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Anti-urolithiatic Property of *Crataeva nurvala* Root and Bark from Nepal on Ethylene Glycol induced Urolithiatic Mice

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Abstract

Introduction

Due to unavailability of potent drug for treatment of urolithiasis and its reoccurrence, the interest is increasing for study of natural products with anti-urolithiatic activity having fewer side effects. The present study was aimed to examine the anti-urolithiatic property of methanolic extract of *Crataeva nurvala* root and bark for in vivo, in comparison to popular herbal standard Cystone.

Method

Mice were divided into seven groups. Group I act as normal control group and group II serve as negative control. Group II to group VII were given normal diet and pure drinking water containing ethylene glycol and ammonium chloride. Group III was taken as standard whereas Group IV to VII were taken as test group. Test group were fed with 150 mg/kg and 300 mg/kg of both bark and root extract where as a standard group was supplied with Cystone at dose of 750 mg/kg. At 11th day of treatment, urine was analyzed for total biochemical parameters namely uric acid, calcium and magnesium along with total urinary volume per day.

Result

The result showed that both root and bark extract of plant have effective antiurolithiatic effect. Among them, root extract at the concentration of 300 mg/kg performed most significant activity which is almost similar to standard.

Conclusion

Our study revealed that, *C. nurval* root can be the best alternative for ayurvedic drug as it alone can show the potent anti-urolithiatic activity almost similar to poly herbal drug .

Key words: *Crataeva nurvala*, urolithiasis, Ethylene glycol, Ammonium chloride, Cystone.

INTRODUCTION

Deposition or development of stones in any part of the urinary system i.e. the kidney, ureters, or the urinary bladder is called urolithiasis. A stone is formed after deposition of solute materials such as calcium, oxalate, phosphate and uric acid inside the kidney [1]. Urinary stone diseases (urolithasis) are one of the most common complaints of modern society that has been described since time immemorial. According to recent data, urolithiasis has become third most common disorder of the urinary tract. Major causing factor for this disease are due complex physiochemical process, calculogenesis, urinary super saturation and matrix formation [2]. Globally, calcium oxalate is considered as the chief constituent in the renal calculi. Calcium containing stones, principally calcium oxalate monohydrate (COM), calcium oxalate dehydrate (COD) and basic calcium phosphate are the most frequently arising stones to an extent of 75-90% which is followed by magnesium ammonium phosphate uric acid and cystine. Silicate stones also known as drug induced stones are very hardly seen, and can be a result of taking certain medications or herbal products and the subsequent build-up of chemicals from those products in the urine. Some of these drugs are loop diuretics, acetazolamide, topiramate, zonisamide, ciprofloxacin, indinavir etc [3]. No new treatments have been established in decades, and these that are available have inherent problems related to patient compliance, cost, effectiveness and side effects. Therefore study of the potency of herbal drugs used in the traditional medicine has attained great attention due to safe and effective result at low cost [1].

Crataeva nurvla is a medium sized branched evergreen tree indigenous to India. It is commonly known as Varuna. It is distributed throughout the river banks of southern India and other tropical, sub-tropical countries of the world, which is wild or cultivated [4]. Traditionally the stem bark is used as stomachic, laxative, anthelmintic, expectorant and anti-pyretic [5]. From the ancient time therapeutic effect of plant extract and their bioactive constituents have been proved to be precious remedial recipe to treat various acute and chronic disorders [6]. Pharmacological study reveals the potentiality of *C. nurvala* extract and its active principle, particularly lupeol as diuretic, anti-inflammatory, anti-oxidant, cardio-protective, hepatoprotective, lithonotriptic, anti-rheumatic, anti-periodic, contraceptive, anti-protozoal, rubifaciant and vesicant [5, 7]. Preliminary phytochemical screening reveals that the plant is rich in secondary metabolites like alkolides, saponins, triterpenes, tannins, flavonoids, glycosides, glocosinolates and phytosteroids. Various bioactive constituents such as cadabicine, cetyl alcohol, betulinic acid, friedelin, diosgenin, diacetate etc have already been isolated from the stem bark [5]. Various biological activities such as urolithic propery [8],

antifertility activity [9], analgesic activity [10], antiarthritic [11], cardioprotective [12], antidiabetic activity [13] etc. have been reported from the bark of *C. nurvala*. Although antiurolithiatic activity of bark extract has been proved in previous research, there is no any scientific study for root *of C. nurvala*. Both root and bark of this plant are used traditionally to treat kidney stone in Salyan district of Nepal [14]. Therefore, this study was aimed to evaluate anti-urolithiatic Property of *C. Nurvala* root and bark of Nepalese origin on Ethylene Glycol induced urolithiatic mice and to compare with herbal standard Cystone.

MATERIALS AND METHODS

Chemical Required

Ethylene glycol (Merck specialties private limited, Mumbai) and Ammonium chloride (Merck specialties private limited, Mumbai), Cystone (Himalayan drug company at: Makali, Bangalore – 562 123), Acetone (Thermo fisher scientific India Pvt. Ltd., Mumbai), Methanol (Thermo fisher scientific India Pvt. Ltd., Mumbai) . Water was prepared in the laboratory with distilled water plant, herbal standard Cystone was brought from market.

Instruments and glass wares

Rotary evaporator (R-210/215, BUCHI Labor technok AG, Switzerland), Digital balance (ATX224, SHIMADZU Corporation, Philippines), Rotary evaporator (R-210/215, BUCHI Labor technok AG, Switzerland), Hot air oven (S.M. Scientific Instruments (P) Ltd., Delhi), Grinder and Distilled Water (DW) plant, Sonicator (INDOSATI Scientific Lab Equipments), Autoclave (S.M. Scientific Instruments (P) Ltd., Delhi). beakers (50 ml, 100 ml), volumetric flasks (1000 ml), micropipette, round bottom flask (1000 ml) cotton, plastic bottle, aluminium foils, detergents, butter paper, conical flasks, measuring cylinders, spatulas (stainless steel), paper sheets, glass rods, washing brush, watch glass, funnels, filter paper (Whatman no. 1), scale, map, marker, feeding tube, injection, test tube, gloves, mask, cage, mortar and pestle were used in experiment.

Plant Materials

The plant was collected from Bhalchaur, Salyan district, Rapti Zone, midwestern Nepal. The selection of the species used in this study was mainly based on their ethno medical evidences (literature) of use for condition such as diarrhea, inflammation, fever, diabetes and in urolithiasis. The Plant identification was done by Botanist (Homnath Pathak from Prithvi Narayan campus, Nepal) and also compared with the literatures

Table 1: Details of Plant collection date, area, used part and its local and scientific name

Collection date	July, 2018
Plant name	*C. nurvala*
Local name	Siplican
Parts used	Stem bark and root
Collection site	Bhalchaur, Salyan

Preparation of plant extract

Collected plant materials were cleaned and shade dried at room temperature followed by communition to fine powder. Extraction was done by triple cold maceration in which 500g root and bark dried powder was soaked with 2500 ml of methanol with occasional shaking for 48 hours and then liquids were strained and the marcs were pressed. The liquids were then filtered. The process is repeated up to triple maceration and filtrate was mixed and dried using rotary vacuum evaporator evaporated at a temperature of 40°C. The gummy concentrate was kept in glass vials and the percentage yields of the extract were calculated. Then, the gummy concentrate kept in vials was covered with aluminum foil and stored in the refrigerator at temperature of 4°C until use.

Animals and Ethical Approval

Both male and female Swiss Albino mice weighing 25–30 g were brought from the National Herbarium and Plant Laboratories, Phulchoki Hill Nepal .They were supplied with standard feed and water ad libitum. They were placed in clean polypropylene under constant room temperature (22 ± 1◦C), with continuous cycle of dark and light up to equal time throughout the experiment Animal handling and care and all experimental procedure were completed under official ethical guidelines of Nepal [15, 16]. All experimental animals were submitted to 35% CO_2 euthanasia after finishing the study. The experimental procedures was verified by the Institutional Review Committee of, Pokhara University (Ref: CCT/IRC/058/23) and it was approved at 15 September 2018.

Acute Toxicity Studies

Acute toxicity study was done following the guidelines of Organization of Economic Cooperation and Development (OECD) [17]. There were total seven groups of Swiss Albino mice, each group containing five mice (n = 5). Among them, six were test groups and one was normal control group. Normal control group was given only saline, whereas test groups were feed with plant extract (500, 1000 and 2000mg/kg, p.o.) at 10 mL/kg The any symptoms of adverse effects and mortality were observed every 1 h for the next 6 h and body weight was taken on day 1, 7 and 14 after treatments.

Anti-urolithiatic activity test

Experimental model

Anti-urolithiatic activity was evaluated by adopting previously established method in ethylene glycol and ammonium chloride induced urolithiatic rats [18, 19]. Total thirty five Swiss Albino mice were divided into 7 groups each group containing 5 rats. Animals of all groups were given normal diet and drinking water.

Group-I: (Control group) animal received normal diet and drinking water for 10 days.

Group-II: Urolithiasis induced group was given with drinking water containing 0.75 % (V/V) ethylene glycol and 2 % (W/V) ammonium chloride for 10 days.

Group-III: The standard group received drinking water containing 0.75 % (V/V) EG and 2 % (W/V) AC as well as Cystone 750 mg/kg as standard for 10 days.

Group-IV: The test group also received drinking water containing 0.75 % (V/V) EG and 2 % (W/V) AC with treatment of *C. nurvala* bark extract 150 mg/kg for 10 days.
Group-V: Received drinking water containing 0.75 % (V/V) EG and 2 % (W/V) AC with treatment of *C. nurvala* bark extract 300 mg/kg for 10 days.
Group-VI: Received drinking water containing 0.75 % (V/V) EG and 2 % (W/V) AC with treatment of *C. nurvala* root extract 150 mg/kg for 10 days.
Group-VII: Received drinking water containing 0.75 % (V/V) EG and 2 % (W/V) AC with treatment of *C. nurvala* root extract 300 mg/kg for 10 days.

Collection and urine analysis
At 11th day, 5 mice from each group were kept in single cage and urine was pooled for 24 hours for each and HCl was added before storing at 6^0C. Total urine volume was measured and analyzed for magnesium, calcium and uric acid.

Statistical analysis
The calculations of the average concentration of calcium, magnesium and uric acid for the anti-urolithiatic was based on the expression of numerical data as mean ± SD and were evaluated by two tailed student t- test. The results obtained were compared with the control group. The $p< 0.05$ was considered to be statistically significant.

RESULTS

Extractive yield value
The crude drug of *C. nurvala* was extracted in methanol solvent. The extract yields of crude drugs are given in the table.

Yield value of each extract was calculated as:
Yield value (%)
= Extracts obtained/ Total amount of crude drug x 100

Table 2 : Extraction Yield of *Crataeva nurvala* bark and root.

Plant	Extract Yield (%)	
	Bark extract	Root extract
Crataevea nurvala (Root)	0.3 %	0.27 %

Acute toxicity test
There was no any indication of toxic effect and mortality after the administration of methanolic extract of plant at the doses of 200, 400, 800 and 1000 mg/kg up to 14 days.

Anti-urolithiatic activity test
All the data obtained for anti-urolithiatic activity experiment with methanolic extract of *C. nurvala* root and bark is shown in Table 3 and Figure 1. During experiment we observed significant increase ($p < 0.05$) in urinary excretion of uric acid and calcium where as there is significant decrease of urine output and magnesium in toxicant group as compare to normal control group. However administration of CNBME and CNRME extract in all the dose exhibited dose dependent significant ($p < 0.05$) reduction in urinary calcium and magnesium excretion along with increase of urinary output and magnesium level as compare to toxicant group. Between two extract, antilithiatic effect observed after treatment with root extract was found to be very much effective and equivalent to standard drug Cystone in terms of reduction in the urolithiasis and increase in urinary output.

Table 3: Effect of plant extract on urinary Uric acid, Magnesium and Calcium in EG (0.75%) and AC (2%) induced urolithiatic mice.

S.N	Group	Urine output (ml/day)	Uric acid (mg/dl)	Magnesium (mg/dl)	Calcium (mg/dl)
1	Normal Control	12.29± 0.029	5.428±0.39	4.67±0.44	8.87±0.71
2	Toxicant+ EG Plus AC	6.87± 0.027	12.28±0.34	2.33±0.036	18.05±0.38
3	Standard + EG Plus AC	11.16± 0.02*	7.33±0.40*	3.21±0.16*	7.38±0.523*
4	CNBME 150 mg/kg + EG Plus AC	8.12±0.017*	10.19±0.025*	2.44±0.022*	14.01±0.032*
5	CNBME 300 mg/kg + EG Plus AC	9.44± 0.021*	8.15±0.29*	2.66±0.28**	10.04±0.19*
6	CNRME 150 mg/kg + EG Plus AC	8.53±0.021*	9.55±0.027*	2.51±0.039*	12.38±0.020*
7	CNRME 300 mg/kg + EG Plus AC	9.95±0.018*	7.90±0.016*	2.94±0.046*	8.25±0.065*

Values are expressed as mean ± SD, n = 5 * statistically significant at $p<0.001$, ** statistically significant at $p<0.05$, EG: Ethylene Glycol, AC: Ammonium chloride, SD: Standard Deviation, CNBME: *C. nurvala* bark methanolic extract, CNRME: *C. nurvala* root methanolic extract

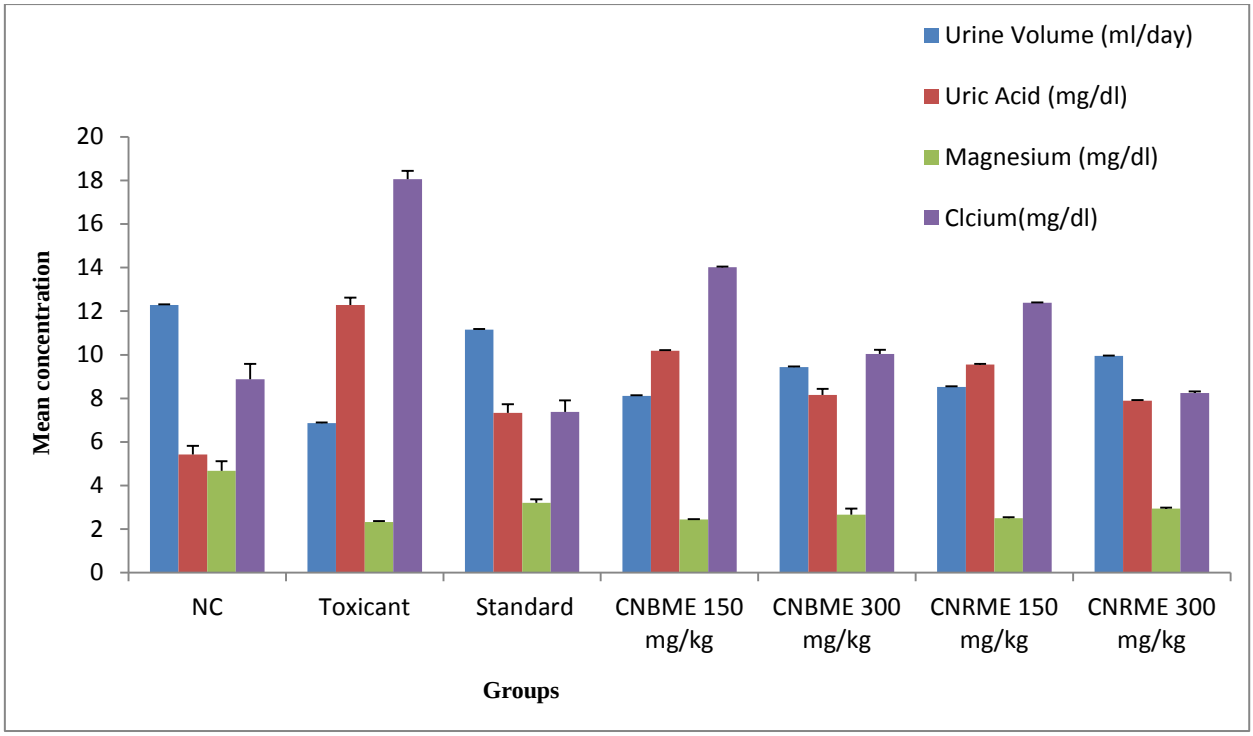

Figure 1 : **Effect of *C. Nurvala* Extract on Urinary parameters in control and experimental urolithiatic mice**.
Note- NC: normal control, CNBME: *C. nurvala* bark methanolic extract, CNRME: *C. nurvala* root methanolic extract.

DISCUSSION

In this study, we investigated the protective effects of CNBME and CNRME in ethylene glycol induced urolithiasis swiss albino mice. Urinary super saturation of different stone-forming elements is considered to be one of the major etiological factors in the formation of stone. A number of models have been used for the study of nephrocalcinosis and nephrolithiasis. Rats or mice have been a suitable species for study of anti-urolithiatic activity because of its urinary system resembling closely that of the human [8]. Urolithaisis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, hydroy-L-proline and glycolic acid. Kidney being the principle target for EG induced toxicity. It's administration to the experimental animal result in substantial excretion of oxalate and deposition of microcrystals in kidney. EG is degraded inside the body into four organic acids namely glycolic acid, glycolaldehyde, glycooxalic acid and oxalic acid leading to hyperoxaluria which is the main initiative factor for lithiasis [2]. Mainly EG is responsible for generation of lithiasis, further AC was co-administered in order to augment lithiaisis effect of EG.

Calcium, phosphate and oxalate are key component to play significant role in renal calculogenesis. Administration of EG in rats increase urinary excretion and renal deposition of calcium, phosphate and oxalate significantly. This effect could be due to retardation in tubular reabsorption of kidney. The inhibitory effect of magnesium on crystallization in urine and formation of calcium oxalate crystals is by virtue of ability of magnesium to form a complex with free oxalate in the urine thereby forming a soluble complex thus reducing the availability of free oxlate to complex with calcium [2].

Normal urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low level of magnesium in urine is associated with stone formation. Increased excretion of uric acid has been reported in stone formers. Uric acid decrease calcium oxalate solubility and it binds and reduces the inhibitory activity of glycosaminaglycans. The increase level of uric acid crystal in calcium oxalate stones and binding capability of uric acid binding proteins to calcium oxalate greatly enhance crystallization of calcium oxalate and result stone formation [20]. Therefore plant extract might have successfully managed the level of oxalate by inhibiting the oxalate synthesis.

As shown in Table 3 and Figure 1.The mean urinary concentration of uric acid, calcium, urinary volume and magnesium in toxicant group reached to 12.28 mg/dl, 18.05 mg/dl, 6.87 ml/day and 2.33 mg/dl and respectively which were very much changed as compared to that of normal control group. In the group treated with standard i.e., Cystone, the concentration of uric acid and calcium decreased to 7.33 mg/dl and 7.38 mg/dl respectively while the concentration of urinary volume and magnesium increased to 11.16 ml/day and 3.21 mg/dl respectively. In the treatment group, all extract of *C. nurvala* has shown positive result as compare to toxicant group. Among them, CNRME at doses of 300 mg/kg showed most effective result. The concentration uric acid and calcium was decreased from 12.28 to 7.9 mg/dl and 18.05 to 8.25 mg/dl respectively. In contrast, concentration of urine volume and magnesium was increased from 6.87 to 9.95 ml/day and 2.33 to 2.94 mg/dl respectively as compared to

toxicant. The result obtained from the ethylene glycol and ammonium chloride induced urolithiasis showed that urolithiasis was markedly inhibited by the administration of the extract, thus indicating that the extract can inhibit the lithiasis process and that the extract is orally active. The diverse class of phytochemicals especially lupeol present in the extract may be responsible for thid activity [8]. It may be the best alternative for ayurvedic drug Cystone as it contains many plant's extracts but *C. nurvala* alone can show the anti-urolithiatic activity.

CONCLUSION

In this study, the ethylene glycol and ammonium chloride induced urolithiatic rats, the root extracts of *C. nurvala* at concentration 300 mg/kg showed the significant prevention of crystal deposition on urinary tract. The standard showed best result at 750 mg/kg. Future research of *C. nurvala* by using extracts of different parts of plant in different solvent can give further information regarding its potential. Its easy availability, efficacy, safety and potency can be used as an alternative with the available anti-urolithiatic agents.

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Authors Contribution

Jitendra Pandey and Rubee Pantha act as First author. Ravin Bhandari act as Corresponding author.

Conflict Of Interest

We declare that we have no conflict of interest.

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