ORIGINAL PAPER

doi: 10.5455/medarh.2020.74.421-427
MED ARCH. 2020 DEC; 74(6): 421-427
RECEIVED: SEP 02, 2020 | ACCEPTED: OCT 13, 2020

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Antihyperglycemic, Antioxidant and Antiapoptotic Effect of Rhizophora Mucronata and Avicennia Marina in Streptozotocin-induced Diabetic Rats

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ABSTRACT

Introduction: Diabetes mellitus is a common disease worldwide. It is considered as the third leading cause of death, in the developed countries followed by heart diseases and cancer. **Aim:** The aim of this study was to assess the effectiveness of the aqueous fraction of R. mucronata and A. marina leaves grown in Saudi Arabia alone or in combination as antidiabetic agents and explore its effect on the antioxidants status. **Methods:** One hundred and twenty male Wistar albino rats were divided into 8 groups were utilized in this study. Streptozotocin (STZ) was utilized for induction of diabetes. The effects of daily oral administration of aqueous extract from the leaves of R. mucronata (400 mg/kg BW), A. marina (400 mg/kg BW) and the combination of both plant extracts for 6 weeks were evaluated on blood glucose, insulin, tissues' antioxidants as well as pancreatic immunohistochemistry in normal, (STZ)-induced diabetic rats. **Results:** Oral administration of the plants extracts significantly reduced ($p \leq 0.001$) serum glucose, insulin and improved the antioxidants status in the liver compared to the untreated rats. Immunohistochemically, the pancreas of diabetic rats treated with R. mucronata revealed a few islets β-cells (2-3%/ HPF) with positive caspase-3. **Conclusion:** The extract of R. mucronata exhibited a promising antidiabetic, antioxidant and tissue enhancing effects compared with A. marina alone or in combination.

Keywords: Mangrove, diabetes mellitus, insulin, tissue antioxidants, pancreas.

1. INTRODUCTION

The World Health Organization (WHO) has reported that Saudi Arabia ranks the second highest in the Middle East, and is seventh in the world for the rate of diabetes (1). Overall, one fourth of the adult population is affected by diabetes mellitus (DM), which is predicted to increase to more than double by the year 2030. The most distressing is possibly the acceleration propensity of diabetes, in recent years, where a nearly ten-fold increase has been witnessed over the past thirty years in Saudi Arabia (2).

Diabetes mellitus is characterized by hyperinsulinemia, hyperglycemia, hyperlipidemia, hyperaminoacidemia, and metabolic upset of carbohydrate, protein and lipid metabolism due to abnormalities in insulin secretion, insulin action, or both (3). The disease is usually associated with a high level of lipid peroxides, reactive oxygen species (ROS), and a decreased level of the antioxidant enzymes, which play an important role in scavenging toxic intermediate radicals formed by incomplete oxidation or regulating the production of ROS and the overall tissue antioxidant efficiency (4).

Marked development has been observed in the management of DM using synthetic medications; however, the management of DM and its complication is still an unsolved mystery. Remedies that were developed on the principles of chemical or conventional medication are not very effective and usually have an increased risk of adverse side effects. Additionally, these drugs are typically very expensive, especially for third world populations. Consequently, the treatment of DM with plant-derived phytochemicals appear to have an extreme potential, as they are easily available and do not require exhaustive pharmaceutical synthesis (5).

Several investigations were conducted to explore the effectiveness of plant extracts on streptozotocin (STZ)-triggered diabetes in the tissue organs of

many experimental animals (3). Current natural or experimental trials are now inclined to find natural resources that are effective in the treatment of DM and its complications, as there are many natural products that can suppress the enzyme activities responsible for the production of glucose, its absorption, and insulin effectiveness (6). Other studies have clarified that various components present in plant extracts have a positive effect on DM, which can modify apoptosis of β-cell and promote insulin action (7).

Rhizophora mucronata and Avicennia marina are two prominent genera of mangrove plants that exist at the interface of the earth and the waters in both the humid and subtropical latitudes (8). Such plants have been used in traditional medicine in the coastal regions of Asian subcontinents for treating health ailments such as diabetes, diarrhea, hepatitis, and inflammation (9).

2. AIM

The aim of this study was to assess the effectiveness of the aqueous fraction of R. mucronata and A. marina leaves grown in Saudi Arabia alone or in combination as antidiabetic agents and explore its effect on the antioxidants status.

3. MATERIAL AND METHODS

Preparation of plant Extraction

Rhizophora mucronata and A. marina leaves were collected from the areas Farasan Island, Jizan and Shuaiba area respectively, Kingdom Saudi Arabia (KSA) during January 2018. The collected leaves of the studied plants were scientifically identified and authenticated by a plant taxonomist at the Department of Arid Land Agriculture, Faculty of Meteorology, Environment and Arid Land Agriculture, King Abdulaziz University, Jeddah, KSA. The aqueous extracts of leaves of R. mucronata and A. marina were prepared according to the methods of Mohamadi and Havasian (10). The yields crude plant extract required in this experiment for a period of 6 weeks was 151.2 g for each plant. The plant extracts were prepared freshly each time and administered orally by stomach tube.

Animals

One hundred and twenty male Wistar albino rats (200-250 g BW), aged six weeks were purchased from the Animal Experimental Unit, King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. All animals were acclimatized to laboratory conditions for 2 weeks before the initiation of the experiment. The animals were fed with normal commercial chow and water ad libitum. The animals were handled according to the ethical guidelines for the care of laboratory animals and all experimental procedures were approved by the Animal Care and Use Committee of the King Abdulaziz University.

Induction of DM

Prior to diabetes induction, the experimental adult male Wistar rats were fasted for 12 hours and diabetes was induced by administering a single intraperitonial injection of freshly prepared STZ (Sigma Chemical Co., St. Louis, MO, USA) at a dose of 60 mg/kg BW in normal physiological saline solution (0.9% NaCl). For this purpose, 100 mg of STZ was dissolved in 5ml normal saline to reach a final concentration of 20 mg/ml just before use (11). After three days STZ injection, the fasting blood glucose (FBG) levels were measured from tail blood samples by using a One Touch Ultra Glucometer (Lifescan, Johnson and Johnson, Milpitas, CA, USA). Animals with blood glucose levels ≥250 mg/dl were considered diabetic (60 rats) and used for subsequent experiments (12). This day was considered the first day of the experiment.

Experimental Design

The experimental animals were divided into 8 groups, 15 rats for each group. The treatment was given orally to the respective groups once a day for a period of 6 weeks. Group I (negative control) group included normal rats received water and fed ad libitum throughout the experiment. Group II (diabetic positive control) group included rats that were intraperitoneally (IP) injected with a single dose of STZ (60 mg/kg BW). Group III (R. mucronata-treated) group included diabetic rats that received an aqueous extract of R. mucronata leaves at a dose of 400 mg/kg BW/day. Group IV (A. marina-treated) group included diabetic rats that received an aqueous extract of A. marina leaves orally at a dose of 400 mg/kg BW/day.

Group V (Combined extract-treated) group included diabetic rats that received a mixture of aqueous extract of R. mucronata (200 mg/kg BW) and A. marina (200 mg/kg BW) leaves (at a final dose of 400 mg/kg BW/day). Group VI included non-diabetic rats received an aqueous extract of R. mucronata leaves at a dose of 400 mg/kg BW/day. Group IIV included non-diabetic rats received an aqueous extract of A. marina leaves at a dose of 400 mg/kg BW/day. Group IIIV included non-diabetic rats received a mixture of aqueous extract of R. mucronata (200 mg/kg BW) and A. marina (200 mg/kg BW) leaves (at a final dose of 400 mg/kg BW/day).

The daily treatment was started on the 4th day after STZ injection and this was considered as the first day of treatment. The extract was given orally using stomach tube. The experiment was continued for a period of 6 weeks.

Biochemical assessment

Blood was collected from retro-orbital venous plexus of rats at the 6th week. It was then left for clotting at room temperature and serum was separated by centrifugation at 3000 rpm for 20 min. Serum glucose was evaluated in normal and diabetic rats by an autoanalyzer (Cobas 6000 analyzer series) using diagnostic kits according to the manufacturer's instruction (Roche Cobas Diagnostics, USA). Serum insulin levels were measured using insulin ELISA kits which includes an enzyme immunoassay for the quantitative determination of insulin in sera of rats (Cat. no. ezrmi-13kelisa, Billerica, MA, USA) according to the method of Thulesen et al. (13).

The homeostasis model assessments of insulin resistance (HOMA-IR) and β-cells function (HOMA-B) were calculated using the following equations (14): HOMA-IR= fasting insulin (MIU/L) × fasting glucose

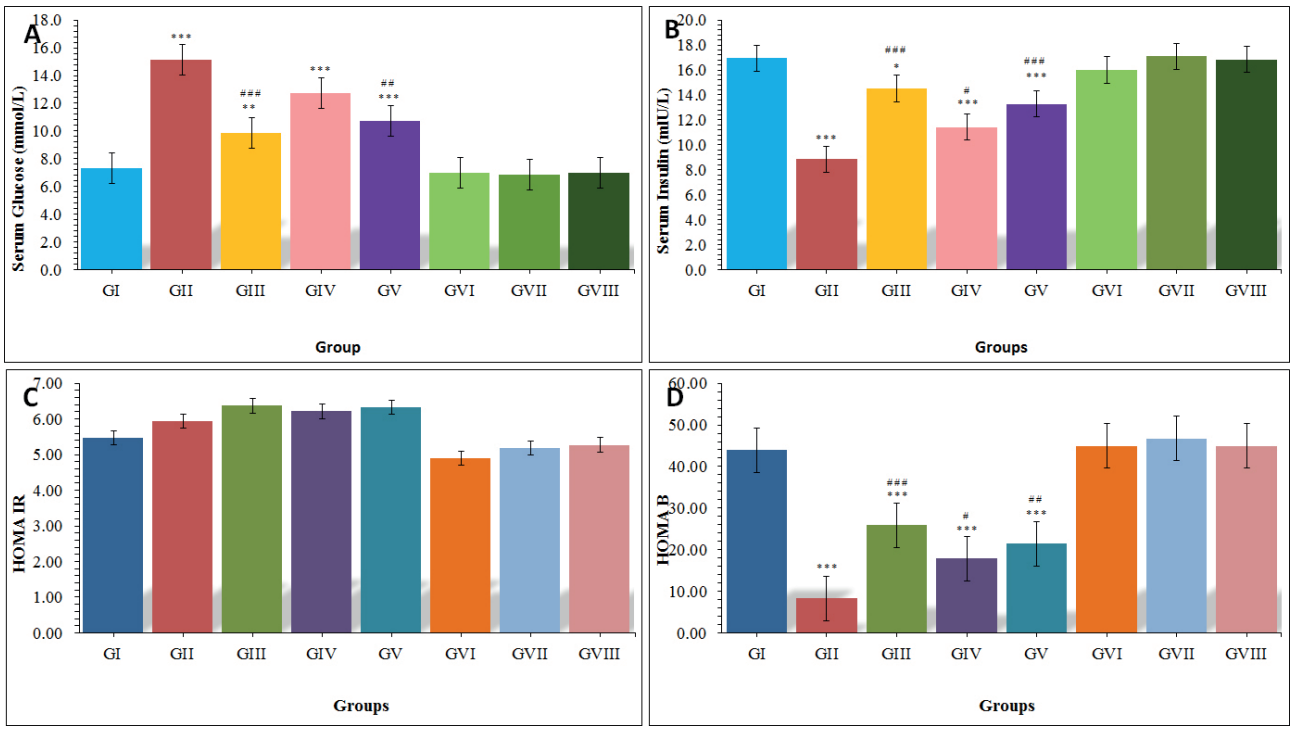

Figure 1. Blood glucose, insulin levels and HOMA in the studied groups. Results are expressed as mean ± SEM (n=15). Mean values are significantly different at p ≤ 0.001*; p ≤ 0.01** compared to normal control group. Mean values are significantly different at p ≤ 0.001###; p ≤ 0.01## compared to STZ-induced diabetic group. GI (Normal control), GII (STZ-induced diabetic), GIII (STZ + R. mucronata), GIV (STZ + A. marina), GV (STZ + R. mucronata + A. marina), GVI (Non-diabetic + R. mucronata), GVII (Non-diabetic + A. marina), GVIII (Non-diabetic + R. mucronata + A. marina).**

(mmol/L) /22.5 HOMA-B = insulin (MIU/L) × 20 /fasting glucose (mmol/ml) – 3.5.

At the end of the experiment, animals were sacrificed under anesthesia and the abdomen was open in order to dissect the liver and pancreas. Liver tissues were excised from rats of all groups, rinsed with isotonic saline solution, blot-dried and weighed. The tissues were cut into small pieces and minced. A homogenate was prepared with 5% (w/v) potassium phosphate buffer (0.1 M, pH 7.4) using a homogenizer (Model silent crusher-M; Heidolph Instruments, Donau, Germany). Homogenate was then centrifuged at 16,000 g for 20 min to remove nuclei and cell debris (15). The supernatant obtained was used for measuring the levels of malondialdehyde (MDA) (16), catalase (CAT) (17), reduced glutathione (GSH) (18) and superoxide dismutase (SOD) (19).

Immunohistochemical study

The dissected pancreas was fixed in neutral buffered formalin and processed for obtaining of paraffin blocks. The latter were sectioned at 4-6 μ and stained using the standard immunohistochemical methods for the detection of apoptotic caspase-3 in pancreatic tissue. Diaminobenzidine (DAB) was used as chromogen since it allows a permanent preparation. Hematoxylin was used as a counterstain.

Statistical Analysis

The obtained data in this study were expressed as mean ± standard error (SE). Statistical significance of the difference between groups, with more than two categories, was determined by one-way analysis of variance (ANOVA) followed by Least Significant Difference (LSD) post-hoc test. The statistical software package used for analysis was Statistical Package for Social Sciences (SPSS 24). The values were considered to be significantly different when the P value was < 0.05.

4. RESULTS

Serum glucose, insulin, HOMA-β and HOMA- IR levels. Administration of STZ resulted in an increased levels of serum glucose level, compared the control rats while administration of R. mucronata or A. marina alone or in combination to STZ-induced diabetic rats resulted in a significant decrease ($p \leq 0.001$) in serum glucose levels. Among the two studied extracts, R. mucronata showed the potent hypoglycemic effect compared with the untreated diabetic rats. Non-diabetic rats that received R. mucronata, A. marina extract and mixture of the two plants extracts revealed non-significant changes in serum glucose level when compared with normal rats (Figure 1A).

It was noticed that serum insulin level significantly decreased ($p \leq 0.001$) in of STZ-induced diabetic rats compared to the control group. Administration of R. mucronata, A. marina or their mixture to diabetic groups resulted in a significant increase ($p \leq 0.001$; $p \leq 0.05$; $p \leq 0.001$ respectively) in the serum insulin levels compared to untreated diabetic rats (G2). The normal groups that received R. mucronata, A. marina or their mixture showed non-significant differences in serum insulin levels compared to the control group (Figure 1B).

When it came to HOMA calculation, it was noticed that induction of diabetes in rats using STZ resulted in a non-significant increase in HOMA-IR compared to the control group. In addition, treating the diabetic groups with R. mucronata, A. marina or their mixture did not significantly reduce HOMA-IR (Figure 1C). On the oth-

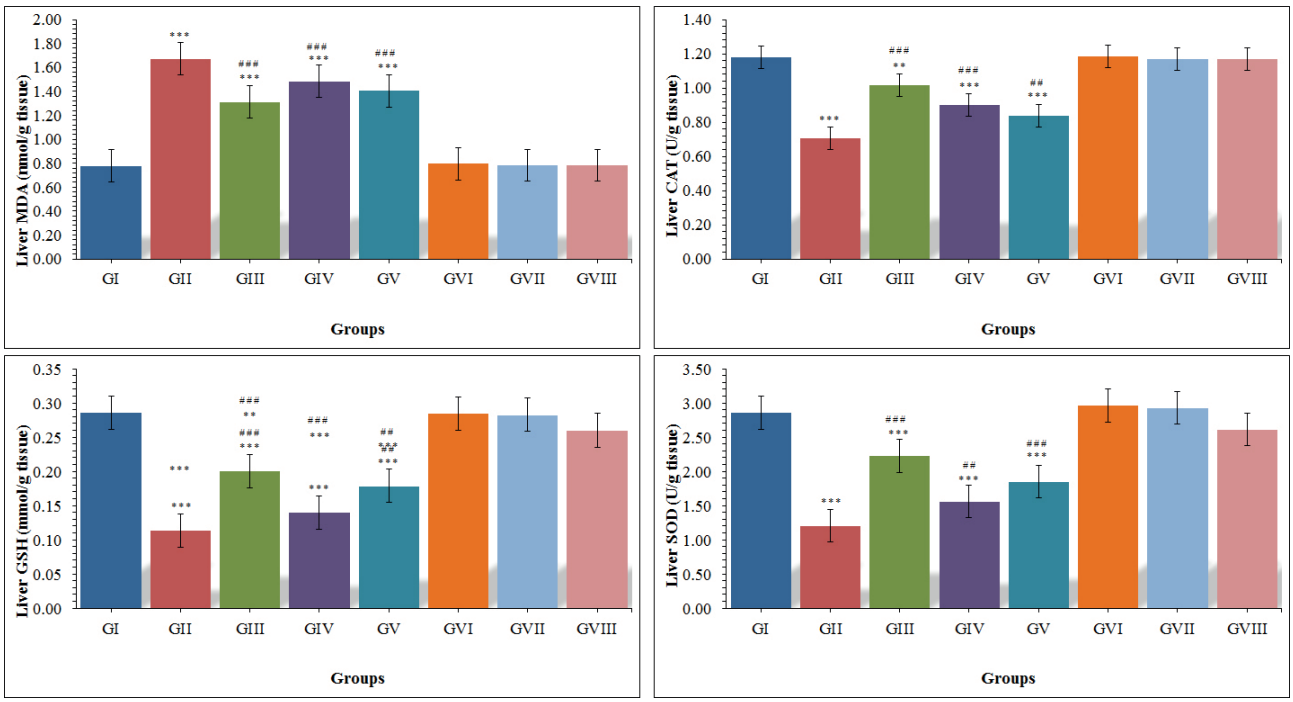

Figure 2. MDA, CAT, GSH and SOD in the studied groups. Results are expressed as mean ± SEM (n=15). Mean values are significantly different at p ≤ 0.001*; p ≤ 0.01** compared to normal control group. Mean values are significantly different at p ≤ 0.001###; p ≤ 0.01## compared to STZ-induced diabetic group. GI (Normal control), GII (STZ-induced diabetic), GIII (STZ + R. mucronata), GIV (STZ + A. marina), GV (STZ + R. mucronata + A. marina), GVI (Non-diabetic + R. mucronata), GVII (Non-diabetic + A. marina), GVIII (Non-diabetic + R. mucronata + A. marina). MDA (Malondialdehyde), CAT (catalase), GSH (reduced glutathione) and SOD (superoxide dismutase).**

er hand, STZ-induced diabetes resulted in a significant increase (p≤ 0.001) in HOMA-β compared to the control group. Treating the diabetic groups with R. mucronata, A. marina or their mixture significantly increased it (p≤ 0.001, p=0.03, p=0.01) respectively compared to the untreated diabetic group (Figure 1D).

MDA and antioxidant levels in the liver

The liver MDA level, which is the end-product of LPO, of STZ-induced diabetic and treated diabetic groups (GII-GV), showed highly significant increase (P ≤ 0.001) compared to control group. However, the treatment of the diabetic groups with R. mucronata, A. marina and their mixture induced a highly significant decrease (p ≤ 0.001) in the liver MDA levels compared to STZ-induced diabetic rats (Figure 2A).

The level of liver CAT in untreated diabetic group showed highly significant decrease (p ≤ 0.001) compared to the control group. The treatment or the diabetic groups (GII-GV) with R. mucronata, A. marina and their mixture revealed a significant increase (p ≤ 0.001; p ≤ 0.001; p ≤ 0.01 respectively) in the liver CAT levels if compared with the diabetic control group (Figure 2B).

Regarding the level of GSH in the liver, it showed a highly significant decrease (p ≤ 0.001) in STZ-induced diabetic group compared to the control group. Daily treatment of diabetic groups (GII-GV) with R. mucronata, and a mixture of R. mucronata and A. marina induced a significant increase (p ≤ 0.001; p ≤ 0.01 respectively) in the liver GSH levels compared to the diabetic group (Figure 2C).

The liver SOD level in untreated diabetic rats was highly decreased (p ≤ 0.001) compared to control group. However, daily administration of R. mucronata, A. marina and their mixture to STZ-induced diabetic rats (GII-GV) induced a significant increase (p ≤ 0.001; p ≤ 0.01; p ≤ 0.001 respectively) in the liver SOD levels compared to the diabetic group (Figure 2D).

The results of this study indicated that the levels of MDA, CAT, GSH and SOD in the non-diabetic rats (GVI-GVIII) revealed non-significant differences compared to control group (Figures 2).

Immunohistochemical assessment

Pancreatic tissues of control rats showed negative expression of caspase-3 in islets cells on the pancreas apart from few caspas-3 positive cells. The pancreas of STZ-induced diabetes (GII) showed about 65-75 %/HPF of the β-cells with a positive caspase-3 (Figure 3). The pancreatic tissue reactivity to apoptotic marker caspase-3 in STZ-induced diabetic rats treated with R. mucronata (GIII) was comparable to that of the control pancreas. A few islets β-cells in some sections (2-3%/HPF) showed positive caspas-3 cells. The pancreas of (GIV), however, showed some of the islets β-cells (20-25 %/ HPF) with caspas-3 positive cells (Figure 3).

The pancreatic islets of STZ induced -diabetic rats treated with plant extract mixture (GV) revealed about 7-9 % of the cells with weak positive caspase-3 reaction (Figure 4). Immunohistochemical changes of apoptotic marker caspase-3 in non-diabetic rats that received R. mucronata, A. marina extract and a mixture of the two plant extracts (G6-8) revealed unremarkable changes when compared with normal rats (Figures 4).

5. DISCUSSION

Oxidative stress plays an important role in the development and progression of DM due to higher free

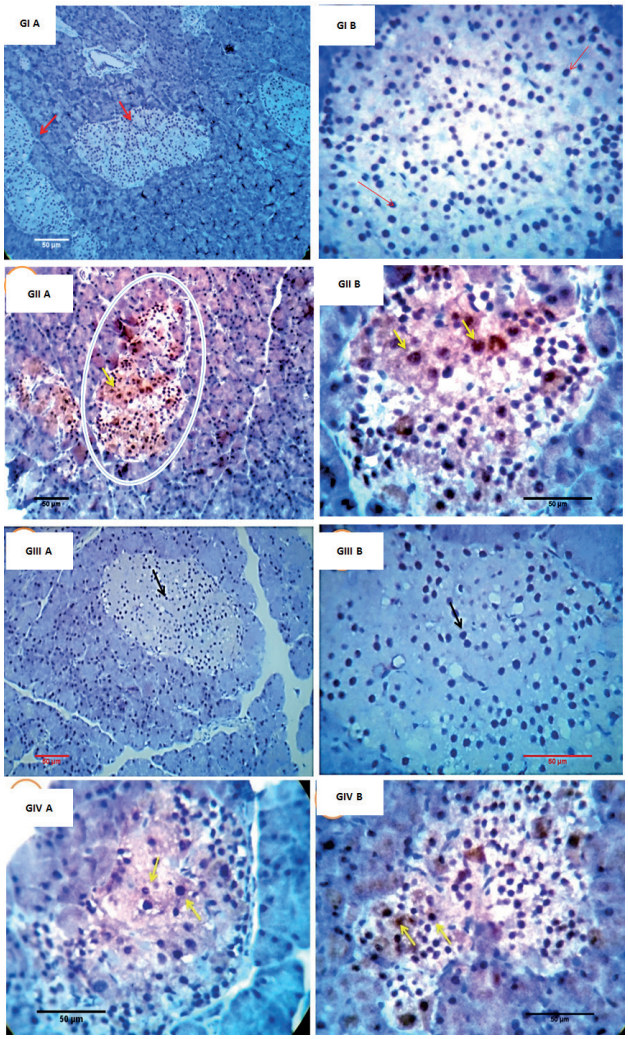

Figure 3: Photomicrographs of rat's pancreas of (GI, A, B) showing normal islets of langerhans β-cells with few apoptotic cells (red arrows) while that of (GII, A, B) showing about 65-75 %/HPF of the β-cells with a positive caspase-3 reaction yellow arrows). Pancreas (GIII A, B) showing no Caspas-3 positive apoptotic cells in the islets while that of (GIV A, B) showing some of the islets β-cells (20-25/ HPF) with Caspas-3 positive reaction (yellow arrows). Scale bars (A, 100, B, 50). GI (Normal control), GII (STZ-induced diabetic), GIII (STZ + R. mucronata), GIV (STZ + A. marina).

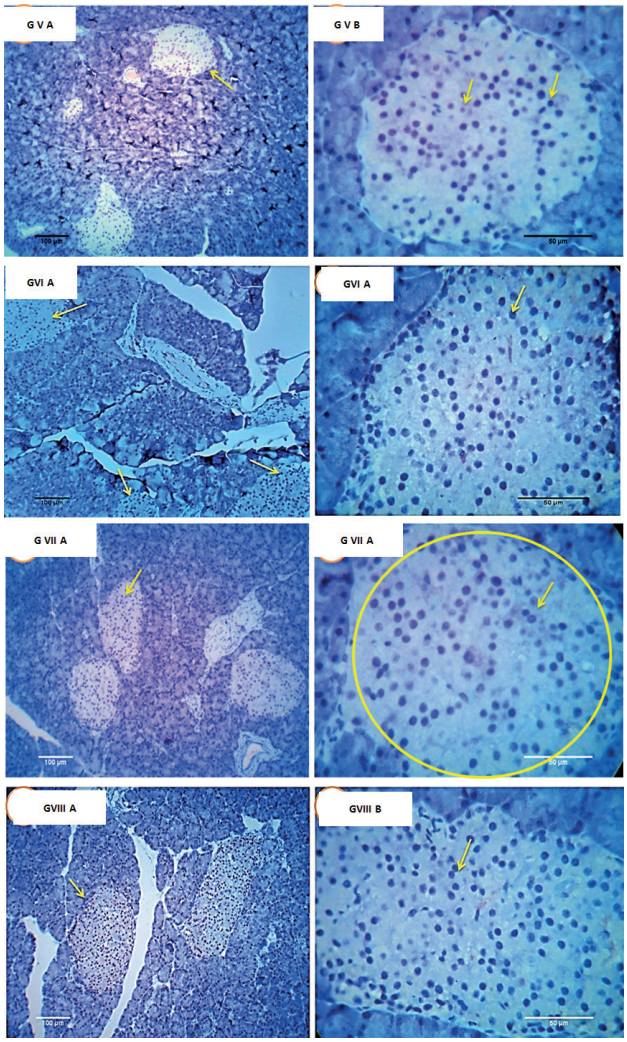

Figure 4: Photomicrographs of rat's pancreas (GV A, B) showing about 7-9% of the islet cells with weak positive to caspase-3 reactivity (yellow arrows) while that of (GVI A, B), (GVII A, B) and (G8 A, B)appear free from any apoptotic reactivity to caspase-3 (yellow arrows). Scale bar (A, 100, B, 50). GV (STZ + R. mucronata + A. marina), GVI (Non-diabetic + R. mucronata), GVII (Non-diabetic + A. marina), GVIII (Non-diabetic + R. mucronata + A. marina).

radical production, damage to cell constituents, and impairment in the antioxidant defense enzymes (20, 21). Naturally and experimentally induced diabetes is usually accompanied with increased level of lipid peroxides, ROS, as MDA and a decreased levels of the key antioxidant enzymes, CAT, SOD and GR-peroxidase (GSH-Px) which play an important role in scavenging the toxic intermediates of incomplete oxidation or regulating the production of ROS and the overall tissue antioxidant capability (22).This study aimed to assess the effectiveness of the aqueous fraction of R. mucronata and A. marina leaves grown in Saudi Arabia alone or in combination as antidiabetic agents and explore its effect on the antioxidants status.

In the present study, oxidative stress and antioxidant parameters were evaluated based on the rats' liver tissues; MDA and GSH levels; and CAT and SOD activities that were significantly decreased in STZ-induced diabetic rats compared to normal rats. Treatment of STZ-induced diabetic rats with the aqueous extracts of R. mucronata and A. marina singly or in combination significantly lowered lipid peroxidation (LPO) as reflected by decreased concentration of MDA and increased concentrations of antioxidant enzymes (CAT, SOD and GSH) in liver, heart and muscle tissues compared to STZ-induced diabetic rats. The over generation of ROS, LPO, and diminished tissue concentrations of SOD, CAT, and GSH were reported in both clinical and experimental models of diabetes (23). Similar findings were reported in different tissues, such as diabetic rat's liver, kidney, and pancreas (24), heart (25), as well as blood (26).

The anti-oxidant effect of the plant extracts under investigation was more pronounced in the diabetic rats-treated with R. mucronata extract. Such results could be due to the polyphenol-rich compounds in R. mucronata extract, which restricts the oxidative stress in liver tissues as marked by reduced levels of lipid peroxides, and raised level of GR (27). Observations of the current study are comparable to that of Mansouri et al.

(15). The authors found that treatment of STZ-induced diabetic rats with grape seed proanthocyanidin decreased the amount of LPO and increased the activity of antioxidant enzymes, SOD, CAT and GPX.

Good scavenging activity, antioxidant probability and ROS lowering action were observed in diabetic rats treated with aqueous extract of R. mucronata against STZ-induced diabetic rats. Prospective results were also distinguished in diabetic rats received aqueous extract consisting of a mixture of R. mucronata, and A. marina. Moreover, the levels of MDA, GSH, CAT and SOD in muscle tissue were completely restored to the normal values in diabetic rats administered R. mucronata. Such plant extracts could possibly reduce the potential glycation of oxidative enzymes or they may reduce reactive oxygen free radicals and improve the activities of antioxidant enzymes.

The results of the current study also point out that the plant extract can either increase the biosynthesis of GSH or reduce the oxidative stresses leading to less degradation of GSH, or have both effects. Similar observations established, the protective, ameliorative and enhancing potentials of R. mucronata and/or A. marina in reducing oxidative stress by either increasing antioxidants activity (28) or reducing MDA levels in tissues (29). Significant decrease in the serum glucose levels, serum and hepatic MDA concentrations and increased the total antioxidant capacity were observed in diabetic rats treated with green tea extract (200 mg/kg) (30).

Many research works and investigations have had been spotted the light on the phytochemical properties of R. mucronata leaves principally, phenolics, flavonoids, and quercetin which have antioxidative, antidiabetic, and cardioprotective activities. Promising antioxidant properties of R. mucronata was induced by inhibiting 1,1-diphenyl-2-picrylhydrazyl (DPPH) and radical scavenging that might be due to the high amount of quercetin (31).

Similarly, positive effects of A. marina extracts on MDA and antioxidants activities were explored in the current investigation. Dose-dependent administration of aqueous extract of A. marina (100 or 200 mg/KG .BW) to STZ-induced diabetic rats significantly increased the enzymatic activities of SOD, GPX and CAT, concurrently extracts of A. marina, significantly decreased the MDA concentration compared to STZ-induced diabetic rats (28). Moreover, Hamzevi et al. showed that administration of A. marina extract (100 and 300 mg/kg BW, IP) to STZ-induced diabetic rats significantly lowered the level of MDA in liver tissue while the activities of the antioxidant enzymes, SOD, GSH, CAT were increased. (29) The extract of A. marina leaves also showed potential antioxidant property based on the free radical scavenging activity assay, such antioxidant property in turn might have prevented the formation of trichloromethyl peroxy radical, thereby reduced tissue damage (32). SOD is involved in the direct elimination of ROS, through dismutation of superoxide radicals (33). CAT is located in peroxisomes and converts H2O2 to water and oxygen. Moreover, GSH function via GPX which is located in the mitochondria for the detoxification of H2O2. GSH has a multifactorial role in antioxidant defense. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by GPX. Increased GSH level might have been sustained to counteract fast-generating oxygen radicals and concomitantly protect tissue cells from ROS and peroxides (24).

Immunohistochemical investigations of STZ-induced diabetic rats treated with R. mucronata, A. marina or mixture of them revealed that, the number of apoptotic pancreatic islet cells revealed markedly reduced signals, with the best result obtained by R. mucronata where apoptotic changes in the pancreatic tissue were nearly similar to that of normal rats. Excitingly, A. marina was less significantly effective and a mixture from both plant extracts was of moderate significance regarding this concept. This may be attributed to the flavonoids compounds in R. mucronata which could play an important role in the prevention of β-cell apoptosis, promotion of β–cell propagation beside secretion and enhancement of insulin activity (8). Similar findings were obtained by Abd Eldaim et al. who reported that Moringa oleifera leaves extracts prevented changes to the histoarchitecture of pancreatic tissues of alloxan-induced diabetic rats, with reduction in the percent of caspase-3 reactive cells, contrary to the STZ-induced diabetic rats where significant increase in apoptotic rate of pancreatic islet cells occur (34).

6. CONCLUSION

In conclusion, the aqueous extracts of R. mucronata, A. marina, and their mixture were able to improve the antioxidant defense system in STZ-induced diabetic rats. Moreover, the findings of this investigation are important in pharmaceutical industry and scientific research for the development of more preventive and effective therapeutic novel plant-derived anti-diabetic drugs.

- **Acknowledgments**: This work was supported by the King Abdulaziz City for Science and Technology in the Kingdom of Saudi Arabia, Grant No. (1-17-01-009-0091). The authors also, acknowledge assistance from the Science & Technology Unit, Deanship of Scientific Research and Deanship of Graduate Studies, and also, acknowledge support from the Dept. of Biological Sciences, Faculty of Science, King Abdulaziz University (KAU), Jeddah, KSA..
- **Author's contribution**: Each author gave a substantial contribution in acquisition, analysis, and data interpretation. Each author had a part in preparing the article for drafting and revising it critically for important intellectual content. Each author gave the final approval of the version to be published and agreed to be accountable for all aspects of the work, ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.
- **Conflicts of interest**: The authors declare no conflicts of interest.
- **Financial support and sponsorship**: All authors have not received any funding for the production of this manuscript.

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