

Effect of Stevia rebaudiana and exercise on fatty liver in type 2 diabetic rats

**Reem M. Emam, Abdelaziz M. Hussein, Asaad A. Elmileegy, Fayza R. El-Menabawy,
and Gad El Mawla A. Gad**

Department of Medical Physiology, Faculty of Medicine, Mansoura University, Egypt.

Submit Date: Oct 29,2020
Revise Date: March 23, 2021
Accept Date : March 26, 2021

Keywords

- NAFLD
- T2DM
- Stevia
- Exercise

Abstract

Introduction: Nonalcoholic fatty liver disease (NAFLD) is a prevalent chronic liver disease, which threatens the health of both adults and children. It is strongly associated with type 2 diabetes mellitus, obesity and insulin resistance. In the present study, we evaluate the effect of Stevia rebaudiana and exercise on fatty liver in T2DM. **Methods:** Thirty-two male Sprague Dawely rats were divided into normal control, diabetic, Stevia treated diabetic, and exercise treated diabetic rats. Biochemical parameters, oxidative stress markers, and histopathological examination for liver tissue were done. **Results:** in diabetic group, blood glucose level, HOMA index, cholesterol level, triglycerides level, bilirubin level, liver enzymes (ALT& AST), and MDA were significantly increased. While insulin level, GSH, and CAT activity were significantly decreased when compared to normal control group. Biochemical parameters and Oxidative stress markers were improved in the treated groups, the improvement was more significant in exercise treated group than Stevia treated group. Histopathological examination confirmed our results. **Conclusion:** Stevia rebaudiana and exercise could protect against liver injury induced by T2DM, as they improve glyceemic state, liver enzymes, and oxidative stress markers

Corresponding author: Reem M. Emam, Department of Medical Physiology, Mansoura faculty of Medicine, **Email:** re_mohie@yahoo.com, Phone #0502782496

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is defined as macro vesicular steatosis in $\geq 5\%$ hepatocytes, in the absence of a secondary cause such as alcohol or drugs. Prevalence of NAFLD is about 25% globally, ranging from 13% in Africa to 23% in Europe, and 32% in the Middle East. There is a close association between NAFLD and type 2 diabetes (23%), central obesity (51%), dyslipidemia (69%), and the metabolic syndrome (43%) [1]. NAFLD is now considered a health problem because it is a risk factor, which contributes to type 2 diabetes and some cardiovascular diseases [2].

T2DM is a progressive disorder characterized by insulin resistance and a progressive insulin secretory defect. Glycemic control reduces the risk for diabetes-related morbidity and mortality. In order to control glucose levels as the disease progresses, patients require lifestyle changes, dietary modifications, exercise, weight loss, and pharmacologic treatment, often with multiple classes of diabetes medications [3].

Exercise is a cornerstone of treatment for people with T2DM [4]. Single bout of exercise lowered the blood glucose concentration of patients with diabetes and improved glucose tolerance temporarily [5]. It is known that everyday physical activity, not exercise, is associated with health. Cross-sectional studies suggest that people with NAFLD have lower levels of physical activity than those without, and are also more prone to fatigue. Physical activity is a key determinant of metabolic control and is commonly recommended for people with NAFLD [6].

In modern medicine, herbal plants are preferred as a therapy because of cost, having fewer side-effects and anti-oxidant activities. *Stevia rebaudiana* Bertoni as a herbal plant is famous due to its sweet taste and beneficial effects in blood glucose regulation. In Japan and Brazil, extract of *Stevia* is used as food additives and non-caloric sweeteners. *Stevia* leaves contain eight glycosides including dulcosides A, rebaudiosides A-E, stevioioside and stevioside. Stevioside is the sweetest glycoside found in *Stevia* with antioxidant, antimicrobial, and anticancer properties [7].

Materials and methods

Experimental animals

Thirty-two male Sprague Dawley rats, aged 8-10 weeks, weighting 150-200g were included in this study. Rats were purchased and housed at the Medical Experimental Research Centre (MERC), faculty of medicine, Mansoura University. They were housed in a controlled environment with a temperature of 25°C ($\pm 3^\circ\text{C}$). The rats were allowed free access to water and diet. This research was approved by Mansoura Institutional Research Board (IRB), which follows the Declaration of Helsinki.

Experimental design

Animals were divided into 4 groups of 8 rats each. Group 1: (Normal control): normal rats received isotonic saline for 4 weeks. Group 2: (T2DM): rats with T2DM and received isotonic saline for 4 weeks. Group 3: (DM + *Stevia*): rats with T2DM and treated with *Stevia* at a dose of 400 mg/Kg orally once daily for 4 weeks [8]. Group 4: (DM + exercise): rats with T2DM and maintained on exercise (swimming) for 30 mins/day, 5 days/week for 4 weeks [9].

Type 2 DM was induced by feeding rats high fat diet (60% fat, 20% proteins, 20% carbohydrates) for 4 weeks then single intraperitoneal injection of streptozotocin (35 mg/kg body weight) freshly prepared in cold 0.1 mol/L citrate buffer (pH 4.5) [10]. Rats with blood glucose > 200 mg/dl was considered diabetic and used in this study.

Collection of blood and harvesting liver tissue

At the end of the experiment, rats were anesthetized and blood samples were collected from the heart by cardiac puncture. The blood was put in a clean test tube and left to coagulate and then centrifuged to obtain serum. The serum was stored at -20° C for biochemical analysis. After exposure of the abdominal cavity of the rat, about 5 ml saline was perfused in the hepatic circulation. Then a small part of the liver was dissected to be preserved in cryo-tube in liquid nitrogen for oxidative stress markers. The rest of the liver was dissected in two halves. The first one was preserved in 10 % formalin for histopathological examination; the second one was preserved in -20 °C to be used as a frozen section for histopathological examination by red oil stain.

Biochemical parameters

Plasma glucose was measured using commercial kit. Insulin level was determined by utilizing rat insulin ELISA kit purchased from (Sun Red biological technology company, China) following the manufacturer's instructions. Homeostasis Model Assessment (HOMA) index is calculated after measuring fasting blood glucose and fasting insulin level in the same rat by this equation: $HOMA = \frac{FBG_{(mg/dl)} \times \text{fasting insulin}_{(U/ml)}}{405}$ [11]. Cholesterol and triglycerides were measured by using commercial kit (SPIN REACT). ALT and AST were determined by using

Kit purchased from Sigma Chemical Co. (St. Louis, MO, USA). Bilirubin assay was done using commercial kit (Diamond Diagnostics).

Oxidative stress markers

A small part of the liver was taken, homogenized in 0.02 M sodium phosphate buffer, pH 7.4 (1:4 water/volume) using an Ultra-Turrax smooth glass homogenizer with a motor driven , then centrifuged. Supernatant was used for assay of malondialdehyde (MDA), reduced glutathione (GSH), and catalase (CAT). Tissue MDA was measured by the modified technique. Kits obtained from company of Bio-diagnostic, Egypt. Reduced glutathione (GSH) was measured using the kits purchased from Bio diagnostic, Giza, Egypt. Catalase reacts with a known quantity of H₂O₂. The reaction is stopped after exactly one minute with catalase inhibitor.

Histopathological examination

Liver specimen was divided into two halves; the first part was fixed in 10% buffered neutral formalin solution. After that it was embedded in paraffin, then sectioned and stained with Hematoxylin and Eosin (H&E). The second part was kept frozen in -20° C to be stained with Red Oil stain. This stain is to detect the fat vacuoles in the tissue and differentiate it from glycogen droplet. Immune stain (caspase 3) was used to detect apoptosis, as it gives brown color with apoptotic tissue. Kit for Mouse and Rabbit Antibodies, Purchased from GBI Labs Company, USA was used.

Quantification of Caspase 3 (calculation the % ratio)

The software routine of quantification includes:

Step 1: Image acquiring from the camera using a u-tech® frame grabber.

Step 2: Enhancing color tones of the image depending on the hue of target areas.

Step 3: Holding the image at the level of the desired hue range to form a binary mask that represent target areas.

Step 4: Define binary mask as region of interest (ROI).

Step 5: Apply area measurement routine to obtain results expressed as % area of positively stained area in relation to all field area.

Step 6: All results appeared in Excel files.

Statistical analysis

Data were entered and statistically analyzed using the Statistical Package for Social Sciences (SPSS) version 16. Quantitative data were described as means (SD) or medians, as appropriate. They were tested for normality by Shapiro-Wilk test. One way ANOVA with LSD post-hoc multiple comparisons was used for comparison between groups. "p value ≤ 0.05 " was considered to be statistically significant.

Results

Biochemical parameters and Oxidative stress markers:

DM group showed significant increase in blood glucose level, HOMA index, cholesterol level, triglycerides level, bilirubin level, liver enzymes (ALT& AST), and MDA when compared to normal control group ($P < 0.0001$). Significant decrease was showed in insulin level, GSH, and CAT activity in DM group when compared to normal control group ($P < 0.0001$). These parameters were improved in the treated groups (DM+Stevia), (DM+Exercise); the improvement

was more significant in DM+Exercise group than DM+Stevia group ($P < 0.05$) (tables 1-4).

Histopathological examination of liver specimen stained by Hematoxylin and Eosin showed abnormal swollen vacuolated hepatocytes with eccentric nuclei, and area of focal necrosis in DM group when compared to normal control group. Moderate improvement occurred with DM+Stevia group, while the marked improvement occurred with DM+ Exercise group (Fig. 1).

Liver specimen stained with Oil Red O stain showed severe fatty infiltration in DM group when compared to normal control group. DM+Stevia group showed moderate improvement (moderate fatty cells infiltration). The best improvement showed in DM+Exercise group, as it showed mild fatty cells infiltration (Fig.2).

Immunohistochemical examination of liver specimen stained with caspase 3 showed strong staining reaction in liver specimen of DM group that means severe apoptosis. Mild to moderate brown staining reaction of caspase 3 was shown in treated groups (DM+Stevia, DM+Exercise) that means moderate improvement. No brown reaction was shown in normal control group (Fig.3).

The percentage ratio of apoptosis in the liver tissue was analyzed by image analysis as shown in figure 4 and it showed the following, high percentage in DM group when compared to other treated groups as it was about 1%. It showed moderate improvement in DM+Stevia group as the ratio was about 0.5%. The best improvement was shown in DM+Exercise group as it was about 0.2% (Fig. 4).

Table (1): Glycemic parameters in different groups in experiment

	Normal control	DM	DM+Stevia	DM+ Exercise
Blood glucose (mg/dl)	100.2±14.1	368.2±36.3*	222.1±34.7*#	181.1±10.8*#S
Insulin level (IU/mL)	10.9 ±2.1	5.7 ±0.7*	4.6 ±0.3*	5.7 ±0.1* ^S
HOMA index	2.6±0.7	6±1.9*	4.9±0.6*#	3.7±0.2*# ^S

Test used: One-way ANOVA followed by post-hoc Tukey. Values are expressed as means ± SD. DM= diabetic group, DM+Stevia=diabetic treated with stevia, DM+Exercise= diabetic treated with exercise. (*) significant vs normal control group, (#) significant vs DM group, (\$) significant vs DM+ Stevia group

Table (2): Lipid profile in different groups in experiment

	Normal control	DM	DM+Stevia	DM+ Exercise
Cholesterol level (mg/dL)	100.5±9.02	371.5±19.4*	139.3±3.01*#	126.5±3.6*# ^S
Triglycerides level (mg/dL)	111±9.1	266.1±10.9*	146.1±17.8*#	130±1.7*# ^S

Test used: One-way ANOVA followed by post-hoc Tukey. Values are expressed as means ± SD. DM= diabetic group, DM+Stevia=diabetic treated with stevia, DM+Exercise= diabetic treated with exercise. (*) significant vs normal control group, (#) significant vs DM group, (\$) significant vs DM+ Stevia group

Table (3): liver function tests in different groups in experiment

	Normal control	DM	DM+Stevia	DM+ Exercise
Bilirubin level (mg/dL)	0.59±0.09	0.79±0.04*	0.59±0.08#	0.61±0.07#
ALT level (U/L)	39.5±10.8	72.3±20*	54.3±12.5*#	37.5±2.8# ^S
AST level (U/L)	32.3±7.1	51.6±6.6*	47.3±8.1*	33.8±6.1# ^S

Test used: One way ANOVA followed by post-hoc tukey. Values are expressed as means ± SD. DM= diabetic group, DM+Stevia=diabetic treated with stevia, DM+Exercise= diabetic treated with exercise. (*) significant vs normal control group, (#) significant vs DM group, (\$) significant vs DM+ Stevia group

Table (4): Oxidative stress markers in different groups in experiment

	Normal control	DM	DM+Stevia	DM+ Exercise
Malondialdehyde (MDA) level (m.mol/g liver tissue)	4.6±1.2	16.7±3.2*	7.9±1*#	5.9±0.7# ^S
Reduced glutathione (GSH) level (m.mol/g liver tissue)	21.7±6.2	4.9±0.8*	12.9±0.2*#	16.5±2.7*# ^S
Catalase activity (U/g liver tissue)	13.4±1.7	6.9±0.7*	10.2±0.3*#	9.1±0.7*# ^S

Test used: One-way ANOVA followed by post-hoc Tukey. Values are expressed as means ± SD. DM= diabetic group, DM+Stevia=diabetic treated with stevia, DM+Exercise= diabetic treated with exercise. (*) significant vs normal control group, (#) significant vs DM group, (\$) significant vs DM+ Stevia group

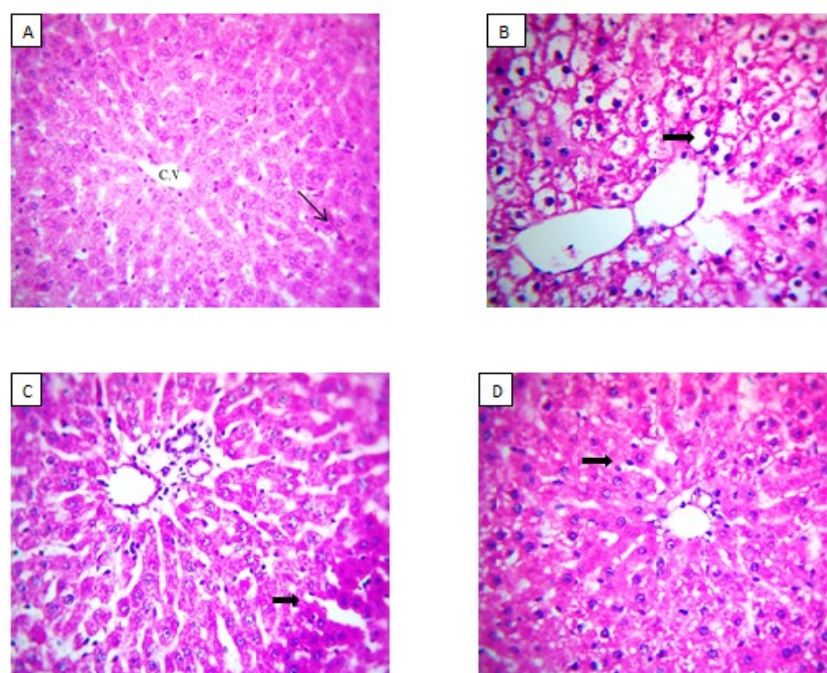


Figure (1): **A) Control group.** Liver specimen of normal control group, the arrow showed normal hepatocytes with central nuclei with no fat infiltration **(B): DM group.** Liver specimen of DM group, the arrow show severe fatty infiltration with eccentric nuclei and areas of focal necrosis **C) DM + Stevia group.** Liver specimen of DM+Stevia group, show moderate improvement, the arrow show mild fatty infiltration and near normal hepatocytes **D) DM + Exercise group** Liver specimen of DM+Exercise group, show marked improvement, the arrow show slight fatty infiltration and near normal hepatocytes (H&E, 400x).

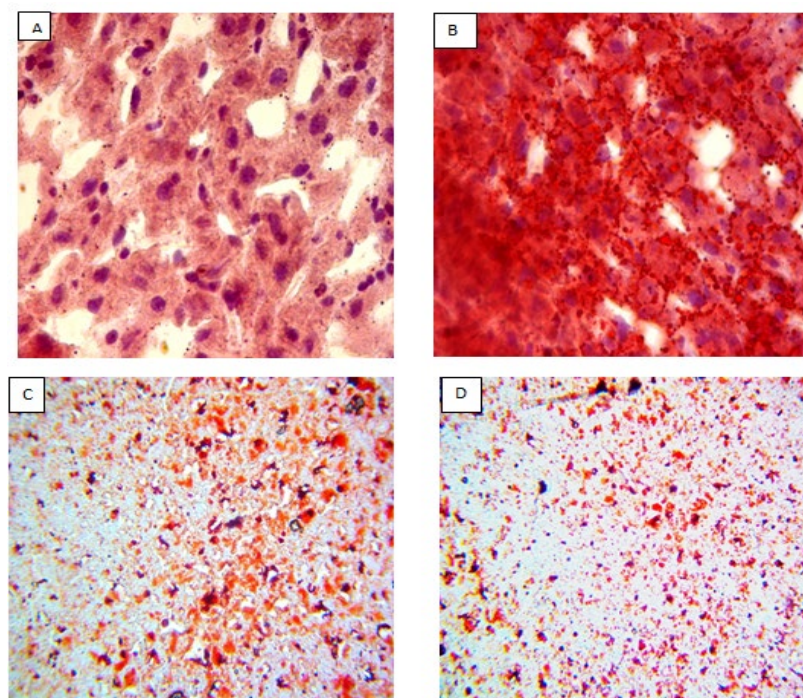


Figure (2): **A) Control group.** Liver specimen of normal group, shows negative Oil Red stain (no fat cells) **(B): DM group.** Liver specimen of DM group, show positive Oil Red stain (fat cells infiltration) **C) DM + Stevia group.** Liver specimen of DM+Stevia group, show moderate Oil Red stain (moderate fatty cells infiltration) **D) DM + Exercise group** Liver specimen of DM+Exercise group, show mild Oil Red stain (mild fatty cells infiltration) (O.Rx400).

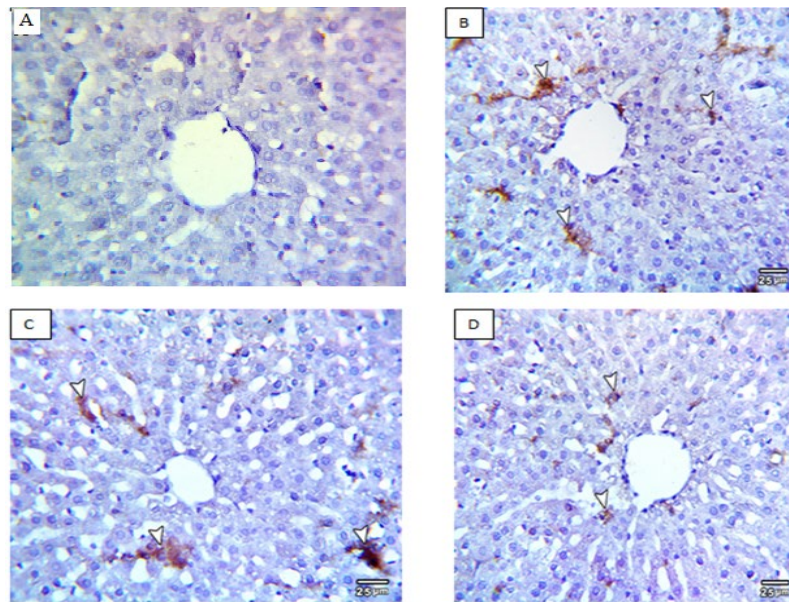


Figure (3): **A) Control group.** Liver specimen of normal group, showed no brown reaction of caspase 3 **(B): DM group** the arrow showed strong staining brown reaction of caspase 3 in liver specimen. **C) DM + Stevia group** the arrow showed the brown staining reaction of Caspase 3 in liver specimen. **D) DM + Exercise group** the arrow showed the brown staining reaction of Caspase 3 in liver specimen.

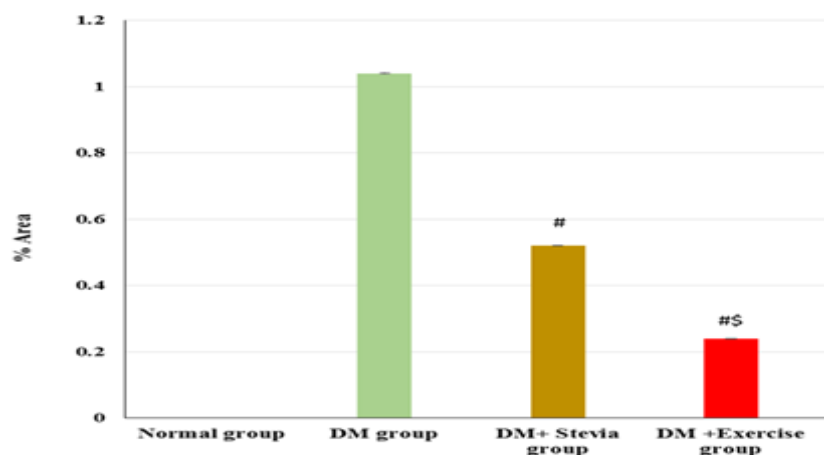


Figure (4): % Area of immunohistochemical stain of caspase 3 in different groups. (#) significant vs DM group, (\$) significant vs DM+ Stevia group.

Discussion

Nonalcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) commonly exist together. The principle behind the management of NAFLD with T2DM involves an indirect effect through improvement in insulin resistance and hyperglycemia and thus is used for the treatment of T2DM as well [12]. One of the medicinal plants widely used as an alternative medicine especially in the treatment of hyperglycemia is Stevia rebaudiana and its extracts which have

pharmacological and therapeutic features that include antioxidant, antihypertensive, antimicrobial, anticancer and antidiabetic effects [13]. In the liver, exercise increases fatty acid oxidation, decreases fatty acid synthesis, and prevents mitochondrial and hepatocellular damage through a reduction of the release of damage-associated molecular patterns [14].

In the current study, development of T2DM was confirmed by the presence of significant increase in blood glucose, and HOMA index in DM group compared to normal group. This was previously

illustrated by [15]. Significant decrease in insulin level in DM group compared to normal group also confirms development of T2DM. This was illustrated in a previous study [16]. At the same time, it contradicts with another previous study [17] that claimed that insulin level was elevated in diabetic group when compared to normal group. Low level of insulin in diabetic group in this study may be due to STZ destroyed significant portion of β -cells resulting in less insulin release in presence of hyperglycemia as illustrated in previous literature [18].

Interestingly, these effects were significantly improved by *Stevia* aquatic extract, and this is in agreement with the study of [19]. *Stevia* may stimulate the beta cells to release insulin, leading to improvement in the carbohydrate metabolizing enzymes and thus establishing normal blood glucose level [19]. Swimming exercise in this study significantly decreased FBG, and HOMA index when compared to diabetic group, suggesting that exercise relieve T2DM, and this is in agreement of the study of [17].

Stevia treated group in this study showed non-significant decrease in insulin level when compared to diabetic group, and this contradict with a previous study which illustrated that *Stevia* is able to decrease insulin level significantly [20]. Exercise treated group in this study showed non-significant change in insulin level when compared to diabetic group and this contradicts with a previous study [17].

Regarding to oxidative stress markers, it was known that oxidative stress plays a key role in the pathogenesis of diabetes and NAFLD [24]. In this study, we found that diabetes reduced GSH, and

Regarding cholesterol and triglycerides levels, they showed significant elevation in diabetic group when compared to normal group. These findings were significantly improved with *Stevia* treatment and this is in agreement of the study of [19]. Exercise in this study showed significant decrease in cholesterol and triglycerides levels when compared to diabetic group and this is in agreement of the study of [21].

Serum bilirubin in this study was elevated significantly in diabetic group when compared to normal group; this may be due to hepatocellular injury and biliary cirrhosis. Oral primary bile acid replacement may effectively reverse liver failure and restore liver functions [22]. Treated groups showed significant decrease in serum bilirubin when compared to diabetic group and this is in harmony with their hepato-protective effect. Liver enzymes (ALT and AST) showed significant elevation in diabetic group when compared to normal group suggesting liver injury. ALT was decreased significantly and AST was decreased non-significantly with *Stevia* treatment and this is in agreement of the study of [19]. Therefore, the hepato-protective properties of *Stevia* may be useful as a treatment. Exercise also in this study showed significant decrease in liver enzymes either ALT or AST when compared to diabetic group and this is in agreement of the study of [23].

CAT significantly in the liver tissue. The opposite trend was observed in MDA level, which is a marker for lipid peroxidation, with respect to the normal group. Similar results are available in the literature [25]. ROS may be generated and causes

Ca²⁺ leakage into the cytosol through inositol trisphosphate receptor, and consequent Ca²⁺ influx in the nuclei and mitochondria resulting in altered calcium homeostasis, ROS generation, cytoskeletal damage and mitochondrial dysfunction [26]. These findings lead to glucolipotoxicity and β -cell dysfunction during HFD-STZ-induced type 2 diabetes in rats [27].

Stevia aquatic extract in this study improved these finding as it caused significant elevation in the level of GSH, and CAT, and significant reduction in MDA level when compared to diabetic group, and this is in agreement of previous study done by [19]. Consistent with the results of the current study, the anti-oxidant effects of Stevia has been evaluated. The result showed that Stevia extract has a significant antioxidant potential [28].

Exercise in this study caused significant elevation in GSH, and CAT levels, and significant reduction in MDA level when compared to diabetic group, and this is in agreement of previous study done by [17]. These findings indicate that exercise may be beneficial to type 2 diabetic rats via attenuating oxidative stress in the liver. High level of MDA leads to diabetic liver damage and other complications [24]. Exercise may relieve diabetes-induced inhibition of SIRT1 gene expression. This SIRT1 gene has been identified in various metabolic tissues, such as liver that regulates hepatic glucose, lipid metabolism, insulin secretion, and oxidative stress. SIRT1 is decreased in rat liver of T2DM with NAFLD [17].

According to the literature, glucose is oxidized to produce reactive ketoaldehyde and superoxide radicals prone to complications in diabetes. Therefore, excessive production of free radicals causes the destruction of macromolecules

including carbohydrates, proteins, lipids, and DNA [29]. In addition, ROS production further leads to hepatic structural and functional disorders [30]. MDA significantly correlated with hepatic histo-architectural distortion and making a strong circle between oxidative stress and NAFLD [31]. It was indicated that neutralization of reactive species has significantly been able to inhibit the development of several organ damages in diabetes. For this purpose, antioxidant defense mechanism like CAT is available in cells [32]. However, high levels of free radicals production and the simultaneous decline of endogenous antioxidant reserves may result in cell injury and in the development of insulin resistance [29].

Furthermore, according to histopathological findings, distortion of liver architecture, cytoplasmic vacuolization, hepatic sinusoidal dilation, inflammatory cell infiltration, unclear cell boundaries with acidophilic cytoplasm, and obvious degeneration of nuclei and signs of apoptotic cell death (nuclear pyknosis) were observed in diabetic animals in slides stained with H&E. However, these finding showed improvement with Stevia treated group, and this is in agreement of a previous study [33]. It is cleared that Stevia affect the metabolic pathway associated with lipotoxicity, including steatosis, hepatitis and steatohepatitis. Not only that, but also exercise treated group showed histological improvement in the form of decreased steatosis and inflammatory cells. These data are in accordance with previous results [17] and suggest that exercise training is very effective for the management of diabetes mellitus.

Regarding fat cells, slides stained with Oil Red stain in this study showed severe positive reaction

that means severe fatty infiltration in diabetic group when compared to normal group. Stevia treated group showed moderate reaction that means moderate fatty infiltration indicating the ability of Stevia in improving hepatic steatosis [34]. Exercise treated group showed mild fatty infiltration, and this was illustrated in a previous literature [35].

Regarding apoptosis, there were strong reactions against Caspase-3 monoclonal antibodies in hepatic parenchyma observed around areas of steatosis, with significant increase in % immune staining area (figure 4) with image analysis in

untreated NAFLD group when compared with normal rats which had no staining reaction or expression in image analysis suggesting occurrence of apoptosis in diabetic group. These results are in consistent with a previous study [36]. Stevia treated group showed significant reduction of apoptotic reaction when compared to diabetic group and this cleared the Stevia protective effect. Also, exercise treated group showed significant reduction in percentage ratio of apoptotic marker (caspase 3) when compared to diabetic group and this in harmony of a previous literature [37].

Conclusion

It is possible to conclude that Stevia and exercise has many beneficial effects on non-alcoholic fatty liver disease (NAFLD) of diabetic rats. Their efficacy is mainly through oxidative stress attenuation and hypoglycemic effect.

Acknowledgements: MERC is acknowledged for its significant contribution to the experimental part of the study.

Conflict of interest: The authors declare that there is no conflict of interest.

References

1. Younossi, Z. M., Koenig, A. B., Abdelatif, D., Fazel, Y., Henry, L., & Wymer, M. Global epidemiology of nonalcoholic fatty liver disease-Meta-analytic assessment of prevalence, incidence, and outcomes. *Hepatology*. 2016; 64(1), 73-84. doi:10.1002/hep.28431
2. Dharmalingam M, Yamasandhi P. Nonalcoholic fatty liver disease and Type 2 diabetes mellitus. *Indian Journal of Endocrinology and Metabolism*. 2018; 22(3):421.
3. **Standards of Medical Care in Diabetes—2016:** Summary of Revisions. *Diabetes Care*. 2015;39 (Supplement 1).
4. **Colberg SR, Sigal RJ, Fernhall B, Regensteiner JG, Blissmer BJ, Rubin RR, et al.** Exercise and Type 2 Diabetes: The American College of Sports Medicine and the American Diabetes Association: joint position statement executive summary. *Diabetes Care*. 2010; 33(12):2692–6.
5. Huebschmann, A. G., Schauer, I. E., Bauer, T. A., Regensteiner, J. G., & Reusch, J. E. Exercise Performance Impairments and Benefits of Exercise Training in Diabetes. *Contemporary Diabetes Diabetes and Exercise*. 2017; 83-108. doi:10.1007/978-3-319-61013-9_6
6. **Romero-Gómez, M., Zelber-Sagi, S., & Trenell, M.** Treatment of NAFLD with diet, physical activity and exercise. *Journal*

- of Hepatology. 2017; 67(4), 829-846. doi:10.1016/j.jhep.2017.05.016
7. **Kumar H., Singh K., and Kumar S.** 2C-methyl- d- erythritol 2,4-cyclodiphosphate synthase from Stevia rebaudiana Bertoni is a functional gene. *Molecular Biology Reports*. 2012; 39(12), 10971-10978. doi:10.1007/s11033-012-1998-9.
 8. **Bayat E., Dastgheib S., Egdar S., and Mokarram P.** Effect of the Aquatic Extract of Stevia on the Serum Level of Interleukin-6 in Streptozotocin-Nicotinamide Induced Diabetic Rats. *Shiraz E-Medical Journal*. 2017; 18(2). doi:10.17795/semj45015.
 9. **Leme J, Silveira R, Gomes R, Moura R, Sibuya C, Mello M, et al.:** Long-term physical training increases liver IGF-I in diabetic rats. *Growth Hormone & IGF Research*. 2009; 19 (3): 262-6.
 10. **Hussein AM., Abdel-Aziz A., Gabr M., and Hemmaid KZ.** Myocardial and metabolic dysfunction in type 2 diabetic rats: impact of ghrelin. *Canadian Journal of Physiology and Pharmacology*. 2012; 90(1), 99-111. doi:10.1139/y11-103.
 11. **Salgado, A. L. F. d. A., Carvalho, L. d., Oliveira, A. C., Santos, V. N. d., Vieira, J. G. & Parise, E. R.** Insulin resistance index (HOMA-IR) in the differentiation of patients with non-alcoholic fatty liver disease and healthy individuals. *Arquivos de gastroenterologia*. 2010; 47(2), 165-169.
 12. **Dharmalingam, M., & Yamasandhi, P.** Nonalcoholic fatty liver disease and Type 2 diabetes mellitus. *Indian Journal of Endocrinology and Metabolism*. 2018; 22(3), 421. doi: 10.4103/ijem.ijem_585_17.
 13. **Carrera-Lanestosa A, Moguel-Ordóñez Y, Segura-Campos M.** Stevia rebaudiana Bertoni: A natural alternative for treating diseases associated with metabolic syndrome. *J Med Food*. 2016;20:933- 43. DOI:1089/jmf.2016.0171. PMID:28792778
 14. **Windt, DJVD., Sud, V., Zhang, H., Tsung, A., & Huang, H.** The Effects of Physical Exercise on Fatty Liver Disease. *Gene Expression*. 2018; 18(2), 89–101. doi: 10.3727/105221617x15124844266408
 15. **Ghiasi, R., Soufi, F. G., Hossein Somi, M., Mohaddes, G., Babil, F. M., Naderi, R., Alipour, M. R.** Swim training improves HOMA-IR in type 2 diabetes induced by high fat diet and low dose of streptozotocin in male rats. *Adv. Pharm. Bull.* 2015; 5, 379.
 16. **Gaballah HH, Zakaria SS, Mwafy shorouk E, Tahooun NM, Ebeid AM.** Mechanistic insights into the effects of quercetin and/or GLP-1 analogue liraglutide on high-fat diet/streptozotocin-induced type 2 diabetes in rats. *Biomedicine & Pharmacotherapy* . 2017; Aug;92:331–9.
 17. **Ghiasi, R., Naderi, R., Mozaffar, A., & Alihemmati, A.** The effect of swimming training on oxidative stress, SIRT1 gene expression, and histopathology of hepatic tissue in type 2 diabetic rats. *Biologia*

- Futura. 2019; 70(3), 167–174. doi: 10.1556/019.70.2019.21
18. **Kabil, S. L., & Mahmoud, N. M.** Canagliflozin protects against non-alcoholic steatohepatitis in type-2 diabetic rats through zinc alpha-2 glycoprotein up-regulation. *European Journal of Pharmacology*. 2018; (828) 135–145. doi: 10.1016/j.ejphar.2018.03.043
19. **Assaei R, Mokarram P, Dastghaib S, Darbandi S, Darbandi M, Zal F, et al.** Hypoglycemic Effect of Aquatic Extract of Stevia in Pancreas of Diabetic Rats: PPAR γ -dependent Regulation or Antioxidant Potential. *Avicenna J Med Biotechnol*. 2016; Jun;8(2):65-74.
20. **Holvoet, P., Rull, A., García-Heredia, A., López-Sanromà, S., Geeraert, B., Joven, J., & Camps, J.** Stevia-derived compounds attenuate the toxic effects of ectopic lipid accumulation in the liver of obese mice: A transcriptomic and metabolomic study. *Food and Chemical Toxicology*. 2015; 77, 22–33. doi: 10.1016/j.fct.2014.12.017
21. **Moazami, M., & Askari, A.** Effect of Six Months of Aerobic Exercise on Lipid Profile, Inflammatory Markers and Risk Factors of Cardiovascular Disease in Obese Women. *Medical Laboratory Journal*. 2018; 12(2), 32–37. doi: 10.29252/mlj.12.2.32
22. **Chen, H.-L., Wu, S.-H., Hsu, S.-H., Liou, B.-Y., Chen, H.-L., & Chang, M.-H.** Jaundice revisited: recent advances in the diagnosis and treatment of inherited cholestatic liver diseases. *Journal of Biomedical Science*. 2018; 25(1). doi: 10.1186/s12929-018-0475-8
23. **Katsagoni, C. N., Georgoulis, M., Papatheodoridis, G. V., Panagiotakos, D. B., & Kontogianni, M. D.** Effects of lifestyle interventions on clinical characteristics of patients with non-alcoholic fatty liver disease: A meta-analysis. *Metabolism*. 2017; 68, 119–132. doi: 10.1016/j.metabol.2016.12.006
24. **Klasic, A., Isakovic, A., Kocic, G., Kavarić, N., Jovanovic, M., Zvrko, E., Skerovic, V., Ninic, A.** Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes*. 2018; 126, 371–378.
25. **de Bem, G. F., da Costa, C. A., Cordeiro, V. D. S. C., Santos, I. B., de Carvalho, L. C. R. M., de Andrade Soares, R., Ribeiro, J. H., de Souza, M. A. V., da Cunha Sousa, P. J., Ognibene, D. T.** Euterpe oleracea Mart. (açai) seed extract associated with exercise training reduces hepatic steatosis in type 2 diabetic male rats. *J. Nutr. Biochem*. 2018; 52, 70–81.
26. **Koliaki, C., & Roden, M.** Alterations of Mitochondrial Function and Insulin Sensitivity in Human Obesity and Diabetes Mellitus. *Annual Review of Nutrition*. 2016; 36(1), 337–367. doi: 10.1146/annurev-nutr-071715-050656
27. **Govindaraj, J., & Pillai, S. S.** Rosmarinic acid modulates the antioxidant status and protects pancreatic tissues from

- glucolipototoxicity mediated oxidative stress in high-fat diet: streptozotocin-induced diabetic rats. *Molecular and Cellular Biochemistry*. 2015; 404(1-2), 143–159. doi: 10.1007/s11010-015-2374-6
28. **Singh S, and Garg V.** Antidiabetic, antidyslipidemic and anti-oxidative potential of methanolic root extract of *Stevia rebaudiana* (Bertoni) on alloxan induced diabetic. *J Pharm Pharm Sci*. 2014; 3 (7): 1859– 1872.
29. **Padiya, R., Khatua, T. N., Bagul, P. K., Kuncha, M., Banerjee, S. K.** Garlic improves insulin sensitivity and associated metabolic syndromes in fructose fed rats. *Nutr. Metab.* 2011; 8, 53.
30. **Iskender, H., Dokumacioglu, E., Sen, T. M., Ince, I., Kanbay, Y., Saral, S.** The effect of hesperidin and quercetin on oxidative stress, NF- κ B and SIRT1 levels in a STZ-induced experimental diabetes model. *Biomed. Pharmacother*. 2017; 90,500–508.
31. **Klasic, A., Isakovic, A., Kocic, G., Kavacic, N., Jovanovic, M., Zvrko, E., Skerovic, V., Ninic, A.** Relationship between oxidative stress, inflammation and dyslipidemia with fatty liver index in patients with type 2 diabetes mellitus. *Exp. Clin. Endocrinol. Diabetes*. 2018; 126, 371–378.
32. **Naderi, R., Mohaddes, G., Mohammadi, M., Ghaznavi, R., Ghyasi, R., Vatankhah, A. M.** Voluntary exercise protects heart from oxidative stress in diabetic rats. *Adv. Pharm. Bull*. 2015b 5, 231
33. **Holvoet, P., Rull, A., García-Heredia, A., López-Sanromà, S., Geeraert, B., Joven, J., & Camps, J.** Stevia-derived compounds attenuate the toxic effects of ectopic lipid accumulation in the liver of obese mice: A transcriptomic and metabolomic study. *Food and Chemical Toxicology*. 2015; 77, 22–33. doi: 10.1016/j.fct.2014.12.017
34. **Zhao, L., Wang, X., Xu, M., Lian, Y., Wang, C., Yang, H., & Mehmood, A.** Dietary intervention with Stevia residue extracts alleviates impaired glucose regulation in mice. *Journal of Food Biochemistry*. 2018; 42(6). doi: 10.1111/jfbc.12651
35. **Baek, K.-W., Gim, J.-A., & Park, J.-J.** Regular moderate aerobic exercise improves high-fat diet-induced nonalcoholic fatty liver disease via monoacylglycerol O-acyltransferase 1 pathway suppression. *Journal of Sport and Health Science*. 2018; doi: 10.1016/j.jshs.2018.09.001
36. **Mohamed, A.K., & Magdy, M.** Caspase 3 role and immunohisto-chemical expression in assessment of apoptosis as a feature of H1N1 vaccine-caused Drug-Induced Liver Injury (DILI). *Electron Physician*. 2017; 9(5), 4261–4273.
37. **Shi, G.J, Zheng, J, Wu, J, Qiao, H.Q, Chang, Q, Niu, Y, et al.** Beneficial effects of *Lycium barbarum* polysaccharide on spermatogenesis by improving antioxidant activity and inhibiting apoptosis in streptozotocin-induced diabetic male mice. *Food Funct.*, 2017; 8, 1215-1226.