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ORIGINAL ARTICLE

Combination of Shengmai San and *Radix puerariae* ameliorates depression-like symptoms in diabetic rats at the nexus of PI3K/BDNF/SYN protein expression

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Abstract

Background: Hyperglycemia is a characteristic feature of diabetes that often results in neuropsychological complications such as depression. Diabetic individuals are more vulnerable to experience depression compared to the normal population. Thus, novel treatment approaches are required to reduce depressive symptoms among diabetic individuals. Traditional Chinese medicines (TCMs) such as Shengmai San (SMS) and *Radix puerariae* (R) are usually widely used to treat ailments such as neurological complications since ancient time.

Methods: In this study, SMS was combined with R to prepare an R-SMS formulation and screened for their antidepressant activity in diabetic rats. The antidepressant potential of the prepared combination was evaluated behaviorally using open field test, novelty-induced hypophagia, and forced swim test in diabetic rats with biochemical and protein expression (PI3K, BDNF [brain-derived neurotrophic factor], and SYN [presynaptic vesicle protein]) analysis.

Results: Diabetic rats (streptozotocin, 45 mg/kg) showed elevated fasting blood glucose (FBG) >12 mM with depressive symptoms throughout the study. Treatment with R-SMS (0.5, 1.5, and 4.5 g/kg) significantly reverted depressive symptoms in diabetic rats as evinced by significantly ($p < 0.05$) reduced immobility time with an increased tendency to eat food in a novel environment. Treatment with R-SMS also significantly

Ayaz Ahmed and Guirong Zeng share first authorship; both authors were involved equally in experimentation and preparation of the manuscript.

increased the protein expression of PI3K, BDNF, and SYN protein, which play a crucial role in depression.

Conclusion: This study showed that R-SMS formulation antagonized depressive symptoms in diabetic rats; thus, this formulation might be studied further to develop as an antidepressant.

KEYWORDS
BDNF, depression, Shengmai San, STZ induced diabetes, traditional Chinese medicine

1 | INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by hyperglycemia, impaired insulin secretion, or its resistance. Diabetes has a global impact and has been declared as a rapidly growing health emergency of the 21st century.[1] According to the estimates of the International Diabetic Federation, 537 million people are living with diabetes, and this number is expected to increase up to 783 million by 2045.[1] The World Health Organization has suggested that diabetes-associated mortality will be ranked seventh by 2030. Diabetes is associated with various micro- and macrovascular complications such as retinopathy, peripheral neuropathy, stroke, coronary heart diseases, and neurodegenerative (learning and memory loss with depression) disorders.[2–4] Overwhelming evidence has suggested the bidirectional involvement of diabetes with major depressive disorders (MDD).[5,6] Studies highlighted that diabetic individuals are 30%–40% more prone to acquire extreme depression.[7,8] Literature supports the fact that depression is a frequent comorbid condition of both type 1 and type 2 DM with common pathophysiological mechanisms.[7–11] The pathophysiology of depression in diabetic patients is multifactorial, which includes insulin resistance, inflammation, oxidative stress, decreased activity of norepinephrine and serotonin (5-HT), and brain-derived neurotrophic factor (BDNF).[12–14] Diabetes was reported to enhance insulin resistance in brain, thus facilitating neurodegenerative complications such as depression. Comorbid depression increases both micro- and macrovascular complications, the major cause of multiorgan damage and mortality.[15–17] Diabetes-associated depression intensifies mortality risk by 1.5-fold compared to nondiabetic individuals.[18]

Neurons express BDNF, a specific neurotrophin responsible for synaptic plasticity by complementing neuronal growth, its maturation, and synapse development.[19] Neurotrophin hypothesis states that depression is associated with BDNF reduced expression and function.[20,21] BDNF provides neuroprotection by inducing long-term potentiation by binding to its receptor i.e., either tropomyosin-related kinase B (TrkB) or low-affinity nerve growth factor receptor.[22] BDNF expression is reduced in diabetic brain, which is a key indication of MDD.[23,24] BDNF administration in hyperglycemic or hyperlipidemic (db/db) mice significantly improves insulin sensitivity, energy, and glucose homeostasis with reduced hyperphagia and body weight.[19,25] Presynaptic vesicle protein (SYN) is mainly present in the synaptic vesicle membrane and is responsible for the release of neurotransmitters via phosphorylation. It can be used as a specific marker to indicate synaptic density, distribution, and their number, thus measuring the plasticity of synaptic structure and function.[26] Usually, BDNF binds to Ig-C2 domain of TrkB to activate TrkB receptor, leading to dimerization, tyrosine autophosphorylation, and adaptor protein recruitment.[20,27–29] This further activates the PI3K–Akt pathway and maintains insulin sensitivity.[28,30] During MDD, the downstream pathway of TrkB receptor is interrupted.[31,32] Therefore, depression associated with diabetes needs to be addressed to improve the quality of life of diabetic individuals.

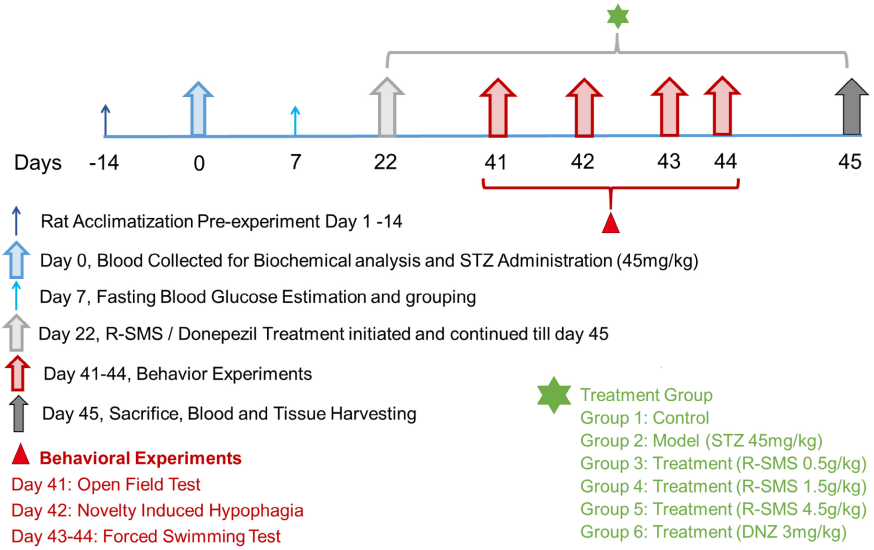
The main remedial aim in treating diabetes comorbid with depression is to reduce depressive symptoms and improve hyperglycemia using antidepressants and hypoglycemic interventions.[33–35] However, a study reported the moderate effect of antidepressants used during diabetes.[36] Already-approved antidepressant drugs exhibited major cardiometabolic effects; citalopram and mirtazapine were found to induce appetite, resulting in weight gain, which further elevates hyperglycemia.[37] Therefore, this situation demands novel pharmacophores as antidepressant formulations with least toxic effects.[38] Recently, traditional Chinese medicine (TCM) has been emerging globally by virtue of its importance given to herbal formulations, that is, extracts or isolated compounds for therapeutics against a variety of diseases. Several TCM formulations, decoction of Pinellia and Magnolia bark, and Chinese Angelica decoction have been used for replenishing blood, lowering glucose level, and relieving depression. Furthermore, decoction for tonifying the kidney-yin has shown to improve depression comorbidities in diabetes.[39,40] Recently, our group has shown that a combination of Shengmai San and *Radix puerariae* (R-SMS) significantly improves learning and memory deficit in diabetic rats.[41] Therefore, this study was designed to explore the curative potential of R-SMS to reverse diabetes-associated depressive disorder in streptozotocin (STZ) rats and to elucidate its molecular mechanism at the nexus of PI3K/BDNF/SYN expression.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Sixty Sprague–Dawley male rats (average weight: 200–220 g) were purchased from Hunan SJA Laboratories Animal Company Ltd. The rats were housed in an environmentally controlled condition (24 ± 1°C and 40%–50% humidity). They were acclimatized for 2 weeks and provided a high-fat diet and water ad libitum. The

FIGURE 1 Experimental design.



protocol was designed in accordance with the National Institute of Health guidelines, and the study was approved by the Ethical Committee of Hunan Drug Safety Evaluation (study number: 2016016). The rats were anesthetized using xylazine and ketamine (1:2) to collect blood and brain after behavioral experiments.

2.2 | Diabetes induction

Diabetes was induced in the rats by a single intraperitoneal administration of STZ (Ahmed et al., 2019 and 2020). STZ (45 mg/mL) was prepared in 0.1 M sodium citrate buffer (pH 4.5). The rats were not fed for 16 h before administration, and they were intraperitoneally injected with freshly prepared STZ. Fasting blood glucose (FBG) was measured using a blood glucometer (GA-6, SANNUO) after 7 days of STZ administration from the tail vein. Rats with an FBG ≥ 10.0 mM were considered diabetic and included in the study.

2.3 | Preparation of R-SMS formulation

Medicinal herbs were procured from Beijing Tong Ren Tang and validated by Professor Bengang Zhang at IMPLAD. The R-SMS formulation was prepared by mixing SMS (composed of *Panax ginseng*, *Ophiopogon japonicus*, and *Schisandra chinensis*) and *Radix puerariae* in the ratio of 3:3:2:4. The preparation of R-SMS powder formation and its chemical analysis is reported earlier (Ahmed et al., 2021). Sodium carboxymethyl cellulose (CMC-Na, 0.5%) was used to mix R-SMS formulation.

2.4 | Study design

Rats ($n = 10$) on high fat diet and without STZ administration served as the normal control group. However, diabetic rats ($n = 10$) were randomly divided into five groups, that is, STZ (no treatment, diabetic control), STZ + R-SMS (0.5 g/kg), STZ + R-SMS (1.5 g/kg), STZ + R-SMS (4.5 g/kg), and STZ + DNZ (donepezil, 3 mg/kg). Treatment with R-SMS and DNZ in the respective groups was started after 3 weeks of diabetes induction. On the contrary, 0.5% CMC-Na was administered to the diabetic and the control groups. The therapeutic regime comprised 3 weeks, and drugs were administered intragastrically once daily between 10:00 and 11:00 a.m. After 3 weeks of treatment, the rats in each group were subjected to behavioral experiments. During the behavioral experiments, drug treatment continued, and the drug was administered half an hour before the behavioral tasks. The detailed experimental design is shown in Figure 1.

2.5 | Biochemical profile

Blood was collected for serum biochemical profile analysis before induction of diabetes and at the end of the experiment. Serum insulin levels were determined for each group using an ELISA (enzyme-linked immunosorbent assay) kit according to the manufacturer's protocol (Solarbio Science & Technology Co.). Lipid profile (i.e., low-density lipoprotein [LDL], high-density lipoprotein [HDL], total glycerides [TG], and total cholesterol [CHO]) of the diabetes and treated groups was analyzed using an automated biochemist analyzer (MODULAR P800, Roche). Similarly, the body weight of rats in each group was measured before STZ administration and at the end of the experiment. The data were presented as ratio change in comparison with the control or the diabetic group.

2.6 | Locomotion and explorative behaviors

Changes in rat movement profile in the control, diabetic, or treated group were evaluated using a camera-operated open-field rectangular arena (Shanghai Xin-Ruan Information Technology Co.). The rectangular box (100 × 100 × 40 cm) was enclosed in a metallic chamber with illumination (120 lx). The rats from each group were placed in the center of the arena and allowed to acclimatize for 2 min.

Movement patterns such as speed and time of movement were recorded for 10 min after acclimatization and analyzed using the supermaze software (Shanghai Xin-Ruan Information Technology Co.).

2.7 | Forced swim test

The forced swim test (FST) was conducted consecutively for 2 days in a plastic cylinder (depth, 50 cm; diameter, 20 cm). On day 1, the rats were placed in a cylinder filled with water (40 cm) for acclimatization and allowed to swim for 15 min. On day 2, swimming pattern was recorded for a period of 5 min using a mounted camera. Individual rats were monitored for immobility time (i.e., floating without any movement or struggle) and mobility time (i.e., struggling to keep head above water level).[42] The data were recorded by two individuals who were blinded to the experiments.

2.8 | Novelty-induced hypophagia test

Hypophagia refers to the inhibition of feeding produced by exposure to novelty.[43] After 24 h of food deprivation, the rats were placed in a new plastic cage. The latency of feeding (time elapsed until the rat started to eat) was recorded manually.

2.9 | Western blot analysis

Hippocampi from rat brain were dissected after behavior tests and homogenized in RIPA lysis buffer (Solarbio Science & Technology Co.) containing a cocktail of protease and phosphatase inhibitor (Thermo Scientific). Debris from the resulting homogenate was removed by using refrigerated (4°C) centrifugation at 12000 rpm for 10 min. The protein concentration of the individual sample was determined using bicinchoninic acid protein assay kit (Solarbio Science & Technology Co.). The protein expression of PI3K and SYN was utilized in the prepared protein homogenate of the control, diabetic, and treated groups. Proteins in the homogenate were separated using 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) electrophoresis. The proteins were transferred to a polyvinylidene difluoride membrane, and the expression of the mentioned proteins was determined using antibodies for SYN, PSD-95, and actin (1:1000, Abcam), respectively, as described earlier.[2]

2.10 | Statistical analysis

Data are presented as mean ± SEM. Statistical significance between the groups in behavioral experiments was analyzed using one-way analysis of variance (ANOVA) and least significant difference test, whereas the significance of protein expression and other indexes between the groups was determined by Student's *t* test using SPSS version 21 (IBM). $p \leq 0.05$ was considered as the significant level. The data were analyzed for normality using Shapiro–Wilk test, and the homogeneity of variance was analyzed using Levene's test.

3 | RESULTS

3.1 | Diabetes confirmation and metabolic profile

The diabetic (STZ alone) and treated (STZ + R-SMS) rats showed significant increase in blood sugar level compared to the control rats, suggesting R-SMS treatment has no profound effect on hyperglycemia (Figure 2A). STZ-treated diabetic rats showed a significant decrease (30 g) in body weight after STZ administration compared to the control group (without STZ, $p < 0.001$), which gain weight exponentially (Figure 2B). On the contrary R-SMS-treated group showed significant dose-independent weight gain compared to the diabetic group (Figure 2B, $p < 0.05$). Dyslipidemia is a characteristic feature of diabetes, and the STZ-induced diabetic rats showed one- to twofold increase in LDL, TG, and CHO, with reduced HDL levels (Figure 2C). Treatment of STZ-treated diabetic rats with R-SMS (0.5, 1.5, and 4.5 g/kg) significantly improved dyslipidemia, as evinced by the reduced levels of LDL, TG, and CHO and increased HDL levels ($p < 0.05$, $p < 0.01$, and $p < 0.001$; Figure 2D).

3.2 | Effect of R-SMS on locomotory and explorative behaviors

In the open field test (OFT), STZ-treated rats displayed significantly reduced movement time compared to the control and R-SMS-treated rats ($p < 0.01$ and $p < 0.001$; Figure 3A). Similarly, the total distance covered by the control rats was significantly ($p < 0.001$) higher (18404.1 ± 1330.7 mm) compared to the STZ diabetic group (7268.8 ± 816.9 mm). Treatment with R-SMS (0.5, 1.5, and 4.5 g/kg) and DNZ (3 mg/kg) significantly restored mobility in diabetic rats, as exhibited by the significant increased distance traveled with enhanced speed, that is, in the range of 20–30 mm/s compared to 12.1 mm/s by diabetic rats (Figure 3B,C). Moreover, STZ-treated rats covered a significantly reduced distance in the central area (560.74 ± 122.94 mm) compared to the control and R-SMS-treated rats (2000–4000 mm) (Figure 3D).

3.3 | Effect of R-SMS on novelty-induced hypophagia

STZ-treated diabetic rats showed significant ($p < 0.001$) prolonged latency of feeding in the new environment (193.7 s) compared to the controls (42.3 s; Figure 4). Treatment with DNZ (3 mg/kg) and R-SMS (0.5, 1.5, and 4.5 g/kg) significantly ($p < 0.001$) declined the latency of feeding in the diabetic rats, that is, 55–60 s, respectively (Figure 4).

3.4 | Effect of R-SMS on FST

The immobile time for the STZ-treated diabetic rats was significantly higher (239 ± 24.1 s) compared to the control group ($p < 0.001$;

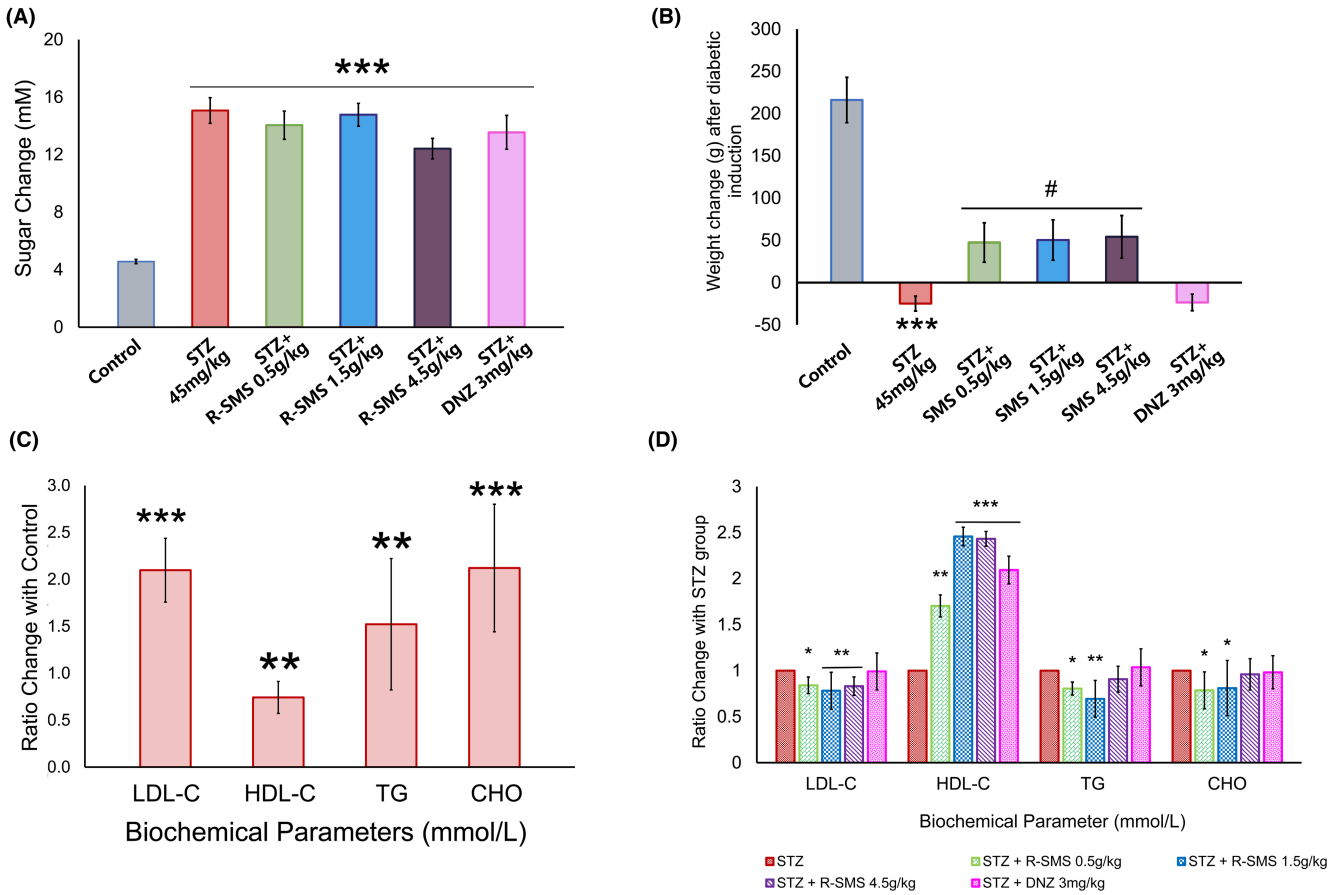


FIGURE 2 Effect of R-SMS (Shengmai San and *Radix puerariae*) on weight and biochemical parameter of control, diabetic, R-SMS, and DNZ (donepezil) treated rats. (A) Sugar change among rats in STZ (streptozotocin) and treated groups compared to the control rats. (B) Weight change after STZ administration. (C) Ratio of lipid profile in STZ-administered rats compared to normal rats. (D) Ratio of lipid profile in R-SMS- and DNZ-treated rats compared to STZ-administered rats. Each bar represents value as means ± SEM. $n = 8$ rats per group. Statistical significance of the data is represented as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the STZ model.

Figure 5). In DNZ (3 mg/kg) and R-SMS (0.5, 1.5, and 4.5 g/kg) treated rats, the immobile time significantly decreased compared to the diabetic rats (Figure 5).

3.5 | Effect of R-SMS on BDNF expression using ELISA

BDNF was considerably reduced (216.3 ± 16.01 pg/mL, $p < 0.01$) in the hippocampi of diabetic rats compared to those of the controls (326.7 ± 16.9 pg/mL; Figure 6). The BDNF concentration significantly increased in DNZ (3 mg/kg) and R-SMS (0.5, 1.5, and 4.5 g/kg) in the range of 250–285.3 pg/mL ($p < 0.05$; Figure 6).

3.6 | Effects of R-SMS on the protein expression of PI3K and SYN in the hippocampus of diabetic brain

The protein expression of PI3K and SYN in the hippocampi of the diabetic rats after treatment with R-SMS was evaluated for the first time. The expression of PI3K and SYN was significantly downregulated compared to the nondiabetic control rats ($p < 0.01$; Figure 7A–D). Treatment with 4.5 g/kg R-SMS and 3 mg/kg DNZ significantly upregulated the protein expression of the aforementioned proteins ($p < 0.01$; Figure 7A–C). However, low doses of R-SMS (0.5 and 1.5 g/kg) also restored the protein activation of PI3K but failed to restore the expression of SYN significantly (Figure 7B,C).

4 | DISCUSSION

The modernization of the world and the changing routine promote a sedentary lifestyle due to which there is a gradual increase in the number of diabetic patients every year. The International Diabetes Foundation has predicted that the number of diabetic individuals would increase up to 783 billion by 2045. Clinical studies have highlighted a close correlation between diabetes and depression, and data suggest that diabetic individuals are 30%–40% more vulnerable to acquire depressive symptoms.[7,44] These symptoms may be the result of biochemical changes in diabetic individuals, such as enhanced inflammation, activation of the hypothalamic–pituitary–adrenal axis, and reduced BDNF expression.[45] Depression during diabetes further

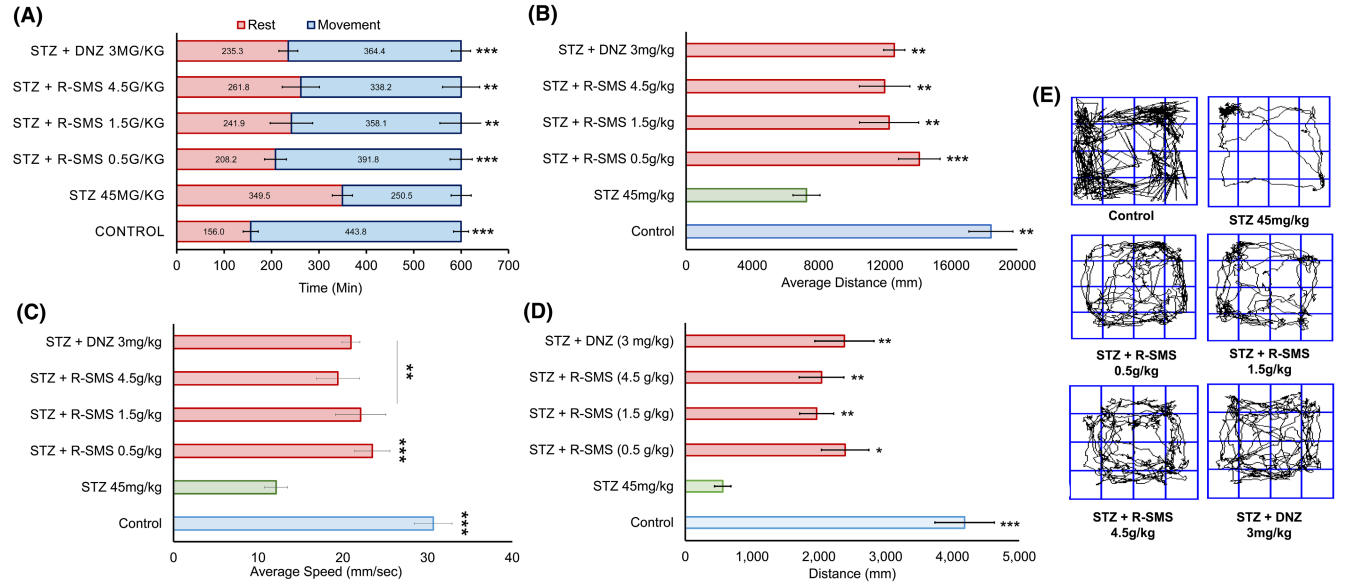


FIGURE 3 Effect of R-SMS (Shengmai San and *Radix puerariae*) on rat autonomic activity using open field arena among control, diabetic, R-SMS, and DNZ (donepezil) treated rats. (A) Rest and movement time. (B) Average speed. (C) Average distance traveled. (D) Distance moved in the central area. (E) Pictorial view of rat movement in the open field arena. Data are expressed as mean ± SEM. $n = 8$ in each group. $^{*}p < 0.05$ and $^{**}p < 0.001$ compared with the diabetic model.

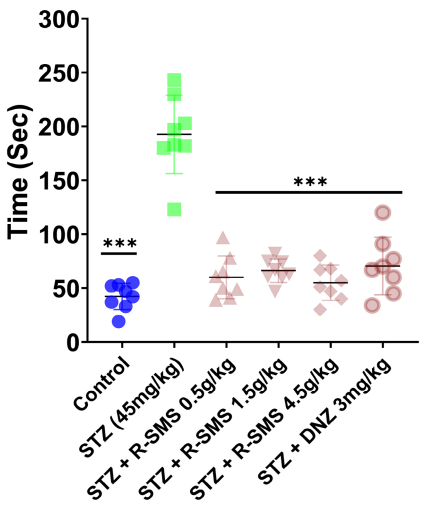


FIGURE 4 Effect of R-SMS (Shengmai San and *Radix puerariae*) on novel inhibition of feeding in rats. Each bar is expressed as mean ± SEM. $n = 8$ animals in each group. Statistical significance is represented as $^{***}p < 0.001$ compared with the diabetic model.

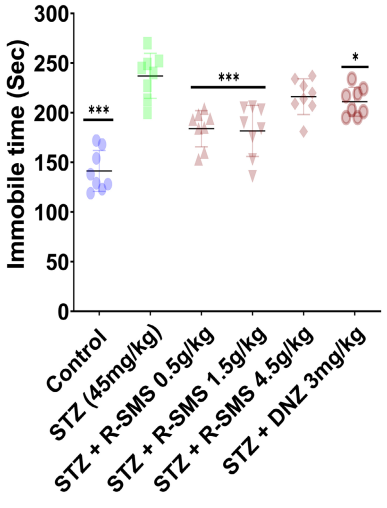


FIGURE 5 Effect of R-SMS (Shengmai San and *Radix puerariae*) on diabetic rats on forced swim test. Each bar is expressed as mean ± SEM. $n = 8$ animals in each group. Statistical significance is represented as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the diabetic model.

intensifies the diabetic condition by promoting insulin resistance and heavy bleeding in diabetic individuals.[46] In contrast, a diagnosis of depression in patients with diabetes exacerbates depression. The relation between the two reflects common etiologies that involve a complex two-way interaction between multiple variables such as autonomic and neurohormonal disorders, weight gain, inflammation, and changes in brain tissue.[17] But still the complete complex molecular mechanism is unknown. The cure is directed to reduce depression in individuals with diabetes to help them socialize, thus improving their health status. TCMs have been reported to cure many ailments, including diabetes-associated depressive symptoms.[39,40]

Thus, this study was designed to formulate R-SMS that comprises four bioactive components, namely Puerarin, Ginsenosides, Schizandrin, and Ruscogenin.[41] All the components of R-SMS have been shown to be neuroprotective, especially antidepressant activity among chronic unpredictable mild stress mouse models.[47–50] During diabetes various major end organs, including the brain, have exhibited enhanced inflammation and oxidative stress. Ginsenosides,

a constituent of SMS, were shown to reduce inflammation in diabetic neuropathy by downregulating TGF-β, KIM1, and advance glycation endproducts.[51] Ginsenosides were also shown to play a neuroprotective role in diabetes-associated depression rats by downregulating BDNF.[52] Puerarin has shown anti-inflammatory and antioxidative potential, thus alleviating type II diabetes, obesity, hypertension, and so forth.[53] It has also been shown to ameliorate depression in diabetic rats by promoting neurogenesis.[54] Therefore, the ingredients in the formulation exhibited high antioxidant, anti-inflammatory, and neurogenic activities that might be responsible for reversing diabetes-associated depression in rats. No study has reported their activities in diabetes-associated depression-like symptoms. Thus, this study was designed to evaluate the antidepressant effect of R-SMS in diabetic rats using a battery of behavioral tests.

The diabetic rats showed three- to fourfold increase in blood glucose (i.e., 13 mM). Diabetes is often accompanied by dyslipidemia, which mainly includes decreased HDL and increased LDL, TG, and CHO.[55] The diabetic rats in this study also showed that LDL-C, TG, and CHO significantly increased up to 1.5–2-fold and decreased HDL-C level and bodyweight during the study, which is the characteristic feature of STZ-induced diabetic rats.[2,55] Treatment with R-SMS had no effect on blood glucose, as evidenced by the high blood sugar level in each treated group, but significantly improved body weight and biochemical profile among diabetic rats. These results suggest that R-SMS treatment has no effect on blood glucose; however, it significantly improves dyslipidemia and weight loss among diabetic rats. Similarly, Yao et al[56] also showed that SMS restored dyslipidemia in high-fat-diet rats.

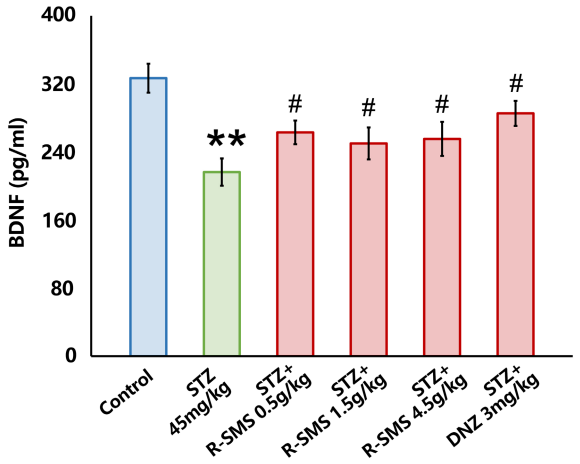


FIGURE 6 Effect of R-SMS (Shengmai San and *Radix puerariae*) on diabetic rats' hippocampal BDNF (brain-derived neurotrophic factor) protein level. Each bar is expressed as mean ± SEM. $n = 8$ animals in each group. Statistical significance is represented as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared with the diabetic model.

Diabetic animal models were reported to have neuropsychological complications such as learning or memory deficit and depression.[57] In this study, according to the literature survey, behavioral tests (i.e., OFT, novelty-induced hypophagia [NIH], and FST) were conducted to evaluate depression in diabetic rats with or without R-SMS treatment.[42,43] OFT was employed to observe rodent locomotory or anxious behavior. FST is the most frequent method used to validate depression-like symptoms in animal models.[58] It works on the following principle: when a rat is introduced in a water-filled container, it makes an effort to either escape or remain immobile, an indicator of behavioral deficit, that is, depression. Similarly, NIH is usually employed to understand depressive behavior in animal models. Moreover, it is also used to evaluate the efficacy of antidepressant treatments. Principally, it used the rat's innate fear to estimate the time utilized and amount of food eaten in the new environment.[43,59] The results showed that diabetic rats exhibited prolonged immobility time with reduced distance traveled and latency of feeding in the OFT, FST, and NIH, respectively, which reflect their depression-like behaviors. However, treatment with R-SMS considerably reversed depressive symptoms, as indicated by the significantly improved mobile time and latency of eating in each behavioral test. The results are comparable with the standard drug used, that is, DNZ. Furthermore, our results are comparable with other studies, which showed improvement in depressive symptoms in the diabetic animal model when treated with TCM, that is, Banxiahoupu decoction, Chaihu-shugan, and Kai-Xin-San.[39,60–62] The

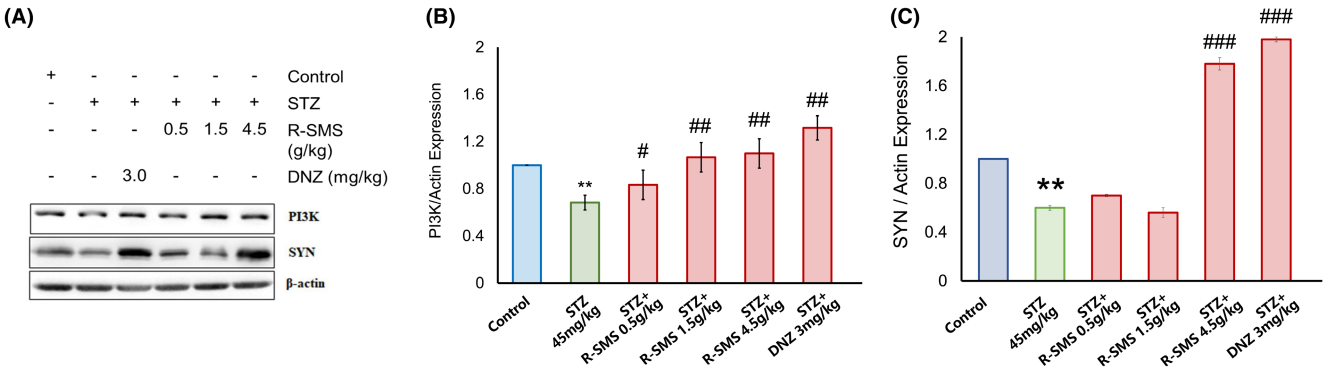


FIGURE 7 Effects of R-SMS (Shengmai San and *Radix puerariae*) on hippocampal PI3K and SYN (presynaptic vesicle protein) expression in diabetic rats. (A) Western blot image of PI3K and SYN expression. (B) Differential expression of PI3K. (C) Differential expression of SYN in diabetic and treated rats. Statistical significance of the date is represented as $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to the diabetic group.

dyslipidemia-associated inflammation and oxidative stress might be responsible for depressive behavior in diabetic rats, which was reversed after R-SMS treatment.

Clinical and animal studies have long established the role of BDNF in major depressive and anxiety syndrome. BDNF plays a crucial role in increasing synaptic generation, neuronal protection, survival, and improving neuroplasticity. Reduced expression of BDNF is a characteristic feature of depressive brain, which will increase in response to antidepressant therapy.[48] Diabetic brain was also reported for reduced expression of BDNF, which confirms its involvement in depression-like behavior in diabetic individuals based on neurotrophin hypothesis.[20,21,63] BDNF on the downstream was found to activate PI3K–Akt pathway, which maintains insulin sensitivity, neuronal cell maturation, and growth.[28,30] On the contrary, PI3K inhibitor was found to delay neuronal differentiation from the first stage to the third stage, which further strengthens the role of PI3K in neuronal growth.[64] BDNF-related activation of PI3K was found to promote the proliferation of hippocampal neurons in mice and improve depression-like behavior and neuroplasticity.[65] Presynaptic vesicle (SYN) protein is responsible for the release of neurotransmitters through phosphorylation, and its density, distribution, and number of synapses define synaptic plasticity structurally and functionally.[26] The results of this study showed that BDNF, PI3K, and SYN were notably downregulated in the hippocampi of diabetic rats, which might be the reason for depressive behaviors in diabetic rats. R-SMS significantly upregulated the expression of BDNF, PI3K, and SYN at high doses in the hippocampi, thus improving depression-like behaviors in diabetic rats.

5 | CONCLUSION

In conclusion, diabetic rats exhibited depressive symptoms after 3 weeks of diabetes induction. The expression of BDNF, PI3K, and SYN proteins was also significantly downregulated in the diabetic brains, which might be the reason for depressive symptoms. However, our formulation significantly mitigates depressive symptoms in diabetic rats, as evidenced by enhanced mobility in the OFT, NIH, and FST. R-SMS also stimulates neuronal plasticity at the nexus of BDNF, PI3K, and SYN protein expression, thus reverting depression in diabetic rats.

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AUTHOR CONTRIBUTIONS

Ayaz Ahmed and Guirong Zeng performed the experiments, finalized, analyzed results and wrote manuscript; Mudassar Azhar helped to perform the experiment; Fengzhong Wang, Jingru Wang, and Bei Fan helped to finalize the manuscript; Xinmin Liu, Dejiang Jiang, and Qiong Wang helped for conceptualization of study, result analysis and manuscript finalization.

CONFLICT OF INTEREST STATEMENT

All authors read the manuscript and declared no conflict of Interest.

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