# The hypoglycemic effect of the aqueous extract of the fruits of *Balanites aegypticea* in Alloxan-induced diabetic rats

**Abdella Emam Abdella Baragob, Waleed Hassan AlMalki, Imran Shahid, Fatimah Abdullah Bakhdhar, Hanouf Saeed Bafhaid, Omar Muhammad Izz Eldeen[1]**

*Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al Qura University, P. O. Box 13174, Makkah, The kingdom of Saudi Arabia, [1]Faculty of Science and Technology, Omdurman Islamic University, Khartoum, Republic of Sudan*

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## ABSTRACT

**Background:** *Balanites aegypticea* is used medically for many purposes e.g. anti-spasmodic, stomach pain, malaria, and yellow fever. The extract of the fruit is also used to reduce the blood glucose levels. **Objectives:** The objective of this study was to investigate the hypoglycemic effects of the aqueous extract of the fruits of the *Balanites aegypticea* in alloxan-induced diabetic rats. **Materials and Methods:** Twenty-five adult male Vistar rats were used in this study. The rats were randomly collected and divided into 5 groups (5 rats in each group). The untreated rats (negative control group) received basal diet and tap water only for 15 days. The experimental rats became diabetic by intraperitoneal injection of alloxan (150 mg/kg body weight). The fruit of *Balanites aegypticea* was powdered, extracted, and dried using organic solvents. The diabetic rats received aqueous extract 200 mg/kg, 400 mg/kg, and 800 mg/kg, respectively, for 2 weeks. Plasma glucose levels were measured by using Glucose GOD-PAP method through spectrophotometer. **Results:** The results showed that 800 mg/kg aqueous extract decrease significantly the plasma glucose level ($P \leq 0.05$) in diabetic rats, and there is a considerable gain in body weight ($P \leq 0.05$) compared to the diabetic control group. Four-hundred mg/kg aqueous extract has a mild effect on body weights and plasma glucose levels, while 200 mg/kg aqueous extract has no significant effect on plasma glucose level and a little effect on body weight. **Conclusions:** The results of the presented study revealed that the aqueous extract of *Balanites aegypticea* has hypoglycemic properties. It can decrease the plasma glucose level and can improve weight in diabetic experimental animals.

**Key words:** *Balanites aegypticea,* diabetic rats, hypoglycemic effect, plasma glucose level

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## INTRODUCTION

Diabetes mellitus is a serious metabolic disorder characterized by disarrangement of the metabolism of the carbohydrates, proteins, and lipids.[1] It is caused by a defect in complete or relative insufficiency of insulin secretion, insulin action, or both.[2] It has been estimated that more than 200 million people are affected with diabetes worldwide.[3] Insulin and oral hypoglycemic agents are used in the treatment of diabetes mellitus, but these agents have some undesirable side-effects, which limit their therapeutic efficacy.[4] Medicinal plants have a traditional role in the cure, treatment, and prevention of a number of diseases, and their uses are considered safer than synthetic drugs.[5] Similarly, herbal plants and dietary traditions play a pivotal role to regulate and attenuate metabolic disorders. The use of medicinal plants to treat diseases is authenticated centuries ago and opening a new horizon now-a-days in diabetes and cancer treatment by providing the clues for more investigation in this field.[6] *Balanites aegypticea* (locally known as Lalob, Higlig) belong to the family *Balanitaceae*. It is basically grown in Egypt and Sudan, but also found in India and Iran. The fruit of the tree is composed of outer pulp, which contains sugars, saponins, and the internal nuclei contain oils and proteins. The oil of the fruit is used to treat headache

**Address for correspondence:**
Dr. Imran Shahid, Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al Qura University,
P. O. Box 13174, Makkah, The kingdom of Saudi Arabia.
E-mail: iyshahid@uqu.edu.sa

and to improve lactation.[7] Similarly, the non-volatile oil of the fruit also contains the sapogenins, diosgenin, and yamogenin, which are used as an abortifacient and an antidote for arrow-poison in traditional medicine.[8] Some studies have been described the use of the fruit as an anti-spasmodic, stomach pain, and treatment of diabetes.[9] Many researchers have been trying to find the hypoglycemic effect of the internal layer and outer pulp of the Higlig fruit and described that the fruit of the Higlig may be used to treat diabetes. Considering the above mentioned facts, the main objective of this study was to evaluate the hypoglycemic effects of the aqueous extract of the internal layers (nodes) of the Higlig fruits in the diabetic rats.

## MATERIALS AND METHODS

### The animals

Twenty-five male Vistar rats, 4-8 weeks old, weighing 200-250 g, were used in this study. The rats were placed in suspended bracket cages in an air-conditioned room with a temperature of 25 ± 5°C and photoperiod of 12L: 12D. All animals were carefully maintained under standard animal house conditions with free access to food and water. The rats were fasted 16 hrs before injection. The approval of the ethical committee of the Umm Al Qura University was also obtained.

### *Balanites aegypticea* extraction

One-hundred grams of the nuclei of Higlig fruit were mixed and grinds with 2 liter of ethanol, and extraction was carried out by using soxhelt apparatus. The extraction was carried out 3 times to ensure complete extraction while maintaining the temperature at 40°C. The extracted sample was collected in a condenser. This process continued for 4-6 hours. The extracted sample was dried using a magnetic stirrer and finally evaporated under the vacuum at 70°C to reduce the solution volume to 1/8 of initial value. The aqueous solution of the extract was prepared by mixing the ethanol extract with 2000 ml of distilled boiling water for 60 minutes under continuous stirring. The obtained mixture was filtered twice through a mesh, then 2 times through a Whatman paper No. 2, and the obtained liquid was dried using a magnetic stirrer and finally, evaporated under the vacuum at 70°C to reduce the solution volume to 1/8 of initial value. This aqueous extract was maintained in 4°C until used.

### Diabetic induction

The rats were not fed 16 hours before injection. The alloxan solution (alloxan monohydrate from Sigma Co., USA) was prepared in distilled water, and 150 mg/kg dose was injected intraperitoneally to the rats in a positive and experimental group. The rats with more than 200 mg/dl plasma glucose level in fasting were considered as diabetic group.

### Animals' treatments

A group of 5 rats was considered as a negative control group, which received basal diet and tap water only for 15 days. Twenty diabetic rats were divided randomly and equally into 4 groups: One group considered as sham group (positive control group) and 3 groups as treatment animals, which received different concentrations (200 mg/kg, 400 mg/kg, and 800 mg/kg of body weight) of the aqueous extract for 2 weeks, respectively.

### Blood sampling

Blood samples were collected from orbital sinuses at the start and end of the experimental procedure after 0 hr, 2 hrs, and 4 hrs time interval by using the retro-orbital method by means of a micro capillary glass heparinized tubes. Blood was collected into a clean, dry centrifuge tube and left to clot in a water bath at room temperature for half an hour. The blood was centrifuged for 1 minutes at 3000 rpm to separate the serum. Serum was carefully aspirated and transferred into clean quit plastic tubes and kept frozen at -20°C until the time of analysis.

### Determination of plasma glucose levels

The plasma glucose level in the experimental animals was measured by using Glucose GOD-PAP method according to the manufacturer's protocol (mti-diagnostics GmbH, Germany). The absorbance (Abs.) was determined at 546 nm in spectrophotometer (Shimadzu, Japan), and the following equation was used to determine the plasma glucose concentrations.

$$\text{Sample glucose conc. (mg/dl)} = \frac{\Delta\text{Abs. Sample}}{\Delta\text{Abs. Standard}} \times \text{Standard conc. (100 mg/dl)}$$

Where $\Delta A$ sample = (A sample - A blank)
$\Delta A$ standard = (A standard - A blank)

Statistical Analysis

The data were analyzed as mean ± SEM. A $P$ value less than 0.05 ($P \leq 0.05$) was considered as the significant level in different groups using one-way ANOVA followed by Tukey's post-test.

## RESULTS

Before the start of the experiment, healthy rats in a narrow weight range (200-250 g) were selected; so, there was no significant difference between the animal's weight in different animal groups. After the induction of diabetes (2 weeks after the administration of Alloxan solution), the diabetic rats showed a significant weight loss compared to untreated non-diabetic rats (150 ± 20.5 vs. 225 ± 18.4, $P \leq 0.05$, Table 1). However, 2 weeks after the

start of treatment with aqueous extract, experimental rats showed a significant recovery in body weight (195 ± 20.7, 225 ± 16.4, and 260 ± 12.5 at 200 mg/kg, 400 mg/kg and 800 mg/kg concentrations, respectively, vs. 150 ± 20.5 in untreated diabetic rats, $P \leq 0.05$, Table 1 and Figure 1).

The analysis of plasma glucose level by Glucose GOD-PAP method is summarized in Table 2 at different time intervals in diabetic-treated animals. As shown in Table 2, there are significant differences in plasma glucose level between the healthy and diabetic rats at different time intervals. According to Table 2, after 2 weeks of experimental procedure, in group 3 and 4, rats treated with 200 mg/kg and 400 mg/kg extract conc. showed a mild reduction in blood glucose levels compared to diabetic control group (190.65 ± 13.4 and 160.33 ± 13.1 vs. 200.25 ± 5.2, respectively, $P \leq 0.05$). However, the reduction in blood glucose level was much significant in treated diabetic rats (group 5) than the diabetic control group at 800 mg/kg extract conc. (120.66 ± 7.5 vs. 200.25 ± 5.2, respectively, $P \leq 0.05$). Similarly, we observed that the decreased plasma glucose level was much more significant immediately after the treatment and there was a gradual increase in plasma glucose level at the time increased as shown in Figure 2.

## DISCUSSION

The limitations, side-effects, and cost of the currently available oral anti-diabetic agents to control blood glucose have stimulated and sparked the researchers to discover and develop novel anti-diabetic agents with fewer side-effects and potential therapeutic outcomes.[10] Many plant species in traditional medicines are known to be used

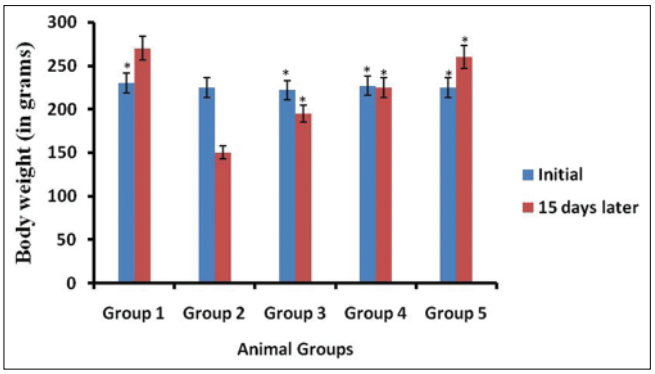


**Figure 1:** The effect of the aqueous extract of Balanites aegypticea on the body weight of treated diabetic animal groups compared to untreated diabetic animals. Data are presented as mean ± SEM. A *P* value less than 0.05 ($P \leq 0.05$) was considered as a significant difference compared to the group 1, using one-way ANOVA followed by Tukey's post test

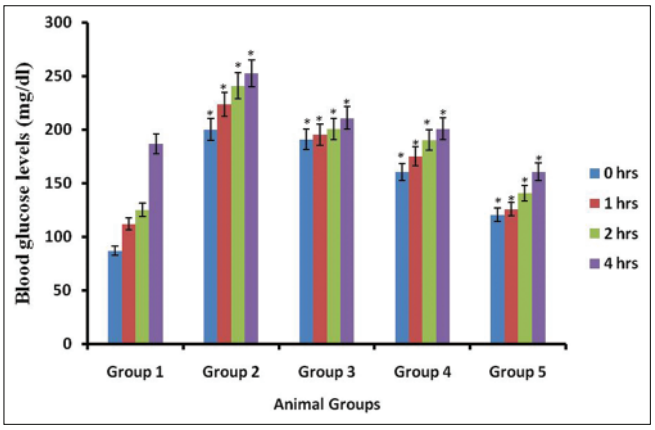


**Figure 2:** The hypoglycemic effect of the aqueous extract of Balanites aegypticea on the blood glucose level of treated diabetic animal groups (Group 3, 4 and 5) as compared to positive animal group (Group 2). Data are presented as mean ± SEM. A *P* value less than 0.05 ($P \leq 0.05$) was considered as a significant difference compared to the group 1, using one-way ANOVA followed by Tukey's post test

**Table 1: The mean body weight of normal and diabetic animal groups before and after the treatment.**

| Groups | Group 1 | Group 2 | Group 3 | Group 4 | Group 5 |
|---|---|---|---|---|---|
| Weight (in grams) | Negative control (Healthy) | Positive control (Diabetic) | Diabetic plus (200 mg/kg) aqueous extract | Diabetic plus (400 mg/kg) aqueous extract | Diabetic plus (800 mg/kg) aqueous extract |
| Initial | 230* ±25.2 | 225±18.4 | 222* ±20.5 | 227* ±30.5 | 225* ±26.5 |
| 15 days later | 270±10.3 | 150±20.5 | 195* ±20.7 | 225* ±16.4 | 260* ±12.5 |

(*n*=5 rats in each group). Data are presented as mean ± SEM. A *P* value less than 0.05 ($P \leq 0.05$) was considered as a significant difference compared to the negative control using one-way ANOVA followed by Tukey's post test and indicated by *.

**Table 2: The comparison of mean blood glucose levels in untreated and treated diabetic groups at different time intervals**

| Groups | 0 hr | 1 hrs | 2 hrs | 4 hrs |
|---|---|---|---|---|
| Group 1 (Negative control) | 86.99±13.6 | 112.06±17.5 | 124.94±17.5 | 186.75±22.0 |
| Group 2 (Positive control) | 200.25* ±5.2 | 223.50* ±16.0 | 240.85* ±17.8 | 252.45* ±15.7 |
| Group 3 Diabetic plus (200 mg/kg) aqueous extract | 190.65* ±13.4 | 195.45* ±15.5 | 200.55* ±15.3 | 210.83* ±21.5 |
| Group 4 Diabetic plus (400 mg/kg) aqueous extract | 160*.33±13.1 | 175.05* ±14.8 | 190.16* ±16.9 | 200.66* ±15.1 |
| Group 5 Diabetic plus (800 mg/kg) aqueous extract | 120.66* ±7.5 | 125.83* ±15.3 | 140.58* ±13.7 | 160.65* ±15.5 |

(*n*=5 rats in each group). Data are presented as mean ± SEM. A *P* value less than 0.05 ($P \leq 0.05$) was considered as a significant difference compared to the positive control using one-way ANOVA followed by Tukey's post test and indicated by *.

for their hypoglycemic effects with scanty experimental evidences. Researchers are working with medicinal plants like *Nelumbo nucifera,*[11] *Rosmarinus officinalis, and Heseachlamys sdulis*[12] to evaluate their hypoglycemic effects. Bosch *et al.* reported the anti-diabetic effect of bitter melon in experimental animals.[13] Similarly, Shahraki *et al.* observed that the serum glucose decreased significantly in diabetic rats after receiving 50 mg/kg *Teucrium polium* for a month.[14] Esmaeili and Yazdanparast showed a significant decrease in plasma glucose level in streptozotocin-induced hyperglycemic rats after 6 weeks of consecutive oral treatment with aqueous extract of *Teucrium polium*.[15]

Alloxan is the most commonly used agents for the induction of diabetes in experimental animal models like rats. There is some conclusive evidence that alloxan cause rapid depletion of beta cells, DNA alkylation, and accumulation of cytotoxic free radicals in pancreatic tissue, which cause initial inflammation and after that, infiltration of activated macrophages and lymphocytes that leads to reduce insulin release resulting in sustained hyperglycemia state.[16] In this study, rats were injected with alloxan solution (150 mg/kg body weight) to achieve hyperglycemia. The animals with more than 200 mg/dl plasma glucose level in fasting were considered diabetic for this study.

The fruits of *Balanites aegypticea* can be used as an anti-spasmodic, for stomach pain, and treatment of diabetes. There are few scientific evidences, which describe the hypoglycemic potential of various aqueous extracts of *Balanites aegypticea*.[17] The hypoglycemic effect was thought due to increased peripheral metabolism of glucose, an increase in insulin release from β-cells,[18] and decreased absorption of glucose from intestine.[19] Considering the above mentioned facts, the present study was designed to evaluate the hypoglycemic effect of aqueous extract of the fruit of *Balanites aegypticea* in diabetic-treated rats at different conc. at different time intervals. Our result shows clearly that the aqueous extract has hypoglycemic effects in alloxan-induced diabetic rats after 2 weeks treatment. These findings also support the use of this plant in traditional medicine as a hypoglycemic agent as reported in literature.[20]

Our results showed that the body weight of the diabetic rats was significantly raised when treated with 400 mg/kg and 800 mg/kg aqueous extract (Group 4 and 5), whereas gain in body weight was mild to group 3 (200 mg/kg) after 2 weeks treatment. These findings are in accordance with Ramesh *et al.*[21] and Erenmemisoglu *et al.*[22] studies who showed that *Teucrium polium* and *Rosmarinus officinalis* have hypoglycemic effects in normoglycemic and diabetic mice. The absence of weight loss or gain in weight, in treatment groups, probably may be due to some of the components of *Balanites aegypticea* aqueous extract, which may increase serum leptin levels,[23] and this increased serum leptin level ultimately rise circulating insulin as a direct correlation has been approved between leptin and insulin.[24] The results also showed a very significant decrease in the plasma glucose level in group 5 immediately after the treatment, where the diabetic rats were treated with 800 mg/kg aqueous extract. As the time passed, the levels of plasma glucose levels increased although the differences were not much more significant at different time intervals compared to the diabetic control group. Similarly, the plasma glucose levels were also decreased in diabetic rat groups 3 and 4 where the diabetic rats were given 200 mg/kg and 400 mg/kg aqueous extract, respectively. However, this decrease in plasma glucose level was not so much significant compared to the diabetic control group as shown in Table 2 and Figure 2. One of the possible reasons for the increase in plasma glucose level with the passage of time may be the metabolism of the active ingredient of the extract responsible for the hypoglycemic effect.

The phytochemical investigation of the fruit of *Balanites aegypticea* revealed that it may contain rutin, interketones, organic constituents, oils (volatile oils and fatty acids) present in the internal kernel.[25] It may also assume that the hypoglycemic effect of the aqueous extract may be attributed to its constituents such as rutin, saponins, and organic constituents. However, further studies are needed to find out the active ingredients of the extract. Complementary studies about the role of each active ingredient and its mechanism of action will be helpful to determine the role of each component in reducing plasma glucose levels. Further research is needed to know the histological changes in the pancreas and liver of the diabetic rats with or without aqueous extract. Similarly, either the lowering of plasma glucose level in treating animals is therapeutic or preventive after stopping the treatment dose or ingestion of the extract should be elucidated further in the near future.

## CONCLUSION

The results of the presented study showed that the aqueous extract of *Balanites aegypticea* when administered to alloxan–induced diabetic rats had a hypoglycemic effect. The best results were obtained at an aqueous extract concentration of 800 mg/kg after a short time interval. The study also suggests that there is a further need for more biological investigations and phytochemical studies with different doses of the extract to find the active ingredient of the extract.

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