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Full-length Research Article

Modulatory Effects of Ethanol Extract of *Dissotis rotundifolia* Whole Plant on Metabolic Syndrome-Induced Hepato-Renal Dysfunctions in Rats

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Summary: Metabolic syndrome has been associated with increased incidence of liver and chronic kidney damage. This study aimed at exploring the mitigatory action of *Dissotis rotundifolia* whole plant extract on metabolic syndrome-induced hepato-renal dysfunctions. Fifty (50) adult male Wistar rats weighing between 150-180 g were randomly distributed into five equal groups as follows: Group 1 (Control), Group 2 (MetS control), Group 3 (MetS + 100 mg/kg ethanol extract of *Dissotis rotundifolia*), Group 4 (MetS + 200 mg/kg ethanol extract of *Dissotis rotundifolia*), and Group 5 (MetS + 20 mg/kg Rosuvastatin). Metabolic syndrome was induced using a 40% high-fat diet and 20% fructose in drinking water for 8 weeks. Biomarkers of hepatic [alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)] and renal [blood urea nitrogen (BUN) and creatinine] damage, oxidative stress [malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), antioxidant parameters [glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx) and glutathione S-transferase (GST)], lipid profiles [Low Density Lipoprotein cholesterol (LDL-c), High Density Lipoprotein cholesterol (HDL-c), Triglycerides (TG), and Total Cholesterol (TC)], and immunohistochemistry of the liver [Neutrophil Gelatinase-Associated Lipocalin (NGAL) and kidney [Angiotensin Converting Enzyme (ACE)] tissues of rats were determined. The results showed that metabolic syndrome caused a significant ($P<0.05$) increase in biomarkers of oxidative stress, MDA, H_2O_2 generation, LDL-c, HDL-c, TG, TC, but significantly reduced hepatic and renal GSH, SOD, GPx, and GST in comparison with the control. Furthermore, biomarkers of renal and hepatic damage were significantly ($P<0.05$) elevated in MetS untreated rats. Higher renal immune reactivity of NGAL but lower expression of ACE was recorded for MetS untreated rats. *Dissotis rotundifolia* extract mitigated biomarkers of oxidative stress, hepato-renal dysfunctions, and improved the antioxidant defense system. The observed protective effects of *Dissotis rotundifolia* on metabolic syndrome-induced hepatic and renal damage could be due to the amelioration of lipid peroxidation and increased antioxidant defense system.

Keywords*: Dissotis rotundifolia*, metabolic syndrome, Antioxidant, oxidative stress, hepato-renal dysfunction, rats.

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INTRODUCTION

Metabolic syndrome (MetS) is a complex medical condition comprising the occurrence of obesity, diabetes, and hypertension as comorbidities (Yang *et al*., 2022). The disease with a global prevalence of 25% is usually characterized by increased triglycerides, decreased high-density lipoprotein (HDL) cholesterol, insulin resistance, and hyperglycaemia (Saklayen, 2018; Kwitek, 2019). Currently, MetS is a principal contributor to disease burden and deaths in both developed and underdeveloped countries of the world (Rodríguez-Correa *et al*., 2020). Unfortunately,

effective management and treatment continue to pose great challenges to the medical community due to the multifactorial etiologies of the syndrome. Generally, the pathogenesis of MetS involves the dysregulation of the physiological mechanisms concerned with the metabolism of energy substrates, resulting in excessive fat storage in various tissues and organs (Grabner *et al*., 2021). Pathogenic mechanisms contributing significantly to the development of MetS include reduced total antioxidant capacity, impaired glucose uptake and utilization to altered insulin sensitivity by cells, and disturbed fatty acid metabolism (James *et al*., 2012; Monserrat-Mesquida *et al*., 2020). Increased oxidative stress associated with MetS results in accelerated generalized inflammatory responses, decreased vascular compliance, atherosclerosis, and hypertension (Grandl and Wolfrum, 2018).

The pathophysiology of MetS has been closely linked with abnormalities in the liver and the kidneys. As a result, these organs have been suggested to hold great potential as therapeutic targets in the management of MetS (Kotronen and Yki-Järvinen, 2007). In most cases, MetS is associated with Non-Alcoholic Fatty Liver Disease (NAFLD), characterized by the accumulation of fat in more than 5% of hepatocytes in the absence of excessive alcohol consumption (Wang *et al*., 2022). The strong association between NAFLD and MetS is highlighted by the presence of at least one component of MetS in 90% of NAFLD patients and at least three components of MetS in 33% of NAFLD patients (Almeda-Valdés *et al*., 2009). Moreover, hepatic lipid deposition positively correlates with several features of insulin resistance, a central factor in the pathophysiology of MetS, and low hepatic insulin sensitivity is associated with increased liver adipose tissue content (Rector *et al*., 2008). Likewise, chronic kidney disease is commonly associated with MetS, with severe histopathological lesions including glomerulonecrosis and loss of renal function reported in many patients (Alexander *et al*., 2009; Thomas *et al*., 2011; Carbone *et al*., 2013; Grupper *et al*., 2022).

In both renal and hepatic tissues, MetS-induced oxidative stress resulting from excessive production of reactive oxygen species has been implicated in the toxic mechanisms leading to tissue damage (Fortuno *et al*., 2006). For instance, the NADPH oxidase (NOX) family of enzymes, including NOX1, 2, and 4, which are highly expressed in the kidney, stimulate the production of reactive oxygen species in renal tissues, thereby aggravating oxidative stress with consequent induction and exacerbation of renal disease progression (Panday *et al*., 2015). Also, oxidative stress is considered one of the causative factors of liver damage, and MetS associated excessive lipid deposition in the liver may predispose to steatosis and NAFLD, generally recognized as the manifestation of MetS in the liver (Perdomo *et al*., 2019; Palladini *et al*., 2019). As a result, recent therapeutic strategies involved in the management of MetS are often geared towards the reduction of exaggerated oxidative stress in various tissues and organ using synthetic and natural antioxidants, as well as medicinal plants with high phytochemical constituents and validated antioxidant efficacies.

Dissotis rotundifolia is a medicinal plant used traditionally for the treatment of many medical conditions in several African countries including Nigeria (Gill, 1992). The plant belongs to the family Melastomataceae and is commonly referred to as pink lady (Baba and Onanuga, 2011). In different parts of Nigeria, *D. rotundifolia* is locally called Nkpisi-nku in Igbo, Ebafo in Benin and Awede in Yoruba (Friday *et al*., 2009). *D. rotundifolia* has potent antioxidant activity and has been reported to upregulate enzymatic and non-enzymatic antioxidants in *in vivo* studies (Adinortey *et al*., 2020; Djehoue *et al*., 2020). This study was designed to evaluate the probable ameliorative effects of *D. rotundifolia* whole plant on hepatic and renal toxicities associated with MetS in male Wistar rats.

MATERIALS AND METHODS

Collection and identification and preparation of *Dissotis rotundifolia* extract*:* *Dissotis rotundifolia* whole plants were collected from a farm at Erunmu, Oyo state. The plant was identified and deposited at the University of Ibadan Herbarium (UIH 22906). The plant sample was washed, cut into smaller pieces and air dried at room temperature (27°C–30°C) for four weeks. The dried plant was thereafter pulverised into its powdery form, and 1000g of this powder was extracted in 10 L of 95% ethanol (analytical grade) for two weeks using the cold extraction method (Gbadamosi *et al*., 2022). The extract was concentrated at 40 °C using a rotary evaporator, and the concentrated extract was defatted using n-hexane. The defatted ethanol extract was then stored in the refrigerator (4^{o}C) before experimental use.

Chemicals: The biochemical analyses in this study involved the use of several chemicals such as 1, 2-dichloro-4-nitrobenzene (CDNB), 5, 5'-dithio-bis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), thiobarbituric acid (TBA), glutathione, hydrogen peroxide, sodium hydroxide, epinephrine, and xylenol orange from Sigma (St. Louis, MO). Also, normal goat serum, biotinylated antibody, and horse radish peroxidase (HRP) System were purchased from Kirkegaard & Perry Lab Inc (Gaithersburg, MD). NF-kB anti-body was purchased from Bioss Inc. (Woburn, MA), while diaminobenzidine (DAB) tablets were purchased from AMRESCO (LLC. OH). All other chemicals used were of analytical grade - British Drug Houses (Poole, Dorset, UK).

Antibodies: Biotinylated secondary antibodies: 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution and primary antibodies against Angiotensin Converting Enzyme1 Polyclonal Antibody (E-AB-16159: 1:500 Dilution) and Neutrophil Gelatinase-Associated Lipocalin (NGAL), Polyclonal Antibody (E-AB-16061: 1:200 Dilution) were purchased from Elabscience Biotechnology®, China).

Ethical Approval

All experiments and protocols were carried out in accordance with the guidelines of the Faculty of Veterinary Medicine, and University of Ibadan Animal Care and Use Research Ethical Committee (UI-AUREC/19/0119).

Experimental Animals Procurement and Acclimatization: Fifty (50) male Wistar rats (150 -180 g) were obtained from the Experimental Animal Unit of the Faculty of Veterinary Medicine and transported to the

Animal house of the Department of Veterinary Biochemistry and Physiology, University of Ibadan. The animals were kept in pathogen-free cages at room temperature (25-27 ºC) in a well-ventilated house under natural environmental light conditions for the period of acclimatization and throughout the experiment. They were acclimatized for two (2) weeks, fed with standard animal diet (commercial pelletized rat finisher) and allowed access to drinking water *ad libitum.*

Induction of metabolic syndrome and Grouping of animals: Metabolic syndrome was induced by feeding the rats with high carbohydrate (20%) fructose in drinking water and high fat diet (40%) for 8 weeks as earlier described by previous authors (Kohli *et al*., 2010; Mahmoud and Elshazly, 2014; Barrios-Ramos *et al*, 2014). The defatted ethanol extract of *Dissotis rotundifolia* and the standard drug for metabolic syndrome (Rosuvastatin) were administered by oral gavage daily for eight weeks, respectively. Rosuvastatin (Ros) is a member of the satin family with higher efficacy in reducing bad cholesterol (LDL cholesterol) than other statins due to a higher number of binding interactions with 3-hydroxy-3methyl-glutaryl-coenzyme A reductase (HMG-CoA), a rate limiting enzyme in cholesterol synthesis (Li *et al*., 2023).
Fifty adult male Wistar rats weighing between 150-180g were divided into five groups of ten rats each for the experiments as follows: Group 1 (Control), Group 2 (MetS control), Group 3 (MetS + 100 mg/kg ethanol extract of *Dissotis rotundifolia*) (Adinortey et al., 2020), Group 4 (MetS + 200 mg/kg ethanol extract of *Dissotis rotundifolia*) (Adinortey et al., 2020), *and* Group 5 (MetS + 20 mg/kg Rosuvastatin).

Serum Preparation and Isolation of Post-Mitochondrial Fraction: Approximately 3 mL of blood was collected from the retro-orbital venous plexus of the animals into plain sample bottles before they were sacrificed by cervical dislocation. The blood was centrifuged at 4000 rpm for 15 min to obtain the serum, which was preserved at 4ºC, and used for biochemical analysis. The liver and kidney were harvested on ice, rinsed, and homogenized in aqueous potassium buffer (0.1 M, pH 7.4) and the homogenate centrifuged at 10,000 rpm (48C) for 10 min to obtain the supernatant fraction.

Biochemical Assays: The post-mitochondrial fractions of the liver and kidney were used for the estimation of reduced glutathione (GSH) as described by Beutler *et al*. (1993). Glutathione S-transferase (GST) was measured by the method of Habig *et al*. (1974) and Glutathione peroxidase (GPx) activity was determined as described by Rotruck *et al*. (1973). Superoxide dismutase (SOD) was determined by measuring the inhibition of auto-oxidation of epinephrine at pH 10.2 as described by Misra and Fridovich (1972) and with modification from our laboratory (Oyagbemi *et al*., 2015). The MDA level was calculated as described by Varshney and Kale (1970). Hydrogen peroxide (H_2O_2) generation was estimated as described (Wolff, 1994). Protein concentration was determined by Biuret method as described by Gornall *et al*. (1949).

Immunohistochemistry

Immunohistochemical staining and imaging: Immunohistochemistry was performed as described by Oyagbemi *et al.* (2023). Antibodies against renal angiotensin-converting enzyme (ACE) and Neutrophil gelatinase-associated lipocalin (NGAL) were probed in the kidney with slight modification using 2-step plus Poly-HRP Anti Mouse/Rabbit IgG Detection System with DAB solution (Catalog number: E-IR-R217 from Elabscience Biotechnology®, China). The kidney samples were fixed with 10% neutral buffered formalin, embedded in paraffin wax, and sectioned at a thickness of 5 µm. The slides were subsequently dewaxed in xylene (100%) solution for 2 minutes and afterward, hydration was carried out in different concentrations of ethanol (100%, 90%, and 80%) for 2 minutes each. The hydrated slides were rinsed and put in a PBS buffer tank for 5 mins. The antigen retrieval was performed with citrate buffer solution containing 2.1 g of citric acid monohydrate and 14.75 g of trisodium citrate dehydrate adjusted to pH 6.0 in microwave oven. Endogenous peroxide (H_2O_2 block) was carried out following the manufacturer's instructions as directed on the kit (E-IR-217C). Drops of H_2O_2 were added to cover the sections and incubated in a humidifying chamber at room temperature for 10 min. The slides were rinsed afterwards and put back in the PBS tank for 5 min. Goat serum (E-1R-R217A) was added onto the slides to prevent nonspecific binding and incubated in humidifying chamber at room temperature (35ºC) for 30 mins. After 30 mins of incubation, the tissues were probed with primary antibodies viz-a-viz Angiotensin Converting Enzyme1 Polyclonal Antibody (E-AB-16159: 1:500 Dilution) and Neutrophil gelatinase-associated lipocalin (NGAL) Polyclonal Antibody (E-AB-16061: 1:150 Dilution) for kidney and were incubated for 2 hours at room temperature. Following incubation, the slides were rinsed with PBS and secondary antibody labelled (E-1R-R217B) was added, and the slides were incubated in humidifying chamber at room temperature for 20 min. Thereafter, the slides were rinsed and immersed in PBS tank for 5 min. Finally, a few drops of the substrate diaminobenzidine (DAB) was added at room temperature for 10 s; 50 µL of DAB concentrate (E-1R-R217D) + 1 mL DAB solution (E-1R-R217E) in the dark. The reaction was terminated with deionized water and slides were immersed in haematoxylin for 3 s before rinsing with PBS. The slides were placed in 80%, 90%, and 100% of ethanol, and then xylene (100%) for 2 minutes each. Slides were removed, allowed to dry and a DPX mountant was applied. Sections were examined using a digital camera and a Leica software application package version 3.4 light microscope (Leica LAS-EZ®).

Statistical analysis

All values are expressed as mean ± standard deviation (SD). The test of significance between two groups was estimated by Student's t-test. One way Analysis of Variance (ANOVA) with Tukey's post-hoc test with p-values < 0.05 considered statistically significant.

RESULTS

Effect of *D. rotundifolia* on Kidney and Liver function Tests: The result of the effect of *D. rotundifolia* extract on markers of kidney and liver function in MetS induced rats is

presented in Figure 1. The blood urea nitrogen (BUN) and creatinine increased significanly ($p<0.05$) in the MetS group. *D. rotundifolia*at 100 mg/kg caused significant ($p<0.05$) decrease in the urea compared with the MetS rats without *D. rotundifolia* treatment. However, *D. rotundifolia* extract at 200 mg/kg did not confer significant protection against renal damage caused by MetS. From the experiment, significant ($p<0.05$) increase was obtained in serum ALT and AST in MetS untreated rats compared ~~to~~ with the control. The extract of *D. rotundifolia* at 100 mg/kg and 200 mg/kg, and Ros caused a significant ($p<0.05$) decrease in ALT activity, while there were no significant change~~s~~ in the activity of AST following treatment with *D. rotundifolia* at 100 mg/kg and 200 mg/kg and Ros in comparison ~~to~~ with the control, respectively..

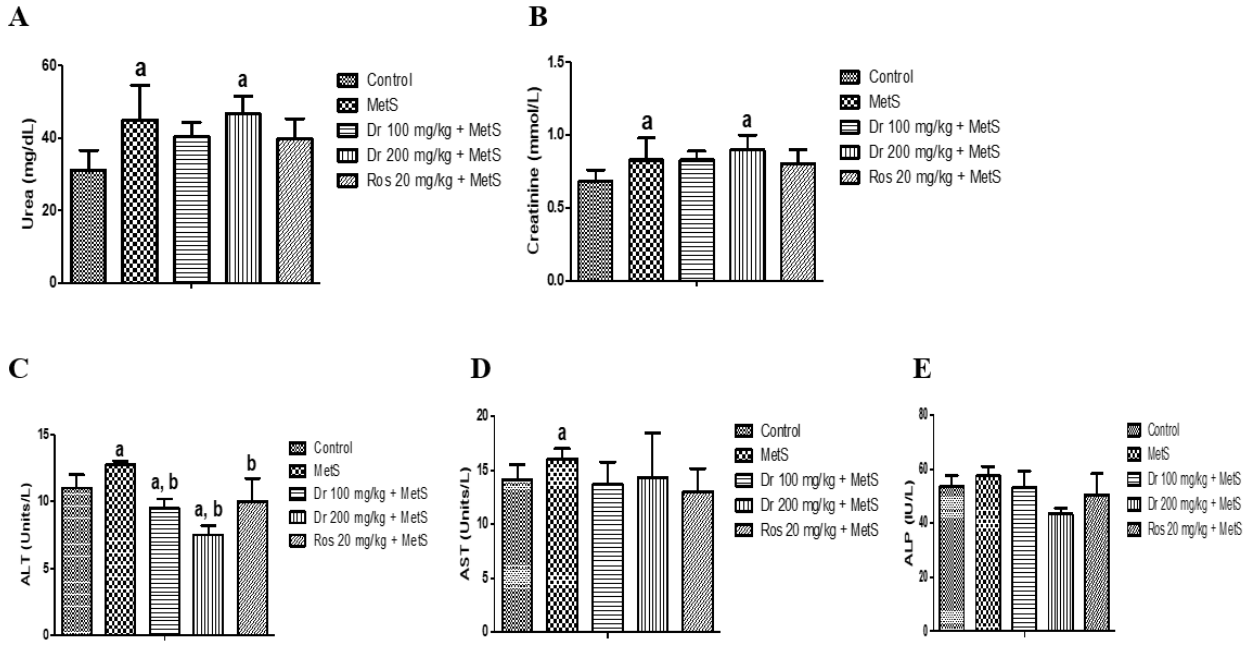

Figure 1:
The ameliorative effects of *Dissotis rotundifolia* on serum kidney and liver function tests.
Superscript (a) indicates significant difference when compared to the control and Superscript (b) indicates significant difference when compared to metabolic syndrome. $P < 0.05$ was taken as statistically significant difference. Mean ± SD (n= 5).
Abbreviations: MetS (Metabolic syndrome), Dr (*Dissotis rotundifolia*), Ros (Rosuvastatin).

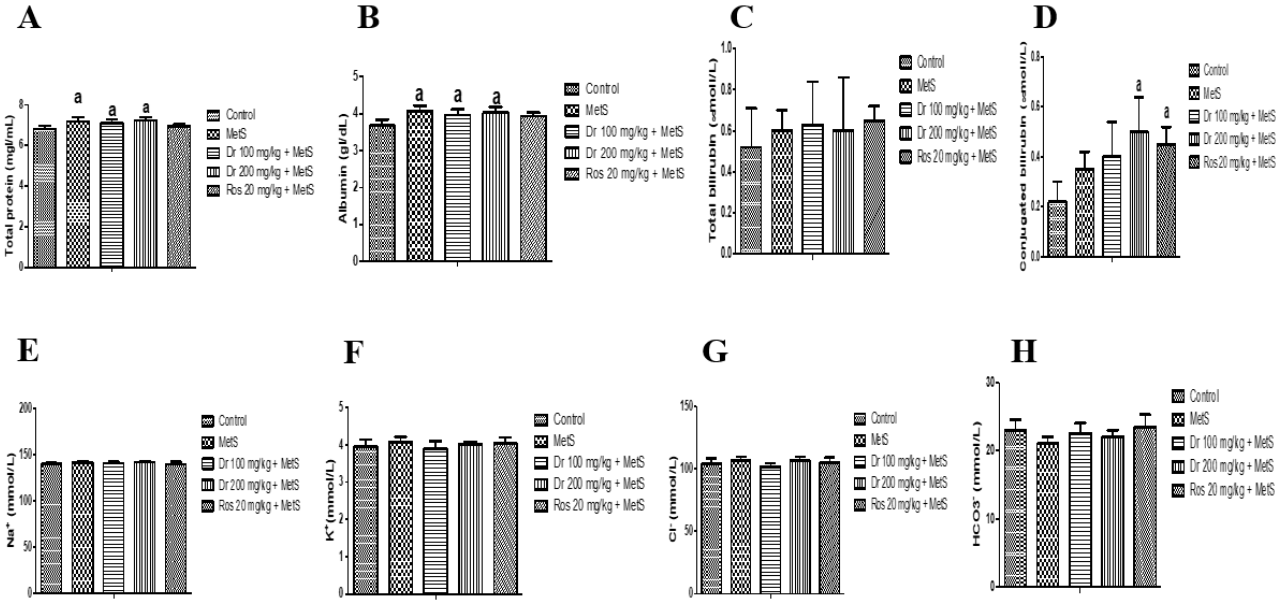

Figure 2:
The ameliorative effects of *Dissotis rotundifolia* on serum proteins and electrolytes. Superscript (a) indicates significant difference when compared to the control and Superscript (b) indicates significant difference when compared to metabolic syndrome. $P < 0.05$ was taken as statistically significant difference. Mean ± SD (n= 5). **Abbreviations:** MetS (Metabolic syndrome), Dr (*Dissotis rotundifolia*), Ros (Rosuvastatin).

Effect of *D. rotundifolia* on Serum Proteins and Electrolytes: The findings from serum proteins show a significant increase in the values of total proteins and albumin recorded in MetS rats and *D. rotundifolia*-treated

rats at 100 mg/kg and 200 mg/kg, relative to the control, respectively (Figure 2). For total bilirubin, there was no statistically significant difference in the values recorded for serum total bilirubin across treatment groups and the MetS untreated rats. However, treatment of MetS rats with *D. rotundifolia* 200 mg/kg and Ros (20 mg/kg) significantly increased the values of conjugated bilirubin compared with the control. There was no statistically significant difference in the serum sodium, potassium, chloride, and bicarbonates across all groups . However, the value of serum bicarbonates was significantly lower in MetS untreated group compared with the control.

Effect of *D. rotundifolia* on lipid profile: The results of *D. rotundifolia* extract on lipid profiles of MetS-induced rats is presented in Figure 3. MetS caused significant ($P<0.05$) increase in the total cholesterol (TC), triglyceride (TAG), low density lipoprotein-cholesterol (LDL-c), and high-density lipoprotein-cholesterol (HDL-c) when compared to the control and other treatment groups. Also,*D. rotundifolia* caused significant reduction in serum TC, TAG, and LDL-c. The HDL-c values of MetS rats were significantly higher that of the control and MetS rats treated with the extract of *D. rotundifolia* and Ros. The values of HDL-c for *D. rotundifolia* and Ros treatment groups were significantly lower than that of the MetS untreated rats.

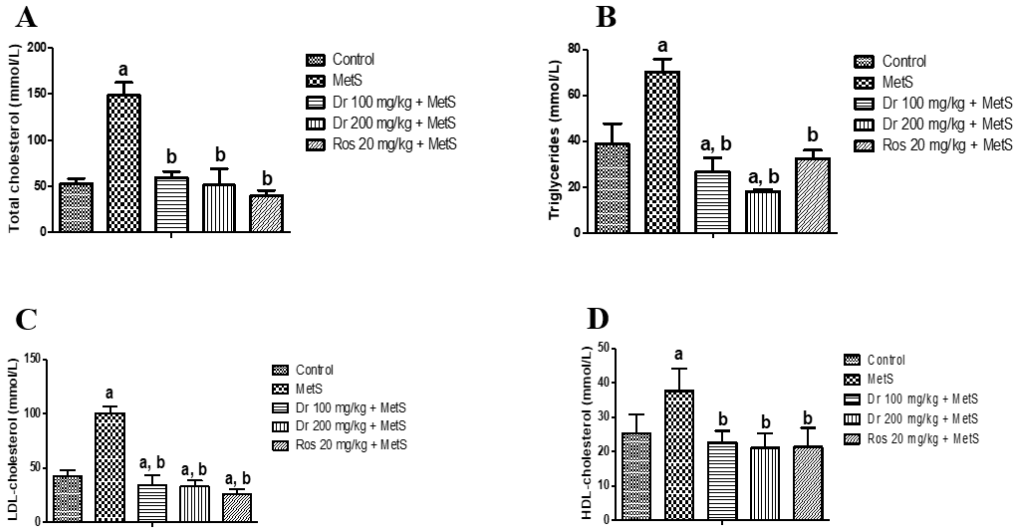

Figure 3:
The ameliorative effects of *Dissotis rotundifolia* on serum lipid profile. Superscript (a) indicates significant difference when compared to the control and Superscript (b) indicates significant difference when compared to metabolic syndrome. $P < 0.05$ was taken as statistically significant difference. Mean ± SD (n= 5). **Abbreviations:** MetS (Metabolic syndrome), Dr (*Dissotis rotundifolia*), Ros (Rosuvastatin).

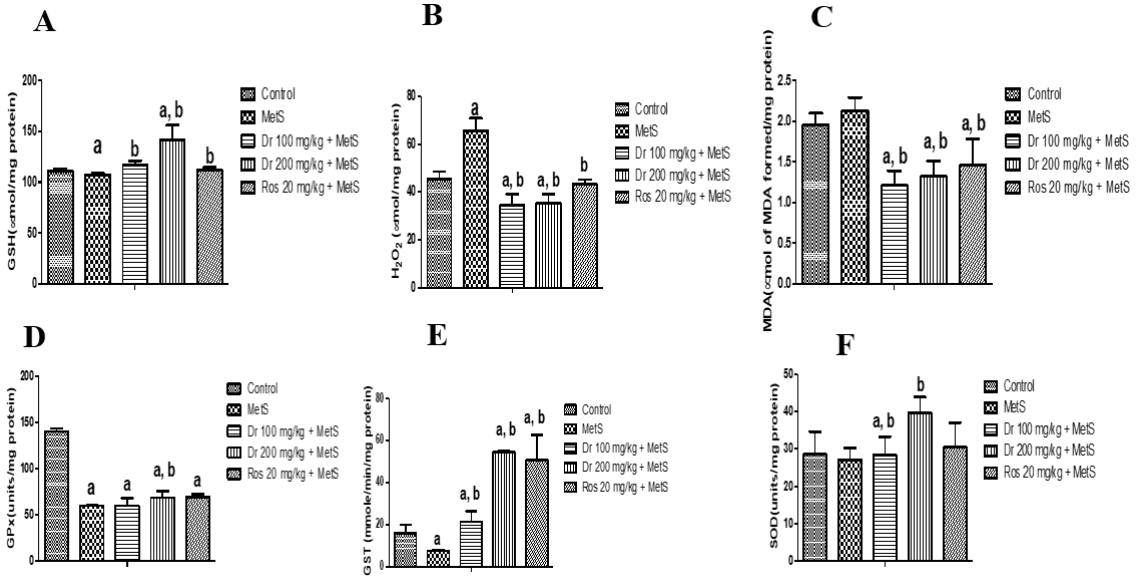

Figure 4:
The ameliorative effects of *Dissotis rotundifolia* on renal oxidative stress biomarkers. Superscript (a) indicates significant difference when compared to the control and Superscript (b) indicates significant difference when compared to metabolic syndrome. $P < 0.05$ was taken as statistically significant difference. Mean ± SD (n= 5). **Abbreviations:** MetS (Metabolic syndrome), Dr (*Dissotis rotundifolia*), Ros (Rosuvastatin).

Effect of *D. rotundifolia* on biomarkers of renal oxidative stress: The renal reduced glutathione (GSH) content in MetS untreated was significantly lower ($p<0.05$) than that in the control (Figure 4). Furthermore, hepatic GSH of rats treated with *D. rotundifolia* extract and Ros was significantly higher relative to the MetS rats. A statistically

significant increase in the values of renal hydrogen peroxide (H_2O_2) generation was recorded for MetS rats when compared to the control. *D. rotundifolia* extract and Ros significantly reduced renal biomarkers of oxidative stress in MetS treated rats. The antioxidant activity of superoxide dismutase (SOD) was enhanced by *D. rotundifolia* extract (100 mg/kg and 200 mg/kg) when compared to the MetS untreated rats. Also, MetS rats had a significant reduction in the antioxidant activities of glutathione peroxidase (GPx) and glutathione S-tranferase (GST) in comparison to the control. On the other hand, treatment of MetS rats with *D. rotundifolia* extract (100 mg/kg and 200 mg/kg) caused a significant increase in the GPx and GST activities in a dose-dependent manner.

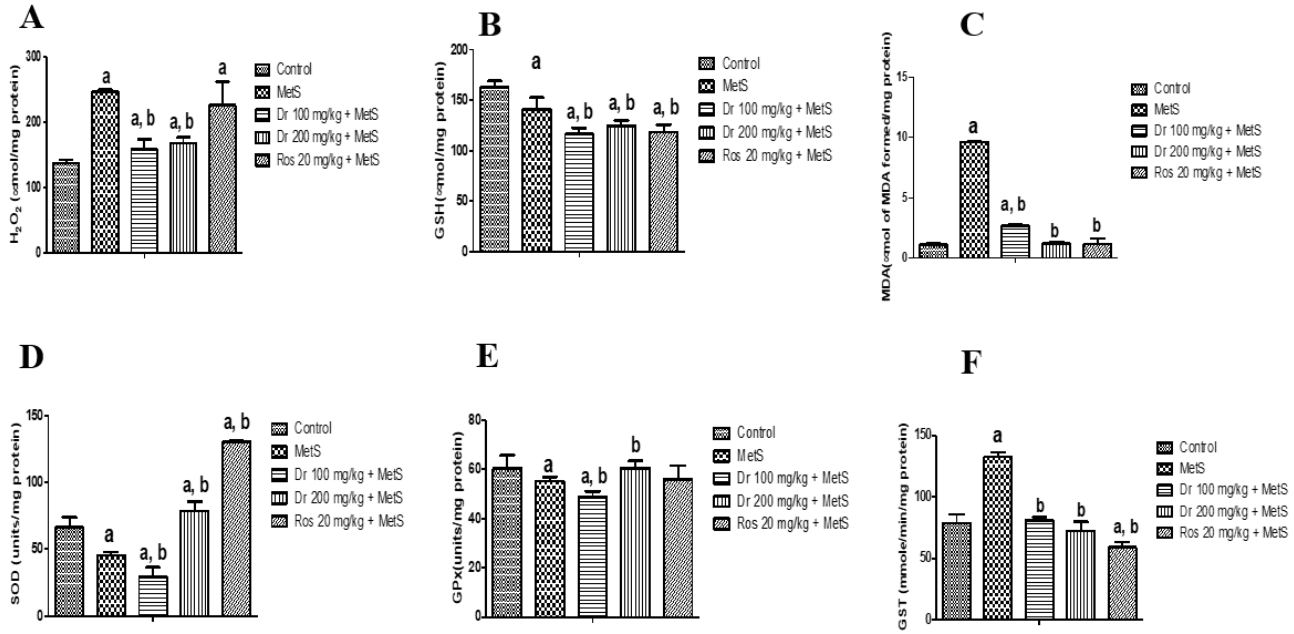

Figure 5:
The ameliorative effects of *Dissotis rotundifolia* on hepatic biomarkers of oxidative stress. Superscript (a) indicates significant difference when compared to the control and Superscript (b) indicates significant difference when compared to metabolic syndrome. $P < 0.05$ was taken as statistically significant difference. Mean ± SD (n= 5). **Abbreviations:** MetS (Metabolic syndrome), Dr (*Dissotis rotundifolia*), Ros (Rosuvastatin).

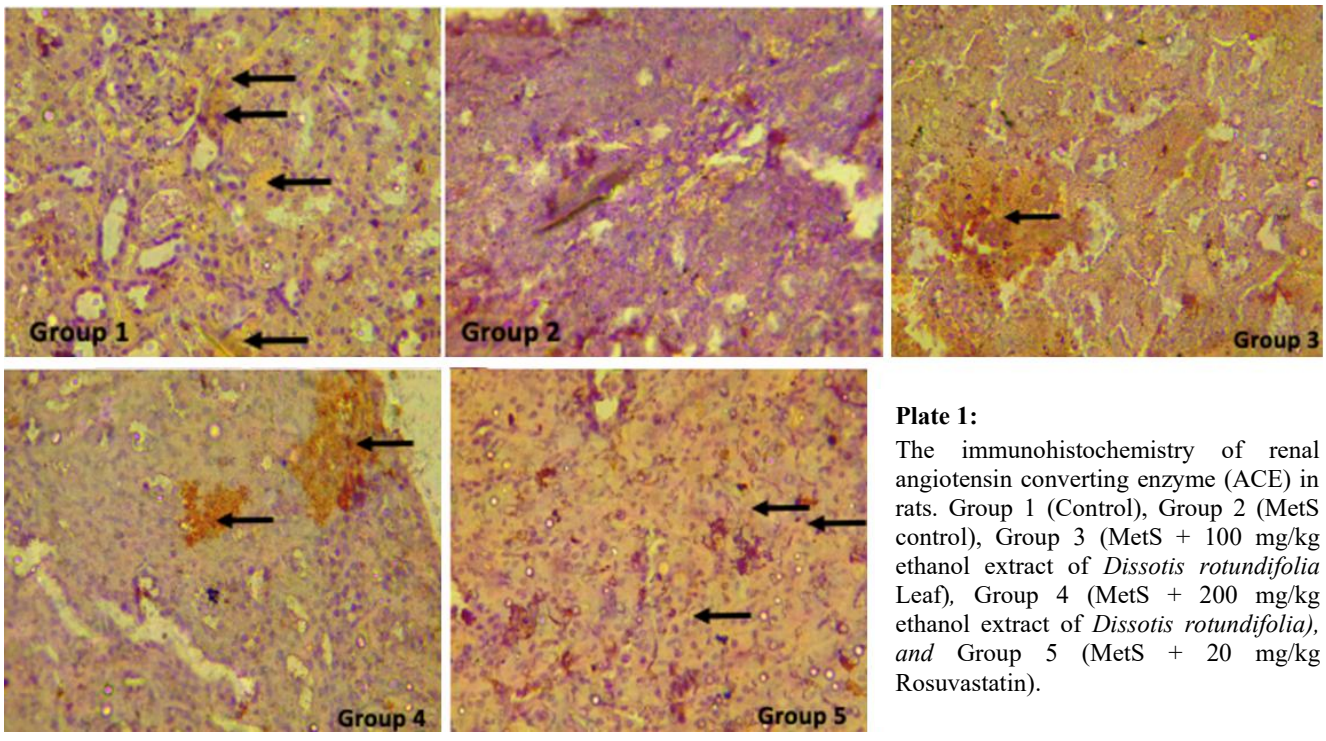

Plate 1:
The immunohistochemistry of renal angiotensin converting enzyme (ACE) in rats. Group 1 (Control), Group 2 (MetS control), Group 3 (MetS + 100 mg/kg ethanol extract of *Dissotis rotundifolia* Leaf), Group 4 (MetS + 200 mg/kg ethanol extract of *Dissotis rotundifolia), and* Group 5 (MetS + 20 mg/kg Rosuvastatin).

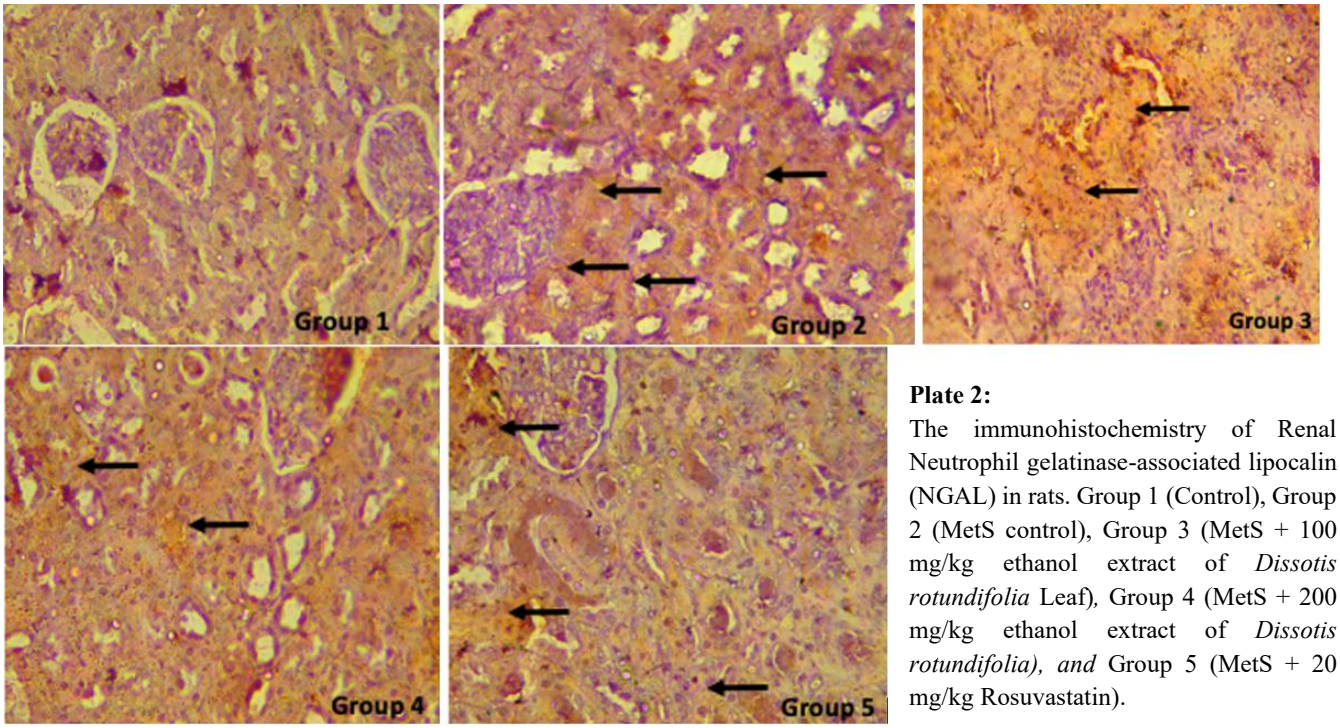

Plate 2:
The immunohistochemistry of Renal Neutrophil gelatinase-associated lipocalin (NGAL) in rats. Group 1 (Control), Group 2 (MetS control), Group 3 (MetS + 100 mg/kg ethanol extract of *Dissotis rotundifolia* Leaf), Group 4 (MetS + 200 mg/kg ethanol extract of *Dissotis rotundifolia), and* Group 5 (MetS + 20 mg/kg Rosuvastatin).

Effect of *D. rotundifolia* on biomarkers of hepatic oxidative stress: In this study, a significant reduction in hepatic GSH content, with a concomitant significant increase in hepatic H_2O_2 generation and MDA were recorded in the MetS group compared with the control and *D. rotundifolia* extract (100 mg/kg and 200 mg/kg) treated groups (Figure 5). Furthermore, *D. rotundifolia* extract caused a significant reduction in hepatic H_2O_2 generation and MDA in comparison to the MetS untreated rats. However, MetS caused a significant reduction in hepatic SOD and GPx activities when compared to the control. Also, *D. rotundifolia* at 200 mg/kg dose significantly increased hepatic SOD and GPx activities relative to the control and MetS rats. MetS significantly caused a significant increase in hepatic GST activity when compared to the control and other treatment groups. However, the extract of *D. rotundifolia* (100 mg/kg) gave a higher value of hepatic GST activity in comparison to the control.

Immunohistochemistry of renal angiotensin-converting enzyme (ACE) and neutrophil gelatinase-associated lipocalin (NGAL): In this study, immunohistochemistry revealed lower expression of angiotensin-converting enzyme (ACE) in MetS-untreated rats relative to the control and *D. rotundifolia-treated* rats at 100 and 200 mg/kg doses. However, *D. rotundifolia* at 100 and 200 mg/kg and Ros showed higher immune-positive reactions of ACE relative to the control and MetS untreated rats, respectively (Plate 1). The immunoreactivity of neutrophil gelatinase-associated lipocalin (NGAL) was higher in MetS untreated rats in comparison to the control rats (Plate 2).

DISCUSSION

Oxidative stress has been severally reported in the pathogenesis and pathophysiology of MetS and the individual components of the syndrome (Vona *et al*, 2019). As a result, in-depth investigations on oxidative-mediated organ-specific damage in disease conditions such as obesity, diabetes, and hypertension, which are clustered in MetS are currently ongoing globally (Carrier, 2017). Specifically, MetS-associated abdominal adiposity promotes inflammation and oxidative stress and other complications such as insulin resistance, hypertension, and hyperlipidemia (Rani *et al*., 2016; Francisqueti *et al*., 2017). In this study, the establishment of metabolic syndrome was manifested as elevated levels of total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) in the plasma, with decreased levels of high-density lipoprotein cholesterol (HDL-C), as earlier reported (He *et al*., 2022). Increased biomarkers of oxidative stress (OS) and decreased antioxidant defenses have been measured in the blood of patients with MetS, suggesting an *in vivo* overproduction of oxidizing species (Spahis *et al*., 2017). Increased oxidative stress can result from several variables, such as high-fat and high-carbohydrate diets, and persistent undernutrition, by activating intracellular pathways such as nicotinamide adenine dinucleotide phosphate (NAPDH) oxidase (NOX), oxidative phosphorylation in mitochondria, glycoxidation, protein kinase C (PKC), and the polyol pathway (Korac *et al*., 2021). Furthermore, changing dietary intake from organic healthy foods to highly processed foods may lead to increased exposure to advanced glycated end products aged garlic extracts (AGEs) by a non-enzymatic chemical reaction called glycation (Fallavena *et al*., 2022).

Diets with high antioxidant content have been reported to provide beneficial effect in the management of MetS-associated oxidative stress and inflammation (Castro-Quezada *et al*., 2014; Casas *et al*., 2014). Similarly, medicinal plants and plants derived phytochemicals have been reported to positively modulate the pathophysiology and disease progression in metabolic syndrome patients (Rochlani *et al*., 2017). The observation of increased hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) levels in the MetS group of rats, in this study, suggests the induction of oxidative stress, whereas, the significantly

increased level of the antioxidants reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathione transferase (GST) in rats treated with the defatted ethanol extract of *D. rotundifolia* suggests a potent antioxidant effect of the plant. . Observation in this study corroborates an earlier report of Adinortey *et al.* (2020), who reported a conservation of GSH levels, reduced MDA levels, and enhanced SOD activity in rats administered flavonoid-rich extract of *D. rotundifolia* (Adinortey *et al*., 2020). Medicinal plants, some of which have been used for thousands of years, serve as an excellent source of bioactive compounds for the treatment of MetS because they contain a wide range of phytochemicals with diverse metabolic effects (Graf *et al*., 2010). A wide array of bioactive phytochemicals from medicinal plants, such as turmeric, garlic, cinnamon, ginger, grapes, onions, and broccoli have demonstrated a positive modulatory role in the management of MetS (Rochlani *et al*., 2017). *Dissotis rotundifolia* has potent antioxidant activity that may be attributable to the high levels of flavonoids, phenols and saponins (Djehoue *et al*., 2020; Ezeabara *et al*., 2022; Gbadamosi *et al*., 2022). Flavonoids are distributed in foods and have been severally reported to provide beneficial effects in the management of many diseases by significantly modulating several metabolic parameters, such as lipid profile, blood pressure, and blood glucose (Gouveia *et al*., 2022). Moreover, natural polyphenols generally have antioxidant and anti-inflammatory effects, and have been reported to aid vascular functioning, promote gastrointestinal digestion, lower blood lipids, prevent atherosclerosis, and lower blood pressure (Cai *et al*., 2015; Bruno and Ghiadoni, 2018 *l*., 2018; Zhang *et al*., 2021). Furthermore, saponin containing medicinal plant products such as ginseng has been shown to increase fasting insulin sensitivity index and exert anti-insulin resistance as well as anti-obesity activity (Luo *et al*., 2020). Therefore, the normalisation of the lipid profile in rats treated with the defatted ethanol extract of *D. rotundifolia* compared with the control group, in this study, may be attributable to the high polyphenolic antioxidant effects of this plant, as earlier reported (Darkwah *et al*., 2018).

The liver enzymes aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are routinely used clinically to assess the functional status of the liver. In this study, the induction of MetS in rats caused elevated serum levels of AST, ALT, and ALP, compared with the control, and the MetS rats that were treated with the defatted ethanol extract of *D. rotundifolia*, thus suggesting a hepatoprotective effect of the plant in MetS. Elevated ALT in the serum often suggests specific damage to hepatocytes and has been reported to be linked with various risk factors for metabolic syndrome, diabetes, and cardiovascular diseases such as obesity, hyperglycemia, dyslipidemia and increased blood pressure (Sanyal *et al*., 2015; Kathak *et al*., 2022, Gbadamosi *et al*., 2020). Thus, the observed reduced levels of the hepatic enzymes in rats administered *D. rotundifolia* extract strongly suggest hepatoprotective effects, and a desirable modulatory effect of the whole plant extract in MetS.

The elevation of creatinine and blood urea nitrogen observed in MetS-induced rats without *D. rotundifolia* treatment strongly suggests an induction of kidney damage. Blood urea nitrogen often increases due to inadequacies in its removal by the kidney, but may also be associated with several physiological conditions, such as high protein intake, intestinal bleeding, infection, fever, dehydration, medications, burns, and poisoning (Weiner et al., 2014). However, an increase in blood urea nitrogen in the presence of concomitant increased creatinine levels often signifies renal damage resulting from MetS-associated cardiovascular dysfunctions or impaired renal blood flow (Rivadeneyra-Domínguez *et al*., 2018). Likewise, increased serum creatinine levels, often associated with kidney damage, have been reported to increase in metabolic syndrome patients (Wang *et al*., 2015). Several reports have suggested significant increased likelihood of the development of chronic kidney disease characterized by changes in renal structure, decreased glomerular filtration rate (GFR), and increased urinary microalbumin in patients with MetS (Zhang and Lerman, 2017; Kawamoto *et al*., 2019).

The involvement of chronic kidney disease recently, has become one of the major risk factors in MetS (Kazancioğlu, 2023; Lin *et al*., 2023; Zohara *et al*., 2023; Scurt *et al*., 2024). The neutrophil gelatinase-associated lipocalin (NGAL) is a 25-kDa protein that is secreted mostly by immune cells such as neutrophils, macrophages, and dendritic cells. Its production is stimulated in response to inflammation (Romejko *et al*., 2023). The NGAL is known mainly as a biomarker of acute kidney injury, chronic kidney disease, and is released after tubular damage and during renal regeneration processes (Voth *et al*., 2023). Also, NGAL is useful in the diagnostic processes of cardiovascular diseases because it is highly expressed in injured heart tissue and atherosclerostic plaque (Yewale *et al*., 2023; Voth *et al*., 2023). The data from the immunohistochemistry revealed renal damage associated with MetS as indicated with highly immune-positive reactions of NGAL. The protection of the renal tissues from MetS could be associated with reduction in the NGAL immune reactivity of MetS rat treated with *D. rotundifolia* extract. Another major risk factor for MetS is hypertension. Previous research findings have reported positive association between hypertension and MetS (Das *et al*., 2023; Stanciu *et al*., 2023; Soleimani *et al*., 2023). For this reason, the immunoreactivity of angiotensin converting enzyme (ACE) was assessed. Also in this study, the immune reactivity of MetS rat treated with defatted *D. rotundifolia* extract ACE was higher than the Mets untreated rats. The angiotensin-converting enzymes (ACE and ACE2) are highly expressed in renal tubules and play an important role in the regulation of renal function by the intrarenal renin-angiotensin system (Larrinaga *et al*., 2010). ACE 2 degrades angiotensin (Ang) II to Ang (1–7) and Ang I to Ang (1–9) (Ye et al., 2004) and may reduce the incidence of hypertension since angiotensin II is a potent vasoconstrictor. Therefore, the mechanism of nephroprotection and the antihypertensive effects of *D. rotundifolia* could be through the inhibition of NGAL and upregulation of ACE signaling pathway.

In conclusion, the defatted ethanol extract of *D. rotundifolia* whole plant offered protection against metabolic syndrome-induced liver and kidney damage and hepatorenal oxidative stress, improved antioxidant defence status, and mitigated hyperlipidemia.

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