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EVALUATION OF THE DIURETIC ACTIVITY FOR THE ETHANOLIC EXTRACT OF *BALANITES AEGYPTIACA* (L.) DELILE FRUIT BY USING MODIFIED LIPSCHITZ METHOD

KULKARNI KM[1*] AND KONDAWAR MS[2]

1: PhD scholar, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

2: Professor and Head of Department of Pharmaceutical Chemistry, Appasaheb Birnale College of Pharmacy, Sangli, Maharashtra, India

***Corresponding Author: Mr. Kiran M.Kulkarni: E Mail: kulkarnikiran986@gmail.com**

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ABSTRACT

Yet *Balanites aegyptiaca* is most commonly preferred folk fore medicinal plant, there is no scientific finding still exists to express such diuretic potential. So present study was deal with investigation of the diuretic potential as well as lethal hazaradness for *Balanites aegyptiaca* (L.) Delile Fruit extract by using Wistar rat. Diuretic potential of alcoholic fruit extract investigated on albino rats within five different groups by using modified Lipschitz method in normal saline was used to hydrate test animal rats. Control group was treated with Normal saline (25mL/kg), 20mg/kg of furosemide was provided to the reference group, and various quantities of the ethanolic extract (i.e., 100, 250, and 500 mg/kg) were given to the test groups, respectively, through intra-peritoneal method. Statistical data was evaluated through Annova test with a threshold of significance of 0.05 was considered statistically significant. Test groups were shown positive diuretic activity with significant increase in a dose. While, urine's pH unchanged. Ethanolic extract was interpreted for diuretic activity with the help diuretic index. At dose of 500 mg/kg, Lipschitz values express 46% diuretic activity in comparison to standard furosemide. There was no

hazardous signs were observed in test animals during acute toxicity study yet at maximum dose up to 5000mg/kg.

Keywords: *Balanites aegyptiaca* (L.) Delile Fruit, diuretic effect, modified Lipschitz method

INTRODUCTION

A diuretic agent promotes not only the rate of urine formation but also maintains body's fluid-electrolyte balance. It is considered crucial fact for the treatment of many chronic heart and kidney related diseases. Now days most of diuretics preferred for treatment have been possessed much more adverse effects as like disturbed electrolyte balance, metabolic changes, onset of diabetes and sexual dysfunction. As a result, it's critical to take into account alternatives with higher clinical potential and minimum negative impact. Much of plants employed in traditional medicament have not yet undergone scientific testing for their safety and efficacy **[1, 2]**.

On literature review it is revealed that there are some species like *Balanites aegyptiaca* (L.) Delile from Zygophyllaceae family and Balanites Genus which shows the presence of many potent chemical constituent like alkaloids, glycosides, saponin glycosides, flavonides along with some secondary metabolites. Due to presence of these chemical constituents in the plants, hence there will be the possibility of having effective Diuretic activity. Intended approach for current investigation is to evaluate Diuretic potential of any mostly neglected species Plant belongs to Zygophyllaceae family **[3, 4]**.

MATERIAL AND METHOD

Plant material

Dried ripe fruits of *Balanites aegyptiaca* (L.) Delile were collected when plant in greatest period of prosperity from rural reagion of bhilavadi village. Respective plant material was authenticated from Botanical survey of India, Pune. (Ref.No-No.BSI/WRC/100-1/Tech./2020/116).

Preparation of extract [5, 6]

Fruits were carefully cleaned with clean, running water before being dried in the shade and ground into a powder. It was extracted using hexane, chloroform, ethyl acetate, ethanol, and water in that increasing order of Polarity. Soxhlation was used to complete it. 200gm of finely powdered fruit sample were transferred to soxhlet device, and (95%) ethanol was employed for separation of chemical constituents. It was taken more than 17hrs to complete entire cycles of extraction. The solvents recovered from extract by using rotary evaporator. Percentage gain was calculated in relation to the entire portion of fruit material utilized. Through maceration at

room temperature for 7 days, water extract was produced. The extract's phytochemical composition was then assessed. Based on findings that indicated increased acute dieresis activity, ethanol extract was examined.

Phytochemical Investigation:

Balanites aegyptiaca (L.) Delile's alcoholic fruit extract was the subject of a phytochemical study. The tests were conducted using the accepted procedures outlined by Dr. C.K. Kokate and Dr.K.R. Khandelwal in practical Pharmacognosy. **[7]**

Detection of Flavonoids:

Preparation of test solution: Alcohol is used to dissolve a little amount of extract before filtering. The filtrate is concentrated and put through a number of tests to see if flavonol glycosides are present.

a. Shinoda test:

. To the test solution, add a small amount of strong hydrochloric acid and a few magnesium turnings. After some time, crimson red and pink scarlet appear; flavonoids are present.

b. Ammonia test:

Filter paper immersed into an ethanolic drug portion was subjected to ammonical vapors. A yellow-colored patch appears on the filter paper.

c. Alkaline reagent test:

Add several droplets of sodium hydroxide solution to the test solution. A bright golden glow develops. It turns colorless when a few drops of weak acid is poured. Flavonoid presence is detected.

d. Lead acetate solution needs to be introduced to the little quantity of residual. It contains precipitate which is yellow in color**.**

Experimental animals [8]

The study was conducted by using albino rats, 200–300 g in weight, of either sex, from the Central Animal House at the Appasaheb Birnale College of Pharmacy in Sangli, Maharashtra. In compliance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) rules, the experiment carried out. The institutional Animal Ethical Committee granted permission to the experimental protocol. (IAEC/ABCP/20/2020-21). These animals were utilized as test subjects to check diuretic impact. Prior to conducting the studies, the animals were housed in typical husbandry settings for a duration of 15 days to enable for an adapt. Every rat was kept in six metallic cages at a constant temperature of 22±2°C.

Acute toxicity study [9, 10, 11]

Determination of LD50: Using albino rats (16–22g) of Mail or female gender were maintained as per regulations of

CPCSEA.The lethal dose was assessed. For minimum period of 3hrs prior to start experimental procedure, no food & water were given to experimental animals. Extract was administered in one dosage portion then mortality rate was monitored within 2 days while running experimental procedure. The next dose of the extract was calculated using the up-and-down approach with preference of OECD recommendations No. 423. The highest dosage tested (5000 mg/kg) for LD50, at the moment when the animals exhibited no acute poisoning symptoms. There were no obvious alterations in the treated rats' viscera. After a whole day, the animals' mortality was noted.

Evaluation of diuretic activity- (Modified Lipschitz test) [12, 13, 18, 19]

The animals will be kept in typical humidity and temperature conditions that endure a three-day adaption period. There will be five distinct groups (n = 6) developed from the animals. Group 2 will receive the standard diuretic, furosemide (Lasix, SANOFI AVENTIS, i.p.20mg/kg). Group 1 will receive common saline sample (25 ml/kg, orally) as the negative control. The ethanolic extract of Balanite aegyptiaca will be administered to Groups 3, 4, and 5 in a normal saline solution, while the diuretic efficacy will be assessed by modified Lipschitz index where normal saline was administered as a hydrating fluid. After ethanolic extract treatment in groups 3, 4, and 5 at minimal (100 mg/kg), midal (250 mg/kg) & elevated (500 mg/kg) doses in normal saline. Normal saline (15 ml/kg) was provided to test animals for hydration purpose and put in experimental carrier (3/cage) these were created specifically to keep urine and feces separate while being maintained at 21°C±0.5°C. In the end, the entire quantity of urine recovered after 5 and 24 hours was counted. While course of activity exposure of water or food was strictly prohibited in testing animals. Numerous parameters were measured and estimated, including the volume of urine as well as the concentrations of potassium, sodium, and chloride in the urine.

Preparation of Kidney homogenates [14]

Towards end of Experimental procedure, with help of decapitation experimental animals were sacrificed. Specialized heparinized tube was preferred to collect blood from arterial vein then allow for centrifugation. Further biochemical analysis was assessed with collected plasma sample which was maintained properly at -20^0c.Kidney portion was dissected properly then adopt appropriate cleaning to remove any debris and properly stored for further concern biochemical analysis.

Determining urine electrolyte content

Quantitative estimation of electrolyte (Sodium, Potassium & chlorine) from urine sample of experimental animals was done with Ion sensitive electrode.

Statistical evaluation

Experimental results were expressed with help of different statistical parameters like mean, SEM etc. Using Graph Pad Prism software, a one-way ANOVA and the Dunnett's t method was implemented on view of stat investigation. Whenever p value was less than 0.001, a correlation was deemed statistically significant.

RESULT

Phytochemical screening

Phytochemical investigation of Ethanolic extract of Balanite aegyptiaca fruit revealed the presence for alkaloids, flavonoids, saponins & tannins. It was blind for coumarins.

Acute toxicity

At a stage of elevated 5000 mg/kg dosage per body weight, no overt toxicity signs or other unusual behavior were seen in the test animals in the acute toxicity investigation. For the purpose of further examination, doses such as 1/20th, 1/10th, and 1/5th were selected from lethal dose & believed to 100 mg/kg, 250 mg/kg, and 500 mg/kg, as a minimum, middle and high elevated as per concern manner.

Diuretic effects

The ethanolic extract of Balanite aegyptiaca fruit delivered intraperitoneally (i.p.).urine formation was increased linearly with rising dose concentration **(Figure 1)**. At 250 mg/kg and 500 mg/kg doses, respectively, there was a 2. and 3.0 times increase in urine output as compared to the control group **(Table 1)**. The extracts' diuretic index values at 250 and 500 mg/kg doses, respectively, were 2.06 and 2.66, suggesting a good diuretic efficacy at this dose level **(Table 3)**. In lieu of furosemide, the Lipschitz values showed that the ethanolic extract of Balanite aegyptiaca fruit exhibited 5%, 33%, and 50% diuretic efficacy at dosages of 100, 250, and 500mg/kg.

Table 1: The impact of Balanite aegyptiaca fruit on urine volume

Sample (mg/kg)	Urine output
Control	3 ± 0.256
100mg/kg	4 ± 0.256
250mg/kg	6 ± 0.256*
500mg/kg	9.5 ± 0.256**
Standard	20 ± 0.256**

The data is expressed as the mean standard error; *p < 0.001 relative for vehicle, **p < 0.001 relative for standard

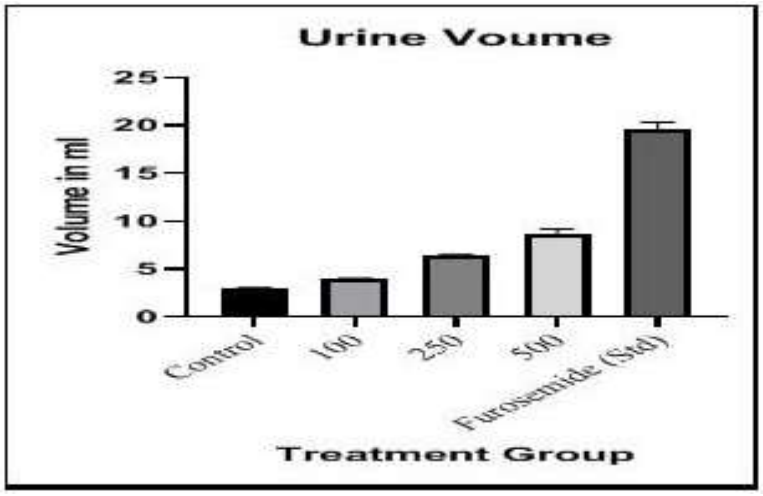


Figure 1: Graph of Urine volume Vs treatment group

Urinary electrolyte excretion and Urine pH

Significant natriuretic effects were induced by an ethanolic extract of Balanite aegyptiaca fruit with linear increase in dosage concentration. This effect peaked at 500 mg/kg **(Figure 2)**. After test extracts was administered intraperitoneally, there was a dose-dependent rise in removal of urine Sodium, potassium & Chlorine **(Figure 2)**. Particularly, the potassium excretion concentration was nearly identical at the 500 mg/kg dose while combined with the group that received furosemide treatment. The removal of electrolytes such as sodium, potassium etc in the urine samples of the treated groups increased gradually, as indicated by the saliuretic index values as well. In order to figure out how much each electrolyte excretes in relation to the others, the Na+/K+ ratio was also estimated. The Na+/K+ ratio results **(Table 3)** demonstrated that as the dosage increased, K+ excretion was comparatively larger than Na+ excretion. There was no apparent difference in the pH of fresh urine samples between the treatment and control groups **(Table 4)**. Additionally, serum parameters were found within the usual range **(Table 5)**. While there was no any considerable alteration in normal size and shape of kidney fraction within different groups (Control, Test group & standard) of Experimental animals **(Figure 4)**.

Table 2: Efficacy of oral *Balanite aegyptiaca* fruit's ethanolic extract rehabilitation on urine electrolyte excretion

Sr. No.	Sample	Na^+ (mmol/L)	K^+(mmol/L)	Cl^-(mmol/L)
1	Control	126.33 ± 9.92	53.83 ± 3.26	83.66 ± 3.19
2	100mg/kg	135.66 ± 5.53	59.83 ± 5.93	86.5 ± 5.35
3	250mg/kg	161.51 ± 7.28*	77.83 ± 5.12*	106.5 ± 5.51*
4	500mg/kg	165.52 ± 7.86 **	79 ± 6.06**	118.16 ± 6.9**
5	Standard	183.33 ± 8.96**	88.16±5.67**	127.66 ± 7.3**

Values are offered as the SEM of the mean. ANNOVA by using student's t test, with *p < 0.001 related to control and **p < 0.001 related to standard furosemide

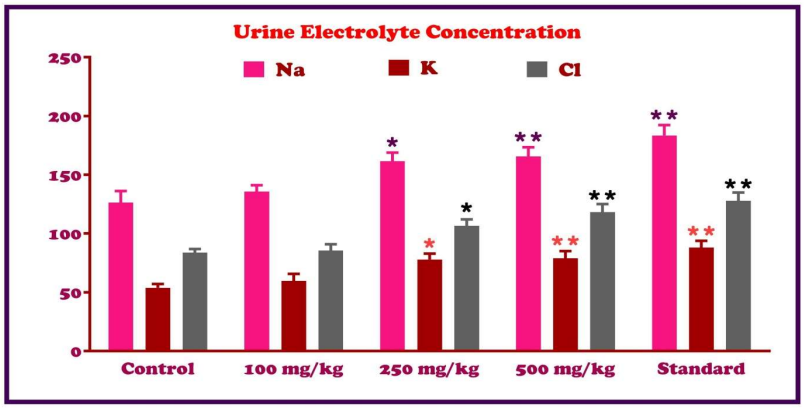


Figure 2: Graph of Urine electrolyte concentration Vs treatment group

Table 3: Impact of Balanite aegyptiaca fruit on urinary volume index & electrolyte concentration index

Drugs (mg/kg)	Diuretic index	Lipschitz index	Na^+ index	Cl^- index	K^+ index
Control	1	-	1	1	1
100mg/kg	1.33	5	1.16	1.13	1.16
250mg/kg	2.06	3.3	1.30	1.34	1.39
500mg/kg	2.66	2.10	1.35	1.46	1.75
Standard	6.66	-	1.44	1.52	1.84

Diuretic index = Urine amount in the test group / the amount of urine in control group.
Lipschitz index = test group's mean urine volume / the reference group's mean urine volume.
Na+ index compares the amount of sodium excreted by the test group and the Vehicle group.
K+ index = compares the amount of Potassium excreted by the test group and the Vehicle group Cl− index = compares the amount of Chloride excreted by the test group and the Vehicle group

Table 4: Urine P^H of treatment group

Sample (mg/kg)	Urine P^H
Control	6.1 ±0.02867
100mg/kg	6.5±0.02867
250mg/kg	6.6 ±0.02867*
500mg/kg	6.6 ±0.02867**
Standard	7.1 ±0.02867**

Values appear as the SEM of the mean. ANOVA by using student t test, with *p < 0.001 related to control and **p < 0.001 related to standard Furosemide.

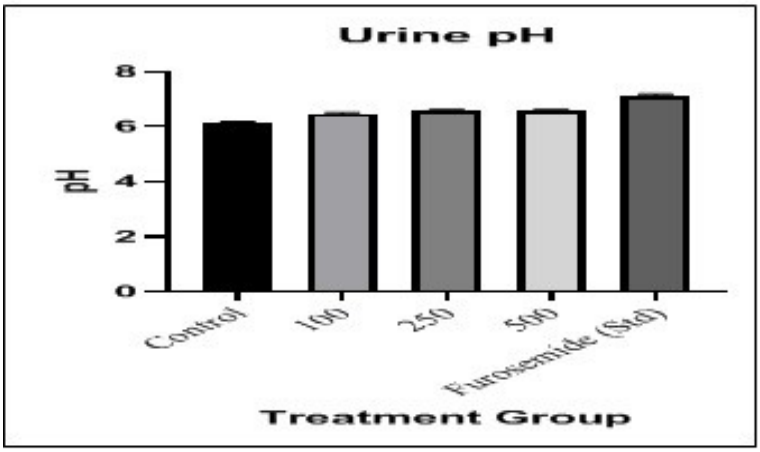
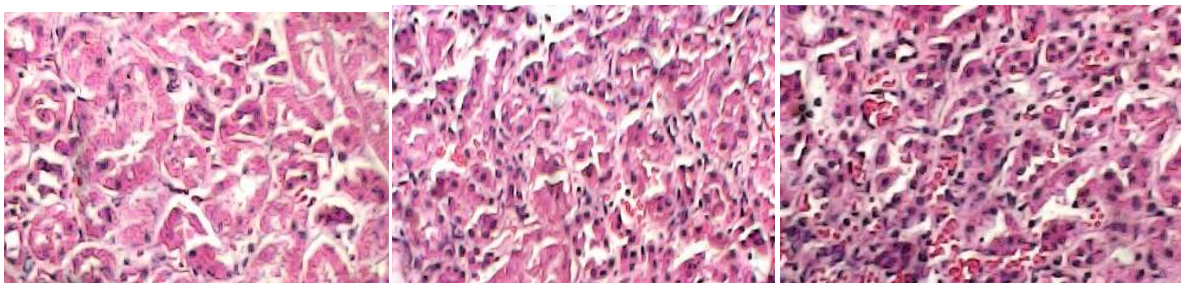


Figure 3: Graph of Urine P^H of treatment group



Control Test Standard

Figure 4: Kidney homogenates for different groups of test animals rats

Table 5: Impact of Balanite aegyptiaca fruit's ethanolic extract on serum parameters

Sr. No.	Sample	Albumin	Creatinine	Urea	Glucose
1	Control	41.7±0.03018	0.616 ±0.02462	22.13 ± 0.01441	94.3 ± 0.01379
2	100mg/kg	43.2±0.03018	0.636 ±0.003464*	24.3 ± 0.01441	97.33 ±0.01379*
3	250mg/kg	44.4±0.03018*	0.764 ± 0.0867	24.58 ±0.01441*	96.24 ±0.01379*
4	500mg/kg	44.8±0.03018**	0.846 ±0.003464**	25.82 ±0.01441**	98.16 ±0.01379**
5	Standard	44.4±0.03018**	0.764 ±0.003464**	24.87 ±0.01441**	98.12 ±0.01379**

Values are presented as the SEM of the mean.ANOVA by using student t test, with *p < 0.001 related to control and **p < 0.001 related to standard.

DISCUSSION [13, 15, 16, 17, 19]

In healthy adult male Wistar rats, Balanite aegyptiaca fruit assessed for diuretic potential and contrasted with that of furosemide. It is a common agent who targets ascending loop of henle from urinary tract.Diuresis is characterized by two things: Promotes urinary excretion of electrolytes and potentiate urinary output. These processes occurred due to inhibition of renal tubule reabsoption of not only electrolytes but also water. According to Table no.1and 2, there was considerable rise in urine volume and measurable fall in electrolyte reabsorption (p 0.001) in dose dependent manner, suggesting much more chances for favorable and causative activity, probably mediated through receptor interaction. Values from Table no 2 were clearly suggested that it was affected on Sodium, potassium and Chlorine symporter. So much more possibility for possessing similar kind of MOA to that of Loop diuretics. It is also quite capable to show interaction with active target receptors sites from ascending loop of henle.

Balanite aegyptiaca fruit is widely studied for a considerable presence of active chemical constituents such as alkaloids, flavonides etc along with wide range of medicinal applications and exceptionally safe nature. Consistent with the previously described research, our initial phytochemical analysis verified the existence of alkaloids, anthraquinones, flavonoids, saponins, and tannins in the fruit of Balanite aegyptiaca. Dieresis has been demonstrated to be produced by alkaloids, flavonoids, saponins, and organic acids; this suggests that the

secondary metabolites may be the cause of the diuretic effect of Balanite aegyptiaca fruit in our investigation.

If the treated groups' diuretic index values are more than 1.50, the extract is said to have good diuretic activity. The treated groups (III and IV) in our study had diuretic index values of 2.06, 2.66, respectively. This suggests that Group IV had a 3-fold increase in urine volume. Additionally, Lipschitz values demonstrated that the plant exhibited 46% of the diuretic efficacy with comparison of furosemide at the maximum dose (500 mg/kg) (Table no 3). Further fractionation and separation of the pure active chemical constituent responsible for this plant extract's diuretic activity is required due to the crude extract's weak diuretic activity in comparison to the conventional medication.

One of the primary causes of the abnormalities in arterial blood pressure and one of the significant extrinsic variables in idiopathic hypertension is thought to be an excess of Na+ in bodily fluids. Our research shows that the intraperitoneal (i.p.) administration of Balanite aegyptiaca fruit caused notable natriuretic effects upon increased in dosage concentration when compared to control. This discovery emphasizes the fruit of Balanite aegyptiaca's medical application in the management of hypertension. On the other hand, the amount of Na+ excretion at the 500 mg/kg dose was significantly less than standard furosemide, suggesting a decreased likelihood of causing hyponatremia.The urine excretion of potassium electrolyte, particularly upon exposure of 500 mg/kg dose, was similar to standard, indicating Balanite aegyptiaca fruit will quite capable to target ascending loop of henle.Similarly, urinary excretion of Potassium was also shown direct proportion to that dosage concentration.

CONCLUSION

According to the current study, there is a great chance that Balanite aegyptiaca fruit will be employed as a diuretic. However, in order to extract the powerful diuretic phytochemical ingredients, activity guided fractionation is necessary.

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