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Evaluation of acute oral toxicity study of essential oils (Eos) from *Pogostemon benghalensis* and *P. cablin* in Wistar rats

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ABSTRACT

The use of crude herbal decoctions in the traditional treatment of diseases is a common practice. *Pogostemon benghalensis* and *P. cablin* are commonly used for treatment of diverse categories of diseases such as infectious and non-infectious disease. Native people use the crude decoctions as bactericidal, antimalarial, anti-leshimania, anti-diarrheal and insecticidal activities. Its safety profile is not yet elucidated and therefore, this study was to analyze the acute toxicity of essential oils (Eos) from *P. benghalensis* and *P. cablin* as medicinal. Methods include acute toxicity study using male and female Wistar albino rats with single oral dose and followed up to 14 days as per the guidelines of OECD. Visual observations were carried regularly during the experimental period while body weight was measured weekly. Organ weight, clinical chemistry and hematology data were collected on the 7th and 14th days. Results were presented as mean ± standard deviation. One-way analysis of variance (ANOVA) was carried. Oral administration of Eos from *P. benghalensis* and *P. cablin* revealed no treatment-related mortality in female rats up to the dose of 5000 mg/kg. In acute toxicity studies, no remarkable treatment related anomalies were observed compared to negative controls. Food consumption, body weight, organ weight, hematology did not showed sound variation between controls and treatment groups. However, creatinine, triglycerides, and monocytes were lower in the treated groups in 7th day as compared to control groups. No significant variations between male and female groups in relative organ weight, hematology were noticed. In conclusion, the Eos from *P. benghalensis* and *P. cablin* showed LD_{50} > 3000 mg/kg in acute toxicity studies.

Keywords: *Pogostemon benghalensis, P. cablin,* traditional medicine, safety, plant medicine, adverse effect, acute oral toxicity

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INTRODUCTION

Globally, secondary metabolites from plants are employed as therapeutics in primary health care system. Most of the crude extracts used by the native communities are non-validated scientifically. The plant extracts can be harmful at certain concentrations, but may be safe and curative in other doses. Therefore, there is a range of possible impacts from acute to sub-acute level of toxicity. The plants synthesize the pool of phytochemicals like protein, sugar, lipids, polyphenols, alkaloids, saponins, nitrogen conjugates etc. as a part of defense mechanisms against grazing animals or invading pathogens. Many synthetic compounds that have been proven as toxic even in minute quantities, while the plant-based drugs have been employed to prevent, diagnose, and treat diverse disorders. This therapy has been practiced out for the past several decades. Exposure of a phytochemical in human beings can be studied by analyzing the cumulative effects and concentrations that may be carcinogenic, mutagenic, teratogenic, skin or eye irritation and others[1]. Toxicity testing is essential to quantify the damage level caused by the molecules to biological and non-biological compounds of the cell system. This acute oral toxicity test is usually attempted on prospective products to develop novel drugs and to determine their therapeutic potentials[2].

Lack of evidence-based approaches like the legal and regulatory framework, pharmacovigilance, non-standardization and lack of toxicological profiling of herbal products form the greatest concern of medicinal plants usage[3, 4]. Plants synthesize diverse metabolites that form complex molecules that may be beneficial or harmful to humans[5, 6]. The general view is that natural plant based products are safe and without side effects and is proven false in some cases[7]. Investigations carried out on many plant

species revealed toxic substances. Despite the widespread crude usage of *Pogostemon benghalensis* and *P. cablin* in traditional medicine, there is a lack of experimental data on its possible toxicity. In addition, allelopathic enthusiasts criticize herbal specialties in terms of organ damage. To eliminate these issues and to ensure the safety of the herbals like *P. benghalensis* and *P. cablin*, the present study was carried in terms of acute toxicity analysis of essential oils extract *via* administered orally in male and female Wistar rats.

MATERIALS AND METHODS

Plant Material

Fresh leaves and twigs of *Pogostemon benghalensis* and *P. cablin* used in the present study were collected from the natural habitats of Munnar Hills of Idukki district, Kerala, India on July 2019. The collected species were identified using flora and were authenticated at the Herbarium of Jawaharlal Nehru Tropical Botanic Garden and Research Institute (JNTBGRI), Palode, Trivandrum, Kerala, India. The voucher specimens were numbered (MGH 1456 and 1457 for *Pogostemon benghalensis* and *P. cablin* respectively) and kept in the herbarium of the Department of Botany, Mahatma Gandhi College, Trivandrum, Kerala, India. The collected plants were washed thoroughly with running tap water and the hydro-distillation of fresh leaves were carried out using a Clevenger-type apparatus to extract the essential oil.

Animals

The healthy Wistar rats of 8 to 10 weeks aged with the body weight of 120–140 g were used for the present acute oral toxicity studies. The rats were caged in standard lab conditions for 5 days at the temperature of 23°C (± 1°C), with 12 h light/ dark cycle. They were given standard diet and water *ad libitum*[8, 9].

Rats were randomly categorized in to control and experimental groups. A total of 30 rats containing 6 rats per group (three male and three female, I to V group) were used for acute toxicity study a control (water alone), tween-20 (solvent) and six treatment classes (of Eos from *P. benghalensis* and *P. cablin* solubilized i.e., 500, 1500 and 3000 mg/kg b.w). All classes of the rats were fasted overnight prior to Eos administrations. After the fasting period, all rats were weighed, and the concentrations were carried based on their body weight. The extracts were prepared in tween-20. Eos of the two species were separately mixed in tween-20 at the ratio of 1: 1 v/v in glass tubes and labelled carefully. Rats in groups III to V were given single oral dose of the respective oil by using feeding needle and rats were kept without food for 3–4 h prior to administration. Initially, the rats were closely observed for first 60 min, intermittently for 4 h and thereafter once every 24 h for 14 days. Food was given after 1–2 h of dosing. The same procedure was carried for the vehicle group of 6 rats to whom tween-20 was administered in same volume as that of treated groups. Both the groups were observed closely for any toxic effect at regular intervals for a total period of 14 days. Surviving rats were observed to determine the toxic reactions onset. Weights of animals were monitored and documented as well. At the end of study, rats were weighed and blood samples were collected by cardiac puncture under anesthesia with isoflurane and serum was separated for biochemical and hematological evaluations. Vital organs were excised after killing mice by cervical dislocation and the weight of organs was noted.

Biochemical assay

The biochemical parameters include serum urea, serum creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphate (AP),total bilirubin, total protein, albumin, globulins, cholesterol, triglycerides (TGA), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were measured by using Randox kits.

Hematological evaluations

The blood samples from animals (both treated and vehicle control groups) were collected in EDTA containing tubes for hematological study. Hemoglobin (Hb), total red blood cells RBC, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelet count, white blood cells count (WBC), neutrophils, lymphocytes, monocytes and eosinophils were determined with automatic hematology analyzer.

Statistical analysis

Experimental results were presented as mean ± SD and the statistical significance between the groups was analyzed by means of one way ANOVA followed by Tukey's multiple comparison test. $P \leq 0.05$ was considered as statistically significant.

RESULTS AND DISCUSSION

The doses of Eos from *P. benghalensis* and *P. cablin* employed were 500, 1500 and 3000 mg/kg body weight of rats in the test groups. Control class (I and II) received water and tween-20 respectively. After feeding the Eos, the rats were kept under close vision continuously for 1 h and intermittently for 4 h and thereafter once every 24 h for 14 days.

During this course of experimental periods, medical observations were carried for mortality, behavioral changes, neurological features and other anomalies. The body weight was measured weekly. Finally, the gross physical examinations were attempted. The animals were then anesthetized under isoflurane. After sacrificing, gross pathological features of the rats were attempted on the vital organs.

The acute toxicity analyzes did not revealed any toxic symptoms or signs among the experimental groups of male and female rats from 500 to 3000 mg/kg b.w. No morbidity or mortality was noticed in the administrated rats at the above specified doses during the acute toxicity studies. As a result, the LD_{50} of the Eos from *P. benghalensis* and *P. cablin* should be higher than 3000 mg/kg body weight.

The gross pathological analyzes of the kidneys and liver of Eos from *P. benghalensis* and *P. cablin* administrated rats revealed no anomalous physical changes in terms of size, shape, color, and texture as compared with the control groups. The absolute mean liver weights were 5.52 g (at 1500 mg/kg *P. benghalensis*), 5.67g (at 3000 mg/kg *P. benghalensis*) compared with the control (5.43 g). Similarly, the absolute mean kidney weights were 1.18 g (at 1500 mg/kg *P. benghalensis*) and 1.17 g (at 3000 mg/kg *P. benghalensis*) as compared with the control group (1.17 g) (Fig.1). Similarly, the liver weights were 5.59 g (at 1500 mg/kg *P. cablin*) and 5.71g (at 3000 mg/kg *P. cablin*). Similarly, the absolute mean kidney weights were 1.21 g (at 1500 mg/kg *P. cablin*) and 1.22 g (at 3000 mg/kg *P. cablin*) (Fig.1).

There was a gradual increase in the body weight of both the treated and control rats (Table 1). The initial mean body of control rats was 123.67g; at the end of the experiment their final mean body weight was 136.21 g. The mean body weight gain for the control rats was 12.54 g. The initial mean body weights of rats treated with doses of 1500 mg/kg, and 3000 mg/kg of Eos from *P. benghalensis* were 126.2 g, and 127.8g respectively. At the end of the experiment (after 14 days) the final mean body weight of rats treated with 1500 mg/kg and 3000 mg/kg was 139.1 g and 140.4 g, respectively. The mean body weight gain for rats treated with 1500 mg/kg, and 3000 mg/kg of *P. cablin* was 140.2 g and 141.1 g respectively (after 14 days) (Table 1).

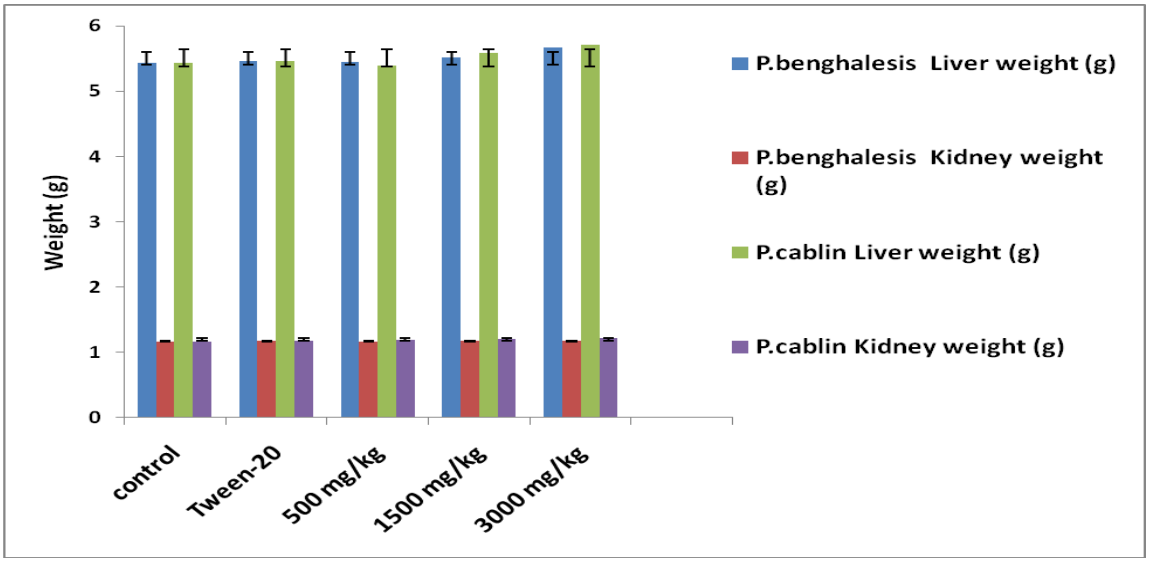

Figure 1: Liver and kidney weights of rats treated with Eos from *P. benghalensis* and *P. cablin*

Table 1: Body weights of rats treated with Eos from *P. benghalensis* and *P. cablin*

Treatment groups	*P. benghalensis*		*P. cablin*	
	1 weak	2 weak	1 weak	2 weak
Control	123.67 ±0.56	136.21 ±0.18	123.67 ±0.23	136.21±0.44
Tween-20	127.45 ±0.71	134.5 ±0.87	127.45±0.25	134.5±0.03
500 mg/kg	125.1 ±0.27	134.4 ±0.34	126.3±0.23	133.9±0.99
1500 mg/kg	126.2 ±0.67	139.1 ±0.24	127.0 ±0.18	140.2±0.21
3000 mg/kg	127.8 ±0.38	140.4 ±0.31	127.93±0.26	141.1±0.36

Acute toxicity analysis is a pre-requisite to evaluate the adverse impacts that happen within a short time of administration of a herbal drug. Principally, such tests are carried in rats/mice and are usually attempted in designing a new drug or crude product to gain amble knowledge on its toxicity potentials[4].

For acute toxicity study, 500, 1500 and 3000 mg/kg of Eos from *P. benghalensis* and *P. cablin* were given to rats. The rats did not show any signs/symptoms of toxicity at all the tested concentrations. No mortality was seen in the administrated rats, i.e., even at 3000 mg/kg during the course of the study period. Thus, the LD_{50} of the Eos could be higher than 3000 mg/kg. The Eos from *P. benghalensis* and *P. cablin* was considered safe on acute dose exposure.

Biochemical parameters

No sound serum creatinine level change was noticed; meanwhile serum urea level in acute toxicity group was slightly higher as compared to vehicle control group (Table 2). Biochemical markers of liver function test revealed significant ($p < 0.05$) changes (Table 2).There was no remarkable variation in the creatinine and urea level in the control and treated groups. Alanine aminotransferase (ALT), aspartate aminotransferase (AST) levels were increased. Meanwhile, alkaline phosphatase (AP) was reduced in acute toxicity group as compared to vehicle control group.

Table 2: Biochemical parameters of rats treated with Eos from *P. benghalensis* and *P. cablin*

Parameters	Vehicle control group	Acute toxicity group (Eos from *P. benghalensis* - 3000 mg/kg b.w)	Acute toxicity group (Eos from *P. cablin* - 3000 mg/kg b.w)
Creatinine serum (mg/dl)	0.396 ±0.065	0.392 ±0.01	0.402 ±0.05
serum urea (mg/dl)	17.86 ±0.14	18.05 ±0.28	18.84 ±0.43
ALT (U/L)	210.0 ±2.55	248.7 ±1.54	251.0 ±2.65
AST (U/L)	336.6 ±1.89	339.0 ±2.38	340.6 ±2.55
AP (U/L)	165.2 ±0.56	124.3 ±0.09	128.4 ±0.54

(ALT- Alanine aminotransferase, AST- Aspartate aminotransferase, AP- Alkaline phosphatase)

Total protein and globulin levels were increased. However, no remarkable changes were seen in total bilirubin and albumin among the tested groups. It was also noticed that when the Eos treated groups was compared with vehicle control group in terms of HDL and VLDL showed no sound variations, however there was significant ($p < 0.05$) increase in cholesterol, triglycerides (TGA) and LDL (Table 3).

Table 3: Biochemical parameters of rats treated with Eos from *P. benghalensis* and *P. cablin*

Parameters	Vehicle control group	Acute toxicity group (Eos from *P. benghalensis* - 3000 mg/kg b.w)	Acute toxicity group (Eos from *P. cablin* - 3000 mg/kg b.w)
Total bilirubin (mg/dl)	0.91 ±0.07	0.915±0.006	0.920±0.013
Total protein (U/dl)	6.7 ±0.21	7.59±0.09	7.96±0.25
Albumin (U/dl)	4.62±0.054	4.59 ±0.21	4.69±0.05
Globulin (U/dl)	2.4±0.38	2.64 ±0.12	2.72 ±0.021
Cholesterol (mg/dl)	164.36±0.89	173 ±0.02	174.89 ±0.12
TGA (mg/dl)	126.2 ±0.18	148 ±0.31	150 ±0.27
HDL (mg/dl)	31 ±0.21	35 ±0.29	36.2 ±0.33
LDL (mg/dl)	111 ±0.056	129.56 ±0.18	131±0.65
VLDL (mg/dl)	24.5±0.34	28.9 ±0.21	29.2 ±0.28

(TGA- Triglycerides, HDL- High density lipoprotein, LDL- Low density lipoprotein, VLDL-Very low density lipoprotein)

In acute toxicity evaluation of Eos from *P. benghalensis* and *P. cablin*, the health status of the animal was evaluated by biochemical parameters including serum biomarkers activity levels. Ramaiah[10] and Ozer[11] reported that liver damages due to hepatotoxic chemicals can elevate the ALT, AST and total proteins levels. Remarkable elevation in ALT, AST, total proteins and globulin levels were seen in the present study (Table. 2). Adedapo[12] reported the toxic effects of *Euphorbia* species on hematological and biochemical parameters of rats. Similarly, Adeoye[13] correlated the genotoxic and systemic toxicity of pharmaceutical effluent in Wistar rats with oxidative stress. Ogunlana[14] evaluated the toxicological profile of the *Caesalpinia bonduc* extract. Ali[15] documented cytogenetic damage among the female agricultural workers of Pakistan exposed to pesticides. Hepato cellular damage may leads to increased cell membrane permeability and release of amino transferases into the blood stream. AP is treated as the indicator of biliary tract obstruction[16]. In the present study, there was remarkable reduction in AP levels was noticed (Table. 2) which is a sign of herbal hepato protective effect. Multiple hyperlipidemias are often secondary to many factors like over diet, alcohol intake, therapies or to diseases like nephrosis, diabetes, hypothyroidism or tumors[17]. Enhanced cholesterol, triglycerides and LDL levels noticed in the experimental group suggests the multiple hyperlipidemic impacts of Eos of *Pogostemon* species. Goldstein[18] reported the hyperlipidemia in coronary heart disease in 176 families and delineation of a new inherited disorder. Travlos[19] reported that renal function impairment is reflected by increased levels of serum creatinine and urea. In the present data, serum urea levels were found elevated (Table 2) revealing that there is a marginal renal injury.

Hematological data

Blood parameters are sensitive indicators of the physiological changes in response to any pollutant or stress in animals[20]. Blood platelets have key role in blood clotting processes. This study revealed significant increase in platelet count levels as compared to control vehicle group (Table 4) reflecting hemostatic potential of tested Eos. Li[21] reported similar stand on the hemostatic activity of the Tibetan herb *Lamiophlomis rotate*. In the present study increased level of mean corpuscular volume (MCV) and reduced mean corpuscular hemoglobin concentration (MCHC) was noticed (Table 4). Elevated WBC count and lymphocytes refers the potentiality of Eos defending nature against the pathogens and also its contribution to enhance cellular inflammatory events. The hematological data of vehicle group and Eos treated group are illustrated in Table 4.

Table 4: Hematological parameters of rats treated with Eos from *P. benghalensis* and *P. cablin*

Parameters	Vehicle control group	Acute toxicity group (Eos from *P. benghalensis* - 3000 mg/kg b.w)	Acute toxicity group (Eos from *P. cablin* - 3000 mg/kg b.w)
Hb (g/dl)	12.1±0.21	12.78 ±0.23	12.9 ±0.31
Total RBC (10^{12}/l)	7.42 ± 0.04	7.89 ±0.08	7.92 ±0.06
HCT (%)	33.5 ±0.11	41.69 ±1.32	42.3 ±1.28
MCV (fl)	45.4 ±0.25	54.3 ±0.78	55 ±0.99
MCH (pg)	16.02 ±0.45	16.09 ±0.21	16.08 ±0.33
MCHC (g/dl)	35.3 ± 0.254	27.8 ±0.28	29 ±0.93
Platelet count (x10^9/l)	246 ±1.43	490 ±2.34	493 ±3.01
WBC Count (10^9/l)	3.3 ±0.09	5.23 ±0.76	5.4 ±0.54
Neutrophil (%)	9.9 ±0.03	10.3 ±0.73	10.5 ±0.27
Lymphocytes %	85 ±0.76	87 ±0.58	89 ±0.87
Monocytes %	2.9 ±0.1	3.9 ±0.03	4.09 ±0.04
Eosinophils %	1.02 ±0.04	2.03 ±0.21	2.06 ±0.06

(Hb-Hemoglobin, RBC-Red blood cells, HCT-Hematocrit, MCV-Mean corpuscular volume, MCH-Mean corpuscular hemoglobin, MCHC-Mean corpuscular hemoglobin concentration, WBC-White blood cells)

The present results from the acute toxicity study of Eos from *P. benghalensis* and *P. cablin* supports the nontoxic nature of herbal extracts and was comparable with the reports by other researchers[10, 11, 12]. Further, the results of Eos extracts showed no symptom of toxicity or mortality even at 3000 mg/kg concentration of the species. Ior[22] showed that the LD_{50} of ethanolic extract from the leaves of *Syzygium guineense* was 4000 mg/kg.

Variations in body weight are a marker character for evaluating the toxicity of plant drugs. In the present work, there was no remarkable increase in the absolute mean body weight of the treated rats as compared to control groups. The absolute mean body weight for the Eos treated rats was 140.4 and 141.1 g as compared to control 136.2 g (after 14 days). The mean body weight gain for rats treated with 1500 mg/kg and 3000 mg/kg were statistically insignificant.

Liver and kidneys of animals are employed by many researchers to analyze the safety or toxicity of drugs or phytochemicals. In the present acute toxicity study, gross pathological results of the kidneys and liver of treated rats did not showed much visual variation in terms of size, shape, color, and texture when compared with control groups. Further, there were no remarkable variations in the absolute weight of liver and kidneys of Eos administrated animals as compared to control groups. Vahalia[23] analyzed the chronic toxicity versus generic ayurvedic mineral formulation tamra bhasma in laboratory animals and substantiated its safety as drugs. Abba[24] analyzed the hemorrhagic centrolobar necrosis and cytoplasmic vacuolation of the hepatocytes in *Syzygium guineense* treated mice. Loha[25] authenticated methanolic leaf extract of *Syzygium guineense* in terms of histology of the liver, kidney and biochemical compositions of blood in rats used in acute and sub acute toxicity studies. *Chlorophytum alismifolium* tuber extract's non toxic nature was established by acute and sub-acute toxicity studies in Wistar rats by Abubakar[26]. Chebaibi[27]reviewed the acute toxicity of plant mixture employed in traditional treatment of kidney diseases in Morocco. Jatsa[28] evaluated the acute and sub-chronic oral toxicity studies of the aqueous leaf extract of *Clerodendrum umbellatum* on mice. Hemalatha[29] confirmed the safety of *Trema orientalis* methanol extract in rats by acute and sub-acute toxicity studies. Gebrehiwot[30] proved the non-toxic potential of root extract of *Carissa spinarum* by *in vivo* acute and sub-acute toxicity studies in Swiss albino mice. Abotsi[31] compared the acute and sub-acute toxicity of the ethanolic extract of the aerial parts of *Hilleria latifolia* in rodents. Jothy[32] documented the acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Kpemissi[33] proved the acute and sub chronic oral toxicity assessments of *Combretum micranthum* in Wistar rats. Pal and Mishra[34] evaluated acute toxicity of the methanolic extract of *Dhatryadi ghrita* in Wistar rats. Acute and sub-acute toxicity of *Echinops kebericho* decoction in rats was validated by Deyno[35]. Gaelle[36] documented acute and sub acute toxicity profiles of the methanol extract of *Lycopersicon esculentum*. All the above plant based works justify the present data on rats treated with Eos from *P. benghalensis* and *P. cablin.*

CONCLUSION

In the background of findings of acute toxicity studies it was possible to suggest that Eos from *P. benghalensis* and *P. cablin* is safe and non toxic based on the mortality, behavioural evaluation, mass of organs, biochemical and hematological parameters. However, the results should be further evaluated for long term usage and repeated dose effects to ensure safety of these aromatic species.

Conflict of interest

The authors declare that they have no conflict of interest.

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