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Evaluation of Acute Toxicity of the Ethanolic Root Extract of *Coleus vettiveroides* in Wistar Rats

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Abstract: In this study, we evaluated the oral acute toxicity study of ethanolic root extract of *Coleus vettiveroides* in Wistar rats. The acute oral toxicity study was carried out per OECD 423 guidelines. The study was approved by the Institutional Animal Ethics Committee (IAEC). In an acute toxicity study, a single oral dose (800, 1600 and 3200 mg/kg) of *Coleus vettiveroides* root extract was administered and observed for 14 days. On the 15th day, body weight, histological, haematological, and serum hepatic biochemical parameters were evaluated and compared to the standard group by sacrificing all group animals. *Coleus vettiveroides* treated groups revealed neither mortality nor any significant changes. The result indicates that the oral administration of ethanolic root extract of *Coleus vettiveroides* plant did not produce any significant toxic effect in Wistar rats. The extract can be utilized safely for therapeutic use in pharmaceutical formulations.

Keywords: Acute toxicity, *Coleus vettiveroides*, Hematology, Liver, Kidney, Lung, Spleen

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Introduction

During the past few decades, traditional system of medicine has received marvellous attention for *in vivo* studies (Harborne, 1984). Toxicology is the essential part of pharmacology which deals with the undesirable effect of phytochemicals on

living organisms before their use as a drug or chemical in clinical use (Ohkawa *et al.*, 1979). Several studies are concentrated on toxicity analysis to determine the safety of medicinal plants and their products. Toxicity analysis is

essential, as some herbs consumed might have toxic effects, and many reports have been published for toxicity caused due to prolonged-term consumption of herbs. The toxicity mechanism could differ depending on the cell membrane and chemical properties of the toxicants in human beings. It might happen within the cell membrane, on the cell surface or tissue underneath, and at the extracellular matrix. According to OECD guidelines, toxicological studies are highly significant in animals like mice, rats, guinea pigs, dogs, rabbits, and monkeys to ascertain the protection and effectiveness of a new drug. Toxicological studies aid in extending the decision of whether a new drug could be adopted for clinical use. OECD guidelines such as 401, 423 and 425 do not permit the use of drugs clinically without clinical trials or toxicity studies (Sedlak and Lindsay, 1968). Depending on the period of drug exposure to animals, toxicological determination could be acute, sub-acute and chronic toxicological studies. The acute toxicity test uses a single dose in each animal on one occasion only to determine gross behaviour and also LD₅₀ or median lethal dose.

The chronic tests in which two species, one rodent and one non-rodent, are dosed daily for six months. The sub-acute trials wherein animals (typically rats and dogs) are dosed daily, beginning around the expected therapeutic level and increasing stepwise every two to three days until toxic symptoms are observed (Misra and Fridovich, 1976; Takahara and Ogata, 1978; Blais *et al.*, 1993). *Coleus* is a member of the Lamiaceae family, a perennial, branched, aromatic herb. The *Coleus* plant grows in tropical to subtropical situations and in a warm temperate climatic zone in the mountains of India, Nepal, Burma, Sri Lanka, Thailand and Africa. The essential oil in this plant's roots is used to prepare Ayurvedic formulations. Its antioxidant, anticancer, antidiabetic and hepatoprotective activities were demonstrated. It is used as a single medicine or as one of the ingredients in ayurvedic/herbal preparations.

To assess the toxic nature of bioactive compounds present in the plant root extract, acute oral toxicity is the first step to be carried out. Acute toxicity testing involves estimating the lethal dose that kills 50% of the tested group of animals. In the present investigation, as a part of safety evaluation, acute toxic effects of ethanolic root extracts prepared from *Coleus vettiveroides* have been studied in Wistar rats (Broadhead and Combes, 2001; Botham, 2004). After the treatment, it was also investigated for its haematological, biochemical, and histopathological changes in liver, kidney, lung and spleen tissues.

Materials and Methods

Chemicals and reagents:

GSH, MDA, NBT and SDS were obtained from Sigma Chemical Company, St. Louis, MO, USA. Folin's - Ciocalteau reagent, H and E stain and TBA were obtained from Sisco Research Laboratories, Mumbai - India. Acids, bases, solvents and salts used for the investigation were of analytical grade (AR) obtained from SRL, Mumbai, India and the diagnostic kits were purchased from Enzo Life Sciences.

Animals:

Wistar albino rats of either sex (150 - 200 g) were used for the present studies. They were housed in clean polypropylene cages and maintained under standard laboratory conditions at 22 ± 2°C and 12 h alternate light-dark cycle. They were allowed free access to a normal pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum* (Cronin, 2002; Aneela *et al.*, 2011). The Institutional Animal Ethics Committee (IAEC), Saveetha Institute of Medical and Technical Sciences, Chennai, Tamilnadu, India approved the experimental protocol for the animal studies (BRULAC/SDCH/SIMATS/IAEC/12-2019/036).

Plant materials:

The roots of *Coleus vettiveroides* were collected from a forest area in Coimbatore, India, and the sample was identified and authenticated by botanist Dr. Rama Bhat. The material was kept

under shade drying, pulverized by a mechanical grinder and passed through a 30-mesh sieve.

Extract preparation:

500 g powder of *Coleus vettiveroides* (CV) root was taken and soaked in 2 L of absolute alcohol in a 5 L round flask for about 24 h, and reflux condensation was done at 60-800 °C for 3 h. It was cooled, and the alcohol was drained. Reflux condensation was repeated twice with 2 L of alcohol each time. The filtrate was concentrated by distillation till a syrupy consistency of extract was obtained. The extract was evaporated to dryness in a China dish in a water bath. The extract was filtered and concentrated to dryness in a vacuum and stored in an airtight container for further use (Lorke, 1983; Loomis and Hayes, 1996; Ansari, 2007). CV ethanolic root extract was suspended in distilled water and administered.

Experimental design:

The acute oral toxicity study followed OECD 423 guidelines (OECD, 2001). Animals were given a normal diet and had regular access to water. The animals were divided into four groups, each group comprising six animals. Group I rats served as control, and Group II, III and IV rats were orally administered with a single dose of 800, 1600 and 3200 mg/kg b.w. of root extract of *Coleus vettiveroides*, respectively. The rats were monitored for behavioural and morphological changes daily for 14 days and sacrificed on the 15th day, and various biochemical and histopathological changes were observed.

Qualitative estimation of Phytoconstituents:

The collected root extracts were analyzed to explore the chemical profile using standard phytochemical tests qualitatively.

Mortality and Toxic Signs:

The visual observations of mortality, changes in physical appearance, behaviour (sleepy, salivation, lethargy), and any injury or illness were conducted once daily for 14 days.

Relative Organ Weight:

On the 15th day, all the animals were anaesthetized by an intraperitoneal injection of ketamine. Blood samples were collected by cardiac puncture into EDTA-containing and non-heparinized tubes for haematological and biochemical analysis. Rats were then euthanized after blood collection, and the internal organs (liver, spleen, kidney and lungs) were removed, weighed, and observed for gross lesions.

Blood analysis:

The biochemical analysis included liver function markers (AST, ALT), bilirubin (total), and nephrotic markers (urea, creatinine and uric acid) were analyzed using Enzo Life Sciences diagnostic kits and measured using Robonik semi-auto analyzer. Hematologic parameters included red and white blood cells, haemoglobin, hematocrit, and platelets using the unit XN1000 (Sysmex).

Antioxidant parameters:

Estimation lipid peroxidation:

This method depends on the formation of MDA as an end product of lipid peroxidation which reacts with thiobarbituric acid producing a thiobarbituric acid reactive substance (TBARS), a pink chromogen which can be measured spectrophotometrically at 532 nm, MDA standard was used to construct a standard curve against which readings of the samples were plotted (Ohkawa *et al.*, 1979). Briefly, 0.2 ml of tissue homogenate, 0.2 ml of SDS, 1.5 ml of acetic acid, and 1.5 ml of TBA were added. The mixture was made up to 4 ml with water and then heated in a water bath at 95°C for 60 min. After cooling, 1 ml of water and 5 ml of n-butanol/pyridine mixture were added and shaken vigorously. After that, it was centrifuged at 4000 rpm for 10 min; then, the organic layer was taken and measured at 532 nm absorbance. The level of MDA is expressed as nmoles/mg protein.

Estimation of reduced glutathione:

Reduced glutathione was determined by the method of Sedlak and Lindsay (1968). 0.5 ml tissue homogenate was mixed with 0.2 M Tris

buffer at pH of 8.2, and then contents were mixed with 0.1 ml of 0.01 M Ellman's reagent (5,5'-dithiobis-(2-nitro-benzoic acid)) (DTNB), then centrifuged at 3000 g for 15 min. The absorbance was read at 412 nm. A series of standards treated similarly also run to determine the glutathione content. The amount of glutathione is expressed as μM of GSH/mg protein.

Assay of superoxide dismutase:

Superoxide dismutase was assayed following the method of Misra and Fridovich (1972). The tissue homogenate (0.1 ml) was mixed with reaction mixtures that contained sodium carbonate (1 ml, 50 mM), nitroblue tetrazolium (0.4 ml, 25 μM), and hydroxylamine hydrochloride (0.2 mL, 0.1 mM). The mixture was observed at 560 nm using UV-spectrophotometer (MINDRAY 91).

Assay of catalase:

The catalase activity was assayed according to the method of Takahara and Ogata (1978). Phosphate buffer (1.2 ml) and 0.2 ml of tissue homogenate were mixed, and the reaction was started by adding 1.0 ml of H_2O_2 solution. A decrease in the absorbance was measured at 240 nm at 30-sec intervals for 3 min. For the enzyme blank, distilled water was used instead of hydrogen peroxide. The activity of the enzyme was expressed as μM of H_2O_2 decomposed/min/mg of protein.

Histopathological examinations:

After processing, the vital organs isolated from sacrificed rats were fixed in 10% formalin and embedded in paraffin wax. Paraffin sections were made at 5 μm and stained with haematoxylin and eosin. The slides were studied under a light microscope (Olympus model) and photomicrographs were taken.

Statistical analysis:

Values were expressed as Mean $\pm$ SD. One-way ANOVA followed by Post Hoc t-tests were used to determine the significance of inter-group differences. $P < 0.05$ was considered statistically significant.

Results

Preliminary and qualitative analysis of Phytoconstituents:

The qualitative phytochemical analysis of Ethanolic Root Extract of *Coleus vettiveroides* (ERECV) has been shown in Table1.

Acute toxicity:

The ethanolic root extract was determined per the OECD guideline 423, where the limit test dose of 3200 mg/kg was used. Treatment-related toxic symptoms or mortality were not observed after a single dose of oral administration of ethanolic root extract at 800, 1600 and 3200 mg/kg b.w. The general behaviour of animals treated with extract and control group was observed first for a short period of 4 h followed by a long period of 72 h. The animals treated with extract showed no changes in behaviour, breathing, skin effects, water consumption, food intake, impairment and temperature compared with the control group. Therefore, the extract seems safe at a dose level of 3200 mg/kg, and the LD_{50} was considered to be >3200 mg/kg.

Effect of ERECV on Relative Organ and Body Weight:

The experimental rats did not die, and no evidence of poisoning could be seen in the experimental animals' skin, fur, eyes, sleep, salivation, diarrhoea, or behaviour. Rats that received ERECV did not show any significant differences in their food or water intake compared to control rats during the experiment. The effects of ERECV on body weight were evaluated, and there was a significant increase in body weight which showed a significant difference when compared with control and after 14 days ($p < 0.001$). The liver's relative organ weights were increased in animals treated with an ERECV dose of 800 mg/kg b.w. ($p < 0.001$) compared with the control. There was no significant difference in liver weights in animals treated with ERECV doses of 1600 mg/kg and 3200 mg/kg b.w compared with the control. The kidney's weight has increased in animals treated with an ERECV dose of 800 mg/kg b.w.

Table 1: Qualitative phytochemical analysis of Ethanolic Root Extract of *Coleus vettiveroides* (ERECV)

S.No.	Phytochemicals	ERECV
1	Alkaloids	+
2	Tannins	+
3	Proteins	+
4	Saponins	-
5	Aminoacids	+
6	Flavanoids	+
7	Phenols	+
8	Carbohydrates	+
9	Glycosides	-
10	Sterols	+
11	Terpenoids	+

Table 2: Effect of oral administration of ethanolic root extract of *Coleus vettiveroides* (ERECV) on the rat's average body weight (g)

Body weight (g)	Control	ERECV (800 mg)	ERECV (1600 mg)	ERECV (3200 mg)
Day 1	144.12 ± 2.63	146.61 ± 2.43	144.00 ± 2.62	141.30 ± 0.85
Day 14	172.6 ± 2.51***	181.0 ± 1.0***	179.3 ± 1.15***	178 ± 2.64***

Values are expressed as mean ± STD. ***P<0.001 when compared to control group

Table 3: Effect of oral administration of ethanolic root extract of *Coleus vettiveroides* (ERECV) on rat's average organ weight (g)

Organ	Control	ERECV (800 mg)	ERECV (1600 mg)	ERECV (3200 mg)
LIVER	9.28 ± 0.08	9.52 ± 0.03***	9.45 ± 0.23 ^{NS}	9.14 ± 0.16 ^{NS}
KIDNEY	2.10 ± 0.06	2.21 ± 0.02***	2.10 ± 0.03 ^{NS}	2.13 ± 0.03 ^{NS}
LUNG	1.45 ± 0.01	1.47 ± 0.02 ^{NS}	1.46 ± 0.02 ^{NS}	1.42 ± 0.01 ^{NS}
SPLEEN	0.76 ± 0.03	0.82 ± 0.07 ^{NS}	0.72 ± 0.02 ^{NS}	0.72 ± 0.02 ^{NS}

Values are expressed as mean ± STD. ***P<0.001, NS: Not Significant when compared to control group

compared with the control group. There was no significant difference in kidney weight in animals treated with an ERECV dose of 1600 mg/kg and 3200 mg/kg b.w. compared with the control group. There was no significant difference in the weight of the lung and spleen in ERECV-treated groups compared with the control. The effect of the plant extract on body weight and organ weight is shown in Tables 2 and 3.

Effect of ERECV-induced Changes on haematological parameters:

Compared to control rats, the total RBCs, WBCs, and PCV levels in ERECV-treated rats did not differ significantly from those of control rats after 14-days. There was an increase in platelet counts in animals that received the ERECV dose of 3200 mg/kg b.w. (p<0.001). Also, compared with the control group, there was a slight increase in haemoglobin levels in animals receiving the dose of ERECV 800mg/kg and 1600 mg/kg (p<0.01). The effect of the plant extract on haematological parameters is shown in Table 4.

Table 4: Effect of oral administration of ethanolic root extract of *Coleus vettiveroides* (ERECV) on haematological parameters of the rat

BLOOD PARAMETERS	Control	ERECV (800 mg)	ERECV (1600 mg)	ERECV (3200 mg)
RBC (10 ⁶ / L)	7.54 ± 0.03	7.59 ± 0.04 ^{NS}	7.51 ± 0.07 ^{NS}	7.39 ± 0.11 ^{NS}
WBC (10 ⁹ / L)	7.40 ± 0.14	7.43 ± 0.03 ^{NS}	7.48 ± 0.05 ^{NS}	7.49 ± 0.07 ^{NS}
PLATELETS (10 ³ /μl)	229.00 ± 2.94	236.00 ± 3.92 ^{NS}	233.00 ± 2.83 ^{NS}	239.75 ± 1.71 ^{***}
Hb (g/dl)	14.38 ± 0.19	15.62 ± 0.70 ^{**}	15.21 ± 0.45 ^{**}	14.26 ± 0.12 ^{NS}
PCV (%)	35.75 ± 1.50	36.50 ± 1.91 ^{NS}	34.50 ± 1.29 ^{NS}	33.50 ± 1.29 ^{NS}

Values are expressed as mean ± STD. **P<0.01, NS: Not Significant when compared to control group.

Effect of ERECV induced Changes on Kidney and liver markers:

There was a decrease in aspartate transaminase (AST) in the animal group treated with an ERECV dose of 800 mg/kg b.w. (p<0.001) compared with the control, There was no significant difference in groups treated with 1600 and 3200 mg/kg b.w. compared to the control group. There was an increase in alanine transaminase (ALT) in the animal group treated with an ERECV dose of 1600 mg/kg b.w. (p<0.01) compared with the control. There was no significant difference in groups treated with 800 and 3200 mg/kg b.w. compared to the control group. Compared to control rats, oral administration of ERECV did not significantly alter alkaline phosphatase (ALP) in the serum. No significant changes were observed in the activity of lactate dehydrogenase (LDH), GGT, bilirubin and protein level in rats treated with ERECV compared to control rats. Kidney function measures like glucose, creatinine, and uric acid exhibit no significant alterations between the control and ERECV-treated groups. Compared with control, urea level was increased in ERECV-treated animal groups like 1600 mg/kg (p<0.01) and 3600 mg /kg (p<0.001) b.w. The effect of the plant extract on kidney and liver markers is shown in Table 5.

Effect of ERECV-induced changes on Oxidative stress:

ERECV treated group has no significant changes in CAT compared to the control group. There was a significant increase in MDA level treated with 3200 mg ERECV compared to the control group (p<0.001). There was a significant increase in SOD of rats treated with 800 mg ERECV (p<0.05) and 1600 mg ERECV (p<0.01) compared with the control group. GSH level has increased significantly in animals treated with ERECV compared to the control group (p<0.001). The effect of the plant extract on Oxidative stress is shown in Table 6.

Histopathology:

Liver:

Control (Group I): No pathological damage was observed in normal liver architecture. There was no degeneration and inflammation in hepatocytes.

ERECV 800 mg (Group II): Normal peripapillary lining and hepatocytes were observed. No fatty degeneration of hepatocytes was observed in the lobular and radiating pattern.

ERECV 1600 mg (Group III): Normal portal triad with the bile duct, hepatic artery, and portal vein in the centrilobular region was observed.

Table 5: Effect of oral administration of ethanolic root extract of *Coleus vettiveroides* (ERECV) on biochemical parameters of the rat

PARAMETERS	Control	ERECV (800 mg)	ERECV (1600 mg)	ERECV (3200 mg)
A.S.T. (IU/L)	70.54 ± 0.78	60.83 ± 2.75***	62.79 ± 6.49 ^{NS}	69.41 ± 4.07 ^{NS}
ALP(IU/L)	134.97 ± 1.91	133.09 ± 3.31 ^{NS}	137.48 ± 2.86 ^{NS}	129.60 ± 3.01 ^{NS}
ALT(IU/L)	74.09 ± 2.14	74.67 ± 2.07 ^{NS}	79.15 ± 1.15**	78.42 ± 2.60 ^{NS}
GGT(IU/L)	30.08 ± 1.15	31.03 ± 1.85 ^{NS}	31.05 ± 1.35 ^{NS}	30.23 ± 1.11 ^{NS}
LDH(IU/L)	222.03±11.98	238.08 ± 4.87 ^{NS}	212.65 ± 6.52 ^{NS}	201.95 ± 22.41 ^{NS}
BILIRUBIN (mg/dl)	0.75 ± 0.04	0.76 ± 0.14 ^{NS}	0.82 ± 0.07 ^{NS}	0.87 ± 0.04 ^{NS}
PROTEIN (g/dl)	7.92 ± 0.19	7.97 ± 0.09 ^{NS}	8.46 ± 0.62 ^{NS}	7.51 ± 0.46 ^{NS}
GLUCOSE (mg/dl)	116.88 ± 6.15 ^{NS}	118.48 ± 6.51 ^{NS}	107.93 ± 13.73 ^{NS}	111.18 ± 11.32 ^{NS}
UREA(mg/dl)	19.04 ± 0.28	20.28 ± 1.11 ^{NS}	20.19 ± 0.38**	23.58 ± 1.33***
URIC ACID (mg/dl)	2.20 ± 0.26	2.20 ± 0.22 ^{NS}	2.00 ± 0.43 ^{NS}	2.10 ± 0.26 ^{NS}
CREATININE(mg/dl)	0.50 ± 0.04	0.50 ± 0.07 ^{NS}	0.54 ± 0.05 ^{NS}	0.55 ± 0.05 ^{NS}

Values are expressed as mean ± STD. **P<0.01, ***P<0.001, NS: Not Significant when compared to the control group.

Table 6: Effect of oral administration of ethanolic root extract of *Coleus vettiveroides* (ERECV) on Oxidative stress in rat liver tissue

OXIDATIVE STRESS	Control	ERECV(800mg)	ERECV(1600mg)	ERECV(3200mg)
MDA (nmol/mg protein)	1.20 ± 0.19	1.24 ± 0.36 ^{NS}	1.66 ± 0.31 ^{NS}	2.24 ± 0.29***
SOD (Units/mg protein)	9.58 ± 0.56	11.53 ± 1.13*	11.25 ± 0.70**	9.78 ± 0.26 ^{NS}
CAT (Units/mg protein)	82.45± 0.87	83.93 ± 1.22 ^{NS}	83.03 ± 1.29 ^{NS}	81.85 ± 0.39 ^{NS}
GSH (Units/mg protein)	43.15± 0.61	44.88 ± 0.17***	47.23 ± 1.38***	46.98± 1.20***

Values are expressed as mean ± STD. *P < 0.05, ***P<0.001, **P<0.01, NS: Not Significant when compared to the control group.

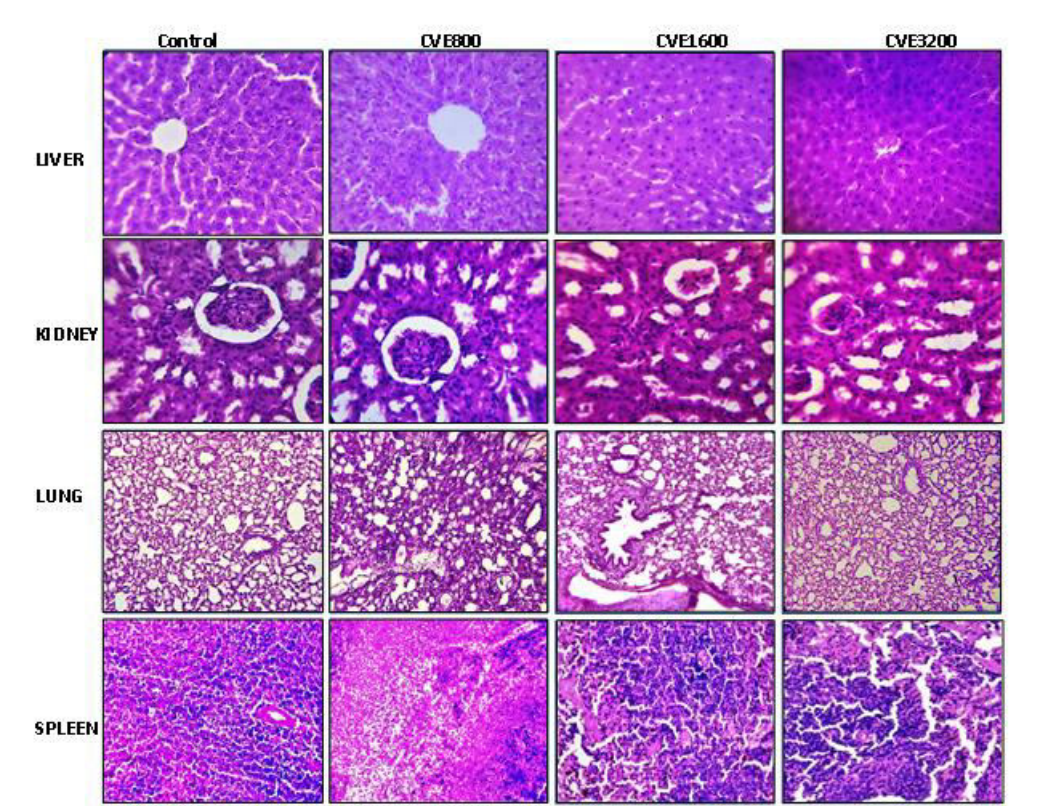


Fig. 1: Histopathological changes in various tissues of rats after ERECV treatment.

ERECV 3200 mg (Group IV): No histopathological changes were observed in liver architecture. Hepatocytes are normal, with no infiltration of inflammatory cells in the perivascular region (Fig. 1).

Kidney:

Control (Group I): Cytoplasmic vacuolation in renal tubules with no inflammatory signs was observed. Normal kidney structure with Bowman's capsule, distal and proximal tubules was observed in the control group.

ERECV 800 mg (Group II): No decrease in the thickening of the cellular structure of the Bowman's capsule. No abnormalities were noted in the histo architecture of the kidneys.

ERECV 1600 mg (Group III): The glomerulus showed squamous epithelium, a glomerular tuft of capillaries with normal thickness of endothelium in the Bowman's capsule was observed.

ERECV 3200 mg (Group IV): There is no degeneration of glomerular capillaries, no

thickening in renal tubules, and no change in the elasticity of afferent and efferent arterioles of the kidney was observed (Fig. 1).

Lung:

Control (Group I): Normal lung architecture within septa with normal alveolar walls and the vascular bed was observed.

ERECV 800 mg (Group II): Alveolar wall is lined by pseudostratified ciliated columnar epithelium with goblet cells.

ERECV 1600 mg (Group III): No changes in the pulmonary morphological characteristics and infiltrates inside the alveoli were observed.

ERECV 3200 mg (Group IV): Alveoli are thin-walled spaces lined by simple squamous epithelium. Smaller bronchioles are lined by simple columnar epithelium, and no pathological changes in alveoli were observed (Fig. 1).

Spleen:

Control (Group I): Normal spleen architecture with

red and white pulp in parenchyma was observed.

ERECV 800 mg (Group II): No abnormalities were observed in the spleen parenchyma's architecture in the extract-treated group.

ERECV 1600 mg (Group III): Normal thickness of spleen capsule and no change in the architecture of spleen was observed.

ERECV 3200 mg (Group IV): Normal spleen architecture and normal thickness of spleen capsule with red and white pulp were observed similar to the control group (Fig. 1).

Discussion

Toxicology tests are used to observe products such as individual compounds, mixtures of compounds, crude extract, pesticides, medications, food additives, packing materials or their chemical ingredients. World Health Organization (WHO) recommends that medicinal herbs be the dominant source to obtain various drugs. Therefore, such medicinal plants must be investigated to understand better their medicinal properties, safety and effectiveness (Ansari, 2007). The safety of plant extract is evaluated mainly by acute oral toxicity analysis. In the present study, even a higher dose of plant extract, i.e. 3200 mg/kg, did not show any signs of toxicity or mortality to animals. Thus, even at 3200 mg/kg, plant extract may be considered safe. This observation is in agreement with Pooja *et al.* (2016). who assessed the acute toxicity of ethanolic extracts of this plant using three concentrations, i.e. 800 mg/kg, 1600 mg/kg and 3200 mg/kg. They reported no behavioural changes, and no mortality was observed in animals when these concentrations were used.

The ethanolic extract of the plant contains alkaloids, tannins, proteins, amino acids, flavonoids, phenols, carbohydrates, sterols, and terpenoids as phytochemical constituents.

A significant increase ($p < 0.001$) in the average body weight in rats treated with ERECV and control on the 14th day compared to the 1st day. It was also observed that there was an increase in

liver and kidney weight in animals treated with ERECV 800 mg/kg b.w. No significant differences were found in the spleen and lung weights of the treated rats compared to the control groups. Animals' haematological parameters are delicate indicators of the physiological changes brought on by any environmental contaminant or toxic stress (Jain *et al.*, 2009). it is also observed that haematological values shown in the treated groups were normal compared to the control group except for Hb and platelets. Platelet counts were elevated, suggesting that the tested rat had hemostatic activity, and rats given 3200 mg/kg of ERECV had significantly higher platelet counts than the healthy control group. These findings imply that ERECV can cause thrombocytopenia in rats by raising platelet production when used at the highest dose (Tchoumtchoua *et al.*, 2014; Kale *et al.*, 2019). Apart from thrombocytosis, increased levels of Hb may also be associated with inflammation arising from the assault on vital organs.

The liver and kidneys play essential roles in metabolic processes, and multiple blood biomarkers are needed to evaluate the health state of various organs, including the liver and kidneys (Yang *et al.*, 2014). The liver and kidneys frequently come under assault by toxic substances because of their essential roles. Although AST, ALT, urea, and creatinine evaluation are reliable indicators of liver and kidney health, their sensitivity and specificity are restricted (Fassett *et al.*, 2011; Campion *et al.*, 2013; Yang *et al.*, 2014). The level of AST has declined in rats given 800 mg/kg of ERECV, and the level of ALT has increased in rats treated with 1600 mg/kg b.w. The level of ALP did not show any significant difference compared with the control group. No significant changes were observed in LDH, GGT, bilirubin and protein levels in treated with ERECV compared to control rats. The kidney parameters of creatinine, uric acid, and glucose in control and ERECV-administered rats did not alter significantly. There was an increase in urea levels in animals treated with ERECV 1600 mg/kg b.w.

The estimated significant increases in the levels of MDA in rats administered with ERECV 3200 mg and increased levels may be due to oxidative damage (El-Demerdash, 2004; Abdel Wahab, 2012). There was no significant difference in SOD level in the rats treated with ERECV 3200 mg and the control group. SOD level in rats receiving ERECV 800 mg and 1600 mg has declined compared to the control group, and a decrease in SOD activity is a sensitive indicator of oxidative stress-induced hepatic injury. Hepatic catalase activity was not altered with different doses (8000, 1600 and 3200 mg/kg) of ERECV compared to the control group. There is an increase in GSH levels in rats treated with ERECV compared to the control group. The main metabolic target of any toxic substance is the liver, kidney, lungs, and spleen, which are the vital organs (Gregus, 2001). In contrast to the control group, no lesions/least effects were noticed on macroscopic inspection of the kidney, lung, spleen, and liver when animals were treated with ERECV.

Conclusion

The non-toxic nature of ethanolic root extract prepared from *Coleus vettiveroides* was confirmed by an acute oral toxicity test conducted as per the OECD guidelines. The normal behaviour of animals during the observation of fourteen days suggests the safety and harmless nature of ethanolic root extract even up to 3200 mg/kg body weight of animals. Further studies are warranted, including sub-acute and chronic toxicological evaluations, to confirm the safe use of this extract.

The oral doses of *Coleus vettiveroides* root ethanolic extract can be considered safe as they did not exhibit any lethality or adverse effects in the acute toxicity studies in rats. The present study provides supportive data on using *Coleus vettiveroides* in traditional medicine and could lend credence to its medicinal use. Chronic toxicity, mutagenicity and carcinogenicity evaluations should be performed to understand the plant's safety profile better.

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