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Study of the Effect of Stevia With or Without Metformin on Streptozotocin – Nicotinamide Induced Diabetes in Adult Male Albino Rats

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Abstract

There is growing evidence that detects a pathological vicious circle relationship between complications of diabetes mellitus (DM) and oxidative stress as well as abnormal inflammatory response. This study aimed to elucidate possible effects of stevia, with or without metformin, on glycemic indices, redox state, and inflammatory state in DM induced by streptozotocin and nicotinamide in adult male albino rats. For this purpose, fifty adult male albino rats were equally divided into five groups: group 1 (control), group 2 (diabetic), group 3 (diabetic metformin-treated), group 4 (diabetic stevia-treated), and group 5 (metformin and stevia-treated). The experiment continued for four weeks. Then fasting blood glucose (FBG), insulin, Homeostasis Model Assessment of Insulin Resistance (HOMA-IR), malondialdehyde (MDA), catalase, tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) were measured. The results of the study showed that metformin as well as stevia either as a monotherapy or combination significantly improved glycemic profile in parallel with balancing redox status and ameliorating inflammatory response in diabetic adult male albino rats. Co-administration of stevia with metformin appeared to have a synergistic effect. It could be concluded that stevia increases metformin's anti-inflammatory and antioxidant properties. and can be used as a complementary therapy for the management of oxidative stress and inflammatory insult in DM.

Keywords: Diabetes, Streptozotosin, Nicotinamide, Metformin, Stevia.

1. Introduction

Worldwide, around 463 million individuals have DM, which has serious economical and medical consequences. It has been ranked as one of humanity's most urgent general health issues [1]. It is a severe, protracted metabolic disorder that triggers both macro and microvascular consequences, including heart disease, cerebrovascular disorders, neuropathy, nephropathy, and retinopathy. [2]. Hyperglycemia, which is a hallmark of type 2 diabetes mellitus (T2DM), is caused by advanced decline in insulin secretory β cell function and frequently coexists with variable degrees of insulin resistance. Other glucoregulatory abnormalities, such as inappropriate hyperglucagonemia and a compromised incretin response, frequently coexist with these two primary pathogenic processes [3]. Sedentary lifestyles, bad dietary habits, and environmental pollution are major causes of increasing the prevalence of T2DM which accounts for 90% or more of diabetic patients [4]. These predisposing factors induce oxidative stress and abnormal inflammatory response that has been determined to be a crucial component of the pathogenesis of T2DM and its complications [5]. Thereby the management of diabetes must include

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not only medications that have a hypoglycemic effect but also medications that can attenuate oxidative stress and abnormal inflammatory response.

T2DM is usually treated by insulin secretagogues which stimulate pancreatic β cells to secret more insulin (sulphonylureas and meglitinides), or insulin sensitizers which increase the sensitivity of body tissues, particularly muscles and adipose tissue to insulin (thiazolidinediones and biguanides including metformin). Also, dipeptidyl peptidase inhibitors which preserve glucagon like peptide 1 (GLP1) from being hydrolyzed. GLP1 is an incretin hormone secreted from gastrointestinal tract in response to oral glucose then it stimulates insulin secretion from pancreatic β cells in an amount proportion to glucose content in food. Sodium-glucose cotransporter inhibitors are another class of drugs used in treatment of T2DM. They inhibit glucose reabsorption via renal tubules thus excreted in urine [5]. Metformin is indicated as first-line therapy in treating type 2 diabetic patients if a diet regimen and exercise program are not enough [7]. It has a favorable safety profile, high dependability, and low cost, and is strongly advised by guidelines released by the European Association for the Study of Diabetes and the American Diabetes Association [8]. Metformin (dimethyl biguanide) is linked to an ancient herbal medication, Galega officinalis, found to be rich in guanidine and was globally used as an oral hypoglycemic drug before its molecular mechanisms of action or its target pathway was understood [9].

Some researchers reported the ability of metformin to reduce inflammatory insult and counteract oxidative stress [10]. Although it is one of the favored antidiabetic drugs, it has some adverse effects like severe GIT disturbance, lactic acidosis, and pernicious anemia [11].

Stevia rebaudiana Bertoni Family: Asteraceae (stevia) is a famous global herb that is used in different parts of the world as a natural sweetener. It contains many biologically active compounds such as phenols, flavonoids, and diterpene glycosides. Such compounds have antihyperglycaemic, anti-inflammatory, and antioxidant effects [12].

This work was elucidated to evaluate the ability of stevia either alone or in combination with metformin to modulate hyperglycemia, inflammatory response, and oxidative stress in streptozotocin–nicotinamide induced diabetes in adult male albino rats.

2. Materials and methods

2.1. Materials

Streptozotocin and Nicotinamide were bought from Sigma Chemical Company Inc. (St Louis, MO, USA). **Metformin:** was obtained as a powder from Algomhoria chemical company (Cairo, Egypt). **RA60** was purchased from HYET Sweet B.V. (Breda, the Netherlands). It is an extraction product from the leaves of Stevia rebaudiana Bertoni Family: Asteraceae. This extract contains 95.48% total steviol glycosides, which include the following ingredients: rebaudioside A (63.43%), stevioside (22.85%), rebaudioside C (8.21%), 0.73% are dulcoside A (0.73%), and other steviol glycosides (0.26%). **Ketamine and Xylazine** were purchased from Nile Pharmaceutical (Cairo, Egypt) and were used for anesthesia.

2.2. Experimental design

This experimental study was conducted between September and November 2021 at the animal house of the department of Medical Physiology, Al-Azhar Faculty of Medicine, Cairo Egypt. The experiment was approved by the local Ethics Committee of Al-Azhar Faculty of Medicine. All procedures followed the Institutional Guidelines and observed the essential ARRIVE (Animal Research: Reporting of In-Vivo Experiments) guidelines for animal research. Fifty adult male albino rats of a local strain weighing between 80 and 120 grams were used for this study. The animals were kept in adequate cages (40 x 32 x 40 cm for every 5 rats) with wide-meshed raised flooring to prevent coprophagia under similar standard environmental conditions. They were fed with ordinary rat chow and allowed a free access to tap water. To acclimate, the rats were housed for 15 days at room temperature. Five groups of animals were divided equally and at random:

- **Group 1 (control group):** rats were gavaged 2 ml of 0.9% NaCl solution daily for 4 weeks.
- **Group 2 (diabetic group):** A single IP (intra-peritoneal) injection of streptozotocin (45 mg/kg BW) freshly prepared in a citrate buffer was used to induce diabetes in overnight fasted rats. This was done 15 minutes after nicotinamide was dissolved in 0.9% NaCl and given IP (110 mg/kg BW). The presence of hyperglycemia was established by blood's high glucose (> 250 mg/dl), which was detected 3 days after the injection [13]. After that rats were gavaged 2 ml of 0.9% NaCl solution daily for 4 weeks
- **Group 3 (diabetic-metformin treated):** after induction of diabetes, 1% of metformin solution was prepared and rats of this group were administered 200 mg/kg BW of metformin by oral gavage daily for 4 weeks [14].

- **Group 4 (diabetic-stevia treated):** after induction of diabetes, Rats were given 200 mg/kg BW of RA60 dissolved in 0.9% NaCl then given by oral gavage daily for 4 weeks [15].
- **Group 5 (diabetic- metformin & stevia treated):** after induction of diabetes, rats were treated with both 200 mg /kg BW of metformin and 200 mg /kg BW of RA60 by oral gavage daily for 4 weeks.

2.3. Biochemical serum analysis: At the completion of the experiment, and after twelve hours overnight fasting, rats were deeply anesthetized, according to protocols of the American Association of Laboratory Animal Science, with ketamine (100 mg/kg BW) and Xylazine (10 mg/kg BW). Then 4 cm midline abdominal incision was done, and blood immediately was withdrawn slowly from the left ventricle using a 21-gauge needle [16]. Collected blood was centrifuged at 5000 rpm for 20 minutes for serum separation. Serum was then put in Eppendorf tubes and stored frozen at -20^{o} C until analysis.

2.3.1. Assessment of glycemic indices: Serum glucose level was estimated by using biochemical assay kit ab65333(Abcam) and serum insulin level was measured using an Enzyme-linked immunosorbent assay (ELISA) kit (10-1250-01, Mercodia AB, Uppsala, Sweden) according to manufacture protocols. HOMA-IR was calculated by HOMA Calculator, version 2.2.3 developed by the diabetes trial unit, at Oxford University.

HOMA-2 IR=$\frac{[(Glycaemia(\ mg/dl)/18.2)\times Insulin\ (mU/ml)]}{x22.517}$.

2.3.2. Assessment of inflammatory state: Serum TNFα, as a pro-inflammatory marker, and serum IL-10, as an anti-inflammatory marker, was measured using ELISA kits (RayBio Rat, RayBiotech, Norcross, GA, USA) according to the manufacturer guide.

2.3.3 Assessment of redox state: Serum MDA, as a pro-oxidant marker, was measured according to ***Erdelmeier (1997)***[17] and serum CAT, as an antioxidant marker, was measured according to ***Zamocky and Koller (1999)***[18].

2.4. Histopathological examination: after the collection of blood via cardiac puncture, while the rats were still under general anesthesia, the pancreas was carefully dissected. Half of the pancreas that contains the tail portion was promptly fixed for 48 hours at room temperature in 10% neutral buffered formalin. The Dehydration was then carried out by running the tissue in 70% ethyl alcohol followed by 90% and two cycles of absolute ethyl alcohol. Samples were cleared in several changes of xylene. The specimens were infiltrated in molten Paraffin in the oven at 600^{o}C. Paraffin blocks were sectioned with the rotatory microtome at 5µm thickness and mounted on a clean albumenized glass slide.

Hematoxylin and Eosin (H&E) stains were applied to some sections to analyze the general structure. Masson's trichrome dye was applied to other sections to analyze the collagen fibers and blood vessels [19 – 20].

2.5. Statistical analysis: Utilizing the statistical package of the social sciences (SPSS) computer program version 24, statistical analysis was carried out. The mean and standard deviation (SD) were used to express the results. To compare the means of the various research groups, one-way analysis of variance (ANOVA) and Bonferroni post hoc multiple comparison tests were performed. A significant difference was deemed to exist when the P value was less than 0.05.

3. Results

The results of this study are represented in the following table and figures. When comparing the diabetic group (group 2) with the control group (group 1), there was a significant elevation in FBS, insulin, and insulin resistance (IR) ($P \leq 0.05$). When comparing each of the diabetic metformin-treated group (group 3), the diabetic stevia-treated group (group 4), and the diabetic metformin & stevia treated group (group 5) with the diabetic non-treated group (group 2); there was a significant improvement of the glycemic indices where FBS, insulin, and IR were significantly lower in these groups when compared with the diabetic group except for IR in the metformin-treated group (group 3) where the improvement was not significant. FBS in the diabetic metformin-treated group (group 3) was significantly lower than FBS in the diabetic stevia-treated group (group 4). FBS was significantly lower in the diabetic metformin & stevia treated group (group 5) than either the metformin-only treated group or the stevia-only treated group ($P \leq 0.05$) indicating the synergistic effect on FBG when using both metformin and stevia together rather than using each agent alone (Fig. 1).

Table (1)
Mean ± SD of studied parameters in different groups of the study

Parameters / Groups	FBS (mg/dl)	Insulin (µU/L)	IR	MDA (mMol/L)	Catalase (U/L)	TNF- α (pg/ml)	IL-10 (pg/ml)
Group 1 [Control]	107.43 ± 17.45	39.71 ± 1.53	9.81 ± 0.48	9.07 ± 1.29	32 ± 1.95	5.46 ± 1.42	61.44 ± 5.99
Group 2 [Diabetic]	299.57 ± 7.57	20.6 ± 1.6	15.06 ± 1.3	16.43 ± 1.55	23.22 ± 3.55	45.7 ± 4.6	53.55 ± 6.17
Group 3 [Metformin treated]	187.43 ± 3.75	29.93 ± 1.49	13.69 ± 0.9	12.13 ± 1.35	25.8 ± 3.11	21.3 ± 2.1	71.12 ± 5.32
Group 4 [Stevia treated]	240.57 ± 4.44	25.09 ± 2.25	14.74 ± 1.59	13.62 ± 1.33	24.7 ± 2.99	33.7 ± 2.5	70.12 ± 3.54
Group 5 [Metformin and Stevia treated]	170.14 ± 4.91	31.97 ± 5.56	12.28 ± 0.77	10.9 ± 1.4	25.7 ± 3.45	17.5 ± 3.6	81.11 ± 10.99

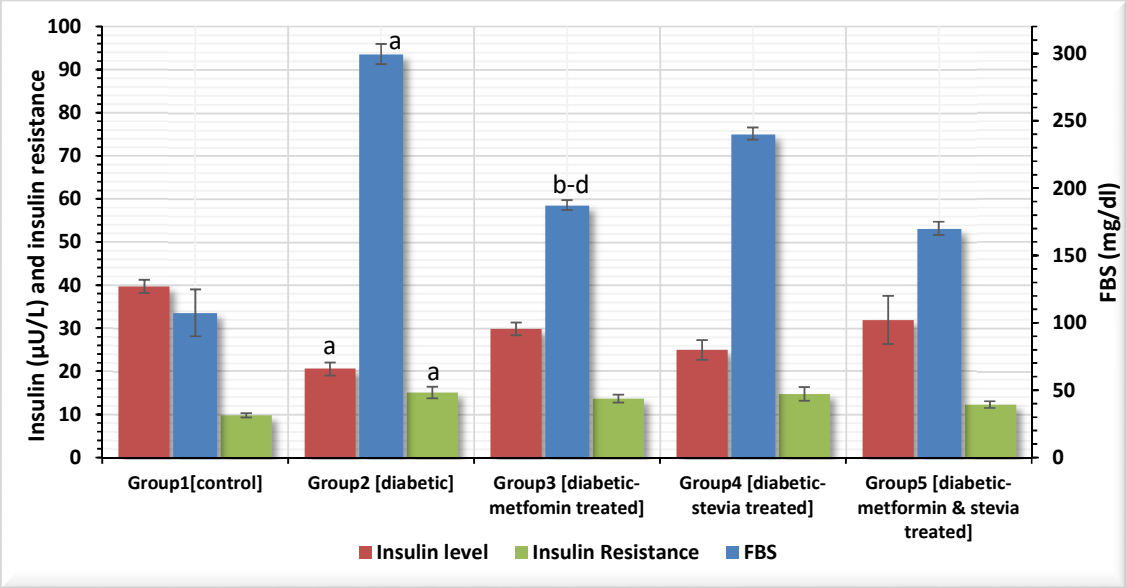

Fig. 1: comparing FBS, insulin, and insulin resistance in studied groups: a = significant in comparison with control group (group 1); b = significant in comparison with diabetic group (group 2); c = significant in comparison with diabetic-metformin treated group (group 3); d = significant in comparison with diabetic-stevia treated group (group 4)

When comparing the diabetic group (group 2) with the control group (group 1), there was a significant elevation in MDA and a significant reduction in catalase ($P \leq 0.05$) indicating a disturbance in the redox state in the diabetic group. When comparing each of the diabetic metformin-treated group (group 3), the diabetic stevia-treated group (group 4), and the diabetic metformin & stevia treated group (group 5) with the diabetic non-treated group (group 2), there was a significant reduction in MDA levels. Also, there was an elevation in catalase levels in the three treated groups however; this elevation in catalase was not significant. There were no significant difference in-between diabetic treated groups whether treated with metformin alone or stevia alone or both together as regard MDA and catalase levels (Fig. 2).

In the diabetic group (group 2), inflammatory insult was pronounced by a significant elevation in TNF-α and a significant reduction in IL-10 ($P \leq 0.05$) when compared with the control group (group 1). This inflammatory insult was significantly improved in the treated groups whether treated by metformin alone (group 3) or stevia alone (group 4) or both together (group 5) as detected by the significant reduction in TNF-α and significant elevation in IL-10 ($P \leq 0.05$) in these groups when compared with the diabetic non-treated group. TNF-α was significantly lower ($P \leq 0.05$) in the metformin-treated group (group 3) when compared with stevia treated group (group 4). There was a significant synergistic anti-inflammatory effect when using metformin and stevia together compared with using each agent alone. This is evidenced by the significant reduction in TNF-α and significant elevation in IL-10 ($P \leq 0.05$) in group 5 (metformin & stevia treated) when compared with group 3 (metformin-treated) or group 4 (stevia treated) (Fig. 3).

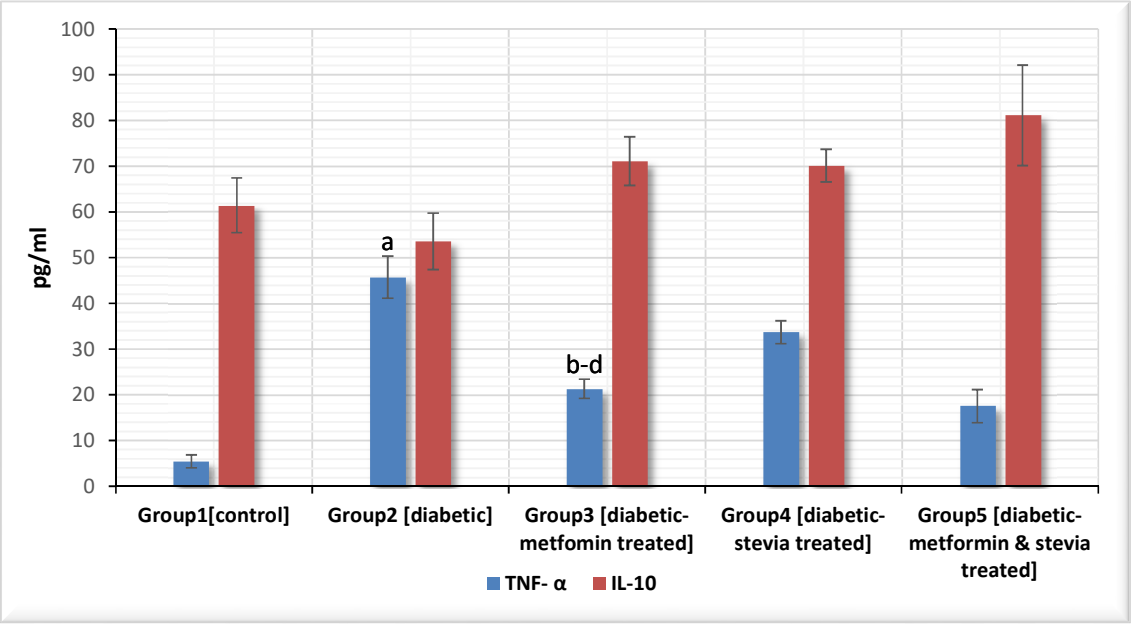

Fig. 2: comparing MDA and catalase in studied groups: a = significant in comparison with control group (group 1);b = significant in comparison with diabetic group (group 2)

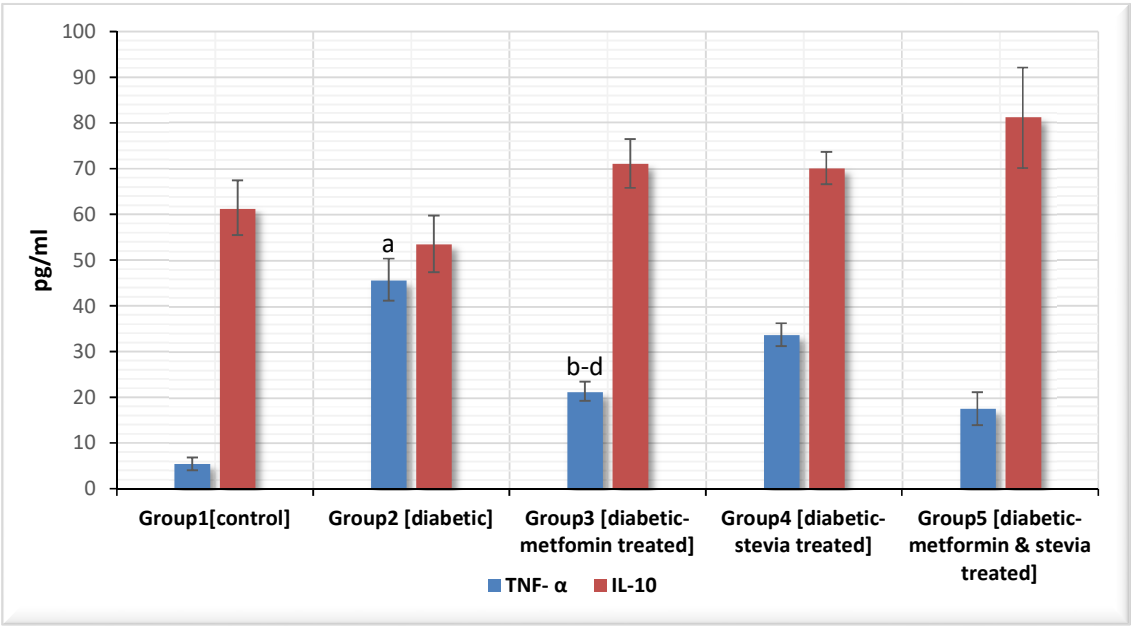

Fig. 3: comparing TNF-α and IL-10 in studied groups: a = significant in comparison with control group (group 1); b = significant in comparison with diabetic group (group 2); c = significant in comparison with diabetic-metformin treated group (group 3); d = significant in comparison with diabetic-stevia treated group (group 4)

Examining histological sections of the control group stained by H&E showed that the parenchyma of the pancreas was divided into exocrine and endocrine parts. The secretory acini that make up the exocrine portion of the pancreas are separated from one another by flimsy connective tissue. The shapes of pancreatic acini were oval or rounded, and their lumens were quite small. The lining cells had irregular cell borders and were pyramidal in shape. Their cytoplasm showed basal basophilia and had rounded basal nuclei with visible nucleoli and apical acidophilic zymogen granules. The blood vessels

might be distributed in between the pancreatic acini. Among the pancreatic exocrine acini, the pancreas had a large number of islets of Langerhans. The islets of Langerhans are composed of masses and cords of secretory cells and contain numerous fenestrated capillaries. While some endocrine cells had highly acidophilic cytoplasm and dark nuclei located mostly at the islet's periphery (α-cells), other endocrine cells showed pale cytoplasm and pale conspicuous nuclei that tended to be located towards the core (*β*-cells) (Fig. 4).

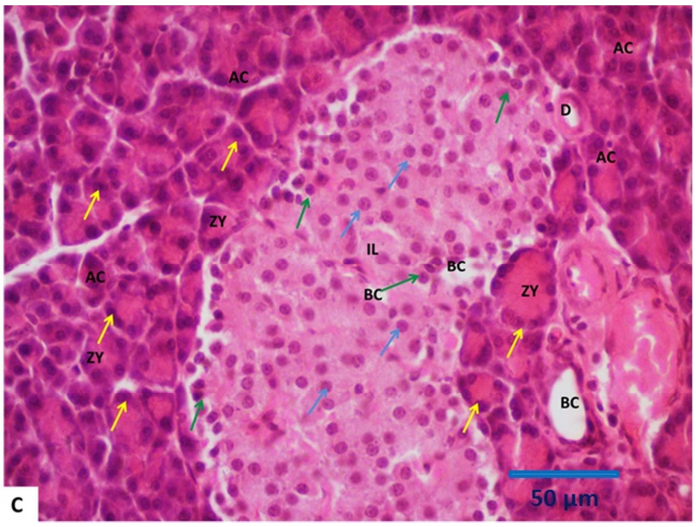

Fig. 4: Photomicrograph of a pancreas section from the control group showing islet of Langerhans (IL) surrounded by the exocrine pancreatic acini (AC). The islet contains cords of spherical cells separated by blood capillaries (BC). The cells of the periphery are α cells (green arrows) and those in the center are *β* cells (blue arrows). The cytoplasm of the AC is backed with acidophilic zymogen granules (Zy) and the nuclei are rounded and vesicular (Yellow arrows), D: ducts

However, the diabetic sections (group 2) showed changes in both exocrine and endocrine parts with an indistinct border between them. Some exocrine acini revealed reduced lumen, others were swollen with focal cytoplasmic vacuolation and pyknotic nuclei. In almost all acinar cells eosinophilic degeneration was observed. Interlobular ducts were lined with flattened epithelium. Blood capillaries of the pancreas showed congestion, and dilatation and were filled with eosinophilic material. Inflammatory cell infiltration was seen surrounding ducts, in the connective tissue septa, in between the acini, and inside the lumen of blood vessels. The pancreatic islets showed an apparent decreased number of β-cells in the central part of the islet, α-cells were more prominent with mononuclear cellular infiltration around the periphery of the islets. Some islet cells had vacuolated cytoplasm, and apoptotic pyknotic nuclei or nuclear fragmentation. Some islet cells were destroyed leaving empty spaces (Fig. 5).

Diabetic rats treated with metformin (group 3) showed that the pancreatic tissue architecture is not markedly distorted as in the diabetic non-treated group (group 2). There was a partial restoration of pancreatic islet cells together with decreased blood vascularity,decreased acinar vacuolation, and partial restoration of acinar size at the border between the exocrine and endocrine pancreas (Fig. 6). Similar improvement happens in group 4 (diabetic stevia treated group) (Fig. 7).

When both metformin and stevia were used together (group 5) better improvement in the exocrine and endocrine pancreas was observed than using each agent alone (Fig. 8).

By using Masson trichrome stain, the control pancreas revealed tiny collagen fibers around Langerhans islands, blood vessels, and around the acini. The diabetic pancreas displayed dense collagen fibers around the acini, islands, and blood vessels. The amount of collagen started to decrease in group 3, and group 4, and become close to normal in group 5 (Fig. 9).

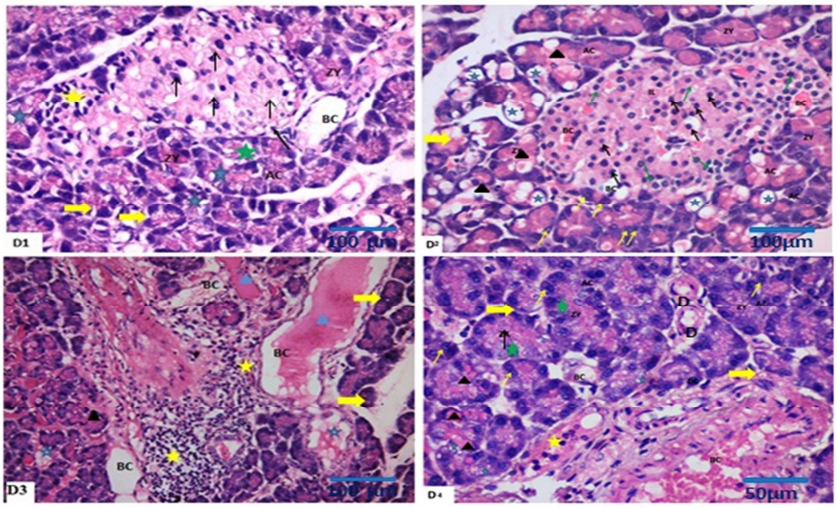

Fig. 5: Photomicrographs of group 2 (diabetic group) showed ill district border between exocrine and endocrine pancreas. The acini (AC) either reduced in size (Thick yellow arrow) or swollen (green stars). Some AC appeared vacuolated (blue star) and some AC has vesicular nuclei (small yellow arrow) others filled with eosinophilic material (black triangle). Ducts (D) are lined with flat epithelium. The islets showed destruction of β cells leaving empty spaces (D1), many apoptotic cells (black arrow). The blood capillaries (BC) in D3&D4 were congested and filled with eosinophilic material (Blue triangle). Cellular infiltration is seen around blood vessels and at periphery of islet (yellow star). Zy: zymogen granules

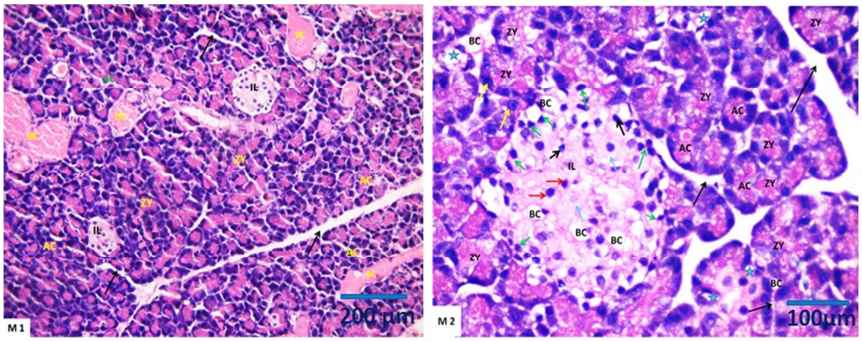

Fig. 6: photomicrographs of group 3 (diabetic metformin-treated) showed that most of the acini (Ac) restore their normal size, Less vacuolated with vesicular nuclei (yellow arrow). Some vacuolated acini appeared (blue star). The connective tissue septa are dilated (long black arrows). The islet of Langerhans (IL) showed α-cells (green arrow), β cells (blue arrow), and a few apoptotic cells (short black arrow). BC: Blood capillaries, Zy: Zymogen granules, d: Ducts.

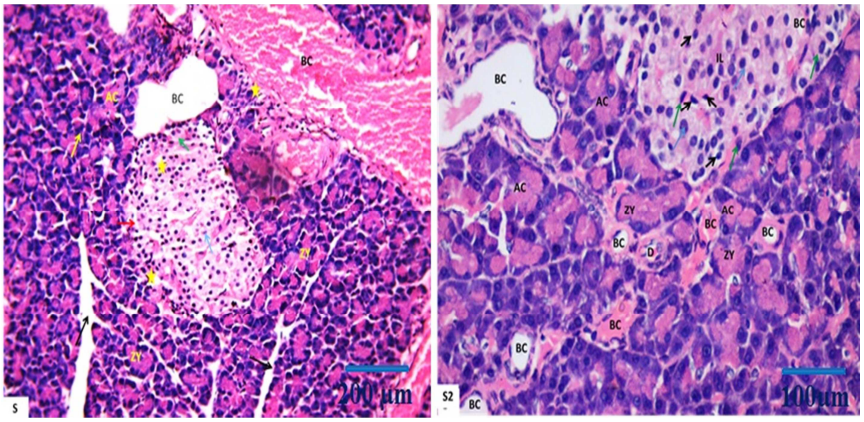

Fig. 7: photomicrographs of group 4 (diabetic stevia treated) showed that showed that most of the acini (Ac) restore their normal size, with wide CT septa (long black arrows). The islet of Langerhans (IL) showed α-cells (Green arrow), *β* cells (blue arrow), and apoptotic cells (short black arrow). The number of α-cells and *β*-cells started to increase. Cellular infiltration (yellow star). Some blood capillaries (BC) filled with eosinophilic material. d: Ducts, Zy: zymogen granules.

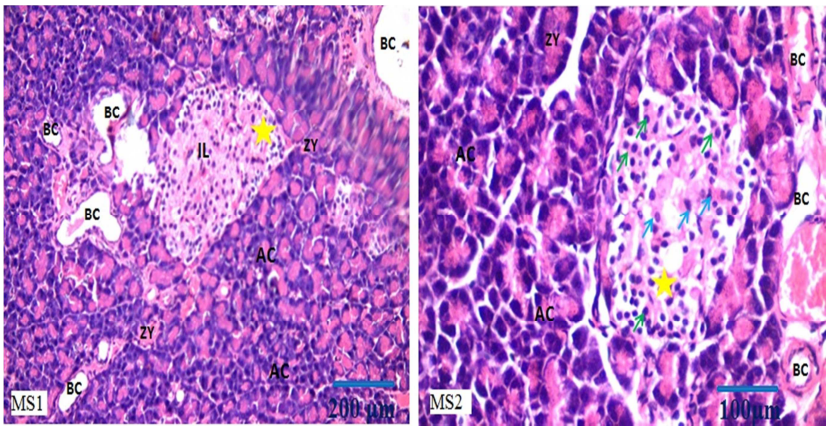

Fig. 8: photomicrographs of group 5 (diabetic stevia & metformin-treated) showed most of the acini (Ac) restore their normal size. The islet of Langerhans (IL) showed α-cells (Green arrow), *β* cells (blue arrow). Cellular infiltration (yellow star), Blood capillaries (BC), Zymogen granules (Zy), Ducts (d)

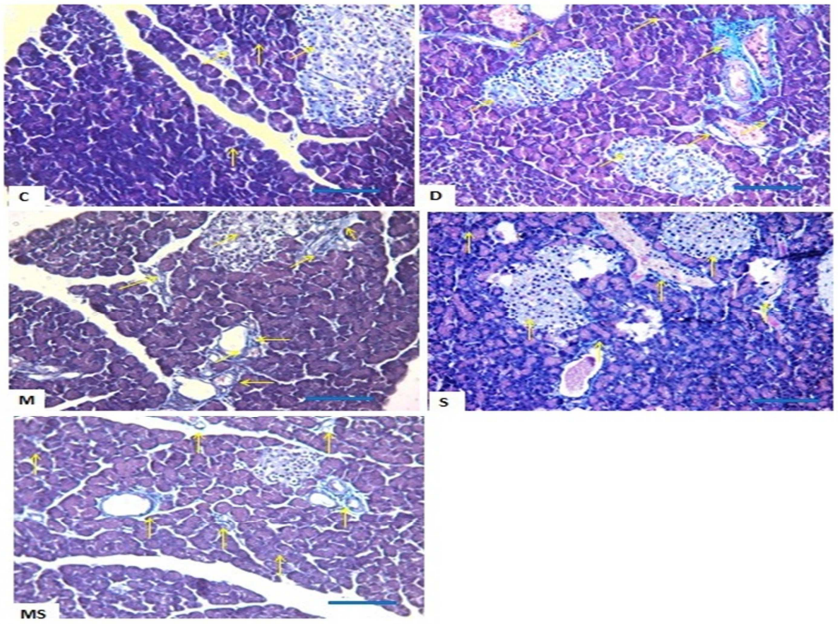

Fig. 9: photomicrographs of pancreatic sections of studied groups stained with Masson trichrome stain showed that the diabetic group pancreas (section D) showed dense collagen fibers around the acini, islands, and congested blood vessels (Arrows). The amount of collagen started to decrease in diabetic-treated groups whether treated with metformin (section M), stevia (section S), or both (section MS) with the most remarkable improvement in group 5. (C: control group).

4. Discussion

In the current study, streptozotocin successfully induced diabetes in rats and this was evidenced biochemically by elevated FBS & lowered insulin levels and histologically by the destructive effect of streptozotocin on β cells of the pancreas in the diabetic group. Nicotinamide was used before streptozotocin injection to ameliorate its destructive effect on β cells aiming to produce an experimental rat model of type 2 diabetes mellitus which is the prevalent type of diabetes.

This is in agreement with Sathibabu Uddandrao et al.who reported that streptozotocin – nicotinamide induced DM is an appropriate animal model for the initial evaluation of treatments for T2DM. Rats are given nicotinamide to somewhat protect insulin-secreting cells from streptozotocin, whose well-documented ability to cause pancreatic-cell death [13].

A single intraperitoneal injection of nicotinamide

is administered before streptozotocin to lessen its toxicity on β cells. In cells exposed to streptozotocin, the DNA fragments. Poly ADP-ribosylation is promoted by DNA damage. Nicotinamide, a poly ADP-ribosylation inhibitor, can somewhat reduce streptozotocin toxicity. However, the ability of nicotinamide to scavenge free radicals rather than a restriction of poly ADP ribosylation is what primarily accounts for this ameliorative impact on streptozotocin toxicity [21].

The current study showed an increase in oxidative stress in the diabetic rats compared to the control ones as evidenced by high MDAand low catalase levels. Also, a significant increase in the inflammatory marker TNF-α and a decrease in the anti-inflammatory IL-10.

MDA is a sign of lipid peroxidation. MDA is created when highly reactive oxygen metabolites interact with unsaturated fatty acids in membrane phospholipids [22]. One of the most significant antioxidant enzymes is catalase. Almost all aerobic organisms include it. Two molecules of hydrogen peroxide are broken down by catalase into one oxygen molecule and two molecules of water [23].

Interleukins are a group of cytokines secreted by white blood cells which interfere with immune responses and play a role in the pathogenesis of T2DM and its complications including for example retinopathy, neuropathy, nephropathy and atherosclerosis. TNF-α has various metabolic activities and induces diabetes progression, whereas cytokine IL-10 (which is a key anti-inflammatory mediator) alleviates type 2 DM [24 – 25].

Inflammation and oxidative stress interact to cause diabetes complications. Increased production of intracellular advanced glycation end products, the production of reactive oxygen species (ROS) in the mitochondria, the activation of protein kinase C, and the polyol pathway flow are all facilitated by intracellular hyperglycemia. ROS directly and through these routes stimulates the expression of inflammatory mediators [26].

Diabetes, induced oxidative stress, inflammation, and the onset and progression of diabetes mellitus and its complications are all strongly correlated. Oxidative stress stimulates the generation of inflammatory mediators which in turn enhances the production of reactive oxygen species. One of the key pathogenic factors in the emergence of diabetic complications is the connection between diabetes, oxidative stress, and inflammation [3].

Type 2 diabetes is associated with subclinical systemic inflammation due to raised plasma levels of pro-inflammatory cytokines such as TNF-α and decreased levels of the anti-inflammatory IL-10 [27].

The current study showed that treatment with metformin significantly reduced FBS and IR together with an increase in insulin levels in the diabetic metformin-treated group when compared to the diabetic non-treated group.

These results are concordant with Wu and his Co-workers who referred to the hypoglycemic effect of metformin as the suppression of hepatic glucose output in addition to its role in the gut where it decreases glucose absorption [28].

Zhou et al. stated that metformin has been reported to increase the secretion of the incretin hormone as glucagon-like peptide-1 which explains the effect of metformin as an insulin secretagogue [29].

Metformin is still the first-line medication for T2DM recommended despite the development of new classes of glucose-lowering medications, some of which are linked to cardiovascular protection. This is because of its excellent safety profile, which includes a low risk of hypoglycemia, the ability to cause modest weight loss, cardio-protective properties, and low cost [28].

Our study showed that in the metformin-treated group, MDA and TNF-α levels were both significantly lower whereas IL-10 level was significantly higher than in the diabetic untreated group. These findings point to the anti-oxidant and anti-inflammatory properties of metformin.

This is in agreement with Dehkordi and his coworkers who stated that all available evidence points to metformin's antioxidant and anti-inflammatory effects in several illnesses. Numerous disorders whose pathophysiology involves inflammatory processes and oxidative stress may benefit from metformin treatment [30].

Also, this is in concordance with Sabzali and coworkers who reported that metformin reduces ROS and ROS-induced damage to DNAs. It also protects the antioxidant defense system and upregulates glutathione [31].

The antioxidant and anti-inflammatory effects of metformin have been reported in many studies including Abbaszadeh et al. [32], Hasanvand [33], Yasmin et al. [34], and many others. It produces antioxidant and anti-inflammatory properties by activating the adenosine monophosphate-activated protein kinase (AMPK) signaling system [33].

The current study showed that stevia significantly improved glycemic indices in stevia treated group when compared to the diabetic non-treated group. FBS and IR were significantly lower whereas insulin level was significantly higher.

These outcomes are consistent with Kurek &Krejpcio who stated that both in the scientific community and the food industry, stevia, and its

glycosides are gaining popularity, primarily as an alternative sweetener. The stevia formulations include antioxidant, anti-inflammatory, and anti-hypertensive properties. Additionally, they assist in controlling blood sugar levels by influencing glucose absorption, enhancing insulin secretion, or raising the concentration of glucose transporters. [35].

Chowdhury et al. reported that over many years, stevia has been utilized as a safe sweetener because it possesses antihyperglycemic properties. stevia has antihyperglycaemic, insulin-mimetic, insulinotropic, and glucagon-static qualities that play a significant role in the therapy of diabetes, although there is a paucity of properly randomized control trials [36].

Also, the results of the study clarified the anti-oxidant and anti-inflammatory properties of stevia as detected by the significantly lower levels of MDA and TNF-α and higher levels of IL-10 in stevia treated group.

Multiple studies have reported the antioxidant and anti-inflammatory effects of stevia for example, but not limited, Ruiz-Ruiz et al. [37], Lemus-Mondaca et al. [38], Ramos-Tovar et al. [39]and Peteliuk et al. [40].

The well-known antioxidant and anti-inflammatory properties of stevia make this medicinal herb, mainly used to treat diabetes, an excellent therapeutic option [41].

It has been suggested that Stevia Rebaudiana has a variety of therapeutic characteristics that could be utilized in functional foods. Most of the polyphenols present have antioxidant properties. By inhibiting the two main signaling pathways, nuclear factor kappa B and mitogen-activated protein kinase signaling, stevioside demonstrates anti-inflammatory characteristics. Stevioside promotes the synthesis of the anti-inflammatory cytokine IL-10 while decreasing the production of pro-inflammatory cytokines including IL-6, IL-1, and TNF-α [42].

Stevia not only has anti-inflammatory, antioxidant, and hypoglycemic effects, but it also contains important nutrients like vitamins A, B3, and C, as well as minerals like magnesium, potassium, selenium, and zinc, which contribute to its antioxidant characteristics [43].

Concomitant use of both stevia and metformin showed significant synergistic hypoglycemic and anti-inflammatory effects as indicated by more reduction in FBS and TNF-α and more elevation in IL-10 levels in stevia & metformin-treated group than corresponding levels in metformin only or stevia only treated groups.

Using medicinal plants and functional foods in the prevention and treatment of diabetes has received increasing interest in recent years. One such natural substance is Stevia, which has a significant amount of different phytochemicals that can aid in lowering cholesterol, blood pressure, and blood sugar. Not only this but also provides anti-oxidant and anti-inflammatory properties which may have important roles in preventing and managing microvascular and macrovascular diabetic complications [44].

Improvement of the pancreatic structure was clear in groups 3, 4, and 5, which may be due to the protective effect of metformin and stevia, as they suppress oxidative stress and the inflammatory response thus protecting the pancreatic cells.

Uncontrolled hyperglycemia can cause apoptosis, widespread oxidative damage, and inflammation. According to several reports, DM significantly increases oxidative stress in several tissues, which in turn drives tissue destruction. Free radicals produced by the auto-oxidation of glucose and protein glycosylation in the pancreas can cause oxidative stress in β cells, putting them at risk for harm. In light of the aforementioned, the search for drugs that are not merely hypoglycemic or anti-hyperglycemic becomes important, even though hyperglycemia appears to be the main problem [45].

5. Conclusion: stevia has a significant hypoglycemic effect. Added to that, are the anti-oxidant and anti-inflammatory properties which may be very beneficial in managing diabetes and its complications. Using stevia, as an add-on therapy to traditional anti-diabetics may be very useful not only in controlling blood glucose levels but also in handling other pathogenic mechanisms involved in diabetic complications namely oxidative stress and chronic subclinical inflammatory state. Metformin, an old and inexpensive drug, is still ahead of others in managing type 2 diabetes. Its origin is a plant and studies should be continued in phytochemicals attempting to find out new anti-diabetic agents with pleiotropic effects against the pathogenesis of diabetic complications.

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9. References

1- Leigh, P., Jay, S.S. and Julio, R.; Novel therapies with precision mechanisms for type 2 diabetes mellitus, Nat. Rev.journal, 17, (364-377) 2021.

2- Ran, N., Kaori, H. and Hiroshi, I.; Effects of High Glucose and Lipotoxicity on Diabetic Podocytes, MDPI Nutrients J., 241, (1-11) 2021.

3- Oguntibeju, O.O.; Type 2 diabetes mellitus, oxidative stress, and inflammation: examining the links, International Journal of Physiology, Pathophysiology, and Pharmacology. 11, 3, (45) 2021.

4- Al Kuraishy, H.M., Hamada, M.T. and Al Samerraie, A.Y.; Effects of metformin on omentin levels in a newly diagnosed type II diabetes mellitus: Randomized, placebo-controlled study, Mustansiriya Med J., 15, 7 2018.

5- Dwiyanti, H., Setyawati, R., Prihananto, V. and D Krisnansari, R.; The effect of feeding antioxidant-rich coffee on glucose blood response, MDA and SOD on diabetic rats induced with streptozotocin, IOP Conf. Series: Earth and Environmental Science, 653, (1- 6) 2021.

6- Chaudhury A., Duvoor, C., Reddy Dendi, V.S., Kraleti, S., Chada, A., Ravilla, R., Marco, A., Shekhawat, N.S., Montales, M.T., Kuriakose, K., Sasapu, A., Beebe, A., Patil, N., Musham, C.K., Lohani, G.P. and Mirza, W. Clinical Review of Antidiabetic Drugs: Implications for Type-II Diabetes Mellitus Management, Front. Endocrinol., 8, 6 2017.doi: 10.3389/fendo. 00006. 2017.

7- Foretz, M., Guigas, B. and Viollet, B. Understanding the glucoregulatory mechanisms of metformin in type 2 diabetes mellitus, Nature Reviews. Endocrinology, 15, (569-589) 2019.

8- Davies, M.J. Management of hyperglycemia in type-II diabetes. A consensus report by the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD), Diabetes Care, 41, (2669–2701) 2018.

9- Mo, D., Liu, S., Ma, H., Tian, H., Yu, H., Zhang, X., Tong, N., Liao, J., and Ren Y. Effect of acarbose and metformin on the inflammatory state in newly diagnosed type-II diabetes patients: a one-year randomized clinical study, Drug Design, Development, and Therapy, 13, (2769-2776) 2019.

10- Aksoz, E., Gocmez, S.S. and Sahin, T.D.; The protective effect of metformin in scopolamine-induced learning and memory impairment in rats, Pharmacol. Rep., 71, (818–825) 2019.

11- Ranjbar, T. and Masoumi, S.J.; The effect of stevia rebaudiana on nonalcoholic fatty liver disease (NAFLD): A review, Int J Nutr Sci., 3, (2-6) 2018.

12- Abdel-Aal, R.A., Abdel-Rahman, M.S., Al Bayoumi S, and Ali, L.A.; Effect of stevia aqueous extract on the antidiabetic activity of saxagliptin in diabetic rats, Journal of Ethnopharmacology, 265, 113188 2021.
https://doi.org/https://doi.org/10.1016/j.jep.2020.113188

13- Sathibabu Uddandrao, V.V., Brahmanaidu, P., Ravindarnaik, R., Suresh, P., Vadivukkarasi, S., and Saravanan, G.; Restorative potentiality of S-allyl cysteine against diabetic nephropathy through attenuation of oxidative stress and inflammation in streptozotocin–nicotinamide-induced diabetic rats, European Journal of Nutrition, 58, 6, (2425–2437) 2019.

14- Gama, N., Malik, S., Suchal, K., Vasisht, S., and A Tomar, A; Metformin alleviates bleomycin-induced pulmonary fibrosis in rats: Pharmacological effects and molecular mechanisms, Biomedicine & Pharmacotherapy J., 97, (1544-1553) 2018.

15-Masoumi, S.J., Ranjbar, T., Nekooeian, A.A., Tanideh, N., Omid Koohi-Hosseinabadi, O.K., Amanat, S., Azarpira, N., Ahmad Monabati, A.; A comparison of the effects of Stevia extract and metformin on metabolic syndrome indices in rats fed with a high-fat, high sucrose diet, Food Biochem. J., 44, (13242-52) 2020.

16- Hoof, J.; Methods of Blood Collection in the Mouse, Lab Animal(NY), 29, 10 2000.

17- Erdelmeier, I.; Reactions of N-Methyle-2-phenlindole with Malondialhyde and 4-Hydroxyalkenals. Mechanistic Aspects of colorimetric assay of lipid peroxidation, Chemical Research in Toxicology, 11, 10, (1184-1194) 1997.

18-Zamocky, M. and Koller, F.; Understanding the structure and function of catalase. Mol. Biol., 14, 66 1999.

19- Suvarna, K., Layton, C. and Bancroft, J.D.; Bancroft's theory and practice of histological techniques. Eighth edition: Elsevier: Pp 153-197. 2019

20- Drury, R. and Wallington, E.A.; Carleton's histological technique. Oxford University Press, Oxford New York Toronto. (140-142, 496-497). 1980.

21-Shalaby, A., El-Feki, S., Alkot, A., and Mahmoud, A.M.; Effect of garlic (Allium Sativum) water extract and vitamin E on alloxan-nicotinamide adult male diabetic rat, Al-Azhar Med. J., 44, 4, (497–510) 2015.

22-Mohamed Abd El-Galil, M., & Mohammad Farag Alkot, A. Impact of crude aloe vera gel on silver nanoparticle-induced lung cytotoxicity in adult male albino rats: functional, histological and immunohistochemical study, Al-Azhar Med. J., 51, 1, (563–604) 2022.

23-Nandi, A., Yan, L-J., Jana, C.K., and Das, N.; Role of catalase in oxidative stress-and age-associated degenerative diseases, Oxidative Medicine and Cellular Longevity, 1942, 0900 2019. https://doi.org/10.1155/2019/9613090

24- Saxena, M., Srivastava, N., and Banerjee, M.; Association of IL-6, TNF-α and IL-10 gene polymorphisms with type 2 diabetes mellitus, Molecular Biology Reports, 40, 11, (6271–6279) 2013.

25- Saraiva, M., Vieira, P., & O'garra, A.; Biology and therapeutic potential of interleukin-10, Journal ofExperimental Medicine, 217, 1 2020.

26- Yuan, T., Yang, T., Chen, H., Fu, D., Hu, Y., Wang, J., Yuan, Q., Yu, H., Xu, W., and Xie, X.; New insights into oxidative stress and inflammation during diabetes mellitus-accelerated atherosclerosis, Redox Biology, 20, (247–260) 2019.
https://doi.org/https://doi.org/10.1016/j.redox.2018.09.0255

27- Shahbazian, H., Aleali, A.M., Amani, R., Namjooyan, F., Cheraghian, B., Latifi, S.M., Bahrainian, S., and Ghadiri, A.; Effects of saffron on homocysteine, and antioxidant and inflammatory biomarkers levels in patients with type 2 diabetes mellitus: a randomized double-blind clinical trial, Avicenna Journal of Phytomedicine, 9, 5, (436) 2019.

28-Wu T., Horowitz, M. and Rayner, C.K.; New insights into the anti-diabetic actions of metformin: from the liver to the gut, Expert Rev Gastroenterol Hepatol., 11, 2, (157- 166) 2017.

29-Zhou, K., Donnelly, L.A., and Kimber, C.H.; Reduced-function SLC22A1 polymorphisms encoding organic cation transporter 1, and glycemic response to metformin: a Go DARTS study, Diabetes, 58, 6, (1434- 1439) 2009.

30- Dehkordi, A.H., Abbaszadeh, A., Mir, S., and Hasanvand, A.; Metformin and its anti-inflammatory and anti-oxidative effects; new concepts, Journal ofRenal Injury Prevention., 8, 1, (54–61). 2018.

31- Sabzali, M., Eidi, A., Khaksari, M., and Khastar, H.; Anti-inflammatory, antioxidant, and antiapoptotic action of metformin attenuates ethanol neurotoxicity in the animal model of fetal alcohol spectrum disorders, Neurotoxicity Research. 40, 2, (605–613) 2022.

32- Abbaszadeh, A., Koushki, S., Koushki, S., Eskandarzadeh, M., and Hasanvand, A.; Metformin; a mini-review to its antioxidative and anti-inflammatory properties, Journal of Renal Injury Prevention., 7, 1, (7–10) 2018.

33- Hasanvand, A.; Antioxidative and anti-inflammatory effects of metformin; a new look to an old drug, J Renal Endocrinol., 4, 1, (2) 2018.

34- Yasmin, T., Rahman, M.M., Khan, F., Kabir, F., Nahar, K., Lasker, S., Islam, M.D., Hossain, M.M., Hasan, R., Rana, S., and Alam, M.A.; Metformin treatment reverses high-fat diet-induced non-alcoholic fatty liver diseases and dyslipidemia by stimulating multiple antioxidant and anti-inflammatory pathways, Biochemistry and Biophysics Reports., 28, 101168 2021.
https://doi.org/https://doi.org/10.1016/j.bbrep.2021.101168

35- Kurek, J.M., and Krejpcio, Z.; The functional and health-promoting properties of Stevia rebaudiana Bertoni and its glycosides with special focus on the antidiabetic potential – A review, Journal ofFunctional Foods, 61, 103465. 2019.
https://doi.org/https://doi.org/10.1016/j.jff.2019.103465

36- Chowdhury, A.I., Rahanur Alam, M., Raihan, M.M., Rahman, T., Islam, S., and Halima, O.; Effect of stevia leaves (Stevia rebaudiana Bertoni) on diabetes: A systematic review and meta-analysis of preclinical studies. Food Science & Nutrition, 2048, 7177 2022.

37- Ruiz-Ruiz, J.C., Moguel-Ordonez, Y.B., and Segura-Campos, M.R.; Biological activity of Stevia rebaudiana Bertoni and their relationship to health, Critical Reviews in Food Science and Nutrition, 57, 12, (2680–2690) 2017.

38- Lemus-Mondaca, R., Vega-Galvez, A., Rojas, P., Stucken, K., Delporte, C., Valenzuela-Barra, G., Jagus, R.J., Aguero, M.V., and Pasten, A.; Antioxidant, antimicrobial and anti-inflammatory potential of Stevia rebaudiana leaves: effect of different drying methods, Journal of Applied Research on Medicinal and Aromatic Plants, 11, (37–46) 2018.

39- Ramos-Tovar, E., Flores-Beltran, R.E., Galindo-Gomez, S., Camacho, J., Tsutsumi, V., and Muriel, P.; An aqueous extract of Stevia rebaudiana variety Morita II prevents liver damage in a rat model of cirrhosis that mimics the human disease, Annals of Hepatology, 18, 3, (472–479). 2019.

40- Peteliuk, V., Rybchuk, L., Bayliak, M., Storey, K.B., and Lushchak, O.; Natural sweetener Stevia

rebaudiana: Functionalities, health benefits, and potential risks, EXCLIJournal, 20, 1412. 2021.

41- Ramos-Tovar, E. and Muriel, P.; Chapter 51 - Stevia as a Putative Hepatoprotector. (P. B. T.-L. P. Muriel (ed.); pp. (715–727). Academic Press. 2017.https://doi.org/https://doi.org/10.1016/B978-0-12-804274-8.00051-5

42- Mlambo, R., Wang, J., and Chen, C.; Stevia rebaudiana, a Versatile Food Ingredient: The Chemical Composition and Medicinal Properties, Journal of Nanomaterials, 1687, 4110 2022.

43- Lemus-Mondaca, R., Vega-Galvez, A., Zura-Bravo, L., Ah-Hen, K. (2012): Stevia rebaudiana Bertoni, source of a high-potency natural sweetener: A comprehensive review on the biochemical, nutritional and functional aspects, Food Chem., 132, (1121-1132). 2012.https://doi.org/https://doi.org/10.1016/j.jarmap.2018.10.003

44- Bayat, E., Rahpeima, Z., Dastghaib, S., Gholizadeh, F., Erfani, M., Asadikaram, G., and Mokarram, P.; Stevia rebaudiana extract attenuate metabolic disorders in diabetic rats via modulation of glucose transport and antioxidant signaling pathways and aquaporin-2 expression in two extrahepatic tissues, Journal ofFood Biochemistry, 44, 8, e13252 2020.

45- Nna, V.U., Abu Bakar, A.B., M.d. Lazin, M.R.M.L., and Mohamed, M.; Antioxidant, anti-inflammatory and synergistic anti-hyperglycemic effects of Malaysian propolis and metformin in streptozotocin–induced diabetic rats, Food and Chemical Toxicology, 120, (305–320) 2018. https://doi.org/https://doi.org/10.1016/j.fct.2018.07.028