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Hibiscus sabdariffa mitigates hyperlipidemia, cardiac oxidative stress, and inflammatory cytokines in serum and cardiac tissue of adult female Wistar rats with fructose-induced metabolic syndrome

Sameer Mohammed Khan[1], Adamu Imam Isa[1,*], Hanan Eissa[2], Ashraf Salah Metwally[3], Mohamed O'haj Mohamed[4], Ibrahim Gaya Bako[5], and Noura El Adle Khalaf[6]

[1]*Department of Physiology, College of Medicine, University of Bisha*, Bisha, Saudi Arabia
[2]*Department of Pharmacology, College of Medicine, University of Bisha*, Bisha, Saudi Arabia
[3]*Department of Microorganisms and Clinical Parasitology, College of Medicine, University of Bisha*, Saudi Arabia
[4]*Department of Clinical Biochemistry, College of Medicine, University of Bisha*, Bisha, Saudi Arabia
[5]*Department of Human Physiology, Faculty of Basic Medical Sciences, Ahmadu Bello University*, Zaria, Nigeria
[6]*Department of Clinical Pharmacology, Faculty of Medicine, Mansoura University*, Mansoura 35516, Egypt

***Corresponding author**: adamuisaimam@gmail.com

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Abstract: Metabolic syndrome (MetS) is a cluster of metabolic abnormalities that includes central obesity, insulin resistance, high blood pressure, atherogenic dyslipidemia, and chronic low-grade inflammation, all of which together elevate the risk of cardiovascular disease and type 2 diabetes. MetS is more prevalent in women. A study involving 35 female Wistar rats investigated *Hibiscus sabdariffa* (HS) extract's therapeutic effects across five groups: normal control, untreated metabolic syndrome group, three experimental groups with fructose-induced metabolic syndrome receiving 100, 200, and 400 mg/kg HS extract. HS extract at 400 mg/kg significantly improved serum lipid metabolism by reducing total cholesterol, triglycerides, and low-density lipoprotein (LDL) cholesterol, while increasing high-density lipoprotein (HDL) cholesterol. It also improved cardiovascular risk indicators, the Castelli risk indices I and II. HS extract demonstrated potent antioxidant effects in the heart by reducing malondialdehyde (MDA) levels and enhancing the activities of superoxide dismutase (SOD), catalase (CAT), and the concentration of reduced glutathione (GSH). It also had immunomodulatory effects**,** reducing inflammatory markers, tumor necrosis factor (TNF)-α, and interferon (IF)-γ in the serum and increasing brain-derived neurotrophic factor (BDNF) in both serum and heart. IF-γ was increased significantly in the heart. In conclusion, HS extract, especially at higher doses, shows substantial therapeutic potential for managing metabolic syndrome by improving lipid profiles, enhancing cardiovascular health, boosting antioxidant defenses, and supporting immune function.

Keywords: metabolic syndrome, *Hibiscus sabdariffa*, lipid metabolism, antioxidant activity, cytokines

INTRODUCTION

MetS is a complex condition induced by a cluster of modifiable risk factors that significantly contribute to cardiovascular diseases, type 2 diabetes mellitus, and other health complications [1,2]. MetS is a cluster of metabolic abnormalities that includes central obesity, insulin resistance, high blood pressure, atherogenic dyslipidemia, and chronic low-grade inflammation, all of which elevate the risk of cardiovascular disease and type 2 diabetes [2]. The onset of MetS is frequently linked to excessive dietary fructose intake, which triggers metabolic shifts leading to decreased insulin sensitivity, lipid accumulation, and oxidative stress-induced cellular damage [3]. To better understand the progression of MetS and its potential therapeutic interventions, fructose-fed Wistar rat models have been widely utilized, offering valuable insights into disease pathogenesis [4].

Oxidative stress and inflammation play a critical role in the worsening of MetS, particularly in endothelial dysfunction, which increases cardiovascular risks [5]. Persistent inflammation results in elevated

cytokines and adhesion molecules, exacerbating vascular complications [6,7]. Consequently, therapeutic strategies aimed at reducing oxidative damage and inflammatory responses are crucial for managing MetS and mitigating cardiovascular risks.

Hibiscus sabdariffa is a medicinal plant rich in polyphenols, and it exhibits notable antioxidant activity [8]. Its calyces contain anthocyanins, flavonoids, and other bioactive compounds, which have been shown to support blood pressure regulation, lipid metabolism, and cardiovascular health [9]. The therapeutic effects of *H. sabdariffa* have been studied in both human and animal models, demonstrating its ability to counteract oxidative stress by scavenging free radicals and enhancing antioxidant defenses [10,11]. Furthermore, its anti-inflammatory properties are attributed to its ability to modulate pro-inflammatory cytokine activity, notably TNF-α and interleukin-6 (IL-6) [12,13]. The lipid-regulating effects of *H. sabdariffa* also contribute to improved metabolic profiles, with reductions in LDL cholesterol and triglycerides and increases in HDL cholesterol [10].

HS has been shown to reduce serum cholesterol and triglycerides and offer protection against oxidative stress [14]. Further investigation into its effects on fructose-induced metabolic disturbances revealed sex-specific protective mechanisms. Early administration of HS in rats later exposed to a high-fructose diet revealed that female rats were protected from hypertriglyceridemia and hepatic lipid accumulation [15], while male rats exhibited protection against hypercholesterolemia [16]. This highlights the importance of considering sex as a biological variable in therapeutic interventions for metabolic disorders. Beyond its direct antioxidant and lipid-lowering effects, HS also possesses notable anti-inflammatory properties. The natural compounds in HS can regulate cytokine expression and reduce oxidative stress, both key factors in the development of metabolic syndrome [17]. This highlights HS's potential to lower inflammatory cytokines and cardiac oxidative stress, supporting its role in a comprehensive approach to managing the complex features of metabolic syndrome and its associated cardiovascular risks. Specifically, the impact of *H. sabdariffa* on cardiac brain-derived neurotrophic factor (BDNF) and INF-γ in MetS models remains largely unexplored. This study aimed to investigate the effects of *H. sabdariffa* on cardiac markers of oxidative injury, inflammatory signaling, and lipid profile in the serum of female rats with fructose-induced MetS. By elucidating the potential cardioprotective mechanisms of *H. sabdariffa*, this research seeks to contribute to the development of targeted nutritional and botanical interventions for the management of MetS and its associated cardiovascular risks.

MATERIALS AND METHODS

Ethics statement

All experimental protocols were executed in strict accordance with the institutional regulations for animal well-being. This project received ethical clearance from the Ahmadu Bello University Committee for the Ethical Use of Animals (Approval number: ABUCAUC/2023/004)

Materials

The following materials and equipment were utilized in this study: D-fructose (Code: 16485, Batch NO: MCR-12863, Cas NO: 1610-38-1) sourced from Mumbai, India; a Changzhou Xingyun balance (model: XY100C, No. 1404273); an Axiom UK Vis Spectrophotometer S23A; FineTest (Wuhan, China) supplied Rat IFN-gamma (ER1070) and Rat TNF-alpha (ER1393) kits, as well as reagents for total cholesterol (TC) (EU2634), triglycerides (TG) (ER2150), HDL (ER1035), and LDL (ER0311) assays; a GSH Colorimetric Assay Kit (E-BC-K030) from Elabscience, China; and a Ceti Max III-Mono Monocular Compound Microscope from Chalgrove, Oxon, UK

Plant identification and extraction

H. sabdariffa was obtained from a local agricultural site in Samaru in the Sabon Gari district of Kaduna State, Nigeria. To ensure botanical accuracy, the plant was authenticated, and a reference sample (voucher specimen No. 01056) was deposited in the plant collection of the Biological Sciences Department at Ahmadu Bello University, Zaria. The extraction procedure was conducted according to the methodology detailed in [14].

Animal handling and care

Thirty-five fully grown Wistar rats, each weighing 180-200 g, were acquired from the animal facility in the Department of Human Physiology at Ahmadu Bello University, Zaria, Nigeria. The animals (5 per cage) were kept in see-through, plastic enclosures for a 14-day adjustment period, during which they received unlimited standard laboratory food and fresh water.

Induction of MetS

A freshly constituted 20% w/v fructose solution, equivalent to 0.2 g/mL of fructose, was prepared immediately before administration to induce metabolic syndrome (MetS) [19,20]. To preclude potential confounding effects from microbial activity, the solution was stored in light-impermeable vessels wrapped in metallic foil [21].

Experimental design

Thirty-five female Wistar rats were randomly divided into 5 discrete groups each comprising 7 animals; group I served as the normal control and was allowed access to distilled water, group II served as the negative control (MS-untreated) and was given 20% fructose throughout the period of the experiment. Groups III, IV and V, while simultaneously exposed to the 20% fructose solution, received daily oral gavage of the plant extract at doses of 100, 200, and 400 mg/kg, and were designated as MS+HS 100 mg/kg, MS+HS 200 mg/kg and MS+HS 400 mg/kg, respectively. A baseline control group maintaining normal glucose homeostasis was given unrestricted access to distilled water.

Blood and tissue sample collection

Animals were anaesthetized by intraperitoneal injection of pentobarbital at a dosage of 60 mg/kg [21]. Following this, blood samples were collected directly from the heart via cardiac puncture and immediately transferred to tubes without anticoagulants. The resulting serum was used for analysis. Subsequently, the heart tissue was carefully extracted, its mass precisely measured, and then rinsed in ice-cold phosphate-buffered saline (PBS) to remove residual blood. The heart tissue was then homogenized, achieving a uniform suspension by combining 9 mL of 0.01 M PBS (pH 7.4) per g of tissue, centrifuged for 10 min at 5,000 × g, and used for biochemical analyses.

Biochemical assays

The lipid profiles, including serum total cholesterol (TC, EU2634), triglycerides (TGs, ER2150), HDL (ER1035), and LDL (ER0311), were measured using reagent kits from FineTest (Fine Biotech, Wuhan, China). The derived indices were calculated using the formulas provided in [22,23]: the atherogenic coefficient (AC = log [TG/HDL-C]), Castelli's risk index I (CRI-I = TC/HDL), and Castelli's risk index II (CRI-II = LDL/HDL). Inflammatory and neurotrophic markers were then assessed. TNF-α (ER1393), IFN-γ (ER1070), and BDNF (ER0008) levels in the cardiac tissue were determined using commercial enzyme-linked immunosorbent assay (ELISA) kits (FineTest, FineBiotech, Wuhan, China), strictly adhering to the manufacturer's instructions. Oxidative stress parameters were evaluated using established methodologies. MDA concentration, a marker of lipid peroxidation, was quantified following the protocol described by Ohkawa et al. [24]. GSH levels were determined using a commercially available assay kit (E-BC-K030, Elabscience, Houston, TX, USA). SOD activity was measured based on the method developed of Misra and Fridovich [25], while CAT activity was assessed using Claiborne's method [26]. Total protein concentrations were determined using the Bradford assay, with Coomassie Brilliant Blue G-250 as the dye and bovine serum albumin serving as the calibration standard [27]. Absorbance readings were collected using a Vis Spectrophotometer S23A (Axiom UK).

Data analysis

The quantitative findings of this study are given as the mean±SEM. Statistical evaluations were performed using IBM SPSS version 23. To ascertain intergroup variations, one-way ANOVA was initially employed, followed by Tukey's post hoc test for pairwise comparisons when the ANOVA yielded significant results. The significance level was $P<0.05$.

RESULTS

This study evaluated the effect of *H. sabdariffa* on hyperlipidemia, cardiac oxidative stress, and inflammatory

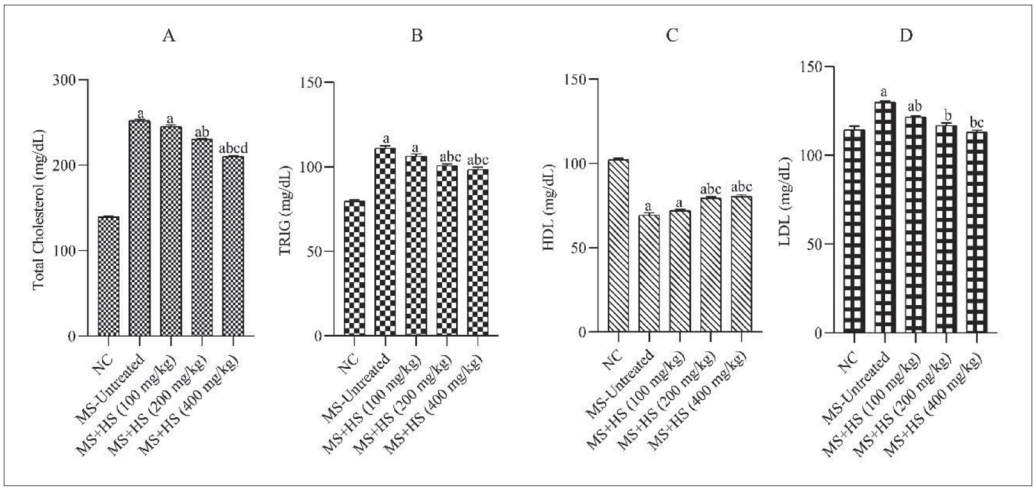

Fig. 1. Effect of *H. Sabdariffa* on serum **A** – total cholesterol, **B** – triglycerides, **C** – HDL, **D** –LDL. MS – MetS, HS – *Hibiscus sabdariffa*. [a]P<0.05 compared with NC, [b]P<0.05 vs MS-untreated, [c]P<0.05 vs MS+HS (100 mg/kg), [d]P<0.05 vs MS+HS (200 mg/kg).

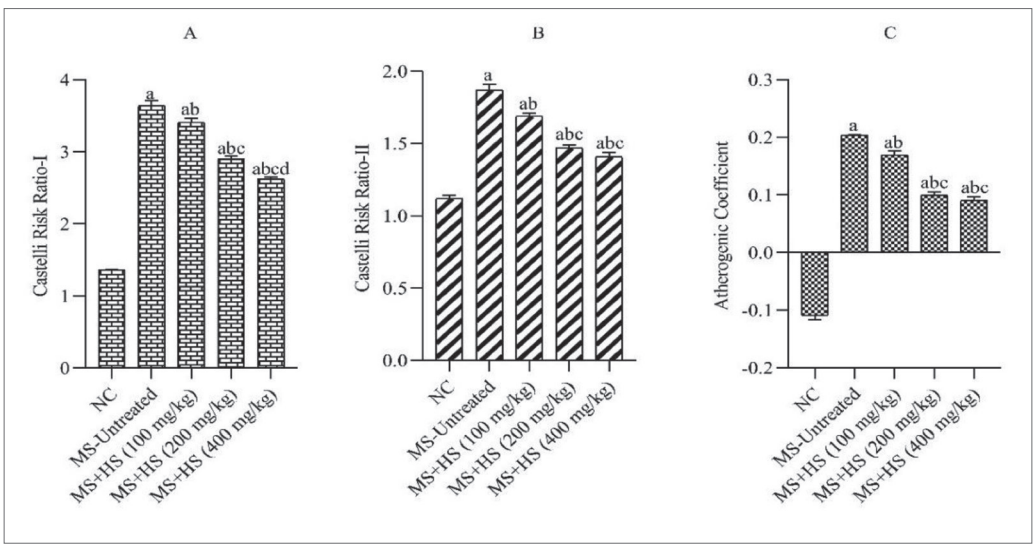

Fig. 2. Effect of *H. Sabdariffa* on Castelli risk indices I and II, and atherogenic coefficient. **A** – Castelli risk index I, **B** – Castelli risk index II, C – atherogenic coefficient. MS – MetS, HS – *H. sabdariffa*. [a]P<0.05 compared to NC, [b]P<0.05 vs MS untreated, [c]P<0.05 vs MS+HS (100 mg/kg), [d]P<0.05 vs MS+HS (200 mg/kg).

cytokines in adult female Wistar rats with fructose-induced metabolic syndrome

Effect of *H. sabdariffa* on serum TC, TG, HDL, and LDL

Fig. 1A, B, and D illustrate the observed marked increases in TC, TG, and LDL cholesterol levels in the MS-untreated group compared to the normal control (NC) group (P<0.05). Conversely, Fig. 1C highlights a significant decrease in HDL cholesterol levels in the untreated MetS group compared to the NC group (P<0.05). Treatment with HS extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant reduction in TC, TG, and LDL levels (P<0.05) compared to the MS-untreated group. These doses also significantly elevated HDL levels relative to the MS-untreated group (P<0.05). Among the tested doses, 400 mg/kg of HS extract was the most effective, producing significantly greater reductions in TC, TG, and LDL levels compared

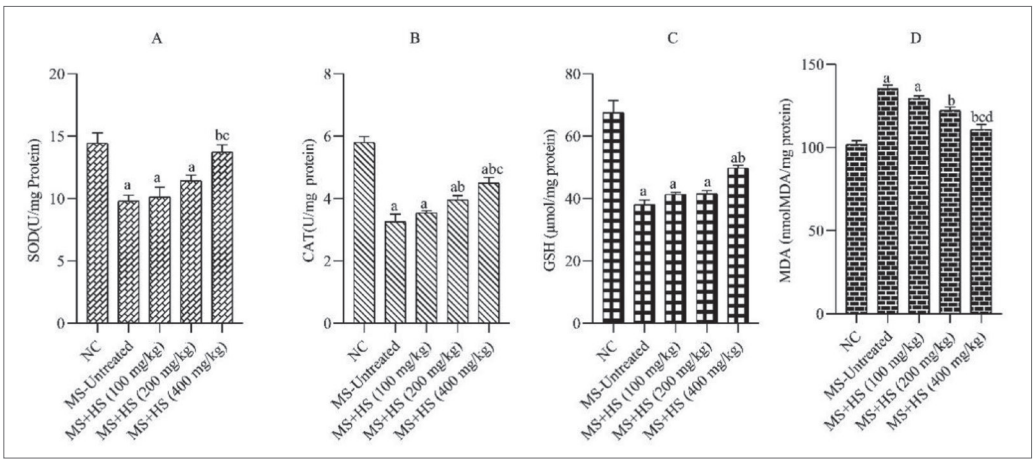

Fig. 3. Effect of *H. Sabdariffa* on cardiac tissue, superoxide dismutase, catalase, reduced glutathione, and malondialdehyde. **A** – SOD, B – CAT, C – GSH, D – MDA. MS – MetS, HS – *H. sabdariffa*. [a]P<0.05 compared to NC, [b]P<0.05 vs MS untreated, [c]P<0.05 vs MetS+HS (100 mg/kg), [d]P<0.05 vs MetS+HS (200 mg/kg).

to the 100 mg/kg dose (P<0.05). In the case of TG, the 400 mg/kg dose also outperformed the 200 mg/kg dose (P<0.05). Although the 100 mg/kg dose of HS extract showed some potential in improving lipid profiles, the reductions in TC and LDL were not statistically significant (P>0.05).

Effect of HS on Castelli risk index (CRI) I, and the atherogenic coefficient (AC)

In Fig. 2A, it can be seen that the CRI-I was significantly elevated in the untreated MetS group compared to the NC group (P<0.05). Treatment with HS extract at doses of 200 mg/kg and 400 mg/kg significantly reduced CRI-I relative to the MS-untreated (P<0.05). Furthermore, the 400 mg/kg dose showed a significant decrease in CRI-I compared to both the MS+HS 100 mg/kg group (P<0.05) and the MS+HS 200 mg/kg group (P<0.05). Similarly, the CRI-II was higher in the untreated MetS group than in the NC group (P<0.05) (Fig. 2B). Treatment with HS extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant reduction in CRI-II compared to the untreated MetS group (P<0.05). The 400 mg/kg dose also led to a significant decrease in CRI-II compared to the MS+HS 100 mg/kg group (P<0.05) and the MS+HS 200 mg/kg group (P<0.05). The AC was significantly higher in the untreated MetS group compared to the NC group (P<0.05) (Fig. 2C). HS extract at 200 mg/kg and 400 mg/kg significantly lowered the AC compared to the untreated MS group (P<0.05). Additionally, the 400 mg/kg dose significantly reduced AC levels in comparison to both the MS+HS 100 mg/kg group (P<0.05) and the MS+HS 200 mg/kg group (P<0.05).

Effect of *H. sabdariffa* on cardiac SOD, CAT, GSH, and MDA

Fig. 3A-D demonstrates the presence of oxidative stress in the untreated MetS group, characterized by significantly reduced (P<0.05) SOD, CAT and GSH compared to the NC. MDA was elevated significantly (P<0.05) in the MS-untreated group compared to the normal control. Administration of HS extract at doses of 200 mg/kg and 400 mg/kg significantly alleviated these adverse effects, leading to increased SOD, CAT, and GSH activity, along with a marked reduction in MDA levels compared to the untreated MetS group. The 400 mg/kg dose proved to be particularly effective, showing significant improvements in oxidative stress markers compared to the 100 mg/kg dose (P<0.05).

Effect of *H. sabdariffa* on TNF-α, BDNF, and IF-γ in the heart

Fig. 4A shows a pronounced increase in TNF-α in the MS-untreated group compared to the NC group (P<0.05). Administering HS extract at concentrations of 200 mg/kg and 400 mg/kg effectively diminished TNF-α concentrations when compared with the MS-untreated

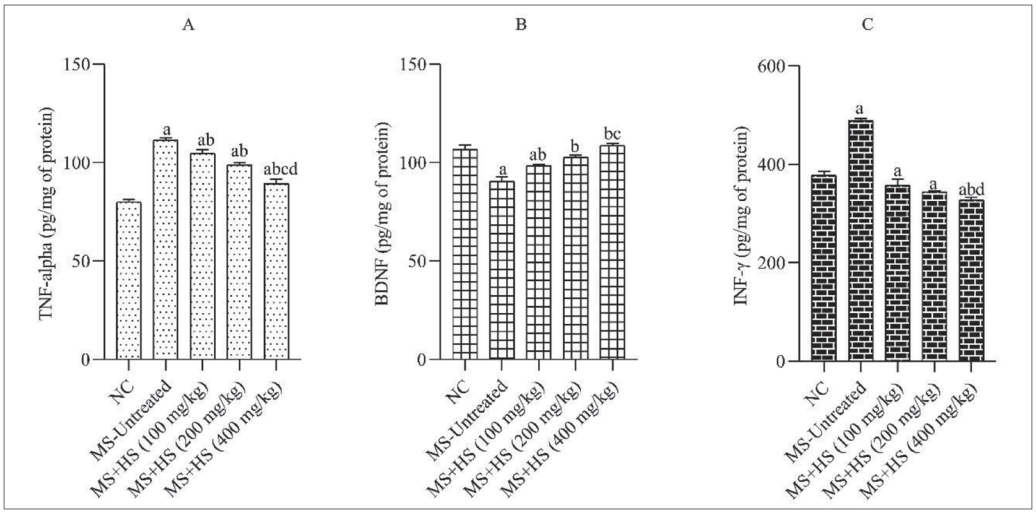

Fig. 4. Effect of *H. Sabdariffa* on **A** – TNF-α, **B** – BDNF, **C** – INF-γ. MS – MetS, HS – *H. sabdariffa*. [a]P<0.05 compared to NC, [b]P<0.05 vs MS untreated, [c]P<0.05 vs MetS+HS (100 mg/kg), [d]P<0.05 vs MetS+HS (200 mg/kg).

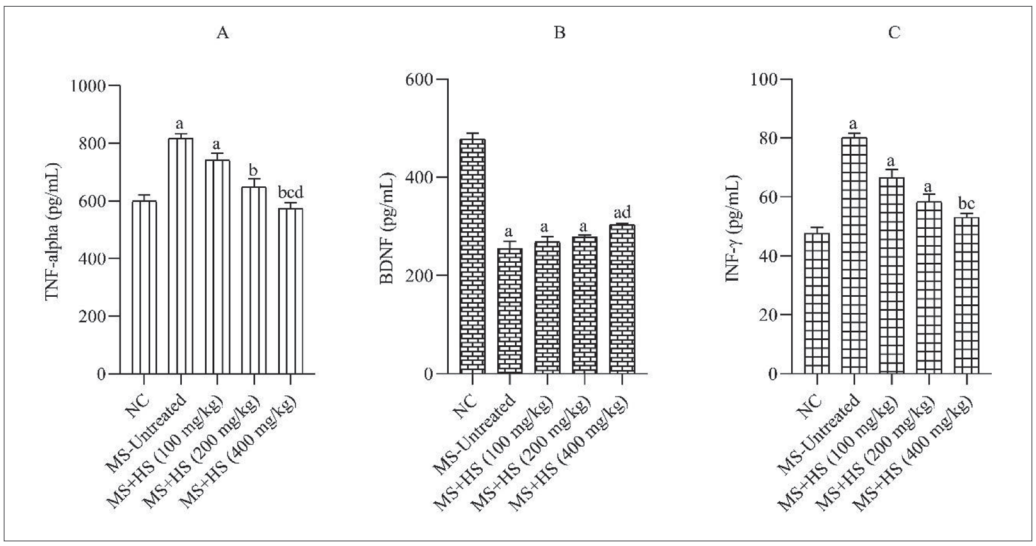

Fig. 5. Effect of *H. Sabdariffa* on serum **A** – TNF-α, **B** – BDNF, **C** – INF-γ. MS – MetS, HS – *H. sabdariffa*. [a]P<0.05 compared to NC, [b]P<0.05 vs MetS untreated, [c]P<0.05 vs MetS+HS (100 mg/kg), [d]P<0.05 vs MetS+HS (200 mg/kg).

(P<0.05). The 400 mg/kg dose induced a substantial drop in TNF-α levels, exceeding the reductions observed in the MetS group treated with 100 mg/kg HS (P<0.05) and 200 mg/kg HS (P<0.05). As can be seen in Fig. 4B, the untreated MetS group exhibited a notable decrease in BDNF compared to the NC group (P<0.05). Administration of HS extract at doses of 200 mg/kg and 400 mg/kg significantly increased BDNF levels compared to the MS-untreated group (P<0.05). The 400 mg/kg treatment led to a significant rise in BDNF, surpassing the levels seen in both MetS groups that received 100 mg/kg HS (P<0.05) and 200 mg/kg HS. Fig. 4C shows the elevation of INF-γ in the MS-untreated group relative to the NC group (P<0.05). The administration of HS extract at 200 mg/kg and 400 mg/kg yielded a substantial decline in INF-γ when compared with the MS-untreated group (P<0.05). Application of 400 mg/kg produced a considerable decrease in INF-γ relative to the MetS groups treated with 100 mg/kg HS (P<0.05), 200 mg/kg HS (P<0.05).

Effect of *H. sabdariffa* on serum TNF-α, BDNF, and IF-γ

Fig. 5A shows a marked elevation of TNF-α in the untreated MetS group when compared to the NC group (P<0.05). Administration of HS extract at dosages of 200 mg/kg and 400 mg/kg effectively lowered TNF-α concentrations relative to the untreated MetS group (P<0.05). The 400 mg/kg dose produced a notable decline in TNF-α, surpassing that of the MetS groups treated with 100 mg/kg HS (P<0.05) and 200 mg/kg HS (P<0.05). Fig. 5B reveals that a substantial reduction in BDNF was evident in the untreated MetS group when compared to the NC group (P<0.05). The administration of HS extract at concentrations of 200 mg/kg and 400 mg/kg significantly raised BDNF levels in comparison to the MS-untreated group (P<0.05). Additionally, the group receiving 400 mg/kg of HS extract displayed a significant rise (P<0.05) in BDNF, exceeding that of the MetS groups given 100 mg/kg HS and 200 mg/kg HS. Interferon-γ concentrations were markedly elevated in the MS-untreated group compared to the NC group (P<0.05) (Fig. 5C). Administration of HS extract at doses of 200 mg/kg and 400 mg/kg resulted in a significant drop in IF-γ levels when compared to the MS-untreated group (P<0.05). The administration of 400 mg/kg yielded a substantial decrease in IF-γ, surpassing that of the MetS groups treated with 100 mg/kg HS (P<0.05) and 200 mg/kg HS (P<0.05).

DISCUSSION

Persistent, low-grade inflammation is a characteristic of metabolic syndrome. This condition involves signaling molecules that interfere with lipid metabolism, prompting the liver to overproduce cholesterol and triglycerides. Previous research has established this association [28,29]. The elevated levels of TNF-α observed in the untreated MetS group in the present study strongly indicate an underlying inflammatory state. This inflammatory environment may impair lipid metabolism, thereby promoting increased cholesterol synthesis. The *H. sabdariffa*-treated groups exhibited a dose-dependent reduction in total cholesterol levels. The maximum dose (400 mg/kg) brought cholesterol levels closer to those of the healthy control group. This outcome implies that *H. sabdariffa* may possess the ability to lower cholesterol. This effect may be attributed to the inhibition of hydroxymethylglutaryl-CoA (HMG-CoA) reductase, a key enzyme in hepatic cholesterol synthesis, thereby leading to reduced cholesterol levels. Evidence suggests that plant-derived substances with strong antioxidant qualities can inhibit HMG-CoA activity [30,31]. This potential is linked to the existence of flavonoids and polyphenols in *H. sabdariffa* [32,33]. Additionally, *H. sabdariffa* may promote the removal of cholesterol through bile acids, thereby assisting in the lowering of cholesterol concentrations in an organism [34]. Chronic inflammation is associated with increased cholesterol levels and the progression of atherosclerotic plaque formation [35,36]. In the current study, *H. sabdariffa* displayed anti-inflammatory attributes that may assist in mitigating inflammation, thus contributing to a reduction in cholesterol. IFN-γ can impede the expression of enzymes involved in cholesterol biosynthesis, such as HMG-CoA reductase [37]. This may lead to reduced cholesterol synthesis, as demonstrated in this study. Additionally, IFN-γ can modulate the expression of genes involved in lipid metabolism, including those regulating cholesterol transport and storage. This alteration can impact overall cholesterol equilibrium [38]. MetS is associated with increased TG levels [39], as demonstrated in the MetS-untreated group in this study. Treatment with *H. sabdariffa* reduces TGs. Bioactive molecules such as polyphenols and flavonoids can inhibit TG synthesis in the liver, a process known as lipogenesis, leading to decreased TG levels in the circulation [40].

Insulin resistance can cause the body's cells to become less responsive to insulin. This resistance can inhibit the liver's capacity to produce HDL cholesterol, resulting in lower levels in the bloodstream [41]. This could account for the HDL result in the MetS-untreated group. HDL levels improved with HS administration in this study. This effect could be attributed to the mitigation of insulin resistance and enhanced HDL production. Additionally, it may also be due to the bioactive compounds in *H. sabdariffa*, such as anthocyanins and phenols, which can inhibit cholesterol absorption in the intestines. This inhibition leads to a reduction in LDL cholesterol and a relative increase in HDL cholesterol [42,43]. Flavonoid-containing substances like *H. sabdariffa* can promote cholesterol efflux from cells to HDL particles. This process helps increase HDL cholesterol levels in the bloodstream

[44]. However, this effect of *H. sabdariffa* was only significant in the groups receiving higher doses. LDL was notably diminished with *H. sabdariffa* treatment in the current study compared to the MS-untreated group. High fructose consumption leads to increased lipogenesis in the liver. This process results in the production of TGs, which are then packaged into very low-density lipoprotein (VLDL) particles. VLDL particles are released into the bloodstream and can be converted to LDL cholesterol [56], as observed in the LDL results of this study. Treatment with HS reduced LDL levels. By reducing inflammation, HDL can help slow the progression of plaque formation and reduce LDL levels [46,47]. The higher HDL observed in the *H. sabdariffa*-treated groups in this study could have contributed to the reduced inflammation and LDL levels noted in these groups.

A rise in overall cholesterol levels and a decline in HDL cholesterol concentrations consequently caused an increase in CRI-I, signaling an amplified likelihood of cardiovascular complications [23]. Administration of *H. sabdariffa* lowered CRI-I in this study, reflecting the decrease in total cholesterol and the increase in HDL in the treated groups. CRI II was markedly greater in the MetS-untreated group compared to the NC. Treatment with *H. sabdariffa* reduced CRI-II, which is consistent with the decreased LDL and elevated HDL seen in the treated group. Thus, *H. sabdariffa* reduced the risk of heart-related diseases in animal models of metabolic syndrome. Metabolic syndrome significantly influences the atherogenic coefficient (AC), a metric used to evaluate the potential for developing cardiovascular illnesses [48]. In the present study, the AC was significantly higher in the untreated MetS group compared to the NC. In the *H. sabdariffa-treated* groups, AC was notably reduced compared to the untreated MetS group. This effect is attributed to the cholesterol reduction observed in the treated groups.

Lipid peroxidation was increased in the MetS-untreated group compared to the healthy control. Metabolic syndrome is characterized by heightened oxidative imbalance, which stems from increased levels of reactive oxygen species (ROS) [49]. Treatment with *H. sabdariffa* in this study significantly decreased MDA levels. This reduction is linked to the antioxidant capacity of *H. sabdariffa* through the enhancement of endogenous antioxidant activities, such as SOD, GSH, and CAT, as observed in the HS-treated groups. MetS is associated with persistent, low-grade inflammation. Inflammatory signaling molecules, such as TNF-α and IL-6, can amplify the creation of ROS, further contributing to oxidative imbalance and lipid breakdown [50]. Therefore, by reducing TNF-α levels, the extract suppressed ROS production and consequently prevented lipid damage. Elevated LDL cholesterol is especially prone to oxidation, resulting in the formation of oxidized LDL, which can trigger lipid degradation in the heart [51]. Therefore, *H. sabdariffa*'s protective effect against lipid damage in the heart may be due to its ability to lower LDL levels. The antioxidant enzymes were significantly reduced in the MS-untreated group compared to the NC. MetS is linked to reduced activity of key antioxidant enzymes such as SOD, CAT, and glutathione peroxidase (GSH-Px). These enzymes play critical roles in neutralizing ROS and protecting cardiac cells from oxidative damage [52]. The synthesis of GSH can be impaired in individuals with metabolic syndrome due to altered metabolic pathways and increased demand for antioxidants [53]. *H. sabdariffa* is rich in polyphenols, flavonoids, and other antioxidants [42]. These compounds help neutralize reactive oxygen species (ROS) and reduce oxidative stress, which in turn can upregulate the expression and activity of antioxidant enzymes like SOD, GSH, and CAT, as observed in the present study. The bioactive compounds in *H. sabdariffa* can influence the expression of genes involved in the antioxidant defense system. This modulation leads to increased synthesis of antioxidant enzymes, enhancing the body's ability to combat oxidative stress [55]. By reducing inflammation, *H. sabdariffa* helps enhance antioxidant activity, as evidenced by the decreased TNF-α levels observed with its treatment in this study. TNF-α was significantly elevated in the MetS-untreated group compared to the NC in both serum and heart tissue. As adipose tissue expands in metabolic syndrome, it becomes dysfunctional and inflamed, leading to increased production and release of TNF-α [56]. This could explain the higher TNF-α concentration observed in the MetS-untreated group in this study. Administration of *H. sabdariffa* reduced both serum and tissue TNF-α, potentially due to its antioxidant activity. By neutralizing ROS, *H. sabdariffa* helps lower TNF-α levels. *H. sabdariffa* has inherent anti-inflammatory properties that can directly reduce

the production of inflammatory cytokines, including TNF-α. The bioactive compounds in *H. Sabdariffa* can inhibit the signaling pathways that lead to TNF-α production, thereby reducing inflammation [57]. In this study, *H. sabdariffa* has been shown to improve lipid profiles by reducing TC, LDL cholesterol, and TGs, while increasing HDL cholesterol. Improved lipid profiles can reduce the inflammatory response and lower TNF-α levels, as observed in the *H. sabdariffa*-treated groups. By reducing TNF-α levels, *H. Sabdariffa* helps mitigate inflammation in the heart, promoting overall cardiovascular health.

In this study, heart tissue and serum BDNF levels were significantly lower in the MS-untreated group compared to the NC. Brain-derived neurotrophic factor (BDNF) has been linked to the development of cardiometabolic risk factors in some populations [58]. Treatment with *H. sabdariffa* in this study improved both serum and cardiac BDNF levels. H. sabdariffa may have achieved this by mitigating oxidative stress, dyslipidemia, and inflammation, while simultaneously upregulating antioxidant defenses, as demonstrated in this study. Chronic inflammation can lead to decreased BDNF levels in various tissues, including the heart [59]. Dyslipidemia can negatively impact the expression of BDNF in the heart. Oxidative stress can damage cells and tissues, including those in the heart, and reduce the expression of BDNF. Recent studies indicate that IFN-γ may play a key role in the inflammatory response associated with obesity [60]. In this study, serum and heart homogenate IFN-γ levels were evaluated. IFN-γ was significantly higher in the MetS-untreated groups compared to the NC. Inflammation has been shown to increase IFN-γ production. Oxidative stress can damage immune cells and impair their ability to produce interferons. Thus, the reduced IFN-γ levels observed in the *H. sabdariffa*-treated group in this study indicate a reduction in inflammation and oxidative stress.

CONCLUSIONS

H. sabdariffa improves lipid profiles in MetS by increasing HDL and reducing LDL levels in treated groups compared to untreated MetS animals, thereby lowering Castelli's risk index and the atherogenic coefficient. Additionally, it lowers TC and TGs, contributing to better cardiovascular health. It enhances the activity of antioxidant enzymes such as SOD, CAT, and GSH. This suggests a protective effect against oxidative damage, promoting overall cellular health. *H. sabdariffa* decreases levels of the pro-inflammatory markers TNF-α and INF-γ, contributing to its anti-inflammatory effects. Furthermore, it boosts heart and serum BDNF levels, indicating additional cardiovascular benefits.

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Conflict of interest disclosure: The authors declare no conflict of interest.

Data availability: The data presented in this study are openly available at: https://doi.org/10.5281/zenodo.15615393

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