WORLD JOURNAL OF PHARMACY AND PHARMACEUTICAL SCIENCES
SJIF Impact Factor 7.421
Volume 7, Issue 6, 76-87 Research Article ISSN 2278 – 4357

ANTIDIABETIC ACTIVITY OF BALANITES AEGYPTIACA (L.) DELILE LEAVES IN EXPERIMENTAL DIABETIC RATS

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Article Received on
03 April 2018,

Revised on 24 April 2018,
Accepted on 14 May 2018,

DOI: 10.20959/wjpps20186-11654

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ABSTRACT

Screening of antidiabetic activity of methanolic extract of *Balanites aegyptiaca* (L.) Delile (leaves) was done in rats by conducting glucose tolerance test (GTT) study and evaluating their effects (single dose and multidose treatment study) on blood glucose level, serum lipid profile levels in alloxan diabetic rats. The plant extract significantly ($p<0.01$) improved the glucose tolerance test up to 4 hour. The plant extract showing 15 and 29% reduction in blood glucose level from control values at the end of 2hrs and 4 hrs respectively. The administration of the plant extract was found to reduce blood glucose level in alloxan induced diabetic rats in single dose study. The plant extract exhibited significant ($p<0.05$) antihyperglycemic efficacy from 1 hr after its oral administration, the effect lasted up to 6 hrs when compared with normal rats and diabetic control rats. Blood glucose lowering potential percentage of the plant extract was 21% at 6 hr after administration, while the standard drug Glibenclamide (2.5 mg/kg) caused 20% reduction of blood glucose at the same time interval when compared with diabetic control rats. The observation indicates that, The *B.aegyptiaca* extract was beneficial in enhancing HDL cholesterol and lowering TG, TC and LDL cholesterol, thereby reveals its useful therapeutic value. It is evidenced that glucose lowering activity of the *B.aegyptiaca* extract is responsible for controlling and correcting the altered lipid profile, this effect may be due to the presence of active constituents in the leaves of *B.aegyptiaca*.

KEYWORDS: *Balanites aegyptiaca* (L.) Delile; Antidiabeteic.

INTRODUCTION

Medicinal herbs are widely employed by greater number of people because seeking for side effect free of treatment since using synthetic drugs can produce side effects. Overall, 80% of the world's population has dependability in traditional medicine, chiefly based on plant drugs for their primary healthcare (Sivananthan &Elamaran, 2013a). The active constituents are typically extracted from all herbal/ plant structures, but the quantity of these components are varying from structure to structure. Highest quantity of active principle within the part is favored to therapeutic purposes (Sivananthan, 2013b).

Diabetes mellitus (DM) is a metabolic disease characterized by hyperglycemia resulting from defects in insulin secretion and /or action. This disease, which has been a disease of minor significance to world health until a few decades ago, is now one of the main threats to human health in the 21th century. It is most common non-communicable disease worldwide and the fourth to fifth major cause of mortality in developed countries. The global figure of people with diabetes is set to rise from the current estimate of 150 million to 220 million in 2010 and 300 million in 2025 (Alberti & Shaw, 2004). Diabetes is defined as a state in which homeostasis of carbohydrate and lipid metabolism is improperly regulated by insulin. This results primarily in elevated fasting and postprandial blood glucose levels. If this imbalanced homeostasis does not return to normalcy and continues for a protracted period of time, it leads to hyperglycemia that in due course turns into a syndrome called diabetes mellitus. Besides hyperglycemia, several other factors including dyslipidemia or hyperlipidemia are involved in the development of micro and macro vascular complications of diabetes which are the major cause of morbidity and death (Kameswar *et al.*, 2003).

In spite of the presence of known antidiabetic medicine in the pharmaceutical market, remedies from medicinal plants are used with success to treat this disease. Many traditional plant treatments for diabetes are used throughout the world. Plant drugs and herbal formulation are frequently considered to be less toxic and freer from side effects than synthetic one (Bailey & Day, 1989). Based on the WHO recommendations, hypoglycemic agents of plant origin used in traditional medicine are important. Moreover, continuous use of the synthetic antidiabetic drugs cause side effects and toxicity. Therefore, seeking natural and non-toxic antidiabetic drug therapy is of prime importance (Ashok *et al.*, 2010). In the present

research work, the methanolic extract of the leaves of the plant *Balanites aegyptiaca* Del had been examined scientifically for its antidiabetic activity.

Balanites aegyptiaca Del., also known as 'Desert date' in English, and "Heglig" in Arabic, a member of the family Zygophyllaceae, is one of the most common but neglected wild plant species of the dry land areas of Africa, the Middle East and South Asia (Hall & Waljer,1991). It is multibranched, spiny shrub or tree up to 10 m tall, crown spherical, in one or several distinct masses. It can be found in many kinds of habitat, tolerating a wide variety of soil types, from sand to heavy clay, and climatic moisture levels (Ndoye, 2004).

Traditional Uses

Aqueous extract of fruits showed spermicidal activity without local vaginal irritation in human being, up to 4% sperms becoming sluggish on contact with the plant extract and then immobile within 30 seconds; the effect was concentration-related. Protracted administration of the fruit pulp extract produced hyperglycemia-induced testicular dysfunction in dogs. Seed is used as expectorant, antibacterial, and antifungal. Fruit is used in whooping cough, also in leucoderma and other skin diseases. Bark is used as spasmolytic (Khare, 2007).

The root extracts have proved 'slightly effective' against experimental malaria (Karel & Roach, 1951). In Egyptian folk medicine, the fruits are used as an oral hypoglycaemic (Kamel, 1998) and an antidiabetic; an aqueous extract of the fruit mesocarp is used in Sudanese folk medicine in the treatment of jaundice (Sarker *et al*., 2000). Latex of the plant is used in epilepsy, administered through intranasal route (Seifu, 2004). Fruits are used to treat dysentery and constipation. The seed oil is used to treat tumours and wounds (Khalid *et al.*, 2010). Used as laxative, also used in treatment of haemorrhage, stomach aches, jaundice, yellow fever, syphilis, and epilepsy (Ojo *et al.*, 2006). A fruit is used to treat liver disease and as a purgative, and sucked by school children as a confectionary in some countries (Barley *et al.*, 1962). The bark is used in the treatment of syphilis, round worm infections, and as a fish poison. The aqueous leaf extract and saponins isolated from its kernel cakes have antibacterial activity (Zarroug *et al.*, 1988). In Libya and Eritrea, the leaves are used for cleaning infected wounds. In Sudan and Chad the bark of *B. aegyptiaca* is used as soap for washing (Oliver, 1960). The Leaves contain saponin, furanocoumarin, and flavonoid namely quercetin 3-glucoside, quercetin-3-rutinoside; 3-glucoside, 3-rutinoside, 3-7-diglucoside and 3 rhamnogalactoside of isorhamnetin (Samuelsson *et al.*, 1991).

MATERIALS AND METHODS

The selection of plant

The leaves of *Balanites aegyptica* were collected and taxonomically identified and authenticated by taxonomy expert, at Herbarium of Medicinal and Aromatic Plants Research Institute (MAPRI), National Center for Research (NCR), Khartoum, Sudan where the voucher specimen has been deposited.

Animals

Wistar albino rats of either sex were used. The normoglycemic rats weighing from 80-120 g and the diabetic rats weighing from 200-250 g. The rats were kept on a fixed diet so as to stabilize the fasting plasma glucose level which was fixed at 70-110 mg/dl level.

Methanolic extraction of the plant

Solid-liquid MeOH extraction of the leaves of *Balanites aegyptica* plant (500 g) was performed. Extraction was carried out according to method described by Harborne (1984). Methanol was selected as extraction solvent due to its ability to dissolve a vast variety of compounds in it. The plant powder (500 g) was soaked in crude methanol (1.0 L) in large container and was regularly shaked for 2 hours using shaker apparatus and then kept overnight at room temperature (28°C). Then filtered through Whatmann filter paper and the re-extraction of the residue was repeated twice till the colour of the solvent returned colorless. Solvent was evaporated under reduced pressure using rotary evaporator apparatus at 40°C to yield concentrated dry extracts. The yield percentage was calculated as follows:

$$\text{Yield percentage} = \frac{\text{Weight of the dry residue x 100}}{\text{Weight of the plant material}}$$

Dose selection and finalizing LD_{50} cut off values

The plant extract was prepared as a suspension by triturating with water and 1% gum acacia. It was mixed with required quantity of distilled water and 1% gum acacia for preparation of suspension. This suspension was administered orally to rats by intragastric tube. Prior to dosing, animals were kept for 12 hours of fasting. After the dose was administered food was withheld for a further 3-4 hours. Three animals were used and the study was begun at 2000 mg/kg body weight. Animals were observed initially after dosing at least once during the first 30 minutes and periodically during the first 24 hours. $1/10^{th}$ of this lethal dose (i.e.200mg/kg) was taken as effective dose (Therapeutic dose) for antidiabetic activity.

Antidiabetic Activity Study

Screening of antidiabetic activity of the plant extract was done in rats by conducting glucose tolerance test (GTT) study and evaluating their effects (Single dose and Multidose treatment study) on blood glucose level, serum lipid profile levels in alloxan diabetic rats.

Glucose tolerance test (GTT)

Fasting blood glucose level of each rat was determined at zero time after overnight fasting with free access to water. Rats were divided into three groups containing five rats each:

Group I: Control group: Rats received 1ml of 1% gum acacia suspension orally.

Group II: Standard drug-Glibenclamide (2.5mg/kg).

Group III: Methanolic extract of *Balanites aegyptiaca* (200mg/kg).

These animals received their doses by oral route using a gavage syringe. 50% Glucose (2g/kg) was orally administered 30 minutes after the administration of the extract or Glibenclamide or gum acacia suspension. Blood samples were collected from the orbital plexus under ether anaesthesia just prior to and after 60, 120 and 240 min after glucose loading. Glucose levels were estimated using Glucose Oxidase Method.

Effect of the plant extract on blood glucose levels in aloxan induced diabetic rats (single dose-Acute treatment)

The animals were selected and weighed then marked for individual identification. The rats were injected with alloxan monohydrate in saline (0.9% NaCl) at a dose of 120mg/kg body weight intraperitonially to induce diabetes in overnight fasted male wistar albino rats weighing 120-150g. After one hour of alloxan administration the animals were given feed *ad libitum*. After 72 hours, animals with blood glucose levels higher than 250mg/dl were considered diabetic and were included in the study. Rats were divided into four groups containing five rats each:

Group I: Normal control group: Rats received 1ml of 1% gum acacia suspension orally.

Group II: Diabetic control group: 1ml of 1% gum acacia suspension orally.

Group III: Diabetic rats receiving standard drug-Glibenclamide (2.5mg/kg).

Group IV: Methanolic extract of *Balanites aegyptiaca* (200mg/kg).

Blood samples were collected from the orbital sinus under ether anaesthesia just prior to and at 2, 4 and 6 hours intervals after the administration of the extract and blood glucose levels were estimated using Glucose Oxidase Method.

Effect of the plant extract on blood glucose levels in aloxan induced diabetic rats (Multi dose-Sub acute treatment)

The animals were selected and weighed then marked for individual identification. The rats were injected with alloxan monohydrate in saline (0.9% NaCl) at a dose of 120mg/kg body weight intraperitonially to induce diabetes in overnight fasted male wistar albino rats weighing 120-150g. After one hour of alloxan administration the animals were given feed *ad libitum*. After 72 hours, animals with blood glucose levels higher than 250mg/dl were considered diabetic and were included in the study. Rats were divided into four groups containing five rats each:

Group I: Normal control group: Rats received 1ml of 1% gum acacia suspension orally.

Group II: Diabetic control group: 1ml of 1% gum acacia suspension orally.

Group III: Diabetic rats receiving stadard drug-Glibenclamide (2.5mg/kg).

Group IV: Methanolic extract of *Balanites aegyptiaca* (200mg/kg).

These rats were given the same doses of the extracts once daily for 15 days in this study. Blood samples were collected from the orbital plexus of non-fasted rats under ether anaesthesia on days 0, 5, 10 and 15 of extract administration and blood glucose levels and serum lipid profile levels on day 15 were measured.

Sample Collection

1-2 ml of blood were drawn out by capillary tubes in fluorinated test tubes from the orbital plexus of rats under inhalation anesthesia using halothane and centrifuged at 3000 r.p.m for 5 minutes to separate plasma. The plasma prepared was used to estimate the glucose, cholesterol and triglyceride concentrations.

Measurement of biochemical parameters

The blood glucose, total cholesterol, triglycerides, HDL cholesterol and LDL cholesterol concentrations were measured using commercial kits by enzymatic spectrophotometric method.

RESULTS

Screening of antidiabetic activity of the methanolic extract of the leaves of *Balanites aegyptiaca* was done in rats by conducting glucose tolerance test (GTT) study and evaluating their effects (single dose and multidose treatment study) on blood glucose level and serum lipid profile in alloxan diabetic rats.

Glucose tolerance test (GTT)

The plant extract significantly ($p<0.01$) improved the glucose tolerance test up to 4 hour (Table 1). The plant extract showing 15 and 29% reduction in blood glucose level from control values at the end of 2hrs and 4 hrs respectively. The Glibenclamide also improved the glucose tolerance test up to 4 hrs.

The effect of the plant extract on the blood glucose level in alloxan-diabetic rats (Single dose treatment/Acute study)

The administration of the plant extract was found to reduce blood glucose level in alloxan induced diabetic rats in single dose study. The plant extract exhibited significant ($p<0.05$) antihyperglycemic efficacy from 1 hr after its oral administration, the effect lasted up to 6 hrs when compared with normal rats and diabetic control rats. Blood glucose lowering potential percentage of the plant extract was 21% at 6 hr after administration, while the standard drug Glibenclamide (2.5 mg/kg) caused 20% reduction of blood glucose at the same time interval when compared with diabetic control rats (Table 2).

Multi dose treatment/Sub acute study

A) **The effect of the plant extract on blood glucose level in alloxan diabetic rats**

During the multidose treatment period, administration of the methanolic extract of *Balanites aegyptiaca* leaves (200mg/kg/day) caused a significant decrease of 29%, 38% and 52% in blood glucose levels on 5^{th}, 10^{th} and 15^{th} day intervals, respectively, when compared with diabetic control group. When comparison between initial blood glucose level and 15^{th} day blood glucose level was made in the same group animals, it was found that, there is a reduction of 55.50% in the plant extract whereas 47.10% reduction was observed in standard drug-Glibenclamide (2.5mg/kg) treated animals (Table 3).

B) The effect of the plant extract on serum lipid profile in aloxan induced diabetic rats

Observations of hypoglycemic effect of the plant extract in alloxan-induced hyperglycemic rats serum lipid profile is shown in table 4. These serum levels were measured on 15^{th} day of treatment with different extract treated groups. These levels were expressed as mg/dl and were given in mean$\pm$ SD.

Total cholesterol (TC): There was a significant increase in total cholesterol levels in the diabetic control animals (83.00$\pm$2.00 mg/dl) compared to that of normal control animals (55.50$\pm$1.6 mg/dl). Total cholesterol levels in the plant extract 200 mg/kg (58.50$\pm$2.7 mg/dl)

showed a highly significant ($p<0.01$) reduction in the total cholesterol level in the serum compare to that of diabetic control animals (83.00±2.00 mg/dl).

Triglycerides (TG): There was a significant increase in triglyceride in the diabetic control animals (123.5±2.4 mg/dl) compared to the normal control animals (85.25±1.5 mg/dl). Triglyceride level in the plant extract 200mg/kg (94.00±9.3 mg/dl) showed a high significant ($p<0.05$) reduction in triglyceride level when compared to that of diabetic control triglyceride level (123.5±2.4 mg/dl). Glibenclamide (2.5 mg/kg) (92.25±8.0 mg/dl) also showed high significant ($p<0.01$) reduction in the triglyceride level in the serum compare to that of diabetic control animals (123.5±2.4 mg/dl).

High Density Lipoprotein (HDL): There was a significant decrease in HDL cholesterol in the diabetic control animals (30.00±1.4 mg/dl) compared to that of normal control animals (37.00±1.5 mg/dl). HDL cholesterol in the plant extract showed a highly significant ($p<0.01$) rise in HDL cholesterol level in the serum compare to that of diabetic control animals (30.00±1.4 mg/dl)

Low Density Lipoprotein (LDL): There was a significant increase in low density lipoprotein cholesterol levels in the diabetic control animals (34.42±3.7 mg/dl) compared to that of normal control animals (16.25±0.4 mg/dl). Low density lipoprotein cholesterol levels in the plant extract (23.00±4.9 mg/dl) showed a significant ($p<0.05$) reduction in low density lipoprotein cholesterol levels when compared to that of diabetic control low density lipoprotein cholesterol levels (34.42±3.7 mg/dl). Glibenclamide (19.00±1.9 mg/dl) also showed significant ($p<0.05$) rise in the LDL levels in the serum compare to that of diabetic control animals (34.42±3.7 mg/dl).

Table 1: The effect of the plant extract on the blood glucose level in glucose loaded rats.

Group	Treatment	Blood glucose concentration (mg/dl) (mean±SD)			
		In fasting	60 min	120 min	240 min
I	Normal control (1% gum acacia)	93.50±3.4	163.3±2.2	160.5±3.9	131.3±3.8
II	Glibenclamide (2.5 mg/kg)	95.00±2.8	145.5±1.1*	138.0±2.8*	99.50±1.6*
III	*B. aegyptiaca* 200mg/kg	91.00±5.2	136.5±2.3*	117.5±4.2*	97.83±2.5*

*$p<0.01$ significant, compared to normal control.

Table 2: The effect of the plant extract on the blood glucose level in alloxan-diabetic rats (Single dose treatment/Acute study).

Group	Treatment	Blood glucose concentration (mg/dl) (mean±SD)			
		0hr	60 min	120 min	240 min
I	Normal control (1% gum acacia)	83.25±2.6	87.50±2.0$^{\#\#}$	89.75±1.8$^{\#\#}$	92.25±2.4$^{\#\#}$
II	Diabetic control	287.5±5.0	294.50±5.2*	290.5±4.1*	292.0±3.5*
III	Glibenclamide (2.5 mg/kg)	297.3±7.2	274.0±9.7*	258.8±12.9$^{*\#}$	252.0±13.1$^{*\#\#}$
IV	*B. aegyptiaca* 200 mg/kg	275.5±7.7	263.0±4.1$^{*\#\#}$	262.0±5.3*	248.3±6.1$^{*\#\#}$

*p<0.01 significant, compared to normal,$^{\#}$p<0.05 & $^{\#\#}$p<0.01 Significant compared to diabetic control.

Table 3: The effect of the plant extract on the blood glucose level in alloxan-diabetic rats (Multi dose treatment/Subacute study).

Group	Treatment	Fasting blood glucose concentration (mg/dl)			
		0th Day	5th Day	10th Day	15th day
I	Normal control (1% gum acacia)	85.25±2.6	85.75±1.8$^{\#\#}$	85.25±1.3$^{\#\#}$	87.25±1.1$^{\#\#}$
II	Diabetic control	287.5±5.2	274.3±7.1**	264.3±5.3**	255.8±5.1**
III	Glibenclamide (2.5 mg/kg)	299.3±6.9	217.3±14.3$^{**\#\#}$	188.3±13.8$^{**\#\#}$	158.3±15.3$^{**\#\#}$
IV	*B. aegyptiaca* 200 mg/kg	275.5±7.7	194.2±16.0$^{**\#\#}$	164.4±10.6$^{**\#\#}$	122.6±9.4$^{*\#\#}$

*p<0.05 & **p<0.01 significant, compared to normal,$^{\#}$p<0.05 & $^{\#\#}$p<0.01 Significant compared to diabetic control.

Table 4: The effect of the plant extract on serum lipid profile in alloxan-diabetic rats after 15 days of treatment.

Group	Treatment	TCholesterol mg/dl	TGL mg/dl	HDL mg/dl	LDL mg/dl
I	Normal control (1% gum acacia)	55.50±1.6**	85.25±1.5**	37.00±1.5*	16.25±0.4*
II	Diabetic control	83.00±2.0	123.5±2.4	30.00±1.4	34.42±3.7
III	Glibenclamide (2.5 mg/kg)	58.50±2.7**	92.25±8.0**	51.50±1.9**	19.00±1.9*
IV	*B. aegyptiaca* 200 mg/kg	60.0±6.1**	94.0±9.3**	45.66±1.5**	23.00±4.9*

*p<0.05 & **p<0.01 significant, compared to diabetic control.

DISCUSSION

When the methanolic extract of the leaves of *B.aegyptiaca* subjected for antidiabetic activity in GTT, it was observed that this plant possesses antihyperglycemic activity which is confermed by improvement of glucose tolerance test. This indicates the possible action including acting through pancreatic mechanism or by inhibition of glucose absorption through gastrointestinal tract. In this study, a single i.p. injection of alloxan (120 mg/kg of body wt.) to rats resulted in severe hyperglycemia and elevation in cholesterol levels. Alloxaan has been widely used for the induction of diabetes mellitus in various experimental animals, it produce diabetes mellitus by cytotoxic action on pancreatic β-cells results in insulin deficiency (Szkudelski, 2001). The *B.aegyptiaca* extract was given orally with the help of a gastric tube to alloxan induced diabetic rats. Further, samples of blood were collected at 1 hr, 2 hr, 4 hr and 6 hr in single dose treatment study (Acute study), whereas on day 5^{th} , 10^{th} and 15^{th} in multi dose treatment study (Sub acute study). Control animals received equal volume of 1% gum acacia.

The results of blood glucose level estimation obtained from alloxan induced diabetic rats, in single dose treatment study, indicated that the extract of *B. aegyptiaca* ($p<0.01$) and standard drug-Glibenclamide ($p<0.01$) showed highly significant antidiabetic activity at 1 hr, 4 hr and 6 hr. In multi dose treatment study the plant extract and Glibenclamide had shown highly significant ($p<0.01$) antidiabetic activity on 10^{th} and 15^{th} day of study when compared with diabetic control rats. The serum TC, TG and LDL cholesterol levels were elevated in untreated diabetic rats. Extract and standard drug treatment for 15 days in diabetic rats showed significant reduction in all these lipid profiles. The observation indicates that, The *B.aegyptiaca* extract was beneficial in enhancing HDL cholesterol and lowering TG, TC and LDL cholesterol, thereby reveals its useful therapeutic value. It is evidenced that glucose lowering activity of the *B.aegyptiaca* extract is responsible for controlling and correcting the altered lipid profile, this effect may be due to the presence of active constituents in the leaves of *B.aegyptiaca*.

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