



Taibah University  
**Journal of Taibah University Medical Sciences**

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Brief Communication

## Therapeutic effects of balanitoside in streptozotocin-induced diabetic rats



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Received 6 November 2017; revised 30 December 2017; accepted 5 January 2018; Available online 9 February 2018

### 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.<sup>1</sup> 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.<sup>2</sup> 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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Peer review under responsibility of Taibah University.



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glycogen in the liver induces liver enzyme abnormalities in uncontrolled diabetic patients.<sup>2</sup> 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.<sup>3</sup> 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.<sup>4</sup>

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.<sup>5</sup> Saponins exhibit various pharmacological activities, including expectorant, anti-inflammatory, vaso-protective, antioxidant, hypocholesterolemic, immunomodulatory, hypoglycemic, molluscicidal, antifungal, and antiparasitic activities.<sup>6</sup> 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.<sup>7</sup> 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.<sup>8</sup> 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  $\pm$  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.

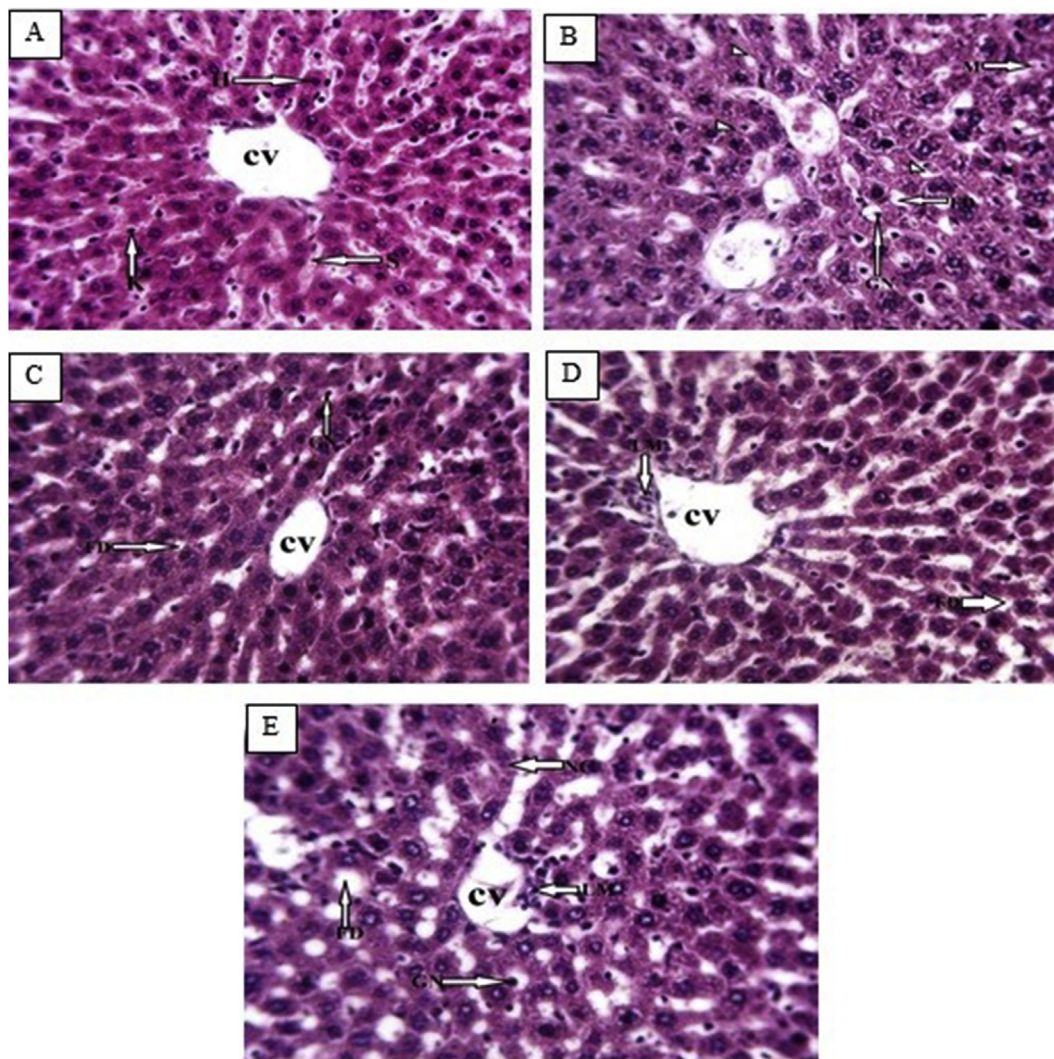
## Discussion

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 $\pm$ 7 <sup>a</sup>	72.2 $\pm$ 3.83 <sup>a</sup>	68.2 $\pm$ 10.42 <sup>a</sup>	75.4 $\pm$ 6.77 <sup>a</sup>	67.2 $\pm$ 1.3 <sup>a</sup>	0.138
ALT (U/L)	40.44 $\pm$ 4.11 <sup>a</sup>	47.2 $\pm$ 2.38 <sup>b</sup>	47.8 $\pm$ 3.34 <sup>b</sup>	56.2 $\pm$ 2.77 <sup>c</sup>	48.4 $\pm$ 4.27 <sup>b</sup>	0.001
AST (U/L)	35.6 $\pm$ 1.14 <sup>a</sup>	43 $\pm$ 3.8 <sup>b</sup>	42.4 $\pm$ 2.07 <sup>ab</sup>	48.2 $\pm$ 8.25 <sup>b</sup>	44.8 $\pm$ 3.7 <sup>b</sup>	0.023
PPC (mg/dL)	107.98 $\pm$ 33.43 <sup>b</sup>	79.22 $\pm$ 11.17 <sup>a</sup>	105.98 $\pm$ 4.56 <sup>b</sup>	92.05 $\pm$ 5.98 <sup>ab</sup>	100.5 $\pm$ 8.39 <sup>ab</sup>	0.01

Values expressed as Mean  $\pm$  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.

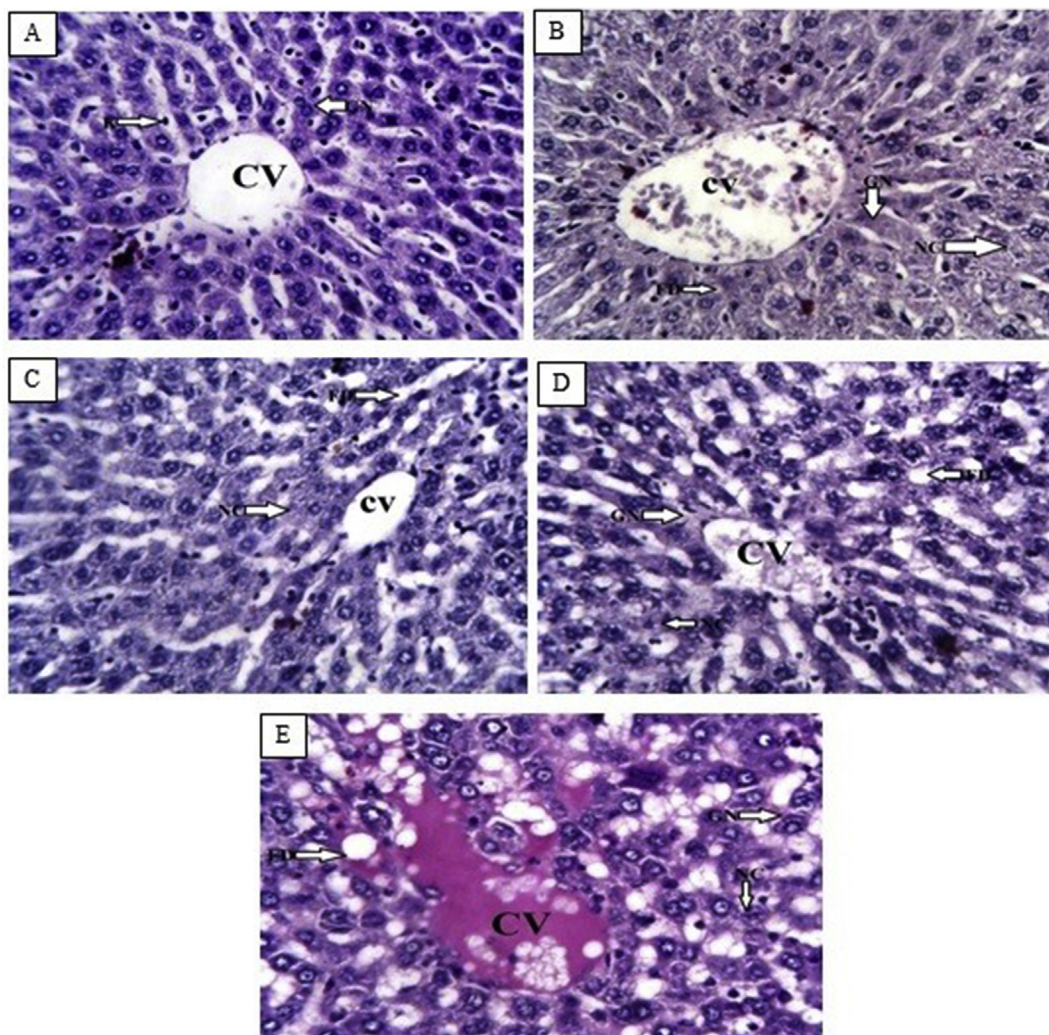


**Figure 1:** Photomicrographs of liver tissues from normal control rats, diabetic control rats, and rats treated with 10 and 20 mg/kg balanitoside and 6 U/kg insulin showing hepatocytes (H), fatty droplets (FD), sinusoid (S), giant mitochondria (GN), necrotic cells (NC), lymphocytes (LM), and central vein (CV) (H & E stain;  $\times 250$ ).

studies.<sup>14</sup> It might be attributed to the increase in the rate of protein catabolism, decrease in the rate of protein synthesis, or microproteinuria, which are important clinical markers of diabetes mellitus. Increased insulin levels usually result in increased protein synthesis, thereby reducing protein metabolism.<sup>9</sup> The protein concentrations in rats treated with balanitoside or insulin were within the normal range (90–110 mg/dL), according to Hoffman's method.<sup>10</sup>

The significant increase in the levels of ALT and AST in the diabetic control rats suggested that diabetes mellitus could induce hepatic injury, which might be owing to the increase in protein concentrations accompanying gluconeogenesis and urea formation observed in the diabetic state. ALT and AST are directly associated with the conversion of amino acids to keto acids and are reported to increase in diabetic conditions.<sup>11</sup> Rats treated with 10 mg/kg balanitoside or insulin showed a mild increase in ALT and AST levels, compared to the rats treated with 20 mg/kg balanitoside, indicating that balanitoside at 10 mg/kg might exhibit therapeutic effects in the hepatocytes by reducing the levels of ALT and AST. However, elevation of ALT and AST levels in the rats

treated with insulin could be attributed to impairment in insulin signaling rather than hepatic injury because ALT and AST gene transcription is suppressed by insulin.<sup>12</sup> The insignificant increase in ALP levels in the rats treated with 10 mg/kg balanitoside or insulin, compared to those in the normal control rats, suggested that balanitoside at 10 mg/kg might exhibit therapeutic effects in the hepatocytes. The presence of fat and glycogen droplets, vacuolated nuclei, and loss of cellular boundaries observed in the liver of the diabetic control rats indicated that diabetes mellitus coexisted with NAFLD, which is in line with the findings of a previous study,<sup>13</sup> which showed that hepatic accumulation of fat and glycogen are common complications of diabetes mellitus reported in 80% of patients with diabetes. Glycogen accumulates in patients with diabetes because of insulin deficiency that may increase synthase activity resulting in increased rate of gluconeogenesis and more glycogen accumulation.<sup>14</sup> Type 1 diabetes is not associated with fat accumulation if blood glucose level is well-controlled; however, fat accumulation occurs in 70% of patients with type 2 diabetes regardless of blood glucose control.<sup>15</sup> The



**Figure 2:** Photomicrographs of liver tissues from normal control rats, diabetic control rats, and rats treated with 10 and 20 mg/kg balanitioside and 6 U/kg insulin showing hepatocytes (H), sinusoid (S), fatty droplets (FD), vacuolated nuclei (GN), necrotic cells (NC), and central vein (CV) (PAS stain;  $\times 250$ ).

improvement in liver histology in the rats treated with balanitioside or insulin indicated that balanitioside might exhibit therapeutic effects in the hepatocytes.

### Conclusion

Balanitioside at 10 mg/kg exerted therapeutic and antioxidant effects in the liver of streptozotocin-induced diabetic rats by the following ways:

1. Decreasing the plasma levels of oxidative stress markers (ALP, ALT, and AST) and correcting streptozotocin-induced hypoproteinemia, and
2. Increasing the rate of regeneration and healing of the damaged liver tissues.

### Recommendation

More studies are needed to investigate the effects of balanitioside on the plasma levels of other oxidative stress markers, such as superoxide dismutase (SOD), catalase

(CAT), and malondialdehyde, as well as on the ultrastructure of the liver in diabetic rats.

### Conflict of interest

The authors have no conflict of interest to declare.

### Ethical approval

The research was conducted in accordance with ABU, Zaria Research and Ethical Committee and the ARRIVE Guidelines (Reporting of *in Vivo* Experiment).

### Authors' contributions

WM, WOH, AAB, and NID conceived and designed the study. WM and SGO provided research materials. WOH and AAB provided administrative support. WM, NID, and SGO conducted the research and collected and organized the data. WM, WOH, AAB, and NID analyzed and interpreted the

data. WM and NID wrote the initial and final draft of the manuscript and provided logistic support. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

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**How to cite this article:** Makena W, Hamman WO, Buraimoh AA, Dibal NI, Obaje SG. Therapeutic effects of balanitoside in streptozotocin-induced diabetic rats. **J Taibah Univ Med Sc** 2018;13(4):402–406.