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Evaluation of acute and subacute toxicity of *Vernonia cinerea* (L.) Less using mice model

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Vernonia cinerea (L.) Less is a medicinal plant distributed throughout India and is used traditionally for treating several diseases. This study aimed to analyze the toxicological effects of ethyl acetate extract (VCEA) of this plant. Preliminary phytochemical screening of VCEA was conducted by conventional methods. For acute toxicity studies, female Swiss albino mice were treated with a single oral dose of 2000 mg/kg body weight of VCEA and observed for any changes in general characters. For the subacute toxicity study, mice were treated (50 or 100 mg/kg body weight) consecutively for 28 days and the haematological, biochemical, and histopathological changes were analysed. From the phytochemical analysis, it was inferred that terpenoid content was more in the VCEA extract. No mortality or toxic effects were observed in the acute study. Repeated dosage of VCEA at 50 mg/kg body weight did not impart any adverse effects in any of the parameters assessed. The higher dosage (100 mg/kg body weight) made the animals slightly anaemic. Therefore a dose of up to 50 mg/kg body weight is recommended as safe for testing the pharmacological properties of VCEA in mice models.

Keywords: Acute toxicity, Ayurveda, Phytochemicals, Subacute toxicity, Swiss albino mice

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Introduction

Phytopharmaceuticals have an important role in the general medical practice due to their intrinsic biological properties. Developing new drugs from herbal sources is often safe and cost-effective[1]. Screening of active extracts from plants, by subjecting them to accurate bioassays followed by purification of phytochemicals are the initial steps to the effective, side-effect free nutraceutical-based therapeutic approaches. However, scientific validation on the safety level of herbal extracts is an inevitable step in developing modern drugs from traditional medicines. Plants produce a variety of secondary metabolites which could be beneficial or toxic to humans. Those drugs which are therapeutically effective at one dose might be toxic at increased doses or on prolonged exposure. Toxicity screening for plant extracts used in traditional medicines to cure diseases is essential for the purpose of determining safe doses[2].

Vernonia cinerea (L.) Less is an ayurvedic medicinal plant found throughout India. The Ayurvedic Pharmacopoeia of India describes its traditional uses such as treatment for intermittent fever, boils, lymphatic filariasis, blisters, vaginal discharges, and psychoneurosis[3]. This plant, in combination with other herbal ingredients, is used to cure breast tumours by the tribal community in the southern region of the Western Ghats of India[4]. It is known as poovamkurunnila in Malayalam and belongs to Family Asteraceae. Various extracts of this plant were widely studied for their anti-inflammatory, anti-diabetic, nephroprotective, antimicrobial and anticancer potentials[5–11].

Sesquiterpene lactones (germacranolides) and steroids are the major classes of chemical compounds found in the genus *Vernonia.* Ethyl acetate soluble fraction of *V. cinerea* was reported to have many sesquiterpenes including vernocinolide A, Vernolide A, Vernolide B, and 8 alpha tigloyl oxyhirsutinolide 13-O acetate(8αTGT)[12]. These sesquiterpenes contribute to the anti-cancer property of the plant *V. cinerea*[13]. Though this plant is known for these therapeutically effective terpenoids, especially sesquiterpenes soluble in ethyl acetate, its toxicity studies in suitable animal models are still lacking. Thus the present study was undertaken to evaluate the acute

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Supplementary tables are available online only.

and subacute toxicity of ethyl acetate extract (VCEA) of *V. cinerea* using the Swiss albino mice. It will be useful for determining the safe doses for studying the pharmacological potentials of this extract *in vivo*.

Materials and Methods

Plant collection, authentication, and extraction

Plant materials of *V. cinerea* were collected from Thrissur district, Kerala (India) (10°36'39.7" N76°02′10.7"E) from June to August 2020. A voucher specimen (Accession number 17685) has been deposited at Kerala Forest Research Institute (KFRI) after authentication by Dr. V. B. Sreekumar, Senior scientist and Head, Forest Botany Department, KFRI, Peechi, Thrissur, Kerala. Shade-dried and powdered plant materials (whole plant) were then extracted separately using ethyl acetate, ethanol and water in an accelerated solvent extractor. Extracts were then concentrated in a rotary evaporator.

Phytochemical analysis

All the extracts were subjected to preliminary phytochemical screening for the detection of different chemical constituents. The presence or absence of various phytoconstituents such as alkaloids, polyphenols, saponins, tannins, terpenes, and sterols was detected by the prescribed methods.

Test for alkaloids

To 0.2 g of plant extract, 5 mL of 1% hydrochloric acid was added and then boiled. This was cooled and filtered. To the filtrate 2 drops of freshly prepared Mayer's reagent was added. The appearance of a creamy white precipitate indicates the presence of alkaloids[14].

Test for polyphenols

To 1 mL of extract, an equal volume of ferric chloride was added. The formation of deep blue colour indicates the presence of polyphenols[15].

Test for saponins

Two mL of the extract was mixed with 4 mL of distilled water and shaken vigorously for 1 min. Froth formation indicates the presence of saponins[14].

Test for tannins

To 2 mL of extract, 10 mL of distilled water and a drop of $FeCl_3$ were added. The development of blue colour indicates the presence of tannins[15].

Test for terpenes

To 2 mL of extract, acetic anhydride and concentrated sulphuric acid were added. Bluish-green ring formation indicates the presence of terpenes[14].

Test for sterols

To 1 mL of acetic anhydride, an equal volume of chloroform was added and then cooled to 0^oC. To this, 1 mL of extract and concentrated sulphuric acid were added. The formation of green, red, orange colour changes with time indicates the presence of steroids[14].

Experimental animals

Adult male and female Swiss albino mice aged 6–8 weeks with an average weight of 25±3 g were obtained from Small Animal Breeding Station, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India. The animals were housed in well-ventilated polypropylene cages under controlled temperature and humidity. The animals were fed on a standard laboratory diet and water *ad libitum*. The studies were done after getting prior permission from Institutional Animal Ethics Committee (IAEC) and as per the instructions prescribed by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forest, Government of India[16]. (Ethical committee approval Number: ACRC/IAEC/20(1)-P15).

Acute toxicity evaluation

The acute toxicity of ethyl acetate extract was assessed by following OECD (Organisation for Economic Co-operation and Development) guideline number 423[17]. Female Swiss albino mice were grouped into two (n=6) after 14 days of acclimatisation. Group I: vehicle control (sunflower oil) and group II: VCEA (2000 mg/kg). All the mice were fasted overnight prior to oral drug/vehicle administration. The animals were then kept under close observation continuously for 1 h and intermittently for 4 h and thereafter once every 24 h for the next 14 days. During this study period, clinical observations were made for mortality, behavioural changes and any other abnormalities and their body weight was measured weekly. Food and water consumption was also recorded during the entire study period. On the 15^{th} day, all the mice were euthanized and the necropsy was done.

Subacute toxicity evaluation

Subacute toxicity determination was conducted in both male and female Swiss albino mice according to the OECD guideline number 407[18]. The animals of each sex were weighed and grouped separately into four groups (n=5) as follows: Group I: untreated, Group II: Vehicle control (sunflower oil), Group III: VCEA Low Dose (50 mg/kg body weight), Group IV: VCEA High Dose (100 mg/kg body weight). The drug was administered orally using an oral gavage consecutively for 28 days. Body weight, food and water consumption per group of animals were recorded every third day during the study period. On the 28th day, the final body weight was taken and the animals were euthanized. Haematological and biochemical parameters were done using the blood samples from the animals. Organs such as the liver, kidney, heart, brain, lungs, stomach, intestine, spleen, and genital organs such as the testis and ovary were carefully isolated, weighed and subjected to histopathological analysis.

Haematological and biochemical parameters

Blood was collected immediately from the sacrificed animals in heparinized tubes by cardiac puncture. Estimation of haematological parameters was done using a fully automated machine (Mindray BC 20s). For testing the biochemical parameters, blood samples were collected in tubes without anticoagulant coating and then centrifuged at 3000 rpm for 10 min to separate the serum. Different biochemical parameters were analysed using commercially available kits (Agappe) according to the manufacturer's instructions.

Haematoxylin and Eosin staining

A small portion of the vital organs was taken immediately after sacrificing the animals and fixed in 10% formaldehyde. After several treatments for dehydration in alcohol, sections of 4 µm thickness were cut and stained with haematoxylin and eosin to observe under the light microscope (Leica DM 500). Photomicrographs were captured using the LAZ software at the magnification of 40X.

Data analysis

All data are expressed as mean±SEM. The values were statistically tested using Student's t-test in Microsoft Excel. p value <0.05 were considered significant.

Results

Phytochemical screening

Ethyl acetate, ethanol, and water extracts of the whole plant were screened for the presence of various categories of phytochemicals. Polyphenols were found in all the extracts. Alkaloids, tannins and saponins were absent in ethyl acetate extract. Terpenoids and steroids were found in both ethyl acetate and ethanol extracts (Table 1). The percentage terpenoids yield from ethyl acetate extract was 67.66±1.45% and that of ethanol extract was only 15.33±0.88% (Supplementary Table S1).

Acute toxicity studies using VCEA extract

No mortality was observed in the animals treated with 2000 mg/kg body weight of VCEA. All animals were found to be normal without any significant changes in body weight, food and water consumption (Tables 2 and 3). The animals exhibited no gross behavioural or morphological changes till the end of the observation period. No abnormalities were found in the necropsy.

Table 1 —Phytochemical screening of various extracts of *Vernonia cinerea* (L.) Less

Phytochemicals	Ethyl acetate	Ethanol	Water
Alkaloid	-	+	+
Polyphenol	+	+	+
Saponins	-	+	+
Sterols	+	+	-
Tannins	-	-	+
Terpenoids	+	+	-

'+' indicate the presence and '-'indicate the absence of the phytochemical

Table 2 — Acute toxicity analysis- Effect of VCEA on body weight of Swiss albino mice

Treatments	Day 1	Day 7	Day 14
Vehicle control	29.13±2.04	29.60±0.86	31.30±1.14
VCEA	29.26±1.41	29.86±1.13	31.46±1.41

Data are presented as the mean±SEM. Comparisons were made between vehicle control with the treated groups separately at respective time points.

Table 3 — Acute toxicity analysis - Effect of VCEA on food and water consumption of mice

Treatments	Food consumption (mL/group of mice/day)		Water consumption (g/group of mice/day)	
	Week 1	Week 2	Week 1	Week 2
Vehicle control	10.26±0.73	11.20±0.57	5.33±0.33	6.00±0.58
VCEA	10.03±0.70	10.26±0.43	5.55±0.60	7.66±0.66

Data are presented as the mean±SEM. Comparisons were made between vehicle control with the treated groups separately

Subacute toxicity studies using VCEA extract

Body weight and relative organ weight measurement

No significant changes in body weight and relative organ weight, food and water consumption level were observed in the mice treated with 50 and 100 mg/kg body weight of VCEA extract when compared with the vehicle control (Fig. 1; Table S2 to S6).

Haematological evaluations

Among the haematological parameters Hb, RBC, PCV, and WBC levels were found to be decreased in the VCEA high-dose treated groups of both sexes when compared with vehicle control. All other parameters did not vary significantly in either treated group (Table 4).

Biochemical evaluations

Different serum biochemical parameters are represented in Tables 5 and 6. In both sexes, the liver enzymes like serum glutamate-oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) levels did not show any significant variations. A significant decrease was found in the cholesterol and triglycerides level, in both sexes. The urea levels did not show considerable changes in both males and females, but creatinine values showed a slight reduction in both doses treated groups of male sex. But, the level of creatinine was found to be normal in the case of females.

Histopathological evaluations

Haematoxylin and eosin stained sections of the organs appeared in normal architecture when compared with that of normal and vehicle control (Fig. 2). Sections of liver from both male and female extract treated animals showed normal portal triads and central veins. Kupffer cells were normal. Sections of kidney from both sexes showed normal glomeruli and renal tubules with normal interstitial tissue. Ovary of either treated mice showed normal graafian follicles. In sections of testis, seminiferous tubules containing normal sertoli cells were observed.

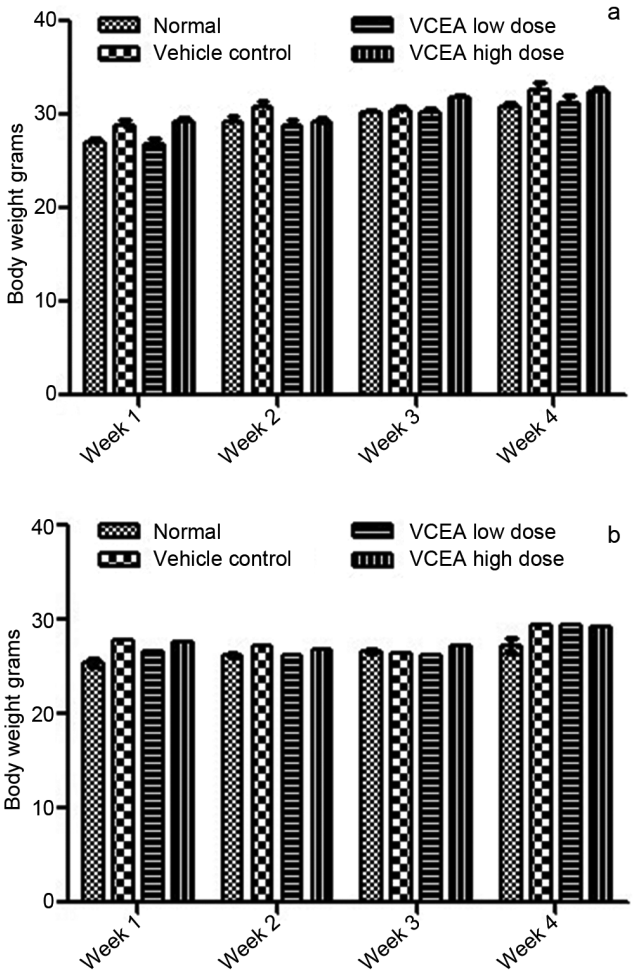

Fig. 1 — Effect of VCEA on body weight of a) male; and b) female mice observed during subacute study.

Discussion

Vernonia cinerea (L.) Less is considered as one among the top 5 most frequently used medicinal species in the genus *Vernonia*. Sesquiterpene lactones (germacranolides) are the chief pharmacologically active phytoconstituents found in this genus. The preliminary phytochemical evaluations on this plant revealed that terpenoid content was much higher in the ethyl acetate extract. Many terpenoid compounds having antitumor potentials were already reported to be present in this plant. Kuo *et al.* reported the cytotoxic potential of the sesquiterpenes such as Vernolide A and Vernolide B against various cell lines[19]. Vernolide A could induce apoptosis in B16F-10 melanoma cells and inhibit its metastatic progression. 8αTGT, another terpene compound from this plant, exhibited antiproliferative activity on oral squamous cell carcinoma. This compound causes G_2/M cell cycle arrest via inhibition of both STAT 2 and STAT 3 phosphorylation[20]. 8αTGT also showed cytotoxicity against HT29 colon adenocarcinoma cells and HepG2 hepatoma cells[21].

Treating illness without causing toxic effects on other parts of the body is an important task in plant based therapeutic approaches. *In vivo* acute and sub acute toxicity profiles of the terpene rich ethyl acetate

Table 4 — Haematological parameters from Swiss albino mice in the sub acute toxicity study with the administration of VCEA

Parameters	Untreated control	Vehicle control (Sunflower oil)	VCEA 50 mg/kg b.wt.	VCEA 100 mg/kg b.wt.
Male				
Hb (g/dL)	14.67±0.35	15.17±0.27	14.07±0.48	14.00±0.06**
RBC (10^6/cu mm)	8.07±0.29	7.73±0.27	7.13±0.15	6.97±0.03*
MCV (10^6/cu mm)	59.00±1.53	62.00±0.58	59.67±1.20	62.67±0.88
MCH (10^6/cu mm)	18.33±0.33	19.33±0.33	19.00±0.00	19.67±0.33
MCHC (10^6/cu mm)	31.33±0.33	32±0.58	33.00±0.00	32.00±0.58
PCV (10^6/cu mm)	47.67±0.67	48.33±1.76	44.00±3.06	43.33±0.88*
WBC (10^3/ cu mm)	7.56±0.52	7.83±0.60	6.16±0.66	5.63±0.84*
Neutrophils (%)	17.0±4.16	11.33±0.33	13.33±0.88	12.67±0.88
Lymphocytes (%)	77.67±3.76	83.33±1.76	81.33±1.86	79.33±0.88
Eosinophils (%)	4.67±0.33	3.33±0.33	4.67±0.67	4.67±0.67
Platelet (10^5/cu mm)	11.0±0.80	10.23±0.20	9.07±1.01	10.80±0.78
Female				
Hb (g/dL)	14.33±0.04	13.90±0.21	14±0.29	12.83±0.12**
RBC (10^6/cu mm)	8.13±0.16	8.50±0.06	8.53±0.15	8.03±0.03**
MCV (10^6/cu mm)	56.67±0.41	56.33±0.33	55.33±0.33	55.33±0.88
MCH (10^6/cu mm)	17.67±0.41	16.33±0.33	15.67±0.33	15.33±0.33
MCHC (10^6/cu mm)	31.00±0.00	28.67±0.33	29.67±0.33	29.00±0.58
PCV (10^6/cu mm)	46.33±0.41	47.33±0.33	47.00±0.58	44.00±0.58**
WBC (10^3/ cu mm)	8.70±0.20	8.83±0.16	8.53±0.57	7.86±0.36*
Neutrophils (%)	14.33±0.41	15.00±1.15	15.33±0.33	15.67±0.88
Lymphocytes (%)	80.33±1.12	80.33±0.88	80.33±0.33	78.00±1.15
Eosinophils (%)	5.00±0.82	4.33±0.33	4.00±0.58	5.33±0.67
Platelet (10^5/cu mm)	10.47±0.73	8.96±1.01	9.17±0.33	9.33±0.09

Data are presented as the mean±SEM. Comparisons were made between vehicle control with the treated groups separately. The symbol (*) represents statistical significance at $P \leq 0.05$, (**) represents statistical significance at $P \leq 0.01$

Table 5 — Biochemical parameters from male Swiss albino mice in the sub acute toxicity study with the administration of VCEA

Parameters	Untreated control	Vehicle control (Sunflower oil)	VCEA 50 mg/kg b.wt.	VCEA 100 mg/kg b.wt.
Liver function				
SGOT (IU/L)	246.67±17.64	304.33±7.54	268.33±27.88	263.67±10.17
SGPT (IU/L)	53.67±2.40	68.00±3.79	57.33±3.76	62±4.00
ALP (U/L)	105.33±1.45	190±0.58	184.1±0.58**	137.67±8.67**
TB (mg/dL)	0.27±0.03	0.33±0.03	0.40±0.00	0.33±0.03
TP (g/dL)	6.10±0.10	6.47±0.18	6.03±0.29	6.20±0.00
Albumin (g/dL)	3.20±0.06	3.30±0.06	3.23±0.15	3.33±0.03
Globulin (g/dL)	2.97±0.03	3.13±0.13	2.93±0.26	2.87±0.03
Lipid profile				
Cholesterol (mg/dL)	146.33±20.17	150.67±3.48	135±0.58**	132.67±1.45**
Triglycerides (mg/dL)	180±15.82	173.33±9.39	141.67±20.76	113.67±25.67*
HDL (mg/dL)	40.33±0.88	38.33±0.33	39.35±0.33	39.67±0.88
LDL (mg/dL)	87±17.39	66.33±3.53	69.67±1.45	68±4.58
VLDL (mg/dL)	19±2.08	30.33±1.45	25.67±2.33	24±3.79
Kidney function				
Urea (mg/dL)	46.33±4.04	43±0.82	39.67±2.03	40±1.53
Creatinine (mg/dL)	0.55±0.00	0.54±0.02	0.48±1.63*	0.47±0.03*
Serum Electrolytes				
Na^+ (mmol/L)	147.33±0.33	143.67±0.33	144±0.53	145.33±1.20
K^+ (mmol/dL)	9.27±0.32	8.87±0.35	8.57±0.41	8.10±0.30
Cl^- (mmol/dL)	105.67±0.33	101±0.00	100.33±0.88	100.33±0.33
HCO_3^- (mmol/dL)	28.33±0.33	23.67±0.33	24.67±0.88	25.33±1.20

Data are presented as the mean±SEM. Comparisons were made between vehicle control with the treated groups separately. The symbol (*) represents statistical significance at $P \leq 0.05$, (**) represents statistical significance at $P \leq 0.01$

Table 6 — Biochemical parameters from female Swiss albino mice in the sub acute toxicity study with the administration of VCEA

Parameters	Untreated control	Vehicle control (Sunflower oil)	VCEA 50 mg/kg b.wt.	VCEA 100 mg/kg b.wt.
Liver function				
SGOT (IU/L)	288.67±6.12	218±6.00	322±78.00	240.33±55.83
SGPT (IU/L)	82.33±3.27	62.33±0.67	76.33±9.33	70.33±5.17
ALP (U/L)	185.33±3.67	105.33±12.67	141.67.±12.33	127.0±0.58
TB (mg/dL)	0.23±0.4	0.30±0.00	0.33±0.03	0.27±0.03
TP (g/dL)	6.63±0.04	6.83±0.17	6.67±0.12	6.87±0.12
Albumin (g/dL)	3.37±0.04	3.13±0.07	3.27±0.07	3.23±0.03
Globulin (g/dL)	3.33±0.08	3.70±0.10	3.50±0.20	3.60±0.10
Lipid profile				
Cholesterol (mg/dL)	109±2.90	151.00±4.88	135.00±0.81*	132.5±2.04**
Triglycerides (mg/dL)	114.67±0.88	175.00±13.07	147.50±28.17*	113.67±25.67*
HDL (mg/dL)	42.00±0.58	38.5±0.41	39.50±0.41	39.5±1.22
LDL (mg/dL)	45.00±2.31	69.50±2.04	69.50±8.16	69.5±6.13
VLDL (mg/dL)	22.00±1.00	30.50±2.04	26.00±3.27	23.5±5.31
Kidney function				
Urea (mg/dL)	46.33±7.34	46.00±3.27	42.67±3.27	42.50±2.04
Creatinine (mg/dL)	0.55±0.00	0.52±0.02	0.53±0.02	0.52±0.00
Serum Electrolytes				
Na^+ (mmol/L)	141.33±1.88	148.67±0.88	147±0.58	147.67±1.20
K^+ (mmol/dL)	9.40±0.12	8.80±0.31	9.47±0.09	8.83±0.18
Cl^- (mmol/dL)	109±0.58	105±1.15	107±1.00	106±1.15
HCO_3^- (mmol/dL)	27±0.58	28±0.58	27.33±0.33	26.67±0.33

Data are presented as the mean±SEM. Comparisons were made between vehicle control with the treated groups separately. The symbol (*) represents statistical significance at $P \leq 0.05$, (**) represents statistical significance at $P \leq 0.01$

extract (VCEA) of *V. cinerea* were investigated in this study. Acute toxicity studies are useful in evaluating the adverse effects of substance that causes upon a single dose administration and this will also be beneficial in selecting dosages for long-term toxicity evaluations[22]. It was found that the single oral dosage of VCEA at 2000 mg/kg body weight was found to be safe in terms of body weight, food and water intake, and general behaviour. No mortality was observed in the treated group. Therefore it is inferred that the LD_{50} of VCEA will be higher than 2000 mg/kg body weight. This result was consistent with a study conducted in the same plant from Malaysia where the methanol extract was found to be safe up to 2000 mg/kg body weight upon a single oral dosage[23].

According to Parasuraman, toxicity screening is essential for calculating the "No Observed Adverse Effect Level" dose which will be useful for therapeutic studies at the clinical stage[24]. OECD guidelines for testing of chemicals Number 407 recommended that the high dose used for the 28-day subacute toxicity studies should be the dose which does not cause mortality and severe suffering but can induce toxic effects in animals and the subsequent lower doses are selected accordingly to attain the dose-response and also the dose at which no observed adverse effect level is obtained. Daily oral administration of sublethal doses such as 50 mg/kg body weight and 100 mg/kg body weight were used for 28 days to investigate the effects of VCEA. Body weight gain was similar in all the animals regardless of treatment. Haemoglobin, RBC, and Packed cell volume (PCV) were significantly decreased in the high-dose treated group of animals of both males and females when compared with the vehicle control indicating an anaemic condition. However, the values of MCV, MCH, and MCHC remained unchanged upon extract treatment. This indicates the VCEA administration did not affect the oxygenation to tissues[25]. In an *in vivo* subacute toxicity study of methotrexate (an anticancer drug) the reduction of PCV level was correlated with the cytotoxic potentials of the drug on the hematopoietic system[26]. Further, some studies have reported the haemolytic potentials of terpenes in erythrocytes[27]. In this study, the total WBC counts deviated significantly upon high-dose treatment indicating the selective action of the extract on haematopoiesis. Haematological parameters

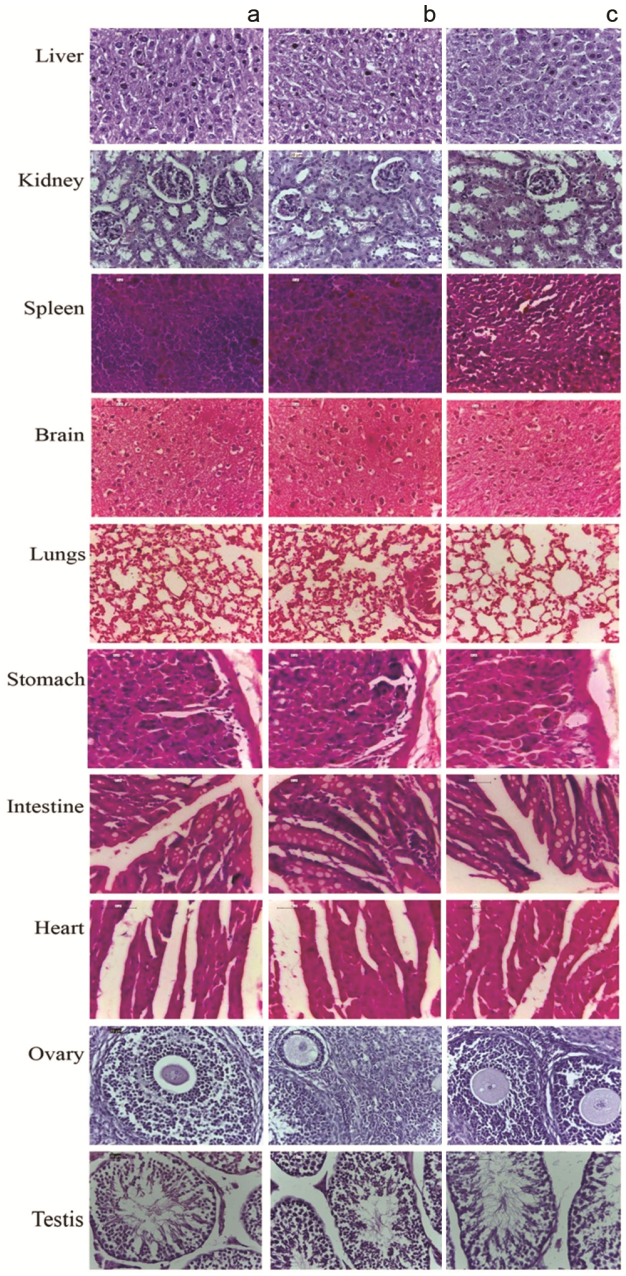

Fig. 2 — Photomicrographs of Haematoxylin and eosin stained sections of organs showing the effect of VCEA in a 28 day subacute study in mice (under 40X magnification power). a) Normal; b) Vehicle control; and, c) VCEA 100 mg/kg body weight.

seemed to be unaltered in animals that received VCEA at the dose of 50 mg/kg body weight.

Increased serum levels of the cellular enzymes SGOT, SGPT and ALP are excellent markers of liver injury[28]. Notably, in the current study with both sexes, VCEA extract did not cause a significant increase in serum levels of these enzymes. Lipid profiles of the animals were not varied significantly in treated male and female groups except for the cholesterol and triglyceride levels, which were found to be lower than that of vehicle control. The lowering of cholesterol and triglycerides may be due to the cardioprotective property of this plant. Another species from the same genus called *Vernonia calvoana* was also reported to exert lipid-lowering and cardioprotective properties in Wistar rats[29]. Urea and creatinine levels in serum is a useful indicator of kidney functioning and there was no increase in the level of these indicated in the VCEA-treated mice of both sexes, which means that their renal functioning was quite good. A slight decrease in serum creatinine level was recorded in males, which is a sign of muscle wastage[30]. However, the reported body weight gain in all the treated groups does not support muscle wastage in animals.

Microscopic assessment of all the stained sections of organs from the treated mice reveals normal tissue architecture. Histopathological findings obtained in this study also support the values recorded in the biochemical evaluations. Relative organ weights of treated animals were also similar to that of controls. These data suggest that VCEA administration at the selected doses does not impart toxic effects at the tissue level except some anaemic response observed in high doses. The dosages up to 50 mg/kg body weight of ethyl acetate extract can be selected safely for further studies in mice determining its promising pharmacological potentials.

Conclusion

Single dosage of VCEA at 2000 mg/kg body weight was found to be fairly non-toxic to mice. Subacute toxicity study revealed that 50 mg/kg body weight and 100 mg/kg body weight of VCEA does not cause much adverse effects to mice according to the haematological, biochemical parameters, and histopathological analysis even though slight anaemic condition observed on the high dose intake. Thus a dose below 50 mg/kg body weight can be taken as more safe doses for further pharmacological studies. Further studies are required to understand major bioactive components of this terpene rich extract.

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Conflict of interest

Authors have no conflict of interest in publishing this original paper.

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