

Diuretic Activity of the Aqueous Bark Extract of *Ziziphus jujuba* Mill. in Wistar Rats

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ABSTRACT

Ziziphus jujuba Mill. (Ber) has long been prized for its antioxidant, antihypertensive, anti-inflammatory, and diuretic qualities. The purpose of this study was to assess *Z. jujuba*'s aqueous bark extract's (AZJBE) diuretic capability in Wistar rats. Thirty male Wistar rats were split into five groups: AZJBE at doses of 100, 200, and 400 mg/kg, standard (furosemide 10 mg/kg), and control. For seven days, urine volume, color, pH, and specific gravity were measured every 12 hours, 24 hours, and every day. The effectiveness and safety of diuretics were evaluated by analyzing renal function measures and urine electrolytes. Alkaloids, flavonoids, phenolics, glycosides, and tannins were found via phytochemical screening. Similar to furosemide, AZJBE increased urine production and salt excretion in a dose-dependent manner while preserving potassium balance. Renal safety was confirmed by the urine's continued pale-yellow color, no discernible changes in pH or specific gravity, and the absence of protein or glucose. The findings confirm the traditional use of AZJBE as a natural, potassium-sparing diuretic for the treatment of hypertension and fluid retention by indicating that it has strong, dose-dependent diuretic activity.

Keywords: *Zizipus jujuba*. Diuretic , wistar rats, bark extract, sodium excretion.

INTRODUCTION

About one in three people worldwide suffer from hypertension, which is a significant risk factor for cardiovascular illnesses and early death. This illness increases the risk of heart failure, stroke, myocardial infarction, renal impairment, and other major health issues. The number of people with hypertension worldwide increased from 650 million to 1.3 billion between 1990 and 2019, affecting approximately 30% of men and 50% of women between the ages of 50 and 75(1). Over 75% of people with hypertension reside in poor nations, where the majority of hypertension-related deaths take place, and over half of them are uninformed that they have the condition. An estimated 100 million people in India had hypertension in 2000; estimates indicate that number would rise to 150 million by 2025, with a particularly high prevalence in Maharashtra (2).

Therapeutic therapies and lifestyle changes are helpful ways to manage hypertension. Diuretics, sympatholytic drugs, renin inhibitors, ACE inhibitors,

calcium channel blockers, β -adrenergic and α/β -adrenergic antagonists, and vasodilators are examples of conventional antihypertensive medications that have been shown to be effective. However, they frequently have side effects, such as myalgia, arrhythmia, visual disturbances, dermal rash, emesis, renal failure, fatigue, cephalalgia, and edema(3). Because of their lengthy history, safety record, and potential usefulness, complementary and alternative therapies—in particular, herbal medicines—are gaining popularity. 75–80% of people worldwide use herbal treatments to treat a variety of ailments, including hypertension. Plants including *Cassia occidentalis*, *Leersia hexandra*, *Adansonia digitata*, *Phyllanthus amarus*, *Hibiscus sabdariffa*, *Ficus carica*, and *Mangifera indica*(4-7). have been shown in ethnobotanical investigations to have diuretic and antihypertensive properties. According to reports, these plants help regulate blood pressure by increasing urine production and promoting sodium excretion.

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ziziphus jujuba Mill., often called Ber, is a perennial plant native to tropical Asia that bears fruit and is a member of the Rhamnaceae family. Burns, inflammation, fever, insomnia, anxiety, cancer (melanoma), bacterial and fungal infections, ulcers, cognitive disorders, spasticity, fertility regulation, hypotension, nephritis, cardiovascular disorders, oxidative stress, immunological dysfunction, and wound healing are just a few of the conditions for which it has historically been used(8). *Z. jujuba* is used empirically as a diuretic in traditional Chinese medicine to treat hypertension. Its pharmacological effects may be attributed to the presence of alkaloids, flavonoids, tannins, phenolics, and cardiac glycosides found in phytochemical investigations of its bark extract. An important benefit over traditional loop diuretics is that preliminary research indicates that herbal diuretics can greatly increase urine volume and salt and chloride excretion without affecting potassium loss. Although *Z. jujuba* bark extract has been used traditionally, there is little experimental data to support its diuretic action, which emphasizes the necessity for rigorous in vivo investigation.

MATERIALS AND METHODS

Chemicals

All chemicals and reagents used in this study were of analytical grade and procured from standard commercial suppliers.

Animals

Animals Wistar rats were obtained from the National Institute of Biosciences, Dhangwadi, Pune, and were maintained at Konkan Gyanpeeth Rahul Dharkar College of Pharmacy and Research Centre, Karjat, Maharashtra, where the study was also conducted. The animals were housed in polypropylene cages under controlled conditions (temperature 24 ± 2 °C) with free access to standard laboratory diet and water. All rats were acclimatized for 7 days prior to the commencement of the study.

Plant Material and Preparation of Aqueous Bark Extract

Plant Material and Preparation of Aqueous Bark Extract The bark of *Ziziphus jujuba* Mill. was

collected from Ambejogai, Maharashtra in August 2025. The plant material was authenticated at Alsarini, Mumbai, Maharashtra. The bark was thoroughly washed with water and shade-dried at room temperature. After drying, it was ground into a fine powder using an electric blender. A total of 500 g of the powdered bark was subjected to extraction using a Soxhlet apparatus with distilled water as the solvent for 48 hours. The resulting extract was filtered through Whatman No. 3 filter paper, and the filtrate was dried in an oven. The final aqueous bark extract yielded 11.68% w/w of solid powder, which was stored in an airtight container in a cool, dry place away from direct sunlight [17].

Phytochemical Evaluation of the Extract

The presence or absence of significant bioactive components was ascertained by qualitative phytochemical screening of *Z. jujuba*'s aqueous bark extract. Using common phytochemical techniques, the investigation concentrated on phenolics, terpenoids, flavonoids, tannins, alkaloids, sterols, anthraquinones, and cardiac glycosides [18].

Experimental Design

The diuretic activity of the aqueous bark extract of *Ziziphus jujuba* Mill. (AZJBE) was evaluated using thirty male Wistar rats, each weighing between 200–230 g. The animals were randomly allocated into five groups ($n = 6$) and housed individually in metabolic cages for acclimatization 24 hours prior to experimentation. All animals were fasted overnight with free access to water.

The treatment groups were as follows:

- Group I (Control): Normal saline (1mL/100g, p.o.)
- Group II (Standard): Furosemide (10 mg/kg, p.o.)



- Group III: AZJBE (100 mg/kg, p.o.)
- Group IV: AZJBE (200 mg/kg, p.o.)
- Group V: AZJBE (400 mg/kg, p.o.)

Urine samples were collected at 12-hour and 24-hour intervals following administration, and cumulative urine volume was recorded.

For the sub-acute diuretic study, a one-day washout period was provided following the acute phase. The treatment continued for seven consecutive days, during which animals had free access to food and water. On the eighth day, the rats were euthanized in a CO₂ chamber. Blood samples were collected via the abdominal aorta and centrifuged at 3000 rpm for 10 minutes at 4 °C. The obtained plasma and urine samples were stored at -20 °C for further biochemical analyses.

Electrolyte concentrations (Na⁺, K⁺, and Cl⁻) were determined using flame photometry and argentometric titration methods. Additional biochemical parameters, including urea and creatinine, were analyzed using standard established procedures(9, 10).



Figure 1: Collected Bark

Diuretic Influence

Based on the mean urine output measured at the 12th and 24th hours (acute study) and from day 1 to day 7 (sub-acute study), the percentage of urine excretion, diuretic action, and diuretic index were computed for each experimental group. These parameters were evaluated using established estimation methods and techniques documented in the literature.

$$\begin{aligned} & \% \text{ Urinary excretion} \\ &= \frac{\text{Total urinary output}}{\text{Total volume of liquid administered}} \times 100 \end{aligned}$$

$$\begin{aligned} & \text{Diuretic action} \\ &= \frac{\text{Urinary excretion of the test group}}{\text{Urinary excretion of the control group}} \end{aligned}$$

$$\begin{aligned} & \text{Diuretic index} \\ &= \frac{\text{Diuretic action of the test group}}{\text{Diuretic action of the control group}} \end{aligned}$$

The potency of the diuretic reaction was evaluated by quantitatively classifying the diuretic index. Strong diuretic activity was defined as a number larger than 1.5, moderate activity as a value between 1.0 and 1.5, light activity as a value between 0.72 and 0.99, and no significant diuretic effect as a value below 0.72(11, 12).

Analysis of biochemistry and Physiochemistry

The methodology outlined by Ntchapda et al. was used to analyze the biochemical parameters related to diuresis(4). A technique called flame photometry was used to determine the sodium (Na⁺) and potassium (K⁺) ion concentrations in urine and plasma. A UV-visible double-beam spectrophotometer was used to determine the amounts of creatinine urea, glucose, albumin, and other electrolytes in biological fluid samples. Urinary osmolarity and natriuresis were measured at the peak diuretic response in both the reference (furosemide) and Z. jujuba extract-treated groups. Aldosterone concentration was evaluated by radioimmunoassay (Aldo RIACT assay kit), while plasma and urine osmolarity was assessed by cryometry using an osmometer.

$$\begin{aligned} & \text{Osmolar clearance} \\ &= \frac{\text{Urinary osmolarity} \times \text{urine flow}}{\text{Plasma osmolarity}} \end{aligned}$$

$$\begin{aligned} & \text{Free water clearance} \\ &= \text{Urine flow} \\ &\quad - \text{Osmolar clearance} \end{aligned}$$

The following standard formulae were used to determine the saluretic index and natriuretic activity:

$$\begin{aligned} & \text{Natriuretic activity} \\ &= \frac{\text{Concentration of Na in the urine of the group}}{\text{Concentration of Na in the urine of the same group}} \end{aligned}$$

Saluretic index

$$= \frac{\text{Concentration of electrolyte in the urine of the test group}}{\text{Concentration of electrolyte in the urine of the control group}}$$

The glomerular filtration rate (GFR) was determined from the creatinine clearance (Ccr) using the following standard equation:

$$\text{Creatinine clearance} = \frac{\text{Urinary creatinine concentration} \times \text{urine volume at 24th}}{\text{Plasma creatinine concentration}}$$

$$\text{Glomerular filtration rate (GFR)} = \text{Creatinine clearance} \times 0.81$$

Analysis of Data

The mean $\pm$ standard error of the mean (SEM) was used to express all data. One-way and two-way analysis of variance (ANOVA) and Tukey's post hoc

test for multiple comparisons were used in the statistical studies. At $P < 0.05$, differences were determined to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical Examination of AZJBE

The aqueous bark extract of *Ziziphus jujuba* (AZJBE) possessed a variety of biologically active substances, including alkaloids, flavonoids, triterpenoids, saponins, and tannins, based on qualitative phytochemical screening. Table 1 provides an explanation of the findings.

Table I: phytochemical components of ziziphus Jujuba's aqueous bark extract

Phytochemical compounds	Results
Total phenolics	+++
Terpenoids	+
Flavonoids	+++
Tannins	+++
Saponins	+++
Anthraquinones	++
Cardiac glycosides	+++

(+) Present, (-) Absent

AZJBE's Impact on Urinary Excretion

Ziziphus jujuba (AZJBE) aqueous bark extract consistently demonstrated a dose-dependent diuretic response (Table II. Table III). In comparison to the control group, the reference medication, furosemide,

increased urine output by about 124% at 12 hours and 109% at 24 hours. By analogy, AZJBE at 400 mg/kg caused a significant rise in urine volume of around 97% at 12 hours and 86% at 24 hours, reflecting a diuretic efficacy that's very similar to furosemide.

Table II: 24-Hour Urine Volume, Diuretic Index, And Diuretic Action In Rats Treated With Azjbe

Groups	12 Hrs. Urine Volume			24 Hrs. Urine Volume		
	Urine (ml)	Diuretic Index	Diuretic Action	Urine (ml)	Diuretic Index	Diuretic Action
Control	2.9 $\pm$ 0.60	1.00 $\pm$ 0.00	0.48 $\pm$ 0.12	5.8 $\pm$ 1.36	1.00 $\pm$ 0.00	0.49 $\pm$ 0.11
Std	6.5 $\pm$ 0.59 ***	3.09 $\pm$ 0.92	1.00 $\pm$ 0.00	12.1 $\pm$ 1.30***	2.59 $\pm$ 0.75	1.00 $\pm$ 0.00
Azjbe 100mg/Kg	2.9 $\pm$ 0.14	1.41 $\pm$ 0.33	0.47 $\pm$ 0.05	4.9 $\pm$ 0.10	1.12 $\pm$ 0.25	0.33 $\pm$ 0.07
Azjbe 200mg/Kg	3.9 $\pm$ 0.13**	1.87 $\pm$ 0.56	0.62 $\pm$ 0.06	7.4 $\pm$ 0.10**	1.69 $\pm$ 0.40	0.60 $\pm$ 0.09
Azjbe 400mg/Kg	5.7 $\pm$ 0.16	2.61 $\pm$ 0.67	0.91 $\pm$ 0.08	10.8 $\pm$ 0.19	2.47 $\pm$ 0.59	0.94 $\pm$ 0.10



Table Iii: 7-Day Cumulative Urine Volume In Experimental Groups

Days / Groups	Control	Std	Azjbe 100mg/Kg	Azjbe 200mg/Kg	Azjbe 400mg/Kg
Day 01	5.76 ± 1.14	12.36 ± 0.57***	4.83 ± 0.08	7.43 ± 0.23*	10.90 ± 0.15***
Day 02	6.16 ± 0.37	13.73 ± 1.47***	5.46 ± 0.08	8.58 ± 0.24**	12.13 ± 0.08***
Day 03	7.66 ± 0.51	15.43 ± 0.98***	6.15 ± 0.08	9.31 ± 0.06**	13.45 ± 0.07***
Day 04	7.21 ± 0.87	17.56 ± 0.93***	6.98 ± 0.10	10.45 ± 0.08**	15.45 ± 0.07***
Day 05	7.76 ± 0.20	23.00 ± 1.21***	9.05 ± 0.09*	13.68 ± 0.06***	20.25 ± 0.07***
Day 06	7.40 ± 0.34	25.50 ± 2.14***	10.15 ± 0.07**	15.25 ± 0.07***	22.25 ± 0.07***
Day 07	7.75 ± 0.79	28.20 ± 1.41***	11.08 ± 0.12***	16.95 ± 0.07***	24.65 ± 0.07***

AZJBE's Impact on Urinary Electrolyte Excretion

When compared to the control, AZJBE dramatically raised the levels of Na⁺, K⁺, and Cl⁻ in the urine (Table IV, Table V, Table VI, Table VII) While AZJBE at 400 mg/kg produced about a 6-fold rise, indicating

high natriuretic and saluretic activity, furosemide significantly increased Na⁺ excretion (~8-fold vs. control). Unlike loop diuretics, which frequently result in hypokalemia, a slight increase in K⁺ levels indicated that the extract maintained a balanced electrolyte profile.

Table Iv: Electrolyte Excretion After 24 Hours Of Treatment

Group	Na ⁺ (Meq/L)	K ⁺ (Meq/L)	Cl ⁻ (Meq/L)
Control	23.69 ± 1.38	21.77 ± 1.12	29.54 ± 2.08
Std	33.16 ± 1.07***	28.18 ± 1.56***	33.79 ± 0.97***
Azjbe 100 Mg/Kg	16.03 ± 0.86***	15.74 ± 1.28***	16.44 ± 1.05***
Azjbe 200 Mg/Kg	17.76 ± 0.37***	17.50 ± 0.66***	20.13 ± 2.28***
Azjbe 400 Mg/Kg	26.36 ± 1.08***	23.82 ± 0.77***	25.17 ± 0.81***

Table II: 7-Day Na⁺ Excretion In Experimental Rats

Days / Groups	Control	Std	Azjbe 100mg/Kg	Azjbe 200mg/Kg	Azjbe 400mg/Kg
Day 01	23.69 ± 1.38	34.18 ± 1.46 ***	13.59 ± 0.18 ***	20.40 ± 0.17 **	30.40 ± 0.16 ***
Day 02	23.46 ± 1.13	37.04 ± 1.22 ***	14.62 ± 0.19 ***	22.19 ± 0.19	32.90 ± 0.15 ***
Day 03	24.95 ± 1.29	41.30 ± 1.87 ***	16.45 ± 0.18 ***	24.74 ± 0.19	36.71 ± 0.13 ***
Day 04	24.15 ± 0.92	47.74 ± 1.83 ***	19.14 ± 0.17 ***	28.62 ± 0.12 ***	42.51 ± 0.17 ***
Day 05	24.33 ± 1.16	54.20 ± 1.95 ***	21.69 ± 0.21 **	32.58 ± 0.22 ***	47.72 ± 0.10 ***
Day 06	24.97 ± 1.65	57.04 ± 1.89 ***	22.71 ± 0.09 **	34.18 ± 0.13 ***	50.81 ± 0.09 ***
Day 07	26.72 ± 1.11	60.28 ± 1.10 ***	24.23 ± 0.15 *	36.16 ± 0.13 ***	53.64 ± 0.10 ***

Table III: 7-Day K⁺ Excretion In Experimental Rats

Days / Groups	Control	Std	Azjbe 100mg/Kg	Azjbe 200mg/Kg	Azjbe 400mg/Kg
Day 01	21.77 ± 1.12	28.30 ± 1.61 *	11.23 ± 0.11 ***	16.79 ± 0.14 **	25.34 ± 0.11 *
Day 02	22.39 ± 1.69	30.41 ± 2.21 **	12.18 ± 0.10 ***	18.36 ± 0.09 **	27.48 ± 0.09 **
Day 03	24.32 ± 1.11	31.68 ± 1.57 **	13.02 ± 0.09 ***	19.08 ± 0.11 **	28.86 ± 0.11 **
Day 04	25.80 ± 1.12	33.29 ± 3.13 **	13.42 ± 0.11 ***	20.46 ± 0.11 **	30.49 ± 0.10 **



Day 05	24.86 ± 0.83	38.85 ± 1.51 ***	15.32 ± 0.12 ***	23.20 ± 0.16 **	34.97 ± 0.07 ***
Day 06	27.09 ± 0.73	41.04 ± 1.42 ***	16.08 ± 0.08 ***	24.84 ± 0.11 ***	36.67 ± 0.14 ***
Day 07	24.40 ± 0.98	43.74 ± 1.43 ***	17.40 ± 0.11 ***	26.26 ± 0.11 ***	39.64 ± 0.10 ***

Table IV: 7-Cl⁻ Excretion In Experimental Rats

Days / Groups	Control	Std	Azjbe 100mg/Kg	Azjbe 200mg/Kg	Azjbe 400mg/Kg
Day 01	23.93 ± 1.04	30.57 ± 1.73*	12.2±0.10***	18.38 ± 0.08**	27.42 ± 0.14*
Day 02	25.47 ± 1.22	32.26 ± 1.33**	13.27±0.10***	19.59 ± 0.08**	29.79 ± 0.10**
Day 03	25.20 ± 1.16	33.12 ± 1.70**	13.77±0.11***	20.37 ± 0.10**	31.09±0.10***
Day 04	25.37 ± 0.81	35.85 ± 2.17**	14.96±0.13***	21.62±0.09***	33.23±0.09***
Day 05	26.56 ± 1.52	39.86±2.29***	16.18±0.13***	24.29±0.12***	37.01±0.12***
Day 06	25.25 ± 1.40	42.38±2.03***	16.81±0.14***	26.07±0.10***	39.61±0.04***
Day 07	23.63 ± 0.82	46.90±1.54***	18.22±0.10***	28.2±0.12***	41.68±0.09***

Table I To Vii: Values Are Mean ± Sem, N = 6; Analyzed By Two-Way Anova Followed By Tukey's Post Hoc Test (*P ≤ 0.05, **P ≤ 0.01, ***P ≤ 0.001 Vs. Control.)

AZJBE's Impact on Urine Physical Properties

Urine Color: The urine had no abnormal color change and all groups produced pale yellow urine.

Urine pH: There was not a significant variance in urine pH across the groups, indicating that the extract had no effect on urine acidity.

Specific Gravity: The result was no apparent change, demonstrating that the extract had no impact on solute density or urine concentration.

AZJBE's Impact on Serum and Urine Biochemical Markers:

Albinuria and Glucosuria

There was no evidence of renal or metabolic impairment in any of the groups' albumin and glucose levels.

Urea and Creatinine

Urinary urea and creatinine excretion raised in a dose-dependent manner after AZJBE administration, whereas comparable serum levels decreased substantially. Results similar to those of furosemide were obtained with the 400 mg/kg dose, confirming accelerated renal clearance and glomerular filtration rate (GFR). These results suggest that AZJBE has

nephroprotective effects and improves the removal of nitrogenous waste.

CONCLUSION

The aqueous bark extract of *Ziziphus jujuba* (AZJBE) indicates strong and dosage-dependent diuretic effect, with the 400 mg/kg dose exhibiting efficacy comparable to furosemide, according to the study's findings. The extract demonstrated both efficacy and safety by successfully raising urine output and electrolyte excretion while preserving normal urine pH, color, and specific gravity. The increase in glomerular filtration rate and creatinine clearance indicates improved renal function without nephrotoxic consequences. These findings highlight *Ziziphus jujuba*'s potential as a safer herbal substitute for treating fluid retention and associated renal problems and validate its historic use as a natural diuretic. It is advised that the active phytoconstituents causing the reported activity be isolated, characterized, and their methods of action investigated through molecular and clinical study.

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