Original article

Effect of sakuranin on carbohydrate-metabolizing enzyme activity modifications in streptozotocin-nicotinamide-induced diabetic wistar rats

Mohamed Farouk Elsadek *, Badreldin Mohamed Ahmed

Community Health Sciences Department, College of Applied Medical Sciences, King Saud University, Saudi Arabia

ABSTRACT

This study is to assess the glucose lowering activity of sakuranin in diabetes induced rats by streptozotocin (STZ) and nicotinamide (NA). Diabetic rats were treated sakuranin for 45 days (20, 40, 80 mg/kg) by orally. Sakuranin (80 mg/kg body weight) was normalized the changes of abnormal blood glucose plasma glucose and plasma insulin levels. Hence, we have continued the further research with this active dose of 80 mg/kg sakuranin. The plasma glucose and glycosylated hemoglobin (HbA$_1$c) reduced and insulin, glycogen and hemoglobin levels increased by Sakuranin administration in diabetic rats. Additionally, hexokinase and glucose-6-phophate dehydrogenase activities increased and glucose-6-phosphatase and fructose-1,6-bisphosphatase activities decreased in diabetic condition while administration of treated compound. In this observed result signified that sakuranin may have potential role of diabetic condition rats by evidenced with reducing glucose and increasing insulin and also protect the carbohydrate metabolic changes.

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1. Introduction

Diabetes is huge accruing disorders in developed and developing countries that can be characterized by increased in blood and urine sugar. Hyperglycemia is having in prolonged uncontrolled conditions that can be affect in normal body metabolism finally results in diabetic complications of morbidity and mortality (Urzúa et al., 2012). Overall, 285 million people affected by diabetes in 2010 and it will be increased about 439 million in 2030 (Chang et al., 2013). A condition of diabetes is defect in insulin utilization and increases in blood glucose. Diabetic patients have found altered metabolic status due to the prolonged alterations of gluconeogenesis (Ahmed et al., 2012). Currently available antidiabetic mostly is having side effects or economically high (Chitra et al., 2010). Therefore, all new researchers are continues finding of antihyperglycemic agents from natural sources such as plants and their products because they might not have side effect and less cost (Gandhi et al., 2012; Pari and Srinivasan, 2010).

Type 2 diabetes (T2DM) is an increasingly frequent condition that develops in people throughout their lifetime, ages of onset ranging from childhood to old age. Worldwide, T2DM is rising as an epidemic and is connected with numerous significant consequences, resulting in a shorter lifespan and decreased quality of life. Both type 2 diabetes (T2DM) and many life-threatening disorders, including gestational diabetes mellitus (GDM), heart disease, hypertension, and certain cancers, are all intricately linked to obesity. Obesity and T2DM both begin due to having metabolic abnormalities that originate in families with comparable criteria (Khan et al., 2015;9:Gc01-5.; Khan et al., 2014). The pancreas produces two toxins, STZ and Alloxan, which can be utilized to produce type 1 and T2DM in animal models. Alloxan was found to be inferior to STZ for causing diabetes. Wistar rats that had become diabetic by means of the STZ-NA model were used. Researchers administered these dosages of STZ (60 mg/kg) and NA (110 mg/kg) to induce T2DM in this investigation (Sayeli and Shenoy, 2021).

Flavonoids are a natural chemical that can be hugely distributed in foods and foods ingredients such as vegetables, fruits, tea and wine. The citrus fruit is having several chronic diseases curable properties because of major source found in flavonoids (Chen

* Corresponding author.
E-mail addresses: mfbadr@ksu.edu.sa (M.F. Elsadek), bmohamed@ksu.edu.sa (B.M. Ahmed).
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et al., 20022002). Sakuranin is a flavanone that can be found in *Daphne giraldii Nitsche* (Thymelaeaceae). This plant has been used in several illness including joint pain and rheumatoid arthritis traditionally at China (Zhang et al., 2014; Jiang et al., 2014).

2. Materials and methods

2.1. Chemicals

All of the chemicals and biochemical kits used in this experiment were of analytical grade and obtained from Sigma Chemicals Coin the United States (see Fig. 1).

2.2. Animal diabetes stimulation procedure

Streptozotocin and nicotinamide were separately dissolved in citrate buffer (pH 4.5) and saline. Diabetes was produced in mice using the previously described methodology Masiello et al., (Masiello et al., 1998) by injecting 45 mg/kg STZ (i.e.) followed by 110 mg/kg nicotinamide after 15 min. Diabetes was confirmed 72 h later by testing plasma glucose levels. For this investigation, animals with plasma glucose levels more than 250 mg/dl were chosen.

2.3. Animals

Sixty (n = 10) healthy adult male albino rats weighing around 180 ± 10gwere obtained from Laboratory of Animal Colony, Helwan, Egypt. The experimental rats were kept in stainless cages; prior to the trial, the rats were fed the basal food for 7 days to allow for acclimatization in a precisely regulated environment. The experiment methodology was carried out in compliance with European Community Directive2010/63/EU. The animal care practices adhered to the National Institutes of Health (NIH) and Helwan University requirements.

2.4. Experimental scheme

Total used animals of this experiment for 36 and they were alienated into 6 sets each of having 6 animals. More details about this experimental scheme are given as follow.
Set 1: consists of normal rats
Set 2: consists of normal rats plus sakuranin (80 mg/kg) of weight of the body.
Set 3: Hyperglycemia (Diabetes) control animals
Diabetic plus sakuranin (20 mg/kg body weight) group IV
Diabetic plus sakuranin (40 mg/kg body weight) group V
Diabetic plus sakuranin (80 mg/kg body weight) group VI
After the 45-day treatment period was completed, the animals were sacrificed by cervical decapitation under fasted overnight
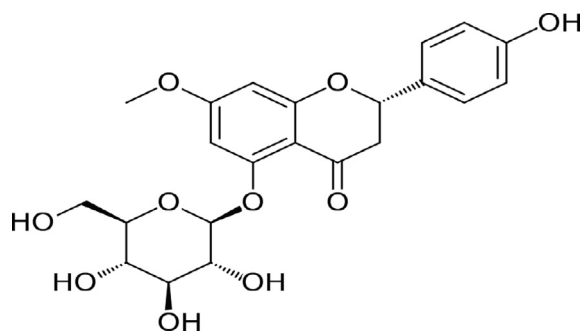

Fig. 1. Structure of sakuranin.

conditions. Blood and required organs were obtained and used to estimate various biochemical and pathological investigations.

2.5. Methods for biochemical estimation assays

2.5.1. Plasma glucose, insulin, hemoglobin, and glycosylated hemoglobin
The glucose ranges were determined using the Trinder technique of a commercial diagnostic kit. The insulin level was assessed using the ELISA technique. The diagnostic kit and the BiSSE method were used to determine hemoglobin and glycosylated hemoglobin.

2.5.2. Enzymes that metabolize carbohydrates and glycogen
The total protein in the utilized tissue was determined using the Lowry et al. (Lowry et al., 1951) technique. The method of Brandstrup et al. (Brandstrup et al., 1957) was used to assess hepatic hexokinase activity. The Ellis and Kirkman (Ells and Kirkman, 1961) approach were used to measure the activity of G6PD. Gancedo et al (Gancedo and Gancedo, 1971) experiment established the activity of fructose-1,6-bisphosphatase technique. G6PD activity was measured using the Koide and Oda et al (Hikaru and Toshitsugu, 1959) technique. The tissue glycogen content was determined using the Morales et al (Morales et al., 1973) technique.

2.6. Statistical analysis

The statistical analysis was performed with SPSS software (version 13.0). This study analysis was represented as mean ± SD for the rats group involved in this study. One-way analysis of variance was performed in this study and then followed with Duncan's multiple range test (DMRT). In this study, the p value which was found to be less than 0.05 is considered as statistical association (Khan et al., 2019).

3. Results

3.1. Glucose and insulin levels in blood

Fig. 2 depicts the estimated circulation glucose and insulin levels. In diabetic conditions, circulating glucose and insulin levels were elevated, but these were dramatically reduced after injection of sakuranin (all doses) in a dose-dependent manner. When compared to others, 80 mg/kg dose produced the best lowering results; thus, this active efficient dose was maintained for the duration of the investigation.

3.2. Body weight

The estimated body weight result is shown in Fig. 3. Body weight results shown a decreased in untreated diabetic states and this was improved upon treatment with sakuranin.

3.3. Hemoglobin and glycosylated hemoglobin

Table 1 displays the hemoglobin and glycosylated hemoglobin levels, together with the urine sugar levels. When rats have diabetes, hemoglobin levels are decreased, and glycosylated hemoglobin and urine sugar levels are increased. This is reversed when sakuranin is injected.
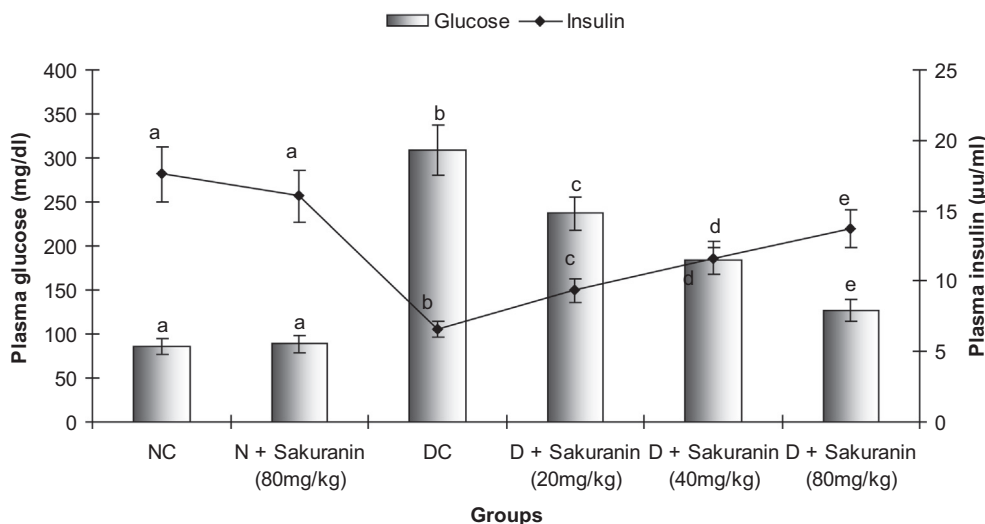
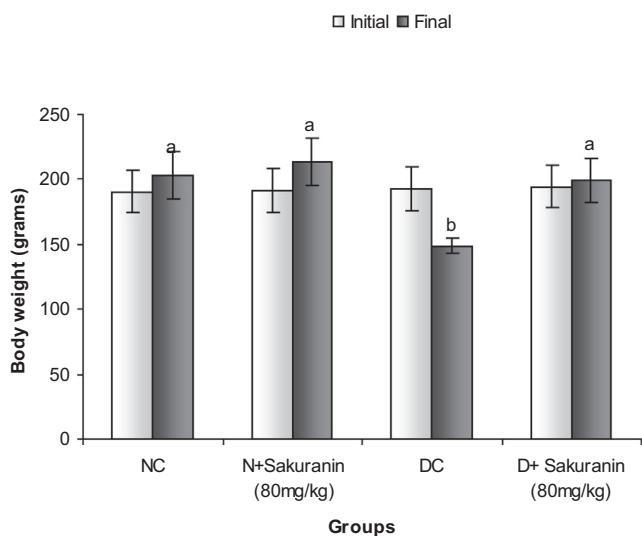**Fig. 2.** Normal and experimental rats plasma glucose and insulin levels changed in the experiments.

Fig. 3. Body weight changes in normal and experimental rats.

Table 1
Shows the effect of sakuranin on hemoglobin, Hb1AC, and urine sugar levels in normal and experimental rats.

Groups	Total hemoglobin	Hb1Ac	Urine sugar
Normal control	11.89 ± 0.81[a]	0.23 ± 0.05[a]	Nil
Normal + sakuranin (80 mg/kg)	12.05 ± 0.97[a]	0.22 ± 0.04[a]	Ni
Diabetic control	6.53 ± 0.58[b]	0.76 ± 0.07[b]	+++
Diabetic + sakuranin (80 mg/kg)	10.61 ± 0.74[c]	0.39 ± 0.04[c]	Nil

3.4. Glycogen stores in the liver and muscles

Table 2 displays the glycogen content of muscle and liver. Untreated diabetic rats had lower muscular and hepatic glycogen levels, which was restored to normal with sakuranin administration.

3.5. Hepatic gluconeogenic and glycolytic enzymes

Hexokinase and glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1,6-bisphosphatase changes of live are

Table 2
Shows the effect of sakuranin on liver and muscle glycogen levels in normal and experimental rats.

Groups	Glycogen (mg/g tissue)	
	Liver	Muscle
Normal control	33.52 ± 2.41[a]	6.83 ± 0.64[a]
Normal + sakuranin (80 mg/kg)	31.95 ± 2.74[a]	6.89 ± 0.677[a]
Diabetic control	19.66 ± 1.89[b]	3.58 ± 0.32[b]
Diabetic + sakuranin (80 mg/kg)	27.57 ± 2.23[c]	5.76 ± 0.56[c]

shown in Table 3. Decreases activities of hexokinase and glucose-6-phosphate dehydrogenase and increases activities of glucose 6-phosphatase and fructose 1,6-bisphosphatase were shown in diabetic states and these were returns to near normal values by administration of sakuranin.

4. Discussion

Currently, T2DM is a major public health concern worldwide (Khan et al., 2015;9:Gc01-5.), and it is caused mostly by a malfunction in insulin action or production from pancreatic β-cells (Ahmed et al., 2012). STZ depleted pancreatic -cells and impaired insulin release or action. The basic function of diabetes conditions is a phenomenal generation of glucose from tissues and a decrease in tissue glucose exploitation (Shirwaikar et al., 2006) and a decrease in insulin action (Shulman, 2000).

Sakuranin dramatically lowered circulation glucose levels in diabetic patients, and this was reflected in urine sugar levels. Flavonoids are recognized to be bioactive anti-diabetic principles that stimulate insulin production in pancreatic -cells and provide resistance to insulin's ability to increase glucose absorption and disposal in tissues (Hii and Howell, 1985). Sakuranin decreased plasma glucose concentrations while increasing insulin levels in diabetics. Sakuranin hypoglycemic effect mechanism could be due to beta cells initiating pancreatic insulin. Body weight loss in diabetics has been seen as a result of protein and fat loss or breakdown. Insulin insufficiency reduces protein content in muscle tissue (Subash Babu et al., 2007). Muscle and protein slaying occur in diabetics due to a lack of glucose for use as an energy source (Musabayane et al., 2005). Sakuranin increased weight reduction in comparison to normal control rats.

Table 3
Effect of sakuranin on the activities of hepatic hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, fructose-1, 6- bisphosphatase in normal control and diabetic rats.

Groups	Normal Control	Normal + sakuranin (80 mg/kg)	Diabetic control	Diabetic + sakuranin (80 mg/kg)
Glycolytic enzyme				
Hexokinase (μmol of glucose phosphorylated/min/g protein)	148.51 ± 12.09[a]	152.74 ± 12.63[a]	112.82 ± 8.93[b]	133.16 ± 11.58[c]
Lipogenic enzyme				
Glucose-6-phosphate dehydrogenase ($\times 10^{-4}$mlU/mg protein)	5.03 ± 0.31[a]	5.18 ± 0.33[a]	3.14 ± 0.19[b]	4.24 ± 0.26[c]
Gluconeogenic enzymes				
Glucose-6-phosphatase (μmol of Pi liberated/min/mg protein)	0.168 ± 0.021[a]	0.164 ± 0.022[a]	0.273 ± 0.032[b]	0.215 ± 0.024[c]
Fructose-1, 6-bisphosphatase (μmol of Pi liberated/h/mg protein)	0.331 ± 0.026[a]	0.345 ± 0.027[a]	0.593 ± 0.041[b]	0.482 ± 0.028[c]

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

Glycosylated hemoglobin was increased due to direct relationship with hyperglycemia. Circulating sugar is reacted with hemoglobin results the formation of glycosylated hemoglobin in diabetic condition (Ahmed and Urooj, 2009). Sakuranin was decreased the glycosylated hemoglobin in diabetic states in this manner rising the hemoglobin level. This designates the competence of sakuranin in glycemic control. Glycogen is the main form of glucose storage in intra cells. The liver glycogen is ideal marker for assess the antihyperglycemic activity of drugs (Grover et al., 2000).

Diabetes has been linked to decreased glucose synthesis in the skeletal muscles and liver, as well as a decrease in glycogen content (Hwang et al., 1996; Welihinda and Karunanayake, 1986). STZ can raise glucose levels while decreasing insulin and glycogen levels in tissues (liver and muscle) (Weber et al., 1966; Vats et al., 2004). Sakuranin may have averted glycogen exhaustion by stimulating insulin action. The liver is an important organ, particularly for glucose and lipid metabolism, which is impacted in diabetic patients in an insulin-dependent manner. During long-term diabetes conditions, essential enzymes involved in carbohydrate metabolism are significantly altered. Hexokinase levels are much lower in diabetes patients, owing to decreased glucose consumption in the system and higher blood sugar levels. Because of insulin shortage, hexokinase and glucose 6-phosphate dehydrogenase levels were lower in diabetics. Sakuranin restored these enzymes to normal function by amplifying insulin emission or activity, which increases glucose utilization in tissues.

Glucose-6-phosphatase and fructose-1,6-bisphosphatase are glucose homeostasis enzymes that play a critical role in gluconeogenesis and are found mostly in the liver and kidney (Nordlie et al., 1999). Insulin inhibits the activity of gluconeogenic enzymes. As a result, the increase in gluconeogenic enzymes could be attributed to insulin insufficiency (Pari and Murugan, 2005).

The gluconeogenic main enzymes in hepatic tissue are tightly connected with gluconeogenesis (van de Werve et al., 2000). Due to deregulation of these liver enzyme activities, G6P and F1,6 BP reduced hepatic glucose consumption and increased hepatic glucose generation during diabetes mellitus. Sakuranin reduced the activity of G6P and F 1,6 BP in diabetics due to decreased endogenous glucose production. Sakuranin modifies and regulates endogenous glucose production in diabetic rats, suggesting that it may play an important role in maintaining glucose homeostasis and defining the role of insulin in the gluconeogenic flux.

5. Conclusion

The administration of sakuranin has a favorable anti-diabetic effect in the presence of STZ-induced diabetes. Thus, the effects could be related to increased glucose consumption and decreased hepatic glucose synthesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgement

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