Scholars Academic Journal of Pharmacy (SAJP)
Abbreviated Key Title: Sch. Acad. J. Pharm.
ISSN 2347-9531 (Print)
ISSN 2320-4206 (Online)

A Unit of Scholars Academic and Scientific Society, India
www.saspublisher.com

Pharmacology

Phytochemical and Invitro Antiurolithiatic Activity of Thlaspi Bursa Pastoris Mother Tincture

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Original Research Article

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Article History
Received: 11.07.2018
Accepted: 23.07.2018
Published:30.07.2018

DOI:
10.21276/sajp.2018.7.7.8

Abstract: A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. *Thlaspi* Q is homeopathic medicine given for urolithiasis; however the mechanism of action remains unknown. The aim of the study was to determine the phytochemical constituents and invitro antiurolithiatic potential of *Thlaspi* mother tincture. Preliminary phytochemical screening was performed .Under invitro condition; kidney stone formation was studied using three assays such as nucleation, growth and aggregation. All the three assays were carried out using spectrometric methods .Nucleation rate were studied at 620nm by after mixingsodium oxalate and calcium chloride at 37°C with stirring. The percentage inhibition of the mother tincture was determined on comparing with control. Similarly growth assay and aggregation assay would carry out by using calcium oxalate monohydrate crystals. And the result was compared with standard cystone tablets. Preliminary phytochemical test of *Thalaspi Q* indicated the presence of flavanoid, carbohydrate, saponins, protein, steroid, phenols etc. The mother tincture shows inhibitory activity in nucleation, growth and aggregation assay but less compared with standard cystone. The present finding gave an experimental evidence to support the invitro efficacy of homeopathic preparation *Thlaspi* Q on modulating the primary events of stone formation.
Keywords: Urolithiasis, Thlaspi Q, CaOx, Nucleation, Aggregation.

INTRODUCTION

Urolithiasisis is the condition where urinary calculi are formed or located anywhere in the urinary system or the process of formation of the stone in kidney, bladder, and/or ureters (urinary tract. Urolithiasis is the third common disorder of the urinary tract. 12% of the population and men are three times more prone than women. It is moreprevelant between the ages of 20 and 40 in both sexes. Etiology is multifactorial and is strongly related to diatery lifestyle habits or practices. Of all kidney stones, 85% contain calcium salts as their main crystalline components [1].

Kidney stone formation is a complex process that is a consequence of an imbalance between promoters and inhibitors in kidneys [2]. The recurrence of urolithiasis represents a serious problem as patients who have one stone are more likely to form another.

The crystallization of stone begins with increased urinary supersaturation, with subsequent formation of solid crystalline particles within the urinary tract. This is followed by nucleation, by which stone forming salts in supersaturated urinary solution coalesce into clusters that then increase in size .the crystal then grow and aggregate with other crystals in solution and ultimately retained and accumulated in the kidney [3].

The synthetic drugs used to prevent urolithiasis are not effective in all patients, and many of them have adverse effects that compromise their long term use. In the present day management of urolithiasis with open renal surgery is an unusual and rarely used one since the introduction of Extracorporeal Shock Wave Lithotripsy (ESWL) which has almost become the standard procedure for eliminating kidney stones. Besides imposing the high cost, shock waves in therapeutic doses may cause acute renal injury, decrease the renal function and an increase in stone recurrence [1].

Now-a-days, however, herbal medicine has gained much popularity because, herbal medicines are more effective, have less side effects and reduce recurrence rate of stone formation, hence search for antilithiatic drug from natural sources has assumed greater importance and is promising[4]. Herbal medicines have many phytoconstituents which may exert their beneficial effect in kidney stone treatment. Plant extracts contain phytochemicals that inhibit stone formation by inhibiting synthesis and agglomeration of crystals. Currently known herbal drugs exert their

antiurolithiatic effect with multidimensional pharmacological actions as angiotensin converting enzyme inhibition, analgesic, antiinflammatory, antioxidant, antispasmodic, astringent, crystallization inhibition, diuretic, demulcent; litholytic, lithotriptic, Phospholipase A2 inhibition and by changing the ions concentrations in urine such as increase magnesium and citrate excretion e.g., decreasing the calcium and oxalates[5]. Herbal remedies are reported to be effective with no side effects. The drug for prevention of the disease or its re-occurrence is of great concern as no drug in clinical therapy is of satisfactory result. Although these herbal medicines are popular in folk culture but rationale behind their efficacy and safety are not well established[6].

Thalaspi Bursa pastoris or Capsella bursa-pastoris (L.) Medik., (Brassicaceae), is found all over the world and it can be eaten raw or cooked commonly known as shepherd's purse and its means is a bag plant, young leaves and roots of this plant have been used as an edible vegetable, eaten raw or cooked in some countries . It contained a wide range of chemicals including flavonoids, polypeptides, choline, acetylcholine, histamine, tyramine, fatty acids, sterols, organic acids, amino acids, sulforaphane, many trace elements, vitamins and many other compounds. *C. bursa-pastoris* has some medicinal properties such as anti-bleeding, anticancer, antithrombin, antioxidant, antidiabetes agents and fever treatment. A homeopathic remedy is made from the fresh plant to be used in the treatment of nose bleeds and urinary calculus[7].

MATERIALS AND METHODS

Drugs and chemicals Homeopathic preparation of *Thlaspi Q* obtained from Central Research Institute for Homeopathy; kottayam. All other chemicals and solvents used in the present study were procured from standard agencies and were of analytical grade. The alcohol content in mother tincture was evaporated under reduced pressure in a rotary evaporator to obtain a semisolid mass then weighed in a rotary evaporator to obtain a semisolid mass that weighed [8].

Preliminary phytochemical screening of thlaspi bursa pastoris mother tincture[9]

Test for carbohydrates

Molish's test: The filtrate was subjected to Molisch's test. Formation of reddish brown ring indicated the presence of carbohydrates.

Fehling's test: Dissolve a small portion of extract in water and treat with Fehling's solution [brown color indicated the presence of carbohydrate.

Test for flavonoids

Shinoda test: To 2 to 3ml of extract, a piece of magnesium ribbon and 1ml of concentrated HCl was added .A pink or red coloration of the solution indicated the presence of flavonoids in the extract.

Test for tannins

To a 2 to 3ml of extract, 10% alcoholic ferric chloride solution was added. Dark blue or greenish grey coloration of the solution indicated the presence of tannins in the extract.

Test for phenols

To 1ml of aqueous extract of sample, 2 ml of distilled water followed by a few drops of 10% aqueous ferric chloride solution was added. Formation of blue or green colour indicated the presence of phenols.

Test for steroid/terpenoid

Liebermann-Burchardt test: To 1ml of extract, 1ml of chloroform, 2 to3ml of acetic anhydride and 1 to 2 drops of concentrated Sulphuric acid are added. Dark green coloration of the solution indicated the presence of steroids and dark pink or red coloration of the solution indicated the presence of terpenoids.

Test for alkaloids

- Draggendorf's test: The extract was treated with few ml of Dragendroff. Orange coloration of the spot indicated the presence of alkaloids.
- Hager's test: The extract was treated with few ml of Hager's reagent. Yellow precipitation indicated the presence of alkaloids.
- Wagner's test: The extract was treated with few ml of Wagner's reagent. The reddish brown precipitation indicated the presence of alkaloids.

Tests for Glycosides

Legal's test: Dissolved the extract [0.1g] in pyridine [2ml], added sodium nitroprusside solution [2ml] and made alkaline with Sodium hydroxide solution. Pink to red color solution indicates the presence of glycosides.

Test for Saponins

Foam test: 1ml of extract was dilute with 20ml of distilled water and shaken with a graduated cylinder for 15 minutes. A 1cm layer of foam formation indicates the presence of Saponins

Test for Anthraquinones

Borntrager's test: About 50 mg of powdered extract was heated with 10% ferric chloride solution and 1ml of concentrated HCl. The extract was cooled, filtered and the filtrate was shaken with diethyl ether. The ether extract was further extracted with strong ammonia. Pink or red coloration of aqueous layer indicated the presence of Anthraquinones.

Test for Amino acids

Ninhydrin test: Dissolved a small quantity of the extract in few ml of water and added 1ml of ninhydrin reagent. Blue color indicated the presence of amino acids.

EXPERIMENTAL PROTOCOL

The effect of mother tincture on inhibition of calcium oxalate formation was determined by nucleation, growth and aggregation assay. The inhibition of calcium oxalate crystals nucleation. Growth and aggregation was studied by the measurement of turbidity by using UV /VISIBLE spectrophotometry.

NUCLEATION ASSAY

The inhibitory activity of the extracts on the nucleation of CaOx crystals was determined by a spectrophotometric assay. Crystallisation was initiated by adding calcium chloride (4 mmol/L) and sodium oxalate (50 mmol/L) , both prepared in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5 and 37°C. 950 µL of calcium chloride solution mixed with 100 µL of herb extracts at the different concentrations . Crystallization was started by adding 950 µL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The Cystone tablets are used as standard solution[10]. The percentage inhibition calculated as (OD (control-sample)/OD (control))/100

GROWTH ASSAY

20 ml eachof 4mMcalcium chloride and 4mMsodium oxalate were added to a 30 ml of solution, containing NaCl (90 mM) buffered with Tris HCl (10 mM) pH 7.2.To this 600 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml acetate buffer) was added. Consumption of oxalate begins immediately after COM slurry addition and was monitored for 600 s by disappearance of absorbance at 214 nm. The Cystone tablets are used as Standard drug solution[11].

The relative inhibitory activity was calculated as follows: % relative inhibitory activity = ((C-S)/C) × 100 Where „C‟ is the rate of reduction of free oxalate without any extract. „S‟ is the rate of reduction of free oxalate with drug extract.

AGGREGATION ASSAY

CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were 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. CaOX crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37 °C in the absence or presence of the plant extract after stopping the stirring [12].

The Cystone tablets are used as Standard drug solution. The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using following formula:

$$Ir = (1 - Turbidity_{sample}/Turbidity_{control}) \times 100$$

RESULTS

The phytochemical analysis of the *Thlaspi* mothertincture is shown in below table 1. The antiurolithiatic activity of Thlaspi mother tincture due to the presence of various secondary metabolites such as alkaloids, flavonoids, glycosides, phenols, steroids, tannins etc.

Table-1: Phytochemical constituents in *Thlaspi* Q

Phytochemical components	Thlaspi mother tincture
Carbohydrates	+
Proteins	+
Alkaloids	-
Flavonoids	+
Glycosides	+
Terpenoids	+
Steroids	+
Phenols	+
Saponins	+

The effect of *Thlaspi* Q in urolithiasis was evaluated in invitro methods. From the studies the percentage inhibition were calculated.

NUCLEATION ASSAY

It is the initial step of renal stone formation. The table 2 and figure 1 showed the effect of different concentration of *Thlaspi* Q on nucleation of calcium oxalate crystal formation. The increase in the concentration of *Thlaspi* mother tincture showed increase in the inhibition of nucleation. Maximum inhibition of nucleation was 67.6% observed at concentration of 100µg/ml.

Table-2: Effect of *Thlaspi* Q and Cystone on nucleation of calcium oxalate crystals

Concentration (µg/ml)	O.D of test	% Inhibition of test	O.D of standard	% Inhibition of standard
20	0.115±0.002	15.1%	0.106±0.002	22.62%
40	0.098±0.005	28.46%	0.087±0.001	36.49%
60	0.084±0.0015	39.5%	0.065±0.002	52.55%
80	0.070±0.0015	48.9%	0.042±0.002	69.34%
100	0.045±0.002	67.6%	0.027±0.002	80.29%
Control	0.137±0.002		0.137±0.002	

Values are mean ±S.D of triplicate

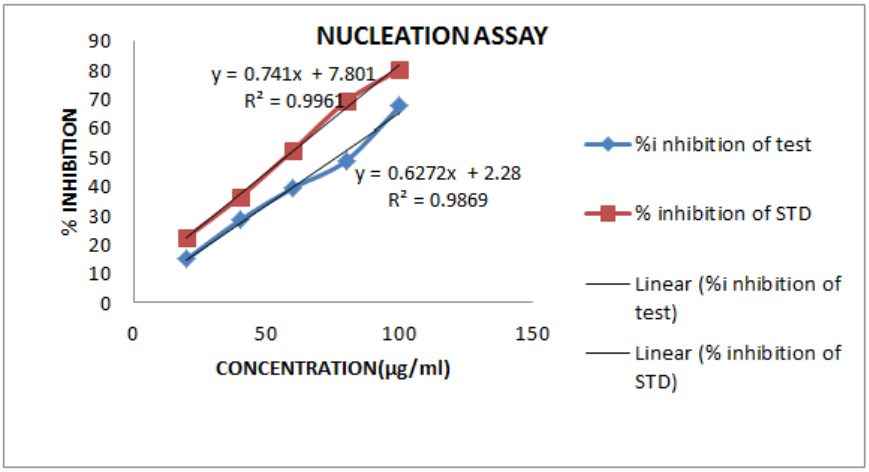

Fig-1: Effect of *Thlaspi* Q and Cystone on nucleation assay

From the above graphical method, the IC_{50} value of standard and test was found to be 56.94 µg/ml and76.10 µg/ml respectively.

GROWTH ASSAY

The table 3 and figure 2 showed the effect of different concentration of *Thlaspi* Q on growth of calcium oxalate crystal formation. The increase in the concentration of *Thlaspi* mother tincture showed increase in the inhibition of growth. Maximum inhibition of nucleation was 66.43%observed at concentration of 100µg/m.

Table-3: Effect of *Thlaspi* Q and Cystone on growth assay

Concentration (µg/ml)	O.D of test	% Inhibtion of test	O.D of standard	% Inhibition of standard
20	0.661±0.003	13.02%	0.536±0.02	29.47%
40	0.571±0.001	24.82%	0.454±0.015	40.26%
60	0.444±0.004	41.57%	0.365±0.01	51.9%
80	0.379±0.002	50.13%	0.285±0.015	62.89%
100	0.254±0.002	66.43%	0.202±0.01	73.42%
Control	0.760±0.002		0.760±0.003	

Values are mean±S.D of triplicate

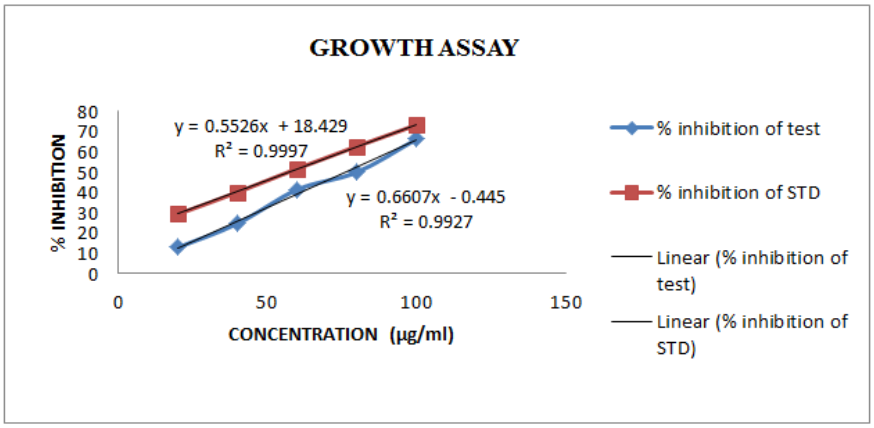

Fig-2: Effect of *Thlaspi* Q and Cystone on growth assay

From the above graphical method, the IC_{50} value of standard and test was found to be 57.18 µg/ml and 76.43µg/ml respectively.

AGGREGATION ASSAY

Table-4: Fig: 2: Effect of *Thlaspi* Q and Cystone on aggregation assay Values are mean ±SD of triplicate

Concentration (µg/ml)	O.D of test	% inhibition of test	O.D of standard	% inhibition of standard
20	0.385±0.004	19.9%	0.275±0.002	42.82%
40	0.365±0.002	24.11%	0.260±0.001	45.94%
60	0.343±0.003	28.69%	0.215±0.002	55.3%
80	0.310±0.002	35.55%	0.166±0.002	65.45%
100	0.285±0.003	40.74%	0.098±0.003	79.62%
Control	0.481±0.002		0.481±0.002	

The table 4 and figure 3showed the effect of different concentration of *Thlaspi* Q on aggregation of calcium oxalates. Crystal formation. The increase in the concentration of *Thlaspi* mother tincture showed increase in the inhibition of aggregation. Maximum inhibition of aggregation was 40.74%observed at concentration of 100µg/ml.

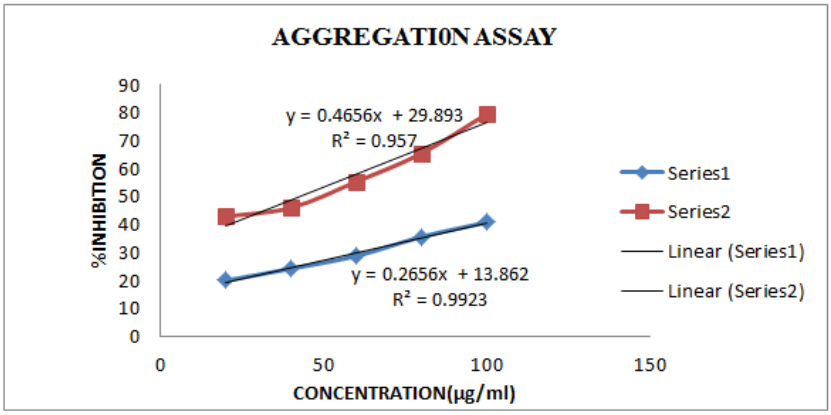

Fig-3: Effect of *Thlaspi* Q and Cystone on aggregation assay

DISSCUSION

Kidney stone are hard solid particles in the urinary tract .In many cases, stones are small and pass out of the body without any problem. If the stone blocks the flow of urine, excruciating pain may result and prompt medical treatment is needed. Kidney stone formation is a complex process of various physico-chemical events including urinary supersaturation, nucleation, growth, aggregation and retension of crystals in the renal tubules. Urinary supersaturation generally considered to be the one of the causative factors of calculogenesis .Calcium oxalate monohydrate crystals are harmful than calcium oxalate dehydrate crystals because of their tendency to attach with kidney epithielial cells resulting in the form of kidney stone.

Supersaturation of urine followed by cluster formation which leads to the process of nucleation. In this process the phase changes of dissolved salts into solid. Then crystal growth and aggregation .Aggregation is the most effective mechanism to increase the size of the particle, composition and structure of urinary crystals. In the present study help to determine the effect of *Thlaspi* mother tincture on crystal nucleation, growth and aggregation. The result shows that the mother tincture possess significant inhibitory activity in crystal formation, it may be due to presence of various phytochemical such as flavanoids, saponins, terpenoids in the mother tincture.

CONCLUSION

The result shows that *Thlaspi* mother tincture shows significant antiurolithiatic activity, but there is a need of detailed invivo study to prove the antiurolithiatic activity.

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