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Experimental Research Article

Stevia and especially Nano-Stevia induced anti-diabetic, antidepressant, and analgesic effects in streptozotocin-induced diabetic rats

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ABSTRACT

Introduction: Diabetes mellitus is a metabolic disease. This disease is almost associated with depression and impaired pain sensation. *Stevia rebaudiana Bertoni* (stevia) has a sweet taste and a useful effect in blood glucose control. This study was designed to evaluate the effect of Stevia and Nano-stevia on psychological problems (e.g. depression and pain) induced by diabetes.

Methods: By a single intraperitoneal administration of streptozotocin, diabetes mellitus was induced in male Wistar rats. Stevia (20 mg/dl per day for 30 days) and Nano-stevia (20 mg/dl per day for 30 days) were administrated intra-gastrically in diabetic rats. Using the forced swim test (FST) and formalin test, depression and pain behaviors were measured, respectively.

Results: The results exhibited that male diabetic rats showed hyperglycemia, depressant-related behavior, and hyperalgesic response. On the other hand, intragastric usage of Stevia (20 mg/dl per day for 30 days) and Nano-stevia (20 mg/dl per day for 30 days) modified the hyperglycemia, depressant-related response, and hyperalgesic effect in diabetic rats. Nano-stevia indicated the maximum significant response rather than *Stevia*.

Conclusion: The results of this study indicate that Stevia and particularly Nano-stevia could be beneficial in managing diabetes and its associated behavioral complications.

Keywords:
Diabetes
Stevia
Nano-stevia
Depression-related behavior
Pain

Introduction

Diabetes mellitus is a metabolic disease observed due to the ineffective insulin production by the pancreas. So, blood glucose rises and causes fatality due to very high blood glucose (Shahid et al., 2022). Diabetes is not only a metabolic illness but also an illness with psychiatric

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aspects (Oguz N 2018). Depression is a mental disorder that is related with diabetes. A combination of genetic and environmental factors might contribute to this disorder (Khan et al., 2019; Knol et al., 2007; Nichols and Brown 2003; Palizgir et al., 2013; Salinero-Fort et al., 2018; Shahi and Mohammadyfar 2017). Depression is also related to adverse clinical profiles such as poor glycemic control (Darwish et al., 2018; Gonzalez et al., 2008; Lin et al., 2004).

Chronic pain is often accompanied by depression, which exacerbates the challenges faced by diabetic patients. (Bair et al., 2010). The high prevalence of pain and its significant association with reduced health-related quality of life may play a crucial role in enhancing diabetes management. The high prevalence of pain and its significant association with reduced health-related quality of life may play a crucial role in enhancing diabetes management. (Molvær et al., 2020). The pain associated with diabetic peripheral neuropathy may be linked to a dysfunction in the endogenous analgesic system within the descending spinal pathways that control pain conduction to the brain (Roy et al., 2017; Tanenberg et al., 2011).

Stevia rebaudiana Bertoni (stevia) is a traditional plant that is well-known because of its sweet taste and useful influences in blood glucose control (Ahmad and Ahmad 2018). It contains proteins, fiber, and carbohydrates (Hossain et al., 2017). Stevia produces anti-inflammatory, antioxidant, anti-stress and membrane-stabilizing effects (Chavushyan et al., 2017; Simonyan et al., 2021). Stevia as a nutraceutical induces therapeutic properties in the modulation of chronic diseases such as diabetes mellitus (Wang et al., 2020). It has been reported that the use of low-calorie sweeteners such as stevia can alleviate numerous etiopathogenic factors related with diabetes (Kumar and Chail 2019). Stevia elicits a positive influence on hyperglycemia by reducing glucose absorption in the duodenum, glycogenolysis, and gluconeogenesis (Jeppesen et al., 2003).

The synthetic agents utilized for the treatment of diabetes cause several side effects. Hence, the utilize of natural sources (e.g. *Stevia rebaudiana Bertoni*) for the treatment of diabetes may be safe (Lemus-Mondaca et al., 2012). Additionally, the usage of drug delivery substances such as niosomes can protect the drug from harsh environments until the drug reaches its target tissue (Jafari-Rastegar et al., 2022). Moreover, the possibility of depression onset in diabetes is about twice that reported in the population (Nouwen et al., 2010). Also, control of diabetic neuropathic pain is important for the improvement of the quality of life in diabetic patients (Molvær et al., 2020). Thus, this study aimed to assess the effect of Stevia and Nano-stevia on the modulation of diabetes complications such as depression and pain sensation impairment, as well as evaluation of the liver tissue morphology.

Methods and Materials

Ethical approval

All procedures in this research were carried out based on the guidelines on the use of laboratory animals and approved by the Tehran Medical Sciences, Islamic Azad University ethical committee for animal research (ID: IR.IAU.PS.REC.1399.188).

Animals

Adult male Wistar rats (obtained from the Tehran University of Medical Sciences (Tehran, Iran)) were used for this research. The body weight of the animals at the beginning of the study were 220–270 g. The rats were housed four per cage in a room with a controlled temperature (23 ± 2 °C) and a 12h light/dark cycle (lights on 07:00 a.m.). Animals had free access to water and food (Behparvar Company, Iran). For each experimental group, eight rats were used.

Nano-stevia synthesis

A standardized stevia-bearing nano-noisome (Nano-stevia) was synthesized by the thin layer hydration procedure based on our previous study (Khakpai et al., 2023). For this process, cholesterol and surfactants (span60 and tween 60) with 1:1 molar ratio was dissolved in 10 ml of chloroform. For the evaporation of chloroform, the rotary evaporator was used. The dried thin films were hydrated by stevia solution in PBS. The samples were sonicated and stored at 4 °C in a refrigerator.

Drugs

Stevia rebaudiana Bertoni powder was obtained from Tame Tabiat company (Iran). Streptozotocin was purchased from Solarbio Science and Technology Co. (Beijing, china CAT No. S8050). All drugs were dissolved in PBS solution.

Induction of diabetes mellitus

According previous investigations, diabetes mellitus was induced by a single intraperitoneal injection of streptozotocin (50 mg/kg) which dissolved in physiological serum (Annapurna et al., 2009; Chundi et al., 2016). Diabetes was confirmed 72h after streptozotocin injection by measurement of serum glucose utilizing a glucose assay kit (Azmoon Co., Tehran, Iran). Rats showing increased serum glucose levels of more than 200 mg/dl were considered diabetic and were utilized for the study.

Experimental design

A total of 40 adult male rats, including 8 healthy rats and 32 diabetic rats were used for the experiments. The rats were divided into 5 groups (n = 8/group) including healthy control rats (saline, 1 ml/kg), diabetic control rats (saline, 1 ml/kg), diabetic rats with intragastric administration of nano-niosome (drug delivery substance; 1 ml/kg per day for 30 days), diabetic rats with intragastric administration of Stevia (20 mg/dl, 1 ml/kg per day for 30 days), and diabetic rats with intragastric administration of Nano-stevia (20 mg/dl, 1 ml/kg per day for 30 days).

Measurement of blood glucose and body weight

Blood glucose and body weight of all rats were recorded weekly. Blood was obtained from the tail vain each time. Diabetes induction was confirmed through determining fasting plasma glucose amount in blood samples of rats. Blood glucose level was measured at the start and at the end of experiments using a glucose assay kit (Azmoon Co., Tehran, Iran).

A fixed similar amount of water and standard dietary food was prepared to all animals to elude any errors.

Behavioral test

Open-field test (OFT)

The locomotor activity of rats was measured using an open-field test (OFT). The device is made of a wooden box measuring 100 cm × 100 cm. The floor of the apparatus was divided into 16 equal squares. The rats were located in the center of the device and were allowed to explore freely. The number of squares crossed with four paws was measured for 6 min (Alijanpour et al., 2019).

Forced swim test (FST)

A forced swim test (FST) is generally utilized to evaluate depressant-related behaviors in rodents. FST was carried out based on our previous studies, and the animals did not have any pretest one day before the main test (Goleij, Youseftabar-Miri, Montazeri, & Khakpai, 2021).This test is based on the hypothesis that when a rat is placed in a container filled with water, it will first try to escape. The rat will finally display immobility state, which might be detected as a sign of behavioral despair. Each rat was located in the FST device, which was made of a cylindrical glass container (height = 30 cm and diameter = 20 cm) filled with water (25 °C). Each animal was individually located in the device for 6 min. Since great agitation is generally observed during the first 2 min, only the immobility times observed during the last 4 min were measured. An animal was identified as immobile when it stopped its swimming efforts and continued floating motionless in the water, as well as making only those movements needed to keep its head above water (Goleij et al., 2021; Yankelevitch-Yahav et al., 2015).

Formalin test

The formalin test allows the assessment of acute and chronic nociception. In the current research, 30 min after FST the formalin test was carried out. In this test, 50 µl of 5% formalin solution was intraplantar administrated into the left hind paw via a 30 gauge needle (Khakpay et al., 2016). The animals were immediately located to the formalin test apparatus, and the duration of flexing and licking behaviors was measured for an hour (Aloisi et al., 1998; Khakpay et al., 2014; Wheeler-Aceto and Cowan 1991). The blinded experimenter measured the data. Pain behaviors were recorded for 60 min, considering the duration of flexing and licking behaviors of the injected paw. Two phases of flexing and licking behaviors were observed: phase 1 (early or acute phase) started instantly after formalin administration to 7 min (0–7 min) and phase 2 (late or chronic phase) started at time16 min to 60 min (16–60 min) (Khakpay et al., 2010; Mahmoudi and Zarrindast, 2002; Roca-Vinardell et al., 2018).

Histological study

At the end of behavioral tests, animals were deeply anesthetized. Then, the livers were removed and washed with saline solution. The liver tissues of all experimental

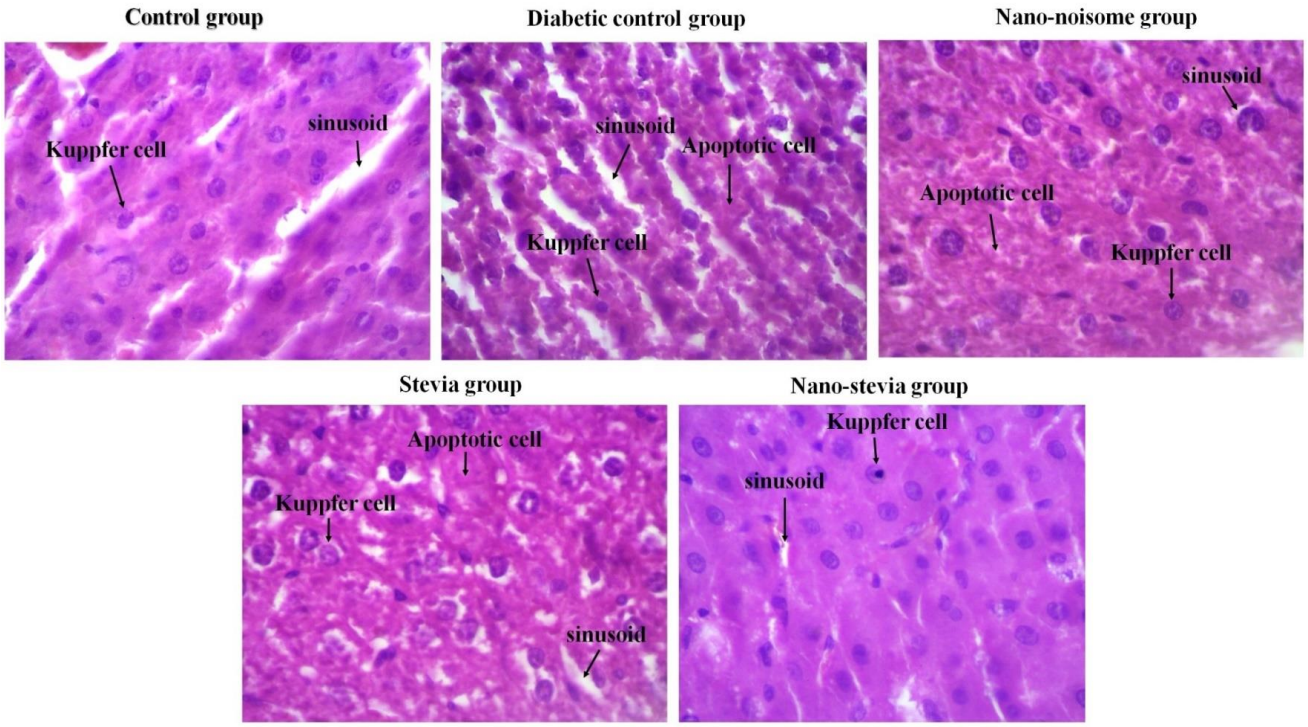

FIGURE 1. Hematoxylin and eosin staining of the liver sections in male diabetic rats. A: Control group, B: Diabetic control group, C: Nano-niosome group, D: Stevia group, E: Nano-stevia group. The diabetic control as well as Nano-niosome groups had a distorted cellular appearance. Stevia and Nano-Stevia groups had a healthy natural arrangement like the control group.

groups were removed and fixed in a 10% formalin solution. The liver tissues were dehydrated in 70%, 80%, 90%, and 100% ethanol and were put in Xylene for more clarity. The samples were embedded in paraffin blocks. The liver sections with a thickness of 5 µm were prepared using a microtome. For morphological evaluation, the liver tissues were stained with hematoxylin and eosin. Then, liver sections were assessed by GraphPad prism.

Statistical analysis

Statistical analyses were evaluated using the IBM SPSS Statistics 26. All results were indicated as means ± standard error of the mean (SEM). Data were analyzed by independent sample t-test and two-way analysis of variances (ANOVA). Values were indicated as statistically significant if P level was lower than 0.05.

Results

Histological results

The liver section from the healthy control group displayed normal cellular cohesion and regularity. Moreover, apoptotic cells were perceived and estimated to be about 5% of the hepatic cells. The liver from the diabetic control and niosome groups showed a dissociated appearance and distorted cellular organization. The percentage of the hepatic cells with a vacuolated nucleus and unclear nucleus membrane was significantly increased compared to the healthy control group ($P<0.05$). In these two groups, the stainless dead cells were assessed at about 50%. The hepatic cells of the Stevia group exhibited a normal shape. The apoptotic and dead cells with fragmented nuclei were assessed at about 30%. The hepatic cells of the Nano-Stevia group showed a normal shape and cells with apoptotic forms were assessed less than 10 % ($P < 0.05$) (Fig. 1).

The effects of intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia on body weight and blood glucose in diabetic rats

Independent sample t-test indicated no significant difference between control rats and diabetic control rats, on body weight [$T(14) = 2.107$, $p > 0.05$; Fig. 2A]. However, the results of independent sample t-test showed a significant difference between control rats and diabetic control rats on blood glucose [$T(14) = 3.357$, $p < 0.001$;

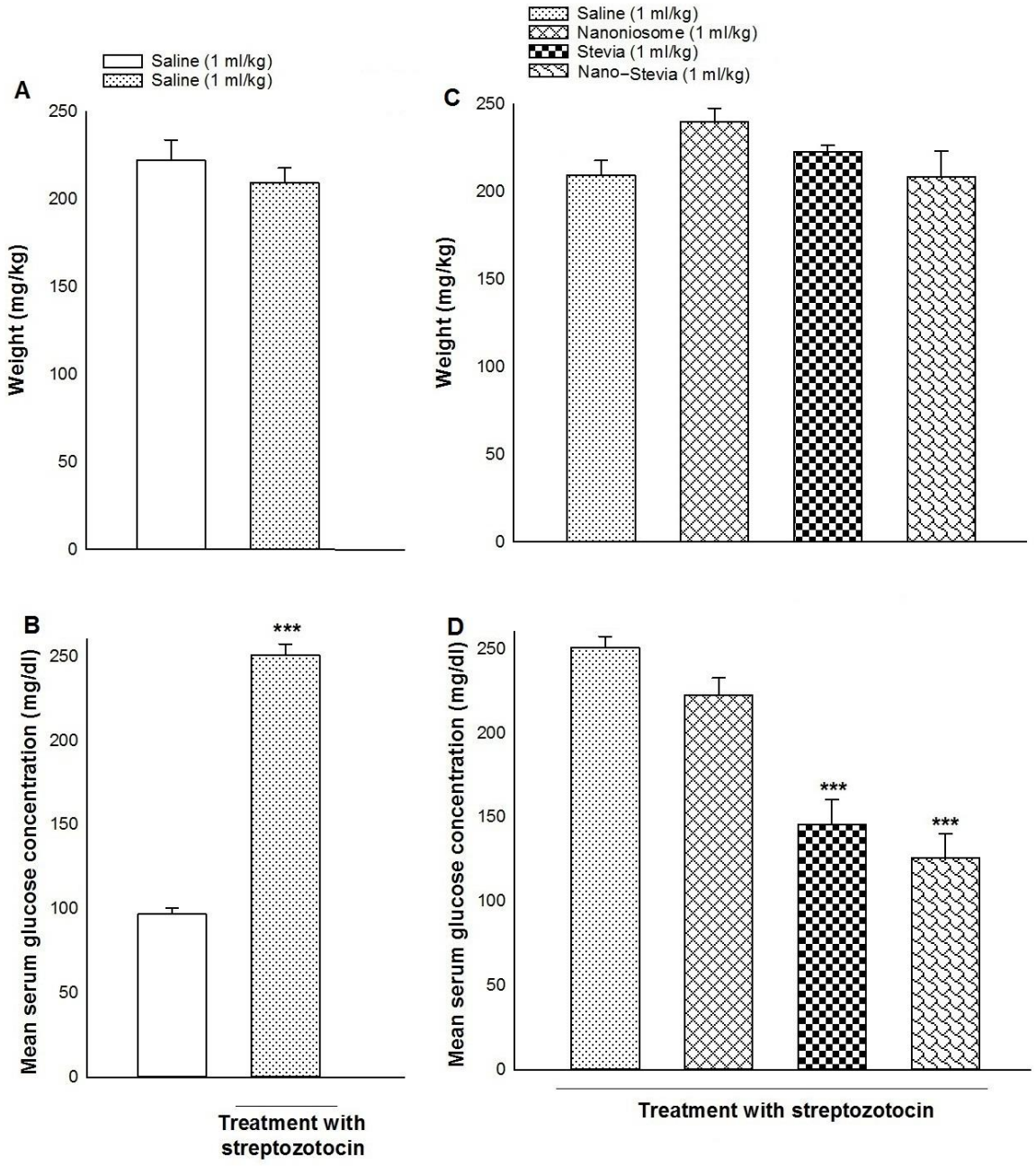

FIGURE 2. The effects of intragastric administration with saline, nano-niosome, Stevia, and Nano-stevia on body weight and blood glucose in diabetic rats. Each bar represents mean ± S.E.M of body weight (A) and blood glucose (B). Significant differences: *** $P < 0.001$ compared to the control group (n = 8).

Fig. 2B]. Indeed, streptozotocin (50 mg/kg) injection caused a significant increase in blood glucose compared to the control group. Therefore, diabetic control rats were used as a control group for next experiments.

The results of two-way ANOVA and post hoc analysis exhibited that intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia for 30 days had no significant effect on body weight [diabetes effect: $F(1, 28) = 0.872$, $P > 0.05$, drug treatment effect: $F(1, 28) = 0.709$, $P > 0.05$, diabetes–drug treatment interaction: $F(1, 28) = 0.126$, $P > 0.05$; Fig. 2C]. Also, the results of two-way analysis of variance and post hoc analysis showed that intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia for 30 days had a significant effect on blood glucose [diabetes effect: $F(1, 28) = 3.890$, $P < 0.05$, drug treatment effect: $F(1, 28) =$

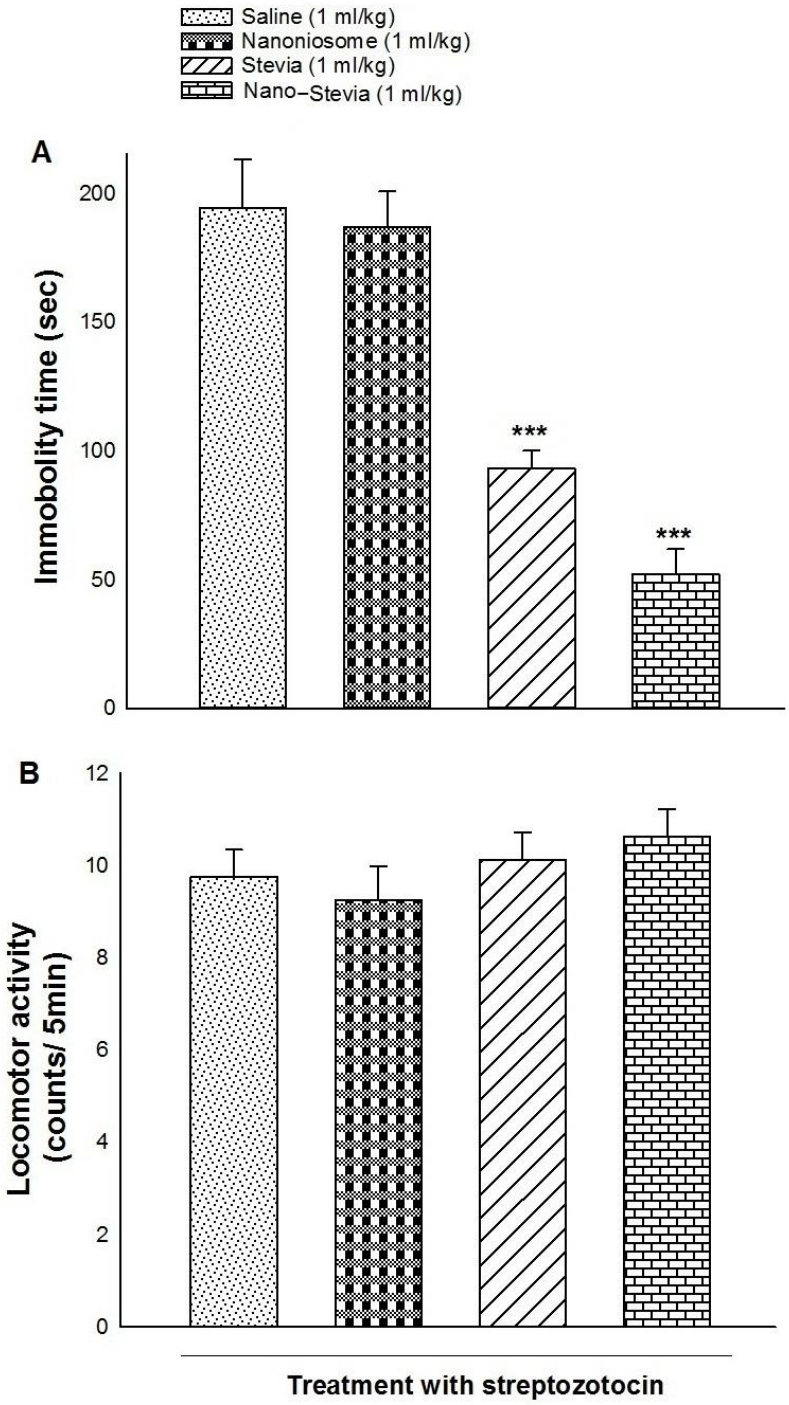

FIGURE 3. The effects of intragastric administration with saline, nano-niosome, Stevia, and Nano-stevia on depression behavior in male diabetic rats. Each bar represents mean ± S.E.M of immobility time (A) and locomotor activity (B). Significant differences: *** $P < 0.001$ compared to the control group (n = 8).

19.001, $P < 0.001$, diabetes–drug treatment interaction: $F (1, 28) = 5.373$, $P < 0.05$; Fig. 2D]. Alone intragastric administration of Nano-nisome could not prevent the increase of blood glucose in the diabetic rats. However, intragastric administration of Stevia and Nano-stevia was able to reduce blood glucose approximately to the normal range.

Effects of intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia on depression-like

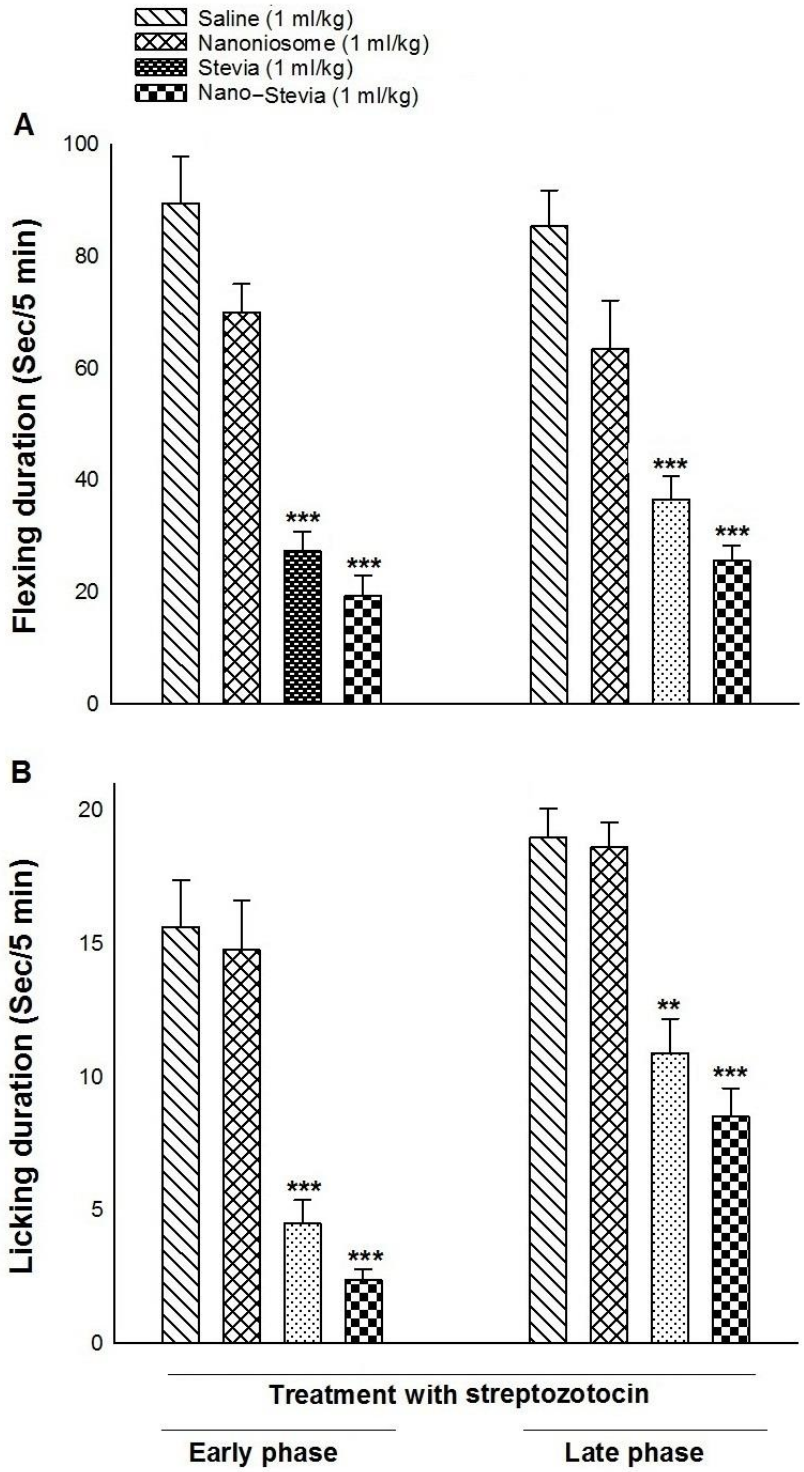

FIGURE 4. The effects of the intragastric administration of saline, nano-niosome, Stevia, and Nano-stevia on pain behavior in male diabetic rats. Each bar shows mean ± S.E.M of the duration of flexing behavior (A) and licking behavior (B). Significant differences: ** $P < 0.01$, and *** $P < 0.001$ compared to the control group (n = 8).

behavior in diabetic rats

Fig. 3 showed the effects of the intragastric application of saline, nano-niosome, Stevia, and Nano-stevia on depression-like behavior in male diabetic rats. Two-way analysis of variance displayed that intragastric application of Stevia (20 mg/dl,1 ml/kg) and nano-niosome (1 ml/kg) + Stevia (20 mg/dl, 1 ml/kg) had no significant effect on immobility time in the FST [diabetes effect: F

TABLE1: Summary of the results.

Fig.	Drug treatments	Effect on body weight (mg)	Effect on blood glucose (mg/dl)	Effect on depression	Effect on pain
2 A and B	Saline (1 ml/kg), saline (1 ml/kg) Streptozotocin (50/kg), saline (1 ml/kg)	No effect	Increased in diabetic rats	-	-
2 C and D	Streptozotocin (50/kg) + Saline (1 ml/kg), or nano-niosome (1ml/kg), or stevia (20 mg/dl, 1 ml/kg), or nano-stevia (20 mg/dl, 1 ml/kg)	No effect	Decreased in stevia and nano-stevia rats	-	-
3 A and B	Streptozotocin (50/kg) + Saline, or nano-niosome, or stevia, or nano-stevia	-	-	Depressant effect in diabetic rats Antidepressant-like effect in stevia and nano-stevia groups	-
4 A and B	Streptozotocin (50/kg) + Saline, or nano-niosome, or stevia, or nano-stevia	-	-	-	Hyperalgesic effect in diabetic rats Analgesic effect in stevia and nano-stevia rats

(1, 28) = 80.200, P < 0.001, drug treatment effect: F (1, 28) = 3.337, P < 0.05, diabetes–drug treatment interaction: F (1, 28) = 1.616, P > 0.05; Fig. 3A] and locomotor activity [diabetes effect: F (1, 28) = 1.954, P > 0.05, drug treatment effect: F (1, 28) = 0.864, P > 0.05, diabetes–drug treatment interaction: F (1, 28) = 0.638, P > 0.05; Fig. 3B]. Post hoc analysis showed that Stevia and Nano-stevia decreased immobility time in the FST, which revealed an antidepressant-like response.

Effects of intragastric administration of saline, nano-niosome, Stevia, and Nano-stevia on pain behavior in diabetic rats

The effects of intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia on pain behavior in male diabetic rats are illustrated in Fig. 4. Two-way analysis of variance indicated that intragastric administration of saline, Nano-niosome, Stevia, and Nano-stevia had no significant effect on the duration of flexing behavior both in the early phase [diabetes effect: F(1, 28) = 105.243, P < 0.001, drug treatment effect: F(1, 28) = 6.304, P < 0.01, diabetes–drug treatment interaction: F (1, 28) = 1.069, P > 0.05; Fig. 4A, left panel] and the late phase [diabetes effect: F (1, 28) = 52.807, P< 0.001, drug treatment effect: F(1, 28) = 7.806, P< 0.01, diabetes–drug treatment interaction: F(1, 28) = 0.871, P> 0.05; Fig. 4A, right panel] of the formalin test. Also, licking behavior did not change both in the early phase [diabetes effect: F (1, 28) = 73.068, P < 0.001, drug treatment effect: F (1, 28) = 1.191, P > 0.05, diabetes–drug treatment interaction: F (1, 28) = 0.207, P > 0.05; Fig. 4B, left panel] and the late phase [diabetes effect: F (1, 28) = 68.383, P<0.001, drug treatment effect: F (1, 28) = 1.553, P > 0.05, diabetes–drug treatment interaction: F (1, 28) = 0.821, P > 0.05; Fig. 4B, right panel] of the formalin test.. Post-hoc analysis displayed that intragastric administration of Stevia and Nano-Stevia decreased the duration of flexing and licking behaviors both in the early and the late phases of the formalin test in comparison to the saline groups, showing analgesic effect in male diabetic rats.

Discussion

In experimental rodents, streptozotocin has been broadly utilized to induce diabetes. As reported by numerous investigations, intraperitoneal injection of streptozotocin (50 mg/kg) caused partial damage in the pancreatic β-cells and liver tissue in rats (Cameron-Smith et al., 1997; Ferner 1992; Punithavathi et al., 2011; Sheikh et al., 2015; Venkateshwarlu E and J, 2013). Then, the synthesis and secretion of insulin decreased, but glucose and lipid levels, fibrosis, and inflammation increased (Markova et al., 2017; Newgard 2012; Tian et al., 2017). Our findings indicated that induction of diabetes in male

rats caused enhancement of blood glucose, depressant-related effect, as well as hyperalgesia response in diabetic rats. Hyperglycemia induced by streptozotocin in the experimental rodents is a valuable experimental model to assess the effect of diverse hypoglycemic agents (Ivorra et al., 1989). The mechanism by which streptozotocin elicits diabetic state includes selective damage to the pancreatic β -cells via DNA alkylation (Szkudelski 2001). This damage caused inadequate insulin secretion and induced a diabetic model (Irudayaraj et al., 2012; Nisha and Mini 2013).

Also, our results showed that intragastric application of Stevia and Nano-stevia reversed the hyperglycemia, depressant-related effect, and hyperalgesic response induced by the injection of streptozotocin in male rats. Shamsaei et al. (2006) reported the existence of a significant link between diabetes and depression (Shamsaei et al., 2006). Accumulating evidence revealed that depression and diabetes share biological origins, mainly overactivation of innate immunity resulting in a cytokine-induced inflammatory response, and possibly via dysregulation of the hypothalamic-pituitary-adrenal axis. Throughout the life, these circuits can cause insulin resistance, cardiovascular disease, depression, enhanced risk of type 2 diabetes, and enhanced mortality. Furthermore, proinflammatory cytokines may directly affect the brain, inducing depressive symptoms (Hood et al., 2012). On the other hand, Stevia revealed therapeutic benefits as a nutraceutical in the controlling of chronic diseases such as diabetes (Wang et al., 2020). This is due to the existence of glycoside stevioside in the Stevia leaves (Ahmad and Ahmad 2018; Brahmachari et al., 2011; Lemus-Mondaca et al., 2012). Regular usage of Stevia glycosides reduces sugar, cholesterol, and radionuclide levels in the blood (Atteh et al., 2008). It shows a high anti-hyperglycemic effect (Chen et al., 2006; Jeppesen et al., 2000). In vivo, investigations display that Stevia enhances glucose tolerance in diabetic rats by maintaining the blood glucose level (Curi et al., 1986). In diabetic patients, it also causes hypoglycemia by lowering both the glycogenolysis and gluconeogenesis activities, as well as absorbing the glucose in the duodenum. Stevia has beneficial properties in the pancreatic tissue by enhancing the insulin amount and increasing anti-diabetic effects (Assaei et al., 2016). Additionally, Kumar and Chail (2019) reported that ingestion of sugar-substitutes may show beneficial effects in the mitigation of brain illness such as depression during diabetes by modulation of oxidative stress and MAO-A activity as well as corticosterone release (Kumar and Chail 2019).

Furthermore, Stevia has many therapeutic properties and has been confirmed safe and effective for hundreds of years (Munro et al., 2000). Preclinical and clinical investigations propose therapeutic and pharmacological usage of stevia and its extracts since they are not toxic and show numerous biological properties. For example, some animal investigations have demonstrated the effects of stevioside for inducing anti-hyperglycemic, insulinotropic, glucagonostatic, antioxidant, anti-inflammatory, antihypertensive, antimicrobial, and anti-tumor properties in diabetic rats (Jeppesen et al., 2003; Ruiz-Ruiz et al., 2017; Sharma et al., 2012; Shivanna et al., 2013). Evidence has revealed that administration of stevia in its raw form, fresh and dried, helps solve numerous health difficulties, for instance, diabetes (Hossain et al., 2017). Moreover, it has been reported that sugar substitutes might decrease the occurrence of psychiatric diseases such as depression in diabetic patients (Kumar and Chail 2019). Stevia has significant neurotrophic properties in comparison to other sweeteners (Villareal et al., 2016). It decreases inflammation by lowering the amount of pro-inflammatory cytokines, NF-κB and IκB, IL-1β, IL-6, and TNF-α (Fengyang et al., 2012; Wang et al., 2014). Stevia and Nano-stevia may neutralize the inflammatory factors contents in the serum of diabetic rats. Stevia could lower fasting blood sugars and inflammatory factors i.e. IL-1β, IL-6, and TNF-α amounts in the diabetic group (Boonkaewwan and Burodom 2013; Fengyang et al., 2012), and so might help in lowering depression-related behavior and pain threshold in diabetic rats.

Conclusion

The properties of Stevia highlighted in numerous studies (Ajami et al., 2020; Kurek and Krejpcio 2019; Lestari et al., 2019; Masoumi et al., 2020; Pallarés et al., 2015; Ruiz-Ruiz et al., 2017; Shahid et al., 2022; Sharma et al., 2012; Simonyan et al., 2021; Wang et al., 2020), along with the demonstrated anti-diabetic, antidepressant, and analgesic effects of Nano-stevia in this study, enhance our understanding of the pathogenesis behind diabetes-related complications and point to promising applications in therapeutic strategies. Never-

theless, further experiments are required to clarify the exact mechanism of Stevia and Nano-stevia in diabetic rats on the modulation of depression-related behavior and pain process.

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Conflict of interest

No financial or other conflicts of interest are declared.

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