Trop J Nat Prod Res, October 2020; 4(10):722-727 **ISSN 2616-0684 (Print)**
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Artemisia judaica Attenuates Hyperglycaemia-Mediated Oxidative Stress and Cardiac Injury in Streptozotocin-Induced Diabetic Rats

Wesam al-Amarat*

[1]*Department of Medical Support, Al-Balqa Applied University, Al-Karak University College, Al-Karak1710, Jordan*

ARTICLE INFO

Article history:
Received 03 September 2020
Revised 28September 2020
Accepted 26 October 2020
Published online 02 November 2020

ABSTRACT

Hyperglycemia-induced oxidative stress is a recognized risk factor for cardiovascular diseases and heart failure. The study investigated the cardioprotective effect of *Artemisia judaica* (*A. judaica*) on diabetes-induced oxidative injury in rats. A rat model of diabetes was achieved by intraperitoneal (i.p.) injection of streptozotocin (55 mg/kg). After confirmation of diabetes, rats were treated with *A. judaica* (300 mg kg^{-1} day^{-1}, p.o.) daily for six weeks. Diabetic rats demonstrated a significant increase in fasting blood glucose and glycosylated hemoglobin (HbA1c) and decrease in insulin levels. Hyperglycaemia-induced cardiac injury was characterised by increased levels of creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) activities in the plasma, along with several histological alterations in the myocardium. The hearts of diabetic rats showed increased levels of malondialdehyde (MDA), with a significant decrease in the content of glutathione (GSH) and the activities of superoxide dismutase (SOD) and catalase (CAT). The oral *A. judaica* treatment ameliorated hyperglycaemia, prevented hyperglycaemia-induced cardiac injury, boosted antioxidants and suppressed oxidative stress. These findings showed that *A. judaica* protected against diabetes-induced cardiac injury through attenuation of oxidative stress. However, the exact mechanism underlying the cardioprotective effects of *A. judaica* undoubtedly deserves further exploration in future studies.

Keywords: *Artemisia judaica*, Oxidative stress, Diabetes, Antioxidants, Cardiac injury.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterised by hyperglycaemia and associated with numerous acute and chronic complications such as nephropathy, retinopathy and cardiomyopathy. Diabetic cardiomyopathy (DCM) as one of the common long-term diabetic consequences involves various pathological changes in the myocardium that eventually lead to heart failure.[1,2]Hyperglycaemia-mediated oxidative stress plays a crucial role in the pathophysiological mechanisms underlying myocardial damage in diabetic cardiomyopathy.[2,3] Excess reactive oxygen species (ROS) stimulate the inflammatory process and apoptotic death pathways activation eventually culminating in myocardial damage and development of DCM.[3-5] Therefore, scavenging of ROS by antioxidants has gained much attention as a promising approach to attenuate DCM and other diabetes complications which are mediated by oxidative damage.

Artemisia judaica L. (*A. judaica*) belongs to the family Asteraceae and Artemisia genera which are widely used in folk medicine being recommended by Bedouins in Middle Eastern countries where it is known by the Arabic name "Al-ba'atharan".[6] Traditionally, it is widely used for the treatment of gastro-intestinal disorders, heart diseases, erectile dysfunction, diabetes mellitus, wound healing, and inflammatory related diseases such as arthritis.[7]Extracts and isolated compounds from *A. judaica* have exhibited various biological and Medicinal characteristics including antibacterial, antifungalinsecticidal, antioxidant, and antidiabetic effects.[8,9] In agreement with the traditional use of *A. judaica* for the treatment of gastrointestinal disorders, cirsimaitin, the flavone isolated from *A. judaica*, inhibited the amplitude of the phasic contractions and reduced the tone of guinea-pig ileum.[10]

Phytochemicals of *A. judaica* from Algeria showed potent antimicrobial and insecticidal effects.[11,12] In addition, piperitone and trans-ethyl cinnamate isolated from *A. judaica* exhibited effective insecticidal and antifungal activities.[13]Piperitone from the plant was also reported to have strong antioxidant activity.[14,15] Furthermore, Liu *et al*. (2004) has demonstrated the pronounced antioxidant capacity of micropropagated *A. judaica* extracts and its flavanols contents.[16] Another *in vitro* study revealed the antioxidant activity of *A. judaica* comparing to the synthetic antioxidant Trolox.[17]Moreover, toxicological study of water and ethanol extracts of *A. judaica* reported that there was no mortalities in mice following oral administration of aqueous extract of *A. judaica* at doses up to 5 g/kg b.wt, while the LD_{50} of ethanol extract was found to be 9.17 g/kg b.wt, concluding that *A. judaica* is non-toxic plant. Authors also reported the hypoglycaemic effects *A. Judaica* extracts in alloxan-induced diabetic rats.[18] This study investigated the possible cardioprotective effect of *A. judaica* on diabetes-induced cardiac injury in rats.

Materials and Methods

Animals

Male Wister rats (200-250 g) were used in the study. The animals were acclimatized for one week. They were housed in stainless steel cages under normal laboratory conditions with a 12 h light/dark cycle and were fed with normal laboratory pellet diet, and the animals had free access to tap water. All the experimental procedures were carried out in accordance with the guide of (NIH Publication No. 8523,

*Corresponding author. E mail: wsam.amarat@bau.edu.jo
Tel: +962772683694

Citation: al-Amarat W .*Artemisia judaica* Attenuates Hyperglycaemia-Mediated Oxidative Stress and Cardiac Injury in Streptozotocin-Induced Diabetic Rats. Trop J Nat Prod Res. 2020; 4(10):722-727. doi.org/10.26538/tjnpr/v4i10.11

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

revised 1996) and were approved by the animal ethics committee of the institution (Reference no. RC/198/2019).

Preparations of A. judaica extract
The fresh plant was collected from Wadi Rum (South Jordan) in April 2020. The dried plant powder (100 g) was extracted using 80% ethanol (1 L) by shaking at room temperature for 42 h. The extract was filtered through filter paper and evaporated to dryness under reduced pressure in a rotatory vacuum evaporator at temperature not exceeding 45°C. The extracts were dissolved in dimethyl sulphoxide (DMSO) for oral administration.[19]

Induction of type 1 diabetes
Experimental diabetes in rats was induced by single intraperitoneal (i.p.) injection of 50 mg/kg streptozotocin (STZ; Sigma, St Louis, MO, USA) dissolved in 0.1 M citrate buffer (pH 4.5).[2] 5% glucose solution in drinking water was provided overnight to overcome the drug-induced hypoglycemia.[20]Hyperglycaemia was confirmed one week later and rats that had fasting glucose levels more than 250 mg/dL were used for the experiments.

Experimental design
After confirmation of diabetes, the rats were divided into four groups of six animals. The first group (control) normal control group received the vehicle (DMSO) orally for six weeks. The second group (*A. judaica* group) was non-diabetic group received *A. judaica* extract (300 mg kg^{-1} day^{-1}) daily orally. The third group (STZ) was untreated diabetic group, while the fourth group (STZ + *A. Judaica)* diabetic group received *A. judaica* extract (300 mg kg^{-1} day^{-1}) daily orally.[8] The dose of *A. judaica* extract used in this study was based on previous studies which revealed the hypoglycaemic, neuro-protective and renal protective effects of *A. judaica* extracts *in vivo* without reporting any toxic effects.[8,18,21]
At the end of the experiment (six weeks), rats were fasted overnight, anaesthetized with diethyl ether and blood sample was collected through cardiac puncture into heparinized tube. The blood sample was centrifuged at 1200 *g* for 10 min and the plasma collected was stored at -20 °C until biochemical analysis. The small amount of blood was mixed with anticoagulant (EDTA) and kept in refrigerator for further HbA1c assessment. The rats were then immediately euthanized; the hearts were excised and cleaned with cooled saline. The left part of the heart was homogenized in cooled buffered saline for biochemical assays, while the right part was fixed in 10% formalin for histological examinations.

Biochemical assay
Plasma glucose levels were estimated using a commercial kit provided by BioMed Diagnostics (White City, OR, USA) based on enzyme colorimetric method utilizing glucose oxidase enzyme.[22] Percentage of HbA1c in blood samples was determined by a kit provided by Biosystems (Costa Brava, Barcelona, Spain). An enzyme-linked immunosorbent assay (ELISA) kit purchased from Ray Biotech (Norcross, GA, USA) was used for detection of insulin levels in the plasma samples.
The activities of three cardiac enzymes, including creatine kinase-MB (CK-MB), lactate dehydrogenase (LDH) and aspartate aminotransferase (AST), were estimated in plasma samples to evaluate the degree of myocardium damage in treated and control groups. Determination of the enzymes activities was performed according to the standard methods using kits purchased from Spinreact (Girona, Spain) and BioVision (Milpitas, CA, USA).[3,23]
The malondialdehyde (MDA) contents were measured in the heart homogenate (10% w/v in 10mM ice-cold Tris–HCl buffer (pH 7.4)) according to the method of Ohkawa*et al*.[24] Reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) were determined according to the methods of Beutler*et al*.,[25]Nishikimi*et al*.[26] and Aebi,[27] respectively. The protein content in the heart homogenate was assayed according to the previously described method of Lowry *et al*.[28]

Histopathological examination
Specimens from the hearts of control and treated rats were collected and fixed in 10% neutral buffered formalin solution for 48 h and processed for paraffin embedding then 5-micron thick sections were sliced using a microtome. The sections were routinely stained with hematoxylin and eosin (H&E) before they were examined under light microscope.[29]

Statistical analyses
All results were expressed as mean ± standard error of the mean (SEM) and all statistical comparisons were performed using GraphPad Prism 5 software (San Diego, CA, USA). Statistical significance among groups was determined by 1-way ANOVA test followed by Tukey's post-hoc analysis. A P value < 0.05 was considered significant.

Results and Discussion

A. Judaica improves body weight and ameliorates hyperglycaemia in type I diabetic rats
As shown in Figure 1A-D, diabetic rats showed a significant decrease in body weight, increased glucose and HbA1c levels and decreased insulin levels. All these changes were ameliorated when rats were treated with *A .judaica* extract during the course of diabetes (Figure 1A-D). Oral administration of *A .judaica* extract for 6 weeks did not affect glucose, HbA1c or insulin levels in normal rats.
STZ-induced diabetes has been widely used as an experimental model of type 1 diabetic rats to investigate therapeutic effects of hypoglycemic agents *in vivo*.[3,30-32] Necrosis of pancreatic *β*-cells and induction of type 1 diabetic rats after STZ administration is attributed to over production of hydrogen peroxide and hydroxyl radicals causing oxidative damage to DNA and cell membrane in addition to induction of protein glycation in the susceptible pancreatic *β*-cells which are characterized by low antioxidants enzymes.[33] STZ-injected group in the study demonstrated significant hypoinsulineamia associated with hyperglycaemia and increased HbA1c%. Furthermore, the prolonged hyperglycaemia in induced diabetic rats continues to stimulate overproduction of oxidative agents causing progressive damage to pancreas and other tissues worsening the hyperglycaemia levels.[34] Treatment of diabetic rats with *A. judaica* extract increased insulin level and attenuated serum glucose and HbA1c% in diabetic rats. Consistently, previous reports have demonstrated the *in vivo* hypoglycaemic action of *A. Judaica*.[18,35]A recent study showed that *A. judaica* suppressed oxidative damage and inflammation in the pancreas along with attenuated pancreatic lesions, which could explain the ameliorated hypoinsulineamia and hyperglycaemiain STZ-induced diabetic rats.[35]*A. judaica* has also been shown to inhibit dipeptidyl peptidase IV and pancreatic/intestinal enzymes of the carbohydrate absorption cascade *in vitro*.[36] Furthermore, phytochemical screening of the ethanolic extract of the aerial parts of *A. judaica* showed that they are rich in flavonoids, saponins, terpenes, and tannins; which can inhibit cAMP phosphodiesterase, which is a modulator of insulin secretion.[18]
Additionally, diabetic rats showed body weight loss which could be a direct result of decreased glucose utilization as an energy source in the cells concomitant with increased protein and fat catabolism.[37]Herein, *A. judaica* administration prevented body weight loss of diabetic animals compared to the untreated diabetic animals. This finding could be attributed to the positive effects of *A. judaica* on insulin and glucose homeostasis.

A. judaica protects against hyperglycaemia-induced cardiac injury
As shown in Figure 2A-C, diabetic rats showed a significant increase in CK-MB, LDH and AST as compared to the control rats. Treatment of the diabetic rats with *A. judaica* extract for 6 weeks attenuated the circulating levels of CK-MB, LDH and AST (Figure 2A-C). Healthy animals treated with *A. judaica* extract for 6 weeks showed no significant changes in plasma levels of the assayed cardiac function markers.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Upon cardiomyocytes injury, the membrane integrity is lost and intracellular enzymes, including CK-MB, LDH and AST, are released into the circulatory system; therefore, high levels of these biomarkers in blood indicate degenerative and necrotic changes in cardiac tissue.[38] Remarkably, treatment of diabetic rats with *A. judaica* ameliorated the circulating CK-MB, LDH and AST activities, and protected against hyperglycaemia-induced cardiomyocytes injury, suggesting its cardioprotective effects. These findings are in agreement with previous reports which showed that *A. Judaica* protected against organs damage and cellular enzymes leakage in rodents through its membrane-stabilizing property .[8,19,39]

The protective effect of *A. judaica* against diabetes-mediated cardiac injury was further confirmed by the histological findings. H&E stained sections in the heart of both control (Figure 3A) and *A. judaica*-administrated rats (Figure 3B) showed normal morphological features microscopically. In contrast, sections in the heart of diabetic rats demonstrated serous atrophy (mucoid degeneration) in coronary and intramuscular fats was noticed in this group (Figure 3C). Hyaline degeneration of myocardial muscle fiber with pyknotic nuclei could be seen. Other section myocardial necrosis infiltrated by inflammatory cells mainly lymphocytes (Figure 3D). Treatment with *A. judaica* ameliorated the diabetes-induced cardiac injury as showed in Figure 3E and F.

A. judaica attenuates myocardial oxidative stress in diabetic rats

Diabetic rats showed a significant increase in MDA (Figure 4A) associated with a significant decrease in GSH (Figure 4B) content and SOD (Figure 4C) and CAT activities (Figure 4D). All these changes were significantly attenuated when rats were treated with *A. judaica* during the course of diabetes (Figure 4A-D).

A substantial amount of experimental evidence suggests that hyperglycaemia-mediated oxidative stress has a pivotal role in diabetes complications.[1,2,4,35] In the present study, diabetic rats showed a significant increase in MDA, a marker of lipid peroxidation, in the myocardium. Lipid peroxidation might impair membrane function and structural integrity, decrease membrane fluidity and inhibit a number of membrane-bound enzymes.[39] Moreover, ROS can diminish the cellular antioxidant capacity by promoting oxidation of the antioxidant enzymes,[1,2] which could explain decreased GSH content and SOD and CAT activities in the diabetic heart. Therefore, maintenance of redox balance in the cells is an important therapeutic strategy to attenuate oxidative stress in various diseases. Remarkably, *A. judaica* treatment attenuated MDA and boosted antioxidants in the hearts of diabetic rats. In this context, *A. judaica* attenuated hyperglycaemia[8,35] and doxorubicin[19] induced tissue injury by suppressing lipid peroxidation and restoration of endogenous antioxidants.[17] These findings indicate that *A. judaica* could protect against cardiac injury by attenuating oxidative stress, which could be attributed to its antioxidant and antiperoxidative properties.

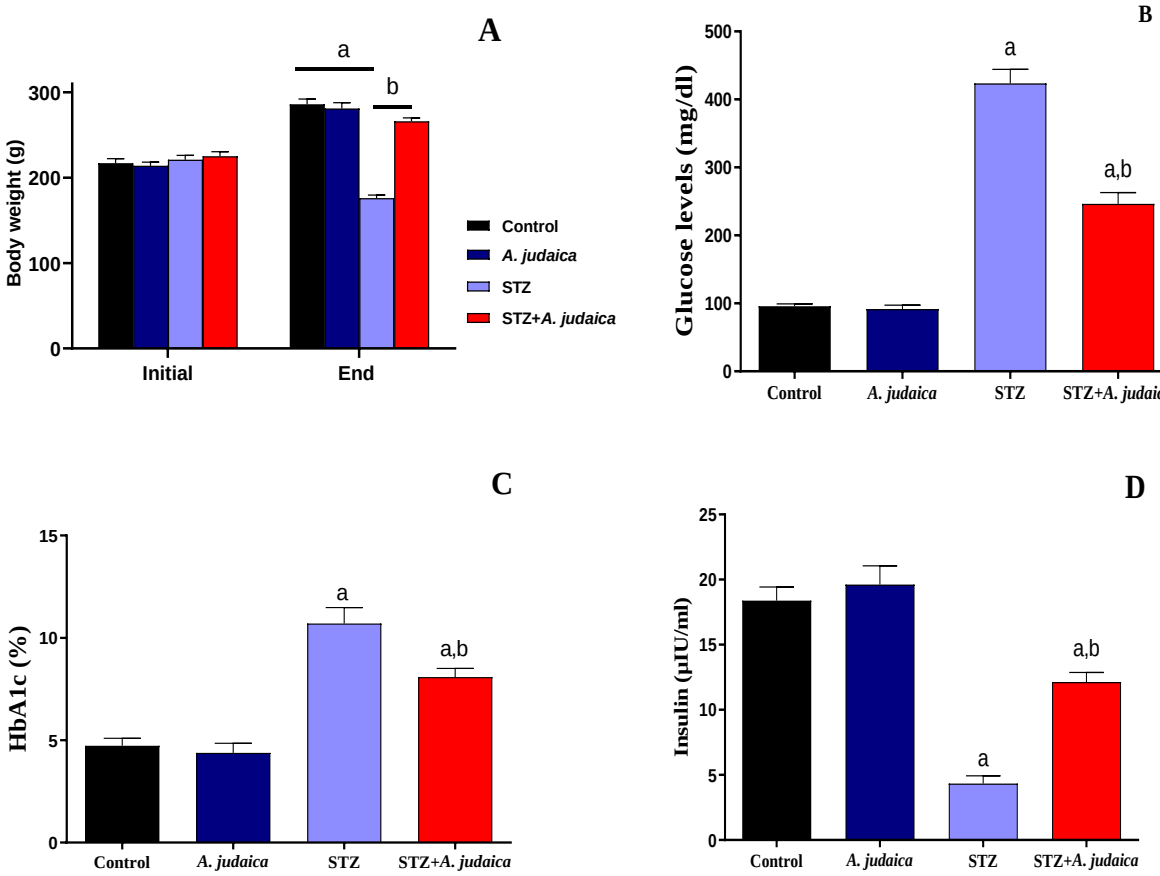

Figure 1: *A. judaica* improves body weight **(A)** and attenuates hyperglycaemia **(B)**, HbA1c% **(C)** and insulin levels **(D)** in STZ-induced diabetic rats. Data are the mean± SEM. (a or b) Significantly different from the control or STZ group respectively at $P < 0.05$.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

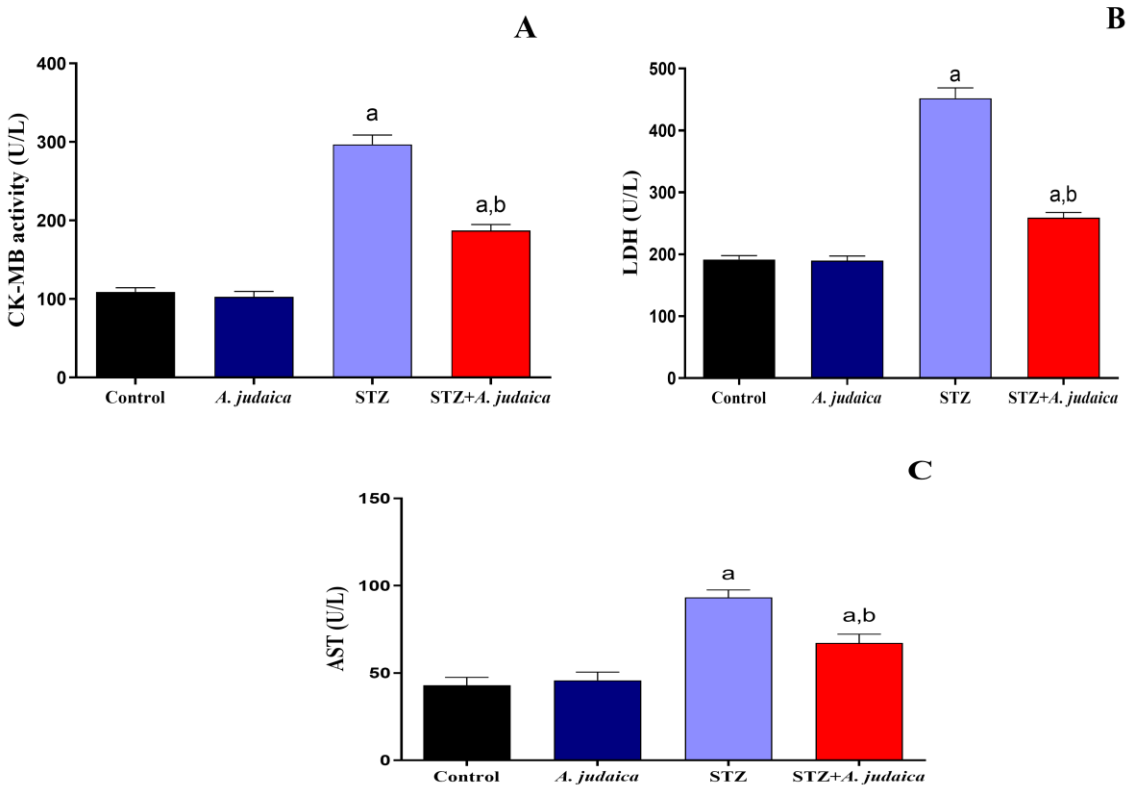

Figure 2: *A. judaica* ameliorates diabetes-induced cardiac damage in rats. Treatment with *A. judaica* for 6 weeks ameliorated serum levels of CK MB **(A)**, LDH **(B)** and AST **(C)** in diabetic rats. Data are the mean± SEM. (a or b) Significantly different from the control or STZ group respectively at $P < 0.05$.

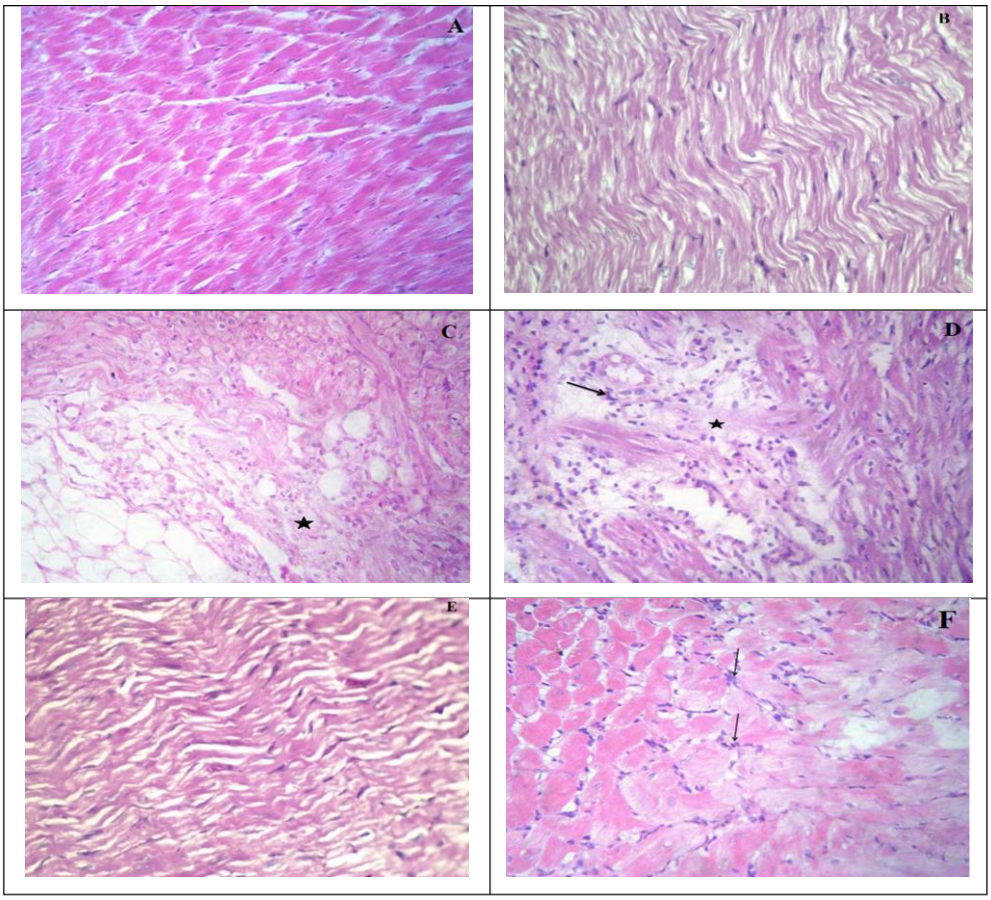

Figure 3: *A. judaica* prevents histological alterations in the heart of diabetic rats. Representative photomicrograph of the heart of the control rats and *A. judaica* showing normal cardiac structures (myocardial striations, spaces, and nuclei) **(A** and **B)**. Sections from the heart of diabetic rats showing serous atrophy of coronary fat (star) **(C)** and necrotic area (star) infiltrated with lymphocytes (arrow) **(D)**. Representative photomicrograph of the heart of diabetic rats treated with *A. judaica* for 6 weeks showing restoration of the normal appearance of cardiomyocytes**(E)** and proliferation of sarcolemma nuclei (arrows) **(F)**.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

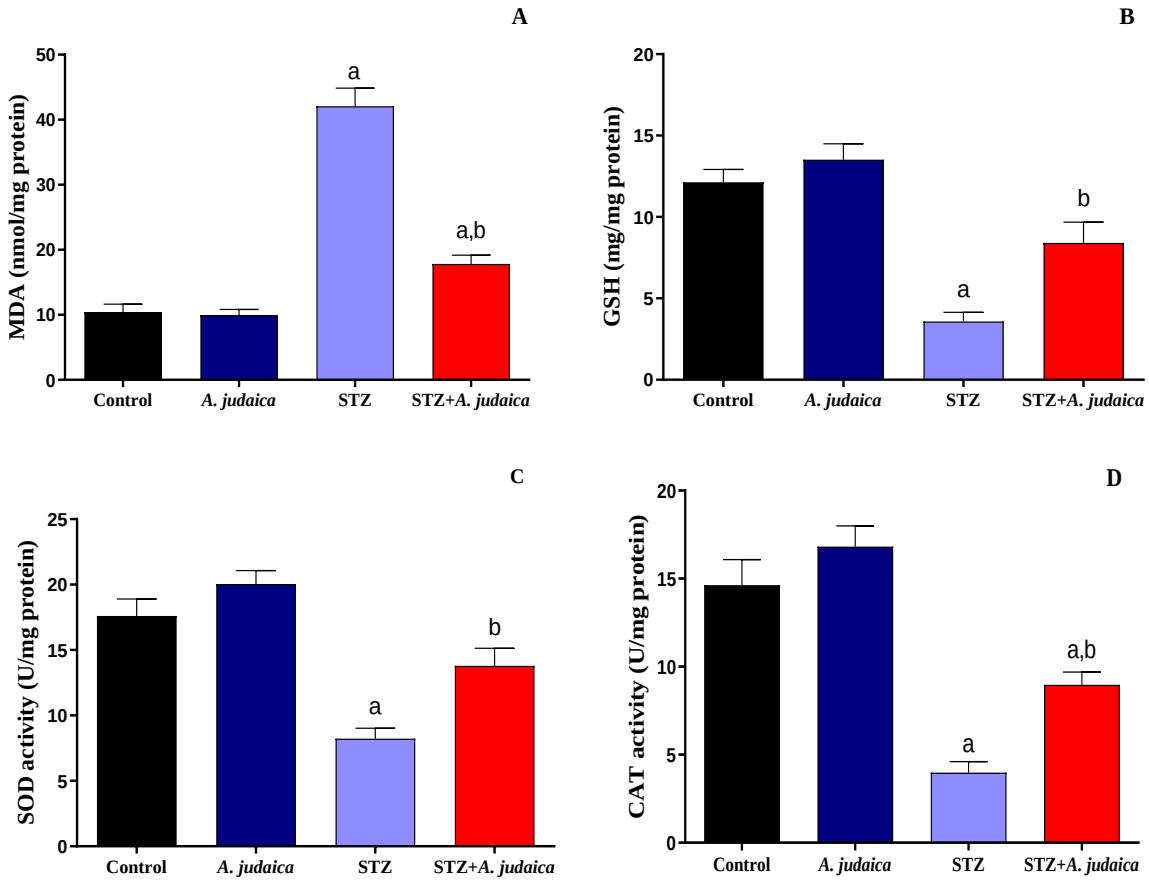

Figure 4:*A. judaica* ameliorates diabetes-induced myocardial oxidative stress in rats. Treatment with *A. judaica* for 6 weeks ameliorated MDA contents **(A)**, GSH levels **(B)** and SOD **(C)** and CAT **(D)** activities in the diabetic myocardium.
Data are the mean± SEM. (a or b) significantly different from the control or STZ group, respectively at $P < 0.05$.

Conclusion

The present study demonstrates that *A. judaica* ameliorates hyperglycaemia and consequently protects against oxidative stress and its mediated cardiac injury *via* elevation of insulin level and posting antioxidant enzymes. Thus, *A. judaica* possesses a therapeutic potential for the treatment of DCM. However, the exact mechanism underlying the cardioprotective action of *A. judaica* undoubtedly deserves further exploration in future studies.

Conflict of Interest

The author declare no conflicts of interest.

Author's Declaration

The author hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgement

The Author is grateful to the authority of Al-Balqa Applied University for the continued support and providing facilities.

References

1. Varga ZV, Giricz Z, Liaudet L, Haskó G, Ferdinandy P, Pacher P. Interplay of oxidative, nitrosative/nitrative stress, inflammation, cell death and autophagy in diabetic cardiomyopathy. Biochim Biophys Acta. 2015;1852(2):232-242.
2. Al Hroob AM, Abukhalil MH, Alghonmeen RD, Mahmoud AM. Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. Biomed Pharmacother. 2018;106:381-389.
3. Althunibat OY, Al Hroob AM, Abukhalil MH, Germoush MO, Bin-Jumah M, Mahmoud AM. Fisetin ameliorates oxidative stress, inflammation and apoptosis in diabetic cardiomyopathy.Life Sci. 2019;221:83-92.
4. Othman AI, El-Sawi MR, El-Missiry MA, Abukhalil MH. Epigallocatechin-3-gallate protects against diabetic cardiomyopathy through modulating the cardiometabolic risk factors, oxidative stress, inflammation, cell death and fibrosis in streptozotocin-nicotinamide-induced diabetic rats. Biomed Pharmacother. 2017;94:362-373.
5. Amin AH, El-Missiry MA, Othman AI. Melatonin ameliorates metabolic risk factors, modulates apoptotic proteins, and protects the rat heart against diabetes-induced apoptosis. Eur J Pharmacol. 2015;747:166-173.
6. Atrooz OM, Abukhalil MH, AlRawashdeh IM. Characterization of β-galactosidase in the Crude Plant

Extract of *Artemisia judaica* L. in Presence and Absence of Some Heavy Metals.Am J Life Sci. 2016;4(5):99-105.
7. Abu-Darwish MS, Cabral C, Gonçalves MJ, Cavaleiro C, Cruz MT, Zulfiqar A, Khan IA, Efferth T, Salgueiro L. Chemical composition and biological activities of *Artemisia judaica* essential oil from southern desert of Jordan. J Ethnopharmacol. 2016;191:161-168.
8. Albasher G, Aljarba N, Al Sultan N, Alqahtani WS, Alkahtani S. Evaluation of the neuro-protective effect of Artemisia judaica extract in a murine diabetic model. Food Biochem. 2020;44(8):e13337.
9. Mesa LE, Lutgen P, Velez ID, Segura AM, Robledo SM. *Artemisia annua* L., potential source of molecules with pharmacological activity in human diseases.AJPCT. 2015;3(5):436-450.
10. Abdalla SS and Zarga MA. Effects of cirsimarit in, a flavone isolated from *Artemisia judaica*, on isolated guinea-pig ileum.Planta Med. 1987; 53(04):322-324.
11. Benmansour N, Benmansour A, El Hanbali F, González-Mas MC, Blázquez MA, El Hakmaoui A, Akssira M. Antimicrobial activity of essential oil of *Artemisia judaica* L. from Algeria against multi-drug resistant bacteria from clinical origin. Flavour Fragr J. 2016; 31(2):137-142.
12. Acheuk F, Lakhdari W, Abdellaoui K, Belaid M, Allouane R, Halouane F. Phytochemical study and bioinsecticidal effect of the crude ethonolic extract of the Algerian plant *Artemisia judaica* L.(Asteraceae) against the black bean aphid, *Aphis fabae* Scop. Poljoprivreda i Sumarstvo. 2017;63(1):95.
13. Abdelgaleil SA, Abbassy MA, Belal AS, Rasoul MA. Bioactivity of two major constituents isolated from the essential oil of *Artemisia judaica* L.Bioresour Technol. 2008;99(13):5947-5950.
14. Mahboubi M and Kazempour N. Biochemical activities of *Iranian Cymbopogon*olivieri (Boiss) Bor. essential oil.Indian J Pharm Sci. 2012;74(4):356.
15. Sitzmann J, Habegger R, Schnitzler WH, Grassmann J. Comparative analysis of antioxidant activities of fourteen Mentha essential oils and their components. Chem. Biodivers. 2014;11(12):1978-1989.
16. Liu CZ, Murch SJ, El-Demerdash M, Saxena PK. *Artemisia judaica* L.: micropropagation and antioxidant activity. J. Biotechnol. 2004;110(1):63-71.
17. El-Sayed MA, BaAbbad R, Balash A, Al-Hemdan NA, Softah A. The potential anti Helicobacter pylori and antioxidant effects of *Artemisia judaica*.Funct Food Health Dis. 2013;3(9):332-340.
18. Nofal SM, Mahmoud SS, Ramadan A, Soliman GA, Fawzy R. Anti-diabetic effect of *Artemisia judaica* extracts.Res J Med Sci. 2009;4(1):42-48.
19. Ahmed ES, Mabrouk DM, Hassanane MM, Khalil WK. Protective effect of *Artemisia judaica* against doxorubicin-induced toxicity in mice. Annu Res Rev. 2017:1-10.
20. Althunibat OY, Al-Mustafa AH, Tarawneh K, Khleifat KM, Ridzwan BH, Qaralleh HN. Protective role of *Punica granatum* L. peel extract against oxidative damage in experimental diabetic rats. Proc Biochem. 2010;45(4):581-585.
21. Abd-Alla HI, Aly HF, Shalaby NM, Albalawy MA, Aboutabl EA. Hunting for renal protective phytoconstituents in *Artemisia judaica* L. and *Chrysanthemum coronarium* L.(Asteraceae). Egy Pharm J. 2014;13(1):46.
22. Mamoru S and Kazuyuki H. A new colorimetric method for determination of serum glucose. Clin Chim Acta. 1977;75(3):387-391.
23. Bae J, Min YS, Nam Y, Lee HS, Sohn UD. *Humulus japonicus* extracts protect against lipopolysaccharide/d-galactosamine-induced acute liver injury in rats. J Med Food. 2018;21(10):1009-1015.
24. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem.. 1979;95(2):351-358.
25. Beutler E. Improved method for the determination of blood glutathione. J Lab Clin Med.1963;61:882-888.
26. Nishikimi M, Rao NA, Yagi K. The occurrence of superoxide anion in the reaction of reduced phenazine methosulfate and molecular oxygen.Biochem Biophys Res Commun. 1972;46(2):849-854.
27. Aebi H. Catalase in vitro. In Meth Enzymol. 1984; 1(105): 121-126.
28. Classics Lowry O, Rosebrough N, Farr A, Randall R. Protein measurement with the Folin phenol reagent. JBiol Chem. 1951;193:265-275.
29. Farshid AA, Tamaddonfard E, Moradi-Arzeloo M, Mirzakhani N. The effects of crocin, insulin and their co-administration on the heart function and pathology in streptozotocin-induced diabetic rats. Avicenna J Phytomed. 2016;6(6):658.
30. Al Hroob AM, Abukhalil MH, Alghonmeen RD, Mahmoud AM. Ginger alleviates hyperglycemia-induced oxidative stress, inflammation and apoptosis and protects rats against diabetic nephropathy. Biomed Pharmacother. 2018;106:381-389.
31. Al-Rasheed NM, Al-Rasheed NM, Hasan IH, Al-Amin MA, Al-Ajmi HN, Mohamad RA, Mahmoud AM. Simvastatin ameliorates diabetic cardiomyopathy by attenuating oxidative stress and inflammation in rats. Oxid Med Cell Longev. 2017; 2017:1-13.
32. Gupta SK, Dongare S, Mathur R, Mohanty IR, Srivastava S, Mathur S, Nag TC. Genistein ameliorates cardiac inflammation and oxidative stress in streptozotocin-induced diabetic cardiomyopathy in rats. Mol Cell Biochem. 2015;408(1-2):63-72.
33. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res. 2001;50(6):537-546.
34. Lenzen S, Drinkgern J, Tiedge M. Low antioxidant enzyme gene expression in pancreatic islets compared with various other mouse tissues.Free Radic. Biol. Med. 1996;20(3):463-466
35. Albasher G, Alwahaibi M, Abdel-Daim MM, Alkahtani S, Almeer R. Protective effects of *Artemisia judaica* extract compared to metformin against hepatorenal injury in high-fat diet/streptozotocine-induced diabetic rats. Environ Sci Pollut Res. 2020; 7:40525–40536.
36. Bhat SH, Ullah MF, Abu-Duhier FMJOP, Medicine E. Bioactive extract of *Artemisia judaica* causes in vitro inhibition of dipeptidyl peptidase IV and pancreatic/intestinal enzymes of the carbohydrate absorption cascade: Implication for anti-diabetic new molecular entities (NMEs). Orient Pharm Exp Med. 2019; 19(1):71-80.
37. Wilcox G. Insulin and insulin resistance. Clin Biochem Rev. 2005;26(2):19.
38. Bodor GS. Biochemical markers of myocardial damage. EJIFCC. 2016;27(2):95.
39. Muthumani M and Miltonprabu S. Arsenic-induced oxidative stress and its possible reversal by chelation therapy. Res Rev J Toxicol. 2012;2:16-37.