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In *Vitro* Antiurolithiatic activity of Hajarul Yahood Bhasm And Hajarul Yahood Pishti

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ABSTRACT: Urolithiasis, kidney stone Formation, is a complex process that results from series of several physiochemical event including super-saturation, nucleation, growth, aggregation retention within the kidney. A study undertaken to evaluate *in vitro* antiurolithiatic activity of some ayurvedic medicine Hajarul Yahood Bhasm (known as sange yahood ber patthar) and Pishti, powder is used. The *In-vitro* activity was determined by inhibition of calcium (Titrimetric analysis). A Medicine Ashmarina, Drotin was used as a drug for comparison. Homogenous precipitation method was used to prepare artificial stone such as calcium oxalate, calcium phosphate and semi-permeable membrane of eggs was used to dissolution bags. Dissolution model where incubated in (37^oc) for 2 hours and after that the entire content in dissolution bag was titrated against KmNo4. The Ayurvedic Medicine has greater capability to dissolve calcium oxalate while Ashmarina and Drotin show less dissolution of calcium oxalate. Therefore, present investigation will supportive to the scientific documentation related *In-vitro* studies. Lastly we conclude that the ayurvedic medicine Hajarul Yahood Bhasm and Ashmarina play a significant role *In-vitro* anti-urolithiatic activity.

KEYWORDS: Hajarul Yahood Bhasm, Hajarul Yahood pishti, Calcium Oxalate, Ashmarina, Drotin Tab

I. INTRODUCTION

A kidney stone is a solid piece of material that forms from crystallization of excreted substance in the urine. Lithiasis is the condition marked by formation of calculi, which is formed by deposition of various calcium and phosphorus salts and anti-lithiasis is prevention of the formation of urinary calculi. The stone may remain in the kidney or break loose and travel down the urinary tract. A small stone may pass all of the way out of the body, but a larger stone can be stuck in ureter, the bladder, or the urethra. This may block the flow of urine and cause great pain. Urolithiasis is the presence of calculi in urinary track. The male to female incidence ratio is 4:1. Kidney stone may contain various combinations of chemicals. The most common type of stone contain calcium in combination in either oxalate or phosphate, this chemical are part of person normal diet and make up important part of the body such as bone muscle. A less common type of stone is called a struvite or infection stone a bit less common stone is the uric acid stone. Urolithiasis is the most common disease of the urinary tract and it is affecting the human beings since the earliest days. The recognition of different types of urinary calculi also resulted in more varieties of medical treatment. Still recurrence rates continue to be high with one of every two patients having another stone within 5 years. So, there is a need to develop new drugs to prevent the recurrence of kidney stones (Anti-urolithiasis medicine). In regard to this, the present study planned *In-vitro* anti-urolithiatic activity using Ayurvedic Medicine Hajarul Yahood Bhasm and Hajarul Yahood Pishti.

II. MATERIALS AND METHODS

Materials: Eggs, *Hajrul Yahood Pishti*, Hajarool Yahood Bhasm, Ashmarina tablet (Bhardwaj), Drotin Tablet (Walter Bushnell), All Ayurvedic medicines were purchased from local market.

Chemicals: Calcium Chloride dihydrate (AR), Sodium Oxalate, Sulphuric Acid, Calcium Oxalate, Disodium Hydrogen Phosphate, Calcium Phosphate, Ammonia solution and Distilled Water (DW), TRIS Buffer, KmNO₄

Sanskrit name / Indian name: - Hajrul yahood Bhasma and Pishti

English name: Lime silicate Calx

Description: Hazrul Yahood Pishti is a grey color round stone with Ber (Indian plum) like appearance *Hazrul Yahood pishti* provides nourishment to kidney cells and support their normal functioning, its properties are virya is *sheeta*, *prabhava* is *diuretic* and *lithontripitic*, *dosha karma* is *kapha* and *pitta*. *HazroolYahood Pishti* to be affected in some organs like kidney, urinary bladder and skin. Hajarul Yahood Bhasma has lithonetripitic and Diuretic action in the body, which help dissolving and pushing kidney stone along the course of ureter.

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Methods:

Step 1: Evaluation for Anti-urolithiatic activity preparation of experimental kidney stone (calcium oxalate stone) by homogenous precipitation:

Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Sodium oxalate (AR) in 10ml of 2N H_2SO_4 were allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium oxalate. Equimolar solution of Calcium chloride dihydrate (AR) in distilled water and Disodium hydrogen phosphate (AR) in 10ml of (2N H_2SO_4), was allowed to react in sufficient quantity of distilled water in a beaker. The resulting precipitate was calcium phosphate. Both precipitates freed from traces of sulphuric acid by ammonia solution, later washed with distilled water and dried at $60^{\circ}C$ for 4 hours.



Step 2: Preparation of semi-permeable membrane from poultry farm eggs:

The semi - permeable membrane of eggs lies in between the outer calcified shell and the inner contents like albumin and yolk. Shell was removed chemically by placing the eggs in 2M HCl for an overnight, which caused complete decalcification. Further, washed with distilled water, and carefully with a sharp pointer a hole is made on the top and the contents squeezed out completely from the decalcified egg. Then egg membrane washed thoroughly with distilled water, and placed it in ammonia solution, in the moistened condition for a while and rinsed it with distilled water. Stored in refrigerator at a pH of 7- 7.4.

Step 3: Estimation of Calcium oxalate by Titrimetry: Weighed exactly 1mg of the calcium oxalate and (10mg) of the Hajrul Yahood Bhasm and Pishti, Ashmarina (used 0.115gm) / Drotin tablet (0.0275) and packed it together in semi-permeable membrane for evaluation. This was allowed to suspend in a conical flask containing 100ml 0.1 M TRIS buffer. One group served as negative control (contained only 1mg of calcium oxalate). The conical flask of all groups placed in an incubator, preheated to 37^{0} C for about 7-8 hours. The contents of semi-permeable membrane removed from each group into a test tube. 2 ml of 1 N sulphuric acid added in each flask and titrated with 0.9494 N KmNO₄ till a light pink color end point obtained. 1ml of 0.9494 N KmNO₄ equivalent to 0.1898mg of 4 Calcium. The amount of un-dissolved calcium oxalate is subtracted from the total quantity used in the experiment in the beginning, to know how much quantity of calcium oxalate actually test substance(s) could dissolve.



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III. RESULTS

In this anti-urolithiatic Model, the Ayurvedic Medicine hajarul yahood Bhasm , and pishti (powder) 10mg concentration produced greater capability to dissolve calcium oxalate while Drotin Tablet (Standard) and Ashmarina has shown better Demineralization for calcium phosphate .yahood bhasm pishti , and hajarool Yahood bhasm exhibit inhibitory action in both of nucleation assay to significant level to evaluate in vitro Anti-urolithiatic activity by comparing different medicine with standard % dissolution of calcium oxalate table is given above. The significant amount of stone deposition on the membrane was observed in all three group quantitative analysis of the stone indicated that stones formed are of magnecium ammonium phosphate type with traces of calcium and oxalate.

Table: 1 % dissolution of calcium oxala

1 Control 0.00	
2 Standard 45.54±14.15*	
3 Hajarul Yahood bhasm 49.11±9.71*	
4 Hajarul Yahood pishti 49.55±17.71*	
5 Ashmarina 74.55±11.26**	

n= 6, mean \pm SE, *p < 0.05

Hajrul Yahood Bhasma and Pishti compared with standard 49.11 ± 9.71 ; 49.55 ± 17.71 and 45.54 ± 14.15 respectively. What is interesting to note is that Ashmarina exhibits high % dissolution of calcium oxalate ($74.55 \pm 11.26\%$) as compared with standard ($45.54 \pm 14.15\%$).

IV. DISCUSSION

Urolithiasis is the most common disease of the urinary tract and it is affecting the human beings since the earliest days. The recognition of different types of urinary calculi also resulted in more varieties of medical treatment. Liathiasis (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 (Ramachandran *et al.*, 2011). A number of medicines have been used in India and elsewhere which claim efficient cure of urinary stones (Mukherjee *et al.*, 1984 al).

In the present study the ayurvedic medicine Hajarul Yahood Bhasma, Hajarul Yahood Pishti inhibited the stone crystal formation thus prove the presence of some active antiurolithiatic compound. Rohan (2015), work on *Kalanchoe pinnata* extract conclude that correlation between *In- vitro* and *In- vivo* studies may helpful to understand the molecular mechanism of litholysis process and reveal phytochemical dissolving of disintegrating renal calculi. The result obtained by them are confirmed by over studies.

V. CONCLUSION

Urolithiasis has been performed for Hajarul Yahood Bhasm, Pishti and Ashmarina Tab works as Antilithiatic in *In-vitro* model for calculating % dissolution of kidney stone. Yahood Bhasm and Pishti gave parallel dissolution of calcium Oxalate crystal. However, best approach of Ashmarina tab which is very effective in kidney stone. This is just because

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of its ingredients includes Hajrul Yahood Bhasma and Pishti along with other herbs. Hence, it may conclude that Ayurvedic formulation having Hajrul Yahood Bhasma and Pishti is effective to control kidney stone.

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