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Evaluation of Anti-Depressant Activity on Bryophyllum Pinnatum Leaves

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ABSTRACT:

The research was aimed Evaluation of Anti-depressant Activity on Bryophyllum Pinnatum Leaves. Levels of these monoamines in the brain either by inhibiting monoamine oxidase or by inhibiting reuptake of these neurotransmitters might be successful in the treatment of depression that has been classified and treated in a verity of ways. The result of our preliminary phytochemistry shows that ethanol leaf extract of Bryophyllum Pinnatum contained alkaloids, glycosides, flavonoids, tannins, saponins, carbohydrate and terpenoids. Glycoside, flavonoids, tannins, terpenoids were detected in the ethanolic fraction phytochemical components especially alkaloids, saponins, flavonoids, carbohydrate have been reported to have antidepressant activity. In the present study, the antidepressant activity of aqueous extract of whole plant of Bryophyllum pinnatum were studied in two classical models for screening animal models for depression the forced swim test and tail suspension test.

Keywords: Anti-depressant, Bryophyllum Pinnatum, Forced-Swim Test Method

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INTRODUCTION

The prevalence of depression in general population is estimated to be around 5%. At present 121 million people are estimated to suffer from depression. An estimated 5.8% of men and 9.5% of women experience a depression episode in their lifetime which suicide being one of the most common outcome of depression (WHO 1998, Stahl SM, et.al., 1998, Richelson E, et.al.,2001). Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is a cost of having to bear the burden of their adverse effects (Tripathi KD 2008, Hardmen JG, et.al. 2007).

GLOBAL STATUS OF HERBAL DRUGS

Herbal drugs constitute a major share of all the officially recognized systems of health in India viz. Ayurveda, Yoga, Unani, Siddha, Homeopathy and Naturopathy, except Allopathy. More than 70% of India's 1.1 billion Population still use these non-allopathic systems of medicine. Currently, there is no separate category of herbal drugs or dietary supplements, as per the Indian drugs Act. However there is a vast experimental–evidence base for many of natural drugs. This offers immense opportunities for Observational Therapeutics and Reverse Pharmacology. Evidence-based herbals are widely used in the diverse systems and manufactured, as per the pharmacopoeial guidelines, by a well-organised industry. Significant basic and clinical research has been carried out on the medicinal plants and their formulations, with the state-of-the-art methods in a number of institutes/Universities. There are some good examples. Indian medicinal plants also provide a rich source for antioxidants that are known to prevent/delay different diseased states. The antioxidants protection is observed at different levels. The medicinal plants also contain other beneficial compounds like ingredients for functional foods. Hence, the global

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knowledge about Ayurveda and Indian herbals will hopefully be enhanced by information on the evidence base of these plants. This will yield rich dividends in the coming years which monitor professionalism. Hence, these systems are not folklore or traditional herbal practices. These are basic axioms of these systems leading to a logical and systematic structure of pathogenesis and diagnosis, which serves also as a determinant for therapy. [2]

MATERIAL AND METHOD

MATERIAL

PLANT PROFILE

Figure 1. *Bryophyllum Pinnatum*

Synonyms

1. Kalanchoe pinnata Lam.
2. Bryophyllum calycinum Salisab
3. Bryophyllum Germinans blanco

Part used Leaf Description

It is astringent, sour in taste, sweet in the post digestive effect and has hot potency. It is well known for its haemostatic and wound healing properties. The plant have considerable

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attention for their medicinal properties and find application in folk medicine as well as in the contemporary medicine.

Experimental Animal

Albino mice of either sex weighing between 25-30gm were used in this study. The animals were procured from animal house of swami vivekanand college of pharmacy Indore M.P. and are acclimatized for 7 days and were housed under standard laboratory condition of temperature (25±2˚C) and relative humidity (55±5%) and were fed with standard pellet diet and water ad libitum. The study was approved by institutional Animal Ethics Committee and conducted as per rules and regulations in accordance to the guidelines of CPCSEA registration no. 1839/PO/ERe/S/15/CPCSEA. [45]

Drugs

Imipramine, Normal saline and other chemical were of analytical grade.

Collection of the plant material

Plant *Bryophyllum Pinnatum* was collected from Jain nursery Indore. Leaves of the plant was washed with running water, dried in shade at room temperature, ground to powder and stored in air tight bag in dry at low temperature.

Preparation of Extract Soxhlet

Extraction

The shade dried leaves (600g), was extracted with Ethanol (90%) and water in a soxhlet extractor. The concentrated material obtained was reduced to a thick mass at room temperature and water was removed by placing it in a desiccator. The weight of the dried mass was recorded and used for experimental studies. The various extracts obtained from the above procedure were used in the form of suspension. [46]

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Acute Toxicity

Toxicity studies of extract were carried out in Swiss albino mice weighing between 25-30g. They were performed according to OECD guideline No. 423. Four group of mice comprising three animals each were treated with 50, 300, 1400 and 2000 mg/kg of the extract orally, via gastric catheter. The animals were then observed continuously for the first 4hrs for any behavioural changes and for mortality if any at the end of 72 hrs. All four doses were found to be safe since no animal died even at the dose of 2000mg/kg when administered orally and the animals did not showed any gross behavioural changes. [47]

Pharmacological Methods:

Experimental protocol

Groups: Group 1- Control (Normal Saline 1ml/kg),
Group 2- Standard (Imipramine 15mg/kg),
Group 3- Received test drug *Bryophyllum Pinnatum* (100mg/kg)
Group 4- Received test drug *Bryophyllum Pinnatum* (200mg/kg)

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Fig: Forced-Swim Test

Methods

Forced-Swim Test:

Forced swim test was proposed as a model to test antidepressant activity. The animals were divided into 4 groups of 6 animals in each, weighing between 25-35 gm. The mice were forced to swim individually in glass jar ($25\times12\times25cm^3$) containing fresh water up to 15 cm height and maintained at 25˚c. After an initial period of vigorous activity for two minutes, each animal assumed a typical immobile posture. A mouse was considered to be immobile when it remained floating in the water without struggling and making only minimum movements of its limbs necessary to keep its head above the water. The total duration of immobility was recorded during the next 4 min of the total test duration of 6 minutes after administering the drugs to the respective group of animals.

The changes in the immobility duration were studied after 30 minutes of administration of the Bryophyllum Pinnatum leaves extract (100,200mg/kg) in the test group, Imipramine (15mg/kg) in the standard group and normal saline (1ml/kg) in the control group.

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Tail Suspension Test:

The changes in the immobility duration were studied after 30 minutes of administration of the Bryophyllum pinnatum leaves extract (100,200mg/kg) in the test group, Imipramine (10mg/kg) in the standard group and normal saline (10ml/kg) in the control group. [49]

Fig 3. Tail Suspension test model

STATISTICAL ANALYSIS

The data obtained in present investigation was subjected to statistical analysis. All results are expressed as mean ± SEM (standard error of mean); Six animals in each group. Statistical analysis was carried out by using Tukey's test. P values <0.0001 were considered significant.

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RESULT & DISCUSSION

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 : 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						
	eatininemg/dl	Urea mg/dl	ric Acid mg/dl	alcium mg/dl	)xalate mg/dl	osphorus mg/dl	gnesium 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***

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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, bp<0.01 and p<0.05 as when compared to normal.

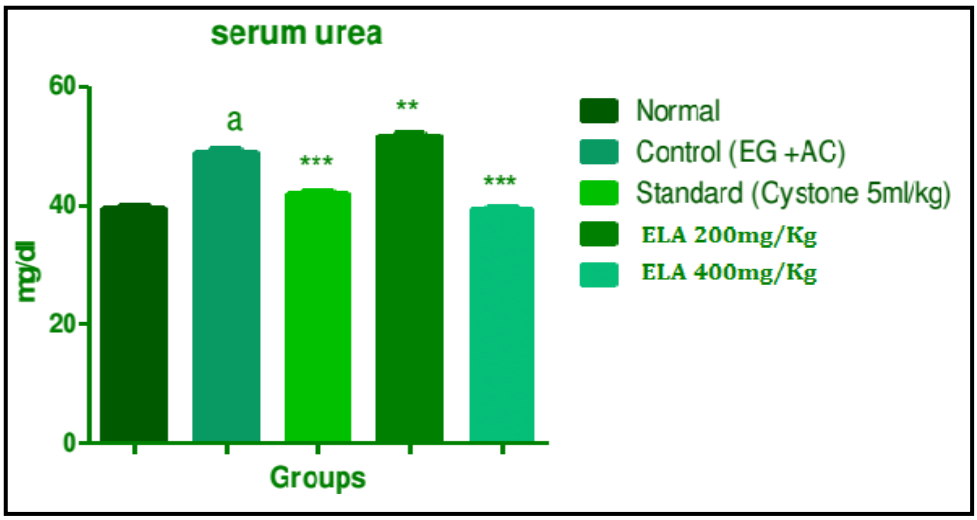

Figure 5: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Urea

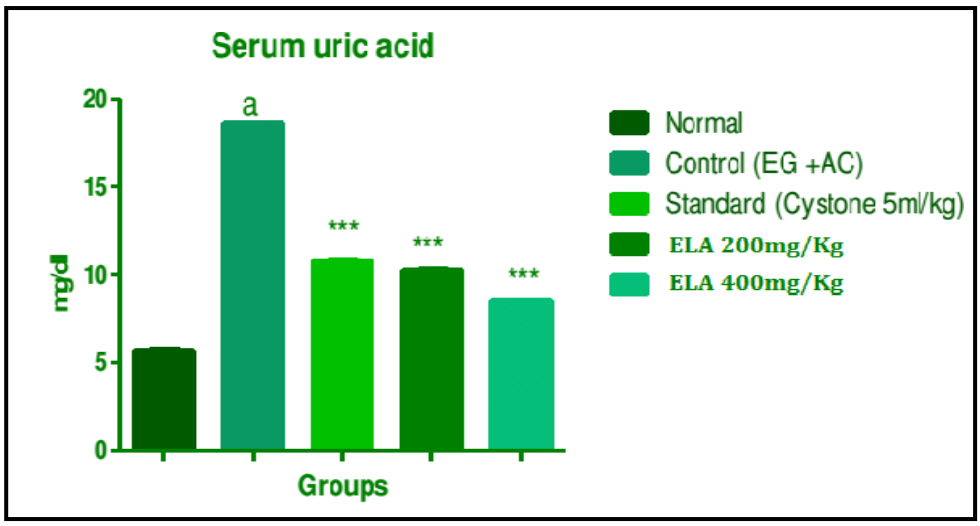

Figure 6: Effect of Ehanolic Extract of *Pascopyrum smithii* on Serum Uric Acid

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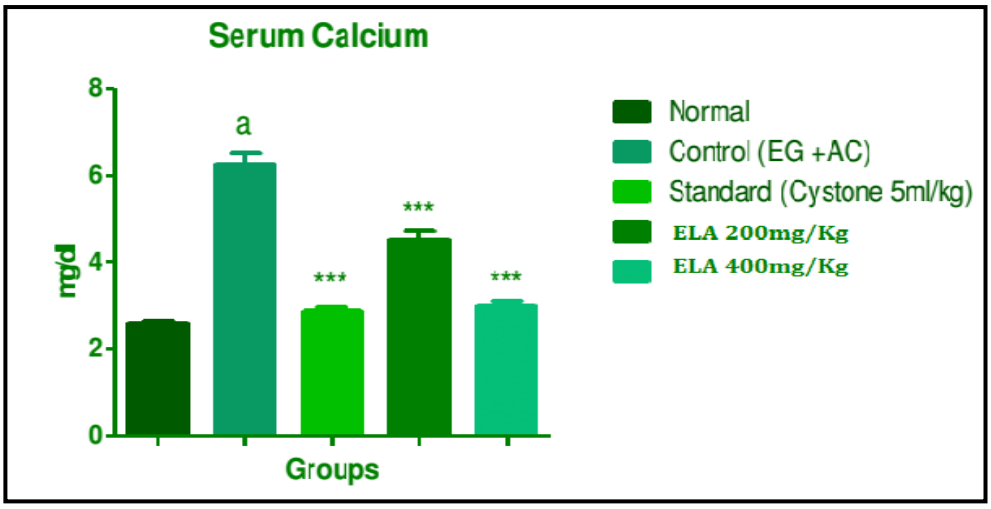

Figure 7: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Calcium

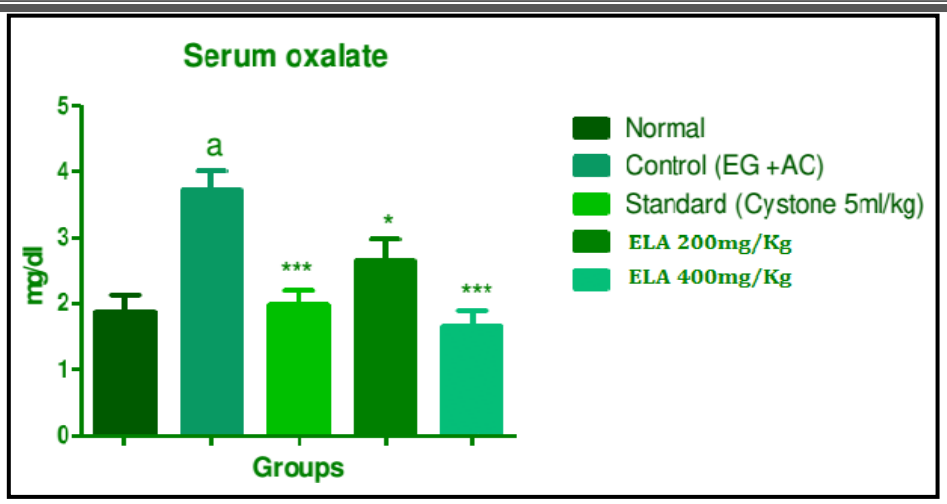

Figure 8: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Oxalate

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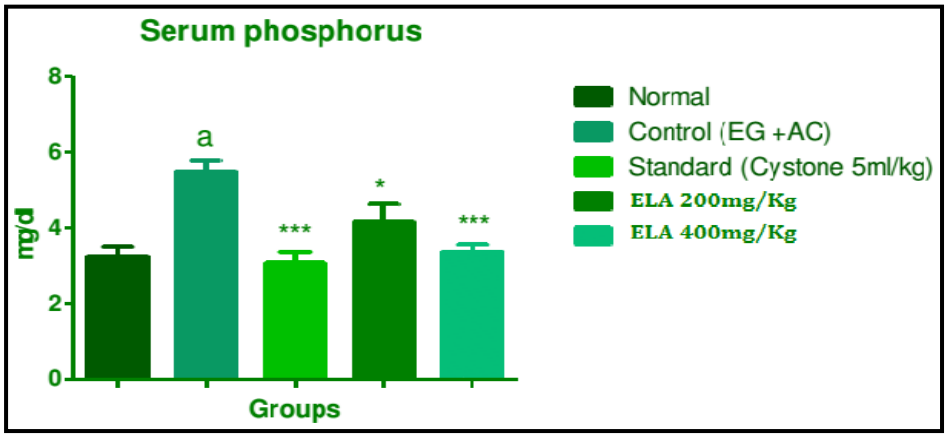
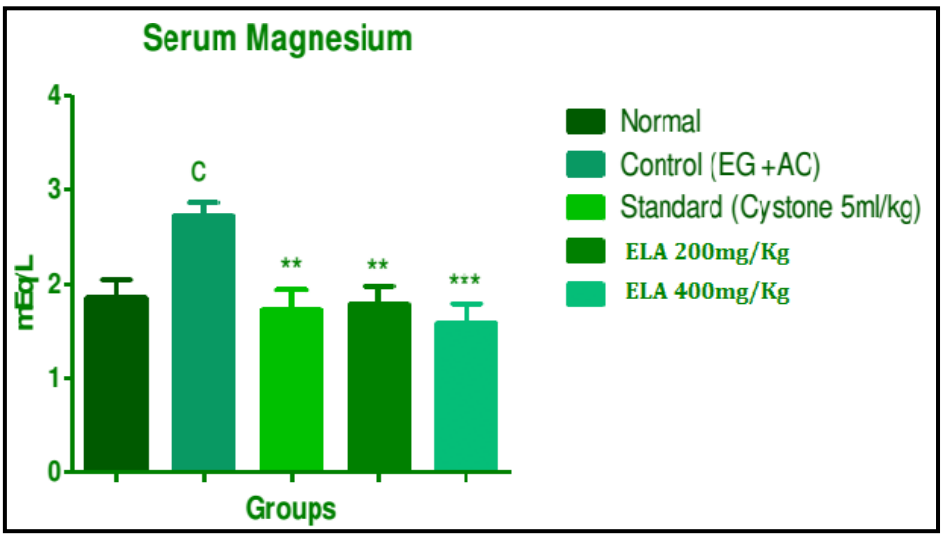


Figure 9: Effect of Ethanolic Extract of *Pascopyrum smithii* on Serum Phosphorus



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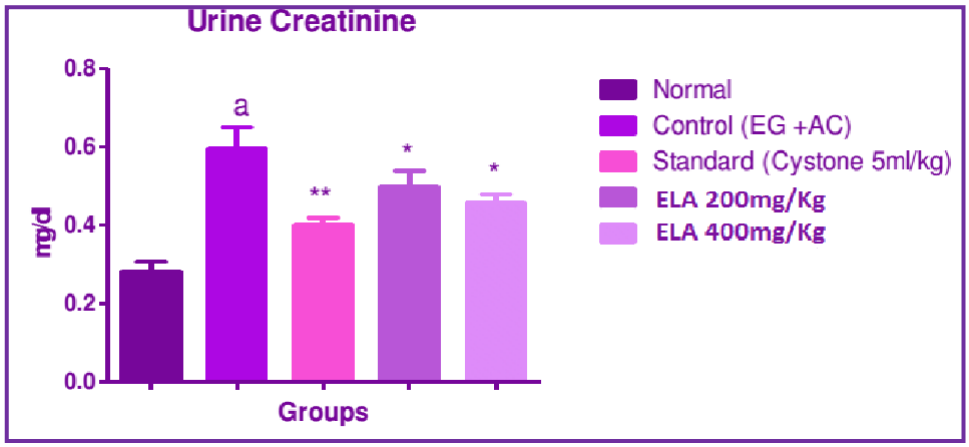

Figure 11: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Creatinine

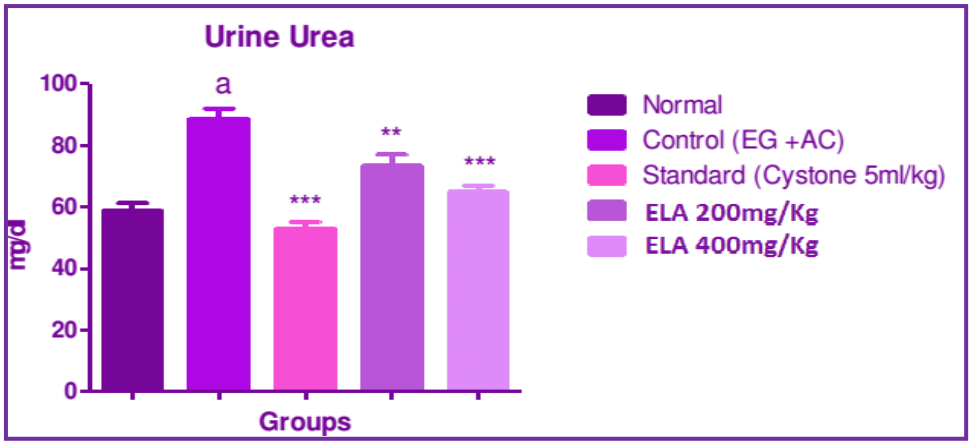

Figure 12: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Urea

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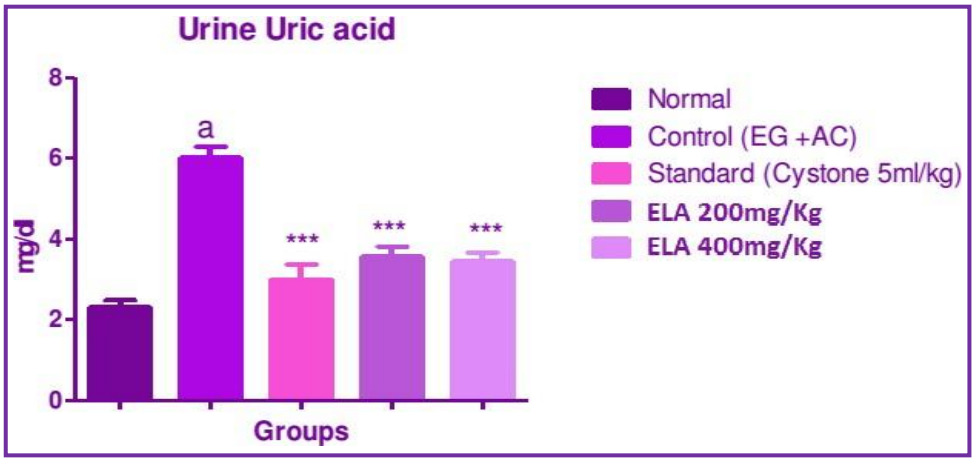

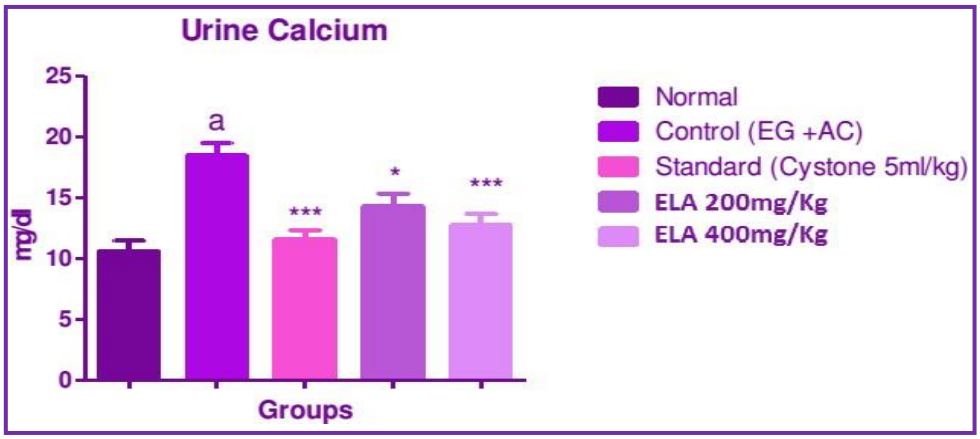

Figure 14: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Calcium

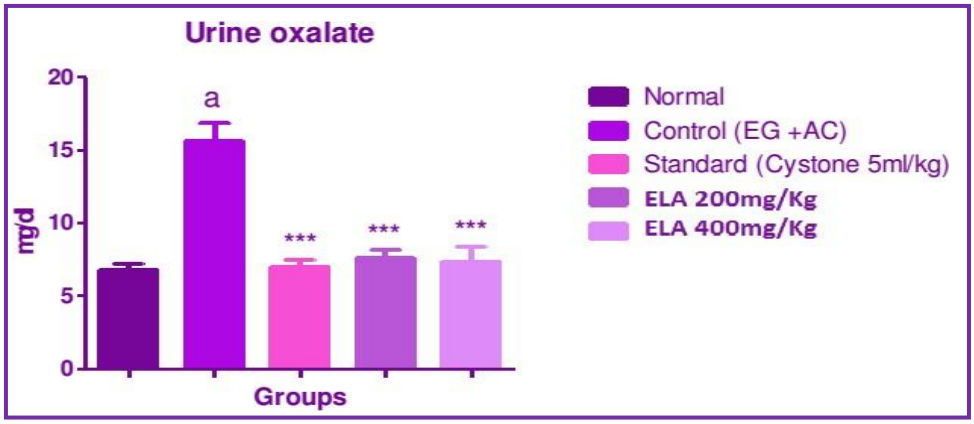

Figure 15: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Oxalate

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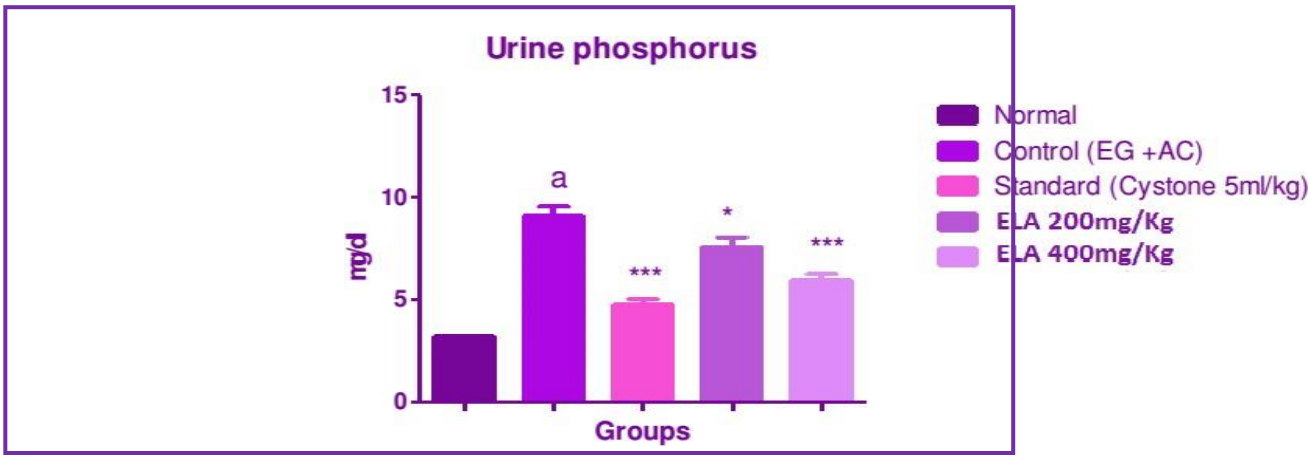

Figure 16: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Phosphorus

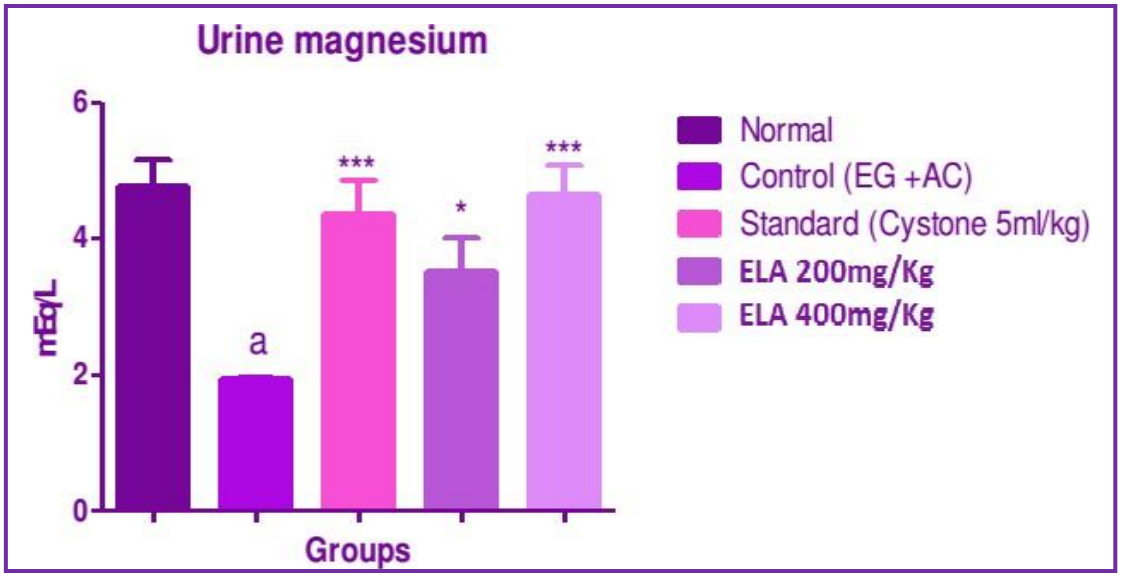

Figure 17: Effect of Ethanolic Extract of *Pascopyrum smithii* on Urine Magnesium

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 nooofficial standard, limits for each parameter was established based on trial and error analysis.

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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)

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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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