

# Toxicological studies and assessment of pharmacological activities of *Abrus precatorius* L. (Fabaceae) ethanolic leaves extract in the management of pain, psychiatric and neurological conditions: An *in-vivo* study

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## ABSTRACT

*Abrus precatorius* L. is an important medicinal plant belonging to family Fabaceae. The present study was conducted to perform pre-clinical safety evaluation and study the pharmacological effects of the ethanolic leaves extract of *A. precatorius* in management of pain, psychiatric and neurological conditions. Acute toxicity was performed to study the general behavioural pattern of mice after treatment with the test extract (single doses of 100, 1000, 1500 mg/kg, body weight) and sub-acute toxicity studies were performed to study the toxic effects of the test extract (500 mg/kg, *per os* for 14 days) on different biochemical and haematological parameters, body and organ weight and histopathology of liver and kidney. The toxicological evaluation of *A. precatorius* ethanolic leaves extract revealed that it has a reasonable safety profile. Analgesic and neuropharmacological effects like muscle relaxant, locomotor, anti-epileptic and anti-depressant activities were also studied on different animal models. The result showed that ethanolic leaves extract of *A. precatorius* at the doses of 300 and 500 mg/kg, *p.o.* possesses significant analgesic and neuropharmacological activities. Thus the present study shows that *A. precatorius* possesses a reasonable safety profile and can be used in the management of pain, psychiatric and neurological conditions.

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## INTRODUCTION

From the dawn of civilizations medicinal plants are considered as part and parcel of human society to combat diseases (Biswas *et al.*, 2002). Numerous present day medicines of the western world have been developed based on traditional knowledge by studying and recognizing the mechanism of action and their receptors (Eldin and Dunford, 1999). Medicinal plants may be beneficial to humans as they have several phytoconstituents to cure diseases but the potential toxicity of these bioactive constituents has not been well established (Rosidah *et al.*, 2009). There is a scarcity in scientific evidence

on the safety and efficacy of herbal drugs to the increase in number of its users which raised concerns regarding toxicity and detrimental effects of these herbal remedies. Thus there is a need to evaluate the safety and efficacy of these plants thoroughly to maximise their benefits for mankind (Mohamed *et al.*, 2011). Pain is considered an unpleasant sensory and emotional experience associated with potential or actual tissue damage which may be induced by an internal or external noxious stimuli (Loeser and Treede, 2008). Mediators such as tumour necrosis factor- TNF- $\alpha$  and interleukin-1 are subsequently elaborated and are believed to promote the synthesis, release and action of autacoid prostaglandin E2 (PGE2) and F2 $\alpha$  by the pericytes and endothelium of brain capillaries that excite pain nerve endings (Panthong *et al.*, 2007). Pain has become the focus of global scientific research due to their implication in virtually all human and animal diseases.

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Drugs mostly used in pain relief in humans are nonsteroidal anti-inflammatory drugs (NSAIDs) and opioids, but these drugs are known to cause adverse effects (Lima *et al.*, 2012).

Depression and mental illness are ranked as the fourth leading cause of mortality, disability and life threatening disease, characterized by a downcast mood, loss of pleasure, negative thoughts, disturbed sleep or appetite, low energy and suicidal ideations (Fava and Kendler, 2000). Epilepsy is a chronic disorder mainly caused due to a recurrent spontaneous abnormal electrical discharge of a group of neurons in the brain and affects about 40 million people worldwide (Ojong *et al.*, 2016). Majority of the commercially available drugs for anti-depressant are effective but causes side effects like tiredness, blurred vision, weight gain, nausea, dry mouth, agitation fatigue and sexual dysfunction (Dhingra and Sharma, 2006). Anti-anxiety drugs like benzodiazepines, diazepam causes side effects like amnesia, sedation, changes in body weight and physical dependence (Moreno *et al.*, 2014).

*Abrus precatorius* L. is a vine belonging to family Fabaceae. It is originally native to India and is now found throughout the tropical and subtropical parts of the world (Morton, 1982). In West Tropical Africa the leaves of *A. precatorius* are used to sweeten foods and are also used as medicine for stomach complaints. They are also used to treat fever, cough and cold (Morton, 1981; Irvine, 1961). They are also applied on cuts, swellings and mouth ulcer and further used as an abortifacient, laxative, sedative, aphrodisiac and nerve tonic (Qadry, 2005). The roots of the plant are used for treatment of gonorrhoea, jaundice and haemoglobinuria. The oil extracted from seeds is said to promote the growth of human hair (Samy *et al.*, 2008). Many phyto-constituents were reported from *A. precatorius* like Glycyrrhizin (Rastogi and Mehrotra, 1998), Abrusosides A–E (Sankaranarayanan *et al.*, 2010), triterpene glycosides, steroids, alkaloids like abrine, hypaphorine, cholin and precatorin, flavonoids like vitexin, toxifolin-3-glucosides (Daniel, 2006).

According to the available literature the leaves of *A. precatorius* is used traditionally as a nerve tonic and also as a pain reliever, however scientific information on its analgesic and neuro-pharmacological properties is still not available or rather scarce. Looking at the present scenario of developing safer drugs to combat pain, psychiatric and neurological disorders, and also to establish scientific evidence of the plant's folkloric use, this work was designed to study the toxicological effects of ethanolic leaves extract of *Abrus precatorius* L. (ELEAP) and also evaluating the analgesic and neuro-pharmacological activities like muscle relaxant, locomotor, anti-epileptic and anti-depressant activities.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of *Abrus precatorius* L. were collected from in and around Sagar Nagar, Visakhapatnam and authenticated by Dr. M. Venkaiah, Professor (Retd.), Department of Botany, Andhra University, Visakhapatnam, Andhra Pradesh,

India. A voucher specimen has been kept in our research laboratory for further reference. The leaves were washed under running tap water to remove the adhering dirt followed by rinsing with distilled water. Then the materials were shade dried for one week and then pulverized in mechanical grinder followed by sieving to obtain a coarse powder.

### Preparation of extract

The powdered leaf materials were defatted with petroleum ether (60-80 °C) in a soxhlet extractor was then air-dried and further extracted with ethanol (90%). The ethanol extract obtained was filtered and was concentrated in rotary evaporator (Evator, Media Instrument Mfg. Co., Mumbai, India) at reduced pressure to obtain a dark greenish brown residue (ELEAP yield 9%).

### Preliminary phytochemical screening

Preliminary phytochemical studies of ethanolic leaves extract of *A. Precatorius* were performed for determination of major phytochemical constituents using standard procedures (Harborne, 1984; Kokate, 1994).

### Experimental animals and housing conditions

Swiss albino mice (20-25 g) of either sex were used for analgesic, muscle relaxant activity, locomotor, anti-epileptic and acute toxicity study. Adult Wistar albino rats (150-210 g) of either sex were used for evaluation of anti-depressant activity, whereas for examination of sub-acute toxicity only male Wistar albino rats of 8-16 weeks old were taken. The animals were housed for at least one week in the laboratory animal room prior to testing in standard polypropylene cages at room temperature of  $34\pm 2^{\circ}\text{C}$  and at 60-65% relative humidity. Food and water were given *ad libitum* unless otherwise specified.

### Ethical approval

The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) of GITAM Institute of Pharmacy, GITAM University, Visakhapatnam, Andhra Pradesh, India (Regd. No. IAEC/GIP-1287/Mpharm/IP/PMK-AKC/11/2011-12)

### Experimental grouping and dosing

The rats were acclimatized for one week prior to use in the experimental models. Twenty four animals were selected and subdivided into four groups of six animals each ( $n = 6$  per group). Group 1 received 3% polyethylene glycol (1 ml/kg, p.o.) and serves as normal control. Group 2 received standard drug which serves as the drug control group. Group 3 and 4 received ELEAP at doses of 300 mg/kg, p.o. and 500 mg/kg, p.o., respectively. For evaluation of analgesic activity Tramadol (30 mg/kg, i.p.) and Aspirin (100 mg/kg, p.o.) was used as the reference standard in hot plate method and in acetic acid induced writhing method respectively. Diazepam (3 mg/kg, p.o.) was used as the reference standard for evaluating muscle relaxant property, locomotor activity and anti-

depressant activity whereas phenytoin (5 mg/kg, p.o.) was used as the reference standard for evaluating anti-epileptic activity. For evaluating sub-acute toxicity twelve male Wistar albino rats were randomly assigned into two groups (n=6/group) where group 1 received distilled water (3 ml/kg, p.o.) and group 2 received ELEAP at a high dose level (500 mg/kg, p.o.) twice daily for 14 days.

#### Acute toxicity studies

The acute toxicity studies were conducted as per the OECD guidelines 423 (Anonymous, 2000), where the limit test dose of 1500 mg/kg, p.o., were used. The test was carried out according to the standard methods (Ganapaty *et al.*, 2002; Baghel *et al.*, 2011). The control group received only vehicle (3 ml/kg, p.o.). The other groups separately received 100, 1000 and 1500 mg/kg, p.o., of the test extract respectively in a similar manner. Immediately after dosing, the animals were closely observed for the initial 4 h after the administration and then once daily during the following days. The behavioural changes closely observed for were hyperactivity, ataxia, salivation, diarrhoea, lethargy, sleep and coma. They were then kept under observation up to 14 days after drug administration to find out the mortality if any. One-fifth and one-third of the maximum tolerated dose of ELEAP (300 and 500 mg/kg, body weight, p.o.) were selected for analgesic and neuropharmacological activities.

#### Sub-acute toxicity studies

Sub-acute toxicity studies were done according to the standard methods with slight modifications (OECD, 2008; Okoye *et al.*, 2012). All rats were treated for 14 days and were observed daily for physiological and behavioural changes. Body weight, food intake and water intake were monitored. Animals were also closely observed for signs of abnormalities during the whole treatment process. On 15<sup>th</sup> day, the animals were anesthetized with pentobarbital sodium 0.035 gm/kg, i.p. and blood samples were collected by retro-orbital puncture using capillary tubes for hematological and biochemical studies. The hematological and biochemical parameters were co-related with the normal range of clinical laboratory parameters (Giknis and Clifford, 2008).

#### Hematological analysis

The blood samples collected by retro-orbital puncture from anesthetized Wistar rats were used for analysis of Hematological parameters like Platelet count, Hemoglobine (Hb) count, Red Blood Cell (RBC) count, White Blood Cell (WBC) count, Differential count (DC) (Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils) (Wintrobe *et al.*, 1976; Jain, 1986).

#### Biochemical analysis

For biochemical analysis blood were centrifuged at 3000 × g for 10 min at 4 °C. Serum was separated from the blood after centrifugation and stored at -20 °C until analysis. Biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT),

serum glutamate-pyruvate transaminase (SGPT), alkaline phosphatase (ALP), total bilirubin, total protein, albumin, serum creatinine, blood urea, total cholesterol, triglyceride and glucose content were assayed using commercial kits (Span Diagnostics Ltd., Surat, India).

#### Evaluation of body weight and organ weight

The evaluation of body weight of the control and treated animals were performed to check for possible toxicity. Macroscopic analysis of target organs of control and treated animals were done to evaluate any abnormalities in weight, texture and shape for determination of possible toxic effects (Dacie and Lewis, 1991). The major targeted organs include rat liver and kidney.

#### Histopathological studies

Histopathological studies were performed on organ samples of liver and kidney. After euthanasia, all animals were autopsied and the major organs like liver and kidney were surgically taken out and were fixed in 20% formalin in normal saline. Sections of 5 µm were obtained on a rotary microtome and then the material was stained by hematoxylin-eosin (HE) (Luna, 1968). The sections were then analysed microscopically for pathological examinations.

#### Analgesic activity

##### Evaluation of Analgesic activity using Acetic acid-induced writhing method

The test was performed according to the standard methods (Sawadogo *et al.*, 2006; Ezeja *et al.*, 2011). Writhing was induced in mice by a single intraperitoneal injection (10 ml/kg) of 0.6% acetic acid. The intensity of nociceptive behaviour was quantified by counting the total number of writhes occurring between 0 and 20 min after stimulus injection. The writhing effect indicated by stretching of abdomen with simultaneous stretching of at least one hind limb. The analgesic activity was expressed as writhing scores over a period of 20 min. The percentage inhibition was also calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control group observation} - \text{Standard or test group observation}}{\text{Control group observation}} \times 100$$

##### Evaluation of Analgesic activity using Eddy's hot pate method

Screening for the analgesic activity was performed in Swiss albino mice according to the standard methods (Siegmond *et al.*, 1957; Bose *et al.*, 2007). Tramadol (30 mg/kg, p.o.) was used as the reference standard for activity comparison. The paws of mice and rats are very sensitive to heat at temperatures which are not damaging the skin. The hot plate consists of an electrically heated surface and the temperature is controlled at 55°C to 56°C. This can be a copper plate or a heated glass surface. Pain stimulus is produced by placing the mice on the hot plate. Paw licking or jumping off the plate was considered as response to the pain stimulus. The reaction time until either paw licking or

jumping occurring in each group was recorded at by a stop-watch. The readings were taken after 60 and 90 minutes of the drug administration. Reaction time of the extract and reference standard was compared with the control.

### Neuropharmacological activities

#### *Evaluation of Muscle relaxant activity by Rotarod apparatus method*

The muscle relaxant activity was evaluated using Rotarod apparatus on Swiss albino mice according to the standard methods (Dunham and Miya, 1957). Diazepam (3 mg/kg, p.o.) was used as the reference standard for activity comparison. The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 5 sections by a plastic disc, thereby allowing the simultaneous testing of 5 mice. The rod is in a height of about 50 cm above the table top in order to discourage the animals from jumping off the roller. Cages present below the sections restrict the movement of the animals when they fall from the roller. A pre-test on mice with a weight between 20 and 30 g were conducted on the apparatus. Only those animals which have demonstrated their ability to remain on the revolving rod for at least 1 minute were used for the test. The test compounds were administered orally. 60 min after oral administration the mice were placed for 5 min on the rotating rod. The time spent in seconds on the rotarod after 60 and 90 min of treatment was recorded for both extracts and reference standards and was compared with the control group.

#### *Evaluation of Locomotor activity by Actophotometer method*

The locomotor activity was performed using actophotometer on Swiss albino mice according to the standard procedures (Turner, 1972). Diazepam (3 mg/kg, p.o.) was used as reference standard for activity comparison. Actophotometer consists of a series of photo cells. When the beam of light from these cells is interrupted by mice, reading is observed on a digital counter in the form of counts. The animals were placed in the cage after 60 and 90 mins of the oral administration of the test and standard compounds. Each animal was observed for 10 min after placing in the cage. The counts were recorded when the beam of light falling on the photocell of actophotometer is cut off by mice. The number of obstructions recorded for extracts and standards were compared with the control group.

#### *Evaluation of Anti-epileptic activity by Maximal electro shock (MES) method*

The anti-epileptic activity was performed using corneal electrodes on Swiss albino mice according to standard methods (Woodbury and Davenport, 1952). Phenytoin (5 mg/kg, p.o.) was used as the reference standard for activity comparison. The test was started 60 min after oral treatment with the test compounds. MES seizures were induced by electroconvulsimeter. Corneal electrodes were used to deliver the stimuli. A 50 Hz alternating current of 12 mA was delivered transauricularly for 0.2 sec in

mice. MES produced various phases of convulsions like flexion, extension and clonus. This current intensity elicited complete tonic extension of the hind limbs in control mice. The animals were observed closely for 2 mins. Disappearance of the hind leg extensor tonic convulsion was used as positive criterion. Percentage inhibition of seizures relative to control was calculated by the following formula.

$$\% \text{ Inhibition} = \frac{\text{Control group observation} - \text{Standard or test group observation}}{\text{Control group observation}} \times 100$$

#### *Evaluation of Anti-depressant activity by Forced swim test (FST) method*

The anti-depressant activity was evaluated using rat swimming apparatus on Wistar rats according to standard methods (Porsolt *et al.*, 1977). Diazepam (3 mg/kg, p.o.) was used as reference standard for activity comparison. Fitness level of each test animal was checked by a pre-test which was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming. During the test, animals were individually placed in a glass cylinder (height 25 cm, diameter 10 cm) containing 10 cm of water and maintained at 23–25 °C. Animals were forced to swim for 6 min. After the initial 2 min of vigorous activity, the total duration of immobility was recorded during the last 4 min of the test. Duration of immobility was recorded for extracts and reference standard which were compared with the control group. The immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water.

### Statistical analysis

The data obtained in the studies were subjected to one way of analysis of variance (ANOVA) for determining the significant difference. The inter group significance was analyzed using Dunnet's t-test. A p-value <0.05 and <0.01 was considered to be significant. All the values were expressed as mean ± SEM.

## RESULTS

### Preliminary phytochemical tests

Preliminary phytochemical screening of the ethanolic leaves extract of *Abrus precatorius* (ELEAP) revealed the presence of flavonoids, saponins, reducing sugar, tannins and triterpenoids as shown in Table 1.

**Table 1:** Preliminary phytochemical screening of the ethanolic leaves extract of *A. precatorius*.

Sl. no.	Phytochemical constituents	Inference
1	Alkaloids	-
2	Flavonoids	+
3	Triterpenoids	+
4	Saponins	+
5	Tannins	+
6	Proteins and amino acids	-
7	Reducing sugar	+

(+) Present, (-) Absent

### Acute toxicity studies

No mortality or morbidity was observed in animals through the 14 day period following single oral administration. Morphological characteristics (fur, skin, eyes and nose) appeared normal. No salivation, diarrhoea, lethargy or unusual behaviours such as self-mutilation, walking backward etc. were observed. Gait and posture, reactivity to handling or sensory stimuli, grip strength was all normal. Food and water intake showed daily fluctuations within the range of control animals. This indicates that the ethanolic extract from *A. Precatorius* leaves was safe to a single dose of 1500 mg/kg, body weight. Hence 300 and 500 mg/kg oral doses of ELEAP were selected to evaluate analgesic and neuropharmacological activities.

### Sub-acute toxicity studies

#### General behaviour of the animals

There was no significant change in body weight over the 14 days of study, since there was no significant difference between the results representing the control group and the test extract group. There were no ELEAP treatment related mortalities recorded in rats after 14 days of dosing. None of the animals after administration of ELEAP showed any obvious morbidity or clinical symptoms of toxicity such as changes in the skin and fur, eyes, respiratory rate, autonomic (salivation, perspiration and piloerection) and central nervous system (ptosis and drowsiness) effects throughout the experimental period of 14 days. The control group also showed no symptoms of toxicity.

**Table 2:** Effects of ELEAP on biochemical parameters in male Wistar rats.

Parameter	Unit	Control (3 ml/kg, p.o.)	ELEAP (500mg/kg, p.o.)	Normal Range
<b>Liver profile</b>				
Total bilirubin	mg/dl	0.11±0.02	0.09±0.01 <sup>ns</sup>	0.05-0.15
Total protein	g/dl	8.2±0.9	6.1±0.5 <sup>ns</sup>	5.2-7.1
Albumin	g/dl	4.1±0.9	3.2±0.5 <sup>ns</sup>	3.4-4.8
SGPT	IU/l	25.6±3.01	28.7±2.3 <sup>ns</sup>	18-45
SGOT	IU/l	78.6±3.85	82.88±4.2 <sup>ns</sup>	74-143
ALP	IU/l	65.8±4.3	88.3±5.4 <sup>ns</sup>	62-230
<b>Renal profile</b>				
Creatinine	mg/dl	0.41±0.02	0.33±0.01 <sup>ns</sup>	0.2-0.5
Urea	mg/dl	20.8±1.91	21.3±1.02 <sup>ns</sup>	12.3-24.6
<b>Lipid profile</b>				
Total cholesterol	mg/dl	40.2±2.09	38.3±3.8 <sup>ns</sup>	37-85
Triglyceride	mg/dl	23.6±1.7	18.1±0.88 <sup>ns</sup>	20-114
Glucose	mg/dl	105.3±2.3	121.8±4.2 <sup>ns</sup>	70-208

Results are expressed as mean ± S.E. from six observations (n = 6). Statistical analysis done by one way ANOVA followed by Dunnet's t-test. \*\*P<0.01, \*P<0.05, ns- not significant when compared to control group. Serum Glutamate-Pyruvate Transaminase (SGPT); Serum Glutamic Oxaloacetic Transaminase (SGOT); ALP: Alkaline Phosphatase.

#### Effect on the haematological and biochemical blood parameters of the animals

In the biochemical parameters evaluated, all parameters remained almost unchanged as non-significant variations were observed. All the values of the biochemical parameters for control group falls within the normal range except for the total protein content which increased slightly, whereas in ELEAP (500 mg/kg,

p.o.) treated group all the biochemical parameters were normal except for the albumin and triglyceride content which decreased slightly than the normal range. All the biochemical parameters of the extract treated group was non-significant when compared with the control group as shown in Table 2. The haematological parameters of rats treated with ELEAP showed almost an unchanged value with non-significant variations when compared with the control group. Further the haematological parameters of rats after treatment of control vehicle and ELEAP shows normal values as they fall within the normal range of haematological parameters of rats (Table 3).

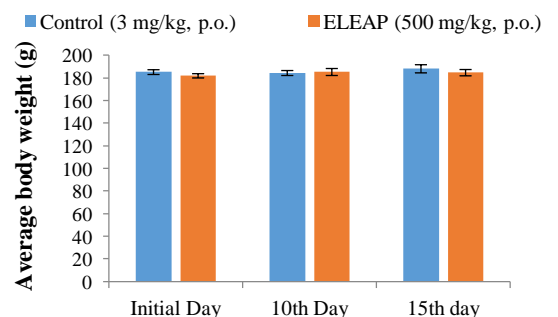
**Table 3:** Effects of ELEAP on hematological parameters in male Wistar rats.

Unit	Control (3 ml/kg, p.o.)	ELEAP (500mg/kg, p.o.)	Normal Range
10 <sup>3</sup> /μL	896±22.03	933±35.6 <sup>ns</sup>	638-1177
g/dl	14.8±0.2	15.1±0.1 <sup>ns</sup>	13.7-17.6
10 <sup>6</sup> /μL	8.23±0.5	8.01±0.1 <sup>ns</sup>	7.27-9.65
10 <sup>3</sup> /μL	6.1±0.5	5.4±0.9 <sup>ns</sup>	1.96-8.25
10 <sup>3</sup> /μL	0.71±0.03	0.63±0.01 <sup>ns</sup>	0.22-1.57
10 <sup>3</sup> /μL	4.33±0.7	3.11±0.9 <sup>ns</sup>	1.41-7.11
10 <sup>3</sup> /μL	0.06±0.01	0.07±0.01 <sup>ns</sup>	0.01-0.16
10 <sup>3</sup> /μL	0.08±0.01	0.09±0.01 <sup>ns</sup>	0.03-0.18
10 <sup>3</sup> /μL	00	~0.01 <sup>ns</sup>	0.0-0.05

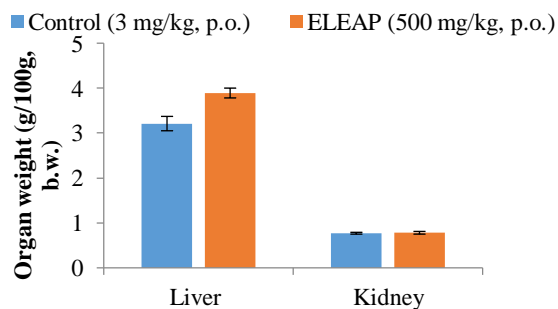
Results are expressed as mean ± S.E. from six observations (n = 6). Statistical analysis done by one way ANOVA followed by Dunnet's t-test. \*\*P<0.01, \*P<0.05, ns- not significant when compared to control group.

#### Effect on body weight and organ weight

The body weight of rats treated with both control and ELEAP treated groups over 14 days period did not cause any significant change when compared with the control (Figure 1A). The organ weights of rats treated with ELEAP decreased slightly when compared with the control treated group (Figure 1B). The organs examined were liver and kidney of rats.



A

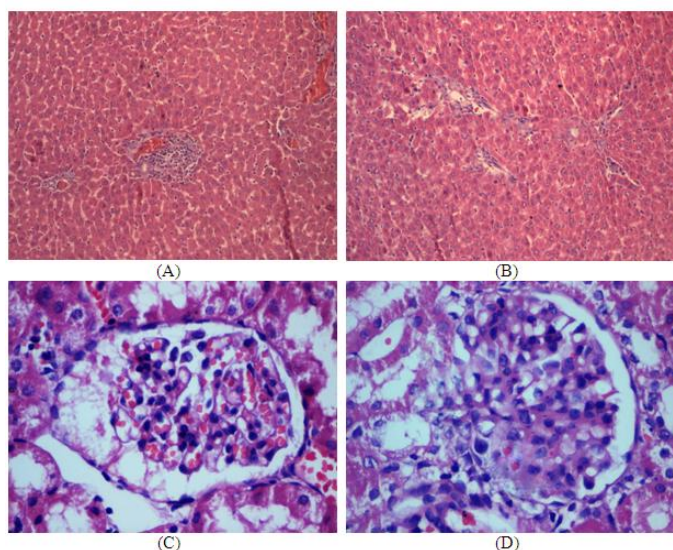


B

**Fig. 1:** (A) Effects on body weight of rats after treatment with ELEAP (B) Effects on organ weights in rats after oral administration of ELEAP.

### Histopathological studies

Histopathological analysis of the liver and kidney were performed on the 15<sup>th</sup> day after administration with control vehicle and ELEAP. Multiple sections of the liver showed normal lobular architecture. The hepatocyte appeared normal and no sign of specific lobular hepatitis was observed with the extract treated group of animals compared with the control treated group (Figure 2: A and B). Similarly the multiple sections taken out from renal biopsy showed normal size and shape of glomeruli, tubules, interstitium and blood vessels. There was no evidence of acute tubular necrosis and glomerular changes (Figure 2: C and D).



**Fig. 2:** Photomicrographs of liver and kidney histopathology (A) Section of rat liver showing normal architecture for control group (10 ×) (B) Section of rat liver treated with ELEAP (500 mg/kg, p.o.) (10 ×) (C) Section of rat kidney showing normal architecture for control group (40 ×) (D) Section of rat kidney treated with ELEAP (500 mg/kg, p.o.) (40 ×)

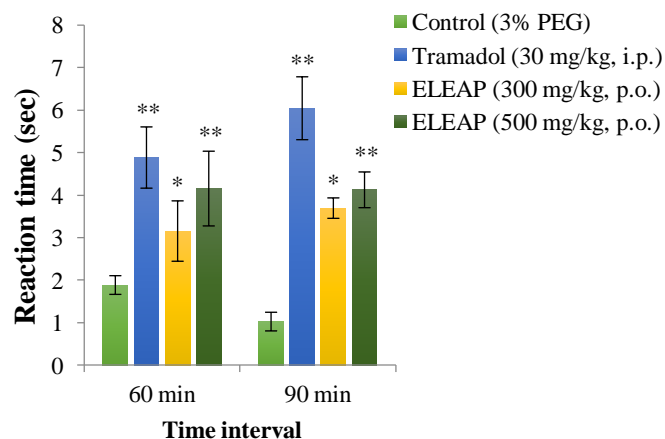
### Effect of ELEAP on Analgesic activity

The ELEAP showed retention time of  $3.16 \pm 0.71$  sec and  $4.16 \pm 0.88$  sec at 60 min at the doses of 300 and 500 mg/kg, p.o., respectively, whereas a retention time of  $3.7 \pm 0.24$  sec and  $4.13 \pm 0.42$  sec were observed at 90 min at doses of 300 and 500 mg/kg, p.o., respectively.

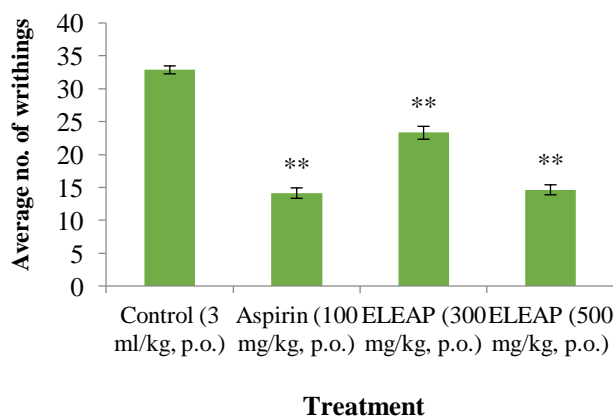
Thus maximum retention time was observed after 60 min at the dose of 500 mg/kg. Tramadol which was used as a reference standard showed maximum retention time of  $6.05 \pm 0.74$  sec at the dose of 30 mg/kg, i.p., at 90 min. The result indicated that the extract raised the pain threshold as compared to control as shown in Figure 3 and the activity was persistent throughout the entire observation period of 90 min.

Assessment of peripheral analgesic effect through acetic acid induced writhing was evaluated on the basis of the average number of abdominal constrictions indicated by the extension of hind paw of animals (mice) during the writhing test (Figure 4). The average number of writhing of the test extract (ELEAP) was

significantly ( $P < 0.01$ ) reduced when compared with the control. The percentage inhibition of aspirin (100 mg/kg, p.o.), ELEAP (300 mg/kg, p.o.) and ELEAP (500 mg/kg, p.o.) was 56.93%, 29.04% and 55.41% respectively.



**Fig. 3:** Analgesic activity of ELEAP using Eddy's hot plate method. Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnet's t-test. \*\* $P < 0.01$ , \* $P < 0.05$  compared to control group.



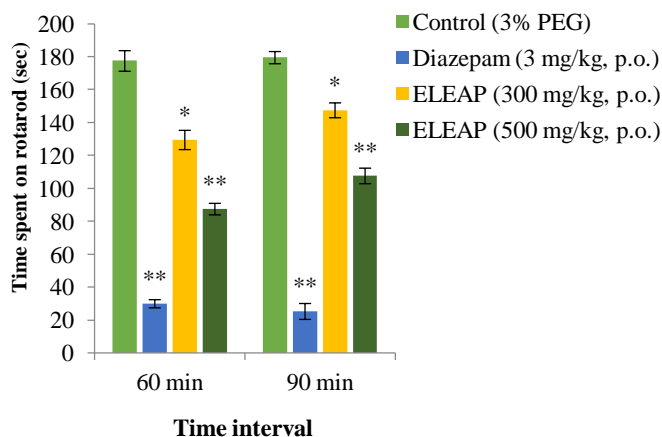
**Fig. 4:** Analgesic activity of ELEAP using Acetic acid-induced writhing method. Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnet's t-test. \*\* $P < 0.01$  compared to control group.

### Effect of ELEAP on Muscle relaxant activity

The muscle relaxant activity of ELEAP is shown in Figure 5. The time spent on the revolving rod was significantly ( $p < 0.01$ ) reduced by ELEAP at the doses of 300 and 500 mg/kg, p.o., when compared with the control group. The time spent on revolving rod was  $129.5 \pm 5.85$  sec and  $87.5 \pm 3.51$  sec at 60 min, at the doses of 300 and 500 mg/kg, p.o., respectively whereas it was  $147.5 \pm 4.51$  sec and  $107.66 \pm 4.73$  sec at 90 min, at the doses of 300 and 500 mg/kg, p.o., respectively. Muscle relaxant activity of the reference standard Diazepam significantly ( $P < 0.01$ ) reduced the



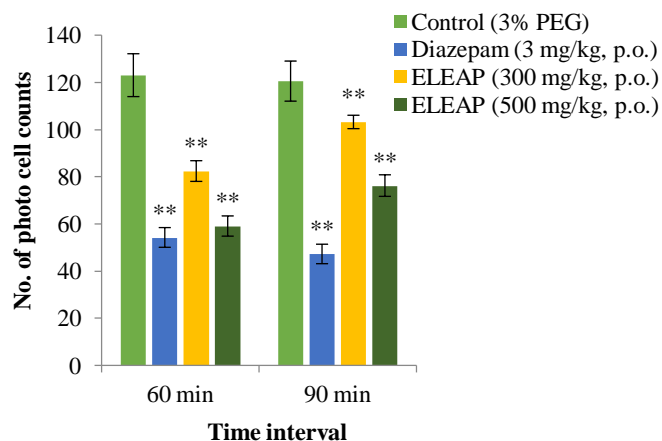
time spent on the revolving rod to  $30 \pm 2.49$  sec at 60 min and  $25.33 \pm 4.84$  sec at 90 min at dose of 3 mg/kg, p.o when compared to the control group.



**Fig. 5:** Muscle relaxant activity of ELEAP using Rotarod apparatus method. Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnett's t-test. \*\*P<0.01, \*P<0.05 compared to control group.

#### Effect of ELEAP on Locomotor activity

The locomotor activity of ELEAP is shown in Figure 6. After the administration of ELEAP at doses of 300 and 500 mg/kg, p.o., there was a significant (P<0.01) reduction in the actophotometer reading (no. of photocells counted) when compared to the control group which was  $123 \pm 9.09$  at 60 min and  $120.05 \pm 8.51$  at 90 min.



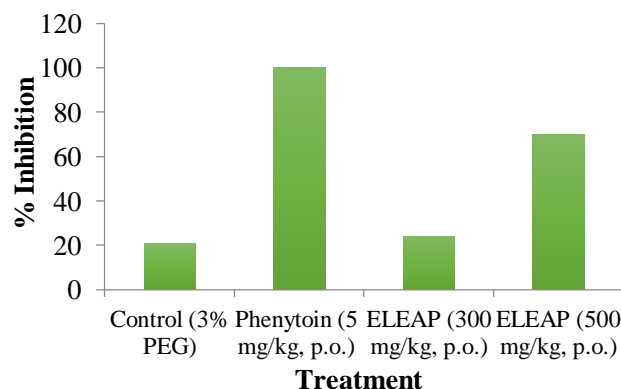
**Fig. 6:** Locomotor activity of ELEAP using Actophotometer method. Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnett's t-test. \*\*P<0.01 compared to control group.

The actophotometer reading was  $82.33 \pm 4.39$  and  $59 \pm 4.28$  at 60 min, at the doses of 300 and 500 mg/kg, p.o., respectively whereas it was  $103.16 \pm 2.84$  and  $76.16 \pm 4.57$  at 90 min, at 300 and 500 mg/kg, p.o., doses of ELEAP respectively. Reference standard diazepam at dose of 3 mg/kg, p.o., significantly (P<0.01) reduced the actophotometer reading when compared to the control group as

the readings were found to be  $54.16 \pm 4.18$  and  $47.16 \pm 4.13$  at 60 and 90 min respectively.

#### Effect of ELEAP on Anti-epileptic activity

The ELEAP at the dose of 500 mg/kg, p.o., and reference standard phenytoin at 5 mg/kg, p.o., showed significant (P<0.01, 0.05) decrease in the duration of extensor phase, whereas ELEAP at the dose of 300 mg/kg, p.o., did not significantly reduced the extensor phase when both the doses of the extracts and the reference standard phenytoin were compared with the control group. The results are demonstrated in Table 4 and Figure 7.



**Fig. 7:** Percentage inhibition of seizures relative to control

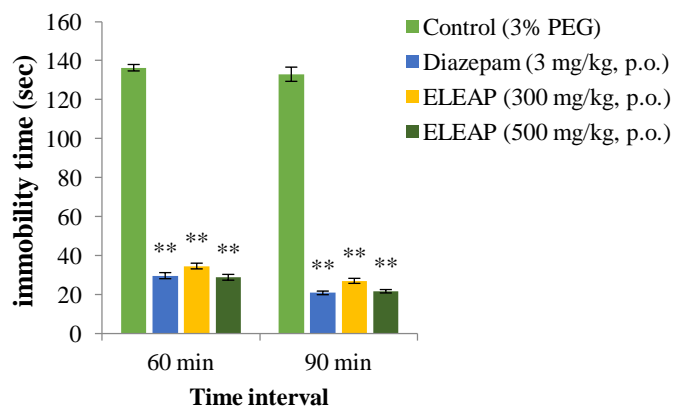
**Table 4:** Effect of ELEAP on Anti-epileptic activity.

Group	Treatment	Dose	Onset of clonus	Duration of tonic flexion	Duration of tonic extension
1	Control (3% PEG)	1 ml/kg p.o.	2.0 $\pm$ 0.28	3 $\pm$ 0.25	12.50 $\pm$ 0.61
2	Phenytoin	5 mg/kg p.o.	4.16 $\pm$ 0.30*	Nil**	Nil**
3	ELEAP	300 mg/kg p.o.	2.1 $\pm$ 0.15 <sup>ns</sup>	4 $\pm$ 0.42 <sup>ns</sup>	9.23 $\pm$ 0.142 <sup>ns</sup>
4	ELEAP	500 mg/kg p.o.	3.15 $\pm$ 0.5*	0.21 $\pm$ 0.25**	1.24 $\pm$ 0.54**

Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnett's t-test. \*\*P<0.01, \*P<0.05, ns- not significant when compared to control group.

#### Effect of ELEAP on Anti-depressant activity

The anti-depressant activity of ELEAP is demonstrated in Figure 8. The immobility time was decreased significantly (P<0.01) by administration of ELEAP at doses of 300 and 500 mg/kg, p.o., when compared with the control group which was  $136.33 \pm 1.66$  sec at 60 min and  $133 \pm 3.65$  sec at 90 min. The duration of immobility was  $34.6 \pm 1.48$  sec and  $28.83 \pm 1.49$  sec at 60 min, at the doses of 300 and 500 mg/kg, p.o., respectively, whereas it was  $27 \pm 1.29$  sec and  $21.66 \pm 0.88$  sec at 90 min at the doses of 300 and 500 mg/kg, p.o., of ELEAP respectively. The immobility time of reference standard diazepam at dose of 3 mg/kg, p.o., was also significantly (P<0.01) reduced to  $29.66 \pm 1.56$  sec at 60 min and  $20.83 \pm 0.94$  sec at 90 min when compared with the control group.



**Fig. 8:** Anti-depressant activity of ELEAP using Forced swim test method. Values are expressed as mean  $\pm$  S.E. (n = 6). Statistical analysis done by one way ANOVA followed by Dunnet's t-test. \*\*P<0.01 compared to control group.

## DISCUSSION

Herbal medicines as well as their derivatives have been used as an alternative to allopathic medicines in treatment of various diseases in many countries. Despite the widespread use of herbal medicine there has been very few scientific studies conducted on herbals to provide knowledge about their efficacy and safety (Graca *et al.*, 2007). Acute toxicity is considered an initial study which provides us the basis for classification and labelling. It also provides initial information about the mode of toxic action of a substance by which we can fix a dose of a new compound and help in dose determination in animal studies (Ukwuani *et al.*, 2012). In our study a single administration of extract with increasing doses did not produce any abnormalities in acute toxicity. Sub-acute ingestion of ELEAP produced behavioural change of very low intensity. The body weight and organ weight shows very little change when compared with the control. Thus ELEAP does not alter much of the behavioural and general morphological changes. The haematopoietic system is one of the most sensitive targets for toxic compounds and an important index of physiological and pathological status in man and animal (Mukinda and Syce, 2007). The haematological profile after treatment with extracts showed values which falls within the normal range. Biochemical parameters of liver and kidney were studied and they showed very little variation when compared with the control and they also fall within the normal range of biochemical parameters of rats. This indicates that the sub-acute administration of ELEAP is not able to produce toxic effects on the haematological and biochemical profile of rats.

For management of pain several analgesic drugs including NSAIDs, opiates, and steroids are available, but these drugs are known to cause severe side effects. Gastric ulceration is caused by NSAIDs, opiates can develop dependence, and steroids are associated with side effects affecting hormonal regulation (Barua *et al.*, 2011). Hot-plate test is a widely used model for neurologic pain. Centrally acting analgesic agents can increase the reaction time in hot-plate test by acting at the spinal cord level (Wigdor and Wilcox, 1987; Vongtau, 2004). Hot plate method is

used to examine supra-spinal analgesia in various compounds and deals with predominantly spinal reflex or behavioural reaction. The plate when heated to a constant temperature it produces two behavioural components that can be measured in terms of their paw licking, reaction times and jumping (Eldahshan and Abdel-Daim, 2015). Structurally tramadol is not considered an opiate, but it exhibits some properties of opioids. Tramadol shows a selective interaction with  $\mu$  receptors, which are responsible for the analgesic effect. It also has weak pharmacodynamic activity on other opioid receptors. At the same time, it acts synergistically on neuroamine transmission by inhibiting synaptic noradrenaline (norepinephrine) reuptake and inducing intrasynaptic serotonin (5-hydroxytryptamine; 5-HT) release (Moore, 1998). Tramadol was used as a reference standard for analgesic activity acting centrally. The evaluation showed that the tested dose of ELEAP (300 and 500 mg/kg, p.o.) raised the pain threshold as compared to the control. The analgesic activity shown by the ELEAP may be due to its ameliorating acting on the central nervous system thus decreasing neurological pain. Aspirin causes mainly the reduction of inflammation, analgesia (relief of pain), the prevention of clotting, and the reduction of fever. These effects caused by aspirin are due to decreased production of prostaglandins and TXA<sub>2</sub>. Aspirin suppresses the production of prostaglandins and thromboxanes by irreversible inactivation of the cyclooxygenase (COX) enzyme. Cyclooxygenase is required for prostaglandin and thromboxane synthesis. Aspirin acts as an acetylating agent where an acetyl group is covalently attached to a serine residue in the active site of the COX enzyme. Thus Aspirin is considered different to other NSAIDs (diclofenac and ibuprofen) which are reversible inhibitors (Toth *et al.*, 2013). Acetic acid induced writhing method is considered a suitable method for determining peripheral analgesic activity and represents pain sensation which acts by triggering localized inflammatory reaction. Acetic acid when injected intraperitoneally pain is produced by liberating endogenous substances and pain mediators through cyclooxygenase, and prostaglandin pathway or irritation of visceral surfaces, which leads to the release of bradykinin, histamine, serotonin and prostaglandins (Altawil *et al.*, 2015). Aspirin was taken as the reference standard for evaluation of peripheral analgesic activity using acetic acid-induced writhing method. The assessment of peripheral analgesic effect of the test drug exhibited significant percentage inhibition in the writhings which were induced by acetic acid in mice at both the tested doses of ELEAP when compared with the control group. The percentage inhibition of writhings indicated the pronounced peripheral analgesic effect in the context of visceral pain which was comparable to the standard drug aspirin (100 mg/kg, p.o.) within 20 min of test.

The assessment of neuropharmacological activity was performed on ELEAP at doses of 300 and 500 mg/kg, p.o. Diazepam and phenytoin were used the reference standards for evaluating neuropharmacological activities. Diazepam or benzodiazepine which works by increasing the efficiency of a natural brain chemical, GABA, to decrease the excitability of



neurons. This reduces the communication between neurons and, therefore, has a calming effect on many of the functions of the brain. GABA controls the excitability of neurons by binding to the GABA<sub>A</sub> receptor (Olsen and Betz, 2006). Different GABA<sub>A</sub> receptor subtypes have varying distributions within different regions of the brain and, therefore, control distinct neuronal circuits. Hence, activation of different GABA<sub>A</sub> receptor subtypes by benzodiazepines may result in distinct pharmacological actions (Rudolph and Mohler, 2006). In the muscle relaxant activity, significant muscle relaxation was produced at 300 mg/kg, p.o. (P<0.05) and 500 mg/kg (P<0.01) when compared with control after 60 and 90 min. Demonstration of marked muscle relaxant effect by rota-rod study indicated that ELEAP induced neurological deficit accompanied with taming or calming effect in mice, supporting its muscle relaxant effect. The muscle relaxant properties of diazepam are produced via inhibition of polysynaptic pathways in the spinal cord (Date *et al.*, 1984). Furthermore in the evaluation of locomotor activity using actophotometer the ELEAP showed significant (P<0.01) decrease in number of photocell count when compared to control. Most of the centrally acting analgesics influence the locomotor activity by reducing the motor activity because of their CNS depressant properties (Dey *et al.*, 2011). Locomotor activity is considered as an index of wakefulness and alertness of mental activity. The decrease in locomotor activity gives an indication of the level of excitability of central nervous system and this decrease may be due to the sedative effect resulting from depression in the central nervous system (Kaur *et al.*, 2010). The ELEAP at dose of 300 and 500 mg/kg, p.o., inhibited the maximal electroshock induced convulsions. This may also suggest that the anti-epileptic action may be mediated by the chloride channel of the GABA / benzodiazepine receptor complex (Husain *et al.*, 2007). Forced swim test is a widely accepted stress model for evaluation of anti-depressant studies. They are useful in screening new anti-depressant drugs as they are sensitive to all major classes of antidepressant drugs including tricyclics, serotonin-selective reuptake inhibitors and monoamine oxidase inhibitors (Porsolt *et al.*, 1977; Steru *et al.*, 1985). In our study the immobility time was decreased significantly (P<0.01) by ELEAP at the doses of 300 and 500 mg/kg, p.o. when compared with the control.

The immobility shown by the mice when subjected to unavoidable stress such as forced swimming test is thought to reflect a state of despair or lowered mood, which is thought to reflect depressive disorders in humans. In addition the immobility time is reduced by treatment with antidepressant drugs (Porsolt, 1981).

## CONCLUSION

The present study demonstrated that the ethanolic extract obtained from the leaves of *A. precatorius* possesses analgesic, muscle relaxant, locomotor, anti-epileptic and anti-depressant activities in the different tested experimental animal models. Thus it can be concluded that ELEAP ameliorates pain, psychiatric and

neurological conditions with a reasonable safety profile. This study can also be taken as a benchmark for further investigation on this plant to identify the chemical entity and characterization of the active constituent. The mechanism of action of the extract or bioactive compounds on CNS activity must also be studied to help improve more efficient and safe neurological drugs.

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