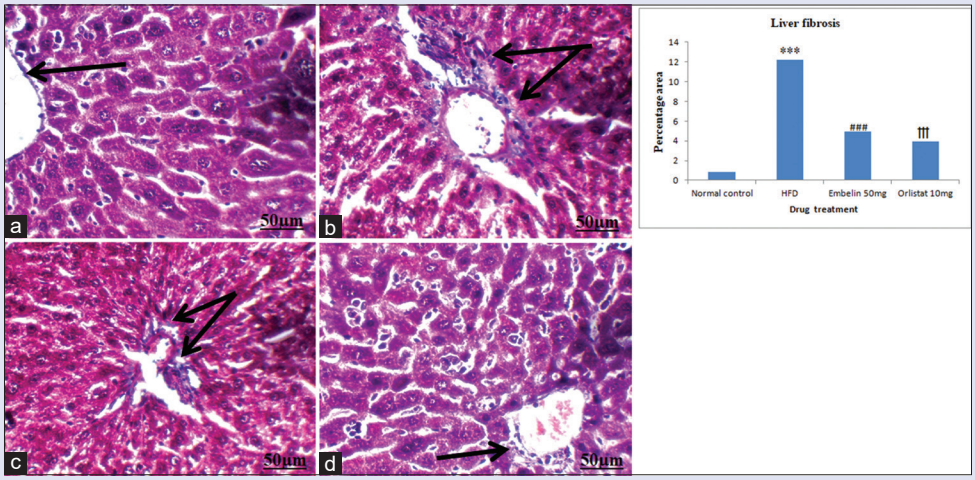

Figure 3: Photomicrographs of liver tissue stained with Masson's trichrome. (a) Mice treated with normal chow diet showing normal liver cells. (b) Mice treated with high fat diet for a period of 8 weeks showing blue colour around the portal area. (c) Treatment of embelin (50 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice showing less colourization around the portal area. (d) Treatment of orlistat (10 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice showing more less colourization around the portal area as compare to embelin. Data are expressed as mean ± standard error of mean ($n = 6$ animals per group). Significance difference was determined by one-way analysis of variance followed by Tukey's multiple comparison test. ***$P < 0.001$ high fat diet versus normal control; ###$P < 0.001$ embelin 50 mg versus high fat diet; †††$P < 0.001$ orlistat 10 mg versus high fat diet

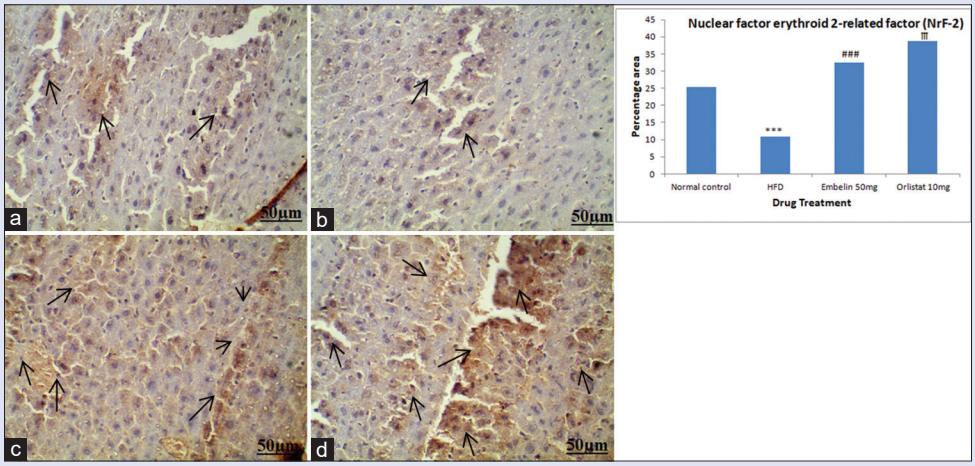

Figure 4: Photomicrograph of immunohistochemical staining revealing NrF-2 activation in liver cells. (a) Mice treated with normal chow diet showing normal liver cells. (b) Mice treated with high fat diet for a period of 8 weeks. (c) Treatment of embelin (50 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice. (d) Treatment of orlistat (10 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice. Black arrows are showing NrF-2 activation. Data are expressed as mean ± standard error of mean ($n = 6$ animals per group). Significance difference was determined by one-way analysis of variance followed by Tukey's multiple comparison test. ***$P < 0.001$ high fat diet versus normal control; ###$P < 0.001$ embelin 50 mg versus high fat diet; †††$P < 0.001$ orlistat 10 mg versus high fat diet

energy expenditure, and consequent excessive fat deposition in the body. Previous studies suggested a valid role of HFD in the induction of obesity.[1]

In the present study, embelin was isolated using the methodology reported by Suthar *et al.* in 2009.[22] After isolation and purification, a yellow colored crystals of embelin was obtained. The characterization of isolated embelin was done by HPTLC, IR Spectra, and LC-MS analysis.

The isolated embelin was further evaluated for its antioxidant potential and its ability to modulate NF-kB and Nrf-2 expression in *in vivo* study. HFD administration caused a significant increase in body weight gain, fat pad weights, BMI with nonsignificant changes in the food intake. Thus, these pathological changes were mainly occurred due to high fat consumption and calories intake but unrelated to the food intake. Hence, the results of our study were in consistence with the findings of the previous research work.[32] Induction of obesity is confirmed by a significant increase in body weight gain, fat pad weights, and BMI with HFD intake. Our literature survey showed a crucial link between modulation of NF-κB, Nrf-2 signaling pathways and obesity. Therefore, it is an interesting way to explore the drug that have the potential to modulate the crosslink between these signaling pathways. It has been well established that oxidative stress plays a critical role in the initiation and progression of obesity. The liver is a site which majorly involves in the free fatty acid (FFA) oxidation and also a potential site of oxidative stress imbalance. According to previous research, oxidative stress plays an important role in HFD-induced obesity. Increase in oxidative stress elevates liver lipid deposition, which further increases the FFAs oxidation and Krebs cycle rate. Furthermore, increased FFA β-oxidation leads to a rise in electron leakage rates from the mitochondrial respiratory chain,

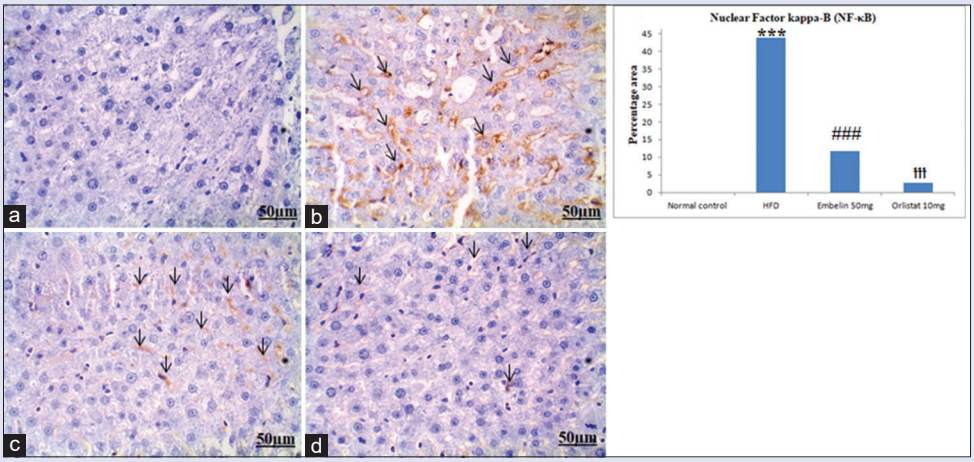

Figure 5: Photomicrograph of immunohistochemical staining revealing nuclear factor kappa-B activation in liver cells. (a) Mice treated with normal chow diet showing normal liver cells. (b) Mice treated with high fat diet for a period of 8 weeks. (c) Treatment of embelin (50 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice. (d) Treatment of orlistat (10 mg/kg/day, p.o.) from 5th to 8th week in high fat diet fed mice. Black arrows are showing nuclear factor kappa-B activation. Data are expressed as mean ± standard error of mean ($n = 6$ animals/group). Significance difference was determined by one-way analysis of variance followed by Tukey's multiple comparisons test. ***$P < 0.001$ high fat diet versus normal control; ###$P < 0.001$ embelin 50 mg versus high fat diet; †††$P < 0.001$ orlistat 10 mg versus high fat diet

caused an increase in the production of free radicals, ROS, and hydrogen peroxide under HFD.[33]

Various natural products are evaluated to protect against oxidative stress-induced by HFD.[34,35] Recent studies revealed that embelin has multiple effects, including hepatoprotective, anti-inflammatory, antibacterial, antioxidative, and anti-obesity effects, but the mechanism of action still unexplored.[36] Therefore, we were motivated to do current research work to evaluate the effect of embelin and its involvement in the alteration of NrF-2 and NF-kB pathways in HFD fed mice.

Treatment with embelin in HFD treated mice caused a significant reduction in body weight gain, fat pad weights, and BMI. Oral administration of embelin did not cause changes in food intake. Thus, the effect of embelin on body weight gain, fat pad weights, and BMI is independent on satiety. In present research work, TBARS levels determined in liver tissue by evaluating the malondialdehyde content were decreased by embelin treatment (50 mg/kg/day, p.o.) for 28 days in HFD treated mice. The production of antioxidant enzymes involved in antioxidant defense and the expression of antioxidant genes was induced by the NrF-2 pathway.[13] Furthermore, in our study, GSH, SOD, and CAT were decreased in HFD treated group as reported by previous studies. Embelin treatment (50 mg/kg/day, p.o.) for 28 days reversed the levels of these enzymes. Thus, it is reasonable to assume that embelin might be improving the liver oxidative stress in HFD-fed obese mice. Several earlier studies reported that NrF-2 plays a vital role in the expression of various antioxidant proteins. Therefore, NrF-2 was a worthy target to investigate in liver tissue in the current study. In this study, we observed a decrease in NrF-2 expression in IHC staining in HFD fed mice, which modulated by treatment with embelin, which indicating that embelin therapy promoted NrF-2 expression to enhance antioxidant potential. The decreased NrF-2 expression provided a statement for the compromised antioxidant defense mechanism/increased oxidative stress induced by HFD in hepatic tissues that are corroborated with the findings of recent publication.[19] Thus, the embelin-dependent modulation of Nrf-2 plays a role in protecting the liver from oxidative stress. As suggested by the previous studies, NF-κB plays a crucial role in modulating a large number of genes involved in inflammatory response. It has been also established that NrF-2-antioxidant signaling pathway activation modulates inflammatory signaling pathways such as NF-κB and its expression accelerates NF-κB signaling pro-inflammatory reactions.[14] On the other way, it has been established that NF-κB could directly repress the NrF-2-mediated pathway at the transcriptional level.[37] Thus the potential crosslink between NrF-2 and NF-κB pathways exists. The present study confirms that embelin could inhibit the activation of NF-κB, as evidenced by immunohistochemical staining. In the present research work, hepatic Masson's trichrome staining of mice with HFD treatment showed fibrosis, which is the major marker of liver pathogenesis. Treatment with embelin (50mg/kg/day, p.o.) showed less fibrosis in liver tissue in HFD fed mice.

On the whole, we have found that embelin treatment (50 mg/kg/day, p.o.) ameliorated body weight gain, fat pad weights, BMI, inflammation, and liver fibrosis induced by HFD in C57BL/6 mice. These ameliorating effects of embelin (50 mg/kg/day, p.o.) are possibly related to a reduction in oxidative stress and inflammation induced by HFD in C57BL/6 mice. The alteration of NF-kB and Nrf-2 protein levels by treatment with embelin probably responsible for its antioxidant and anti-inflammatory effects.

CONCLUSION

This study signifies that HFD increases obesity; the present study also established the role of oxidative stress and inflammation in developing obesity. Therefore, the embelin as an active herbal component can be a potential therapeutic agent for treating or preventing obesity. The importance of NrF-2 and NF-κB expression in managing obesity and liver fibrosis is undeniable.

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Conflicts of interest

There are no conflicts of interest.

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