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ORIGINAL ARTICLE

Hypoglycemic and hepatoprotective effect of Rhizophora mucronata and Avicennia marina against streptozotocin-induced diabetes in male rats

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ABSTRACT

Objectives: Aqueous extracts of *Rhizophora mucronata* and *Avicennia marina* leaves were investigated for their hepatoprotective potential in diabetic rats.
Materials and methods: One hundred twenty male albino rats were randomly assigned to eight equal groups (n = 15). The first group (control) comprised normal healthy rats, while the second to fifth groups were intraperitoneally injected with a single dose of streptozotocin (STZ) [60 mg/kg body weight (BW)] for induction of diabetes. Group 2 was kept as positive diabetic control, while groups 3–5 were orally treated with aqueous extracts of *R. mucronata* (400 mg/kg BW), *A. marina* (400 mg/kg BW) and with a combination of ½ a dose of the two plants, respectively, for six weeks. Groups 6–8 were non-diabetic rats that orally received aqueous extracts of *R. mucronata* (400 mg/kg BW), *A. marina* (400 mg/kg BW), and a combination of ½ a dose of the two plants, respectively, for 6 weeks.
Results: STZ-induced diabetic rats showed a significant reduction in serum glucose and liver enzymes, increased serum insulin, Homeostasis Model Assessment of β-cells (HOMA-β), and Homeostasis Model Assessment of Insulin Resistance (HOMA-IR). Histopathological and immunohistochemical examinations of the liver revealed improved pathologic criteria in the plant extract treated diabetic rats compared with the remarkable changes which had been seen in STZ-induced diabetic rats.
Conclusion: This study suggests that the aqueous extract of *R. mucronata* or its combination with *A. marina* showed potent hypoglycemic and hepatoprotective effects for liver dysfunction, as well as histopathological and immunohistochemical changes in the liver of STZ-induced diabetic rats.

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R. mucronata, A. marina, diabetes, STZ, liver, immunohistochemistry

Introduction

Diabetes mellitus (DM), "the silent killer," is one of the complicated metabolic chronic medical problems as reported in various diabetic patients [1]. The disease occurs due to diminishing tissue response to insulin or production by β-cells of the pancreas, which results in increased blood glucose levels [2]. The number of diabetic patients is in a continuous increase; about 378 million diabetic patients are currently recorded according to the International Diabetic Federation, this number could reach up to 592 million by the end of 2035 [3]. Diabetic patients are usually unable to utilize glucose fully; consequently, it starts to accumulate in the blood, leading to hyperglycemia [4].

The liver is the main organ that plays a role in the synthesis and metabolism of glucose. In diabetes mellitus (DM), along with a total or incomplete shortage of insulin, disturbances in cell metabolism, including carbohydrate, protein, and fat metabolism, also take place [5]. Several investigations have reported that Streptozetocin (STZ) can induce DM. It is considered as an essential chemical to study the pathophysiological mechanisms of DM and the hyperglycemic performance [6]. STZ is toxic to insulin-secreting cells and has been commonly used for the induction of DM with associated insulin insufficiency [7].

Various scientific studies have reported that insulin deficiency can lead to hyperglycemia, which in turn is associated

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with several diabetic complications and disturbances, such as nephropathic and vascular diseases, but only a few pieces of information are available on the possible relationship of diabetic complications and liver functions [8].

Noticeable progress has been documented in the management of DM that usually take different forms, such as diet regimens, exercise, insulin replacement therapy, and use of oral antidiabetic agents. However, the treatment of DM and its complications is still a mystery. Some of the plants' extracts are widely used as oral hypoglycemic agents. *Rzophora mucronata* Lam. of the family *Rhizophoraceae* is a true mangrove plant, which is generally distributed on the river banks of the Indo-Pacific areas and the sea edges. It is the individual mangrove species that can be easily found in East Africa [9]. Plant extracts of mangrove trees are traditionally used in the areas of Asian and African subcontinents for treating health ailments, such as diabetes, diarrhea, hepatitis, inflammation, and cognitive dysfunction [10]. *Avicennia marina* belongs to the family Avicenniaceae, commonly known as grey mangrove. The extracts from *Avicennia* leaves have been known as hypoglycemic compounds [11].

There are no available studies, until now, on the effect of the combination of the two plant extracts on controlling the blood glucose level in STZ-induced diabetic rats and their hepatoprotective potential. Therefore, this study was designed to assess the effect of co-treatment with *R. mucronata* and *A. marina* on blood glucose level, plasma insulin, Homeostasis Model Assessment of β-cells (HOMA β), Homeostasis Model Assessment of Insulin Resistance (HOMA IR), liver function parameters, tissue histopathology, and immunohistochemistry in STZ-diabetic rats.

Material and Methods

Animals

This study was conducted on 120 adult male Wistar rats weighing 200 to 250 gm. The rats were kept at room temperature (24 ± 2)°C with 12 h light/dark cycle and 50 ± 10% relative humidity. Rats were fed a normal commercial chow diet, along with water *ad libitum*. The animals were maintained according to the international ethical guidelines for the care of laboratory animals, and all the experimental procedures were approved by the Animal Care and Use Committee of the King Abdulaziz University, Jeddah, Saudi Arabia, with the reference number (172060302).

Induction of DM

DM was induced by a single intraperitoneal (IP) injection of freshly prepared STZ (Sigma Chemical Co., St. Louis, MO), 60 mg/kg BW, in normal physiological saline solution (0.9% NaCl). For this purpose, 100 mg of STZ was dissolved in 5 ml normal saline to reach the final concentration of 20 mg/ml just before use [12]. Three days later, the level of the blood glucose was assessed and the level ≥250 mg/dl was considered as diabetic.

Plants extraction

The collected leaves of *R. mucronata* and *A. marina* plants were scientifically verified by a plant taxonomist at the Department of Arid Land Agriculture, Faculty of Meteorology, Environment, and Arid Land Agriculture, King Abdulaziz University, Jeddah, Saudi Arabia. Aqueous extracts of plant leaves were prepared according to the previous reports [13].

Experimental design

A total of 120 albino rats were randomly assigned to eight equal groups. Group 1 rats (negative control group) received water and were fed *ad libitum*. Group 2: STZ-induced diabetic rats (diabetic group). Group 3 included the diabetic rats that received aqueous extract of *R. mucronta* leaves (400 mg/kg BW/day). Group 4 included the diabetic rats that received aqueous extract of *A. marina* leaves (400 mg/kg BW/day). Group 5 included the diabetic rats that received aqueous extract of *R. mucronta* (200 mg/kg BW) and *A. marina* (200 mg/kg BW) leaves. Group 6: Non-diabetic rats received aqueous extract of *R. mucronata* leaves in a dose of 400 mg/kg BW/day. Group 7: Non-diabetic rats received aqueous extract of *A. marina* leaves in a dose of 400 mg/kg BW/day. Group 8: Non-diabetic rats received a mixture of aqueous extract of *R. mucronta* (200 mg/kg BW) and *A. marina* (200 mg/kg BW). The treatments were started on the fourth day after the STZ injection, orally by stomach tube, daily for 6 weeks.

Assessment of blood glucose level

To measure the glucose levels, fresh fasting blood samples were obtained from the vein of rats' tail in order to assess the blood glucose level using One Touch Ultra Glucometer (Lifescan, Johnson and Johnson, Milpitas, CA).

Biochemical tests

Blood samples were obtained from the retro-orbital venous plexus of rats at the sixth-week post-treatments. The collected blood sample was let to settle down for 30 minutes at room temperature to form a clot; the serum was then collected by centrifugation at 3,000 rpm for 20 min.

Activities of various serum enzymes like alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT) along with total glucose, total bilirubin (TB), total protein (TP), and albumin were measured by commercial kits (Roche Cobas Diagnostics, USA) using automatic

analyzer (Cobas 6000 analyzer series). Serum insulin levels were assessed using insulin ELISA kits (Cat. no. ezrmi-13kelisa, Billerica, MA) [14].

The steady-state of pancreatic β-cell function was measured by calculating the HOMA-β and HOMA-IR that was computed from fasting serum glucose and fasting serum insulin concentration by the following equations [15]:

$$\text{HOMA - }\beta = \frac{\text{Insulin}\left(\frac{\text{MIU}}{\text{l}}\right) \times 20}{\text{Glucose}\left(\frac{\text{mmol}}{\text{l}}\right) - 3.5}$$

$$\text{HOMA - 1R} = \frac{\text{Glucose}\left(\frac{\text{mmol}}{\text{l}}\right) \times \text{Insulin}\left(\frac{\text{MIU}}{\text{l}}\right)}{22.5}$$

Histopathological examinations

Hepatic tissue samples were collected at the sixth week post-treatments after sacrificing the rats and were fixed in 10% neutral formalin solution overnight. Liver specimens were embedded in paraffin to obtain paraffin blocks. Five-µm sections were prepared and stained with hematoxylin and eosin (H&E) to assess their histopathological structure [16] using light microscopy.

The standard immunohistochemical methods were adopted for the detection of apoptotic marker caspase-3 in liver sections [17]. The tissue sections were routinely microwave-treated to unmistaken the epitopes of antigen [20]. The Biotin-Streptavidin system was used to visualize the apoptotic markers [18]. Diaminobenzidine was used as a chromogen since it allows a permanent preparation. Hematoxylin counterstain was done.

Statistical analysis

All the data were presented as mean ± standard error. Statistical significance was analyzed using a one-way analysis of variance. A $p < 0.05$ was considered as statistically significant.

Results

Effects of plant extract treatments on insulin, glucose, HOMA-β, and HOMA- IR levels

The streptozotocin-induced diabetic rats showed high levels of blood and serum glucose and HOMA-IR, while serum insulin level and HOMA-β decreased, compared to normal rats. See Table 1. The administration of *R. mucronata* or *A. marina* alone or in combination with diabetic rats (Groups, 3, 4, and 5) showed a steadily significant decrease

Table 1. Effect of treatments on the levels of serum glucose, serum insulin and HOMA in different rat groups.

Variable	G1 (Control)	G2 (STZ- induced diabete)	G3 (STZ+ *R. mucronata*)	G4 (STZ+ *A. marina*)	G5 (STZ+*R. mucronata*+ *A. marina*)	G6 (*R.mucronata*)	G7 (*A.marina*)	G8 (*R. mucronata* + *A. marina*)	P value
Serum Glucose (mmol/L)	7.29±0.4	15.14±0.4	9.8± 0.1	12.7±1.7	10.8± 0.3	6.9± 0.5	6.8±0.1	6.9±0.3	0.001
Serum insulin	16.9±0.82	8.8±2.1*	14.5±1.7*#	11.4±2.4*#	13.2±0.7*#	15.9±1.8	17.08±1.2	16.8±2.03	0.001
HOMA-IR	63.5±6.5	111.3±16.6*	74.9±22.1 #	122.5±14.01*	91.3±25.1*	57.9±10.6	60.09±9.4	68.3±7.7	0.08
HOMA-B	43.9±8.1	8.26±3.1*	25.8±3.6*#	17.8±12.1*#	21.3±2.4*#	44.9±15.4	46.7±6.4	44.9±5.6	0.001

Data was presented in the form of Mean ± standard error (SE).
*Significance versus control.
#Significance versus STZ-induced diabetes.

in glucose levels, as well as a significantly increased level of insulin and HOMA-β. The improvement was marked in diabetic rats treated with *R. mucronata*, which had a more potential hypoglycemic effect compared to STZ-induced diabetic rats (G2) (Table 1). Non-diabetic rats received *R. mucronata, A. marina* extract singly, or in a mixture exhibited non-significant changes compared to normal rats (Table 1).

Effects of plant extract treatments on liver enzymes

Serum ALT, AST, ALP, and GGT activities, as well as serum TB levels, displayed a significant ($p \leq 0.001$) augmentation in the STZ-induced diabetic rats compared to normal rats (Fig. 1). Diabetic rats received aqueous extracts of *R. mucronata*, *A. marina,* or their combinations revealed a significant ($p \leq 0.01$) decrease and steady improvement toward the normal values in the aforementioned parameters. Proteinogram analysis, serum TP, albumin, and globulins showed significant ($p \leq 0.01$) reduction in STZ-induced diabetic rats compared to normal rats. Oral administration of plant extracts caused a significant improvement in these parameters. However, the serum albumin did not reduce to the basal level compared to diabetic rats (Fig. 2). Non-diabetic rats received *R. mucronata*, *A. marina* extract singly or in a mixture, (G6-8), revealed non-significant changes compared to normal rats.

Histopathological assessment of the liver

Examined serial sections from the liver of normal rats (G1) revealed typical histo-morphological structures. Liver of STZ-induced diabetic rats exhibited characteristic biliary proliferative reactions, multifocal necrotic areas of coagulative necrosis with a pyknotic or karryoretic nuclei, and deep eosinophilic cytoplasm and hepatocellular degenerative changes of variable degrees, including cloudy swelling, hydropic degeneration, vacuolations, and signet-ring fatty change cells (Fig. 3).

Hepatic sections from diabetic rats treated with *R. mucronta* (G3) revealed normal hepatocytes in most parts of the sections; however, few hepatocytes showed hydropic degeneration and apoptotic changes (Fig. 3). Liver tissues of diabetic rats treated with *A. marina* (G4) revealed hepatic lesions represented by congestion of portal veins, central veins and dilated sinusoids, the associated hepatocytes, showed moderate degenerative changes mainly cloudy swelling and hydropic degeneration beside apoptosis of some cells. Portal biliary hyperplasia, which involved a mild-to-moderate number of bile ducts and

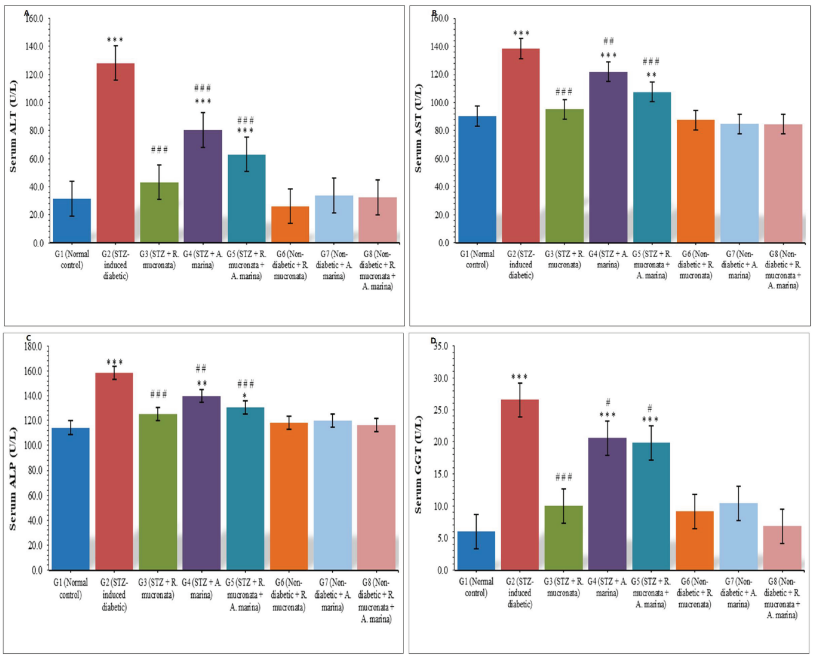

Figure 1. Effect of extracts of *R. mucronata, A. marina* and their mixture on serum ALT (A), AST (B), ALP (C), and GGT (D) levels in the studied groups (n = 15 each), Results are expressed as mean ± SEM. Mean values are significantly different at $p \leq 0.001^{***}$; $p \leq 0.05^{*}$ compared to normal control group. Mean values are significantly different at $p \leq 0.001^{###}$; $p \leq 0.05^{\#}$ compared to STZ-induced diabetic group.

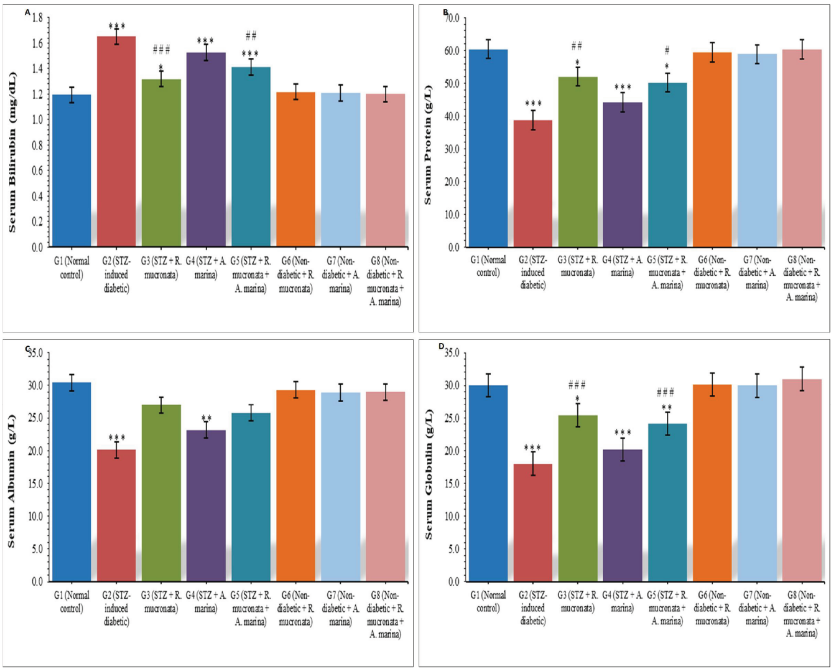

Figure 2. Effect of extracts of *R. mucronata, A. marina* and their mixture on serum bilirubin (A), protein (B), albumin (C) and globulin (D) levels in the serum of the studied groups (n = 15 each), Results are expressed as mean ± SEM. Mean values are significantly different at $p \leq 0.001^{***}$; $p \leq 0.05^{*}$ compared to normal control group. Mean values are significantly different at $p \leq 0.001^{\#\#\#}$; $p \leq 0.05^{\#}$ compared to STZ-induced diabetic group.

ductules and extended to variable distances inside the lobules could be detected. Some of the bile ducts showed periductal fibrosis and leucocytic infiltration (cholangitis) (Fig. 3). Meanwhile, hepatic lesions in some sections of diabetic animals treated with a mixture of *R. mucronta* and *A. marina* (G5) were represented by moderate congestion of the portal blood vessels, sinusoidal dilatation, biliary hyperplasia, chronic cholangitis, portal and interstitial round cells, and eosinophils aggregations beside degenerative changes in some hepatocytes (Fig. 3). Histopathologic changes in non-diabetic rats that received *R. mucronata, A. marina* extract, and a mixture of the two plants extracts revealed unremarkable tissue changes compared to the normal rats.

Immunohistochemical assessment of the liver

Sections from the liver (G1) revealed normal hepatic parenchyma free from any apoptotic changes apart of very few cells (0.5%–1%) /HPF, which showed brownish cytoplasmic reaction (Fig. 4). Sections from STZ-diabetic rats of G2 revealed apoptotic changed in about 5%–7%/ HPF of hepatocytes and Von-Kupffer cells. Positive cells showed characteristic brownish cytoplasmic reaction. Most of the examined liver sections of G3 showed negative reactivity to caspase-3, except very few cells (1%–2%) which revealed early apoptotic changes with light brown cytoplasmic staining (Fig. 4). Liver cells of G4 showed positive apoptotic nuclear and cytoplasmic reactivity in 8%–10% / HPF cells. Liver sections of G5 denoted normal hepatic parenchyma free of apoptosis, however about 2–3 cells /HPF were positive for caspase-3 in a few sections (Fig. 4). Immuno-histochemical changes of apoptotic marker caspase-3 in non-diabetic rats that received *R. mucronata*, *A. marina* extract singly or in a mixture (G6-8) revealed unremarkable changes compared to normal rats.

Discussion

Naturally and experimentally induced diabetes is usually accompanied by an increased level of lipid peroxidation and decreased levels of the key antioxidant enzymes. Oxidative stress has a crucial role in the development and progression of DM due to "higher free radical production, damage to cell constituents, and impairment in the antioxidant defense enzymes." Although the antidiabetic and antioxidant effects of *R. mucronata* extracts and *A. marina* extract have been previously described**,** the antidiabetic and antioxidant effects of the combination of those

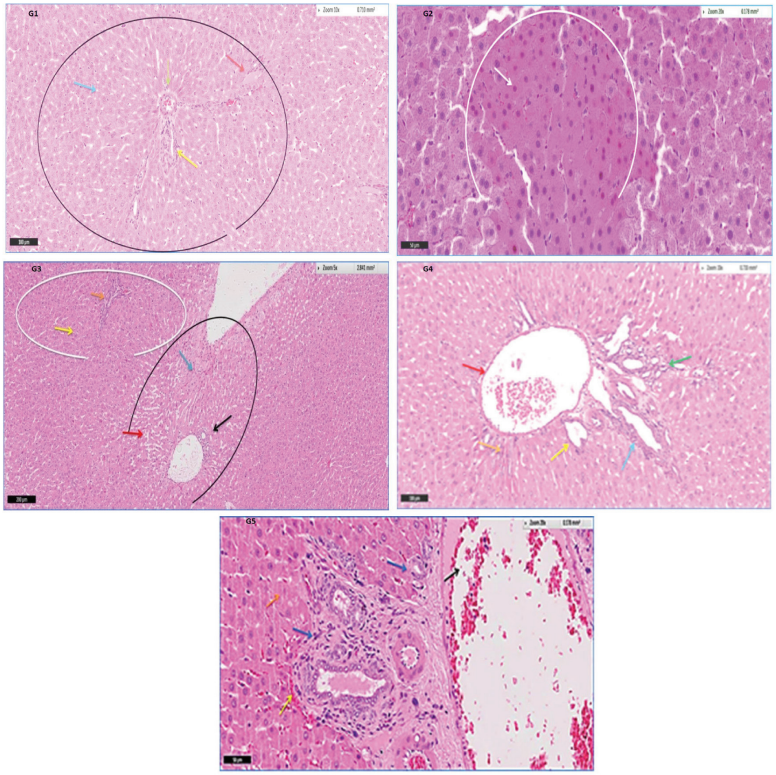

Figure 3. Section in rat's liver of (G1) showing the normal histo-morphology of hepatic lobule (black circle) with preserved hepatic cords (blue arrow), portal blood vessel (green arrow), bile duct (yellow arrow) and lymphatic (brown arrow). H&E. Scale bar = 100 µm. Section in rat's liver of (G2) showing area of coagulative necrosis white circle). H&E. Scale bar = 50 µm. Section of rat's liver (G3) showing apparently normal hepatic parenchyma and portal triad (white circle, golden yellow and yellow arrows), and area with degenerative and apoptotic changes, portal congestion, mild cholangitis and sinusoidal dilatation (black circle, blue, red and black arrows). H&E. Scale bar = 200 µm. Section in rat's liver of (G4) showing congested portal vein (red arrow), biliary hyperplasia and cholangitis (yellow, blue and green arrows) beside apoptosis of a few hepatocytes (golden yellow arrow). H&E. Scale bar = 100 µm. Section in rat's liver of (G5) showing congestion of the portal blood vessels and sinusoidal dilatation (black and yellow arrows), biliary hyperplasia and chronic cholangitis (blue arrows) beside degenerative changes in some hepatocytes (golden yellow arrow). H&E. Scale bar = 50 µm.

extracts were not investigated until now [19]. The current work explored the hepatoprotective effect of *R. mucronata* and *A. marina* and their mixture against STZ-induced diabetes in rats. The results revealed that diabetic markers (Glucose, Insulin, HOMA-β, and HOMA IR) in the current work showed an increase in the fasting blood and serum glucose, HOMA-IR, and a decrease in serum insulin and HOMA-β in STZ-induced diabetic rats of the second group. Hyperglycemia and hypoinsulinemia indicate irreversible destruction of Langerhans islet cells. Because of a similar structure with glucose, STZ is only recognized and transported by GLUT2 to pancreatic islet β-cells, resulting in specific destruction and dysfunction of β-cell [20].

The HOMA model is the most ordinary surrogate measure for the assessment of insulin resistance and β-cell function in clinical and epidemiologic studies [21]. The HOMA model was considered as a structural model of the underlying physiological basis for the feedback loop between the liver and the β-cell in fasting [22]. A significant decrease in fasting serum insulin levels and an increase in HOMA-IR value in diabetic rats indicate an insulin resistance state [23].

Treating diabetic rats using aqueous extracts of *R. mucronata*, *A. marina* alone, or in combination resulted in significant hypoglycemia and an increase in serum insulin, HOMA-β levels than STZ-induced diabetic rats. Results

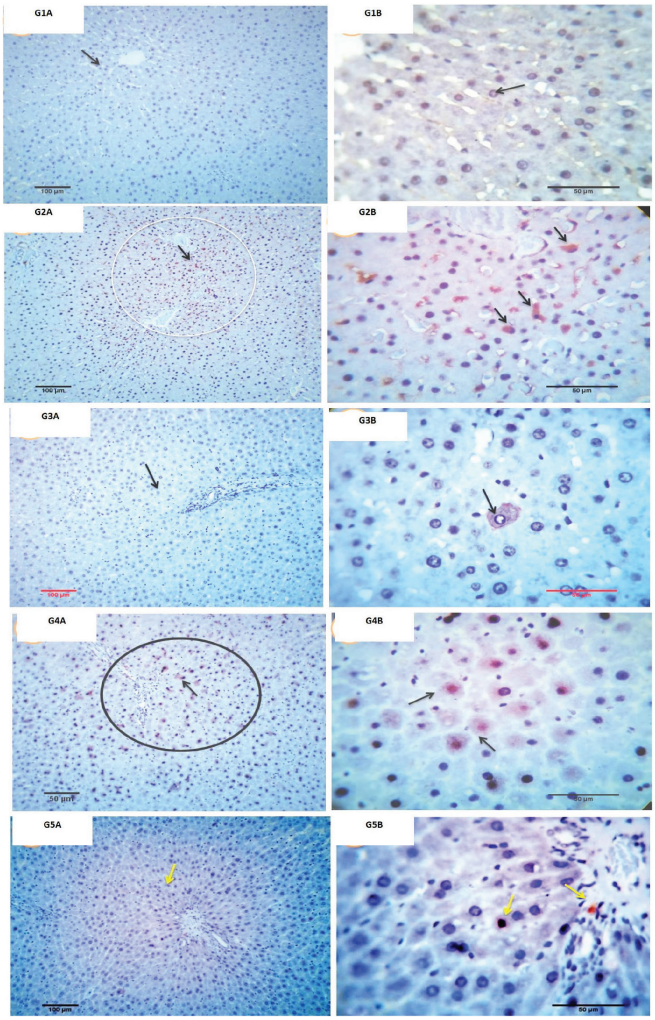

Figure 4. Section in rat's liver of (G1 A, B) showing normal hepatic parenchyma with few apoptotic caspase-3 positive cells (Black arrows). Section in rat's liver of (G2A, B) showing about 5%–7%/HPF of hepatocytes and Von-Kupffer cells positive for caspase-3 apoptotic markers with characteristic brownish cytoplasmic reaction (Black arrows). Section in rat's liver of (G3A, B) showing very few number of cells 1%–2%/HPF (Black arrow). Section in rat's liver of (G4A, B) showing positive apoptotic nuclear and cytoplasmic reactivity (8%–10% / HPF) (circle and Black arrows). Section in rat's liver of (G5) showing about 2–3 cells /HPF positive cells for caspase-3 (yellow arrows). (Immunohistochemical staining with caspase-3 Scale bar (A, 100, B, 50).

obtained in rats treated with *R. mucronata* were encouraging than other groups. Polysaccharides in both plants have antihyperglycemic effects; the impact of which results in increased tolerance to glucose elevated serum insulin levels and lowered blood glucose [24]. Besides, a potent hypoglycemic effect of the extract may be attributed to increased peripheral uptake of glucose, inhibition of hepatic glucose synthesis, and the potent antioxidant effect [25].

Hypoglycemic activity of these plant extracts plays an essential role in improving glucose tolerance. In this concern, Bin-Jumah [19] reported that oral supplementation of hydroethanolic extract of *Monolluma quadrangula*, improves insulin sensitivity and glucose tolerance in a high-fat diet (HFD)/STZ type 2 diabetic rats. The significant increase of serum insulin and HOMA-β level of the diabetic-treated rats compared to the STZ-induced diabetic rats may indicate regeneration of the beta-cells, which could be due to the stimulatory effect of the bio-active phytochemicals present in the plant extracts [26]. Results of diabetic markers were supported by the histopathological and immunohistochemical findings of the pancreas.

Liver enzymes are biomarker enzymes evaluating liver function and integrity. The activities of liver enzymes (ALT, AST, ALP, and GGT) in the present work were significantly ($p \leq 0.001$) upraised in the serum of STZ-induced diabetic rats compared to the normal rats. Such findings might be probably due to the release of these enzymes from the necrotized hepatocytes into the bloodstream, which gives a good indication for the hepatic toxicity of STZ. The increase in oxidative load provoked by STZ-induced diabetes may be responsible for the hepatocellular damage that results in the escape of enzymes from liver cells [27].

The diabetic state leads to a significant increase in serum alkaline phosphatase and gamma-glutamyl transferase activities, as well as total serum bilirubin as compared to the normal rats. Alkaline phosphatase is a biomarker enzyme secreted by the liver, frequently used to affirm the integrity of plasma membrane and endoplasmic reticulum, while gamma-glutamyl transferase constitutes an essential membrane-localized enzyme that plays an important role in glutathione metabolism in the liver. Gamma-glutamyl transferase is a more sensitive marker of bile duct obstruction or intrahepatic cholestasis. Such finding is further supported by the higher total bilirubin concentrations in serum of STZ-induced diabetic rats. The uprising of liver enzymes and bilirubin in STZ-induced diabetic rats had been previously discussed [28].

It can be assumed that *R. mucronata*, *A. marina,* or their mixture might possess an important role in regulating STZ-induced liver injury. It can be suggested that some of the many phytochemicals present in *R. mucronata*, including those with hepatocellular protective effects, play an important role in this concern. The normalization of the liver enzymes and bilirubin after administration of *A. marina* leaf extract establishes its hepatoprotective potential, which was previously proved against CCl_4 induced liver intoxication in rats. This may also be due to the ability of the extract to accelerate the regeneration of liver cells, thus reducing the leakage of marker enzymes into the rat's blood [29].

The present investigation showed that the total serum proteins, albumin, and globulin levels in experimental

STZ-induced diabetic rats, were significantly decreased as compared to the normal rats. The decreased serum total protein level in diabetic rats may be due to the elevated protein muscular breakdown, diminished amino acid uptake, increased glycogenic conversion of amino acid to carbon dioxide, and water, besides reduced protein biosynthesis and protein absorption. The reduced serum albumin level of STZ-induced diabetic rats could be due to massive hepatic necrosis, hepatic dysfunction, intolerance to insulin, and glycogen impairment of oxidative phosphorylation, or due to the effect of diabetic kidney failure. Hypoalbuminemia in diabetic rats is an indication of the reduced synthetic function of the liver [30].

Oral administration of *R. mucronata*, *A. marina* extract singly or in a mixture to diabetic rats restored the total proteins, albumin, and globulin levels to normal values, especially in those administered the former plant and the mix of both plant extracts. The results of the present investigation are also following an earlier report [31]. The biochemical effects for the liver function in diabetic rats with or without treatments were established by the histopathological and immunohistochemical findings. Liver sections in diabetic rats denoted characteristic biliary proliferative reactions with hepatocellular degenerative changes and necrosis. Several morphological and pathological alterations in liver tissues of STZ-induced diabetic (150 mg/kg, IP) mice were associated with lipid deposition, inflammatory cell infiltration, and Kupffer cell hyperplasia [32]. The hepatic degenerative and necrotic changes are usually associated with the inflammatory process. A recent study emphasizes such opinion as to the proinflammatory cytokines. TNF-α and IL-6 markedly increased in the serum of STZ-induced diabetic rats, in comparison to the normal ones. The treatment of the STZ diabetic rats with *M. quadrangula* extract alleviated the levels of TNF-α and IL-6, as compared to the STZ-induced diabetic rats [32].

In the present work, hepatic apoptosis was of a lesser degree as compared with other organs where it was 0.5%–1%, 5%–7%, 1%–2%, 8%–10%, 2%–3% for liver of normal, STZ-induced diabetic rats and diabetics treated rats with *R. mucronata*, *A. marina* extract singly or in mixture, respectively. The non- diabetic rats treated with plant extracts individually or in a mix showed apoptotic free reactivity. The comparative higher levels of apoptosis were recorded in the diabetic rats and diabetic rats treated with *A. marina* (5%–7% and 8%–10%, respectively). Our observations are compatible with previous findings which have reported that apoptosis has an important role in acetaminophen-induced hepatic injury since it inhibits apoptosis and stops the occurrence of acute liver failure [33].

Caspase-3 activity significantly increased in APAP group, whereas chicory leaves hydroalcoholic extract treatment ameliorates this effect. Immunostaining using cleaved caspase-3 might potentially open an exciting experimental advance as apoptotic hepatocytes can already be identified at a very early apoptotic stage where they show "exclusive cytoplasmic staining and an otherwise intact cellular structure." This can allow investigating the role of RAS in its activated form for apoptosis in the liver of different rodent species, which may be related to "the species-dependent activation in liver tumors" [34].

The results of the present data coincide with other findings, which showed that degenerative changes initiated by alloxan in hepatic and pancreatic tissues, enhanced hepatic lipid peroxidation, and unregulated gene expression of Pyruvate carboxylase and caspase-3 [34]. However, treatment with *Moringa oleifera* leaves induces hepatoprotective effects in alloxan-induced diabetic rats, decreased gene expression, and hindered changes to the histo-architecture of liver and pancreas in alloxan-induced diabetic rats, with a reduction in the percent of caspase-3 reactive cells.

Conclusion

The current work spotted the light on the hypoglycemic and hepatoprotective folkloric utilization of the aqueous leaf extract of *R. mucronata* and *A. marina* in diabetes. Further studies concerning the use of purified selective bioactive compounds from the studied plant extract could provide more details on the therapeutic potentials and the side effects.

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Conflict of interest

The authors declared that they have no conflicts of interest.

Authors' contribution

Both the authors have contributed equally in the experimental process, analysis, writing, and editing the manuscript.

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