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Acute Oral Toxicity Study of *Pluchea arguta* Boiss Extract in Mice

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ABSTRACT

Acute oral toxicity of methanol extract of *Pluchea arguta* Boiss was evaluated in Swiss albino mice of both sexes. In this study mice were administered orally with dosages of 1000, 3000 and 6000 mg kg^{-1} b.wt. of methanol extract of *P. arguta* extract. Mortality, signs of toxicity, body weight, food and water consumption, haematological and gross behaviour was observed for 7 days post treatment of *P. arguta* extract. No mortality, signs of toxicity and abnormalities in gross behaviour were observed. In addition, no significant differences were noticed in the body and organ weights between the control and treated groups of both sexes. The methanol extract of *P. arguta* is nontoxic and safe by oral intake.

Key words: *Pluchea arguta*, acute oral toxicity, gross behaviour, mortality, mice

INTRODUCTION

Herbal medicine is gaining popularity in developing countries. Herbal remedies are often believed to be harmless because they are natural and free of side effect (Lopes *et al*., 2000). This increase in popularity and the scarcity of scientific studies on their safety and efficacy have raised concerns regarding toxicity and adverse effects of these remedies (Saad *et al*., 2006). Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor, or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer (Craig 1999). Currently, there is an ongoing world-wide green revolution which is mainly premised on the belief that herbal remedies are safer and less damaging to the human body than synthetic drugs (Willianson *et al*., 1996; Parekh and Chanda, 2006).

According to World Health Organization about 80% of the world population relies on traditional medicine for primary health care and more than 30% of the plant species have been used medicinally (Akerele *et al*., 1991). However, there is limited scientific evidence regarding the safety and efficacy to support the continued therapeutic application of these medicinal plants. Because of this renewed interest in herbal remedies, there is a need for thorough scientific safety evaluation of the medicinal plants (Sofowora, 1982). The increasing usage of plant extracts in food, cosmetics and pharmaceutical industries demands toxicity assessment. No drug is used clinically without its clinical trials and toxicity studies (Anisuzzaman *et al*., 2001).

The evaluation of the toxic action of plant extracts is indispensable in order to consider a treatment safe; it enables the definition of the intrinsic toxicity of the plant and the effects of acute overdose. Laboratory mice are sensitive to toxic substances occurring in plants. The administration of the extracts in increasing amounts enables the evaluation of the toxicity limits and the test

should be carried out in two ways, for three doses and for both sexes, taking into account such factors as age, sex, weight, species, diet and environmental conditions (Parra *et al*., 2001).

Pluchea arguta Boiss (Syn. *Conyza odontophylla* Boiss) is a member of family compositae, about 1000 genera of this family are well known in the world (Jeffrey, 1966). Different species of *Pluchea* are used in Ayurvedic system of medicine in various clinical conditions. Pharmacological studies demonstrated anti-inflammatory and antioxidant activities of different *Pluchea* species (Barros *et al*., 2006; Fernandez and Torres, 2006). This work is carried out to evaluate acute toxicological study of methanol extract of *Pluchea arguta*. The purpose of this study was to evaluate the safety of methanol extract of *Pluchea arguta* in mice by determining oral acute toxicity.

MATERIALS AND METHODS

Plant material: Fresh leaves and stem of *Pluchea arguta* Boiss. were collected in the month of December 2006, from Junagadh Agriculture University, Junagadh, Gujarat, India. It was identified by Dr. P.S. Nagar, Department of Botany, M.S. University, Baroda, Gujarat, India. Fresh plant material was washed under tap water. The leaves plus stem were air dried to a constant weight. The dried leaves plus stem together were homogenized to fine powder and powder was stored in airtight bottles.

Animals: Swiss albino mice of either sex (20-25 g) were used for acute and subacute toxicity studies. The animals were obtained from Sarabhai Research Centre (SRC), Baroda. All the mice were housed standard plastic cages with stainless steel coverlids and wheat straw as bedding material at the animal house of Department of Biosciences, Saurashtra University, Rajkot, Gujarat, India. The animals were kept in group of 5-6 per cage and facilitated with standard environmental condition of photoperiod (12:12 h dark: light cycle) and temperature (27 ± 2°C). They were provided with commercial rat and mice feed (Pranav Agro Industries Ltd., Amrut brand rat and mice pellet feed) and water given ad libitum. The use of these animals and the study protocols were approved by CPCSEA ethical committee.

Extraction: Ten grams of dried crude powder of *Pluchea arguta* Boiss was successively extracted by soxhlet extraction method (Vaghasiya *et al*., 2008). Plant material was defatted by petroleum ether. Defatted material was extracted in methanol. Methanol was evaporated under reduced pressure from the extract. Dried extract was stored in air-tight bottles at 4°C. The extractive yield of *P. arguta* was 10%.

Experimental groups and administration of the extract: In order to study any possible toxic effect or changes in normal behaviour, 4 groups of 10 mice (five male and five female) were used in this experiment. Group I served as control group and the other groups II, III and IV were test groups (1000, 3000 and 6000 mg kg^{-1}, respectively). All the animals were fasted overnight prior to dosing. Before commencing the experiment, the body weight of mice was recorded. Methanolic extract was prepared in distilled water and all the animals except group I were administered a single oral dose of methanol extract of *P. arguta* at 1000, 3000 and 6000 mg kg^{-1} b.wt. The control animals received only vehicle.

Behavioral study: The mice were placed one by one at the centre of three concentric circles drawn on a rubber sheet with diameter of 7, 14 and 21 cm. After dosing all animals were observed

for gross behaviour parameters like CNS depression (Hypoactivity, passivity, relaxation, narcosis and ataxia), CNS stimulation (Hyperactivity, irritability, stereotypy, tremors, convulsions, straub tail and analgesia) and ANS stimulation (Ptosis and exophthalmia) at 1, 2, 3, 24 and 48 h (Morpugo, 1971). The observed results were recorded as sign of toxicity/number of animals studied. Signs of toxicity and mortality were observed daily for 7 days and were monitored daily for changes in body weight.

Food and water consumption: The amount of feed and water consumed were measured daily from the quantity of feed and water supply and the amount remaining after 24 h.

Absolute and relative organ weight: Organs such as liver, kidney, lung, heart, spleen, brain, adrenal gland, thymus, testis and uterus were isolated and weight was measured of all the organs. Relative organ weight was measured and compared with the control group.

Haematological parameters: On 8th day, the animals were made to fast overnight and sacrificed by decapitation and blood was collected. The haematological parameters (haemoglobin, total red blood cell (RBC), leukocyte (WBC), PCV, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils and platelet counts) were determined using an autoanalyzer (System H1, Bayer Diagnostics).

Statistical analysis: All values are expressed as the mean ± SEM. The statistical comparisons were made by means of the student's t test. Values were considered statistically significant at $p<0.05$.

RESULTS AND DISCUSSION

Phytotheraputic products are many times, mistakenly regarded as safe because they are natural (Gesler, 1992). Nevertheless, these products contain bioactive principles with potential to cause adverse effect (Bent and Ko, 2004). Apart from giving a clue on the range of doses that could be used in subsequent toxicity testing, it could equally reveal the possible clinical signs elicited by the substance under investigation. It is also a useful parameter to investigating therapeutic index (i.e., LD_{50}/ED_{50}) of drugs and xenobiotics (Rang *et al*., 2001).

Gross behaviour study: No mortality was observed after the administration of methanol extract of *P. arguta* at the doses of 1000, 3000 and 6000 mg kg^{-1} b.wt. This is an indication that the extract has negligible level of toxicity when administered orally. The behavioral signs of toxicity such as CNS depression (Hypoactivity, passivity, relaxation, narcosis and ataxia), CNS stimulation (Hyperactivity, irritability, stereotypy, tremors, convulsions, straub tail and analgesia) and ANS stimulation (Ptosis and exophthalmia) were also observed. No signs of toxicity were observed, in either sex, in the control or treated groups. In addition, gross necropsy findings did not show any adverse effects in male and female mice of any organs in treated groups as compared to control group. Diarrhoea was observed in male and female mice at the dose of 6000 mg kg^{-1} at 1, 2 and 3 h. After 24 h all the animals were normal. Anti-analgesic effect was seen at the doses of 1000, 3000 and 6000 mg kg^{-1} (Table 1, 2). According to Kennedy *et al*. (1986), substances with LD_{50} higher than 5 g kg^{-1} by oral route are regarded as being safe or practically non-toxic. Similar results were found for single oral dose administration of *P. longifolia* extracts in mice (Nair *et al*., 2009).

Table 1: Acute toxicological study of methanol extract of *Pluchea arguta* at different doses in mice (Males)

				Parameters of gross behaviour															
				CNS depression					CNS stimulation								ANS stimulation		Others
Groups	Doses	Time (h)	Mortality	Hypoactivity	Passivity	Relaxation	Narcosis	Ataxia	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsions	Straub tail	Analgesia	Ptosis	Exophthalmia	Diarrhoea
I	0 (Vehicle)	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		2	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		24	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		48	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
II	1000 mg kg^{-1}	1	0/5	1/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
		2	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		3	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		24	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
		48	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
III	3000 mg kg^{-1}	1	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
		2	0/5	2/5	2/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
		3	0/5	3/5	3/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
		24	0/5	3/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5
		48	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5
IV	6000 mg kg^{-1}	1	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	4/5
		2	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	4/5
		3	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	4/5
		24	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	0/5	0/5	0/5
		48	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5

Data is presented as sign of toxicity/No. animal

Table 2: Acute toxicological study of methanol extract of *Pluchea arguta* at different doses in mice (Females)

				Parameters of gross behaviour															
				CNS depression					CNS stimulation							ANS stimulation		Others	
Groups	Doses	Time (h)	Mortality	Hypoactivity	Passivity	Relaxation	Narcosis	Ataxia	Hyperactivity	Irritability	Stereotypy	Tremors	Convulsions	Straub tail	Analgesia	Ptosis	Exophthalmia	Diarrhoea	
I	0 (Vehicle)	1	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		2	0/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		3	0/5	2/5	0/5	0/5	0/5	0/5	0/5	1/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		24	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		48	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
II	1000 mg kg^{-1}	1	0/5	1/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	3/5	0/5	0/5	0/5	
		2	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
		3	0/5	2/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
		24	0/5	2/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		48	0/5	2/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
III	3000 mg kg^{-1}	1	0/5	0/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		2	0/5	1/5	0/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	
		3	0/5	2/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
		24	0/5	1/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
		48	0/5	2/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
IV	6000 mg kg^{-1}	1	0/5	0/5	1/5	2/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	
		2	0/5	1/5	1/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	
		3	0/5	1/5	1/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	0/5	0/5	0/5	2/5	
		24	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	
		48	0/5	0/5	0/5	1/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	0/5	1/5	0/5	0/5	0/5	

Data is presented as sign of toxicity/No. animals

Food and water consumption: Determination of food consumption is important in the study of safety of a product with therapeutic purpose, as proper intake of nutrients is essential to the physiological status of the animal and to the accomplishment of the proper response to the drug tested instead of a false response due to improper nutritional conditions (Steven and Mylecrdfaine, 1994; Iversen and Nicolaysen, 2003). The food consumption by male mice on each day in control and treated groups was almost same (Table 3) while in female mice there was slight variation in food consumption in both control and treated mice (Table 4). However, these differences were not statistically significant. Similar pattern was observed in water consumption pattern by both male and female mice in control and treated groups (Table 5, 6). The food and water consumption was not affected by administration of methanolic extract of *P. arguta* i.e., it did not induce appetite suppression and had no deleterious effect. It indicates that there was no disturbance in carbohydrate, protein or fat metabolism (Klaassen, 2001).

Generally, the reduction in body weight gain and internal organ weights are simple and sensitive indices of toxicity after exposure to toxic substances. Body weight changes are indicators of adverse effects of drugs and chemicals and it will be significant if the body weight loss occurred is more than 10% from the initial body weight (Raza *et al*., 2002; Teo *et al*., 2002). There was no change in body weight between control and treated groups. The body weight gain was similar in both control and treated groups (Fig. 1, 2). The differences were not statistically significant.

Absolute and relative organ weight: Organ weight also is an important index of physiological and pathological status in man and animals. The relative organ weight is fundamental to diagnose whether the organ was exposed to the injury or not. The heart, liver, kidney, spleen and lungs are

Table 3: Food consumption values of acute toxicity study of methanol extract of *Pluchea arguta* extract in male mice (g/animal/day, mean)

	Feed consumption			
Days	Control	PAM-1000	PAM-3000	PAM-6000
2	11.0	10.3	9.0	9.3
3	10.6	9.6	9.6	9.6
4	13.0	10.2	12.2	7.8
5	12.6	11.4	11.0	9.0
6	10.2	10.2	13.0	11.4
7	11.0	11.0	9.4	11.4

PAM: Methanol extract of *Pluchea arguta*, n = 5

Table 4: Food consumption values of acute toxicity study of methanol extract of *Pluchea arguta* extract in female mice (g/animal/day, mean)

	Feed consumption (Female)			
Days	Control	PAM-1000	PAM-3000	PAM-6000
2	10.2	9.0	11.8	8.6
3	9.8	10.6	12.2	5.8
4	12.2	11.8	14.2	11.0
5	10.6	9.6	11.0	9.0
6	9.0	9.0	10.6	9.0
7	11.0	10.2	12.6	8.6

PAM: Methanol extract of *Pluchea arguta*, n = 5

Table 5: Water consumption values of acute toxicity study of methanol extract of *Pluchea arguta* extract in male mice (mL/animal/day, mean)

	Water consumption			
Days	Control	PAM-1000	PAM-3000	PAM-6000
2	12.0	11.3	11.0	9.3
3	10.6	9.1	9.0	10.0
4	11.0	10.2	12.0	7.6
5	11.6	10.4	8.8	9.2
6	10.2	10.2	13.0	10.4
7	12.0	9.5	10.0	12.0

PAM: Methanol extract of *Pluchea arguta*, n = 5

Table 6: Water consumption values of acute toxicity study of methanol extract of *Pluchea arguta* extract in female mice (mL/animal/day, mean)

	Water consumption			
Days	Control	PAM-1000	PAM-3000	PAM-6000
2	9.8	10.0	11.0	9.0
3	1	9.4	11.4	7.3
4	12.4	12.0	13.7	11.0
5	10.6	9.4	10.0	8.6
6	8.8	10.2	11.6	8.0
7	10	11.2	12.1	8.1

PAM: Methanol extract of *Pluchea arguta*, n = 5

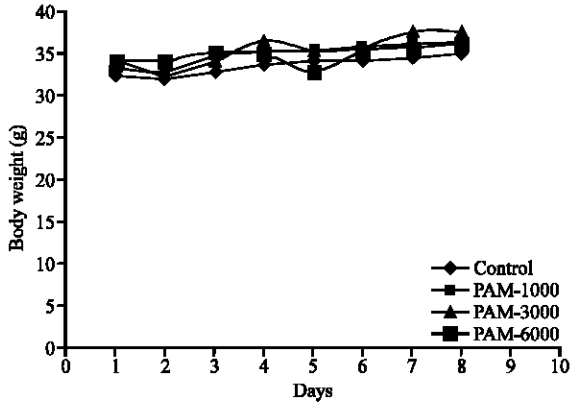

Fig. 1: Effect of *Pluchea arguta* extract on body weight changes in male mice

the primary organs affected by metabolic reaction caused by toxicants (Dybing *et al*., 2002). The absolute and relative heart weight was almost same in control and treated groups in male mice while in female mice, the weight was slightly more in treated groups but it was statistically non significant. The liver weight in both sexes slightly increased in treated groups as compared to control group but the increased weight was non significant. The relative and organ weight of other organs like brain, lung, spleen, kidney, testis, uterus and thymus gland showed a similar trend like that of liver in both sexes of animals (Table 7, 8). Administration of methanolic extract of *P. arguta* did not show any effect on organ weight of all important organs. Moreover, gross

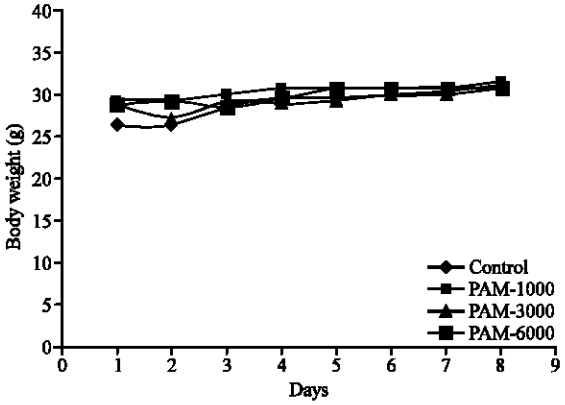

Fig. 2: Effect of *Pluchea arguta* extract on body weight changes in female mice

Table 7: Absolute organ weight (g) and Relative organ weight values (g/100 g b.wt.) of acute toxicity study of *Pluchea arguta* extract in male mice

	Control		PAM-1000		PAM-3000		PAM-6000	
Organs	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight
Heart	0.18±0.02	0.50±0.03	0.18±0.02	0.51±0.04	0.19±0.01	0.51±0.02	0.18±0.004	0.50±0.02
Liver	1.79±0.09	5.16±0.19	1.96±0.09	5.43±0.20	2.02±0.07	5.39±0.25	1.96±0.18	5.41±0.32
Brain	0.5±0.02	1.45±0.09	0.54±0.02	1.51±0.04	0.53±0.02	1.41±0.05	0.51±0.03	1.42±0.09
Lung	0.3±0.03	0.90±0.14	0.36±0.02	0.99±0.04	0.33±0.03	0.87±0.07	0.38±0.03	1.07±0.07
Spleen	0.13±0.02	0.37±0.05	0.18±0.03	0.49±0.08	0.16±0.02	0.43±0.06	0.17±0.04	0.47±0.10
Kidney	0.33±0.3	0.94±0.09	0.36±0.02	1.01±0.04	0.37±0.01	0.99±0.03	0.35±0.02	1.00±0.11
Testis	0.10±0.01	0.29±0.01	0.12±0.01	0.33±0.02	0.11±0.004	0.31±0.02	0.10±0.01	0.26±0.04
Thymus	0.11±0.01	0.62±0.06	0.11±0.01	0.58±0.067	0.13±0.01	0.67±0.051	0.13±0.01	0.74±0.065

Data are expressed as Mean±SEM, n = 5, PAM: Methanol extract of *Pluchea arguta*

Table 8: Absolute organ weight (g) and Relative organ weight (g/100 g b.wt.) of acute toxicity study of *Pluchea arguta* extract in female mice

	Control		PAM-1000		PAM-3000		PAM-6000	
Organs	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight	Absolute organ weight	Relative organ weight
Heart	0.15±0.01	0.47±0.03	0.16±0.01	0.50±0.03	0.16±0.01	0.50±0.02	0.17±0.01	0.55±0.02
Liver	1.59±0.12	5.08±0.32	1.76±0.05	5.64±0.21	1.70±0.08	5.52±0.34	1.74±0.05	5.66±0.15
Brain	0.52±0.02	1.65±0.07	0.52±0.01	1.64±0.08	0.53±0.01	1.73±0.03	0.54±0.01	1.75±0.06
Lung	0.27±0.02	0.86±0.06	0.34±0.03	1.09±0.13	0.35±0.03	1.12±0.08	0.40±0.02	1.30±0.08
Spleen	0.11±0.01	0.34±0.03	0.14±0.02	0.44±0.06	0.15±0.02	0.50±0.06	0.13±0.02	0.41±0.04
Kidney	0.23±0.01	0.73±0.05	0.23±0.01	0.71±0.03	0.24±0.02	0.77±0.06	0.24±0.01	0.79±0.05
Uterus	0.20±0.03	0.66±0.11	0.23±0.03	0.74±0.08	0.24±0.02	0.78±0.06	0.26±0.03	0.85±0.10
Thymus	0.08±0.02	0.53±0.05	0.10±0.02	0.62±0.06	0.11±0.01	0.70±0.05	0.11±0.02	0.73±0.06

Data are expressed as Mean±SEM, n = 5, PAM: Methanol extract of *Pluchea arguta*

examination of internal organs of all mice revealed no detectable abnormalities. Thus, it can be suggested that the methanolic extract of *P. arguta* is virtually nontoxic.

Haematological parameters: Blood parameters analysis is relevant to risk evaluation as the hematological system has a higher predictive value for toxicity in humans (91%) when assay involve rodents and non-rodents (Olson *et al*., 2000). Blood is an important index of physiological and pathological status in man and animals and the parameters usually measured are haemoglobin, total red blood cell (RBC), leukocyte (WBC), PCV, MCV, MCH, MCHC, neutrophils, lymphocytes, eosinophils and platelet counts (Schalm *et al*., 1975). The normal range of these parameters can be altered by the intake of some toxic plants (Ajagbonna *et al*., 1999). The effect of extract on haematological parameters was slightly different in male and female mice. The haemoglobin levels increased in male mice while it decreased in female mice in treated groups as compared to control group of animals. However, these increased or decreased levels were statistically non significant. Similar trend was observed in other blood indices like total RBC, PCV, MCV, MCH and MCHC in both sexes of animals (Table 9, 10). The effect of extract on WBC and its sub population was slightly different in male and female mice. The total WBC level increased in male mice at medium and higher dose level while it decreased in female mice at higher dose level. However, these difference were statistically non significant. Similar trend was observed in other blood indices like neutrophlis and eosinophils in both sexes of animals but in lymphocytes,

Table 9: Hematological parameters of acute toxicity study of *Pluchea arguta* extract in male mice

Hematological parameters	Control	PAM-1000	PAM-3000	PAM-6000
Hemoglobin (g/100 mL)	12.40±1.49	14.45±0.96	15.08±0.21	14±0.58
Total RBC (mL/cu. mm.)	7.70±0.92	9.22±0.73	9.60±0.18	8.73±0.36
PCV (%)	38.02±4.92	44.98±3.15	45.74±0.64	43.08±2.01
MCV (cu. micron)	49.06±0.70	48.97± 0.89	47.76±0.59	49.29±0.48
MCH (Pico gram)	16.10±0.13	15.87±0.21	15.72±0.16	16.03±0.11
MCHC (%)	32.83±0.51	32.43±0.43	32.91±0.14	32.54±0.22
Total WBC (per cu. mm)	4300±1085	3980±1177	5500±777	5640±2386
Neutrophils (%)	26.80±4	40.40±6.78	27.60±3.33	36.40±5.69
Lymphocytes (%)	72.40±4.08	58.40±6.93	71.20±3.68	62.20±6.29
Eosinophils (%)	0.80±0.37	1.20±0.73	1.20±0.58	1.40±0.68
Platelet count (per cu. mm)	1524600±104757	1593600±101299	1712800±100594	1500800±77185

Data are expressed as Mean±SEM, n = 5, PAM: Methanol extract of *Pluchea arguta* RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell

Table 10: Hematological parameters of acute toxicity study of *Pluchea arguta* extract in female mice

Hematological parameters	Control	PAM-1000	PAM-3000	PAM-6000
Hemoglobin (g/100 mL)	15.50±0.32	15.10±0.24	14.68±0.13	15.56±0.25
Total RBC (mL/cu. mm.)	9.68±0.1	9.54±0.15	9.19±0.12	9.51±0.19
PCV (%)	47.08±0.89	45.50±1.02	44.34±0.76	46.34±0.73
MCV (cu. micron)	48.63±0.55	47.70±0.70	48.26±0.60	48.47±0.47
MCH (Pico gram)	16.01±0.24	15.84±0.30	15.99±0.21	16.28±0.16
MCHC (%)	32.93±0.40	33.21±0.45	33.13±0.39	33.59±0.56
Total WBC (per cu. mm)	2420±299	3760±995	3700±701	2660±426
Neutrophils (%)	27.60±2.18	30.40±2.77	24.40±3.54	24.40±3.36
Lymphocytes (%)	72±2.45	69±2.97	75.20±3.53	75.40±3.47
Eosinophils (%)	0.40±0.40	0.60±0.40	0.40±0.40	0.20±0.20
Platelet count (per cu. mm)	1203400±107079	1312000±88786	1177000±39139	1363600±146223

Note: Data are expressed as Mean±SEM, n = 5, PAM: Methanol extract of *Pluchea arguta* RBC: Red blood cell, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, WBC: White blood cell

the trend was completely opposite i.e., it decreased in male mice and increased in female mice at higher dose level as compared to control group. The total platelet levels decreased in male mice at high dose level while it increased in female mice as compared to control group. However, these differences were statistically non significant (Table 9, 10). All the parameters of haematological analysis were statistically not significant when compared with the control group, discarding the possibility of anemia or disturbances in the erythrocytes or hemoglobin production.

CONCLUSION

The methanol extract of *Pluchea arguta* extract was found to be fairly nontoxic when oral acute toxicity study in mice was performed. Detailed experimental analysis on chronic toxicity is essential for further support of this drug.

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REFERENCES

Ajagbonna, O.P., K.I. Onifade and U. Suleiman, 1999. Haematological and biochemical changes in rats given extract of *C. pocera*. Sokoto J. Vet. Sci., 1: 3-12.

Akerele, A., V. Heywood and H. Synge, 1991. Conservation of Medicinal Plants. Cambridge University Press, Cambridge.

Anisuzzaman, A.S.M., N. Sugimoto, G. Sadik and M.A. Gafur, 2001. Sub-acute toxicity study of 5-Hydroxy-2(Hydroxy-Methyl) 4H-pyran-4 One, isolated from *Aspergillus fumigatus*. Pak. J. Biol. Sci., 4: 1012-1015.

Barros, I.M.C., L.D.G. Lopes, M.O.R. Borges, A.C.R. Borges, M.N.S. Ribeiro and S.M.F. Freire, 2006. Anti-inflammatory and anti-nociceptive activities of *Pluchea quitoc* (DC.) ethanolic extract. J. Ethnopharmacol., 106: 317-320.

Bent, S. and R. Ko, 2004. Commonly used herbal medicine in the United States: A review. Am. J. Med., 116: 478-485.

Craig, W.J., 1999. Health promoting properties of common herbs. Am. J. Clin. Nutr., 70: S491-S499.

Dybing, E., J. Doe, J. Groten, J. Kleiner and J. O'Brien *et al*., 2002. Hazard characterization of chemicals in food and diet: dose response, mechanism and extrapolation issues. Food Chem. Toxicol., 42: 237-282.

Fernandez, F. and M. Torres, 2006. Evaluation of *Pluchea carolinensis* extracts as antioxidants by the epinephrine oxidation method. Fitoterapia, 77: 221-226.

Gesler, W.M., 1992. Therapeutic landscape: Medical issues in light of the new cultural geography. Social Sci. Med., 34: 735-746.

Iversen, P.O. and G. Nicolaysen, 2003. Water for life. Tidsskrift for den Norske Laegeforening, 123: 3402-3405.

Jeffrey, S.M.H., 1966. The Flora of Karachi (Coastal West Pakistan). The Book Corporation, Karachi, pp: 375.

Kennedy, G.L., R.L.J. Ferenz and B.A. Burgess, 1986. Estimation of acute toxicity in rats by determination of the approximate lethal dose rather than the LD_{50}. J. Applied Toxicol., 6: 145-148.

Klaassen, C.D., 2001. Casarett and Doulls. Toxicology: The Basic Science of Poison. 6th Edn., The McGraw-Hill Companies Inc., New York.

Lopes L.D.C., F. Albano, G.A.T. Laranja, L.M. Alves and L.F.M. Silva *et al.*, 2000. Toxicological evaluation by *in vitro* and *in vivo* assays of an aqueous extract prepared from *Echinodorus macrophyllus* leaves. Toxicol. Lett., 116: 189-198.

Morpugo, C., 1971. A new design for the screening of CNS-active drugs in mice. Arzneim. Forsch., 11: 1727-1734.

Nair, R., V. Shukla and S. Chanda, 2009. Effect of single dose administration of *Polyalthia longifolia* (Sonn.) Thw. Var. pendula leaf on gross behavioral assessment in mice. Indian Drugs, 46: 116-123.

Olson, H., G. Betton, D. Robinson, K. Thomas and A. Monro *et al.*, 2000. Concordance of toxicity of pharmaceuticals in humans and in animals. Regul. Toxicol. Pharmacol., 32: 56-67.

Parekh, J. and S. Chanda, 2006. *In-vitro* Antimicrobial activities of extracts of *Launaea procumbens* Roxb. (Labiateae), *Vitis vinifera* L. (Vitaceae) and *Cyperus rotundus* L. (Cyperaceae). Afr. J. Biomed. Res., 9: 89-93.

Parra, A.L., R.S. Yhebra, I.G. Sardinas and L.I. Buela, 2001. Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. Phytomedical, 8: 395-400.

Rang, H.P., M. Dale and J. Ritter, 2001. Pharmacology. 4th Edn., Churchill Livingstone, New York, ISBN-13: 9780443059742.

Raza, M., O.A. Al-Shabanah, T.M. El-Hadiyah and A.A. Al-Majed, 2002. Effect of prolonged vigabatrin treatment on haematological and biochemical parameters in plasma, liver and kidney of Swiss albino mice. Sci. Pharmaceut., 70: 135-145.

Saad, B., H. Azaizeh, G. Abu-Hijleh and S. Said, 2006. Safety of traditional Arab herbal medicine. Evid. Based Comp. Alt. Med., 3: 433-439.

Schalm, O.W., N.C. Jain and E.J. Carrol, 1975. Veterinary Haematology. 3rd Edn., Lea and Febiger Publication, Philadelphia, pp: 807-807.

Sofowora, A., 1982. Medicinal Plants and Traditional Medicine in Africa. 1st Edn., John Wiley and Sons, Chichester, New York, ISBN-10: 0471103675, pp: 256.

Steven, K.R. and L. Mylecrdfaine, 1994. Issues in Chronic Toxicology. In: Principles and Methods of Toxicology, Hayes, A.W. (Ed.). 3rd Edn., Raven Press, New York, pp: 673.

Teo, S., D. Stirling, S. Thomas, A. Hoberman and A. Kiorpes and K. Vikram, 2002. A 90-day oral gavage toxicity study of D-methylphenidate and D, L methylphenidate in sprague-dawley rats. Toxicology, 179: 183-196.

Vaghasiya, Y., R. Nair and S. Chanda, 2008. Antibacterial and preliminary phytochemical and physico-chemical analysis of *Eucalyptus citriodora* Hk leaf. Nat. Prod. Res., 22: 754-762.

Willianson, E.M., D.T. Okpako and F.J. Evans, 1996. Selection, Preparation and Pharmacological Evaluation of Plant Material. John Wiley, England.