

Evaluation of *in vitro* Antiuro lithiatic Activity of *Gossypium Herbaceum*

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Abstract:

The present study was undertaken to evaluate the *in vitro* antiuro lithiatic activity of the medicinal plant *Gossypium herbaceum*. Both Ethanolic and Aqueous extracts showed their maximum efficiencies in the dissolution of calcium oxalate crystals. Ethanolic extract was even more efficient than Aqueous extract in dissolution of calcium oxalate crystals. Our results have clearly indicated that the aqueous and ethanolic extracts of *Gossypium herbaceum* were quite promising for further studies in this regard. In this study Neeri was used as standard drug.

Keywords: *In vitro* antiuro lithiatic activity, aqueous extracts, urolithiasis, *Gossypium herbaceum*

INTRODUCTION:

Urolithiasis is derived from the greek words ouron means urine and lithos means stone. Urolithiasis is characterized by the formation of the stone in the kidneys or urinary tract in a large number of people. Nearly 10-15% of the population is currently suffering from kidney stones.

Urinary tract stones (kidney stones) composed of calcium oxalate, either alone or mixed with calcium phosphate. World Health Organization(WHO) estimated that about 12% of men and 55% of women have at least one episode of kidney stone during their life time.[1] The cause of urolithiasis is still unknown but probably positive family history, overweight, obesity or increased body mass index (BMI).[2] Epidemiological studies revealed that urolithiasis is more common in men than in women and is more prevalent between the ages of 20-40 in both sexes. [3] Ammonium urate, mono sodium urate monohydrate, uric acid anhydrous, uric acid mono and di hydrate are commonly existing urate stones. [4,5] Drugs with multiple mechanisms of protective action provide minimizing the diseases.

Plants provide food, raw materials for medicine and various other requirements for the very existence of life from the origin of human beings[6]. The majority of the global population utilizes medicinal plants for their health care. Even the current conventional medicine is using a lot of plant derived chemicals as therapeutic agents. The overuse of synthetic drugs results in higher incidence of adverse drug reactions has motivated humans to return to nature for safe remedies. Herbs and herbal drugs have created interest among the people by its clinically proven effects[7].

*Gossypium Herbaceum*L. belongs to Malveceae and commonly called as cotton plant^[8,9]. It is oldest Indian herbal drug, which is included in our present study is widely used by tribal people. Ayurvedic system has already noticed the importance of this plant. It has several experimentally proven pharmacological activities, which includes Antitumor^[10], Antimutagenic^[11], Anticonvulsant^[12] antibacterial, antihelminthic^[13] and antifungal activities^[14]. The cotton seed has already proved antiUrolithiatic so based on the literature review the present study was carried out antiuro lithiatic activity of leaves of *Gossypium Herbaceum*.

MATERIALS AND METHODS:

PLANT MATERIALS

The leaves of *Gossypium herbaceum* was collected in the month of august 2017 from Maddur village, Medak dist. of Telangana, India. The plant was authenticated by D. Venkateshwara Rao, Deputy Director, Telangana. Forest Academy, Dullapally, Hyderabad, Rangareddy District. The leaves were washed with tap water and dried under shade.

PREPARATION OF PLANT EXTRACT

The leaves were shade dried and powdered. The crude plant extract was prepared by Soxhlet extraction method. 50g of powdered plant material was extracted with 500ml of ethanol and

water individually. The process of extraction was carried out up to 6 cycles, till the solvent in siphon tube of an extractor became colorless. The two extracts were filtered separately, and evaporated to dryness using rotary evaporator. Further the dried extracts were maintained in a refrigerator at 4°C for further antiuro lithiatic activity.

CHEMICALS USED

Neeri, Sodium oxalate, Tris buffer, calcium chloride, Potassium permanganate(KMnO₄), Sulphuric acid(H₂SO₄).

INVESTIGATION OF *IN VITRO* ANTIUROLITHIATIC ACTIVITY TEST BY TITRIMETRY

The experimental kidney stones of calcium oxalate (CaOx) were prepared in the laboratory by taking equimolar solution of calcium chloride dehydrate in distilled water and sodium oxalate in 10 ml of 2N H₂SO₄. Both were allowed to react in sufficient quantity of distilled water in a beaker, the resulting precipitate was calcium oxalate. The precipitate was freed from traces of sulphuric acid by ammonia solution, washed with distilled water and dried at 60°C. The dissolution percentage of calcium oxalate was evaluated by taking exactly 1 mg of calcium oxalate and 10 mg of the extract, packed it together in semi permeable membrane of egg as shown in the model designed given below. This was allowed to suspend in a conical flask containing 100 ml of 0.1M Tris buffer. First group served as blank containing only 1 mg of calcium oxalate. The second group served as positive control containing 1 mg of calcium oxalate and along with the 10mg standard drugs, i.e. Neeri. The 3rd, 4th groups along with 1 mg of calcium oxalate contain methanolic and aqueous, extracts. The conical flasks of all groups were kept in an incubator preheated to 37°C for 2 h. Remove the contents of semi permeable membranes from each group into separate test tubes, add 2 ml of 1N sulphuric acid to each test tube and titrated with 0.9494 N KMnO₄ till a light pink colour end point obtained. The amount of remaining undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning to know the total quantity of dissolved calcium oxalate by various solvent extracts.[15]

RESULTS AND DISCUSSION:

Drug therapy has developed in response to population health care needs. There are many crucial areas in medicine such as liver diseases, arthritis, old age related problems, certain viral infections and cancer where the conventional medicine is devoid of satisfactory treatment. These are among the promising areas of research and development of medicines from the vast highly potential plant resources. Plants are also attractive sources for the development of novel and very effective and safe therapeutic agents against kidney procumbens. Herbal medicines are also in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. Unlike allopathic medicines which target is only one aspect of urolithiatic pathophysiology, most of plant based therapy have been shown to be effective at different stages of stone pathophysiology. About

80% of the world population rely on the use of traditional medicine which is predominantly based on plant material. Plant based drug discovery programmes continue to provide an important source of new drug leads. Lithiasis (stone formation) is an important cause for acute and chronic renal failure, includes both nephrolithiasis (stone formation in kidney) and urolithiasis (stone formation in ureter or bladder or both). Among the various kinds of stones identified, calcium stones occur mainly in Men, while phosphate stones formation is more in women.

This study evaluates the antiurolithiatic activity of Ethanolic and Aqueous extract of *Gossypium herbaceum*. The highest percentage i.e. 87% of calcium oxalate {CaOx} dissolution was observed in ethanolic extract followed by Aqueous extract which had a percentage dissolution of calcium oxalate was 64%(Table 1). Both Ethanolic and Aqueous extracts of *Gossypium herbaceum* were found to be more effective in dissolution of calcium oxalate. From this study, it was observed that aqueous and ethanolic extracts of *Gossypium herbaceum* showed their highest dissolution of calcium oxalate. Ethanolic extract was found to be even more effective than Aqueous extract in dissolution of calcium oxalate. This study has given primary evidence for *Gossypium herbaceum* as the plant which possess lithotriptic property. This in vitro study has given lead data and shown that aqueous and ethanolic extracts are quite promising for further studies in this regard.



Figure 1(a): Decalcification of egg shell in 10% Acetic acid overnight.



Figure 1(b): Decalcified Eggs



Figure 1(c): Egg membrane along with the contents suspended into the 0.1 M Tris buffer.

Figure 1: In vitro experimental model setup to evaluate antiurolithiatic activity.

Table 1: Shows % dissolution of calcium oxalate (CaOx) by *Acacia farnesiana* leaves extracts.

% of dissolution of calcium oxalate		
S.no	Groups	<i>Gossypium herbaceum</i>
1.	Blank	0
2.	Positive Control	81
3.	Ethanolic extract	87%
4.	Aqueous extract	64%

CONCLUSION:

In vitro urolithiasis has been performed on the selected plant *Gossypium herbaceum* by using the standard drug, Neeri. The work was performed by using in vitro antiurolithiatic model for calculating percentage dissolution of kidney stone. Ethanolic leaf extract of *Gossypium herbaceum* shows highest dissolution than standard drug Neeri. This study has given primary evidence for *Gossypium herbaceum* as the plant which possess antiurolithiatic property.

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