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In vivo evaluation of antioxydant potential and antihyperglycemic effect of *Stevia rebaudiana* Bertoni

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Abstract. *Stevia rebaudiana* Bertoni (SR) has a high concentration of phytochemicals that promote health and well-being in conditions such as diabetes. This study aims to assess the antihyperglycemic, antioxidant, and antihyperlipidemic effects of SR on diabetes in male rats caused by Alloxan. Forty adult male rats were divided into five groups. For 28 days, SR was administered by gavage. Spinreact and ELISA kits were used to detect serum levels of blood glucose, insulin, liver function, lipid profiles, antioxidant enzymes, and lipid peroxidation. Histopathology was also investigated. Stevia's free radical scavenging capabilities have an IC_{50} value of between 34.49 and 39.66 mg L^{-1}. Stevia therapy reduced biochemical markers in diabetic rats (DR). After 28 days, SR raised fasting blood glucose, insulin, and lipid peroxidation serum levels by 52%, 40%, and 27%, respectively. Also, in DR treated with SR, there was a substantial increase in high-density lipoprotein cholesterol (35.8%), superoxide dismutase (30%), total antioxidant status (20%), glutathione peroxidase and reductase. SR improves DR's pancreas and liver function by enhancing the endogenous antioxidant system. These findings revealed that SR counteracted Alloxan's necrotic effects by reducing insulin resistance in DR, hence revitalizing pancreatic β–cells.

Key words: HOMA-IR, glycemia, liver, oxidative stress, pancreas, Stevia.

INTRODUCTION

Mellitus diabetes, a major public health problem due to its high incidence and morbidity, is divided into Type 1 and Type 2. It is a prevalent chronic condition characterized by cellular metabolic disruptions such as hyperglycemia and insulinemia, which cause abnormalities in many organs and systems by producing reactive oxygen species (ROS) (Jiménez et al., 2020). Free radicals' induction of oxidative stress may have numerous health consequences, including pathogenic and degenerative processes such as diabetes (Ameer et al., 2020). In Type 2 diabetes patients, oral non-insulin medications are the first-line therapy, along with lifestyle adjustments. Eight

pharmacological categories, anti-diabetic medications (with their action mechanism, benefits and drawbacks), are presently accessible to manage this condition (Abdel-Daim & Halawa, 2014; Hernández et al., 2020). Metformin was the first oral anti-diabetic drug (OAD) approved for treating type 2 diabetes. The research problem is the limited and side-effects of chemical OAD mentioned above, in addition to their high cost. Therefore, the search for an alternative way to treat diabetes is necessary. That's why the cognitive goal of this study is to find a natural remedy for diabetes and has a number of health-promoting properties.

Plants and algae such as *Stevia rebaudiana* (SR) and *Spirulina platensis* are important natural bioactive and antioxidant compounds sources, gradually replacing synthetic antioxidants (Aissaoui et al., 2017; Ameer et al., 2017). The utilitarian goal of the undertaken research study is to demonstrate the effect of SR on Alloxan-induced metabolic and histological problems associated with diabetes in rats.

SR is a perennial shrub of the Compositae family that is cultivated for its leaves in South America. It is grown around the world from March to September. SR propagated by seed or by cuttings or clump division (Peteliuk et al., 2021). Its bioactive components are responsible for several activities and sweetness. Unfortunately, stevia cultivation faces 2–25% yield penalty due abiotic and biotic constraints which further adds to its cultivation cost (Taak et al., 2020; Patel & Navale, 2023).

Agriculture requires mastery of certain parameters (climatic conditions, fertilization, irrigation, optimal harvest periods and geographical dispersion) to assure high stevia yields (Hirich et al., 2022).

Stevia is one of the plants with a nutritional and therapeutic value that has been reported in many traditional pharmacopeias around the world. SR has a history of ethnobotanical applications in various countries, food, and therapeutic medicine. Furthermore, extensive Stevia use has not been shown to have a negative impact on the human body. It is considered safe (Carakostas et al., 2008; Kasti et al., 2022). Stevia bioactive components or extract have been used to reduce oxidative stress and anticipate the aggression of various diseases, including obesity, diabetes, and gut bacteria (Savita et al., 2017; Ameer et al., 2020 & Kasti et al., 2022).

Stevia's bioactive components (stevioside and polyphenols) make it a plant with nutritional, pharmaceutical, and therapeutic value, and it is used to treat metabolic syndrome. Stevia can thus be used to create cosmetic, pharmaceutical, and functional food products (Thomas & Glade, 2010; Ameer et al., 2020). SR has significant curative properties as a natural treatment for metabolic imbalance. The European Food Safety Authority has declared Stevia and the US Food and Drug Administration a natural sweetener and an additive food (4 mg kg^{-1} body weight as acceptable daily intake) (Yu et al., 2017; Thomas & Glade, 2010). To this end, this paper investigates SR's influence on glucose metabolism, cellular and metabolic aggression of Alloxan diabetes in animals.

MATERIALS AND METHODS

Phytochemical profile of SR

In 2020, fresh green leaves were collected from Ouled Fayet, southwest of Algiers. After waste removal, stevia leaves were dried at 25 °C. The mixture was blended in a blender (Miyako BL152, 25,000 rpm, China) and kept at a +4 °C temperature.

Determination of total polyphenols and flavonoids

Pure ethanol was used to extract phenolics from Stevia powder, according to Makkar et al. (1993). The SR powder was broken down (0.2 mg in 10 mL of solvent for 20 min). The solution was centrifuged at 3,000 rpm for 10 min before filtering through Whatman No.1 filter paper.

The ethanol was volatilized at low pressure at approximately 78 °C using a rotary evaporator (SE) to obtain the Stevia extract. Total polyphenols and flavonoids in SE were measured using the Folin-Ciocalteu and $AlCl_3$ methods. Gallic acid and quercetin were used as standards, respectively (Singleton et al., 1999; Chang et al., 2002).

In vivo *antioxydant* profile resolution

The Şahin et al. (2004) technique was used to evaluate the free radical scavenging abilities of Stevia extract against the 2,2–diphenyl–1–picrylhydrazyl (DPPH). However, 100 g mL^{-1} of butylated hydroxyl toluene (BHT) was used as an experimental control. We calculated the anti-free radical activity of the samples using Eq. (1).

was used as a positive control. The antiradical activity of the tested samples was calculated using Eq. (1):

$$\mathrm{DPPH}(\%) = \frac{1 - A_{.SR}}{A_{.DPPH}} 100 \quad (1)$$

where $A_{.SR}$ and $A_{.DPPH}$ represent the absorbances of Stevia and DPPH separately.

The peroxidase reaction of SR and ABTS (2,2–azino-bis (ethylbensothiazoline–6–sulfonic acid ABTS), in comparison to that of positive control BHT (EQ.2) (Sacan & Yanardag, 2010):

$$\mathrm{ABTS}(\%) = \left(A_C \frac{A_t}{A_C}\right) 100 \quad (2)$$

where the absorbance of the tested samples are A_t and A_C with $ABTS^{+}$.

The extract concentration that results in 50% inhibition (IC_{50}) of the DPPH, ABTS and BHT parameters was calculated using the graph correlating the inhibition percentages with the extract concentration of the antioxidant parameters. They were purchased from Sigma Aldrich GMBH in Sternheim, Germany.

Animals and diabetes induction

Forty adult Wistar rats (10–13 weeks; 180 ± 70 g) were obtained from the Pasteur Institute of Algeria for this study. On a standard pellet diet, the rats were kept in polypropylene cells in an animal space (12 h light: 12 h darkness, 24 ± 2 °C and 60% relative humidity) (National Office for Food Livestock, Algiers, Algeria) with free access to water. The Algerian Institutional Animal Care Committee approved the National Administration of Algerian Higher Education and Scientific Research's experimental procedures (Algiers). Before the Alloxan injection, the animals fasted overnight. Alloxan monohydrate was given as a subcutaneous injection at a [150 mg kg^{1} body weight (b.w)] dose (Kameswara Rao et al., 1999). We added a 5% glucose solution to daily water consumption to avoid endocrine and metabolic imbalances and a hypoglycemic crisis. After 72 hours, the animals' fasting blood sugar (FBS) was measured. Diabetic animals had hyperglycemia levels greater than 2 g L^{-1}. Following that, we began data analysis.

Experimental procedure

The Pasteur National Institute (Algeria) provided all the animals under study. Over 28 days, the therapeutic effect of SR was studied. The Stevia plant aqueous extract was collected from an upper part vegetable substance (leaves, 1:100 w v^{-1}) by steeping in distilled water for 30 min and incubating at 40 °C/24 h with low movement on an orbital shaker. The hydrosoluble fraction was centrifuged (6,000 g for 10 min), and the insoluble precipitate was discarded. Whatman No.1 paper was used to filter the supernatant. The rotary evaporator's two pressure/temperature settings (40 °C) concentrated the SR filtrate. Then the water was removed to obtain the SR extract.

Animals in group 1 (normal control rats; G1: NC) and group 2 (diabetic control group; G2: DC) received distilled water. In Group 3 (stevia control), the rats were treated with 400 mg kg^{-1} of Stevia, gavaged daily (G3: SC). Diabetic rats in Groups 4 and 5 were given 200 mg kg^{-1} body weight of metformin (G4: DTM) and 400 mg kg^{-1} body weight of Stevia (G5: DTS), respectively. Over 28 days, the SR and metformin (Met) powders were administered to animals by mouth at the equivalent of 1 mL per day. All animals were fed normally while taking Stevia, metformin, or distilled water.

Biochemical analysis

After fasting the rats for the whole night, blood is collected from the retro-orbital plexus every weekend for 28 days. The blood samples were centrifuged for 900 sec at 25 °C and 6,000 rpm. Then, the plasma was stocked at –20 °C in clean tubes for biochemical interpretations. Serum glucose, triglycerides (TGs), total cholesterol (TC), HDL-c (High-density lipoprotein cholesterol), urea, creatinine, and liver function tests [GPT and GOT] were performed by the automated Random Access Clinical Analyzer PICTUS 200–DIATRON using SPINREACT diagnostic kits (UAA Ctra, Santa Coloma 7 E 17176 Sant Esteve de BAS (GI), Spain). On the 28th day, serum insulin levels were determined using an ELISA kit (Boehringer Manheim Diagnostic, Mannheim, Germany). Insulin resistance was assessed using HOMA–IR (the Homeostasis-Model-Assessment estimate of Insulin-Resistance), and pancreatic β–cells function was evaluated using HOMA–B (the Homeostasis-Model-Assessment insulin β–cells) (Song et al., 2007):

$$\mathrm{HOMA-IR} = \frac{\text{fasting insulin level } \left(\frac{\mu U}{mL}\right) \text{ x fasting blood glucose } \left(\frac{mmol}{mL}\right)}{22.5} \quad (3)$$

$$\mathrm{HOMA-B} = \frac{20 \times \text{Insulin } \left(\frac{\mu U}{mL}\right)}{\text{fasting blood glucose } \left(\frac{mmol}{mL}\right)} \quad (4)$$

Using laboratory kits, the serum oxidative stress enzyme parameters (superoxide dismutase; SOD, glutathione peroxidase; GPx, glutathione reductase; GRx, and total antioxidant status; TAS) were measured (Randox Laboratories Antrim, UK). The serum TBARS levels were determined as described by (Quintanilha et al., 1982).

Body weight gain (BWG)

Every 7 days, the rats' weight was measured. At the end of the experiment (28 days), the rats were sacrificed.

$$\mathrm{BWG\ (g)} = \text{Final BW} - \text{Initial BW} \quad (5)$$

Histology

On the 28th day of the study, pancreatic and liver biopsies were taken to estimate the tissue and organ (pancreas and liver) transformations of Alloxan-induced-diabetic of Forty-rats. In 10% formaldehyde, the samples were fixed and dehydrated. Afterward, paraffin, hematoxylin, and eosin (H & E) were used to embed and stain the samples (Gomori, 1950). These sections were examined in the Anapathology Department, University Hospital Centres, Parnet, Algiers, Algeria, using a Leica microscope that works with light and is equipped with a camera.

Statistical analysis

The results were presented as the mean ± standard error (SE). The ANOVA test was used for the statistical analysis of variance. The statistical significance of the means was determined using the STATESTICA 8.0 software and a Student Test of ANOVA (*t–test*) from the *Newman-Keuls test*. A value of $p < 0.05$ was considered significant, $p < 0.01$ was considered highly significant, and ($p < 0.001$) was considered extremely significant.

RESULTS

Stevia phytochemical and antioxidant characterization

Stevia extract contains 54.57 ± 0.35 mg EAG g^{-1} total polyphenols and 20.35 ± 0.09 mg EQ g^{-1} total flavonoids. Stevia leaves have a higher total phenol content (91 mg g^{-1}). The same results were observed by Serio et al. (2010), Gaweł-Bęben et al. (2015) (60.15 mg EAG g^{-1} of total polyphenols; 20.96 mg EQ g^{-1} of total flavonoids). They have numerous health advantages (Ozola & Dūma, 2020). The most important are antioxidant and anti-diabetic properties (Turkoglu et al., 2007; Molina-Calle et al., 2017). By scavenging free radicals, antioxidants inhibit lipid peroxidation in a relatively short period. DPPH and ABTS tests revealed that Stevia has higher antioxidant activity (79.58 and 70.03%, respectively) than BHT (99.56 and 85.39%, respectively) (Table 1). The percent inhibition of ethanolic Stevia extracts by DPPH-radical inhibition activity was 67.07% and 49.27% (Ahmad et al., 2010, Mohammad AL-Mamun et al., 2018). Stevia's claim to be a natural source of antioxidant compounds with significant antioxidant potential has been confirmed. Stevia extract had a higher tannin content, indicating its antioxidant potential. The lower the IC_{50} value, the greater the DPPH-radical inhibition activity (Shukla et al., 2012; Barba et al., 2015; Savita et al., 2017; Joseph et al., 2019).

Table 1. Antioxidant activities of ethanolic extract of Stevia

Antioxidant activity (%) in 100 (g mL^{-1})				IC_{50} (mg L^{-1})			
DPPH	BHT	ABTS	BHT	DPPH	BHT	ABTS	BHT
79.58 ± 0.43	85.39 ± 0.44	70.03 ± 0.30	99.56 ± 0.05	34.49 ± 0.7	13.33 ± 0.41	39.66 ± 0.33	5.3 ± 0.07

DPPH: 2–2–Diphenyl–1–picrylhydrazyl; BHT: Butyl hydroxytoluene; ABTS: 2.2 Azino–bis (3–ethylbenzthiazoline–6–sulphonic acid) ; IC_{50}: extracts concentration providing 50% inhibition.

Biochemical parameters

In our animal study, Alloxan caused an improvement in the fasting blood glucose level (BGL) and plasma insulin level of diabetic rats, indicating that SR had an antihyperglycemic effect (Figs 1, 2, respectively). The diabetic rats had a 70% increase in BGL compared to the normal rats ($P < 0.001$). When compared to diabetic animals (G2), groups treated with Met or Stevia had a significant decrease in blood glucose ($P < 0.001$). G5: DTS reduced BGL by 52% compared to the untreated diabetic control (G2) ($P < 0.001$), ranging from 3.12 to 1.50 g L^{-1} over 28 days. G4 BGL decreased by 62%, ranging from 3.20 to 1.2 g L^{-1}. Stevia-ed rats (G3) had no decrease in BGL (Fig. 1). Naveen et al. (2012) and Assaei et al. (2016) found similar results.

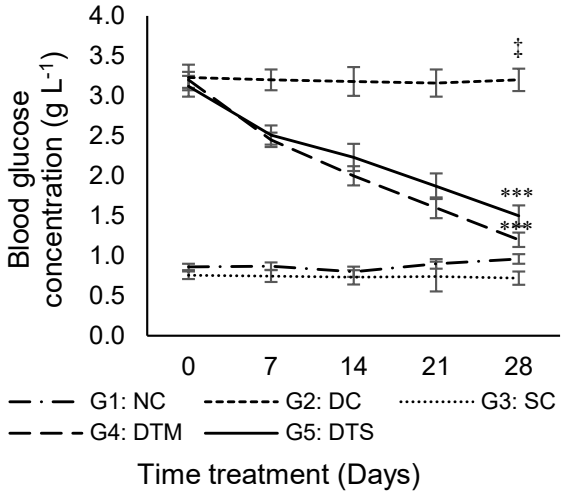

Figure 1. Effect of stevia administration on blood glucose level.

NC: Normal Control; DC: Diabetic Control ; SR: Stevia Control; DTM: Diabetic rats treated with Metformin; DTS: Diabetic rats treated with Stevia. Each value represents mean ± SE ($n=8$). ‡$p < 0.001$, compared with group 1 values ; *** $p < 0.001$ compared with group 2 values at the end of experiment.

The diabetic group had a lower serum insulin level (9.8 U mL^{-1}) than the normal Group (20.01 U mL^{-1}) ($P < 0.001$). During the 28 day study, the serum insulin level in the powder Stevia leaves and metformin-fed groups was higher [11% (G4) versus 40% (G5)] than in the alloxan Group ($P < 0.05$; $P < 0.001$). Normal rats were fed Stevia, and no significant changes were observed (Fig. 2). Except in metformin-treated animals, there was a significant ($p < 0.001$) decrease in HOMA-IR-DC and HOMA-IR-G5 (Fig. 2). As a result, SR has antihyperglycemic activity. Furthermore, in diabetic rats, this plant increased insulin levels. On the other hand, HOMA-B levels in rats treated with Stevia aqueous or metformin increased significantly ($p < 0.001$) compared to diabetic control rats (Fig. 2). Several studies have shown the same result (Naveen et al., 2012; Akbarzadeh et al., 2015; Ahmad & Ahmad, 2018; Han et al., 2022).

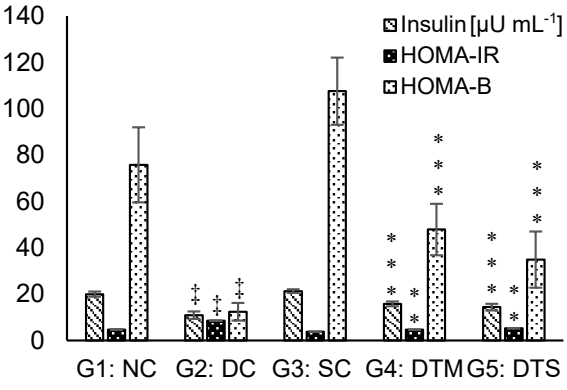

Figure 2. Effect of stevia administration on blood insulin level, HOMA-IR and HOMA-B.

NC: Normal Control; DC: Diabetic Control ; SR: Stevia Control; DTM: Diabetic rats treated with Metformin; DTS: Diabetic rats treated with Stevia. Each value represents mean ± SE ($n = 8$). ‡$p < 0.001$, compared with group 1 values; *** $p < 0.001$ compared with group 2 values at the end of experiment.s

Fig. 3 depicts the effect of SR on the levels of liver enzymes. Serum levels of GOT and GPT were measured (G2) to assess the extent of liver damage caused by Alloxan. Fig. 3 shows that GOT and GPT levels were reduced ($p < 0.001$) in Stevia aqueous extract-fed rats (G5) compared to the diabetic control group (25 and 50%, respectively). After 28 days of OAD treatment, G4 showed a slight decrease in GOT and GPT levels (6 and 17%, respectively) compared to G2 (Fig. 3). Peteliuk et al. (2021) noted the same observations.

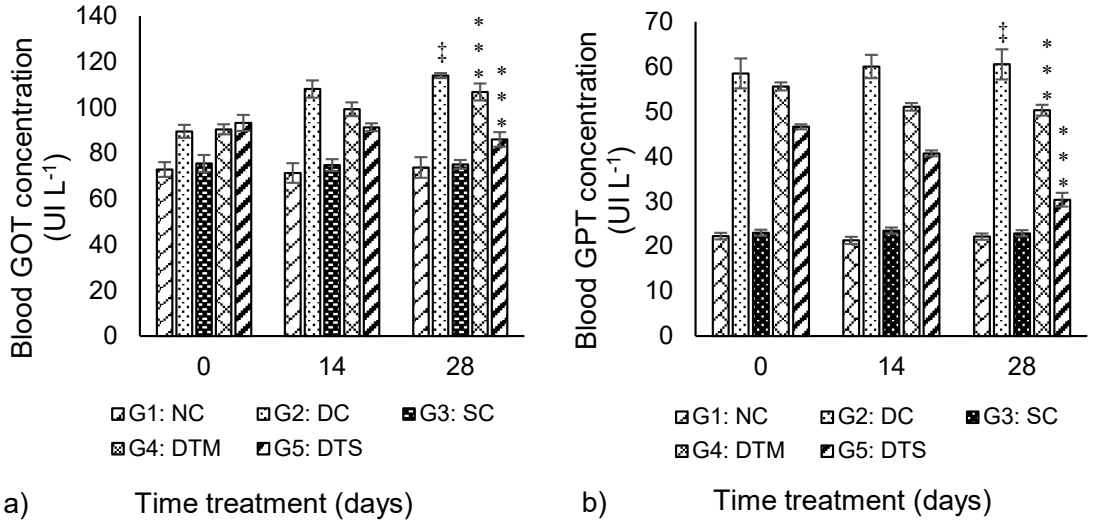

Figure 3. Effect of stevia on serum liver function levels.

GOT (a) : glutamate oxaloacetate transaminase ; GPT (b) : glutamate pyruvate transaminase; NC: Normal Control; DC: Diabetic Control ; SR: Stevia Control; DTM: Diabetic rats treated with Metformin; DTS: Diabetic rats treated with Stevia. Each value represents mean ± SE ($n=8$). ‡$p < 0.001$, compared with group 1 values ; *** $p < 0.001$compared with group 2 values at the end of experiment.

As shown in Table 2, i.p., alloxan injection significantly increased ($P < 0.001$) the levels of TGs (by 66%) and TC (by 49%) when compared to the NC Group. Met and SR treatment, on the other hand, significantly reversed ($p < 0.01$; $p < 0.001$) the increase in plasma TGs and TC by (44% and 35% for SR) and (29.6 and 8% for Met), respectively. G2 had a 51% decrease in HDL-c ($P < 0.001$). On the other hand, SR increased HDL-c levels by 35.8% in G5 and 18% in G4 gavaged with OAD ($p < 0.05$; $p < 0.001$). Similar conclusion were observed by Park & Cha, (2010); Sudha et al. (2017); Ibrahim Ahmed et al. (2019); Peteliuk et al. (2021).

Diabetic animals in G2 had a 64 and 47.5% increase ($P < 0.001$) in urea and creatinine after Alloxan injection, respectively. Met and SR, on the other hand, reduced these effects ($p < 0.01$; $p < 0.001$) by (27.5 and 45% for Met) and (12 and 26% for SR), respectively. No significant differences in TC, TGs, HDL-c, urea, and creatinine levels were found between the SR (G3) and NC (G1) groups (Table 2). The results also show that Alloxan significantly reduced the oxidative stress parameters ($p < 0.001$) compared to the controls. However, after 28 days of Stevia consumption, Group 5 had a significant increase ($p < 0.01$; $p < 0.001$) in TAS (20%), SOD (30%), GPx (27%), and GRx (18%) compared to G2. TAS, SOD, GPx, and GRx levels in G4 were slightly lower than in G2 (4%, 9%, 14%, and 8.6%, respectively) (Table 2).

Table 2. Chemical, oxidative stress parameters and body weight gain at the end of the study

	G1 : NC	G2 : DC	G3 : SC	G4 : DTM	G5 : DTS
Lipid profile					
TG (mg dL^{-1})	57.20 ± 8.56	168.47 ± 14.04‡	58.00 ± 9.23	118.63 ± 12.03***	94.47 ± 8.20 ***
TC (mg dL^{-1})	66.57 ± 11.02	130.51 ± 10.4‡	69.2 ± 11.06	120.06 ± 11.12 **	84.45 ± 8.60 ***
HDL-c (mg dL^{-1})	41.05 ± 4.98	20.14 ± 6.72‡	43.72 ± 5.17	24.6 ± 8.31 *	31.37 ± 7.33 **
Urinary profile					
Urea (mg dL^{-1})	30.89 ± 4.70	85.80 ± 7.61‡	32.95 ± 4.12	62.14 ± 4.05 ***	75.42 ± 2.87 **
Creatinine (mg dL^{-1})	0.64 ± 0.15	1.22 ± 0.23 ‡	0.66 ± 0.29	0.67 ± 0.18 ***	0.90 ± 0.16 ***
Oxidative stress parameters					
TAS [mmol L^{-1}]	1.21 ± 0.11	0.65 ± 0.08‡	1.30 ± 0.04	0.68 ± 0.27	0.81 ± 0.21 **
SOD [U mL^{-1}]	61.78 ± 7.20	40.13 ± 5.71‡	65.31 ± 6.06	44.23 ± 5.52 *	56.75 ± 7.25 ***
GPx [U mL^{-1}]	8.43 ± 0.58	5.13 ± 1.62‡	9.13 ± 0.83	5.96 ± 1.34 *	7.02 ± 1.21 ***
GRx [U g^{-1} protein]	24.32 ± 2.41	17.05 ± 3.42‡	25.11 ± 2.12	18.67 ± 3.73	20.82 ± 3.48 **
TBARS [mmol g^{-1} protein]	24.04 ± 3.12	48.27 ± 6.8‡	23.91 ± 2.17	44.60 ± 4.32 *	35.13 ± 3.75 ***
Body weight gain and loss (g)	28.44 ± 2.50	-13.78 ± 3.13‡	30.18 ± 2.23***	18.30 ± 3.50***	-8.07 ± 3.40***

NC: Normal Control; DC: Diabetic Control; SR: Stevia Control; DTM: Diabetic rats treated with Metformin; DTS: Diabetic rats treated with Stevia. Each value represents mean ± SE (n = 8). ‡$p < 0.001$, compared with group 1 values, (* $p < 0.05$, **$p < 0.01$, *** $p < 0.001$ compared with group 2 values at the end of experiment. TG : triglyceride; TC : total cholesterol, HDL-c : High–density lipoprotein cholesterol. Superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GRx) and total antioxidant status (TAS), TBARS : Thiobarbituric acid reactive substances.

In contrast, Alloxan significantly increased serum TBARS levels in G2 ($p < 0.001$ by 50%) compared to G1. In G5, SR treatment reduced TBARS levels by 27% (Table 2). Body weight gain was significantly reduced ($p < 0.001$) in diabetic rats groups G2, G4, and G5 (–13.78 g, +18.30 g, and –8.07 g, by 7.13%, 8.84%, and 4%, respectively) when compared to the control group (+28.44 g), as shown in Table 2, whereas no significant changes were observed in G3, BWG (30.18 g) for the rats treated with SR. The same results were obtained for animals treated with Streptozotocin, a known diabetogen (Naveen et al., 2012; Assaei et al., 2016).

Histology

In diabetic rats, damage to the liver and pancreatic tissues causes insulin resistance, affecting glucose metabolism. Histological examinations of the pancreas and liver in diabetic animals were performed to determine whether SR could protect these tissues (Fig. 4). Normal (G1) and SR-fed (G3) rats' pancreatic and hepatic histopathology slides revealed no structural changes. The NC rats' pancreas structure had a regular size and shape with full and entire Langerhans islet organization and many pancreatic β–cells (Fig. 4). Diabetic rats in G2 have lower pancreatic β–cell islet mass, cell necrobiosis, and atrophied Langerhans islet.

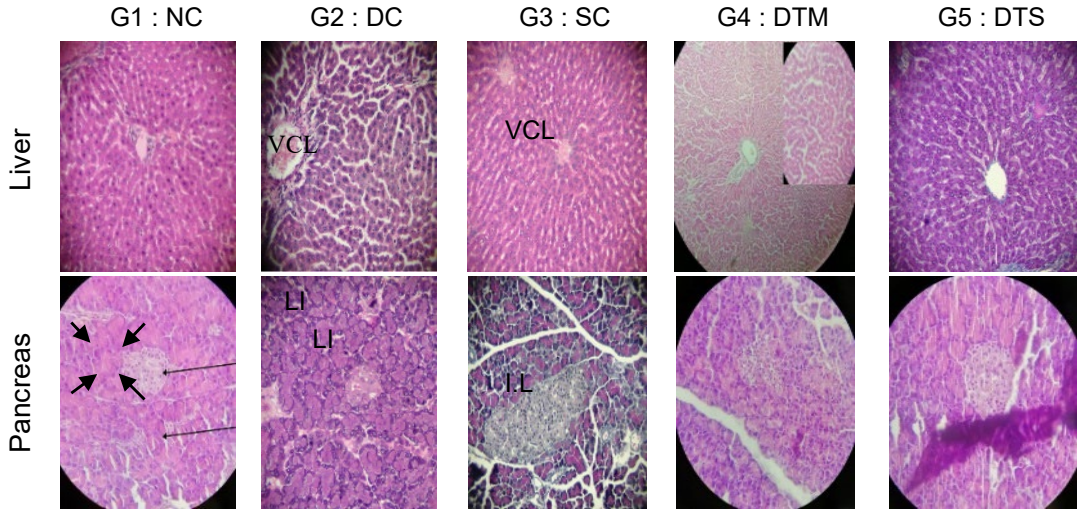

Figure 4. Effect of stevia on the pancreas and liver of normal and diabetic rats H&E, 40x and 10x. Group 1 [NC: the non-diabetic control], group 2 [DC: the diabetic control], group 3 [SC: Stevia control], group 4 [DTM: Diabetic rats treated with metformin], group 5 [DTS: Diabetic rats treated with Stevia], LI: Langerhans islet, AC: Acinus cells.

Treatment with either SR or Met alleviated these pathological changes (Fig. 4). Stevia aqueous extract stimulates active nuclei and regeneration and increases the number of β–cells in pancreatic islet-treated groups (G5). We also saw structural changes, decreased islet size and number, cell necrobiosis, condensed nuclei, and partial damage restoration in G4 pancreases. However, SR or Met treatment alleviated pathological changes (atrophied Langerhans islets, cell rupture, cytolysis, and apoptosis). It was discovered that islets regenerate, particularly in G5 (Fig. 4). As shown in Fig. 4, Stevia extract demonstrates nucleoprotein synthesis as evidenced by big cells

with several cytoplasmic granules, net vesicular nuclei and a high concentration of blood cells called eosinophils that play a role in the immune system stimulation. Studies conducted by Vaiserman et al. (2019a, 2019b); Jeppesen et al. (2000) have detailed the effect of diabetes on the pancreas, liver, glucose and insulin levels.

H&E staining revealed normal liver parenchyma with hepatocytes and central veins in G1 and G3, as shown in Fig. 4. On the other hand, most hepatocytes were damaged and inflamed, with rigid hepatic lobules, empty storage vesicles, and cell injury with central vein congestion, resulting in the premature death of liver cells by autolysis.

Similarly, in diabetic rats treated with Alloxan, the nucleus of hepatocytes showed significant pathological damage (G2). SR treatment, on the other hand, effectively alleviated non–hepatosteatosis and hepatocyte restoration (G5) (Fig. 4). Connective tissue with abnormal hepatocyte structure accompanied by damage and irritants cells in animals of G4.

DISCUSSION

Mesoxalylurea-Alloxan is an organic compound that inactivates islet β–cells and causes chemical diabetes in animals. Diabetes mellitus is a disorder of the metabolic mechanism that slowly sets in with IR, hyperglycemia, and hyperlipidemia. Then, the insulin allows cells to use and convert glucose into energy (Pankaj & Varma, 2013). Substances with no toxic an adverse effect that manages diabetes problems by improving IR and abnormally elevated levels of any or all lipids are needed. *Stevia rebaudiana* Bertoni (family: Asteraceae) commonly known as honey leaf-candy leaf or sweet herb is a perennial shrub of South America. There are 154 species of genus Stevia, and among them *S. rebaudiana* is the only species which synthesize steviol glycosides like stevioside, dulcoside A and rebaudioside (rebaudioside A, B, C, D and E) in its leaves (Taak et al., 2020). SR natural, non-caloric sugar substitute, is a rich source of pharmacologically important glycoside stevioside and has been employed in the world as a treatment for diabetic problems (Akbarzadeh et al., 2015; Ahmad & Ahmad, 2018; Abdel-Aal et al., 2021). The conventional strategies used for stevia cultivation are not reliable because of poor viability of seeds and germination percentage 8. Hence, to overcome such limitations, in vitro propagation is the only remedy that can facilitate large-scale production of genetically identical stevia plants (Jagatheeswari & Ranganathan, 2012; Taak et al., 2020).

In diabetic rats, SR treatment improved the biochemical, histological, and oxidative stress parameters studied. This improvement was also more pronounced in the SR than in the OAD group. Furthermore, most diabetic patients now prefer Stevia, a second option to artificial substances used instead of sugar to sweeten foods and drinks. Steviol glycosides are the most important component of SR because of the intensity of sweetness of a sweetener (300 times sweeter than saccharose) with zero caloric content (Park & Cha, 2010; Khiraoui et al., 2017).

This study aims to demonstrate the effect of Stevia on Alloxan-induced diabetes in rats based on this information. The role of Stevia in lowering glucose levels was investigated using FBG, insulin levels in plasma, and insulin resistance parameters.

When the Stevia treatment rats were compared to the control diabetic group, the results showed reduced blood glucose, insulin resistance (HOMA-IR), and increased insulin level and HOMA-B, as indicated by improved β–cell functions. These findings

support previous research on diabetic rats treated with 4% and 400 mg kg^{-1} b.w. after Streptozotocin injections at 60 and 40 mg kg^{-1} for 5 and 4 weeks, respectively (Naveen et al., 2012; Assaei et al., 2016). More than five mechanisms are leads to reduce BGL by SE through its chemical constituent, namely (Toskulkao et al., 1995; Chang et al., 2005; Naveen et al., 2012; Bender, 2016; Abdel-Aal et al., 2021):

1. Decreasing in the rate of glucose absorption by the intestines
2. Increasing the use of glucose in the muscles by eliminating glucose
3. Changing glucose transport
4. Regulation of gluconeogenesis,
5. Improving insulin sensitivity and/or secretion.

A higher HOMA–IR indicates Insulin-Resistance, whereas a lower HOMA-IR indicates Insulin-Sensitivity. We believe that decreased insulin secretion caused by Alloxan pancreatic damage increased insulin sensitivity. As a result, even at very low serum insulin levels, SR may still sensitize pancreatic system tissues to insulin force, assisting in the reduction of hyperglycemia. According to the DPPH and ABTS tests, stevia leaves have a high antioxidant potential due to polyphenols, flavonoids, and tannins (Krumina-Zemture et al., 2018; Peteliuk et al., 2021). These bioactive molecules with strong antioxidant properties promote the passage of the hormone secreted by the pancreatic ß–cells into the blood or the opposite of the blood glucose to other tissues (Gaweł-Bęben et al., 2015; Molina-Calle et al., 2017; Joseph et al., 2019).

The combination and control of arbuscular mycorrhizal fungi with the addition of P during the growth of stevia allows the modulation of the accumulation of bioactive compounds, improves the nutraceutical value and the exploitation of the raw material as a functional ingredient for foods, dietary supplements and cosmetics (Tavarini et al., 2020).

Previous research has also highlighted the importance of Stevia leaves' hypoglycemic components, which are high in diterpene glycosides and counteract free radicals, Rebaudioside A, stevioside, isosteviol, steviol, and polyphenolic compounds. These ingredients have been shown to help prevent diabetes and its complications (Wheeler et al., 2008; Thomas & Glade, 2010; Ameer et al., 2017; Ameer et al., 2020). Compared to the control, Alloxan elevated BGL contrary to insulin. The addition of MET to diabetic rats reversed these changes, as Met increases glucose utilization by muscles and decreases glucose absorption mechanisms from the intestinal tract (Goodman et al., 2011; Jin et al., 2017; Ibrahim Ahmed et al., 2019).

Some researchers have proposed that, in addition to sweetness and stevioside, the extract obtained from SR-leaves, as well as related compounds such as rebaudioside A, steviol, and isosteviol, may have curative and/or preventive health benefits, including hyperglycemia and blood pressure, oxidation, tumors, diarrhea, stomach, kidney and immune disorders and others (Chatsudthipong & Muanprasat, 2009; Lemus-Mondaca et al., 2012; Periche et al., 2014; Ramos-Tovar et al., 2019). Because the increases in mean GOT and GPT enzymes, urea, and creatinine were relatively higher after Alloxan induction, the findings suggest that the increases in liver and urinary serum parameters were due to physiological rather than toxicological effects. Our findings show that the Stevia supplement improved hepatic and urinary parameters in diabetic rats by significantly lowering GOT, GPT, urea, and creatinine serum levels over 28 days.

Other studies have suggested that Stevia can improve human and animal liver health by lowering serum hepatic biomarkers with daily administration of Stevia (400 mg kg^{-1} b.w.) for four weeks. This could be due to the antioxidant activity of SR combined with free radical–scavenging activity provided by the DPPH and ABTS tests (Assaei et al., 2016; Muriel et al., 2017). According to Carakostas et al. (2008), male rats treated with rebaudioside A have higher plasma urea and creatinine concentrations than female rats. Another study found no significant changes in the blood parameters listed below after long-term stevioside sweetener feeding in male rats (Awney et al., 2010). Anomalies in the lipid and lipoprotein profiles are metabolic factors that contribute to insulin resistance. However, increased total cholesterol, triglyceride serum, and decreased good cholesterol levels in DC rats could be a sign of increased pyruvate dehydrogenase activity (Ford et al., 2008; Latha & Daisy, 2011; Zhang et al., 2013; Castro et al., 2015).

The findings also highlight that taking Stevia aqueous extract daily significantly changes the lipid profile. This spot is reliable with Sharma et al. (2009) research which found that TC and TGs were significantly reduced after one month of daily Stevia extract consumption in women with high cholesterol, while HDL–c was increased in experimental animals (Sudha et al., 2017; Ahmad et al., 2018; Abdel-Aal et al., 2021). The effect of Stevia on lipid characteristics in G2 demonstrates that SR has good hypolipidemic properties. A wealth of information is available regarding using SR to prevent cardiovascular complications in diabetic patients (Khiraoui et al., 2017). As a result, it is possible that consuming SR may help to reduce the prevalence of diabetes and hyperlipidemia and good management of glycemic metabolism. Metformin has also been shown in diabetic animals to have mild hypolipidemic effects. The effect was seen in all parameters studied, including TGs, TC, and HDL-c.

Compared to control male rats, treatment with a high dose of stevioside (1,500 mg kg^{-1} b.w/day), Rebaudioside A and SH significantly increased total cholesterol and HDL-c levels. This is explained by changes in bile acid homeostasis (Nikiforov & Eapen, 2008; Awney et al., 2010). By producing reactive oxygen species, environmental factors and stress levels in animals, Alloxan causes oxidative damage (Sapsuha et al., 2022). As a result, Stevia's hepatoprotective properties and antioxidant effects on pancreatic activity can be used to manage diabetes-related oxidative stress (Kangralkar et al., 2010; Assaei et al., 2016). Stevia aqueous extract significantly increased stress oxidant parameters (TAS, GPx, GRx, and SOD), which are enzymes that degrade free radicals. ABTS levels were significantly reduced. Our conclusions are consistent with those of Naveen et al. (2012), who discovered that feeding diabetic rats with Stevia leaves in their various states reverses the effect of ROS molecules and reactive oxygen species accompanied by lowering MDA levels. Many studies have shown that SR contains numerous biomolecules (*steviosides, polyphenols, flavonoids, alkaloids, water–soluble chlorophylls and xanthophylls, hydroxycinnamic acids, austroinullin, β–carotene, dulcoside, nilacin, rebaudi oxides, riboflavin, steviol and thiamine)* that have antioxidant activity, prevent oxidative DNA damage, inhibit lipid peroxidation in diabetic rats, and significantly increase GSH levels (Gezer et al., 2006; Turkoglu et al., 2007; Jayaraman et al., 2008; Khiraoui et al., 2017; Rotimi et al., 2018).

Serum insulin levels were found to be higher in the Stevia-treated groups in this study. This means some biomolecules in Stevia may stimulate beta cells to release insulin, thereby improving carbohydrate metabolizing enzymes and restoring normal BGL. Moreover, this finding demonstrates that Stevia can improve glucose tolerance and cellular insulin sensitivity (Naveen et al., 2012; Assaei et al., 2016). Rats in the diabetic model Group induced by Alloxan lost weight gradually. Alloxan is toxic, causing pancreatic, hepatic, and renal damage. This type of diabetes causes weight loss, muscle wasting, and the degradation of the muscle protein complex over time (Naveen et al., 2012; Han et al., 2022). In contrast, oral administration of SR, a non-caloric natural sweetener, to G2:DC rats at the last of the experiment highlighted a critical augmentation in BWG, pointing out that Stevia considerably enhanced their health status and she has launched a series of metabolic processes by good blood sugar management and reversed gluconeogenesis (Abdel-Daim & Halawa, 2014; Ameer et al., 2020).

This study also examined how islet endocrine cell populations reorganized during the development of alloxan diabetes in rats. Alloxan is thought to cause Type 1 diabetes by damaging islets of Langerhans β–cells (Aissaoui et al., 2017; Ibrahim Ahmed et al., 2019). In insulin-dependent diabetes, islet α and δ cells are unused autoimmune destruction directed at beta-cells. This result encourages increasing non-beta endocrine cells in the islet core (Plesner et al., 2014). Alloxan is a crystalline compound $C_4H_2N_2O_4$, a toxic glucose analog, which selectively destroys insulin-producing cells in the pancreas when administered to animal species, damaging cell membrane structure, and generating harmful molecules ROS, reactive oxygen species, which are indirectly responsible for causing pancreatic tissue failure, particularly β–cells, and activating protein kinases, hexosamines, and others (Jorns et al., 1997; Ha & Kim, 1999; Brownlee, 2005; Simmons, 2012; Han et al., 2022).

SE may scavenge free radicals and aid in the reconstruction of pancreatic units, allowing them to release more insulin, resulting in an anti–diabetic effect. By revitalizing pancreatic β-cells and antagonizing Alloxan's β-necrotic action, SR synthesized and aggregated insulin in diabetic rats' pancreas tissue (Misra et al., 2011; Assaei et al., 2016). Furthermore, no evidence of pancreas or liver tissue disorder was observed in histo-pathology slides after SE gavage in Group 1. MET did not significantly affect pancreatic islet cell size in the diabetic Group (Jin et al., 2017). After SR treatment, diabetic rats had moderately sized islets of Langerhans with active nuclei cells (G5). Stevia leaves may protect rats from acute and hepatic toxicity, disturbance in the balance between the production of reactive oxygen species (free radicals) and antioxidant defenses, cellular damage and death and a decrease in biliary secretion known by cholestasis by improving the endogenous antioxidant system and exerting anti-inflammatory activity (Shakoori et al., 1994). Stevia leaf primarily benefits from its bioactive, biochemical, and nutritional composition, which are mostly related to the harvest time and P supply. They are original organic phenolic compounds (total phenols and flavonoids as well as antioxidant activities, carbohydrates, protein, and crude fiber), promoting wellness and decreasing metabolic attacks (Najafian & Moradi, 2017; Tavarini et al., 2020).

CONCLUSION

This study investigated the ant-diabetic activity and potential antioxidant mechanism of SR in diabetic rats caused by Alloxan. The findings show that an aqueous extract of Stevia has anti-diabetic effects by lowering abnormal biochemical and histological parameters and oxidative stress markers in rats with Alloxan-induced diabetes. Natural Stevia's antioxidant potential and relevant bioactive properties appeal to consumers.

Further research in the field covering metabolic and health disorders presented in this article are necessary, citing the most important research perspectives, the biological activity of SR and its relationship to human health; the use of other parts of the shrub such as flowers, stems or seeds, and find solutions to stevia cultivation problems to increase yields and reduce costs. The industrial application of SR to prevent diseases that set in over time from food additives and chemical sweeteners. Finally, the use of stevia as a treatment for other diseases. Human applications are desirable too.

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