

Original Article

**IN VITRO ANTIUROLITHIATIC ACTIVITY OF MACERATED AQUEOUS EXTRACT OF
TERMINALIA BELERICA BY USING TURBIDITY METHOD**

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ABSTRACT

Objective: To evaluate the anti-urolithiatic activity of macerated aqueous extract of *Terminalia belerica* by using turbidity method

Method: The present study was used to study the inhibitory effect of the *Terminalia belerica* on urinary stone formation. The aim of study was to examine the *In vitro* antiurolithiatic activity of macerated aqueous extract of *T.belerica* was to estimate inhibitory activity of aqueous extract on the formation of urinary stone. Cystone was used as a positive control. Anti urolithiatic study was performed by turbidity method.

Result: The percentage inhibition shown by aqueous extract at 20µg/ml was 60% and with almost constant inhibition at 100µg/ml and 200µg/ml ranging between 72% and 80%. The percentage inhibition showed by aqueous extract of *Terminalia belerica* has significant compared to standard drug.

Conclusion: In future this drug can be performed in vitro and clinical study beneficial for people with avoiding adverse effect of modern medicinal drugs

Key words: antiurolithiatic activity, aqueous extract, *Terminalia belerica*, cystone,

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INTRODUCTION

Medicinal plants are widely used in non-industrialized societies, mainly because they are readily available and cheaper than modern medicines. The place of plants in medicine was radically altered in the 19th century by the application of chemical analysis. As chemistry progressed, additional classes of pharmacologically active substances were discovered in medicinal plants. In modern medicine, around a quarter of the drugs prescribed to patients are derived from medicinal plants, and they are rigorously tested. In other systems of medicine, medicinal plants may constitute the majority of what are often informal attempted treatments, not tested scientifically. The World Health Organization estimates, without reliable data, that some 80 percent of the world's population depends mainly on traditional medicine; perhaps some 2 billion people are largely reliant on medicinal plants. The use of plant-based materials, including herbal or natural health products with supposed health benefits, is increasing in developed countries. Herbal medicines have been in use since long before modern medicine existed; there was and often still is little or no knowledge of the pharmacological basis of their actions, if any or of their safety.

Nephrolithiasis or renal stone formation in the kidney is one of the oldest and most widespread diseases known to man. In India people living in different states utilize different plants for curing urolithiasis. Urolithiasis is derived from the Greek words "ouron" (urine) and "lithos" (stone). It is considered as the third most common affliction of the urinary tract. Urolithiasis is the deposition of formation of stones in any part of the kidney like ureters or the urinary bladder [1, 2].

Urine is normally supersaturated with most stone-forming salt components, as well as contains chemicals that prevent or inhibit crystal development in urinary tract. However, the presence of certain molecules raise the level of supersaturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation calcium oxalate stones represent up to 80% of analyzed stones. Calcium phosphate account for 15-25%, while 10-15% is mixed stones. The others are struvite 15-30%, cystine 6-10%, and uric acid stones 2-10% [3-5].

Urolithiasis in its different forms, is frequently encountered during urological complications. Some common causes are inadequate

urinary drainage, foreign bodies in the urinary tract, Microbial infections, Diet with excess oxalate and calcium, Vitamin abnormalities, Viz. Vitamin A deficiencies, Vitamin D excess, Metabolic disease like Hyperparathyroidism, Cystinuria, Gout and intestinal dysfunction [6]. Generally, stones of two type's i.e. non calcium and calcium stones are formed [7]. Calcium, Albumin, Creatinine, Urate and oxalate are some necessary analytical markers in serum and urine for clinical diagnosis of this type of urological disorders [8-10].

Terminalia belerica is a well-known medicinal plant and one of the ingredients of popular Ayurvedic formulation "Triphala". It contains numerous phytoconstituents such as Gallo-Tannic acid, bellericanin, ellagic acid, Gallic acid, termilignan, flavones and anolignan B, Tannins, Galloyl glucose and chebulaginic acid, phenyllembin, mannitol, glucose, Fructose and rhamnose, flavanoids and Saponin. These constituents were responsible for its various pharmacological activities like antioxidant, antimicrobial, anti-diarrheal, antidiabetic, antihypertensive, antispasmodic, bronchodilatory, hepatoprotective, anti-carcinogenic and anti-mutagenic properties [11-13].

In Indian traditional system of medicine the plant has been used in the treatment of gall bladder and urinary stone since many years. but its activity has not been reported previously. To justify the ancient and traditional practice of utilizing this plant in renal calculi this research work has been carried out. Therefore, the aim of present study was to examine the *in vitro* antiurolithiatic activity of Macerated *Terminalia belerica* Roxb. (Fam. Combretaceae) using aqueous extract by using turbidity method [14, 15].

MATERIALS AND METHODS

Plant material

The Fruits were collected from the nearby licensed crude drug distributors.

Preparation of plant extracts [16]

The washed and dried crude drug and performed crushing to coarse powdered form *Terminalia belerica* fresh core collected and size was reduced by mechanical grinding and passed through sieves.

Maceration process involves the separation of medicinally active portions of crude drugs, Based on the immersion of crude drug in a bulk of solvent or menstruum. Solid drug material taken in a stoppered container with about 750 ml of menstruum and allowed to stand for at least 3-7 d in a warm place with frequent shaking. The mixture of crude drug-containing solvent is filtered until most of liquid drains off. The filtrate and washing are combined to produce 1000 ml of the solution.

Coarse Solid drug material taken in a stoppered container with about 750 ml of Menstruum and allowed to stand for at least 3-7 d in a warm place with frequent shaking. The mixture of crude drug-containing solvent is filtered until most of liquid drains off. The filtrate and washing are combined to produce 1000 ml of the solution. The crude drug material is charged in the extractor, which is connected with a circulatory pump and spray distributor, along with number of connected tanks to receive the extraction solution. This is known as multiple stage extraction because the solvent added and circulated in the extractor containing drug is removed as extracted solution and is stored in the receiver tanks. This operation is repeated thrice. When the crude drug material is charged in the extractions, the stored solution is once again circulated through fresh drug and then removed as an extract. Likewise, after three extractions, the drug and removed from the extractor, again recharged with fresh drug and the whole cycle is repeated *in vitro* antiurolithiatic activity [17]

The effects of *Terminalia bellerica* extracts on calcium oxalate crystallization was determined by the time course measurement of turbidity changes due to the crystal nucleation and aggregation in the Human urine on the addition of 0.01M sodium oxalate. The precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer

UV/Vis was employed to measure the turbidity of the formation of calcium oxalate.

Study without inhibitor

A volume of 1 ml of human urine was transferred into the cell and 0.5 ml of distilled water to it and blank reading was taken. 0.5 ml of 0.01 M sodium oxalate was added to the previous volume, and the measurement is immediately started for a period of 400 sec.

Study with inhibitor

Extract was re-suspended in distilled water filtered and used at a final concentration of 20, 100 and 200 mg/0.5 ml were prepared. A mixture of 1 ml human urine and 0.5 ml of plant extract solution is versed in the cell. A blank reading was taken and then the volume of 0.5 ml of 0.01 M sodium oxalate was added and the measurement is immediately started for a period of 400 sec. The percentage of inhibition was calculated using the following formula

$$\% \text{inhibition} = \{1 - [Si/Sc]\} \times 100$$

Where; Si: slope of graph in the presence of inhibitor (Plant extract)

Sc: slope of graph without inhibitor (control)

RESULTS AND DISCUSSION

In vitro anti urolithiatic study

Percentage inhibition of the crystallization of calcium oxalate (CaOx) with different concentrations of aqueous extract of *T. bellerica*. It inhibited the crystallization in a concentration-dependent pattern. The percentage inhibition shown by aqueous extract at 20 µg/ml was 60 % and with almost constant inhibition at 100 µg/ml and 200 µg/ml is ranging between 72-80%. Absorbance vs Time of with inhibitor and without inhibitor shown below.

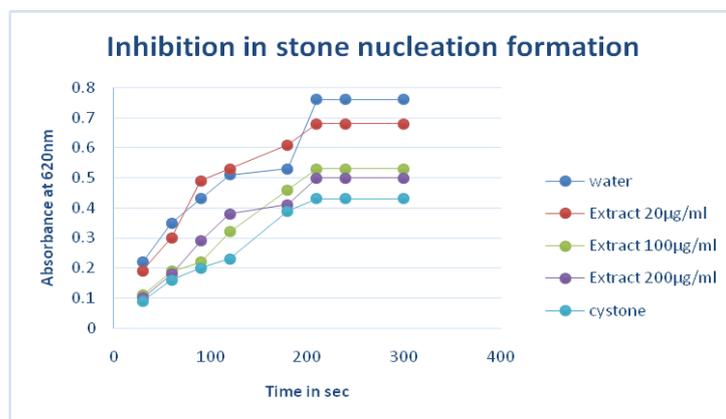


Fig. 1: Graph showing the inhibition in the stone nucleation formation with and without plant extract. Cystone as positive controls

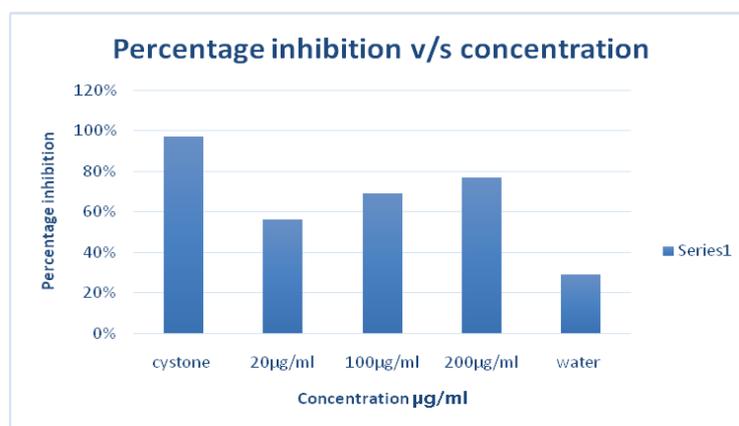


Fig. 2: The percentage inhibition of calcium oxalate of different concentration of aqueous extract of *Terminalia bellerica*

CONCLUSION

Many of the Terminalia species have been reported for antiurolithiatic activity. Fruits of *Terminalia belerica* for this study. Qualitative chemical test showed Alkaloid, Flavanoids, Steroids, Phenols, Saponins and Carbohydrates. The turbidity method has showed extremely significant action on urinary calculi. Turbidimetric method measures the turbidity in terms of calcium oxalate formation in human urine using a spectrophotometer at 620 nm and crystallization inhibition measured by turbidity reduction. The percentage of inhibition showed by aqueous extract of *Terminalia belerica* has significant compared to standard drug. In future this drug can be performed *In vivo* and clinical study beneficial for people with avoiding adverse effects of modern medicinal drugs.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICT OF INTERESTS

Nil

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