

Original Research Article

Protective effect of vanillin in streptozotocin-induced diabetes in neonatal rats via attenuation of oxidative stress and inflammation

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Abstract

Purpose: To evaluate the antidiabetic activity of vanillin in streptozotocin (STZ)-induced diabetic rats.

Methods: Diabetes was induced in 2-day old male pups by intraperitoneal (i.p.) administration of STZ (90 mg/kg). The pups were then randomly assigned to four groups: control group which received citrate buffer only in place of STZ; negative control group, i.e., diabetic group; and vanillin-treated groups which received vanillin (100 or 200 mg/kg, p.o.) continuously from the 6th week of age to the 10th week. The antidiabetic effect of vanillin was determined by measuring the serum levels of insulin, triglycerides and glucose in the diabetic rats. Oral glucose tolerance, kidney and liver function tests were also performed at the end of the protocol. Moreover, the oxidative stress and inflammatory cytokines in liver tissues, and histopathological changes in pancreatic tissues were assessed.

Results: Vanillin treatment significantly decreased serum glucose and triglyceride levels and increased the level of insulin, when compared to the negative control group. There was higher insulin sensitivity in the vanillin-treated group than in the negative control group. In addition, vanillin improved liver and renal functions in STZ-induced diabetic neonatal rats. Hepatic oxidative stress and inflammatory mediators, as well as histopathological changes in pancreas were attenuated by vanillin treatment.

Conclusion: These results reveal that vanillin attenuates hyperglycemia in STZ-induced neonatal diabetic rat model by decreasing oxidative stress and inflammatory cytokines. There, further studies are required to develop the anti-diabetic potentials of vanillin for clinical applications.

Keywords: Vanillin, Streptozotocin, Diabetes, Oxidative stress, Insulin, Neonatal

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INTRODUCTION

Diabetes is one of the most serious metabolic disorders and a leading cause of mortality worldwide. Elevated fasting blood glucose, a characteristic feature of diabetes, occurs due to

insulin resistance or insulin deficiency. The two main types of diabetes are diabetes mellitus type I (DM-I) and type II (DM-II). A study conducted in China in 2008 revealed that about 92.4 million people were diabetic [1].

Several hypoglycemic drugs are used in combination to control blood glucose, but they have several limitations. In the last few decades, studies have shown that alternative medicine possesses promising potential in the management of chronic disorders. Streptozotocin (STZ) is a diabetogenic agent that exerts toxic effects on the β - cells of the pancreas [2]. It was first used to produce acute hyperglycemia in neonatal rats, and it was observed that later in adulthood, the rats suffered from hyperglycemia resembling the features of type 2 diabetes [3]. Thus, the STZ model is ideal for the screening of antidiabetic drugs that enhance the regeneration of pancreatic β - cells.

Vanillin is often used in the industrial preparation of drugs, cosmetics, beverages and foods. It has been reported to exhibit many therapeutic effects such as anti-angiogenic and anti-mutagenic effects [4, 5]. Studies have shown that vanillin enhances the expressions of anti-inflammatory cytokines and decreases the expressions of pro-inflammatory cytokines [6]. It has strong antioxidant, antimicrobial, anticonvulsant and antidepressant activities [7-10]. The present study was carried out to evaluate the antidiabetic effects of vanillin in STZ-induced diabetes in neonatal rats.

EXPERIMENTAL

Animals

Sprague-Dawley rats weighing 150 - 180 g were used for this study. They were used for breeding so as to produce the pups required for the study. The rats were housed under controlled conditions as per the guidelines. The experiments were approved by the Animal Ethical Committee of Huazhong University of Science & Technology, China (approval no. HUST/IAEC/2016/11). The study followed the guidelines of the Association for the Assessment and Accreditation of Laboratory Animal Care International (AAALAC) for experimentation and animal use [11].

Diabetes was induced in 2-day old male pups by i.p. administration of STZ (90 mg/kg, i.p.). Leakage of injected material was prevented by carefully ensuring that the site of injection was under the inguinal fat of the neonates. The pups were weaned at 4 weeks old and selected for screening through oral glucose tolerance test.

The animals were divided into four groups: control group which received citrate buffer only in place of STZ; negative control group i.e. diabetic group; and 2 vanillin groups which were given

vanillin at doses of 100 and 200 mg/kg p.o. for 4 weeks as from the 6th week of age to the 10th week.

Assessment of water consumption and body weight

Water intake was monitored throughout the treatment period i.e. 4 weeks and the rats were weighed daily. The mean body weight and mean water intake of STZ-induced neonatal diabetic rats were recorded.

Evaluation of blood levels of insulin, triglycerides and glucose

The rats were anesthetized and blood was withdrawn from the retro-orbital plexus into non-anticoagulant sample vials and allowed to clot. The serum obtained was used for insulin estimation by radioimmunoassay using a double-antibody radioimmunoassay kit. Glucose and triglycerides were estimated colorimetrically using auto analyzer.

Oral glucose tolerance test

Glucose (2.5 g/kg) was given orally to overnight fasted rats at the end of treatment protocol. Serum levels of insulin and glucose were estimated at 0, 0.5, 1, 1.5 and 2 h after the administration of glucose. Level of insulin and glucose in the serum at 0 and 2 h were used to calculate the ISI value, which is an index used to determine insulin sensitivity.

Assessment of kidney and liver functions

Kidney and liver function tests were carried out in the blood and urine. At the end of the treatment period, blood was withdrawn from the retro-orbital plexus and allowed to clot. Serum aspartate aminotransferase, alanine aminotransferase and creatinine were estimated using assay kits as per the kit instructions. However, 24-h urine sample collected in metabolic cages were subjected to assays for levels of albumin, creatinine and creatinine and albumin clearance.

Determination of oxidative stress parameters

The rats were sacrificed by cervical dislocation at the end of the treatment protocol, and liver tissues were excised. The liver tissues were washed with saline and homogenized in Tris-HCl buffer, pH 7.4. Tissue debris was removed by centrifuging the homogenate at 5000 rpm. The supernatant was used for the estimation of MDA and GSH levels, and the activities of SOD and

CAT using appropriate assay kits as per the manufacturers' instructions.

Assessment of inflammatory mediators

The inflammatory mediators (TNF- α , MCP-1, IL-6 and IL1 β) were assayed in the liver tissue homogenate using Bio-plex Pro-magnetic bead-based Luminex kit as per the instruction of manufacturer. Internally, the dyed magnetic beads bound with specific anti-cytokine primary antibodies were reacted with the tissue homogenate. Then, biotinylated anti-cytokine secondary antibodies were bound to the specific cytokine-bound antibodies. Phycoerythrin-conjugated streptavidin was added to the sample and analysis was done with Bio-Plex Manager software version 6.1.

Histopathological studies

Pancreas tissues were rinsed in saline and dehydrated in increasing alcohol concentrations prior to blocking in paraffin. Tissue slices of 5- μ m thickness were cut from the paraffin blocks using a microtome, and slices were stained with hematoxylin and eosin. Changes in the histology of pancreatic tissue were observed under a trinocular microscope.

Statistical analysis

Data are presented as mean \pm SD ($n = 8$) and were statistically analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test with the aid of SPSS software. Statistical significance was fixed at $p < 0.05$.

RESULTS

Effect of vanillin on water consumption and body weight of STZ-induced diabetic neonatal rats

The effect of vanillin on water consumption and body weight of STZ-induced diabetic neonatal rats is presented in Figure 1. It was observed that mean body weight of the rats was significantly decreased, when compared to control group. However, treatment with vanillin significantly reversed the diabetes-associated weight loss, relative to negative control group on 10th week of age. There was significant increase in the water intake index in negative control group, when compared to control group. However, water intake index was significantly reduced ($p < 0.01$) in the vanillin-treated group of rats, relative to the negative control group.

Effect of vanillin on serum levels of glucose, triglycerides and insulin

Effects of vanillin on serum glucose, triglycerides, insulin and insulin sensitivity index of STZ-induced diabetic neonatal rats are shown in Figure 2. There were significant increases in serum glucose and triglycerides on the 6th week of age, when compared to control group. However, vanillin exposure significantly decreased the serum glucose and triglycerides after the 10th week of age, when compared to the negative control group ($p < 0.01$). Blood insulin was significantly reduced in the STZ-induced diabetic rats after the 6th week of age. However, after four weeks of treatment with vanillin, blood insulin was significantly increased, relative to the negative control group. Insulin sensitivity indices

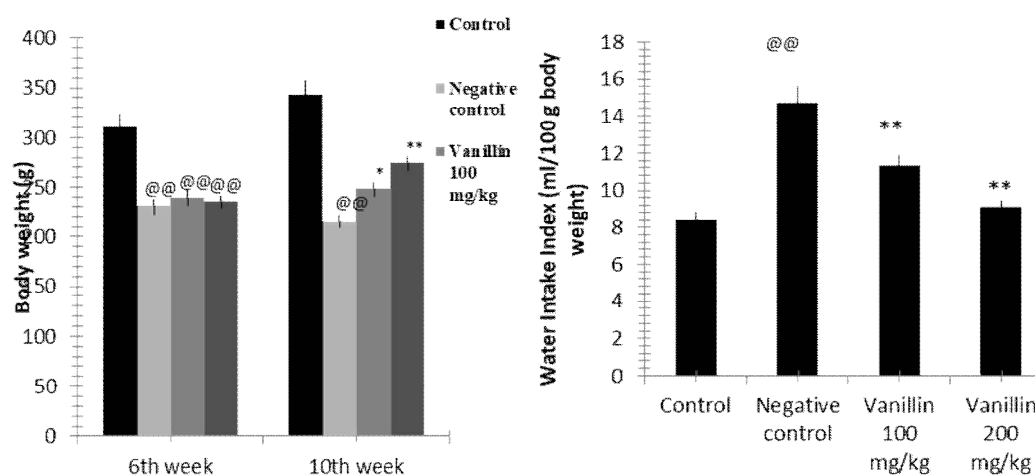


Figure 1: Effect of vanillin on water consumption and body weight of STZ-induced diabetic neonatal rats. Values are mean \pm SD ($n = 8$). @@ $p < 0.01$, compared to control group; * $p < 0.05$, ** $p < 0.01$, compared to negative control group

were used to estimate the sensitivity of insulin. It was observed that treatment with vanillin significantly reversed the altered level of insulin sensitive index, when compared to negative control group.

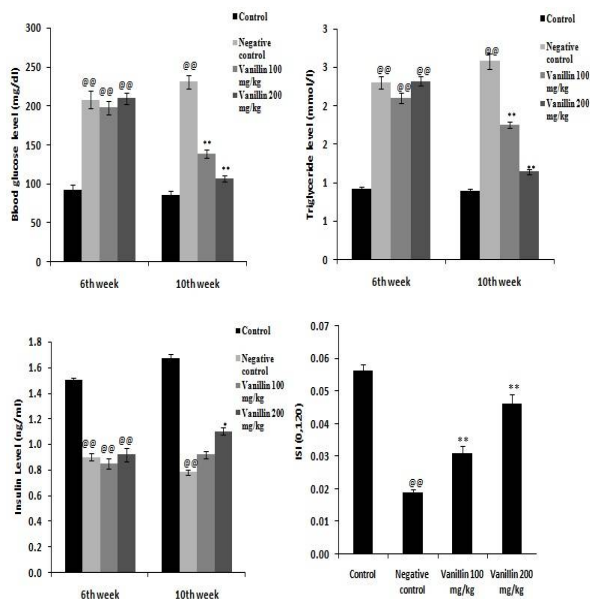


Figure 2: Effect of vanillin on serum levels of glucose, triglycerides, insulin, and insulin sensitivity indices of STZ-induced diabetic neonatal rats. Values shown as mean \pm SD (n = 8). @@ p < 0.01, compared to control group; * p < 0.05, ** p < 0.01, relative to negative control group

Effect of vanillin on oral glucose tolerance test

The effect of vanillin on oral glucose tolerance test in STZ-induced diabetic neonatal rats is shown in Figure 3. It was observed that the AUC increased 2 to 3 folds, reflecting impaired glucose tolerance in the negative control group. However, treatment with vanillin significantly decreased the AUC, relative to the negative control group. Thus, glucose tolerance was enhanced by vanillin in STZ-induced diabetic rats.

Effect of vanillin on kidney and liver functions

Effect of vanillin on kidney and liver functions in STZ-induced neonatal diabetic rats is shown in Figure 4. It was observed that ALT, AST and creatinine levels were significantly increased in the blood of STZ-induced neonatal diabetic rats, when compared to the control group (p < 0.01). However, there were significant decreases in the levels of ALT, AST and creatinine in the vanillin-treated group, when compared to the negative control group (p < 0.01). Urine and creatinine clearance were significantly enhanced in the

negative control group. However, vanillin treatment significantly decreased creatinine and urea clearance, relative to the negative control group.

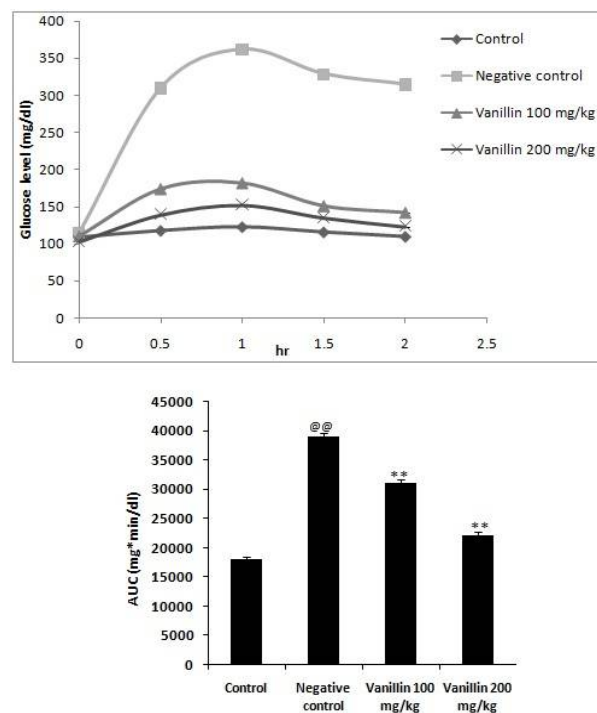


Figure 3: Effect of vanillin on oral glucose tolerance test in STZ-induced neonatal diabetic rats. Results are mean \pm SD (n = 8). @@ p < 0.01, compared to control group; * p < 0.05, ** p < 0.01, compared to negative control group

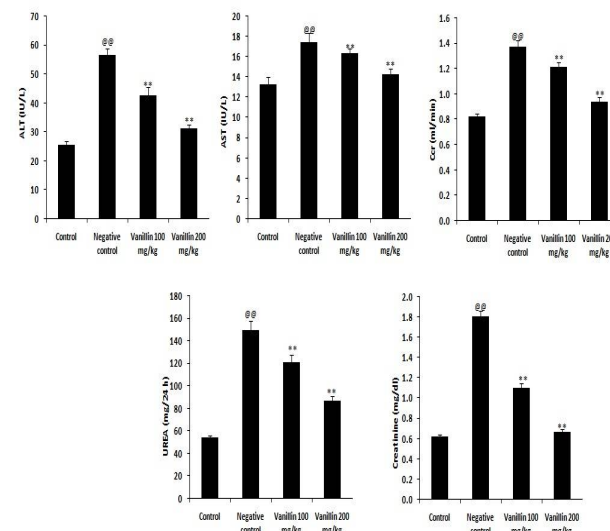


Figure 4: Effect of vanillin on the kidney and liver functions in STZ-induced diabetic neonatal rats. Results are mean \pm SD (n = 8). @@ p < 0.01, compared to control group; * p < 0.05, ** p < 0.01, compared to negative control group

Effect of vanillin on oxidative stress parameters

Table 1 shows the effect of vanillin on the levels of LPO and GSH, and its effect on the activities of SOD and CAT in STZ-induced diabetic rats. There were significant reductions in the activities of SOD and CAT in the negative control group, when compared to control group. Lipid peroxidation (LPO) was enhanced and GSH significantly reduced in the negative control group, when compared to control group. However, treatment with vanillin reversed the

changes in LPO and GSH, and activities of SOD and CAT in the STZ-induced diabetic rats.

Effect of vanillin on inflammatory cytokines

Effect of vanillin on inflammatory cytokines in STZ-induced diabetic neonatal rats is shown in Figure 5. There was significant increase in the levels of inflammatory cytokines IL-1 β , IL-6, TNF- α and MCP-1 in the liver homogenate of the negative control group, when compared to control group. However, treatment with vanillin attenuated the altered levels of inflammatory

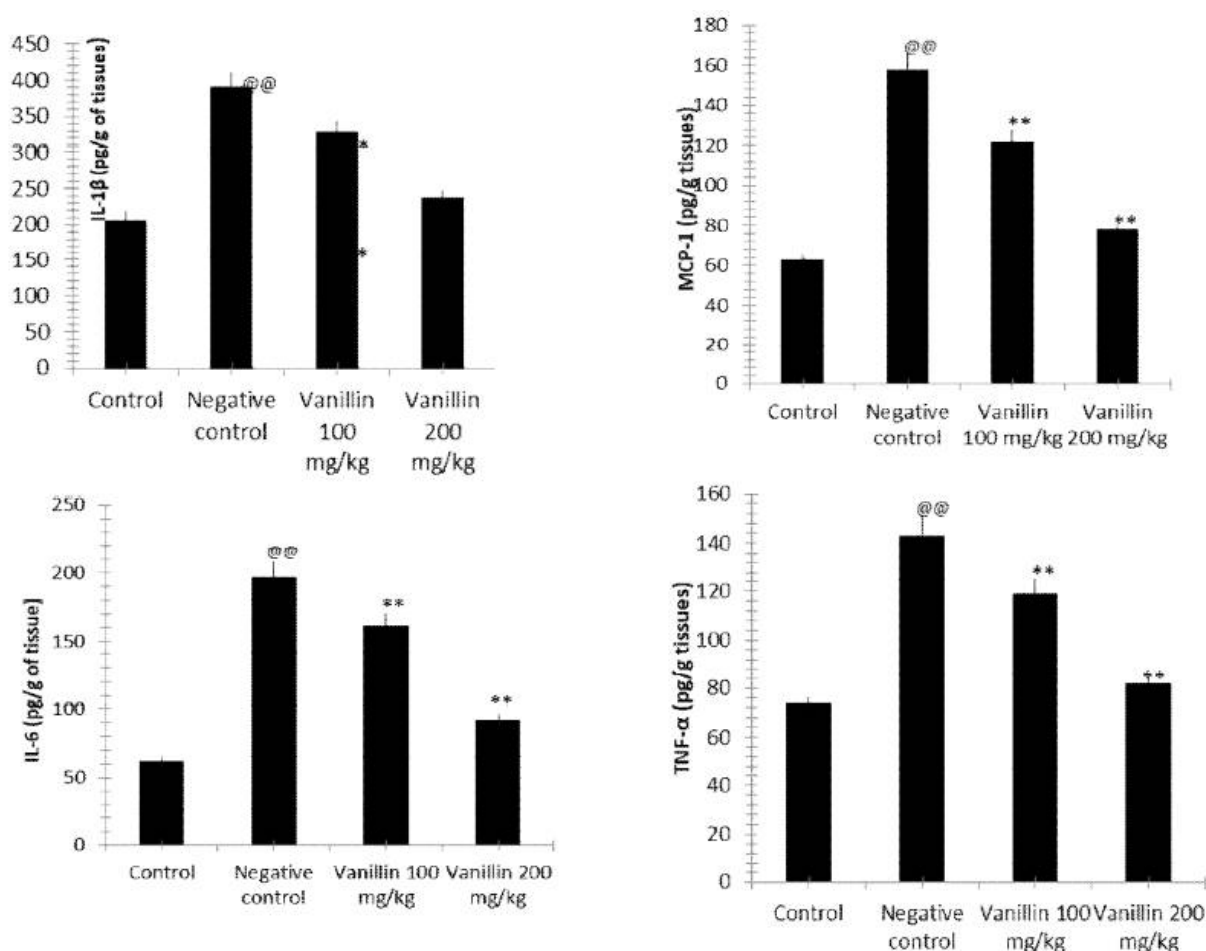


Figure 5: Effect of vanillin on inflammatory cytokines in STZ-induced diabetic neonatal rats. Values are presented as mean \pm SD (n = 8). @@ $p < 0.01$, compared to control group; * $p < 0.05$, ** $p < 0.01$, compared to the negative control group

Table 1: Effect of vanillin on the level of LPO and GSH and activities of SOD and CAT in STZ-induced diabetic neonatal rats (mean \pm SD, n = 8)

Group	SOD (mg/protein)	LPO (nmol MDA/mg protein)	CAT (mg/protein)	GSH (mg/100 g of tissue)
Control	21.2 \pm 1.1	4.2 \pm 0.23	142.4 \pm 12.3	31.62 \pm 1.38
Negative control	2.3 \pm 0.25 ^{@@}	17.3 \pm 1.2 ^{@@}	11.8 \pm 0.7 ^{@@}	8.19 \pm 0.32
Vanillin 100 mg/kg	10.5 \pm 1.1 ^{**}	10.8 \pm 0.82 ^{**}	62.1 \pm 2.93 ^{**}	19.6 \pm 0.96
Vanillin 200 mg/kg	18.1 \pm 1.2 ^{**}	6.1 \pm 0.35 ^{**}	128.9 \pm 10.6 ^{**}	28.47 \pm 1.21

^{@@} $P < 0.01$, compared to control group; * $p < 0.05$, ** $p < 0.01$, compared to negative control group

cytokines in the liver homogenate of the STZ-induced diabetic rats.

Effect of vanillin on histopathological features of the pancreas

The effect of vanillin on the histopathology of pancreas in STZ-induced diabetic neonatal rats is shown in Figure 6. In the control group, the cytoplasm contained several elongated and round islets, and lightly-stained nuclei relative to the surrounding acinar cells. Histopathology of pancreas of the negative control group showed infiltration of lymphocytes, and shrunken and damaged islets. However, the vanillin treatment reduced the changes in size and degree of damage in the islets.

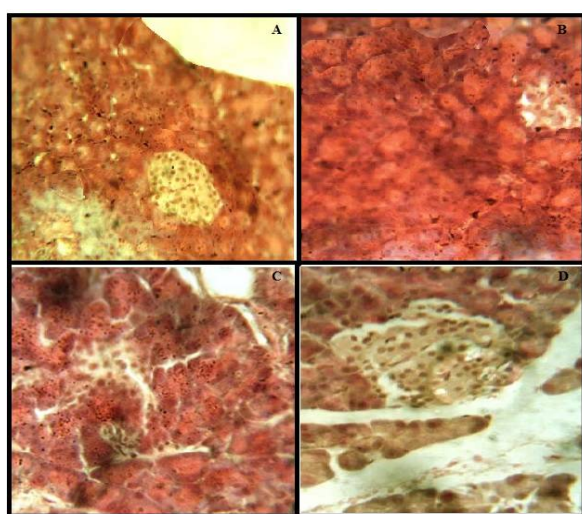


Figure 6: Effect of vanillin on the histopathology of pancreas in STZ-induced diabetic neonatal rats. A: Control, B: Negative control, C: Vanillin 100 mg/kg, D: Vanillin 200 mg/kg (H & E; x400)

DISCUSSION

In this study, the antidiabetic effect of vanillin in STZ-induced diabetes in neonatal rats was investigated. Diabetes was induced in the neonatal rats by injecting STZ, and after 6th week of age, vanillin was given orally for the period of 4 weeks. The antidiabetic activity was assessed by estimating serum glucose, insulin and biochemical parameters of kidney and liver functions.

Previously, postprandial hyperglycemia and glucose intolerance were considered as markers of type II diabetes in which the liver enhances the production of glucose that results in increase in fasting blood glucose [11]. In type II diabetes, blood lipid levels are altered due to a correlation between the metabolisms of glucose and lipids [12]. The results obtained showed that the serum

concentrations of triglycerides and glucose were significantly elevated in the diabetic group, relative to the control group. However, chronic administration of vanillin attenuated the altered concentration of glucose and lipids in diabetic rats.

Insulin promotes the metabolism of glucose and thereby reduces blood glucose levels. Thus, the mechanism of anti-diabetic drugs involves either enhancement of insulin sensitivity or promotion of insulin synthesis [13]. It is known that STZ increases blood glucose by destroying the pancreatic β cells [14]. Uncontrolled hyperglycemia results in enhancement of inflammatory reactions and oxidative stress, leading to destruction of peripheral organs due to glucose toxicity [15]. This decreases the sensitivity of peripheral organs to insulin, thereby exacerbating diabetes. The data obtained in the present study reveal that treatment with vanillin enhances the sensitivity of insulin in diabetic rats. This increase in the sensitivity of insulin occurs by attenuating oxidative stress in STZ-induced diabetic neonatal rats. These findings are consistent with previously published data [16]. Streptozotocin (STZ)-induced destruction of the β cells of the pancreatic tissue causes alteration in the level of insulin which results in diabetes. The histopathology of pancreatic tissue revealed that treatment with vanillin significantly attenuated the STZ-induced pancreatic β cell injury.

CONCLUSION

The results of the present study demonstrate that vanillin exerts anti-hyperglycemic activity in STZ-induced diabetes in neonatal rats by decreasing oxidative stress and enhancing insulin sensitivity. Thus, vanillin has a potential for clinical application in the management of diabetes.

DECLARATIONS

Acknowledgement

This study was supported by West China Second University Hospital, Sichuan University.

Conflict of interest

No conflict interest is associated with this study

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. All the

experiments are performed by Guoyan Lu and Xingling Luo. Zhongqiang Liu and Lin Yang collected the materials. Chao Lin did statistical analysis. Min Xu designed the whole study and supervised the other authors.

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