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Brief Communication

Therapeutic effects of balanitoside in streptozotocin-induced diabetic rats

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Abstract

Objectives: The objective of this study was to evaluate the therapeutic effects of balanitoside in diabetic rats.

Methods: Twenty-five rats were divided into five groups. Rats in groups 2 to 5 were treated with streptozotocin to induce hyperglycemia. In addition, rats in groups 1 and 2 received 1 mL of distilled water, whereas those in groups 3, 4, and 5 received 10 and 20 mg/kg balanitoside and 6 U/kg insulin, respectively, for 14 days. All rats were sacrificed on day 15, blood samples were collected, and serum levels of alkaline phosphatase (ALP), alanine aminotransferase (ALT), and aspartate aminotransferase (AST) were measured. The liver was processed for examination under a light microscope.

Results: The results showed a significant decrease in liver protein concentrations in diabetic control rats, compared to those in the normal control rats and rats treated with 10 mg/kg balanitoside ($p < 0.05$). There was no significant difference in ALP levels among all groups. However, a significant increase in ALT and AST levels was observed in the diabetic control rats, compared to those in the normal control rats ($p < 0.05$). Photomicrographs of the liver of the diabetic control rats showed fat and glycogen droplets, vacuolated nuclei, and loss of cellular boundaries, whereas those of the rats treated with balanitoside or insulin showed a small amount of microvesicular fat droplets and slight infiltration of lymphocytes.

Conclusion: The findings of this study suggest the therapeutic effects of balanitoside in the liver of diabetic rats.

Keywords: Balanitoside; Diabetes mellitus; Hyperglycemia; Liver; Protein concentration

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Introduction

Diabetes mellitus is characterized by hyperglycemia owing to either a decrease in insulin production, insulin resistance, or both.[1] It can be classified into type 1, which results from autoimmune destruction of the pancreatic beta cells, and type 2, which occurs owing to insulin resistance or deficiency.[2] The symptoms of diabetes mellitus include polyuria, polydipsia, and polyphagia. The liver plays an important role in carbohydrate homeostasis, where accumulation of

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glycogen in the liver induces liver enzyme abnormalities in uncontrolled diabetic patients.[2] Non-alcoholic fatty liver disease (NAFLD) is a common complication of diabetes, usually observed in patients with type 2 diabetes and can progress to liver cirrhosis. The prevalence of NAFLD in the general population ranges between 20 and 30%; however, its prevalence is approximately 75% in diabetic patients. In addition, the mortality rate of liver cirrhosis in diabetic patients is more than twice of that in the general population.[3] Three different hypotheses can explain the relationship between diabetes and liver disease: liver disease might induce diabetes, diabetes might contribute to or induce liver disease, or the risk factors for liver disease and diabetes are similar.[4]

Balanitoside is the active pharmacological ingredient of *Balanites aegyptiaca*. It is a saponin that belongs to a family of structurally related compounds of steroids or triterpenoid aglycones linked to one or more oligosaccharide moieties by glycoside linkages; the carbohydrate moiety consists of pentoses, hexoses, or uronic acids. The presence of both polar (sugar) and nonpolar steroid groups provides saponins with strong surface active properties, which distinguish them from other glycosides.[5] Saponins exhibit various pharmacological activities, including expectorant, anti-inflammatory, vasoprotective, antioxidant, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal, antifungal, and antiparasitic activities.[6] In this study, we aimed to investigate the therapeutic effects of balanitoside in diabetic rats.

Materials and Methods

Balanitoside was isolated from *B. aegyptiaca* fruits, as previously described.[7] Twenty-five Wistar rats (150−200 g) were obtained from the animal house, Department of Pharmacology and Therapeutics, ABU, Zaria, Nigeria. They were fed standard food pellets (Vital Feed, Nigeria) and provided water *ad libitum*. They were allowed to acclimatize to the conditions in the animal house for 2 weeks.

Hyperglycemia was induced using streptozotocin, which was freshly prepared in 0.1 M citrate buffer (pH 4.5), as previously described.[8] Rats were divided into five groups ($n = 5$ each). Rats in groups 1 and 2 served as positive and negative control groups, respectively, and received 1 mL of distilled water for 14 days. Rats in groups 3, 4, and 5 intraperitoneally received 10 and 20 mg/kg balanitoside and 6 U/kg insulin, respectively, for 14 days. All rats were killed on day 15, and blood samples were collected in plain bottles via cardiac puncture. The liver was harvested, processed, stained with hematoxylin & eosin (H & E) and periodic acid-Schiff (PAS), and examined under a light microscope.

The blood samples were centrifuged at 2500 $\times g$ for 5 min, and the serum was collected. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were determined using enzyme-linked immunosorbent assay (ELISA) kits.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) software, version 20 (IBM, Armonk, New York, USA). Data are expressed as the means ± standard deviations (SD). One-way analysis of variance (ANOVA) was used to compare the differences among groups (turkey test). $p < 0.05$ was considered statistically significant.

Results

There was no significant difference in ALP levels among all groups ($p = 0.138$). There was a significant increase in the level of ALT between the normal control rats and the diabetic control rats and rats treated with Balanitoside and insulin ($p = 0.001$). There was a significant increase in the level of AST between the normal control rats and the diabetic control rats and rats treated with Balanitoside and insulin ($p = 0.023$). Liver protein concentrations in diabetic control rats was significantly lower than those in normal control rats and rats treated with 10 mg/kg balanitoside ($p = 0.01$). There was no significant difference in protein concentrations between the diabetic control rats and rats treated with 20 mg/kg balanitoside or 6 U/kg insulin (see Table 1).

Photomicrographs of the liver of the normal control rats showed normal hepatocytes, sinusoids, and central vein (Figures 1A and 2A). However, the diabetic control rats showed fatty droplets, vacuolated nuclei, cellular necrosis, glycogen deposits in the central vein and hepatocytes, and hemorrhage (Figures 1B and 2B). Rats treated with 10 mg/kg balanitoside showed normal central vein and hepatocytes with a small amount of microvesicular fat droplets (Figures 1C and 2C). Rats treated with 20 mg/kg balanitoside showed vacuolated nuclei, macrovesicular fat droplets, and infiltration of lymphocytes around the central vein (Figures 1D and 2D), whereas those treated with 6 U/kg insulin showed slight infiltration of lymphocytes within the central vein, degenerated sinusoids, vacuolated hepatocytes, and a small amount of microvesicular fat droplets (Figures 1E and 2E).

This study was limited to measurement of the serum levels of ALP, ALT, and AST, as well as examination of the liver structure using H & E and PAS under a light microscope in diabetic rats.

Discussion

The decrease in liver protein concentrations in the diabetic control rats is in line with the findings of previous

Table 1: Effects of balanitoside on liver enzymes and plasma protein concentrations in streptozotocin-induced diabetic rats.

Parameters	Normal control	Diabetic control	10 mg/kg Balanitoside	20 mg/kg Balanitoside	6 U/kg Insulin	*P*-value
ALP (U/L)	65 ±7[a]	72.2 ± 3.83[a]	68.2 ± 10.42[a]	75.4 ± 6.77[a]	67.2 ± 1.3[a]	0.138
ALT (U/L)	40.44 ± 4.11[a]	47.2 ± 2.38[b]	47.8 ± 3.34[b]	56.2 ± 2.77[c]	48.4 ± 4.27[b]	0.001
AST (U/L)	35.6 ± 1.14[a]	43 ± 3.8[b]	42.4 ± 2.07[ab]	48.2 ± 8.25[b]	44.8 ± 3.7[b]	0.023
PPC (mg/dL)	107.98 ± 33.43[b]	79.22 ± 11.17[a]	105.98 ± 4.56[b]	92.05 ± 5.98[ab]	100.5 ± 8.39[ab]	0.01

Values expressed as Mean ± SD, values in the same row with different superscripts are significantly different at ($p \leq 0.05$). STZ − Streptozotocin, ALP − alkaline phosphatase, ALT − alanine transaminase, AST − aspartate transaminase, PPC − plasma protein concentration.

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