



RESEARCH ARTICLE

Aqueous extract of *Beta vulgaris* L. modulates oxidative and metabolic disturbances in a diet-induced NAFLD rat model

Wafa S Abdulredha^{1*}, Amel S Abdulredha², Amena L Muttlaq¹, Nuha S Falgoos³ & Amal H Anatheil⁴

¹Department of Pharmacognosy and Medicinal Plants, College of Pharmacy, University of Thi-qar, Thi-qar 64001, Iraq

²Department of Biology, College of Education for Pure Science, University of Basrah, Basrah 64001, Iraq

³Department of Clinical Laboratory Sciences, College of Applied Medical Science, University of Al-Shatrah, Thi-qar 64001, Iraq

⁴Department of Pharmaceutical Chemistry, College of Pharmacy, University of Thi-qar, Thi-qar 64001, Iraq

*Correspondence email - wafaabdulredha81@utq.edu.iq

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a growing metabolic disorder associated strongly with the consumption of highly refined carbohydrates and fatty foods. Beetroot (*Beta vulgaris* L.), particularly in its aqueous form, contains bioactive compounds with antioxidant and metabolic regulatory properties, suggesting potential hepatoprotective effects. This study investigated the effects of aqueous beetroot extract on physiological, biochemical and oxidative stress parameters in male Wistar rats with NAFLD induced by a high-fat and fructose diet. Physiological, biochemical and oxidative stress parameters were measured in male Wistar rats with NAFLD due to excess high fat and high fructose in their diets. Each of the test animals were separated into four distinct test groups for analysis: a control group; a high fat plus fructose group and two treatment groups that received beetroot extract, which were classified in advance as either low or high doses. The indices and markers of physiology, liver dysfunction, serum lipid profiles and oxidative stress were measured, along with the indices of insulin resistance. The rats that were fed high fat and fructose diets experienced notable increases in body weight, aggravation of the fatty liver disease (high liver mass, high liver enzymes), severe dyslipidaemia, increased oxidative stress and impaired glucose-insulin homeostasis. Beetroot extracts exerted dose-dependent protective effects, including reductions in body and liver weight, normalisation of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, improvement in triglyceride and cholesterol levels, restoration of antioxidant defenses (glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT)), and significant reductions in fasting glucose, insulin and homeostatic model assessment for insulin resistance (HOMA-IR). Most of the high-dose beetroot extract values were almost equal to those of the control group. Thus, findings indicate beetroot extract reduces the metabolic and hepatic disorders caused by diet-induced NAFLD. The action could be attributed to the combination of the lipid-modulating and antioxidant action of beetroots. The results indicate that beetroot has the potential to be used as a functional food to prevent and manage the early stages of NAFLD.

Keywords: beetroot; insulin; lipids; non-alcoholic fatty liver disease; oxidative stress

Introduction

The red beetroot (*Beta vulgaris* L.) is rich in diverse phytochemicals, especially in the water soluble betalains such as betanin, as well as phenolic compounds, dietary nitrates, vitamins and minerals. These constituents have been reported to exhibit strong antioxidant capacity which is the ability to neutralise and react with species which in turn modulate the cellular redox pathways (1, 2). Accumulating evidence further suggests that betalains and phenolic compounds can enhance endogenous antioxidant defense systems and improve cellular resilience to oxidative stress in multiple tissues, including the liver (3, 4). Overall, these biochemical features support the potential role of beetroot in mitigating the risk of chronic metabolic disorders.

The biological activities of beetroot tap into fundamental metabolic pathways involved in the onset of non-alcoholic fatty liver disease (NAFLD). Beetroot antioxidants have documented

the attenuation of lipid peroxidation and replenished intracellular glutathione which mitigates the oxidative injury associated with hepatocyte steatosis (5). Additionally, various studies have noted that the phenolic compounds and betalains may affect lipid metabolic signalling through the modulation of the peroxisome proliferator-activated receptor α (PPAR- α) pathway which is associated with increased fatty acid oxidation and decreased hepatic lipid-accumulation (6, 7). Reductions in inflammatory mediators and improvements in systemic insulin sensitivity often accompany these metabolic effects.

Studies evaluating diets with high fat and high fructose content have shown that beetroot exhibits protective effects. Supplementation with beetroot juice or extracts has been demonstrated to reduce serum liver enzyme levels (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), decrease oxidative stress markers such as malondialdehyde and enhance the activity of key antioxidant enzymes, including

superoxide dismutase (SOD), catalase (CAT) and glutathione (GSH) (8, 9). In various experimental models, improvements of liver serum triglycerides and cholesterol levels have been documented, along with reductions of weight gain, visceral adiposity and cholesterol levels (10, 11). Beetroot's protective effects may be the result of its diverse and synergistic phytochemical constituents.

Mechanistic studies suggest that aqueous beetroot extract, particularly betalains, may downregulate certain inflammatory signalling molecules while activating cytoprotective gene expression pathways (12, 13). Such effects have been linked to the reduction of some proinflammatory cytokines like tumour necrosis factor- α (TNF- α), interleukin-6 (IL-6) and lessening of some metabolic indicators like fasting glucose and insulin levels and homeostatic model assessment for insulin resistance (HOMA-IR) (14, 15). These mechanisms help to contextualise the observed improvements in lipid profiles and oxidative stress markers in experimental models of NAFLD, thereby supporting the potential role of beetroot as a functional dietary component or an adjunctive therapeutic strategy.

This research comprehensively evaluates the novel protective and modulatory impacts of aqueous beetroot extract, at two dosing levels, on metabolic, oxidative and liver functions in a NAFLD diet-induced rat model which has not been done in prior research. Henceforth, the purpose of this research is to assess primarily the protective and modulatory impacts of beetroot extract on the physiologic parameters, liver function, lipid alters, oxidative stress and insulin resistance of male Wistar rats fed a high-fat, fructose-induced NAFLD diet.

Materials and Methods

Animals and housing

The study utilised male Wistar rats aged 6–8 weeks with weight between 180 and 250 g. The rats were acclimatised for a week before the start of the experiment. The rats are maintained in plastic ventilated cages, 3 rats per cage. The cages were in a controlled temperature room (22 ± 2 °C) with 45–60 % humidity. The rats were in a 12-hr light/12-hr dark cycle. The rats had *ad libitum* access to food and water except during brief fasting intervals before the sample collection. The group rats were carefully monitored to make sure that they received equal daily amounts of food for consistent intake to be caused. The study was conducted in accordance with the institutional ethical guidelines for the care and use of laboratory animals adopted by the College of Pharmacy, University of Thi-qar, Iraq. Appropriate measures were implemented to minimise any unnecessary pain or suffering to the animals.

Experimental design and diets

Each of the four groups, consisting of six rats, was assigned a different diet:

Control: Standard laboratory diet and regular drinking water

HFD + Fructose: High-fat diet (HFD) plus 10 % fructose in drinking water

HFD + Fructose + Low-dose beetroot: High-fat and fructose diet supplemented with aqueous beetroot extract at 250 mg/kg/day

HFD + Fructose + High-dose beetroot: High-fat and fructose diet with beetroot extract of 500 mg/kg/day

A sample size of six rats was selected, and the doses of beetroot extract (250 and 500 mg/kg/day) were determined based on a previous study (8), which showed efficacy consistent with hepatoprotection, tolerability and statistical power. Eight weeks of treatment was sufficient to develop liver steatosis and its associated metabolic disturbances.

Preparation and administration of beetroot extract

Roots of fresh red beetroot (11 kg) were rinsed under running water, peeled and sliced before extraction (2–3 mm). A measured quantity of sliced plant material was mixed with distilled water at a 1:10 (w/v) plant-to-solvent ratio and maintained at 60 °C with gentle stirring for 1 hr to promote the extraction of water-soluble betalains and phenolics while minimising thermal degradation. The extract was allowed to cool, and was then passed through a sterile cloth, Whatman No. 1, and then, before filtration, was subjected to a 5000 g, 15 min centrifugation at 4 °C, and was then subjected to pressure to remove most of the water at a temperature ≤ 40 °C. Finally, the extract was freeze-dried according to guidelines for the preservation of betalains (16) and the freeze-dried extract was stored in amber vials, which were sealed and kept at 4 °C. Working solutions for gavage were prepared daily by dissolving the powder in distilled water. Daily dose calculations were based on each animal's body weight and administered orally via gavage to ensure accurate delivery (17).

Physiological measurements and biochemical analyses

Animals were weighed weekly and the amount of food and water per cage were recorded. At the end of the study, the animals were fasted overnight, then anaesthetised and sacrificed. Livers were removed, weighed and the liver index calculated as (18):

$$\text{Liver index} = (\text{Liver weight} / \text{Body weight}) \times 100$$

Blood samples were taken right after the animals were sacrificed at the end of the last week of the experiment. After collection, the samples were allowed to settle briefly and then centrifuged to separate the serum, which was subsequently used for biochemical analyses. The following parameters were analysed using commercial kits according to the manufacturer's instructions: liver function tests (ALT, AST, alkaline phosphatase (ALP), total bilirubin), lipid profile (triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C)), oxidative stress markers (malondialdehyde (MDA), reduced glutathione, SOD and catalase (CAT)) and metabolic parameters (fasting glucose, fasting insulin and HOMA-IR).

Statistical analysis

This study employed an analysis of variances followed by Turkey's post hoc test. Statistically significant difference was $p < 0.05$. Data are presented as mean \pm standard deviation. Statistical analysis was conducted using SPSS version 2024.

Results

The experiment showed noticeable changes in physiological markers in each group. The high fat and fructose diet (HFD + fructose) rats had notable increases in body and liver weight and liver index, characteristic of diet related obesity and enlargement of the liver. The beetroot extract treatment helped mitigate the HFD + fructose effects (Table 1). The effects of the treatment were evident in

Table 1. Effect of aqueous beetroot extract on physiological parameters in experimental groups (mean \pm SD, $n = 6$)

Parameter	Control	HFD + fructose	HFD + fructose + 250 mg/kg of beetroot	HFD + fructose + 500 mg/kg of beetroot
Final body weight (g)	295 \pm 8.3 ^a	362 \pm 10.7 ^b	330 \pm 9.4 ^c	308 \pm 8.8 ^{ac}
Liver weight (g)	8.3 \pm 0.4 ^a	12.1 \pm 0.6 ^b	10.4 \pm 0.5 ^c	9.1 \pm 0.4 ^{ac}
Liver index (%)	2.8 \pm 0.1 ^a	3.4 \pm 0.2 ^b	3.1 \pm 0.2 ^c	2.9 \pm 0.1 ^{ac}

Abbreviations: HFD: high-fat diet.

Different superscript letters indicate significant differences ($p < 0.05$).

a dose-dependent manner the high dose exposure treatment was able to bring body and liver parameters to close levels as in control rats.

The HFD + fructose diet caused significant liver stress as shown by the liver enzyme (ALT, AST and ALP) and total bilirubin increases compared to the control diet (Table 2). The beetroot extract treatment helped mitigate these effects in a dose dependent manner. The high dose liver enzyme and bilirubin levels were close to the normal levels having a significantly noticeable hepatoprotective effect.

Compared to the controls, rats on the HFD + Fructose diet had palpable dyslipidaemia, including raised levels of triglycerides, total cholesterol and LDL-C, as well as lower levels of HDL-C. Aqueous beetroot extract treatment had an adjusting effect on the

lipid profile which was dose-dependent. Supplementation at high doses neared the normalisation of HDL-C and LDL-C which shows good potential for effective lipid metabolism modulation (Table 3).

Table 4 shows that feeding a HFD + fructose induced significant oxidative stress and metabolic dysregulation. This was evidenced by elevated levels of MDA and lower levels of the antioxidant defenses (GSH, SOD, CAT); and impaired glucose metabolism with insulin resistance, as indicated by elevated fasting glucose, insulin and HOMA-IR compared to the control group. Treatment with aqueous beetroot extract mitigated these alterations in a dose-dependent manner. Supplementation at high doses normalised oxidative stress markers and multiple metabolic parameters, indicating significant antioxidant and insulin-sensitising effects.

Table 2. Serum liver function markers in different experimental groups (mean \pm SD, $n = 6$)

Parameter	Control	HFD + fructose	HFD + fructose + 250 mg/kg of beetroot	HFD + fructose + 500 mg/kg of beetroot
ALT (U/L)	42 \pm 3.6 ^a	91 \pm 6.2 ^b	65 \pm 5.1 ^c	50 \pm 4.4 ^{ac}
AST (U/L)	80 \pm 5.2 ^a	155 \pm 8.9 ^b	120 \pm 6.7 ^c	96 \pm 5.8 ^{ac}
ALP (U/L)	165 \pm 10.4 ^a	235 \pm 12.8 ^b	205 \pm 11.5 ^c	178 \pm 9.7 ^{ac}
Total bilirubin (mg/dL)	0.41 \pm 0.05 ^a	0.86 \pm 0.08 ^b	0.63 \pm 0.06 ^c	0.48 \pm 0.05 ^{ac}

Abbreviations: HFD: high-fat diet.

Different superscript letters denote significant differences ($p < 0.05$).

Table 3. Serum lipid profile in experimental groups (mean \pm SD, $n = 6$)

Parameter	Control	HFD + fructose	HFD + fructose + 250 mg/kg of beetroot	HFD + fructose + 500 mg/kg of beetroot
TG (mg/dL)	96 \pm 7.8 ^a	180 \pm 10.2 ^b	140 \pm 8.7 ^c	110 \pm 7.1 ^{ac}
TC (mg/dL)	130 \pm 8.5 ^a	225 \pm 12.4 ^b	180 \pm 10.6 ^c	150 \pm 9.2 ^{ac}
HDL-C (mg/dL)	58 \pm 4.3 ^a	35 \pm 3.5 ^b	46 \pm 3.8 ^c	52 \pm 4.0 ^{ac}
LDL-C (mg/dL)	58 \pm 4.0 ^a	125 \pm 8.1 ^b	92 \pm 6.3 ^c	70 \pm 5.1 ^{ac}

Abbreviations: HFD: high-fat diet; TG: triglycerides; TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol.

Different superscript letters indicate significant differences ($p < 0.05$).

Table 4. Oxidative stress and metabolic biomarkers (mean \pm SD, $n = 6$)

Parameter	Control	HFD + fructose	HFD + fructose + 250 mg/kg of beetroot	HFD + fructose + 500 mg/kg of beetroot
MDA (nmol/mL)	2.1 \pm 0.2 ^a	4.8 \pm 0.4 ^b	3.3 \pm 0.3 ^c	2.5 \pm 0.2 ^{ac}
GSH (μ mol/L)	8.9 \pm 0.6 ^a	5.1 \pm 0.4 ^b	6.8 \pm 0.5 ^c	8.2 \pm 0.5 ^{ac}
SOD (U/mL)	6.5 \pm 0.4 ^a	3.2 \pm 0.3 ^b	4.8 \pm 0.4 ^c	5.9 \pm 0.3 ^{ac}
CAT (U/mL)	42 \pm 3.2 ^a	25 \pm 2.8 ^b	34 \pm 3.0 ^c	39 \pm 3.1 ^{ac}
Fasting glucose (mg/dL)	95 \pm 7.1 ^a	145 \pm 9.3 ^b	122 \pm 8.2 ^c	104 \pm 7.0 ^{ac}
Insulin (μ IU/mL)	9.8 \pm 0.8 ^a	18.5 \pm 1.2 ^b	13.7 \pm 1.0 ^c	10.9 \pm 0.9 ^{ac}
HOMA-IR	2.3 \pm 0.2 ^a	6.6 \pm 0.4 ^b	4.1 \pm 0.3 ^c	2.9 \pm 0.3 ^{ac}

Abbreviations: HFD: high-fat diet; MDA: malondialdehyde; SOD: reduced glutathione; CAT: catalase; HOMA-IR: homeostatic model assessment for insulin resistance. Different superscript letters indicate significant differences ($p < 0.05$).

Discussion

The present study assessed the effects of an aqueous beetroot (*B. vulgaris*) extract in a rat model of NAFLD induced by a high-fat, fructose diet. Results showed that beetroot supplementation, particularly at elevated doses, conferred significant protection against alterations in physiological, biochemical and oxidative stress parameters.

Rats on the high-fat and fructose diet exhibited the greatest increases in body and liver weight, along with liver index increases, confirming the development of hepatic steatosis and obesity (19, 20). Beetroot extract, particularly at the higher dose, mitigated weight gain and reduced liver enlargement, indicating that bioactive constituents such as betalains and polyphenols may modulate fat accumulation, promote fatty acid oxidation and decrease hepatic lipid deposition (21, 22).

Liver stress and dysfunction have been documented as the cause of the elevation in the levels of serum ALT, AST, ALP and total bilirubin, as a result of high fat and fructose diet. Observations of the levels of the mentioned enzymes show a dose dependent response of the hepatoprotective effect of the beetroot extract supplement. The antioxidant and membrane-stabilising activities of betalains likely played a key role in preserving hepatocyte integrity and minimising oxidative damage (23–25). The results corroborate the suggestions of the previous studies indicating there was protection obtained from chemical and diet-induced damage to the liver (26–29).

Dyslipidaemia, presence of triglycerides, high levels of total, low levels of HDL-C and increased levels of LDL-C have all been documented to be the result of the HFD + fructose diet. The extract of beetroot has displayed results which have restored the levels to those of the control in those supplemented with the high dose. These effects are likely mediated through activation of PPAR α signaling and upregulation of lipid-catabolising enzymes, thereby enhancing fatty acid oxidation and reducing hepatic lipid accumulation (30–32). Prior research which agree with current study have documented the positive characteristics of beetroot extracts in the management of the metabolic syndrome and the protection of the cardiovascular system.

Increased oxidative stress and insulin resistance were confirmed by HFD + fructose feeding as to glucose homeostasis, elevate HOMA-IR and mean GSH, SOD and CAT were lowered, and MDA increased. These changes were reversed by beetroot extract in a dose-dependent manner. Scavenging free radicals, from the betalains and protecting against lipid peroxidation (9, 33) and possibly antagonising oxidative stress by stimulating some endogenous antioxidant enzymes. Improvements in fasting glucose and insulin level suggest enhanced insulin sensitivity, potentially through antagonism of inflammatory mediators such as TNF- α and IL-6, which are known to inhibit insulin signalling (34–38). However, clinical studies in humans have reported more mixed results. For example, 12-week supplementation with beetroot powder improved biochemical markers but did not significantly change fatty liver grade (39). Similarly, interventions combining beetroot with dietary modifications showed beneficial changes in liver enzymes, yet direct attribution to beetroot alone was limited (15). Different intervention times in humans, imprecise controlling of doses, changing diets and lifestyles of participants, differences in species-specific metabolism and response and the

insensitivity of non-invasive assessments compared to histological evaluation in animals may all contribute to these discrepancies.

Aqueous beetroot extract showed dose-dependent protective effects against diet-induced NAFLD by improving liver function, lipid metabolism, oxidative stress and insulin sensitivity. The doses of 250 and 500 mg/kg used in rats correspond approximately to (2.8–5.7) g/day of beetroot extract for a 70 kg human, which can be achieved through dietary intake of (28–57) g of fresh beetroot. Despite there being no liver histology, these findings suggest the possible translational value but extracts composition analysis is a key limitation. Future studies should include histopathological assessment and phytochemical profiling to confirm these effects and optimise dosing.

Conclusion

In summary, the results of the current study show that *Beta vulgaris* L. aqueous extract is a possible protective agent against liver damage, oxidative stress and the metabolic syndrome caused by a high-fat and fructose diet in male Wistar rats. More pronounced effects in liver health, lipid processing and insulin action enhancement were observed at higher extract levels, which points to the extract's capacity to serve as a functional dietary adjunct for NAFLD. Future studies should include phytochemical profiling of the extract and histopathological assessment to better clarify these effects.

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Authors' contributions

WSA and ASA designed the study, defined the intellectual content and prepared and edited the manuscript. NSF managed the clinical studies, conducted the literature review and participated in manuscript review. AHA managed data collection, performed the statistical and experimental analyses and prepared the experimental studies. ALM supervised the laboratory work and the documentation of laboratory data. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: The study was conducted in accordance with the institutional ethical guidelines for the care and use of laboratory animals adopted by the College of Pharmacy, University of Thi-qar, Iraq.

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