



Evaluation of the effects of *Orthosiphon spiralis* (Lour.) Merr., *Cissus javana* DC., and *Upupa epops* L., on kidney stone along with cystone - a herbal drug

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Received: 27 November 2023

Accepted: 27 December 2023

Abstract

Kidney stone cases are common and suffered by many people. It causes health problems such as urinary obstruction, severe pain and infection that adversely affect well-being of individuals. Kidney stone is treatable by traditional methods (traditional healers or folklore medicine) using available medicinal plants. Further, this disease can be treated by using allopathic and herbal drugs, lithotripsy, open surgery, etc. The best practice is treatment of such disease using medicinal plants which are more economic with less side effects. In this study, *Orthosiphon spiralis* (Lour.) Merr., *Cissus javana* DC., *Upupa epops* L., and cystone, a herbal drug, are chosen for evaluating their chemoinhibitory effects for calcium oxalate (COX) and calcium phosphate (CP) stone formation both in the aqueous and urinary media. The results of the study shows that the inhibitory effects on CP stone formation both in aqueous and urinary media was highest by the mixture of *C. javana* and *U. epops*. The mixture of *U. epops* and *C. javana* had no inhibitory effect on COX stone formation in the aqueous medium while *U. epops* has the highest inhibitory effect on COM stone formation in the aqueous medium. Further the inhibitory effect on COM stone formation is the highest by the mixture of *U. epops* and *C. javana* in the urinary medium. And in *in vitro*, the dissolution of kidney stone is digested more by the mixture of *U. epops* and *C. javana*. Hence, *U. epops* and *C. javana* are more effective in the chemoihibition on CP and COX stone formations in *in vitro* condition.

Keywords: Calcium oxalate stone, Kidney stone, Open surgery, Urinary obstruction

1. Introduction

The kidneys are one of the most vital organs of human body (Anthea *et al.*, 1993; Kumar *et al.*, 2005; Really, 2005; Bimola *et al.*, 2014). Malfunction of the kidney (Muharrem *et al.*, 2019) due to the blockage of urine by stone causes unbearable pain (Margaret *et al.*, 2010). Diet containing high calcium and oxalate may also

enhance the formation of kidney stone ie., COX (Marica *et al.*, 1994; Bimola *et al.*, 2016). People between 30 to 50 years are suffered most.

Medicinal plants are used to combat many diseases from the dawn of civilization. These plants are the rich sources of therapeutic agents for the treatment and prevention of many

diseases. These plants and their traditional (Sheikh *et al.*, 2018) uses are more economic having side effects is the choice for treating many diseases and ailments worldwide. Among such plants, some are very effective in the chemoinhibitory action of stone formation in the kidney and its tract.

Upupa epops L., (family: Upupidae) is a colourful bird notable for its distinctive “crown” of feathers. It is the only extent species in the family. It is a medium sized bird and its population is drastically reduced due to climate change like global warming and hunting. For this reason, the species is afforded protection under the law in many countries (Kristin, 2001). The diet of the *U. epops* includes many species which are considered as pests by human, such as the pupae of the recessionary moth, a damaging forest pest (Battisti *et al.*, 2000). *U. epops* is distinctive bird and have made a cultural impact over much of their range. They were considered sacred in ancient Egypt and were depicted on the walls of tombs and temples. They achieved a similar standing in Minoan Crete (Fry *et al.*, 2003). It is also appears in the Quran, known as the “*hudhud*” in Surah Al-Nanl 27:20-22. The *U. epops* is the king of birds in ancient Greek comedy, ‘The bird by Aristophanes’. It was chosen as the national bird of Israel in May 2008 in conjunction with the country’s 60th anniversary. In Morocco, *U. epops* is traded live and as medicinal products in the markets, primarily in herbal shops. This trade is unregulated and a potential threat to the local populations (Manuha, 2020). In olden days, the Manipuris (the people of Manipur, India) hunted this bird for its organ and flesh for using medicinal purposes particularly for the treatment of gall stone (Daniel and Mohammed, 2013). But now this bird is endangered and hunting of this bird is banned under the Wildlife Conservation Act.

One of the easiest ways for the treatment of kidney stone is surgery. But it can be treated by using medicinal plants which are more economic and having fewer side effects as compared to allopathic drugs. In Manipur, the treatments kidney stone by the traditional healers like *meeteis*, Muslims, tribes, etc., is still going on (Vinodkumar *et al.*, 2021). Padmashree awardee (2001), Shri L.Nabakishore Singh (local herbalist) is still treating kidney stone by using

locally available medicinal herbs cut (Mohd *et al.*, 2011) and Manipuri people so far are relieved from kidney stone problems. In the present work, the concept of the treatment of the kidney stone with the mixture of *U. epops* and *C. javana* is chosen to validate folklore medicinal practice which was done by the traditional healers. Even though our research work is against the Wildlife Conservation Act (took permission from the Chief Wildlife Conservator of Forests, Govt. of Manipur), we carry forwarded the work for betterment of the humanitarian service.

2. Materials and methods

Manipur is a state of rich flora and fauna. Healthy plants of *Orthosiphon spiralis* (Lour.) Merr., and *Cissus javana* DC., were collected from different parts of Manipur. The herbarium of the plants were prepared. In the meantime, *Upupa epops* L., was procured from Mr. M. Koireng Meitei. The herbal drug cystone was purchased from the local chemical supplier, Himalaya Company. Table 1 gives the scientific names and parts of the plant/bird used.

Our study is mainly focused on the chemoinhibitory inhibition experiment (Rao *et al.*, 2008) ie. titration method involving the breaking down of the artificial stone like COX and CP (*in vitro*) respectively. Further, digestion of kidney stone is to be conducted with the plant and bird extracts whether digestion is feasible or not. The parts of the plants were washed, dried, chopped and powdered (Bimola *et al.*, 2014; Kamaran *et al.*, 2014). The dried powder parts of the plants were soaked in 50% methanol in a Soxhlet extractor under hot condition. The plant extracts were distilled under reduced pressure using rotary vacuum evaporator (RII) to produce crude mass which further spread in Petridis and dried in the desiccators.

The feathers of *U. epops* were removed and treated with 300 ml aqueous methanol (1:1) for 5 days. Then it was filtered, concentrated under low temperature to get crude mass of feathers of *U. epops*. The kidney stones for the current study was collected from Prof. Sinam Rajen Singh, Department of Urology, Regional Institute of Medical Sciences (RIMS), Lamphel, Manipur and urine sample from a healthy male (~ 30 years) who does not have any stone case collected in sterilized container and camphor was added as preservative. Fresh urine was collected as a solvent to mimic the natural solvent.

2.1. Methodolgy

Calcium oxalate (COX) or Calcium phosphate (CP) stone is allowed to form in aqueous or urinary blank medium as well as in presence of inhibitor (either *U.epops* or plant extract or standard cystone) according

Table 1. Medicinal plants and bird with their particulars

Sl. No	Scientific name	Local name	Part used
1	<i>Orthosiphon spiralis</i> (Lour.) Merr.	<i>Leikhaman</i>	Flower
2	<i>Cussus javana</i> DC.	<i>Kongouyen laba</i>	Leaf
3	<i>Upupa epops</i> L.	<i>Chongaraba</i>	Whole body without feathers

to chemoinhibition experiment. Calcium content after stone formation in presence or absence of inhibitor was observed through the inhibition efficiency (i.e., % inhibition) and it was calculated by the following equations (1) and (2).

$$\% \text{ Inhibition} = \frac{\text{Ca}^{2+} \text{ in centrifugate}}{\text{Total Ca}^{2+} \text{ in the experiment}} \text{ ----- (1)}$$

$$\% \text{ increase of Inhibitor relative in blank} = \frac{\text{Increase of \% inhibition}}{\% \text{ Inhibition by blank}} \text{ ----- (2)}$$

Where the total Ca^{2+} in the experiment equals the Ca^{2+} contents of 50 ml CaCl_2 solution which is determined separately through complexometric titration with standard EDTA solution.

2.1.1. Chemoinhibition experiment: Chemoinhibition experiments were conducted according to Rao *et al.*, (2008). 0.01M each of CaCl_2 and Na_3PO_4 were taken for CP crystallization. 0.01M each of CaCl_2 and sodium oxalate were taken for COX crystallization. 50 ml of plant extract (0.1%) in water or urine was taken as inhibition solutions. Simultaneous blank experiments in water or urine were also carried out for calculating the inhibitory efficiency of plant extract compared to water or urine (Table 2 and 3). All the experiments were conducted at room temperature (25°C). At the end of crystallization, the content of the beaker were digested on a hot water bath for 10 minutes, cooled at room temperature and centrifuged in small volume. The total centrifugates were collected. Calcium content of the centrifugate left after stone had formed was determined by complexometric titration using standard EDTA solution (0.01M), EBT/1% indicator and $\text{NH}_3 - \text{NH}_4\text{Cl}$ as buffer ($\text{pH} 10$). While calculating the Ca content of the centrifugate, a

titre value of EDTA versus inhibition corresponding total inhibition solution was deduced from the total titre value.

3. Results and discussion

Now, the effectiveness of the relative percentage inhibition of *U. epops* and mixture and *O. spiralis* against cystone formation in the aqueous medium is nil while that of the mixture *U. epops* and *C. javana* is 28.0494 (Table 4). On the other hand, in the urinary medium, the relative percentage of inhibition of the mixture of *U. epops* and *C. javana* is found to be the highest i.e., 55.9931 (Table 5). In the case of COX, the relative percentage of inhibition of *U. epops* in the aqueous medium is 33.3409 (Table 6). The relative percentage of inhibition of *U. epops* and *C. javana* in the urinary medium is 200.0037 (Table 7). The chemoinhibitory experiments had shown that some medicinal plants have greater inhibitory power for CP and COX stone formations (Table 8). The chemoinhibitory effect of CP stone formation is found to be the highest in aqueous as well as urinary media by the mixture of *U. epops* and *C. javana* while cystone has no inhibitory effect. The inhibitory effect on COX stone formation by *U. epops* is higher than cystone in the aqueous medium but in the urinary medium cystone is higher than that of *U. epops*. Further, chemoinhibitory effect on COX stone formation is the highest by the mixture of *U. epops* and *C. javana* in the urinary medium but has no chemoinhibitory effect on COX stone formation in the aqueous medium. Therefore, the chemoinhibitory effects on the CP and COX stone formations are the highest by the mixture of *U. epops* and *C. javana*.

In this investigation, we utilized correlation statistics along with their associated P-values to examine the relationships between inhibition (0.1%), the concentration of Ca^{2+} in solution (g) and the concentration of Ca^{2+} in precipitate (g) across seven study subjects namely, Blank-

Table 2. Inhibition experiment CP (blank)

Sl. No.	Water – blank for CP				Urine – blank for CP			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	8.2	8.2	-	0	10.3	10.3	-
2	0	8.2	8.2	8.2	0	10.2	10.2	10.2
3	0	8.2	8.2	-	0	10.2	10.2	-

Table 3. Inhibition experiment COX (blank)

Sl. No.	Water – blank for COX				Urine – blank for COX			
	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)	IR(ml)	FR(ml)	Diff.(ml)	Mean(ml)
1	0	1.2	1.2	-	0	2.1	2.1	-
2	0	1.2	1.2	1.2	0	2.0	2.0	2.0
3	0	1.2	1.2	-	0	2.0	2.0	-

Table 4. Effect of plant extract and bird extract on CP stone formation in aqueous medium

Sl. No.	Plant/bird	Inhibitors 0.1%	Ca ²⁺ in solution(g)	Ca ²⁺ in precipitate(g)	% of inhibition	Diff % of inhibition between sample and blank	Relative % of inhibition
1	Blank-Water	8.2	0.00656	0.7290	0.9240	-	-
2	<i>O. spiralis</i>	4.0	0.0032	0.0703	4.3510	-4.5709	-51.2198
3	<i>C. javana</i>	8.5	0.0068	0.0667	9.2504	0.3365	3.7707
4	<i>U. epops</i> + <i>O. spiralis</i>	7.8	0.0062	0.0673	8.4886	-0.4353	-4.8779
5	<i>U. epops</i> + <i>C. javana</i>	10.5	0.0084	0.0651	11.4270	2.5031	28.0494
6	<i>U. epops</i>	7.8	0.0062	0.0673	8.4886	-0.4353	-4.8779
7	Cystone	8.0	0.0064	0.0711	8.7063	-0.2177	-2.4395

Table 5. Effect of plant extract and bird extract on CP stone formation in urinary medium

Sl. No.	Plant/bird	Inhibitors 0.1%	Ca ²⁺ in solution (g)	Ca ²⁺ in precipitate (g)	% of inhibition	Diff % of inhibition between sample and blank	Relative % of inhibition
1	Blank-urine	10.2	0.0082	0.0653	11.1005	-	-
2	<i>O. spiralis</i>	9.3	0.0074	0.0066	10.0066	-1.0939	-9.8590
3	<i>C. javana</i>	9.76	0.0078	0.0657	10.6217	-0.4788	-4.3133
4	<i>O. spiralis</i> + <i>U. epops</i>	13.8	0.0110	0.0625	15.0184	3.9179	35.2948
5	<i>U. epops</i> + <i>C. javana</i>	15.9	0.0127	0.0608	17.3038	6.2033	55.9931
6	<i>U. epops</i>	10.9	0.0087	0.0648	11.8623	0.7618	6.8628
7	Cystone	4.1	0.0033	0.0702	4.4620	-6.6385	-59.803

Table 6. Effect of plant extract and bird extract on COX stone formation in aqueous medium

Sl. No.	Plant/bird	Inhibitors 0.1%	Ca ²⁺ in solution (g)	Ca ²⁺ in precipitate (g)	% of inhibition	Diff % of inhibition between sample and blank	Relative % of inhibition
1	Blank-water	1.2	0.0010	0.0726	1.3059	-	-
2	<i>O. spiralis</i>	1.5	0.0012	0.07231	1.6324	0.2503	18.1101
3	<i>C. javana</i>	0.9	0.0007	0.0728	0.9795	-0.3264	-24.9942
4	<i>O. spiralis</i> + <i>U. epops</i>	1.3	0.0010	0.0725	1.4147	0.1088	8.3314
5	<i>U. epops</i> + <i>C. javana</i>	1.2	0.0010	0.0726	1.3059	0	0
6	<i>U. epops</i>	1.6	0.0013	0.0722	1.7413	1.6651	127.5060
7	Cystone	1.5	0.0012	0.0723	1.6324	0.3265	25.0019

Table 7. Effect of plant extract and bird extract on COM stone formation in urinary medium

Sl. No.	Plant/bird	Inhibitors 0.1%	Ca ²⁺ in solution (g)	Ca ²⁺ in precipitate (g)	% of inhibition	Diff % of inhibition between sample and blank	Relative % of inhibition
1	Blank-Urine	2.5	0.0020	0.07151	2.7207	-	-
2	<i>O. spiralis</i>	4.1	0.0033	0.07023	4.4620	1.7143	64.0019
3	<i>C. javana</i>	4.6	0.0037	0.0698	5.0061	1.6252	49.1203
4	<i>O. spiralis</i> + <i>U. epops</i>	4.5	0.0036	0.6991	4.8973	2.1766	80.0015
5	<i>U. epops</i> + <i>C. javana</i>	7.5	0.0060	0.0675	8.1622	5.4415	200.0037
6	<i>U. epops</i>	2.8	0.0022	0.0713	3.0472	0.3265	12.0006
7	Cystone	4.1	0.0033	0.0702	4.4620	1.7143	64.0019

Table 8. Comparison of chemoinhibitory effects

Sl. No.	Name of drug, plants & bird	Types of stone	Aqueous medium		Urinary medium	
			% inhibition	% relative inhibition	% inhibition	% relative inhibition
1	Cystone	CP	8.7063	-2.4395	-4.4620	-59.8036
		COM	1.6323	25.0019	4.4620	64.0019
2	<i>O. spiralis</i>	CP	4.3510	-51.2198	10.0066	-9.8590
		COM	1.6324	25.0019	4.4620	64.0019
3	<i>C. javana</i>	CP	9.2504	3.7707	10.6217	-4.3133
		COM	0.9795	-24.9942	5.0061	49.1203
4	<i>O. spiralis</i> + <i>U. epops</i>	CP	8.4886	-4.8779	15.0184	35.2948
		COM	1.4147	8.3314	4.8973	80.0015
5	<i>U. epops</i> + <i>C. javana</i>	CP	11.4270	28.0494	17.3038	55.6631
		COM	1.3059	0	8.1622	200.0037
6	<i>U. epops</i>	CP	8.4886	-4.8779	11.8623	6.8628
		COM	1.7413	33.3409	3.0472	12.0006

Table 9. Treatment of kidney stone with different extracts

Sl. No.	Medium	Extracts	% of crude	Duration (hour)	Mass of kidney stone before treatment with extracts (g)	Mass of kidney stone after treatment with extracts (g)	Amount of kidney digested	Rate of digestion of kidney stone per hour
1	Urine	Cystone	0.1	4	0.7250	0.7139	0.0011	0.0275
2	Urine	<i>U. epops</i>	0.1	4	0.0670	0.0670	0	0
3	Urine	<i>O. spiralis</i>	0.1	4	0.8490	0.8480	0.0010	0.0250
4	Urine	<i>U. epops</i> + <i>C. javana</i>	0.1	4	0.6330	0.6310	0.0020	0.0500

Water, *O. spiralis*, *C. javana*, *U. epops* + *O. spiralis*, *U. epops* + *C. javana*, *U. epops* and cystone. In the context of the effect of plant extract and bird extract on CP stone formation in an aqueous medium, a robust positive correlation (with $P < 0.001$) was observed between inhibition (0.1%) and the concentration of Ca^{2+} in solution (g). However, no significant correlation ($P > 0.05$) was found between inhibition (0.1%) and Ca^{2+} in precipitate (g), nor between the concentration of Ca^{2+} in solution (g) and Ca^{2+} in precipitate (g).

Similarly, when investigating the effect of plant extract and bird extract on CP stone formation in urinary medium, a highly significant linear relationship was observed between inhibition (0.1%) and the concentration of Ca^{2+} in solution (g). Conversely, a strongly significant negative correlation ($P < 0.001$) was identified between inhibition (0.1%) and the concentration of Ca^{2+} in precipitate (g), as well as between the concentration of Ca^{2+} in solution (g) and Ca^{2+} in precipitate (g).

These linear relationships persisted in the analysis of the effect of plant extract and bird extract on calcium oxalate (COX) formation in urinary medium, where a highly significant relationship was found between Inhibition (0.1%) and the concentration of Ca^{2+} in solution (g). However, weak linear relations ($P > 0.05$) were observed between inhibition (0.1%) and Ca^{2+} in precipitate (g), as well as between the concentration of Ca^{2+} in solution (g) and Ca^{2+} in precipitate (g).

In the meantime, kidney stone (collected from RIMS) is treated with cystone, *O. spiralis* and mixture of *U. epops* and *C. javana* separately in the urinary media (*in vitro*). The experimental findings of the observation are shown in Table 9 and it is clear that, *U. epops* has no effect on the digestion of kidney stone. The rate of digestion of cystone is 0.0275 mg per hour that of *O. spiralis* is 0.0200 mg per hour and that of the mixture of *U. epops* and *C. javana* is 0.0500 mg per hour. Hence, it can be concluded that the digestion of kidney stone is highest by the mixture of *U. epops* and *C. javana*.

4. Conclusion

Chemical elements and compounds present in the medicinal plants, *C. javana* and *U. epops* play a significant role in the biological activities directly or indirectly. The mechanism of chemoinhibitory activity is the plant or bird extracts is yet to find out whether either elements or compounds present in the plant extracts and bird extracts or both act as chemoinhibitors. Elements or compounds in the plant/bird extracts enhance the digestion or breaking down of the kidney stone or inhibit the formation of kidney stone.

Since the *C. javana* has very high calcium content (2960 mg/100g) (Bimola *et al.*, 2014), it enhances the chemoinhibitory property of COX because calcium is a competitive inhibitor of oxalate in COX stone formation. Further high calcium content lowers the amount of oxalate absorbed into blood thereby reducing risk of new kidney stone. Further, mixture of extract of *C. javana* and *U. epops* has the highest digestion of kidney stone in the urinary medium. This is due to the fact that the chemical compounds like stigmaterols, cissus javanol (Bimola *et al.*, 2016), etc., present in *C. javana* and chemical compounds like mono-, di- or trimer (Manuel *et al.*, 2009) etc., in *U. epops* helps the digestion of kidney stone. These chemical compounds may either help to remove calcium from COX or break down of oxalate from COX. Hence, validating the traditional claim.

Further investigation is required to find out the chemical compounds that are actually involved in the digestion of kidney stone and their action on kidney stone has to be discovered. If this work is studied together with the pharmaceutical experts, it can be applied to mankind.

Acknowledgements

The authors are thankful to the Head, Department of Chemistry, Modern College, for his moral support. Authors also express their gratitude to the Universities Grants Commission, New Delhi, for the financial support and also to the Chief Conservator of Forests, Government of Manipur, for granting permission to undertake the research work with *Upupa epops* L. Lastly, the authors thank Dr. Naorem Sharat Singh, Associate Professor, Department of Statistics, Dhanamanjuri University, Imphal for helping in the finalization of statistical correlation.

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