

## HEPATOPROTECTIVE AND DIURETIC ACTIVITIES OF ALCOHOLIC AND AQUEOUS EXTRACTS OF *EPALTES PYGMAEA* DC. (ASTERACEAE)

K. AMALA\*<sup>1</sup>A, SARASWATHY<sup>1</sup>, S. AMERJOTHY<sup>2</sup>

<sup>1</sup>Captain Srinivasa Murti Drug Research Institute for Ayurveda (CCRAS), Anna Hospital Campus, Arumbakkam, Chennai 106, <sup>2</sup>Department of Plant Biology & Plant Biotechnology, Presidency College, Chennai 5. India. Email: amalavenkey@gmail.com

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### ABSTRACT

Objective: Alcoholic and aqueous extracts of whole plant of *Epaltes pygmaea* DC. (Family Asteraceae) were screened for hepatoprotective and diuretic activities at the doses of 200 mg and 400 mg/kg body weight of AlEp and AqEp.

Methods: Paracetamol was used to induce liver injury in rats and silymarin was used as the standard drug in hepatoprotective activity. Furosemide was used as reference drug for diuretic activity.

Results: The plant extracts were effective in protecting the liver injury induced by paracetamol in rats. This was evident from significant reduction in serum enzymes aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin. Increased urine volume and urinary electrolytes excretion showed significant diuretic result at tested dose levels.

Conclusion: The alcoholic and aqueous extract of *E.pygmaea* possesses hepatoprotective activity against paracetamol-induced liver damage in rats and also have potent diuretic activity.

**Keywords:** *Epaltes pygmaea*, Asteraceae, Hepatoprotective, Diuretic.

### INTRODUCTION

The family Asteraceae is an advanced and botanically highly specialized family of vascular plants and mainly herbaceous plants, the genera are estimated to number about 1528 and the species probably 22,750 [1]. The genus *Epaltes*, represents about nine species in Tropical Asia, Africa, America and Australia. *Epaltes* is generally used in traditional Ayurvedic medicine in Sri Lanka to cure various ailments like jaundice, urethral discharges and acute dyspepsia. It is also regarded as a diaphoretic, diuretic and a stimulating expectorant [2]. Several eudesmane derivatives have been identified from the genus *Epaltes* such as Eudesmane epoxides in *E. gariepina*, eudesmane ketones in *E. divaricata* and *E. mexicana*, sesquiterpenes in *E. brasiliensis* [3][4][5].

Two species are found in India, of which *E. pygmaea* is one that could possess various activities which still remains unexplored. *E. pygmaea* is a small annual herb, 8 to 20 cm high, minutely winged branched stem with aromatic roots, leaves are alternate, linear, lanceolate to oblong, flower pink, solitary, terminal, heterogamous. It is found in Sri Lanka, India, Java and China, also found in South India, especially towards the coast, gregarious in low lying ground by river banks and paddy field after harvesting in clayey soil [6][7][8][9][10][11]. Few Chemical constituents viz. lupeol acetate, stigmasterol, stigmasterol acetate, apigenin, luteolin, apigenin-7-O-glucoside and luteolin-7-O-glucoside have been isolated from the whole plant of *E. pygmaea* [12]. To our knowledge no previous information is available on their therapeutic action.

In the present study, an effort has been made to establish the scientific validity of the hepatoprotective activity against paracetamol-induced hepatotoxicity in rats and diuretic effect of alcoholic and aqueous extracts of *E.pygmaea*.

### MATERIALS AND METHODS

#### Plant material

The plant material was collected in the month of February 2010 from Thirunelveli district, Tamil Nadu and authenticated by Dr. V. Chelladurai, Ex-Research Officer, Survey of Medicinal Plant Unit-Siddha, Palayamkottai, Tamil Nadu, India. The voucher specimen (00630) was deposited in Captain Srinivasa Murti Drug Research Institute for Ayurveda, Chennai for future reference.

#### Preparation of extracts

The freshly collected plant materials were cut into small pieces, shade dried and coarsely powdered. 100 g of powdered plant material was extracted with alcohol (95%) and aqueous (distilled water) by cold maceration method for 48 hours. The respective extracts were concentrated under reduced pressure to obtain a semi solid mass and were kept under the refrigeration. The yields of alcoholic (AlEp) and aqueous (AqEp) extract of *E. pygmaea* were 11 and 19% respectively.

#### Experimental animals

Healthy albino rats of Wistar strain weighing 100-150 g of either sex were procured from Tamil Nadu Veterinary and Animal Science University, Chennai, Tamil Nadu. The rats were maintained at prevailing ambient temperature, humidity and exposed to natural day and night cycles. The animals were fed with pellet diet *ad libitum* and clean water. The experimental protocol was approved by the Institutional Animal Ethics Committee as per No. IAEC/CSDRIA/04/2010.

#### Chemicals

Test kits for the estimation of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) were procured from E-Merck, India Ltd, Mumbai. Paracetamol (acetaminophen) was procured from Icars Health care, Chennai. Furosemide (Lasix tablet) Silymarin were purchased from local Pharmacy, Chennai manufactured by Micro Labs Limited. All other reagents used for the experiments were of analytical grade.

#### Preliminary phytochemical screening

The prepared extracts were subjected to preliminary phytochemical screening to identify the presence of various phytoconstituents by using the standard phytochemical tests [13][14][15].

#### Acute toxicity study

Acute toxicity study of alcoholic and aqueous extracts of the plant *E. pygmaea* was determined in Wistar albino rats (100-150 g) according to OECD guidelines No. 423 (acute toxic class method)[16]. The animals were fasted overnight and provided with water *ad libitum* and divided into 4 groups of three animals each. The alcoholic and aqueous extracts were administered orally with a starting dose of 2000 mg/kg, to different groups of animals. Animals

were observed on an hourly basis for 24 h and monitored for 14 days for mortality and general behavior of animals and signs of discomfort. Hence the 1/10<sup>th</sup> and 1/5<sup>th</sup> of the dose (200 and 400 mg/kg bw) were selected for further pharmacological studies.

### Drugs

Furosemide was used as reference diuretic drug, silymarin was used as a reference control for hepatoprotective activity against paracetamol-induced model.

### Diuretic activity

The method of Lipschitz *et al.*, 1943 [17] was employed for assessment of diuretic activity. The aqueous, alcohol extracts and furosemide were dissolved in 1% Tween 80 and administered to rats orally. Wistar albino rats of either sex were divided into 6 groups of six animals each, in laboratory cages. They were fed laboratory diet *ad libitum* and allowed free access to drinking water. The animals were fasted for eighteen hours (overnight) with free access to water only before testing. The first group served as normal control received only 1% Tween 80 at 10 ml/kg bw (p.o), the second group served as reference control received furosemide at 10 mg/kg, bw (p.o), rest of the four groups received each extracts viz. alcoholic and aqueous extracts of *E.pygmaea* in a dose level of 200 and 400 mg/kg, bw (p.o).

After administration, the animals were immediately placed in metabolic cages (2 per cage) specially designed (Techniplast (Italy) 3701M081) to separate urine and fecal matter and kept at room temperature of 25 ± 0.5°C. The urine was collected in measuring cylinder up to 5 hours. No water or food was made available to the animals during the period of experiment. The total volume of urine collected was measured for the control and drug treated groups. The parameters studied were total urine volume, Na<sup>+</sup>, K<sup>+</sup>, and Cl<sup>-</sup> ion concentration in urine. Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> ion concentration were measured by Auto analyser [18-23]. The sodium potassium ratio was also calculated.

### Hepatoprotective activity

The animals (rats) were randomly divided into 7 groups of either sex comprising six animals in each group. Hepatoprotective activity was evaluated using paracetamol induced model. The alcoholic and aqueous extracts were suspended with 1% Tween 80, paracetamol and silymarin were suspended with 1% sodium carboxyl methyl cellulose (SCMC). The test drugs were administered to rats orally. Group- I served as normal control and received 1% Tween 80 (10 ml/kg, po) once daily for 12 days. Group-II served as paracetamol control and was administered with paracetamol (1g/kg, po) once daily for first 7 days. Group - III served as reference control received paracetamol (1 g/kg, po) once daily for first 7 days and silymarin was administered from 4<sup>th</sup> day to 12<sup>th</sup> day (40 mg/kg, po). Group - IV and V received paracetamol (1g/kg, po) once daily for first 7 days, *E. pygmaea* aqueous extract (200 and 400 mg/kg, po) was administered from 4<sup>th</sup> day to 12<sup>th</sup> day once daily. Group -VI and VII received paracetamol (1g/kg, po) once daily for first 7 days, alcohol extract of *E. pygmaea* (200 and 400 mg/kg, po) was administered from 4<sup>th</sup> day to 12<sup>th</sup> day, thirty minutes after administration of paracetamol.

On day 13 blood was collected from all animals before sacrifice under light ether anesthesia by retro-orbital puncture and allowed to coagulate for 30 min. at 37°C and centrifuged to obtain serum. Clear serum was used for estimation of various Bio-chemical parameters such as aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and total bilirubin. After collecting blood samples, the animals from all groups were sacrificed by cervical dislocation, and the liver was dissected out, rinsed in ice-cold saline. Portion of liver tissues were fixed in 10% formalin, dehydrated in graded alcohol and then embedded in paraffin. Microtome sections (6 µm thick) were prepared from each liver sample and stained with haematoxylin-eosin dye. The sections were examined for the pathological findings of hepatotoxicity [24].

Liver damage was assessed by the estimation of serum activities of ALT, AST, ALP and bilirubin using suitable standard kits [25-31]. Histopathological assessment of liver damage was done by studying haematoxylin and eosin stained slides of liver tissue.

### Statistical analysis

The result was expressed as mean ± SD. The statistical analysis was carried out using One way ANOVA with Tukey multiple comparison post test. *P* values < 0.05 were considered as significant.

## RESULTS

### Preliminary phytochemical screening

In the present study, alcoholic and aqueous extracts of *E. pygmaea* was subjected to preliminary chemical tests showed the presence of various chemical constituents viz. flavonoid, coumarin, steroid, phenol, tannin, sugar, triterpenoids and amino acids. Both extracts were subjected to pharmacological studies to evaluate acute toxicity studies, diuretic and hepatoprotective activities.

### Acute oral toxicity

In the acute toxicity study, it was observed that both the extracts of *E. pygmaea* up to a dose of 2000 mg/kg po. did not produce any mortality. Hence, 1/10<sup>th</sup> and 1/5<sup>th</sup> of the dose i.e. 200 mg/kg and 400 mg/kg were used for further pharmacological studies.

### Diuretic activity

Alcoholic and aqueous extracts of *E. pygmaea* were screened for diuretic activity, and the extracts were administered orally at the doses of 200 and 400 mg/kg b.w. Urine volume, concentration of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> electrolyte in the urine were recorded. The ratio of the Na<sup>+</sup> and K<sup>+</sup> were calculated to assess the diuretic potential of the extracts. The results obtained for diuretic activity is tabulated in Table 1.

### Urine volume

In the control group, the volume of urine for 5 hours was found to be 1.5 ± 0.20 and in standard group it was found to be 3.63 ± 0.06. In the alcohol extract group (400 mg/kg) the volume of urine for 5 hours was found to be 3.84 ± 0.22. No significant effect observed in the other three extract treated groups (alcohol 200 mg/kg and aqueous 200 and 400 mg/kg).

### Electrolyte excretion

In the control groups, the excretion of sodium for 5 hours was found to be 42 ± 1.00, and in standard group, it was found to be 58 ± 1.0. In the aqueous extracts groups at the dose level of 200 and 400 mg/kg, the excretion of sodium for 5 hours was found to be 70.33 ± 0.98 and 85.33 ± 0.98 respectively. In the alcohol extracts groups at the dose level of 200 and 400 mg/kg, excretion of sodium was found to be 61.66 ± 0.84 and 63.62 ± 0.73 respectively.

In the control groups, the excretion of chloride for 5 hours was found to be 53 ± 1.00, and in standard group, it was found to be 77 ± 1.00. In the aqueous extracts group at the dose level of 200 and 400 mg/kg, the excretion of chloride for 5 hours was found to be 82.66 ± 1.50 and 105.65 ± 0.62 respectively. In the alcohol extracts groups at the dose level of 200 and 400 mg/kg, the excretion of chloride was found to be 69 ± 1.00 and 90.44 ± 0.81 respectively. Both extracts of *E. pygmaea* at the dose levels of 200 and 400 mg/kg showed non-significant in potassium when compared with control group.

The ration of the concentration of sodium and potassium ions in the control groups was found to be 2.67 ± 0.14 and in standard group was found to be 3.69 ± 0.19. In the aqueous extracts groups (200 and 400 mg/kg) was found to be 4.47 ± 0.24 and 5.43 ± 0.31 respectively and in alcohol extracts groups (200 and 400 mg/kg) was found to be 3.85 ± 0.28 and 3.97 ± 0.30 respectively.

**Table 1: Diuretic effect of alcoholic and aqueous extracts of *E. pygmaea***

Group	Dose (mg/kg p.o.)	Total Urine volume (ml/24 h)	Total Na <sup>+</sup> (meq/l)	Total K <sup>+</sup> (meq/l)	Total Cl <sup>-</sup> (meq/l)	Na <sup>+</sup> / K <sup>+</sup> ratio
Normal control	10 ml	1.5 ± 0.20	42 ± 1.00	15.73 ± 1.23	53 ± 1.00	2.67 ± 0.14
Furosemide	10	3.63 ± 0.06***	58 ± 1.0***	15.74 ± 1.09 <sup>NS</sup>	77 ± 1.00***	3.69 ± 0.19***
<i>E. pygmaea</i> (Aq.E)	200	2.04 ± 0.04 <sup>NS</sup>	70.33 ± 0.98***	15.74 ± 1.09 <sup>NS</sup>	82.66 ± 1.50***	4.47 ± 0.24***
<i>E. pygmaea</i> (Aq.E)	400	1.33 ± 0.11 <sup>NS</sup>	85.33 ± 0.98***	15.74 ± 1.10 <sup>NS</sup>	105.65 ± 0.62***	5.43 ± 0.31***
<i>E. pygmaea</i> (Al.E)	200	1.56 ± 0.25 <sup>NS</sup>	61.66 ± 0.84***	16.08 ± 1.44 <sup>NS</sup>	69 ± 1.00***	3.85 ± 0.28***
<i>E. pygmaea</i> (Al.E)	400	3.84 ± 0.22***	63.62 ± 0.73***	16.08 ± 1.44 <sup>NS</sup>	90.44 ± 0.81***	3.97 ± 0.30***

Aq.E. Aqueous extract, Al.E. Alcohol extract: Values are mean ± SD for 6 animals in each group

\*\*\**P* < 0.001 considered significant compare to control value. NS - non significant.

### Hepatoprotective activity

The results of hepatoprotective activity of alcoholic and aqueous extracts of the plant at a dose of 200mg/kg bw and 400mg/kg bw on rats intoxicated with paracetamol is illustrated in Table 2. Assessment of liver function was made by estimating the activities of serum AST, ALT, ALP and total bilirubin. The table also shows the comparison of effects among the untreated (control) and paracetamol treated (negative control), silymarin treated (positive control) group along with the drug treated groups of rats.

*Paracetamol Group (G2)*: There was a significant increase in the serum level of total bilirubin (0.71 ± 0.11 mg/dl), AST (289.33 ± 38.92 IU/L), ALT (113.5 ± 19.36 IU/L), and ALP (747 ± 80.46 IU/L) as shown in Table 2.

*Positive control (silymarin) Group (G3)*: There was a significant decrease in total bilirubin (0.28 ± 0.04 mg/dl), accompanied by significant decrease in level of AST (109 ± 28.92 IU/L) and also significant decrease in ALP (525.33 ± 18.61 IU/L) and ALT (60.5 ± 8.19 IU/L), as compared to the negative control.

*Aqueous low dose Group (G4)*: There was a significant decrease in total bilirubin (0.31 ± 0.04 mg/dl), accompanied by significant

decrease in level of AST (154.5 ± 39.01 IU/L) and also significant decrease in ALT (78.83 ± 25.88 IU/L), as compared to the negative control. ALP showed non significant compared to negative control.

*Aqueous high dose Group (G5)*: There was a significant decrease in total bilirubin (0.42 ± 0.13 mg/dl), accompanied by significant decrease in level of AST (142.53 ± 40.49 IU/L) and also significant decrease in ALP (581.33 ± 28.26 IU/L) and ALT (56.25 ± 4.53 IU/L), as compared to the negative control.

*Alcohol low dose Group (G6)*: There was a significant decrease in total bilirubin (0.3 ± 0.06 mg/dl), accompanied by significant decrease in level of AST (87.61 ± 4.92 IU/L) and also significant decrease in ALT (53.79 ± 11.72 IU/L), as compared to the negative control. ALP non significant compared to negative control.

*Alcohol high dose (G7)*: There was a significant decrease in total bilirubin (0.22 ± 0.04 mg/dl), accompanied by significant decrease in level of AST (106.62 ± 21.79 mg/l) and also significant decrease in ALP (461.16 ± 109.73 IU/l), ALT (53.8 ± 8.13 IU/l), as compared to the negative control.

**Table 2: Effect of alcoholic and aqueous extract of *E. pygmaea* on Biochemical Parameters against Paracetamol induced hepatotoxicity in Rats**

Groups	Dose mg/kg p.o.	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	Total Bilirubin (mg/dl)
Normal control	10 ml	149 ± 32.09	76.5 ± 16.64	518.83 ± 46.87	0.28 ± 0.04
Paracetamol (PCM) control	1 g	289.33 ± 38.92***	113.5 ± 19.36***	747 ± 80.46***	0.71 ± 0.11***
PCM+Silymarin	40	109 ± 28.92***	60.5 ± 8.19***	525.33 ± 18.61***	0.28 ± 0.04***
PCM+ <i>E. pygmaea</i> (Aq.E)	200	154.5 ± 39.01***	78.83 ± 25.88***	619.5 ± 32.62 <sup>NS</sup>	0.31 ± 0.04***
PCM+ <i>E. pygmaea</i> (Aq.E)	400	142.53 ± 40.49***	56.25 ± 4.53***	581.33 ± 28.26 <sup>NS</sup>	0.42 ± 0.13***
PCM+ <i>E. pygmaea</i> (Al.E)	200	87.61 ± 4.92***	53.79 ± 11.72***	671.16 ± 35.10 <sup>NS</sup>	0.3 ± 0.06***
PCM+ <i>E. pygmaea</i> (Al.E)	400	100.62 ± 21.79***	53.8 ± 8.13***	461.16 ± 109.73***	0.22 ± 0.04***

Values are mean ± SD for 6 animals in each group; \**P* < 0.05 \*\**P* < 0.01, \*\*\**P* < 0.001 considered significant compare to control value; NS - non significant. Comparisons were made between Group I vs Groups II & Group II vs Groups III to V

### Histopathological examinations

The histopathological profile of the rat treated with alcoholic and aqueous extracts showed no visible changes confirm in the safety of the extract at selected dose. Histopathological examination of liver section of control group I and silymarin group III showed normal cellular architecture with central vein from which cords of hepatocytes are radiating. In the liver section of the rats intoxicated with paracetamol group II showed disturbance in the lobular arrangement, hepatocytes showed ballooning degeneration and centrilobular necrosis. The liver section of the rats treated with aqueous extracts groups IV & V (200 and 400 mg/kg) showed condensed nuclei, sinusoidal dilatation, ballooning degeneration and normal architecture. The section of alcohol extracts groups VI & VII (200 and 400 mg/kg) showed condensed nuclei, binucleated cells and fatty changes, mild degeneration and normal cellular architecture (Fig. 1).

## DISCUSSION

### Diuretic activity

Diuretics are useful in the treatment of a variety of diseases associated with abnormal retention of salt and water in the extra

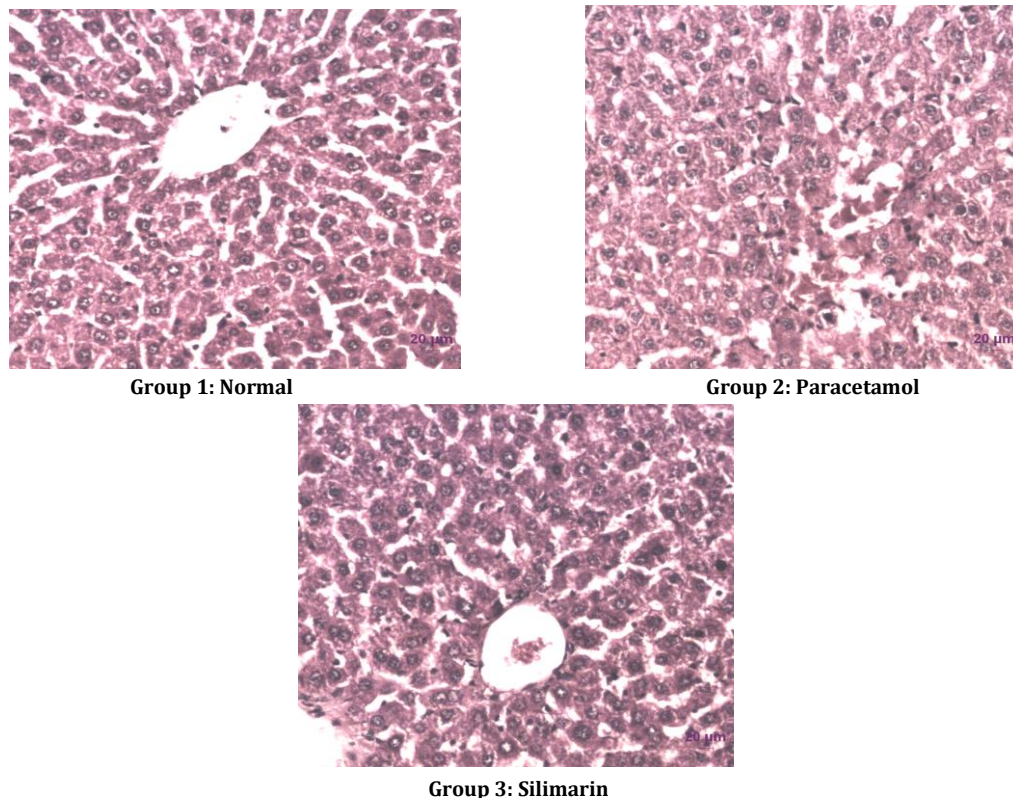
cellular compartments of the body. Diuretic compounds that stimulate the excretion of water are potentially useful in many disorders including most of those exhibiting oedema such as congestive heart diseases, nephritis, toxemia of pregnancy, premenstrual tension, hypertension. And also play an important role in hypertensive patients and pulmonary congestion [32].

From the results, it was observed that the administration of alcohol extract of *E. pygmaea* showed significant (*P* < 0.001) increase in urine volume at the dose level of 400 mg/kg bw when compared with the normal control group. No significant effect was observed in the other three extract treated groups (alcohol 200 mg/kg bw and aqueous 200 and 400 mg/kg bw). Further furosemide showed significant (*P* < 0.001) increase in urine volume with respect to normal control group.

The administration of alcoholic and aqueous extract of *E. pygmaea* treated groups at the dose level of 200 and 400 mg/kg bw showed significant (*P* < 0.001) increase in sodium compared with the normal control group. Both extracts of *E. pygmaea* at the dose levels of 200 and 400 mg/kg showed non-significant in potassium when compared with control group. Both extracts of *E. pygmaea* showed significant increase (*P* < 0.001) in chloride at the dose level of 200

and 400 mg/kg when compared with the normal control group. Urinary sodium and potassium ratio increased significantly ( $P < 0.001$ ) in alcoholic and aqueous extract treated groups of *E. pygmaea* treated groups at the dose levels of 200 and 400 mg/kg bw

when compared with the normal control group. Further furosemide showed significant increase ( $P < 0.001$ ) in sodium, chloride electrolyte content, sodium and potassium ratio of urine with respect to normal control group.



**Fig. 1: Histopathological study of rat liver treated with aqueous and alcoholic extracts of *E. pygmaea*. Photomicrographs of rat liver obtained from different treatment groups. Group 1: Normal - Section of liver showing normal architecture with central vein. Group 2: Paracetamol - Section of the liver showing disturbance in the lobular arrangement, centrilobular necrosis. Group 3: Silymarin - Section of the liver showing normal architecture with central vein from which cords of hepatocytes are radiating. Group 4: 200 mg Aq.E - Section of the liver condensed nuclei, sinusoidal dilatation and ballooning degeneration. Group 5: 400 mg Aq.E - Section of the liver binucleated cells, ballooning and mild degeneration. Group 6: 200 mg ALE - Section of the liver condensed binucleated cells, ballooning, mild degeneration. Group 7: 400 mg ALE - Section of the liver condensed nuclei, binucleated cells and fatty changes, mild degeneration (Haematoxylin and eosin x 20).**

The extracts increased sodium ion excretion to a greater extent than potassium, which is an essential quality of good diuretic with lesser hyperkalaemic side effect [33]. The present study revealed that the alcoholic and aqueous extracts act as hypernatremic and hyperchloremic diuretics (increased sodium and chloride excretion volume) comparable to that of furosemide treated group. Both extracts at the tested doses possess significant diuretic activity and this observation supports the folklore claim of this genus as diuretic.

#### Hepatoprotective activity

A variety of chemicals have been used for the evaluation of hepatotoxicity in rats. This includes thioacetamide, carbon tetrachloride, paracetamol, alcohol, liquid paraffin etc [34]. The present study was carried out with the use of paracetamol for evaluating the hepatoprotective activity.

Paracetamol, an analgesic and antipyretic, is assumed to be safe in recommended doses; overdoses, however produce hepatic necrosis. Small doses are eliminated by conjugation followed by excretion, but when the conjugation enzymes are saturated, the drug is diverted to an alternative metabolic pathway, resulting in the formation of a hydroxylamine derivative by cytochrome  $P_{450}$  enzyme. Hydroxylamine derivative a reactive electrophilic agent reacts non-enzymatically with glutathione depletes, the hydroxylamine reacts with macromolecules and disrupts their structure and function. Extensive liver damage by paracetamol itself decreases its rate of metabolism and other substrates for hepatic microsomal enzymes. Induction of cytochrome  $P_{450}$  or depletion of hepatic glutathione is a

prerequisite for paracetamol-induced toxicity [35]. Product of lipid peroxidation may cause damage to the biological membranes leading to serious cellular injury and leakage of serum marker enzymes like AST, ALT, ALP [36] and finally death [37]. ALT, AST, ALP and total bilirubin are the most sensitive tests employed in the diagnosis of hepatic disease. The degree of rise in SGOT (Serum Glutamate Oxaloacetate Transaminase) and SGPT (Serum Glutamate Pyruvate Transaminase) activity reflects the extent of hepatic damage [38].

In the present study administration of paracetamol led to the increase of serum enzymes AST, ALT, ALP and total bilirubin levels as compared to control group. The rat treated with alcoholic and aqueous extracts at the dose level of 400 mg/kg bw showed a significant reduction ( $P < 0.001$ ,  $P < 0.01$ ) in all the biochemical parameters when compare to paracetamol group. The rat treated with alcoholic and aqueous extracts at the dose level of 200 mg/kg showed a significant reduction ( $P < 0.001$ ) in AST, ALT and bilirubin, ALP showed non significant when compared to paracetamol group.

The activity of the tested samples was comparable to that of standard drug silymarin. Silymarin treated animals showed a significant ( $P < 0.001$ ) reduction in all the tested biochemical parameters when compared to paracetamol group. Histopathological examination of the liver tissues also supported the hepatoprotection.

Paracetamol induced liver necrosis was inhibited significantly by the plant extracts, which confirms the protection action of the alcoholic

and aqueous extract of *E. pygmaea* against experimentally induced liver damage in rats. It can be concluded that the plant of *E. pygmaea* possess hepatoprotective activity.

### CONCLUSION

The therapeutic activities are reported for the first time in the plant *E. pygmaea*. In this present study, both extracts showed significant ( $P < 0.001$ ) hepatoprotective and diuretic activities. The higher dose level (400 mg/kg bw) was more significant ( $P < 0.001$ ) than the lower dose level (200 mg/kg bw) and this exhibited dose-dependent activity.

In the plant *E. pygmaea*, the presence of flavonoids (luteolin, luteolin-7-O-glucoside, apigenin, apigenin-7-O-glucoside), steroids (stigmasterol) and triterpenoids (lupeol acetate) have been reported. A number of scientific reports indicate that luteolin, luteolin-7-O-glucoside and apigenin, apigenin-7-O-glucoside may be responsible for the activities of antibacterial, antioxidant [39], diuretic and hepatoprotective activities [40][41]. The isolation and testing of constituents likely to be responsible for the hepatoprotective and diuretic activity of *E. pygmaea* is under progress in our lab.

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