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Antiuro lithiatic Activity of *Pascopyrum smithii* in a Polyherbal Formulation Inhibition of Calcium Oxalate Crystallization on Urolithiasis Induced Rats

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ABSTRACT

The research was aimed to “To formulate a polyherbal formulation of *Pascopyrum smithii* for Antiuro lithiatic activity on Calcium Oxalate Induced Urolithiasis in Rats” Urolithiasis is defined as the presence of one or more stones at any point within the urinary tract. It is the third most common urinary tract disease; the others are frequent urinary tract infections and benign prostatic hyperplasia. The global incidence of urolithiasis is quite high and, despite enormous progress in the field of medicine, there is no truly satisfactory medication available for the treatment of kidney stones. Most patients still have to proceed for surgery to get rid of this painful disease. Hyperoxaluria is the main trigger for urolithiasis. The present study is to formulate an ayurvedic capsule for treatment of urolithiasis. And to study the role of *Pascopyrum smithii* in preventing and treating kidney stones and to further explore the effective metabolites from the crude extract. The formulated capsules are evaluated as per WHO guidelines and also performed pharmacological activity.

Keywords: Antiuro lithiatic activity, Calcium Oxalate Crystallization, Urolithiasis Induced Rats

INTRODUCTION

Stone disease is one of the commonest diseases and affects 0.131% of the total population. During life, 10-15% of the population can get affected by urolithiasis. After passing a first stone risk of recurrence decreases up to 40% at 5 years and 75% at 20 years,. Due to the low incidence of a reversible metabolic cause, first time stone former do not have a complete



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evaluation of urine and electrolytes. However in more than 90% of recurrent stone formers, reversible metabolic abnormality can be identified. The focus is mainly on the prophylactic treatment of urolithiasis, the dissolution therapy of urolithiasis and the medical treatment of ureteric colic ^[1-3]. Prophylactic Management in a Urinary Stone Former General Medical Advice ^[2-4] All patients with renal colic should receive general medical advice to reduce the risk of further episodes of future stone. They should be advised to increase their fluid intake so that urine output is > L / day. Moreover, the best estimate of urine production is clear urine which prevents urine stagnation and therefore reduces the risk of stones.

By increasing intake of citrus juices, patients should increase their urinary citrate level as urinary citrate is a powerful stone inhibitor. Citrate binds to calcium present in the urine, thereby decreases supersaturation and reduces crystal growth. Furthermore, in patients with normal urinary citrate, an increase in urinary citrate level helps to prevent recurrence of stones. The intake of purine (animal flesh) should be moderate as it increases the urinary secretion of calcium, oxalate and uric acid. By limiting the protein and salt, with maintaining a normal calcium intake, the stones' recurrence rates are reduced compared to a low calcium diet.

A high sodium intake can enhance the risk of calcium oxalate formation and therefore a salt restriction is also recommended. Moreover, obesity also increases the risk of stone disease by raising urinary acidity, hypocitraturia and hyperuricosuria. As a result, weight loss and a low-fat diet should be encouraged. This is particularly important in patients with intestinal disease or malabsorption conditions. Calcium consumption in dietary recommendations should also be continued even the patients is having calcium oxalate stones.



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EXPERIMENTAL WORK

Plant Material:

Pascopyrum smithii was collected from the local market of Indore (Madhya Pradesh). The plant was identified and authenticated by Department of Botany, Holkar Science College, Indore (M.P).

Chemicals and Drugs:

Cystone (Himalaya Pharmaceutical, Bangalore), Ethylene glycol (SRL Mumbai), Tween 80 (Merck Pvt Ltd, B, Mumbai), Anaesthetic ether (SD Finechem Ltd., Mumbai), Chloroform (SD Finechem Ltd .Mumbai). Formaline (SD Finechem Ltd., Mumbai) and all other chemicals and reagents were of analytical grade.

Diagnostic Kits:

Diagnostic kits utilized for creatinine estimation and estimation of Urea, Uric acid, Calcium, Phosphorus, Calcium oxalate was obtained from Robonik Diagnostic Ltd, India.

Instruments:

Instruments used were: Refrigerator centrifuge (MPW-350R), Mini Lyotrap (LTE Scientific Ltd.), Autoanalyzer (Robonik), UV Spectrophotometer (Shimadzu UV 1601), homogenizer (Remi industries, Mumbai) and Research centrifuge (Remi industries, Mumbai).

Experimental Animals:

Wistar albino male (180–220 g) was acquired from the central animal house. The animals were kept at room temperature (22-28 ° C) for 12 hours of darkness and 12 hours of light cycle and were fed a normal laboratory food and water.



Acute Toxicity Study:

Procedure

Acute toxicity studies were conducted based on the Category IV substance of the OECD-423 guidelines (acute toxicity class method). In this study, Swiss albino mice (n = 3) of both sexes were selected using a random sampling technique. The animals were kept on fasting for 4 hours by giving free access to water only. The *Pascopyrum smithii* seed extract was orally administered with a maximum dose of 2000 mg / kg of body weight. Mortality rate was observed for three days and if mortality was observed in 2/3 or 3/3 of the animals, the administered dose was considered a toxic dose. However, if death was observed only in one mouse of every three animals, the same dose was given to confirm the toxic effect. Again if no death was occur, the procedure was continued with higher dose ^[50].

Observations

During the first 30 minutes after administration, the animals were observed separately at least once and then periodically during the first 24 hours (with particular attention during the first 4 hours) and subsequently every day, for a total of 14 days.

S.No.	Code	Toxicity		Time of Death	Observation										
		Onset	Stop		Skin colour	Eyes	Resp	CNS	Tre	Con	Sali	Diah	Sleep	Let	Com
1.	ELA	X	X	X	X	x	x	x	X	X	X	x	x	x	X

(*TRE-Tremor, CON-Convulsions, SALI- Salivation, Diah - Diarrhea, LET)

Selection of Excipients

For the formulation of capsules in addition to the active ingredients, excipients like diluents (filler), binder, disintegrating agent, lubricant and preservatives are required. The choice of excipients was made keeping in mind the current FDA regulations.



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Diluents: Diluents/Fillers are added where the quantity of active ingredient is less (or) difficult to filling. Common tablet/capsule filler include Lactose, Dicalcium phosphate,

S. No	Ingredients	Quantity (mg/capsules)
1.	<i>Pascopyrum smithii</i>	100 mg
2.	Talc	100 mg
3.	Aerosil	2 mg
4.	Dicalcium phosphate	50 mg
5.	Magnesium stearate	2 mg
6.	Sodium Methyl Paraben	0.5 mg
7.	Sodium benzoate	0.5 mg
Total Weight(mg)		250 mg



Formulated capsule



RESULT & DISCUSSION

Preliminary Phytochemical Screening

The extract of drug were analysed for the existence of various constituents. The result of this preliminary phytochemical examination is shown in Table.

Table 13: Qualitative Chemical Examination of Ethanolic Extract of *Pascopyrum smithii*

Phyto-Constituents	Presence or Absence
Carbohydrates	+
Glycosides	+
Fixed oils and fats	+
Gums & mucilage	-
Potein & amino acids	-
Saponins	++
Tannins	+
Phytosterols	+
Flavonoids	+++
Alkaloids	+++

Pharmacological Activity Acute oral toxicity

Acute oral toxicity was performed according to OECD guideline. Extract was safe upto 2000mg/kg.



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Effect of Ethanolic Extract of *Pascopyrum smithii* on Urinary Volume and pH against EG and AC Induced Urolithiasis:

EG and AC (0.75 and 2 %.) administration showed significant ($p < 0.001$) alteration of the output of urine and pH as compared normal group. Administration of Cystone 5ml/kg, EPU 200 and 400 mg/kg caused significantly increased ($p < 0.01$, $p < 0.05$) output and pH of the urine as compared to control (EG and AC) group.

Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Biochemical Parameters against EG and AC Induced Urolithiasis:

Serum Creatinine

Administration of EG and AC (0.75% and 2%) for 10 days caused significant elevation ($p < 0.01$) in serum creatinine concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in serum creatinine concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.05$ and $p < 0.001$) in serum creatinine concentration when compared to EG and AC alone treated group.

Serum Urea

Administration of EG and AC (0.75% and 2%) for 10 days caused significant elevation ($p < 0.001$) in serum urea concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in serum urea concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.01$ and $p < 0.01$) in serum urea concentration when compared to EG and AC alone treated group.

Serum Uric Acid

Administration of EG and AC (0.75% and 2%) for 10 days caused significant elevation ($p < 0.01$) in uric acid concentration of serum compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in uric acid concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.05$ and $p < 0.001$) in serum uric acid concentration when compared to EG and AC alone treated group.

Table 14: Effect of Ethanolic Extract of *Pascopyrum smithii*

Treatment group	Urinary Volume (ml/24hr)	Urine Ph
Normal	18.4±0.97	7.5±2.1
Control (EG+AC)	6.96±0.69a	4.5±1.39a
Standard Cystone (5ml/kg)	14.93±0.57***	8.2±1.32**
ELA 200mg/kg	8.41±0.45*	5.9±1.21*
ELA 400mg/kg	9.9±0.64*	6.14±2.24**

Table 15: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Parameters

Treatment group	Serum Biochemical Parameters						
	Creatinine mg/dl	Urea mg/dl	Uric Acid mg/dl	Calcium mg/dl	Oxalate mg/dl	Phosphorus mg/dl	Magnesium mg/dl
Normal	1.26±0.21	39.48±0.50	5.720±0.02	2.58±0.03	1.88±0.24	3.24±0.26	1.85±0.19
Control (EG+AC)	1.83±0.12b	48.94±0.57	18.64±0.04	6.25±0.25a	3.73±0.28	5.48±0.29	2.72±0.13
Standard Cystone (5ml/kg)	0.53±0.01***	41.99±0.41** *	10.82±0.03* **	2.87±0.10** *	1.99±0.21* **	3.07±0.29** *	1.73±0.20**
ELA 200mg/kg	1.31±0.08*	51.69±0.50**	10.26±0.08* **	4.517±0.20* **	2.65±0.31*	4.16±0.46*	1.79±0.18**
ELA 400mg/kg	0.81±0.01***	39.28±0.49**	8.55±0.03** *	2.99±0.10** *	1.66±0.23* **	3.36±0.19** *	1.58±0.20***

All the values are Mean \pm SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, *p<0.05, **p<0.01, ***p<0.001 as compared to control and ^ap<0.001, bp<0.01 and p<0.05 as when compared to normal.

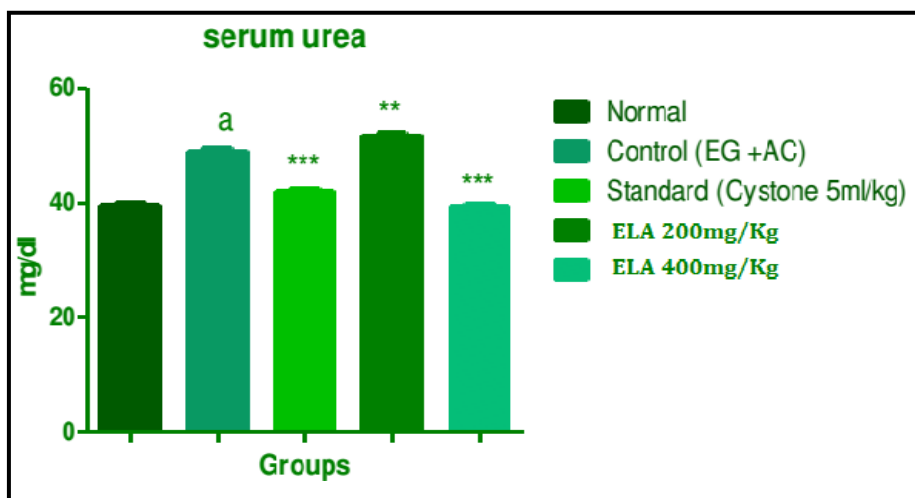


Figure 5: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Urea

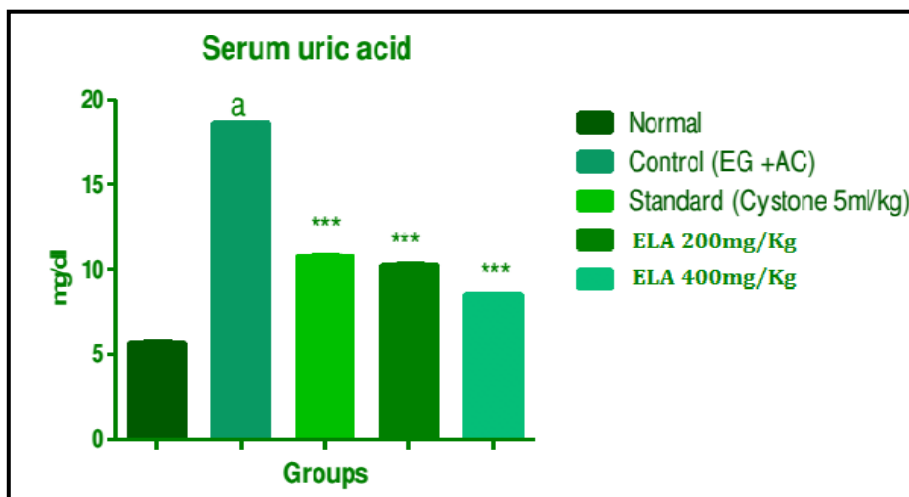


Figure 6: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Uric Acid

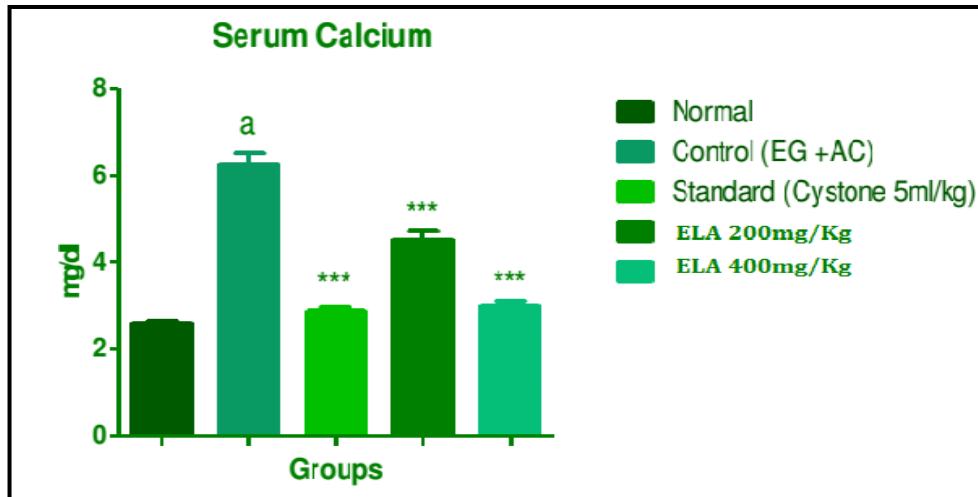


Figure 7: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Calcium

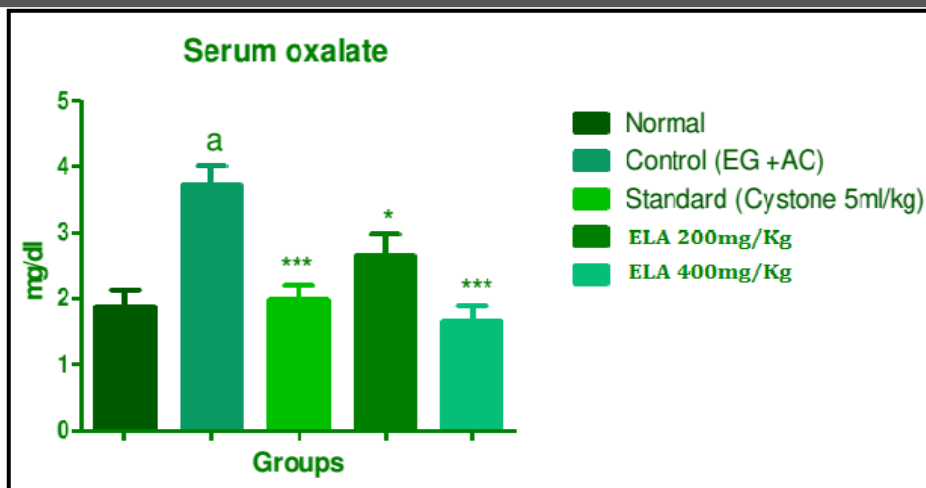


Figure 8: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Oxalate

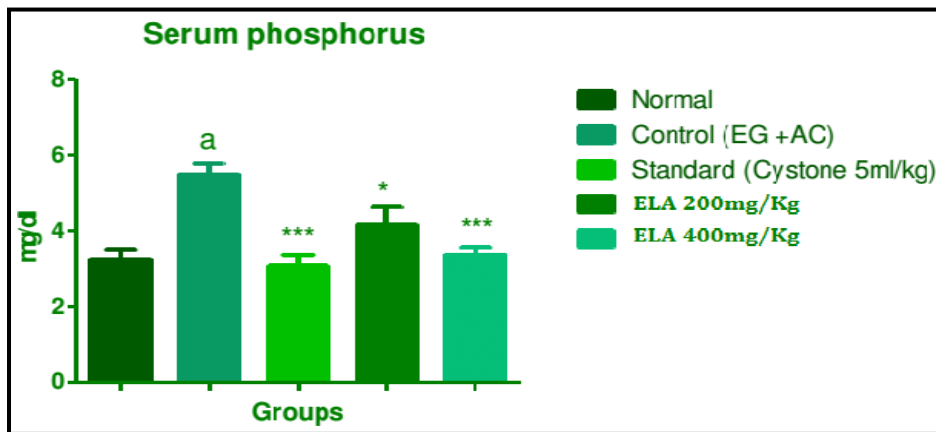
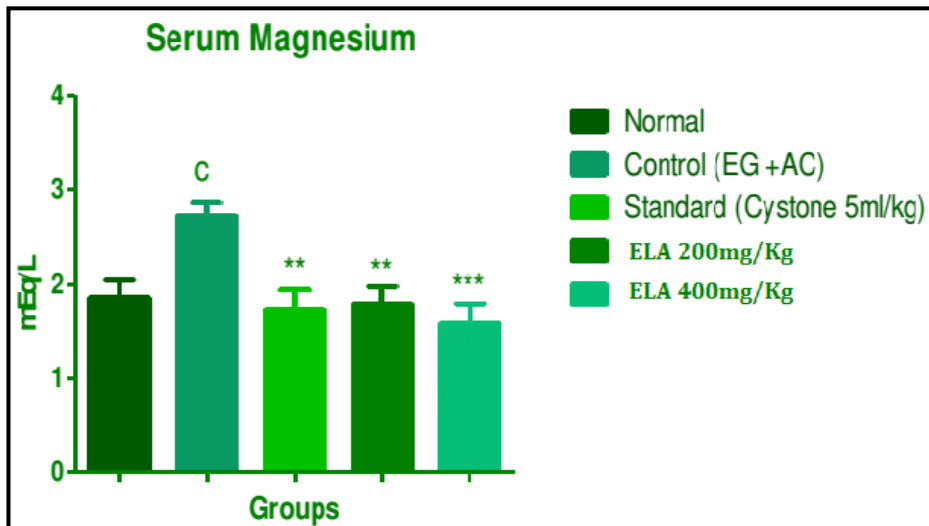


Figure 9: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Phosphorus





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Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Biochemical Parameters: Urinary Creatinine

Administration of EG and AC (0.75% and 2%) for 10 days caused significant increased ($p < 0.001$) in urine creatinine concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.01$) in urine creatinine concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction (not significant and $p < 0.001$) in urine creatinine concentration when compared to EG and AC alone treated group.

Urinary urea

Administration of EG and AC (0.75% and 2%) for 10 days caused significant increased ($p < 0.001$) in urine urea concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in urine urea concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.01$ and $p < 0.001$) in urine urea concentration when compared to EG and AC alone treated group.

Urinary Calcium

Administration of EG and AC (0.75% and 2%) for 10 days caused significant increased ($p < 0.001$) in urine calcium concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in urine calcium concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.05$ and $p < 0.001$) in urine calcium concentration when compared to EG and AC alone treated group.



Urinary Oxalate

Administration of EG and AC (0.75% and 2%) for 10 days caused significant increased ($p < 0.001$) in urine oxalate concentration compared to normal one. Standard cystone 5ml/kg causes significant reduction ($p < 0.001$) in urine oxalate concentration when compared to EG and AC alone treated group. Pretreatment with ELA 200 and 400mg/kg causes significant reduction ($p < 0.001$ and $p < 0.001$) in urine oxalate concentration when compared to EG and AC alone treated group.

Urinary Phosphorus

Administration of EG and AC (0.75% and 2%) for 10 days caused significant

Table 16: Effect of *Pascopyrum smithii* on Urine Biochemical Parameters

Treatment group	Urine Biochemical Parameters						
	Creatinine mg/dl	Urea mg/dl	Uric Acid mg/dl	Calcium mg/dl	Oxalate mg/dl	Phosphorus mg/dl	Magnesium mg/dl
Normal	0.28±0.02	58.94±2.35	2.31±0.16	10.65±0.83	6.81±0.41	3.195±0.01	4.77±0.37
Control (EG+AC)	0.59±0.05 ^a	88.82±3.19	6.01±0.28	18.49±1.03	15.65±1.22	9.093±0.46	1.94±0.01
Standard Cystone (5ml/kg)	0.40±0.01 ^{**}	53.05±2.10 ^{***}	2.99±0.36 ^{***}	11.62±0.72 ^{***}	7.03±0.46 ^{***}	4.758±0.27 ^{***}	4.36±0.48 ^{***}
ELA 200mg/kg	0.49±0.04 ^{ns}	73.48±3.53 ^{**}	3.56±0.25 ^{***}	14.37±0.98 [*]	7.60±0.57 ^{***}	7.58±0.45 [*]	3.51±0.48 [*]
ELA 400mg/kg	0.45±0.02 [*]	64.88±2.10 ^{***}	3.44±0.23 ^{***}	12.81±0.88 ^{***}	7.37±1.01 ^{***}	5.94±0.31 ^{***}	4.65±0.41 ^{***}

All the values are Mean ± SEM, n=6, One way ANOVA followed by multiple comparison of Dunnett's test, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ as compared to control and ^a $p < 0.001$, ^b $p < 0.01$ and $p < 0.05$ as when compared to normal.

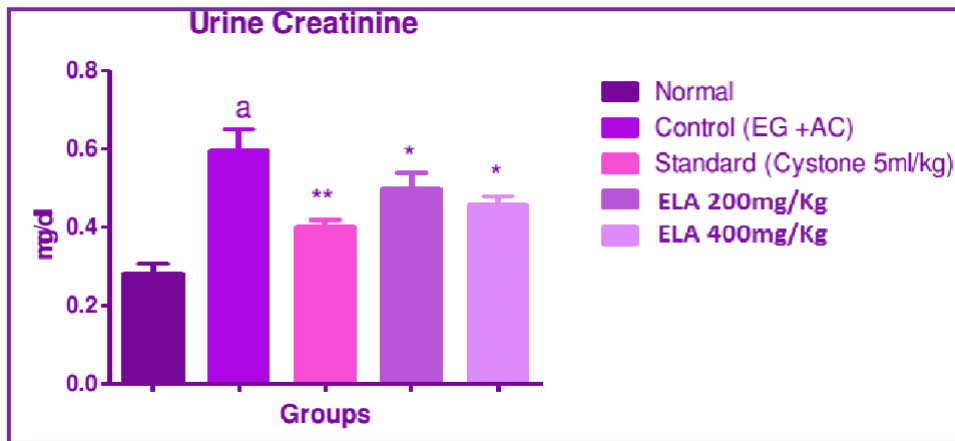


Figure 11: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Creatinine

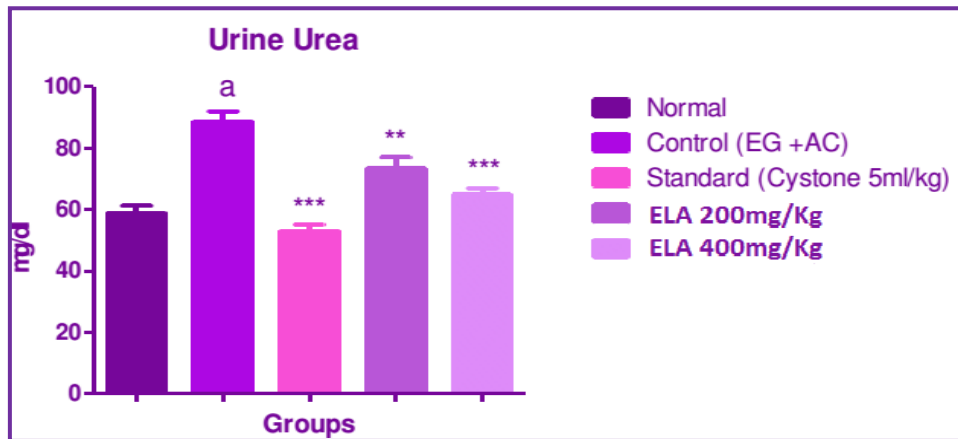


Figure 12: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Urea

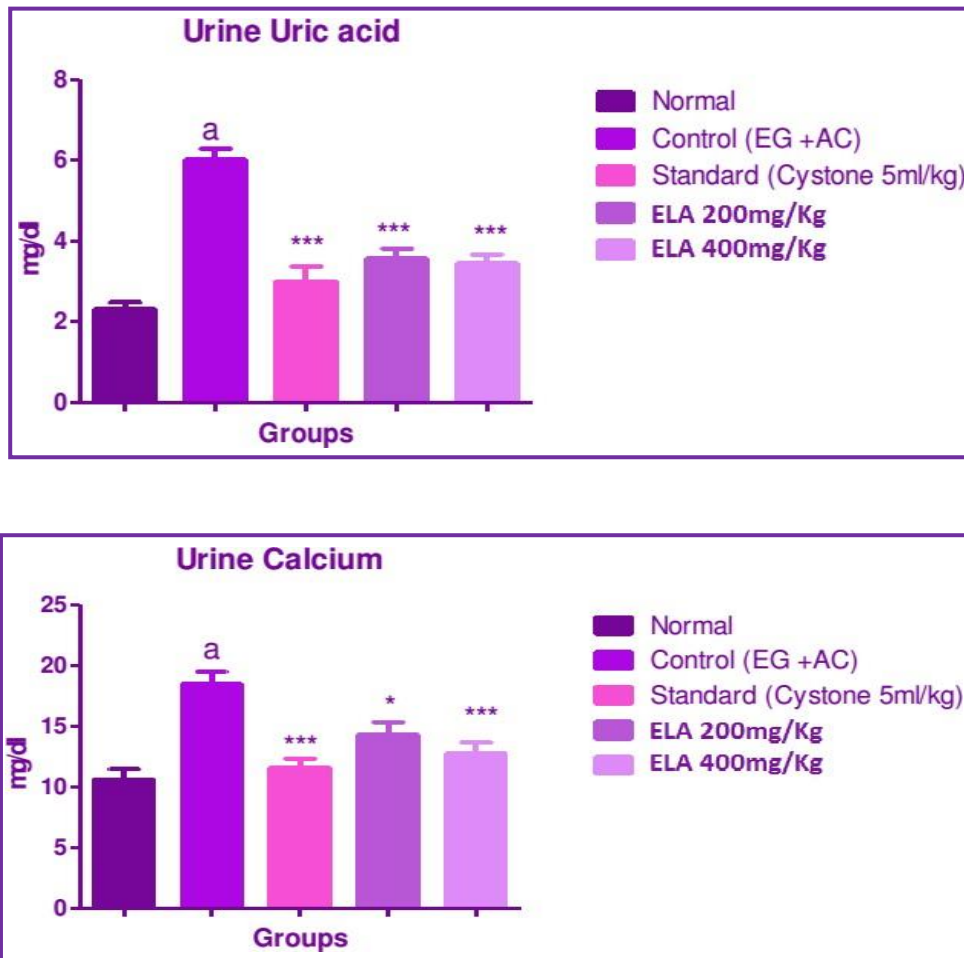


Figure 14: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Calcium

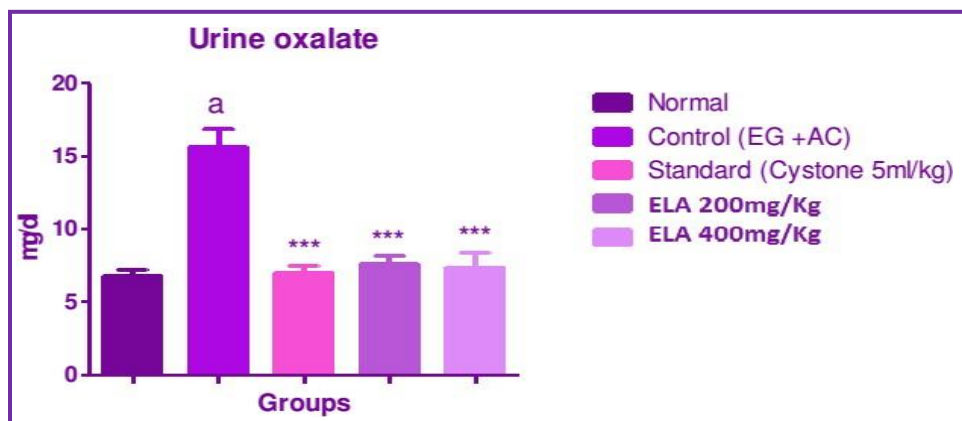


Figure 15: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Oxalate

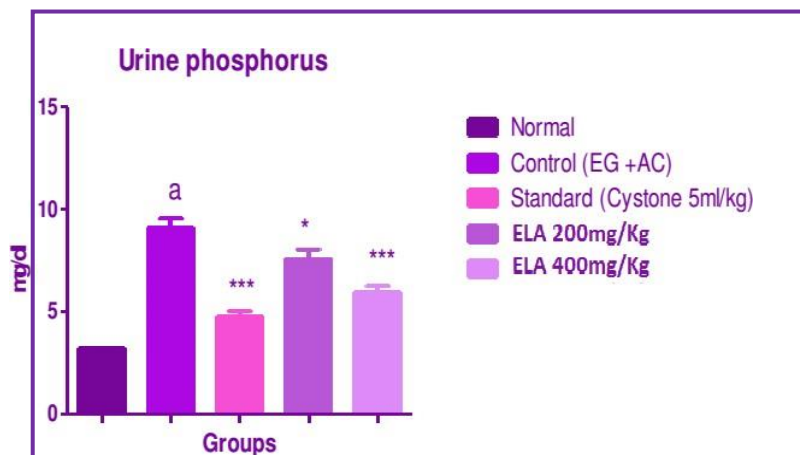


Figure 16: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Phosphorus

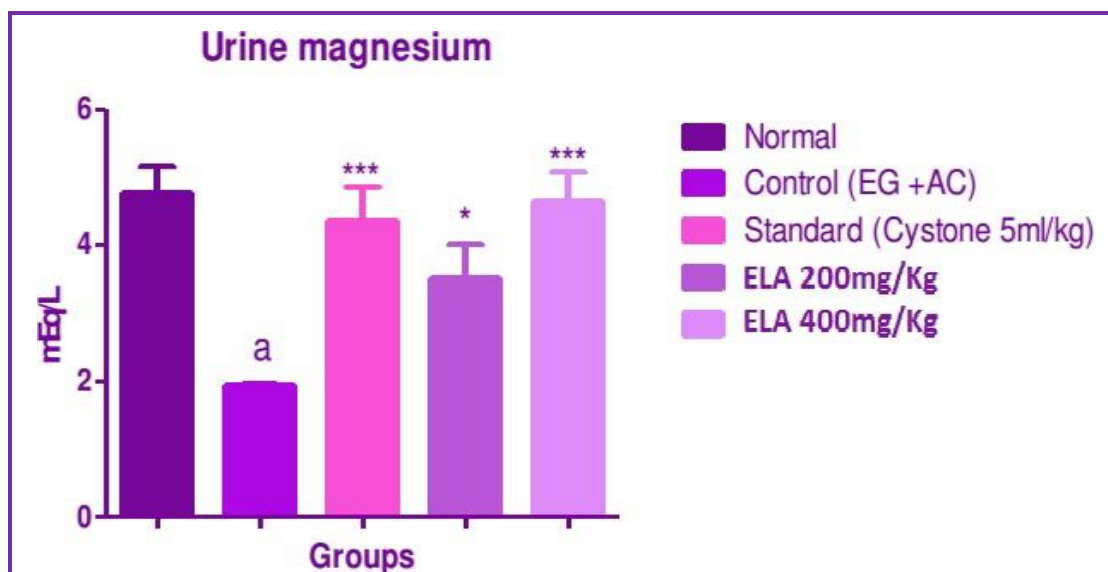


Figure 17: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Magnesium

Standardization of the Finished Product

The final formulation was analyzed for its quality control parameters in three trials. The mean value was obtained and Standard deviation was calculated. Wherever there were no official standard, limits for each parameter was established based on trial and error analysis.

EVALUATION OF CAPSULES

1. Description

“Light brown” coloured granules packed in “0” size Red capsules. The polyherbal capsules were evaluated for organoleptic characters which include colour, odour, taste and nature.



Table 17: Organoleptic characters of capsules

S.No	Parameters	Observations
1	Nature	Powder
2	color	Light brown
3	Odour	Slight aromatic
4	Taste	Characteristic

Uniformity weight of the capsule

Table 18: Uniformity weight of the capsule

S.NO	Average weight/capsule(mg)	IP specification(mg)
1	265	±10%
2	260	
3	255	
Mean ± S.D	260.3±4.5	

Mean ± standard Deviation (n=20)

Disintegration Time

Table 19: Disintegration time

S.NO	Disintegration time(min)	IP specification(min)
1	10.21	NMT 30 Minutes
2	10	
3	11	
Mean ± S.D	10.9±0.5	

Mean ± Standard Deviation (n=3)



Determination of Moisture Content

Table 20: Loss on drying

S.NO	LOD %w/w	IP specification
1	2.1	NMT 5%w/w
2	1.0	
3	2.2	
Mean±S.D	2.1±0.1	

Mean ± Standard Deviation

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