

## STUDY OF *IN VITRO* ANTI-LITHIATIC EFFECT OF *IPOMOEA BATATAS* (L) LEAVES AND TUBEROUS ROOTS

SATHISH R\*, JEYABALAN G

Department of Pharmacy, Sunrise University, Alwar, Rajasthan, India. Email: rsvsathish2000@yahoo.co.in

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### ABSTRACT

**Objective:** The present study was to demonstrate the *in vitro* anti-lithiatic effect of *Ipomoea batatas* (Convolvulaceae) leaves and tuberous roots.

**Methods:** The obtained ethanolic extract of *I. batatas* leaves and tuberous roots (EIBL and EIBR) and aqueous extract of *I. batatas* leaves and roots (AIBL and AIBR) were used for this *in vitro* study. The dissolution method of calcium oxalate by titrimetry method and calcium phosphate by colorimetric method was studied. Nucleation and aggregation of calcium oxalate crystals were determined by a spectrophotometric assay.

**Results:** In the estimation of calcium oxalate by titrimetry method, the *I. batatas* leaves and roots have very significant ( $p < 0.01$ ) capability to dissolve calcium oxalate. Percentage dissolution of calcium oxalate crystals was found to be 37.53%, 22.74%, 39.74%, and 24.28% for EIBL, AIBL, EIBR, and AIBR, respectively. In the estimation of calcium phosphate by colorimetric method, the percentage dissolution of calcium phosphate crystals by EIBL, AIBL, EIBR, and AIBR was found to be 67.15%, 43.17%, 76.74%, and 47.96%, respectively. The *I. batatas* leaves and roots were significantly ( $p < 0.01$ ) dissolved calcium phosphate also. The results were clearly shown that *I. batatas* extracts significantly ( $p < 0.01$ ) inhibited both nucleation and aggregation of calcium oxalate crystals by concentration-dependent manner. The maximum percent inhibition of calcium oxalate nucleation by EIBL, AIBL, EIBR, and AIBR was found to be 59.09%, 50.0%, 84.09%, and 47.73%, respectively, at 1000  $\mu\text{g/ml}$ . The EIBL, AIBL, EIBR, and AIBR were inhibited calcium oxalate aggregation by 63.46%, 36.54%, 84.61%, and 42.3%, respectively, at 1000  $\mu\text{g/ml}$ .

**Conclusions:** The results clearly indicate that *I. batatas* leaves and tuberous roots were found to express *in vitro* anti-lithiatic potential.

**Keywords:** Anti-lithiatic, Calcium oxalate stones, Crystal nucleation and aggregation, *In vitro* Anti-lithiatic, *Ipomoea batatas*, Kidney stones.

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### INTRODUCTION

Urolithiasis, also called renal calculi, is common and an extremely painful condition with recurrent stone formation which is also associated with risk of renal damage. Kidney stone formation is a complex process and it results as a cascade of events, including crystal nucleation, growth, aggregation, and crystal retention within the renal tubules [1]. Usually, kidney stones are yellow or brown with a smooth or gagged structure. Some common types of kidney stones are calcium oxalate, calcium phosphate, struvite, uric acid, and cysteine, among of which calcium stones are the most common kidney stones in both humans and rats [2]. Urolithiasis has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout patient's lifetime, with a recurrence rate of 70–80% in males and 47–60% in females. The current medical management of lithiasis is either costly or not without side effects. Extracorporeal shock wave lithotripsy is widely used for the treatment of urolithiasis and its multiple sessions in recurrent stone formation may cause chronic renal failure. The various therapies including thiazide (diuretic) and alkali citrates are used to prevent the recurrence of hypercalciuria and hyperoxaluria, which induce calculi formation, but evidence for their efficacy is less [3-5]. Therefore, there is a need to establish medical treatment to prevent recurrent stone formation. Nature is the best combinatorial chemistry and has possible answers to all diseases for humankind. The undesirable effect of the modern medicine has already diverted the attention of the people toward herbal medicines. To increase the acceptability and awareness among the people, there is an urgent need to develop trust and faith toward the safer indigenous system by establishing its validity in the treatment for various diseases [6]. Indigenous plants have been used as a potential source of medicine since ancient times. There are more than 13,000 plants have been studied for the various diseases and ailments all over the world in the past few years [7]. The

World Health Organization has shown great interest in documenting the use of medicinal plants from the tribals residing in various parts of the world. *Ipomoea batatas* belongs to the family Convolvulaceae; commonly known as sweet potato is the sixth most important food crop in the world. Sweet potato roots are a good source of carbohydrates, while leaves and stems contain additional nutritional components in much higher concentrations than in many other commercial vegetables. Ethnopharmacological data have shown that sweet potato has been effectively used in herbal medicine to treat inflammatory infections, diabetes, oxidative stress, viral infection, and oral diseases. Recently, studies on sweet potato have focused on its antioxidant capacities due to the increased content of phenols, flavonoids,  $\beta$ -carotene, anthocyanins, and caffeoylquinic acid derivatives [8-11]. The sweet potato roots are sweet, refrigerant, laxative, aphrodisiac, diuretic, and tonic. They are useful in vitiated conditions of pitta, burning sensation, hyperdipsia, constipation, strangury, renal and vesicle calculi, diabetes, and general weakness [12]. The sweet potato is using as traditional medicine against renal calculi, diabetes, and general weakness by the village peoples of Thoppampatti, Dindigul district, Tamil Nadu, India [13]. *I. batatas* (Sakarkand alu) tubers are traditionally given against renal calculi in the districts of West Bengal [14]. It is thus our duty to establish the pharmacological effect of this eco-friendly medicinal plant for the removal of human suffering and miseries from kidney diseases. Hence, this study has evaluated the *in vitro* anti-lithiatic effect of *I. batatas* leaves and tuberous roots.

### METHODS

#### Plant materials collection and extraction

The fully matured dark green leaves and fresh tuberous roots of *I. batatas* (L) lam were collected from the Vadipatti and Dindigul areas, Tamil Nadu, India, in July 2015. The plant was identified and

authenticated by Dr. D. Stephen, Taxonomist, American College, Madurai. The dust-free leaves and sliced tuberous roots were allowed to dry under shade in the laboratory for 20 days. About 1000 g each of pulverized leaves and tuberous root powders were placed, and solvent such as ethanol and water was added individually in the flat bottom flasks. The mouth of flasks was covered with aluminum foil and kept in room temperature for 7 days to dynamic maceration with occasional shaking. The extracts were filtered using Muslin cloth followed by Whatman no 1 filter paper and finally filtered using vacuum and pressure pump. The solvents from the extracts were removed using rotary vacuum evaporator and concentrated under reduced pressure of 300–500 mmHg at 50–60°C [15]. The obtained semi-solid residues of aqueous and ethanolic extracts of leaves and tuberous roots henceforth called ethanolic extract of *I. batatas* Leaves (EIBL), aqueous extract of *I. batatas* leaves (AIBL), ethanolic extract of *I. batatas* tuberous roots (EIBR), and aqueous extract of *I. batatas* tuberous roots (AIBR). All the extracts of *I. batatas* (EIBL) were intended for preliminary phytochemical evaluation [16].

#### Evaluation of *in vitro* anti-lithiatic activity by dissolution method

##### Preparation of semi-permeable membrane from eggs

Apex of eggs was punctured to remove out the entire content, and empty shells were washed thoroughly with distilled water and then placed in a beaker containing 4ml of concentrated HCl in 200 ml distilled water. It was kept overnight for decalcification process; on the next day, semi-permeable membrane was removed carefully, washed thoroughly with distilled water, and placed it in ammonia solution for neutralization of acid traces. Finally, semi-permeable membrane was rinsed with distilled water, stored in refrigerator, and used for calcium oxalate and calcium phosphate dissolution methods [17].

#### Dissolution method of calcium oxalate

##### Synthesis of calcium oxalate by homogeneous precipitation

Exactly 1.47 g of calcium chloride dihydrate was dissolved in 100 ml distilled water and 1.34 g of sodium oxalate was dissolved in 100 ml of 2N sulfuric acid. Equimolar prepared solutions of calcium chloride dihydrate and sodium oxalate were allowed to react in a beaker to precipitate out calcium oxalate with stirring. The resultant calcium oxalate precipitate was freed from traces of sulfuric acid by ammonia solution, washed with distilled water, and dried at 60°C for 2 h [17].

#### Estimation of calcium oxalate by titrimetry method

As per experimental design, dissolution studies of calcium oxalate were carried out in six groups; one group served as negative control (Group I) which consists of 1 mg of calcium oxalate alone, weighed exactly 1 mg of calcium oxalate and packed along with 10 mg of cystone (removing color coating and crushed into powder form) as standard group (Group II). In test groups (Groups III-VI), 10 mg each of test substances EIBL, AIBL, EIBR, and AIBR were packed along with 1 mg of the calcium oxalate. All groups were packed as above in separate egg-based semipermeable membranes tied with thread by suturing at one end. Now, the materials packed in semipermeable membranes were allowed to suspend in separate conical flasks containing 100 ml of 0.1M TRIS buffer. Another end of the thread was tied on the mouth of conical flasks and covered with aluminum foil. The conical flasks of all groups were kept in an incubator, preheated to at 37±1°C for 7 h. After 7 h, flasks with membrane were kept in magnetic stirrer for 5 min. Finally, all the membranes were taken out of the flask and content of each membrane was collected in different test tubes by removing the sutures. 2 ml of 1 N sulfuric acid was added to each test tube and titrated with 0.9494 N KMNO<sub>4</sub> till a light pink color end point obtained. 1 ml of 0.9494 N KMNO<sub>4</sub> is equivalent to 0.1898 mg of calcium. The amount of undissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate was dissolved by the test substances. There is inverse relationship between the dissolution of calcium oxalate and concentration of calcium ions in solutions [17-19]. Each treatment was repeated 3 times and volume of standard KMNO<sub>4</sub> was expressed

as mean±SD. The percentage dissolution of calcium oxalate in various groups was calculated.

#### Dissolution method of calcium phosphate

##### Synthesis of calcium phosphate by homogeneous precipitation

About 1.47 g of calcium chloride dihydrate was dissolved in 100 ml of distilled water and 1.42 g of disodium hydrogen phosphate was dissolved in 100 ml of 2N sulfuric acid. Equimolar prepared solutions of calcium chloride dihydrate and disodium hydrogen phosphate were allowed to react in a beaker to precipitate out calcium phosphate with stirring. The resultant calcium phosphate was freed from traces of sulfuric acid by ammonia solution, washed with distilled water, and dried at a temperature 60°C for 2 h [17].

#### Preparation of reagents

Molybdate sulfuric acid reagent was prepared by 5% w/v of sodium molybdate solution, 13 ml of concentrated sulfuric acid in 80 ml of distilled water, and the final volume was adjusted to 100 ml with distilled water. In reducing solution, 1 g of p-phenylenediamine was dissolved in 100 ml of 3% w/v of sodium metabisulfite solution for reducing solution [17].

#### Estimation of calcium phosphate by colorimetric method

As per experimental design, the dissolution studies of calcium phosphate were carried out in six groups as like dissolution method of calcium oxalate; one group served as negative control (Group I) which containing only 1 mg of calcium phosphate. Exactly 1 mg of the calcium phosphate, 10 mg standard cystone were weighed and packed as standard group (Group II). In test groups (Groups III-VI), 10 mg each of test substances EIBL, AIBL, EIBR, and AIBR were packed along with 1 mg of the calcium phosphate, respectively. All groups were packed as above in egg-based semipermeable membranes, allowed to suspend in separate conical flasks containing 100 ml of 0.1M TRIS buffer, and were kept in an incubator, preheated to at 37±1°C for 7 h. After 7 h, flasks with membrane were kept in magnetic stirrer for 5 min and content of each membrane was collected in different test tubes by removing the sutures. 2 ml of 1 N sulfuric acid, 2.5 ml of molybdic sulfuric acid reagent, 1 ml of reducing solution was added, and final volume was made to 10 ml using distilled water. For standard calibration curve, various dilutions of calcium phosphate were prepared (200, 400, 600, 800, and 1000 µg/ml) containing 2.5 ml of molybdic sulfuric acid reagent, 1 ml of reducing solution, and final volume was made up to 10 ml using distilled water. The optical densities of standard and all the experimental groups were measured by colorimeter at 600–750 nm [17-19]. Each treatment was repeated 3 times and optical densities were expressed as mean±SD. The undissolved calcium phosphate was determined from the standard calibration curve using Microsoft Excel 2007 (Table 1 and Fig. 1). The results of the experimental groups were interpreted as percentage dissolution.

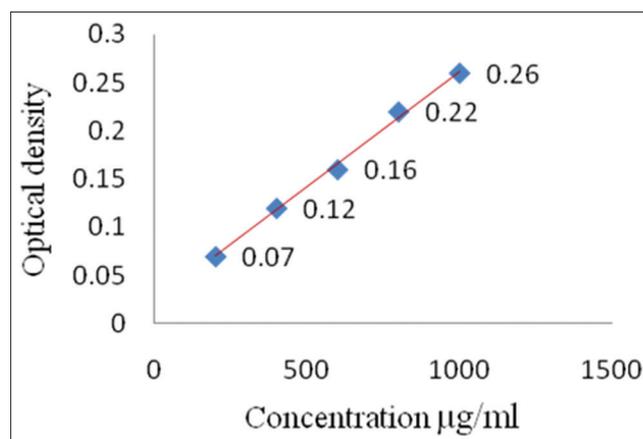


Fig. 1: Standard calibration curve of calcium phosphate

Table 1: Standard calibration curve of calcium phosphate

| Standard calibration curve ( $\mu\text{g/ml}$ ) | Molybdic $\text{H}_2\text{SO}_4$ reagent | Reducing solution | Distilled water (q.s.) | Optical density |
|---|--|-------------------|------------------------|-----------------|
| 200   | 2.5 ml in each                           | 1 ml in each      | Up to 10 ml in each    | 0.07            |
| 400   |  |                   |                        | 0.12            |
| 600   |  |                   |                        | 0.16            |
| 800   |  |                   |                        | 0.22            |
| 1000  |  |                   |                        | 0.26            |

### Nucleation assay (turbidity method)

*In vitro* anti-lithiatic activity was tested in terms of the inhibitory properties of the extracts on the nucleation of calcium oxalate crystals. Nucleation assay was done by following turbidity method, and nucleation of calcium oxalate crystals was determined by a spectrophotometric assay [17,20,21]. The method used was similar to that described by Hennequin *et al.* and Atmani and Khan. Solutions of calcium chloride and sodium oxalate were prepared at a final concentration of 5 mmol/L and 7.5 mmol/L, respectively, in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5; both solutions were filtered 3 times through an 0.22  $\mu\text{m}$  filter. About 950  $\mu\text{l}$  of calcium chloride solution was mixed with 100  $\mu\text{l}$  each of EIBL, AIBL, EIBR, AIBR, and standard cystone at different concentrations (100, 200, 400, 800, and 1000  $\mu\text{g/ml}$ ). Crystallization was initiated by adding 950  $\mu\text{l}$  of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm for 15 min, and the temperature was maintained at 37°C. The optical density of the crystallized suspension was measured at 620 nm in spectrophotometer after addition of calcium-containing solution. All the experiments were performed in triplicate. The rate of nucleation was estimated by comparing the induction time (the delay before the appearance of crystals that have reached a critical size and thus become optically detectable) in the presence and absence of standard and test samples.

$$\% \text{ Inhibition} = \{(\text{OD control} - \text{OD sample}) / \text{OD control}\} \times 100$$

### Aggregation assay

The aggregation of the calcium oxalate crystals was determined by the spectrophotometric assay method similar to that described by Hess *et al.*, with slight modifications [17,20,21]. The calcium oxalate monohydrate crystals were prepared by mixing both the solutions of calcium chloride and sodium oxalate of 50 mmol/L each. The solution was equilibrated to 60°C in a water bath for 1 h and then cooled to 37°C overnight. The crystals were harvested by centrifugation and then evaporated at 37°C. Calcium oxalate crystals were then buffered with 0.5 ml of 0.05 mol/L Tris buffer and 0.5 ml of 0.15 mol/L NaCl solution at pH 6.5 to a final concentration of 1 mg/ml. The experiments were conducted at 37°C in the absence or presence of EIBL, AIBL, EIBR, AIBR, and cystone at different concentrations (100, 200, 400, 800, and 1000  $\mu\text{g/ml}$ ) with constant stirring. After stopping the stirring, optical density was measured at 620 nm in a spectrophotometer, and the percentage inhibition of aggregation was estimated using the same formula as in turbidity method.

### Statistical analysis

The results were expressed as mean $\pm$ SD. The difference among data was determined using one-way ANOVA followed by Dunnett's test as per suitability.  $p < 0.05$  was considered as statistically significant,  $p < 0.01$  was considered as very significant.

## RESULTS

The yields of various extracts were found to be 7.5 % (EIBL), 5.2% (AIBL), 9.4% (EIBR), and 6.1% (AIBR) respectively. From preliminary phytochemical screening of *I. batatas*, we found that the carbohydrates, glycosides, phenols, tannins, proteins and amino acids, saponins, and flavonoids were present in EIBL, EIBR, AIBL, and AIBR (Table 2).

There is inverse relationship between the dissolution of calcium oxalate and concentration of calcium ions in solutions. In the estimation of

Table 2: Qualitative chemical evaluation of *I. batatas*

| Phytoconstituents        | EIBL | AIBL | EIBR | AIBR |
|--------------------------|------|------|------|------|
| Alkaloids                | +    | -    | +    | -    |
| Carbohydrates            | +    | +    | +    | +    |
| Glycosides               | +    | +    | +    | +    |
| Phenols                  | +    | +    | +    | +    |
| Tannins                  | +    | +    | +    | +    |
| Proteins and amino acids | +    | +    | +    | +    |
| Saponins                 | +    | +    | +    | +    |
| Flavonoids               | +    | +    | +    | +    |

+: Present, -: Not present, EIBL: Ethanolic extract of *I. batatas* leaves, EIBR: Ethanolic extract of *I. batatas* roots, AIBL: Aqueous extract of *I. batatas* leaves, AIBR: Aqueous extract of *I. batatas* roots, *I. batatas*: *Ipomoea batatas*

calcium oxalate by titrimetry method, the ethanolic extracts of *I. batatas* leaves and roots have very significant ( $p < 0.01$ ) capability to dissolve calcium oxalate as foremost element for stone forming in the urinary tract. Percentage dissolution of calcium oxalate crystals was found to be 45.47% for cystone, 37.53% for EIBL, 22.74% for AIBL, and 39.74% and 24.28% for EIBR and AIBR, respectively (Table 3).

In the estimation of calcium phosphate by the colorimetric method, the percentage dissolution of calcium phosphate crystals by cystone, EIBL, AIBL, EIBR, and AIBR was found to be 71.94%, 67.15%, 43.17%, 76.74% and 47.96%, respectively. The extracts of both *I. batatas* leaves and roots have very significant ( $p < 0.01$ ) capability to dissolve calcium phosphate as secondary element for stone forming in the urinary tract (Table 4).

The nucleation assay was done by turbidity method, and nucleation of calcium oxalate crystals was determined by a spectrophotometric assay. The changes in the turbidity or optical density of different concentrations of EIBL, AIBL, EIBR, AIBR, and standard cystone (100, 200, 400, 800, and 1000  $\mu\text{g/ml}$ ) were plotted at different time intervals. The turbidity increased linearly up to 5 min, which indicated the nucleation process and then decreased linearly up to 15 min indicating the aggregation process. The maximum percent inhibition of nucleation of calcium oxalate by EIBL, AIBL, EIBR, AIBR, and cystone was found to be 59.09%, 50.0%, 84.09%, 47.73%, and 81.82%, respectively, at 1000  $\mu\text{g/ml}$  (Table 5).

The aggregation of the calcium oxalate crystals was determined by the spectrophotometric assay method. The crystals in solution stick together to form large particles called as aggregates. The maximum percentage inhibition of calcium oxalate aggregation by EIBL, AIBL, EIBR, AIBR, and cystone were found to be 63.46%, 36.54%, 84.61%, 42.3%, and 82.69%, respectively, at 1000  $\mu\text{g/ml}$  (Table 6). *I. batatas* extracts and standard cystone were very significantly ( $p < 0.01$ ) inhibited both the nucleation and aggregation of calcium oxalate crystals (Table 6).

## DISCUSSION

The supersaturation of urine with calcium oxalate, the most common component of kidney stones, attributes to calcium oxalate particles crystallization within the urinary tract with later factors being nucleation, growth, and aggregation. Thus, if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. Although several measures are usually taken to reduce supersaturation such as increasing fluid intake and medical therapy, it is generally accepted that better strategies for preventing kidney stones need to be developed [22-24]. Nucleation is an important first step for

the initiation of crystals, which then grow and form aggregates. Calcium oxalate crystals begin grow, aggregate with other crystals, and retained in the kidney. This is aggregation process which causes renal injury [25]. Stone crystals bind to one another through a process known as aggregation promoted by strong chemicals and electrical forces. Adhered crystals were held in place and cannot be easily separated, and this plays an important role in lithiasis [26]. In this *in vitro* study, the dissolution methods of calcium oxalate and calcium phosphate were used to know the role of *I. batatas* extracts in dissolving the already formed stones nucleus in renal system. As *in vitro* crystallization study was performed, since the results clearly indicate that the crystal nucleation and aggregation were found to express a concentration-dependent inhibition. Sweet potatoes are a nutritious food, low in fat, and protein but rich in carbohydrate. Both tubers and leaves are good sources of antioxidants, fiber, zinc, potassium, sodium, manganese, calcium, magnesium, iron, and Vitamin C [27,28]. Due to the presence of various kind of physiologically active components in both the roots and leaves of sweet potato have potential to maintain human health and mitigate many diseases [29]. The EIBL

and EIBR were shown the maximum percentage of dissolution for the already formed calcium oxalate and calcium phosphate crystals than that of AIBL and AIBR (Tables 3 and 4). The maximum inhibition was recorded for EIBL and EIBR in nucleation and aggregation assays which have shown the remarkable anti-lithiatic activity which was more than that of both aqueous extracts of leaves and roots (Tables 5 and 6). The studies of nucleation and aggregation assay have a great scope to study the mechanism of action of drugs having anti-lithiatic potential. Thus, this study puts forth the possibility of using *I. batatas* as therapeutic agents to treat urolithiasis.

### CONCLUSION

The results clearly indicate that, under *in vitro* conditions, EIBL and EIBR were found to express a concentration-dependent inhibition on the crystal nucleation and aggregation. The ethanolic extracts were also having more capacity to dissolve the already formed kidney stones. These findings substantiate the traditional use of the *I. batatas* in the treatment of urinary stones and kidney problems. To substantiate its

**Table 3: Effect of *I. batatas* on percentage dissolution of calcium oxalate crystals**

| Experimental design | Volume of standard KMNO <sub>4</sub> | Wt. of calcium estimated (mg) | Wt. of calcium reduced (mg) | Percentage dissolution |
|---------------------|--------------------------------------|-------------------------------|-----------------------------|------------------------|
| Group I             | 4.53±0.06                            | 0.8598                        | -                           | -                      |
| Group II            | 2.47±0.15*                           | 0.4688                        | 0.3909                      | 45.47                  |
| Group III           | 2.83±0.12*                           | 0.5371                        | 0.3226                      | 37.53                  |
| Group IV            | 3.51±0.1*                            | 0.6643                        | 0.1954                      | 22.74                  |
| Group V             | 2.73±0.06*                           | 0.5182                        | 0.3416                      | 39.74                  |
| Group VI            | 3.43±0.58*                           | 0.6510                        | 0.2088                      | 24.28                  |

Volume of KMNO<sub>4</sub> was expressed as mean±SD; \*p<0.01 was considered as very significant when compared with control Group I (n=3), *I. batatas*: *Ipomoea batatas*

**Table 4: Effect of *I. batatas* on percentage dissolution of calcium phosphate crystals**

| Experimental design | Molybdic H <sub>2</sub> SO <sub>4</sub> reagent | Reducing solution | Distilled water (q.s.) | Optical density | Wt. of calcium estimated (mg) | Wt. of calcium reduced (mg) | Percentage dissolution |
|---------------------|---|-------------------|------------------------|-----------------|-------------------------------|-----------------------------|------------------------|
| Group I             | 2.5 ml in each                                  | 1 ml in each      | Up to 10 ml in each    | 0.21±0.01       | 0.8657                        | -                           | -                      |
| Group II            |   |                   |                        | 0.08±0.02*      | 0.2429                        | 0.6228                      | 71.94                  |
| Group III           |   |                   |                        | 0.09±0.01*      | 0.2844                        | 0.5813                      | 67.15                  |
| Group IV            |   |                   |                        | 0.14±0.006*     | 0.4920                        | 0.3737                      | 43.17                  |
| Group V             |   |                   |                        | 0.07±0.005*     | 0.2014                        | 0.6643                      | 76.74                  |
| Group VI            |   |                   |                        | 0.13±0.006*     | 0.4505                        | 0.4152                      | 47.96                  |

The optical densities were expressed as mean±SD; \*p<0.01 was considered as very significant when compared with control Group I (n=3), *I. batatas*: *Ipomoea batatas*

**Table 5: Inhibitory effect of *I. batatas* on nucleation assay**

| Concentrations (µg) | Optical densities (percentage inhibition) |                     |                     |                     |                     |
|---------------------|---|---------------------|---------------------|---------------------|---------------------|
|                     | EIBL                                      | AIBL                | EIBR                | AIBR                | Cystone             |
| 100 µg/ml           | 0.39±0.01* (11.36%)                       | 0.41±0.03 (6.81%)   | 0.37±0.01* (15.91%) | 0.42±0.02 (4.55%)   | 0.36±0.02* (18.18%) |
| 200 µg/ml           | 0.31±0.02* (29.54%)                       | 0.37±0.01* (15.91%) | 0.28±0.02* (36.36%) | 0.36±0.04* (18.18%) | 0.27±0.02* (38.63%) |
| 400 µg/ml           | 0.25±0.05* (43.18%)                       | 0.31±0.01* (29.54%) | 0.22±0.07* (50.0%)  | 0.29±0.02* (34.09%) | 0.21±0.06* (52.27%) |
| 800 µg/ml           | 0.21±0.02* (52.27%)                       | 0.26±0.02* (40.91%) | 0.19±0.01* (56.82%) | 0.25±0.01* (43.18%) | 0.17±0.05* (61.36%) |
| 1000 µg/ml          | 0.18±0.06* (59.09%)                       | 0.22±0.02* (50.0%)  | 0.07±0.05* (84.09%) | 0.23±0.03* (47.73%) | 0.08±0.01* (81.82%) |

The optical densities were expressed as mean±SD; \*p<0.01 was considered as very significant when compared with control OD - 0.44±0.01, EIBL: Ethanolic extract of *I. batatas* leaves, EIBR: Ethanolic extract of *I. batatas* roots, AIBL: Aqueous extract of *I. batatas* leaves, AIBR: Aqueous extract of *I. batatas* roots, *I. batatas*: *Ipomoea batatas*

**Table 6: Inhibitory effect of *I. batatas* on aggregation assay**

| Concentrations (µg) | Optical densities (percentage inhibition) |                     |                     |                     |                     |
|---------------------|---|---------------------|---------------------|---------------------|---------------------|
|                     | EIBL                                      | AIBL                | EIBR                | AIBR                | Cystone             |
| 100 µg/ml           | 0.43±0.01* (17.3%)                        | 0.48±0.03 (7.69%)   | 0.42±0.02* (19.23%) | 0.47±0.04 (9.62%)   | 0.39±0.03* (25.0%)  |
| 200 µg/ml           | 0.36±0.02* (30.77%)                       | 0.4±0.02* (23.08%)  | 0.35±0.02* (32.69%) | 0.39±0.01* (25.0%)  | 0.27±0.02* (48.07%) |
| 400 µg/ml           | 0.28±0.05* (46.15%)                       | 0.39±0.02* (25.0%)  | 0.24±0.03* (53.84%) | 0.37±0.02* (28.4%)  | 0.21±0.03* (59.61%) |
| 800 µg/ml           | 0.24±0.03* (53.85%)                       | 0.36±0.03* (30.77%) | 0.19±0.01* (63.46%) | 0.31±0.01* (40.38%) | 0.17±0.02* (67.3%)  |
| 1000 µg/ml          | 0.19±0.04* (63.46%)                       | 0.33±0.01* (36.54%) | 0.08±0.04* (84.61%) | 0.3±0.02* (42.3%)   | 0.09±0.02* (82.69%) |

The optical densities were expressed as mean±SD; \*p<0.01 was considered as very significant when compared with control OD - 0.52±0.01, EIBL: Ethanolic extract of *I. batatas* leaves, EIBR: Ethanolic extract of *I. batatas* roots, AIBL: Aqueous extract of *I. batatas* leaves, AIBR: Aqueous extract of *I. batatas* roots, *I. batatas*: *Ipomoea batatas*

*in vitro* effect, the *in vivo* studies need to be carried out in experimental animals, then this drug can be used for the treatments of kidney stone.

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