

Assessment of the Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on the Liver of Mice

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Abstract

Effects of methanol leaf extract of *Clerodendrum violaceum* on liver function indices of Swiss mice was evaluated. Six groups (A-F) of ten mice each were used. Groups B-F were administered 31.25, 62.5, 125, 250, 500 mg/kg body weight of methanol leaf extract of *Clerodendrum violaceum*, respectively. Group A received 5% DMSO (control). Five animals in each group were sacrificed after 14 days of administration; the remaining were sacrificed after 28 days of administration. Blood was collected for analyses, livers were collected and weighed. Some of the liver samples were homogenized and some preserved in 10% formalin for histopathological examination. After 14 days, there was significant increase ($p < 0.05$) in total and conjugated bilirubin and significant reduction ($p < 0.05$) of albumin and total protein at higher doses. Activities of ALP and γ -GT in serum were significantly elevated ($p < 0.05$) at all doses while liver and serum ALT activity only at lower doses. Liver and serum AST activity were also significantly elevated ($p < 0.05$) at higher doses. Activities of ALP and γ -GT in the liver were significantly reduced ($p < 0.05$) at all doses while ALT activity only reduced at the highest dose in liver and serum. AST activity was reduced at higher doses in liver but only at highest dose in serum. Liver tissue was inflamed with progressive degeneration on day 28. Results showed that methanol leaf extract of *C. violaceum* adversely affected the normal architecture, synthetic and secretory functions of the liver at high doses.

Keywords: *Clerodendrum violaceum*, histopathological screening, liver function indices, organ-body weight ratio.

Introduction

The liver is a large organ made up of chemically reactant pool of cells having a high rate of metabolism. It is responsible for sharing substrates and energy between metabolic systems, processing and synthesizing multiple important substances for transport to other areas of the body and is involved in several other metabolic functions (Dutta et al. 2021). The liver plays a major role in carbohydrate, amino acid and lipid metabolism and plays a key role in the biotransformation of foods, toxic substances, and medicinal products (Arman et al. 2022). Due to these diverse and essential functions carried out by the liver, any change in its normal structure or function will have far reaching consequences. Some of these changes have been shown to occur during disease or exposure to drugs, chemicals, and toxicants, including medicinal plants (Intagliata and Caldwell 2017; Nunes et al. 2022). Monitoring of potential adverse effects of drugs and other compounds on the liver is therefore vital in diagnosis, recovery, and follow-up of many medical conditions (Lilford et al. 2013; Liao et al. 2022).

Medicinal plants have been identified and used to treat various ailments even before the advent of orthodox medicines (Sofowora et al. 2013). Plants produce several chemical compounds, (secondary metabolites) some of which have been shown to have pharmacological effects. However, the presence of these compounds in most medicinal plants may lead to complex and sometimes detrimental effects (Obeten et al. 2017; Okaiyeto and Oguntibeju 2021). The liver is uniquely situated to take up and process all chemical compounds coming into the blood since the metabolism of drugs and other exogenous compounds, including medicinal preparations, mainly takes place there. It is thus, liable to adverse effects from such drugs and their metabolites (García and García 2022).

Clerodendrum violaceum (*C. violaceum*) (Verbenaceae) is commonly called Clerodendrum in English and 'Ewe isedun' in Yoruba (Nigeria). A decoction of its leaves is used for the treatment of fever/malaria in folk medicine. We have previously authenticated the acclaimed antimalarial activity of its leaf extract (Adebayo et al. 2022). We have also reported that its antimalarial activity is augmented by its antioxidant activity (Balogun et al. 2014).

Since *C. violaceum* is taken traditionally for fever and has been shown to have antimalarial activity and considering the physiological roles of the liver in health and disease, it is of interest to investigate its effect on the liver and its function indices.

MATERIALS AND METHODS

Methanol was obtained from BDH Laboratory Supplies, Poole Dorset BH15 UK. Assay kits for enzymes were obtained from Randox Laboratories Ltd. (Co. Antrim, U.K). All other reagents used were of analar grade and prepared in all glass distilled water.

Plant materials

Fresh leaf samples of *C. violaceum* were collected in Oyo town, Oyo State, Nigeria and were botanically authenticated at Forestry Research Institute of Nigeria (FRIN), Ibadan, Oyo State, Nigeria. A specimen with voucher number FHI 108879 was deposited.

Animals

Sixty (60) adult Swiss laboratory mice with an average weight of 20 ± 2 g were obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Jos, Plateau State, Nigeria. The mice were housed in plastic cages and maintained under standard laboratory conditions with free access to rat pellets and tap water *ad libitum*. Animal care and experiments/procedures were carried out according to the ethical guidelines of the NNREC (Norwegian National Research Ethics Committee (2019).

Plant extracts preparation

Fresh leaves of the plant were dried in the shade for seven days at room temperature (25 ± 2 °C) and pulverized to powder using an electric blender (Mazeda Mill, MT 4100, Japan). Four hundred and fifty grams (450 g) of the powder was exhaustively extracted with 4 L n-hexane, 4 L ethyl acetate and 4 L absolute methanol successively for 72 h each. The extracts were filtered using Whatman filter paper No 1 and concentrated under pressure after each extraction period using a rotary evaporator. The concentrates were then exposed to air and allowed to evaporate at room temperature to dryness (Adebayo et al. 2003). In our previous study, methanol extract had the highest antioxidant activity (Balogun et al. 2014) and the best antimalarial efficacy (Adebayo et al. 2022); hence, only the methanol extract was used in this study.

Experimental Design

Sixty Swiss laboratory mice were randomly divided into six groups (A-F) of ten mice each and given the methanol leaf extract of *C. violaceum* orally as follows:

Animals in group A received 5% DMSO and served as control; those in groups B, C, D, E and F received 31.25, 62.5, 125, 250 and 500 mg/kg body weight of the methanol leaf extract of *C. violaceum*, respectively. After fourteen days of extract administration, five animals from each group were sacrificed; blood and liver tissue were collected for analysis. Extract administration continued for another fourteen days after which the remaining animals in all the groups were sacrificed and treated similar to the first batch.

Collection of Blood Samples, Liver Tissue and preparation of Serum and Tissue Supernatants

The mice were sacrificed following diethyl ether anaesthesia. Blood was collected by cardiac puncture into clean, dry test tubes and was allowed to stand for about fifteen minutes at room temperature to clot. It was then centrifuged at 1000 rpm (Gallenkamp Centrifuge 200) for fifteen minutes. The clear supernatant (serum) was carefully collected with a Pasteur pipette. The animals were then dissected, and the liver was removed, cleaned and weighed. The liver samples for each group were then homogenized separately in ice-cold 0.25 M sucrose solution (1:5 w/v). The homogenates were stored frozen overnight before centrifuging. The supernatant obtained after centrifuging was used in the analyses. Parts of the weighed liver tissues were also collected in specimen bottles containing 10% formalin and fixed for histopathological examination.

Organ-body weight ratio

After the mice were sacrificed, their livers were immediately removed, cleaned, and weighed. The relative liver weights were calculated by dividing the weights of livers by the final body weights of the corresponding animals before sacrifice.

Histopathological studies

The method of Krause (2001) which includes several processing steps was used to assess the histopathological effect(s) of the methanol leaf extract of *C. violaceum* on the liver of mice. Briefly, after sacrificing the mice, liver tissue samples were collected and fixed in 10 % formalin to preserve tissues and maintain lifelike structures. The samples were then transported to the Histology laboratory of the Department of Anatomy, College of Health Sciences, University of Ilorin, Kwara State, Nigeria where the processing, preparation, and interpretation of histopathology slides was carried out. The fixed tissue samples were first dehydrated to remove excess water and formalin by immersing them in ascending grades of alcohol. They were then cleared with xylene to remove the alcohol and impregnated with molten paraffin which infiltrates the tissue samples, replaces the clearing agent and provides support. Next, the tissue samples were embedded with more paraffin. Embedding enables careful positioning of the tissue inside a base mould. The tissues were then sectioned from the paraffin block with a rotary microtome and the cut sections were placed on a microscope slide, dried and stained with Hematoxylin and Eosin (H/E) to highlight important features of the tissues and enhance contrast. A Synthetic mountant was added to a coverslip and placed on top of the tissue section on the slide to keep the specimen in place and protect from any accidental contact. Images of the sections were then captured using a camera attached to a microscope (Omax-MD82ES10). Photomicrographs were captured at x400 magnification.

Analysis of biochemical parameters

The method of Ueno et al. (2013) was used to determine albumin concentration. Protein concentrations were determined using the method reported by Zheng et al. (2017). For serum bilirubin, the method reported by Kalakonda et al. 2022 was used. Alkaline phosphatase activity was determined as described by Wright et al. (1972) while the method of Corti et al. (2019) was

used to assay for gamma glutamyl transferase activity. The activities of alanine and aspartate aminotransferases were assayed by the method described by Reitman and Frankel (1957).

Statistical analysis

The group means \pm Standard Deviation (SD) for each parameter was calculated and significant differences were determined by Analysis of Variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used for post-hoc test at 95% confidence level using SPSS-PC programme packages (Version 24.0, SPSS Inc. Chicago).

RESULTS AND DISCUSSION

Liver Function indices

Administration of the extract for 14 days caused a significant reduction ($p < 0.05$) in serum albumin at 500 mg/kg body weight while serum total protein concentration was significantly decreased ($p < 0.05$) at the doses of 250 and 500 mg/kg body weight compared to controls. After 28 days of extract administration, there was an increase in the concentration of albumin at the dose of 250 mg/kg body weight while it was reduced significantly ($p < 0.05$) at 500 mg/kg body weight compared to control. However, serum total protein concentration reduced significantly ($p < 0.05$) at all doses compared to control. There was significant increase ($p < 0.05$) in total bilirubin concentration at the dose of 250 and 500 mg/kg body weight while conjugated bilirubin concentration was increased significantly ($p < 0.05$) at all doses compared to controls after 14 days of extract administration. After 28 days, there was a significant ($p < 0.05$) increase in total bilirubin concentration while conjugated bilirubin concentration was not significantly altered ($p > 0.05$) compared to controls at all doses of the extract administered (Tables 1 and 2).

Table 1: Effects of Methanol Leaf Extract of *Clerodendrum violaceum* on Liver Function Indices of Mice after 14 Days of Administration

Treatment	Albumin (g/dL)	Total protein (mg/ml)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)
Control	13.23 \pm 0.57 ^a	60.18 \pm 1.04 ^a	1.38 \pm 0.11 ^a	0.38 \pm 0.10 ^a
31.25 mg/kg b. wt	12.45 \pm 0.39 ^a	59.28 \pm 2.65 ^a	1.42 \pm 0.15 ^a	0.51 \pm 0.24 ^b
62.5 mg/kg b. wt	11.38 \pm 0.58 ^a	56.85 \pm 2.02 ^a	1.44 \pm 0.29 ^a	0.53 \pm 0.11 ^b
125 mg/kg b. wt	10.30 \pm 0.52 ^a	52.45 \pm 1.14 ^a	1.50 \pm 0.13 ^a	0.59 \pm 0.17 ^b
250 mg/kg b. wt	10.00 \pm 0.10 ^a	48.58 \pm 1.81 ^b	1.55 \pm 0.17 ^b	0.60 \pm 0.31 ^b
500 mg/kg b. wt	6.00 \pm 0.23 ^b	32.25 \pm 1.26 ^c	1.76 \pm 0.13 ^b	0.80 \pm 0.19 ^c

Values are means of 5 replicates \pm SD. Means in the same column with different superscripts for each parameter are significantly different ($p < 0.05$).

Table 2: Effects of Methanol Leaf Extract of *C. violaceum* on Liver Function Indices of Mice after 28 days of Administration

Treatment	Albumin (g/dL)	Total protein (mg/ml)	Total bilirubin (mg/dL)	Conjugated bilirubin (mg/dL)
Control	15.28 \pm 0.34 ^a	67.90 \pm 1.38 ^a	1.39 \pm 0.06 ^a	0.49 \pm 0.07 ^a
31.25 mg/kg b. wt	17.28 \pm 0.65 ^a	54.95 \pm 2.63 ^b	1.46 \pm 0.10 ^a	0.53 \pm 0.03 ^a
62.5 mg/kg b. wt	13.65 \pm 0.56 ^a	53.85 \pm 1.86 ^b	1.48 \pm 0.17 ^a	0.55 \pm 0.05 ^a
125 mg/kg b. wt	15.25 \pm 0.39 ^a	50.50 \pm 1.12 ^b	1.54 \pm 0.12 ^b	0.60 \pm 0.06 ^a
250 mg/kg b. wt	20.35 \pm 0.81 ^b	47.45 \pm 1.15 ^b	1.57 \pm 0.13 ^b	0.73 \pm 0.03 ^a
500 mg/kg b. wt	8.00 \pm 0.24 ^c	25.92 \pm 1.56 ^c	1.70 \pm 0.12 ^c	0.68 \pm 0.04 ^a

Results are means of 5 determinations \pm SD. Means along the same column with different superscripts for each parameter are significantly different ($p < 0.05$).

The concentrations of albumin, total protein and bilirubin in the blood can be used to gain information on the state of the liver (Sawieres, 2022).

Albumin along with other plasma proteins cannot normally diffuse through the thin capillary wall membranes since they are colloidal molecules. Therefore, they remain trapped in the vascular system where they exert a colloidal osmotic pressure which helps to maintain a normal blood volume (Moman et al. 2022). Thus, a decrease in serum albumin concentration which was significant at 500 mg/kg body weight throughout the days of extract administration might be due to a diminished synthetic function of the liver. Any Liver injury can lead to a disturbance in its physiological roles which includes the ability to synthesize albumin at a rate commensurate with catabolism resulting in a reduction in albumin concentration (Ugwu and Suru 2021). Since albumin plays an important role in maintaining an oncotic pressure difference between the plasma and interstitial space,

any decrease in serum albumin concentration if left unchecked will cause the diffusion of water from the blood vessels into the interstitial fluid and tissues (Adebayo et al. 2009; Shi et al. 2022). The significant decrease in the total protein concentration at 250 and 500 mg/kg body weight on day 14 (Table 1) and at all doses after 28 days of extract administration (Table 2) may be for the same reason.

Bilirubin is the main bile pigment formed from the breakdown of haem in red blood cells. The serum bilirubin concentration is considered a true test of liver function because it reflects the ability of the liver to take up, process and secrete bilirubin. The significant increase in serum total bilirubin concentration at 250 and 500 mg/kg body weight and the significant increase at all doses for serum conjugated bilirubin concentration after 14 days of extract administration (Table 1) could be an indication of an impairment in the functional capacity of the liver and possibly haemolysis especially at higher doses (Nunes et al. 2022). Jaundice is caused either due to overproduction of bilirubin or inability of the liver to clear it and is found in several diseases including haemolytic anaemia, cholestasis, Gilbert's syndrome, malaria, and inflammation (Janghel et al. 2019). The increase in serum total bilirubin suggests that higher doses of the extract should be used with caution as it may lead to hyperbilirubinaemia.

Significant elevation of total bilirubin concentration at all doses on day 28 (Table 2) could be a cumulative effect of the extract on the liver leading to an accumulation of bilirubin since the conjugated bilirubin was not affected. These reductions suggest that using this extract at higher doses or for prolonged periods may affect liver function.

Cellular Enzymes

Alkaline Phosphatase

There was a dose-dependent significant increase ($p < 0.05$) in ALP activity in the serum compared to controls after 14 days of extract administration (Figure 1). There was also dose-dependent significant decrease ($p < 0.05$) in liver ALP activity compared to control (Figure 1).

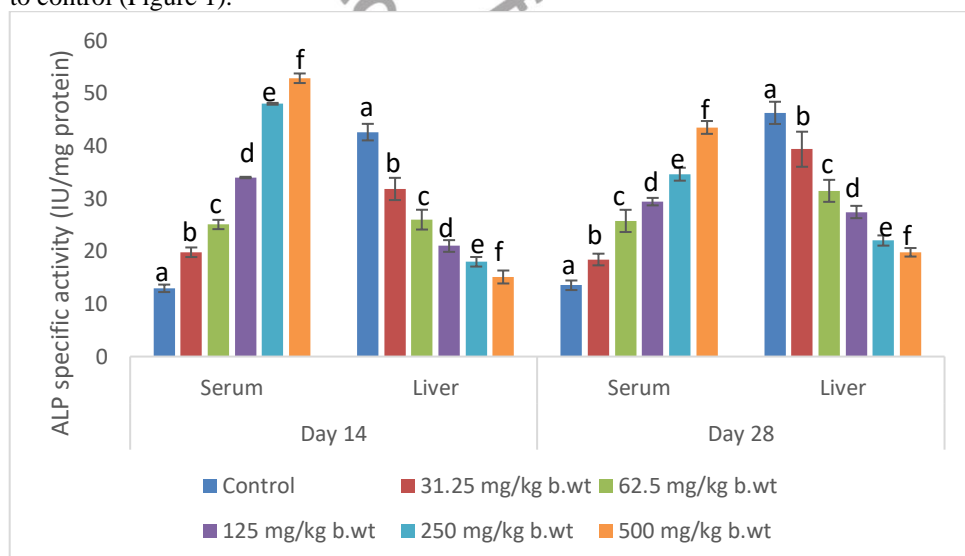


Figure 1: Effects of methanol leaf extract of *C. violaceum* on alkaline phosphatase activities in serum and liver of mice. Values are means of 5 replicates \pm SD. Bars with different letters are significantly different ($p < 0.05$).

After 28 days of extract administration, there was a dose-dependent significant increase ($p < 0.05$) in ALP activities in the serum and a significant decrease in the liver compared to controls (Figure 1).

ALP is primarily located on the hepatocyte membrane and shed into the serum, hence the dose-dependent significant decrease in the activity of ALP in the liver throughout the study period compared to controls (Figure 1) may have resulted from the loss of ALP from the membrane into the serum (Levitt et al. 2022). The corresponding dose specific significant increase in the activity of ALP in the serum (Figure 1) confirms this. The reduction in liver ALP activity would hinder adequate transportation of required ions or molecules across their cell membrane and may lead to starvation of cells (Ayorinde et al. 2008).

γ -GlutamylTransferase (γ -GT)

After 14 days of extract administration, there was a significant increase ($p < 0.05$) in γ -GT activity in the serum at all doses except 31.25 mg/kg b. wt compared to controls (Figure 2). There was also a significant decrease ($p < 0.05$) in its activity in the liver at all doses higher than 31.25 mg/kg body weight compared to controls after 14 days of administration (Figure 2).

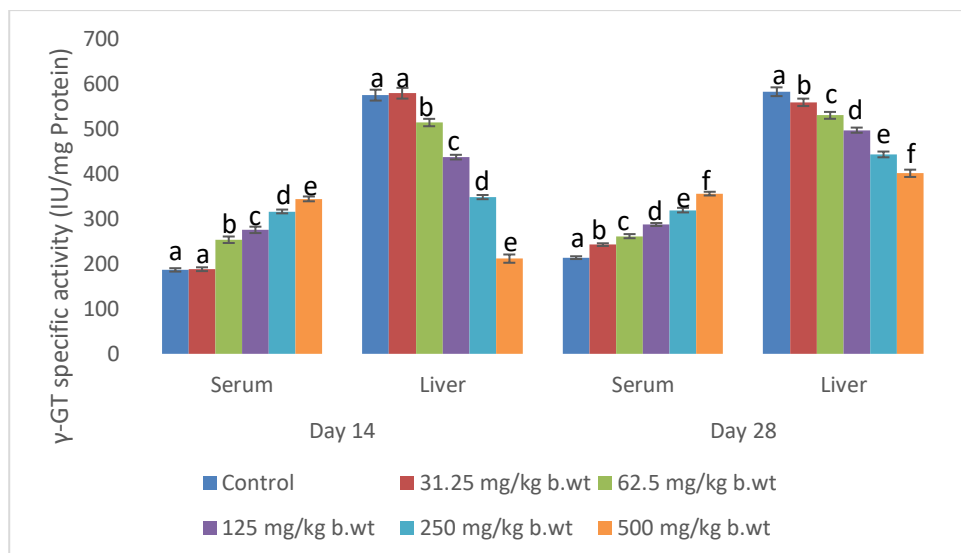


Figure 2: Effects of methanol leaf extract of *C. violaceum* on γ -glutamyltransferase activities serum and liver of mice. Values are means of 5 replicates \pm SD. Bars with different letters are significantly different ($p < 0.05$).

After 28 days of administration of extract, there was a significant increase ($p < 0.05$) in γ -glutamyl transferase activity in the serum at all doses and a significant decrease in the liver at all doses compared to controls (Figure 2).

γ -GT is present in the cell membranes of many tissues. It catalyzes the transfer of amino acids across the cellular membrane, and it is involved in leukotriene metabolism. It also plays a major role in glutathione metabolism (Dillon and Miller 2016). γ -GT is the most sensitive enzymatic indicator of hepato-biliary disease because it allows for differentiation of liver diseases from other conditions in which serum ALP activity is elevated since serum γ -GT activity is usually normal in those diseases (Caravaca-Fontán et al. 2017). The decrease in the activity of γ -GT in the liver throughout the study period (Figure 2) may be attributed to leakage of the enzyme from the liver to the serum through altered membranes or because of structural damage done to the liver by the extract as shown by the changes in the architecture of the liver of experimental animals throughout the study period (Plates 1 and 2); this will account for the corresponding increase in the serum ALP activity. These alterations may adversely affect the metabolism of glutathione and resorption of amino acids from the glomerular filtrate and intestinal lumen.

Aspartate Aminotransferase (AST)

There was significant decrease ($p < 0.05$) in AST activity in the liver at the doses of 250 and 500 mg/kg body weight and at 500 mg/kg body weight in the serum compared to controls after 14 days of administration of extract (Figure 3). There was, however, a significant increase ($p < 0.05$) in AST activity in the serum at the other doses compared to control after 14 days of extract administration (Figure 3).

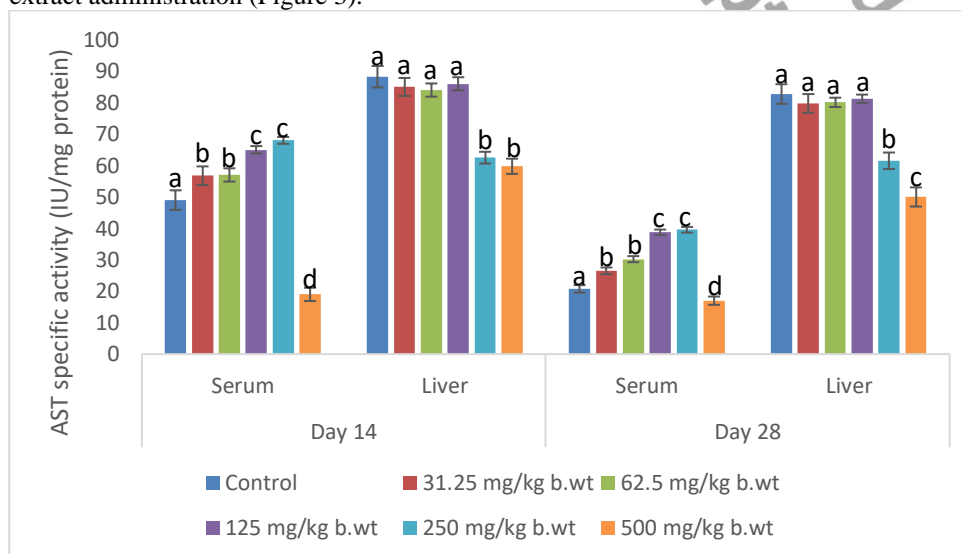


Figure 3: Effects of methanol leaf extract of *C. violaceum* on serum and liver aspartate aminotransferase activity in mice. Values are means of 5 replicates \pm SD. Bars with different letters are significantly different ($p < 0.05$).

After 28 days of administration, there was a significant decrease ($p < 0.05$) in AST activity in the liver at doses higher than 125 mg/kg body weight and at the dose of 500 mg/kg in the serum compared to controls. Its activity in the serum at the other doses was significantly increased ($p < 0.05$) compared to controls (Figure 3).

Alanine Aminotransferase (ALT)

There was significant increase ($p < 0.05$) in ALT activity in the liver and serum at the doses of 62.5, 125 and 250 mg/kg body weight after 14 days of extract administration compared to control (Figure 4). There was, however, significant decrease ($p < 0.05$) in its activity in the liver and serum at the dose of 500 mg/kg body weight compared to controls (Figure 4).

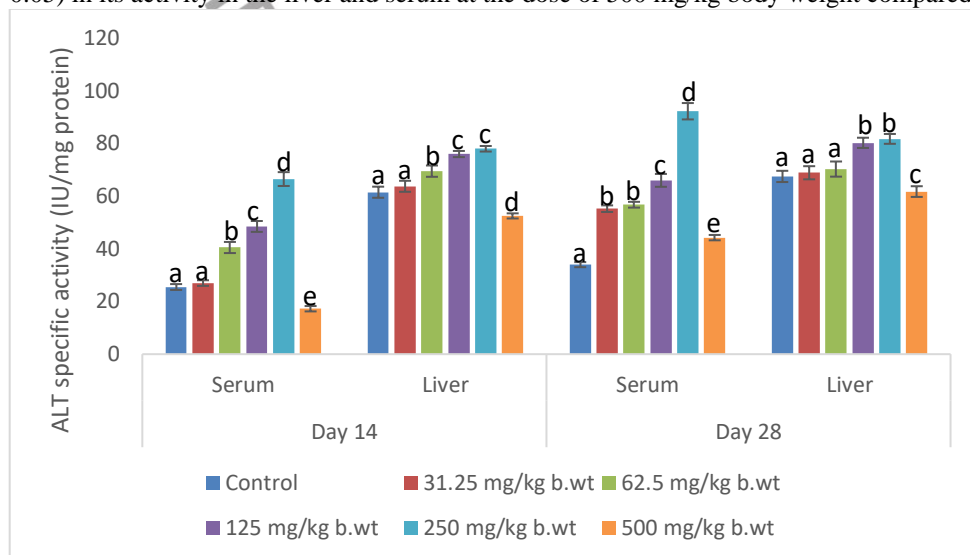


Figure 4: Effects of methanol leaf extract of *C. violaceum* extract on alanine aminotransferase activity in serum and liver of mice. Values are means of 5 replicates \pm SD. Bars different letters are significantly different ($p < 0.05$).

After 28 days of extract administration, there was significant increase in ALT activities at the doses of 125 and 250 mg/kg body weight in the liver and at the lower doses in the serum compared to controls (Figure 4). There was a significant decrease ($p < 0.05$) in the activity of the enzyme at 500 mg/kg body weight in the liver compared to control.

AST and ALT are two closely related enzymes of clinical significance in assessment of liver function. They are normally localized within the cells of the liver, heart, kidney, gills, muscles, and others. They are sensitive indicators of hepatocellular damage which can provide a quantitative evaluation of the extent of damage to the liver within limit (Shrestha et al. 2021). The decrease in activity of AST in the liver may have resulted from leakage of the enzyme from the liver at higher doses into the extracellular fluid due to liver cell membrane damage; it may also have resulted from the inactivation of the enzyme *in situ* by extract components at higher doses since its activity in the serum also decreased at the highest dose. Increased inflammatory cells and haemorrhagic necrosis (Plate 2) observed in the liver could have affected its functionality, thus leading to poor plasma clearance of the enzymes. The significant increase in ALT activities in the liver at the doses observed throughout the study period (Figure 4) indicated that the extract may have stimulated increased synthesis of the enzyme *de novo*, which could be an adaptation mechanism by the liver to offset the stress imposed on it by the extract components thus leading to a higher-than-normal activity (Amiragbaya et al. 2021). The reduction in ALT activity at 500 mg/kg body weight in the liver throughout the experimental period suggests that the rate of synthesis must have reduced or it has been inactivated by extract components at the highest dose since the serum activity was also reduced (Figure 4). Injury to the liver intensifies membrane permeability of the parenchyma cell, and consequently, the activity of AST and ALT in serum increases (Wang et al. 2011).

Organ-Body Weight Ratio

The effects of methanol leaf extract of *C. violaceum* on organ-body weight ratios of mice liver after 14 and 28 days of extract administration is shown in Table 3. There was no significant alteration ($p > 0.05$) in the organ-body weight ratios of the liver at all doses administered throughout the study period except at the dose of 500 mg/kg body weight on day 28 which was significantly increased compared to control (Table 3).

Table 3: Effects of Methanol Leaf Extract of *C. violaceum* on Organ-Body Weight Ratios of Mice Liver after 14 and 28 days of Administration

Treatment	Day 14 ($\times 10^{-2}$)	Day 28 ($\times 10^{-2}$)
Control
31.25 mg/kg b.wt
62.5 mg/kg b.wt
125 mg/kg b.wt
250 mg/kg b.wt
500 mg/kg b.wt

Control	3.74±0.07 ^a	3.61±0.08 ^a
31.25 mg/kg b. wt	4.68±0.02 ^a	3.74±0.07 ^a
62.5 mg/kg b. wt	4.88±0.06 ^a	4.72 ±0.04 ^a
125 mg/kg b. wt	4.23±0.05 ^a	4.77± 0.03 ^a
250 mg/kg b. wt	4.98±0.03 ^a	4.74 ±0.02 ^a
500 mg/kg b. wt	5.09±0.04 ^a	5.99 ±0.06 ^b

Values are means of 5 replicates ± SD. Means in the same column with the same superscripts are not significantly different ($p>0.05$).

An increase in organ-body weight ratio is an indication of inflammation while a decrease maybe due to cell constriction (Nova 2022). The general absence of any significant alteration on liver-body weight ratios of the mice is an indication that the extract did not adversely affect the size of these organs in relation to the weight of the animals. However, the increased liver-body weight ratio at the dose of 500 mg/kg body weight on day 28 (Table 3) suggests inflammation of the liver because of prolonged extract ingestion.

Histopathological Studies

Histopathological investigation of the liver was done for all experimental groups on days 14 and 28 after extract administration. There was a progressive vacuolar degeneration in the liver of mice at all doses on day 14 compared to control (Plate 1). There was progressive vacuolar degeneration in the liver of mice administered 31.25 and 62.5 mg/kg body weight extract on day 28 compared to control (Plate 2). The animals administered 125 mg/kg body weight of extract had mild vacuolar degeneration of the liver with focal areas of lobular lymphocytic infiltration compared to control (Plate 2). The mice treated with 250 mg/kg body weight of extract had moderate vacuolar degeneration of the liver with focal necrosis of hepatocytes (Plate 2); while animals administered 500 mg/kg body weight extract had extensive haemorrhagic necrosis of the liver compared to control (Plate 2).

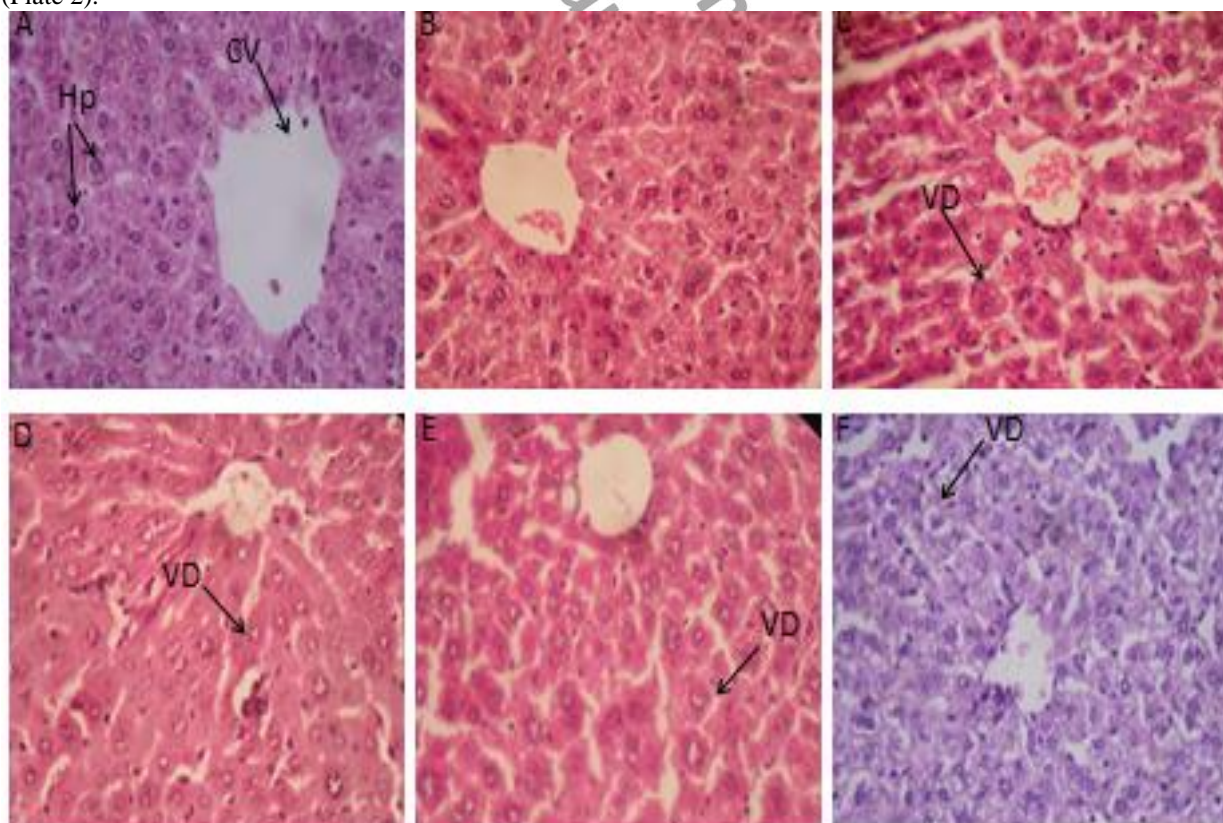


Plate 1: Photomicrographs of the livers of mice administered various doses of methanol leaf extract of *C. violaceum* for 14 days. A, B, C, D, E and F: Control, 31.25, 62.5, 125, 250 and 500 mg/kg b. wt respectively (H and E x400). CV=Central vein, VD=areas with vacuolar degeneration, Hp=normal hepatocytes

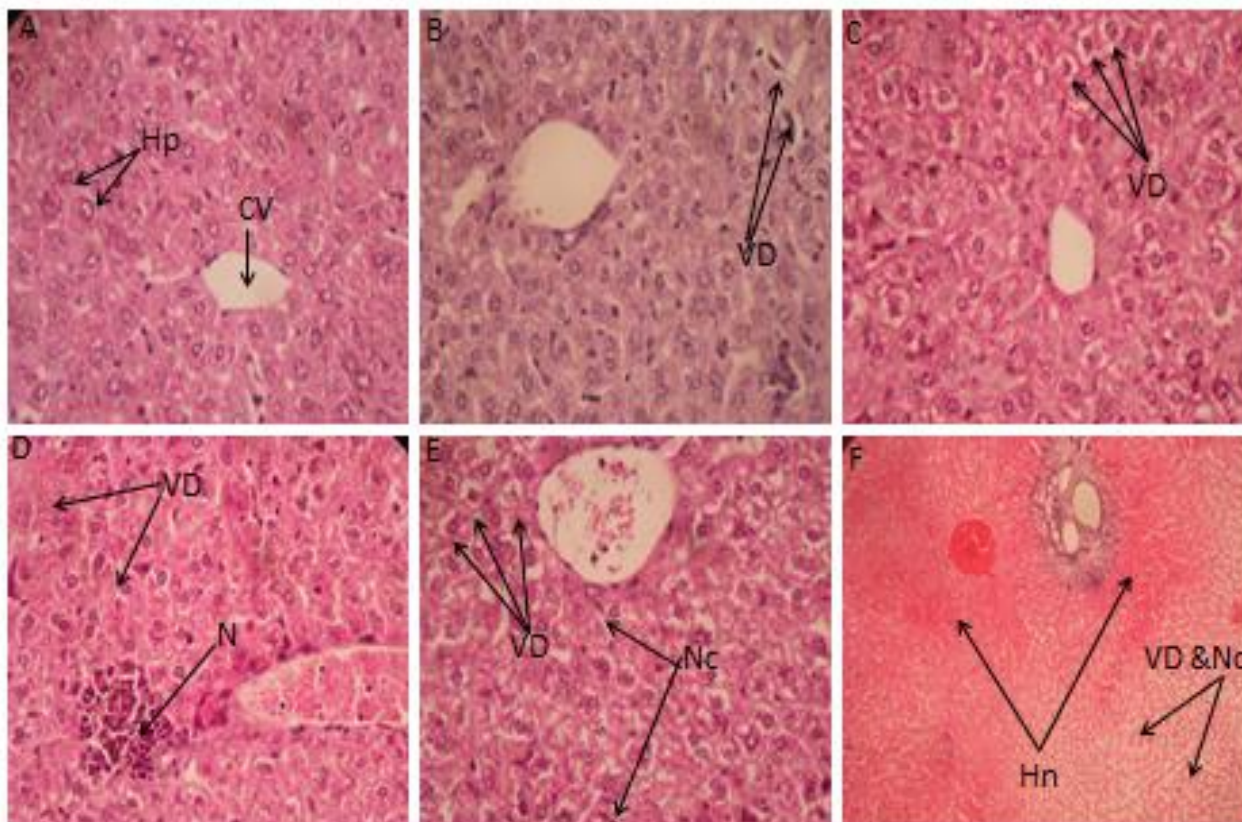


Plate 2: Photomicrographs of the livers of mice administered various doses of methanol leaf extract of *C. violaceum* for 28 days. A, B, C, D, E and F: Control, 31.25, 62.5, 125, 250 and 500 mg/kg b. wt respectively (H and E x400). CV=Central vein, VD=areas with vacuolar degeneration, Hp=normal hepatocytes, N= neutrophils, Nc=necrosis, Hn=haemorrhagic necrosis.

The measurement of biomolecules in serum and tissue homogenates can indicate tissue damage before it becomes apparent in histopathological screening. The changes in the normal architecture of the liver at higher doses and the presence of inflammatory cells, mainly neutrophils (Plates 1 and 2) suggest that the extract had adverse effects on structure and function of the liver. Neutrophils are characteristically present in the early stages of inflammation (Margraf et al. 2022). There was also degeneration and hemorrhagic necrosis of hepatocytes at higher doses suggesting the toxicity at these doses over a long period of usage.

Conclusion

The results of this study showed that the administration of methanol leaf extract of *C. violaceum* adversely affect the normal architecture, synthetic and secretory functions of the liver of experimental animals. This effect was more pronounced at higher doses of the extract when given for a longer period. Therefore, caution should be exercised when using the decoction of the leaves of this plant especially in large quantities and/or for prolonged periods as this may predispose to adverse effects on the liver.

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Journal of Experimental and Molecular Biology
Accepted manuscript - 2023