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Hepatotoxic and haematotoxic potentials of aqueous extract of *Cissus populnea* whole stem in albino rats

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Abstract
Toxicity of aqueous stem extract of *Cissus populnea* (ASECP) on the liver and blood of albino rats was evaluated in this research. Eighty-four (84) male albino rats were divided into three groups that were treated for 7, 14 and 21 days. Each of the groups were divided into 4 of seven rats each (i.e. A – D). Group A received distilled water only and served as the control while groups B, C and D received 100, 200 and 400 mg/kg dose of the extract respectively. Animals were sacrificed 24 hours after the last extract administration and their liver and blood collected for biochemical analysis. Secondary metabolite analysis revealed the presence of flavonoids, phenolics, saponins and terpenoids among others, with flavonoids (131.95 mg/g) and phenolics (172.47 mg/g) especially in abundance. Administration of ASECP significantly decreased ($p < 0.05$) liver aspartate transaminase (AST) and alanine transaminase (ALT) activities, serum total protein and albumin concentrations, red blood cell (RBC), packed cell volume (PCV), haemoglobin (Hb), mean cell haemoglobin (MCH) and monocytes but significantly increased ($p < 0.05$) serum AST and ALT activities and white blood cell (WBC). Histopathological examination of the liver of the animals also revealed enlargement of hepatocytes, diffusion of nuclei within hepatocytes, distortion of hepatocyte radial arrangement and dilution of sinusoids following extract administration. Available results suggest that administration of ASECP at the doses examined can induce alterations in liver function and blood parameters and as such should be used with caution.

Keywords: Hepatotoxicity; Haematotoxicity, *Cissus populnea*; Apartate transaminase; Haemoglobin

INTRODUCTION

Traditional medicine over the years has revealed many ethnobotanicals with therapeutic effects on diverse diseases of man and his livestock [1]. The medicinal properties of these plants have been attributed to their phytochemical constituents, which include secondary metabolites, minerals, vitamins, amino acids, and essential oils [2-4]. However, these plants, along with their therapeutic propensities, possess toxicity tendencies, which is normally overlooked by traditional medicine practitioners. The toxicity of xenobiotics on animal tissues is widely reported in literature. The liver, which is central to the metabolism and detoxification of foreign compounds, is most liable to xenobiotic toxicity [5]. Since the liver is often exposed to the highest concentrations of orally consumed drugs, it is not surprising

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that the liver is often the target organ with ensuing drug-induced liver injury (DILI). DILI is a major challenge for the pharmaceutical industry and public health, since DILI is a common cause of drug development termination, drug restrictions, and post-marketing drug withdrawal [6]. Similarly, the blood has also been reported to be very sensitive to chemical toxicity and the physiological and pathological status of man and animals following contact with a foreign compound can be easily detected through the measurement of blood parameters [7]. Haematotoxicity is an important concern in the administration of pharmaceuticals including many medicinal plant extracts. For example, dapsone (used to treat leprosy) and primaquine (used to treat malaria) can produce a fatal hemolytic anemia in certain genetically predisposed individuals [8]. Ethanolic leaf extract of *Gongronema latifolium*, aqueous stem extract of *Bulbine natalensis* and methanolic leaf extract of *Vernonia lasiopus* among others have been reported to impact negatively on haematological parameters of experimental animals [9-11].

Cissus populnea Guill & Perr belongs to the family Amplidaceae (Vitaceae). It is a semi-climber that grows to 2 to 3 m high and is widely distributed in Senegal, Sudan, Uganda, Abyssinia and Nigeria [12]. *C. populnea* is known in different parts of Nigeria as 'Okoho' (Idoma, Igbo and Igala) 'Dafara' or 'latutuwa' (Hausa) and 'Ajara' or 'Orogbolo' (Yoruba) [13]. This plant, which is used as laxative, cathartic, aphrodisiac and antidote to arrow wounds, also treats sore breasts, indigestion, venereal diseases and intestinal parasites among other ethnomedicinal uses [14]. The stem bark has been reported to contain carbohydrates, tannins, cyanogenic glycosides, anthraquinones, saponins, cardiac glycosides and flavonoids [15-17] reported the extraction of anthraquinones from the stem bark of the plant while it was also reported [18] that *C. populnea* ameliorated flutamide-induced testicular defects. Despite the many medicinal uses of *C. populnea*, there is scarcity of information on its toxicity, which is partly addressed by this work.

EXPERIMENTAL

Experimental animals. Eighty-four male albino rats (average weight 150 ± 10 g) were obtained from the small animal holding unit of the Department of Biochemistry, University of Jos, Nigeria. The rats were housed in well ventilated cages, allowed to acclimatize to housing conditions for 7 days before commencement of experiment and fed with normal rat pellet and tap water throughout the experiment.

Plant material. *Cissus populnea* was collected from Makurdi, Benue State, Nigeria. It was identified at the Department of Plant Science and Biotechnology, Faculty of Natural Sciences, University of Jos, Nigeria. Authentication of plant was done at the Department of Plant Biology, University of Ilorin, Nigeria, where a specimen was deposited and voucher number UILH/001/1019 issued.

Assay kits and reagents. Assay kits for aspartate aminotransferase, alanine aminotransferase, albumin and bilirubin were products of Randox Laboratories LTD, United Kingdom while that of total protein was a product of Fortress diagnostics, United Kingdom. All other reagents were of analytical grade and were prepared in all glass-distilled water.

Methods

Preparation of aqueous stem extract of *Cissus populnea*. *C. populnea* stem was cut into pieces and air-dried to constant weight. The pieces were then pulverized using an electric blender (Super Master® Model SMB-2977). The resulting powder (500 g) was percolated in 3 liters of distilled water, stirred

properly and kept in the refrigerator for 48 hours for proper extraction. The mixture was thereafter filtered using a fine sieve and the filtrate lyophilized (SJIA-18N Branch Manifold Model). The resulting concentrate was weighed to be 40.4 g (i.e. a percentage yield of 8.1 %) and reconstituted to doses (i.e.100, 200 and 400 mg/kg) used in this study.

Secondary metabolites screening and quantification. The prescribed methods from literature were used to determine alkaloids, tannins, anthraquinones, phlobatannins and glycosides [19]; Steroids and terpenoids [20]; saponins [21]; flavonoids and phenols [22]. Alkaloids, saponins, tannins, flavonoids, terpenoids, glycosides, phenols and anthraquinones were quantified using the reported methods [23-29].

Determination of toxicological parameters. Aspartate aminotransferase and alanine aminotransferase activities were assayed by standard methods [30]. Other methods [31-33] were, respectively used to assay total protein, albumin and bilirubin concentrations.

Haematological analysis. Blood was collected from the jugular veins of the animals into EDTA-coated sample bottles 24 hours after extract administration for 7, 14 and 21 days. Blood sample (50 µl) was aspirated into 80 mm high tubes and haematological parameters (RBC, PCV, Hb, MCH, MCHC, MCV, WBC, monocytes, neutrophil and lymphocytes) were determined using automated haematologic analyser [sysmex KX-21 (Japan)].

Histopathological studies. The liver was examined for histological changes using standard procedures [34].

Statistical analysis. Data were expressed as mean (of 7 replicates) ± SEM. Data was subjected to statistical analysis using the IBM® statistical package for social sciences (SPSS) software (version 20). All significant differences were determined by one-way analysis of variance (ANOVA) and post hoc multiple comparison was done using Duncan's multiple range test. The significance level was set at $p < 0.05$.

RESULTS

Secondary metabolite composition of *C. populnea* stem. The concentrations of some secondary metabolites present in *Cissus populnea* stem is presented in Table 1. The stem is abundant in flavonoids (131.95 mg/g) and phenolics (172.47 mg/g). Saponins (28.6 mg/g) and terpenoids (24.04 mg/g) are also present in considerable concentrations. Phlobatannins and steroids were however not detected.

Liver function indices. Figures 1-4 represent activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in the liver and serum of rats treated with ASECP respectively. Extract significantly decreased ($p < 0.05$) liver AST and ALT activities on days 7 and 21 when compared with the control, which was followed by a concomitant significant increase ($p < 0.05$) in serum AST and ALT activities respectively when compared with control.

Shown in figures 5 and 6 respectively are the concentrations of serum total protein and albumin of rats treated with ASECP. The extract significantly reduced ($p < 0.05$) serum total protein concentration on day 1 (400 mg/kg dose) and day 7 (all the doses) while serum concentration of albumin was also significantly reduced ($p < 0.05$) on day 7 (all the doses) and day 21 (400 mg/kg) when compared with control.

Figures 7 and 8 represent serum total and conjugated bilirubin concentrations in rats administered ASECP respectively. There was no significant difference ($p < 0.05$) in the concentrations of serum total and conjugated bilirubin in the rats treated with the extract when compared with the control.

Haematological parameters

Erythrocyte indices. The red blood cell (RBC) and packed cell volume (PCV) of rats administered ASECP are presented in figures 9 and 10. Extract (all doses) on day 7 caused a significant reduction ($p < 0.05$) in RBC when compared with the control while only the animals treated with the 400 mg/kg body weight dose of the extract on day7 showed a significant reduction ($p < 0.05$) in PCV when compared with the control. The haemoglobin (Hb) and mean corpuscular haemoglobin (MCH) of rats administered ASECP are presented in figures 11 and 12 respectively. Extract similarly induced a significant reduction ($p < 0.05$) in Hb on days 1 and 7 (400 mg/kg dose) and also on day 21 (200 and 400 mg/kg doses) when compared with the control. MCH was also significantly reduced ($p < 0.05$) in the extract-treated animals on days 7 and 21 at all doses when compared with control. Figures 13 and 14 represent the mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) in rats administered ASECP respectively. There was no significant difference ($p < 0.05$) in MCHC and MCV of the extract-treated animals when compared with the control.

Leucocyte indices. Shown in figures 15 and 16 respectively are white blood cell (WBC) and monocytes of rats treated with ASECP. The extract at 400 mg/kg dose on day 7 significantly increased ($p < 0.05$) WBC when compared with the control while monocytes were significantly decreased on day 1 and 7 (at all doses) and day 21 (100 mg/kg) when compared with control. Figures 17 and 18 represent the neutrophil and lymphocytes in rats administered with ASECP respectively. There was no significant difference ($p < 0.05$) in neutrophil and lymphocytes of all the extract-treated animals when compared with the control.

Histopathological studies. The photomicro graphs of rat liver following administration of ASECP are presented in plates 1-3. Administration of the extract led to massive enlargement of hepatocytes, diffused nuclei within the hepatocytes, distortion of radial arrangement of hepatocytes and dilation of sinusoids.

DISCUSSION

Plants since ancient times are known to possess therapeutic effects against diverse diseases of mankind and livestock which have been attributed to the presence of secondary metabolites like alkaloids, saponins, phenolics and tannins synthesized by such plants [1].

Table 1: Secondary metabolites composition of *C. populnea* stem

Secondary metabolites	Concentration (mg/g)
Saponins	28.60 ± 0.05
Alkaloid	16.20 ± 0.02
Tannins	7.46 ± 0.02
Flavonoids	131.95 ± 1.80
Terpenoids	24.04 ± 0.09
Glycoside	9.60 ± 0.01
Phenolics	172.47 ± 0.51
Anthraquinones	6.30 ± 0.02
Phlobatannins	Not detected
Steroids	Not detected

Data presented as mean of three replicates ± SD

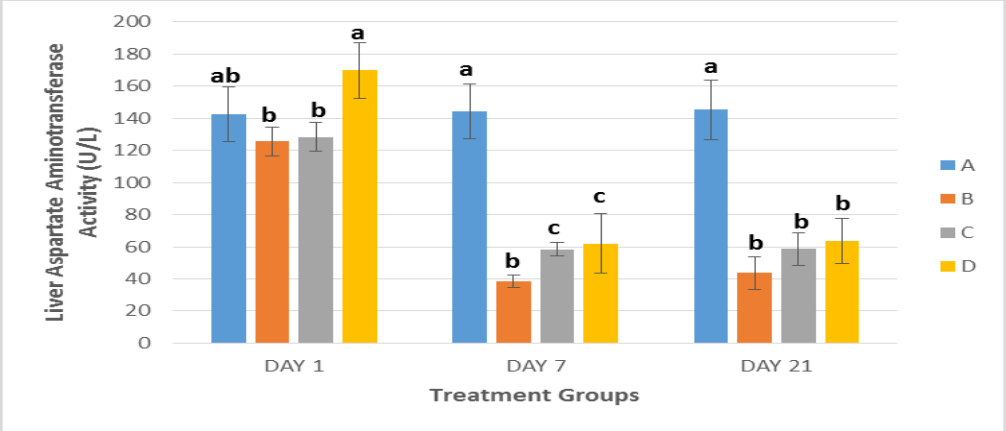

Figure 1: Liver aspartate aminotransferase activity in rats treated with aqueous extract of *Cissus populnea* stem
For this and subsequent figures: Data represent mean of seven replicates ± SEM. Bars with different superscripts are significantly different at $p<0.05$, A = Control, B= Extract (100mg/kg), C= Extract (200mg/kg), D= Extract (400mg/kg)

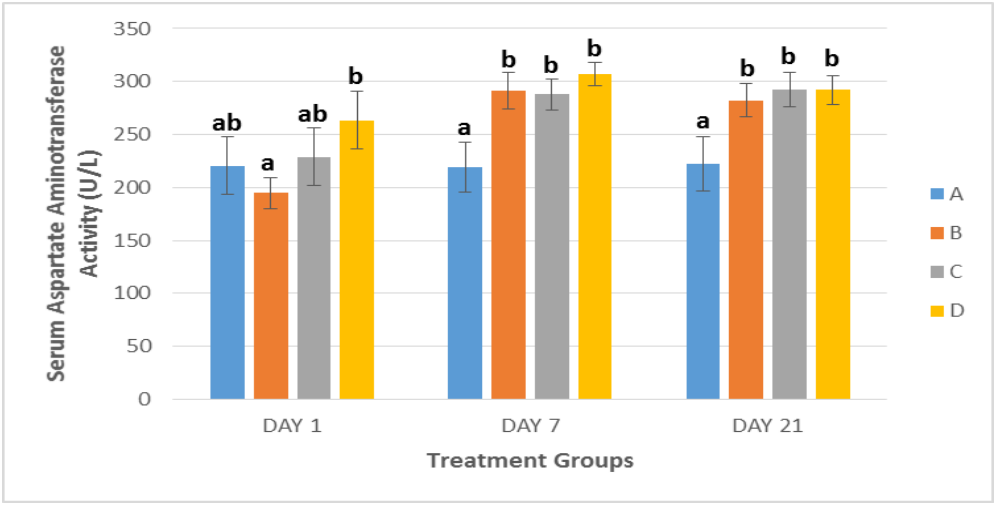

Figure 2: Serum aspartate aminotransferase activity in rats treated with aqueous extract of *Cissus populnea* stem

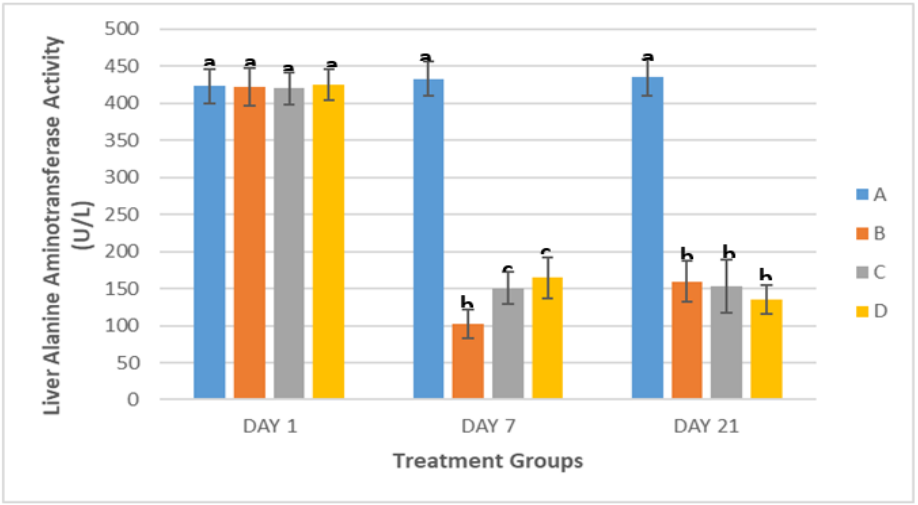

Figure 3: Liver alanine aminotransferase activity in rats treated with aqueous extract of *Cissus populnea* stem

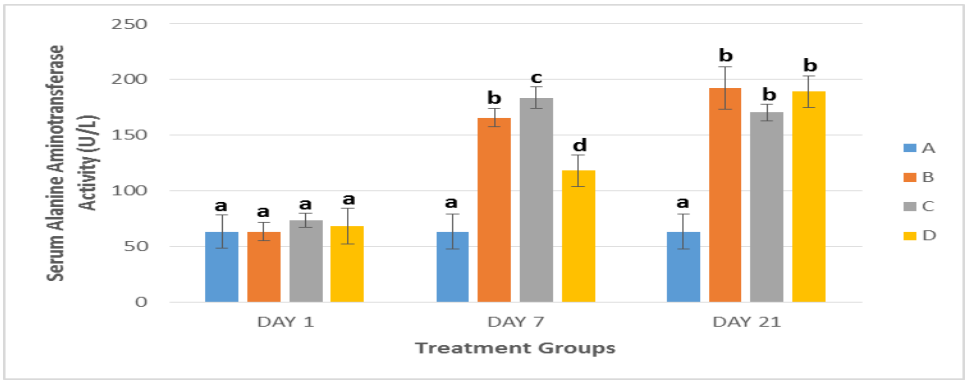

Figure 4: Serum alanine aminotransferase activity in rats treated with aqueous extract of *Cissus populnea* stem

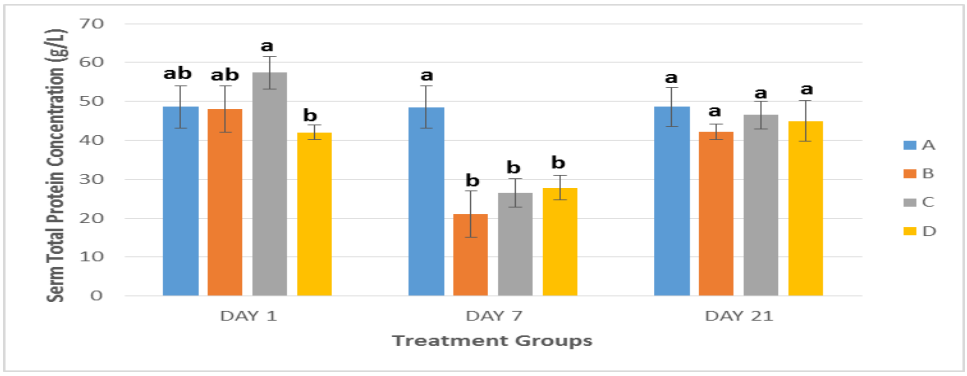

Figure 5: Serum total protein concentration in rats treated with aqueous extract of *Cissus populnea* stem

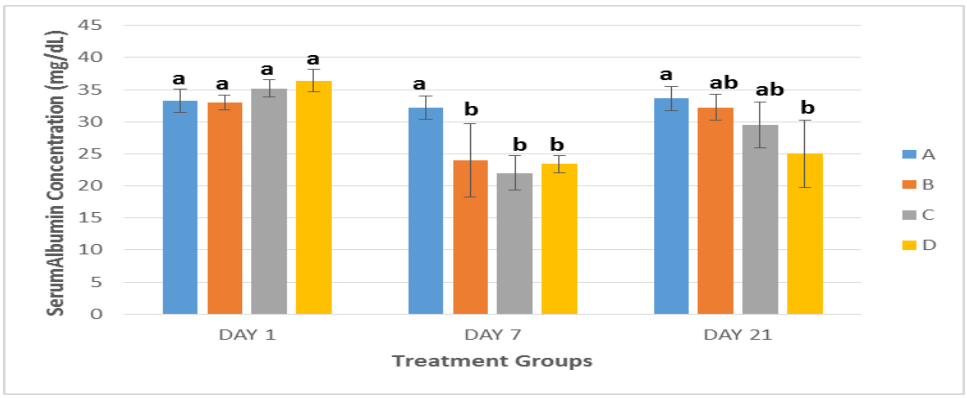

Figure 6: Serum albumin concentration in rats treated with aqueous extract of *Cissus populnea* stem

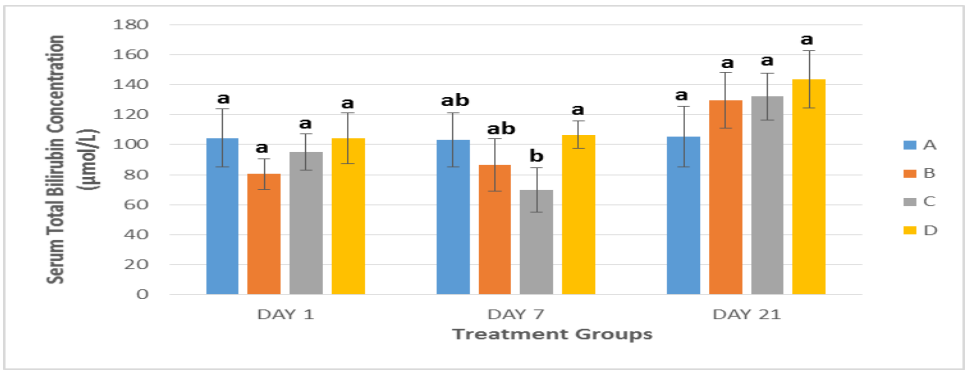

Figure 7: Serum total bilirubin concentration in rats treated with aqueous extract of *Cissus populnea* stem

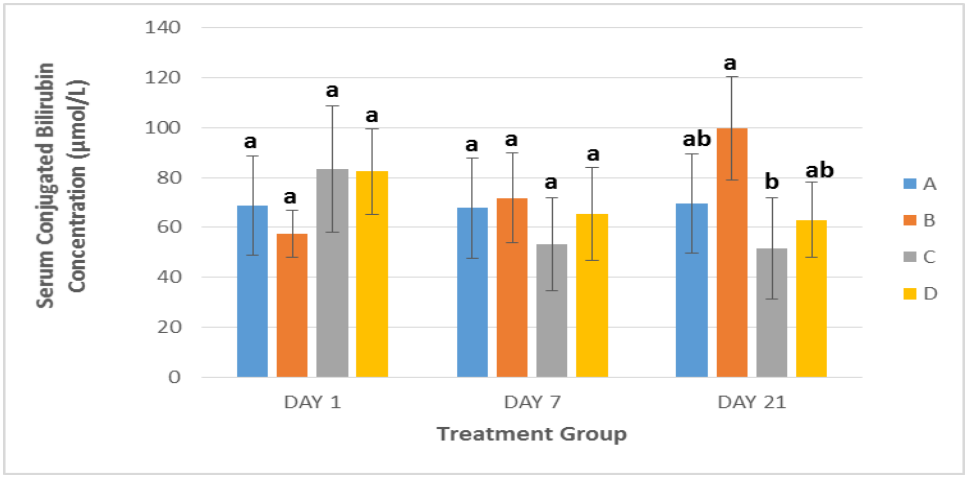

Figure 8: Serum conjugated bilirubin concentration in rats treated with aqueous extract of *Cissus populnea* stem

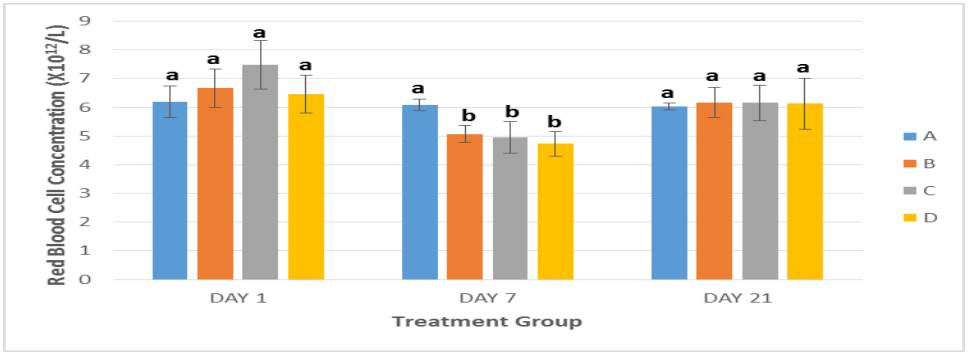

Figure 9: Red blood cell in rats treated with aqueous extract of *Cissus populnea* stem

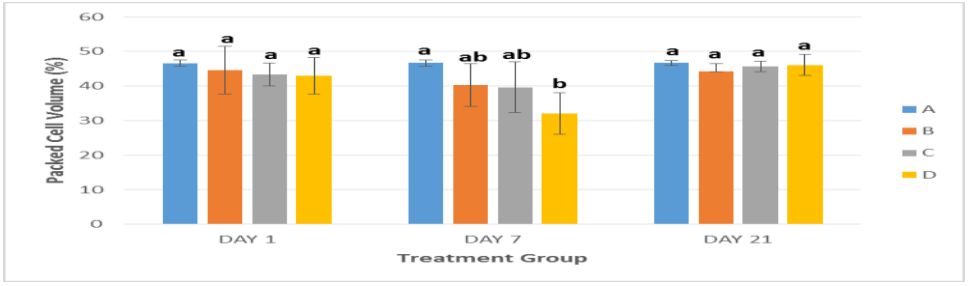

Figure 10: Packed cell volume in rats treated with aqueous extract of *Cissus populnea* stem

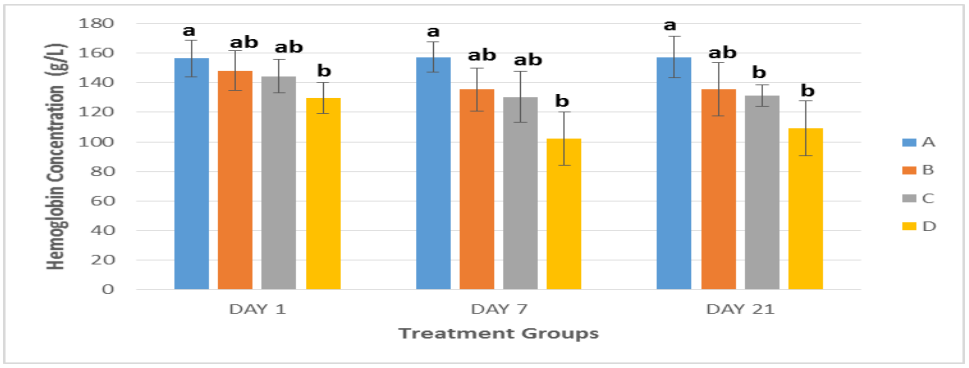

Figure 11: Haemoglobin in rats treated with aqueous extract of *Cissus populnea* stem

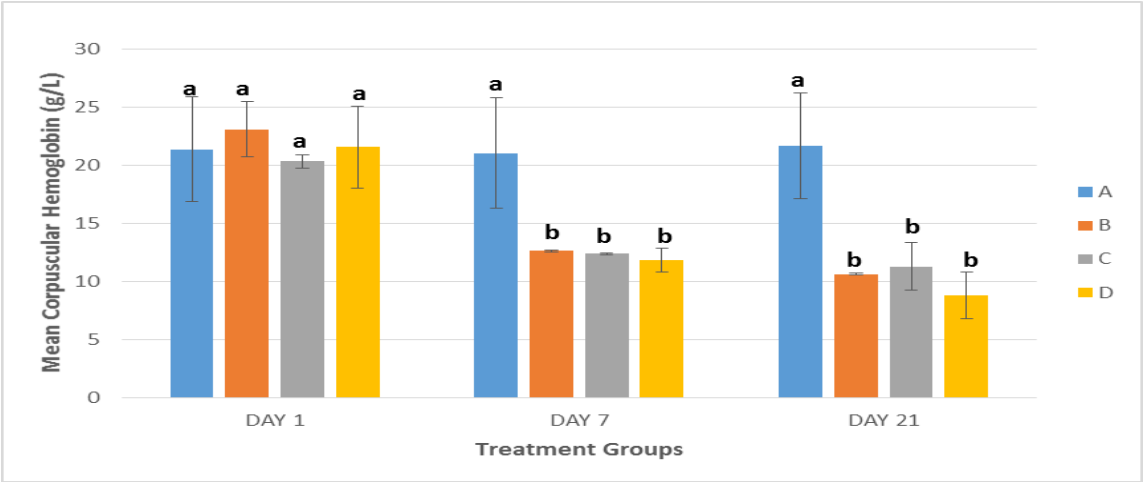

Figure 12: Mean corpuscular haemoglobin in rats treated with aqueous extract of *Cissus populnea* stem

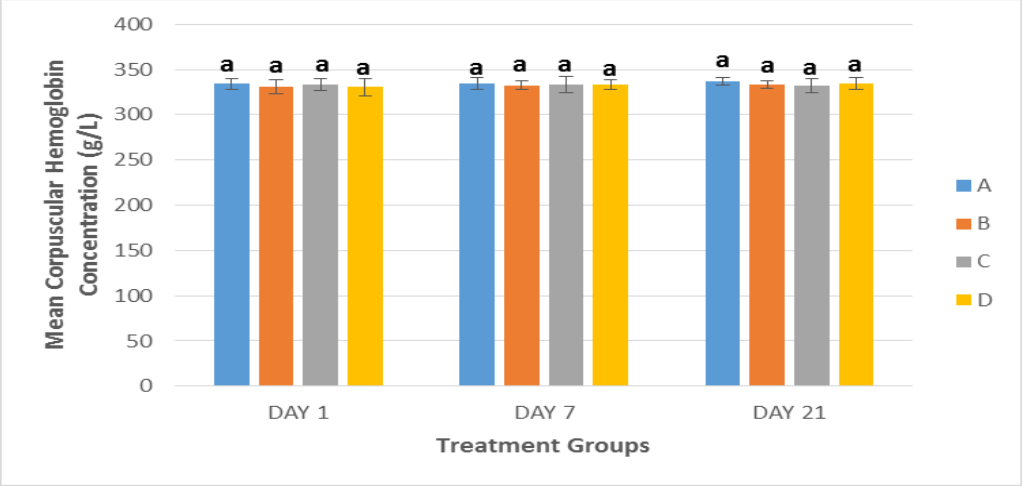

Figure 13: Mean corpuscular haemoglobin concentration in rats treated with aqueous extract of *Cissus populnea* stem

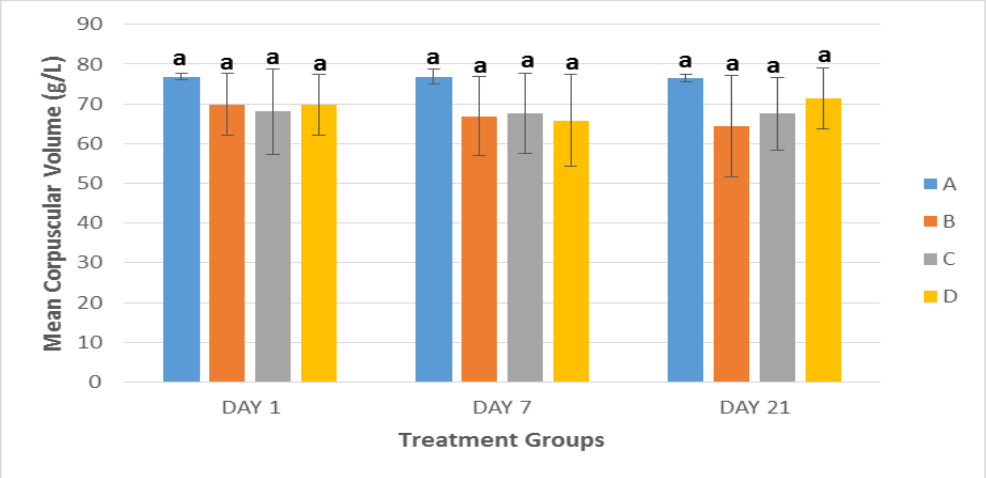

Figure 14: Mean corpuscular volume in rats treated with aqueous extract of *Cissus populnea* stem

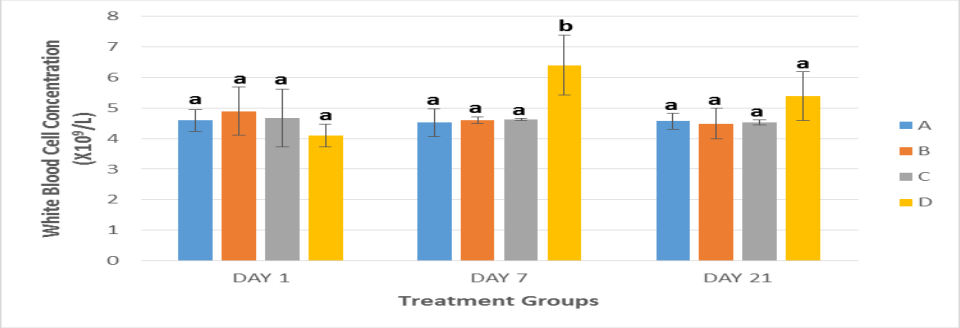

Figure 15: White blood cells in rats treated with aqueous extract of *Cissus populnea* stem

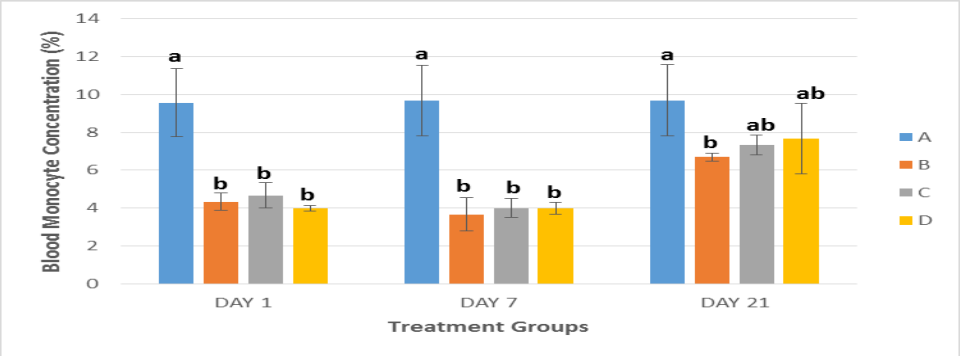

Figure 16: Monocytes in rats treated with aqueous extract of *Cissus populnea* stem

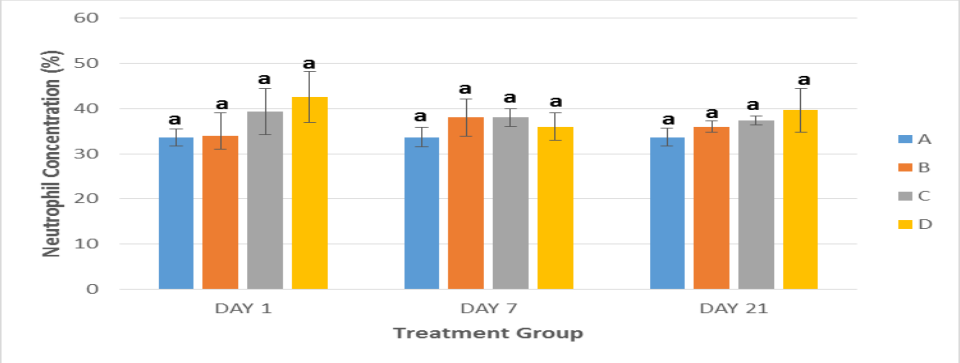

Figure 17: Neutrophil in rats treated with aqueous extract of *Cissus populnea* stem

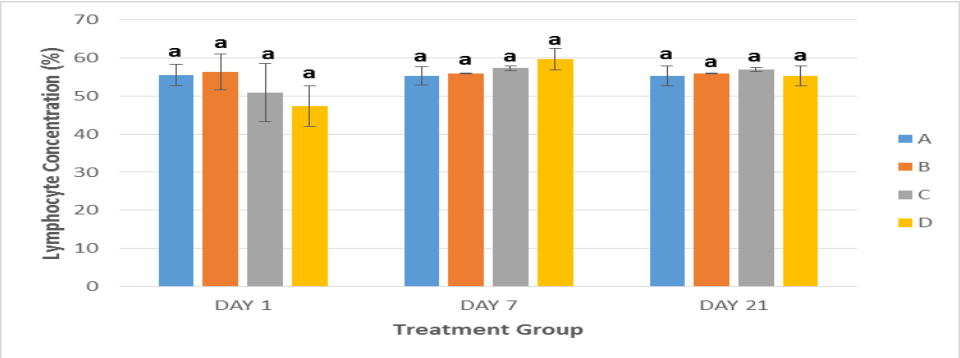

Figure 18: Lymphocyte in rats treated with aqueous extract of *Cissus populnea* stem

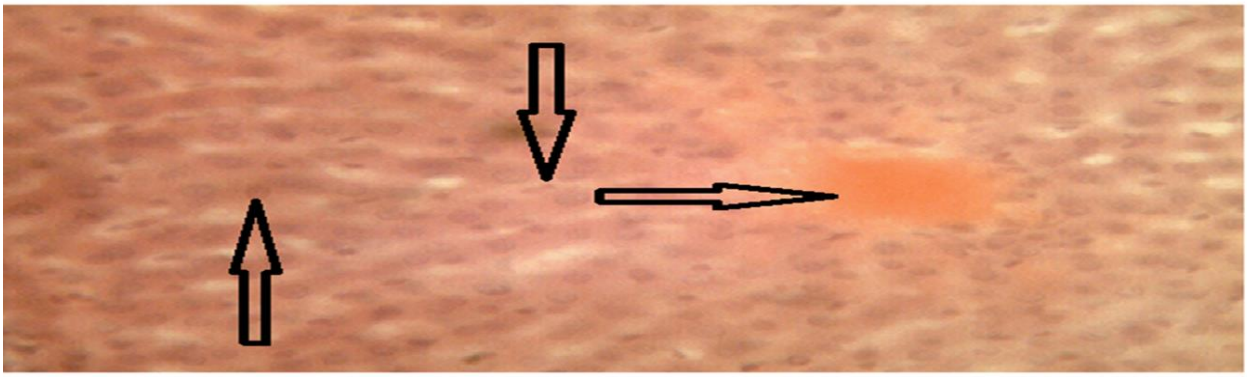

Plate 1: Cross section of liver of control rat administered distilled water (H&E; X400), up arrow: normal nucleus within the hepatocyte, down arrow: normal radial arrangement of hepatocytes, right arrow: red blood cells within the central vein

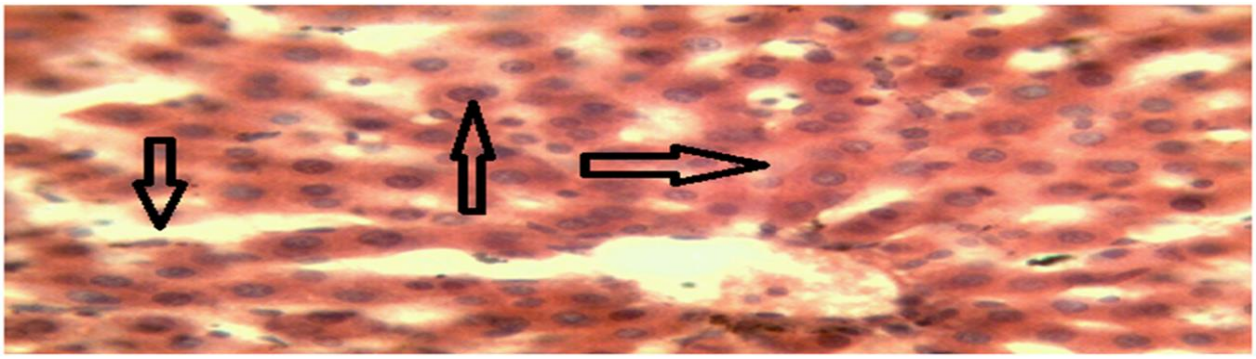

Plate 2: Cross section of liver of rat administered 100 mg/kg body weight dose of aqueous stem extract of *C. populnea* for 21 days (H&E; X400), up arrow: massive enlargement of hepatocytes, right arrow: distortion of radial arrangement of hepatocytes, down arrow: dilation of sinusoids

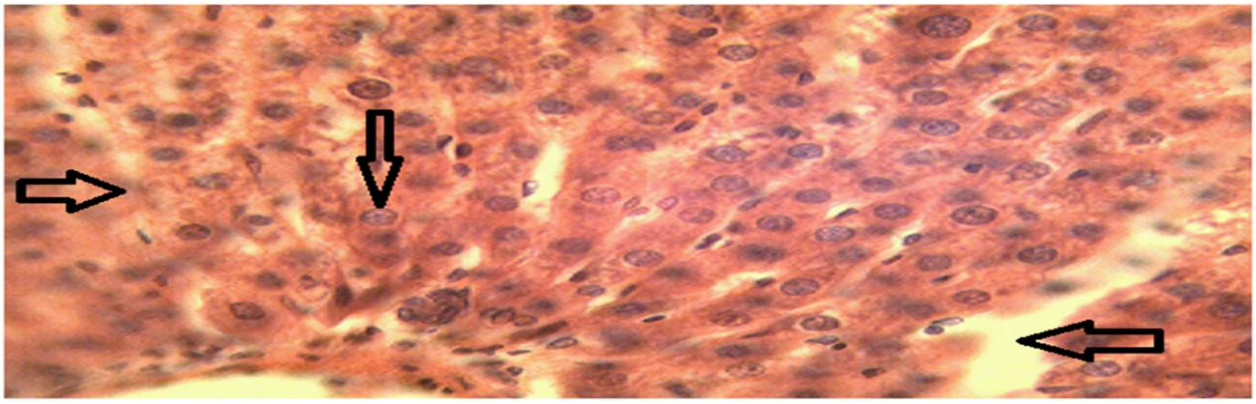

Plate 3: Cross section of liver of rat administered 400 mg/kg body weight dose of aqueous stem extract of *C. populnea* for 21 days (H&E; X400), right arrow: diffused nuclei within the hepatocytes, down arrow: massive enlargement of nuclei, left arrow: dilation of sinusoids

Despite the therapeutic potentials of plant secondary metabolites, many of them are also known to possess toxicity against many mammalian tissues and cells. Previous work [35] reported the absence of alkaloids, phenolics and free anthraquinones in the stem bark of *C. populnea*. All of these secondary metabolites were detected in the present study. Others [17] were also able to isolate anthraquinones from the stem bark of *C. populnea*, which gives credence to the presence of anthraquinones in the plant.

However, the variations in secondary metabolites reported may be attributed to the difference in plant part on which the secondary metabolite analyses was carried out as plant parts may differ in the contents and especially concentrations of secondary metabolites present in them. In addition, variation in plant phyto-constituents have also been attributed to time of harvest of plant and nutrient composition of soil on which plant grows [36].

ALT is a more specific marker of liver disease than AST. Nevertheless, its continued elevation alongside AST indicates rapid destruction of liver cells, which may advance to chronic liver disease [37]. The decreased liver activities of ALT and AST along with their simultaneous increase in the serum as recorded in this study, indicate derangement of hepatocytes cell membranes, which results into leakage of these enzymes into the extracellular fluid. This effect may be attributed to the presence of some phytochemicals in the stem extract and agrees with previous toxicity studies on plant extracts and constituent secondary metabolites [38,39]. Measurement of serum total protein and albumin predicts the status of liver synthetic functions. The temporary alteration of serum concentrations of total protein and albumin following acute exposure to the extract suggests a disturbance of liver synthetic functions. The results of the serum concentrations of total protein and albumin at 21 days revealed there was an attempt at recovery by the animals from the assault caused by the administered extract.

A low MCH may result following blood loss over time, haemoglobinopathy, low iron concentration in the body or microcytic anemia (presence of abnormally small red blood cells). Previous researches have also reported similar results following administration of plant extracts [10]. Since there was no physical blood loss by the animals throughout the experiment, *C. populnea* stem extract might have prevented normal absorption of iron from the provided feed in the gastrointestinal tract or caused microcytic anemia, which may explain the reduction in Hb observed in this study since abnormally small RBCs will not accommodate enough Hb.

The immune system is primarily made up of cells called leucocytes that provide either innate or specific adaptive immunity. There are four different classes of leucocytes namely; lymphocytes, granulocytes, monocytes, and natural killer cells [40]. The reason for the recorded increase in white blood cells in the animals that received the 400mg/kg body weight dose of the extract for 7 days is not understood, but might not be disconnected from an excessive stimulation of white blood cells production in the bone marrow. However, white blood cells level in the animals was restored to control level at 21 days. The reduction in monocytes recorded did not reflect in the total white blood cells count as monocytes only make up 4-8% of total white blood cells [41]. Monocytopenia (low monocytes number in blood) can be caused by anything that decreases the overall white blood cell count like bloodstream infection, chemotherapy or a bone marrow disorder. In this study, monocytes were selectively reduced with other leucocyte parameters not affected. The reason for this is not properly understood but appears the extract might contain a compound acting as a selective inhibitor of monocyte synthesis. Microscopic examination of tissues and cells is an important tool in anatomical pathology. Histopathological investigations can provide diagnostic information through tissue observations. The assault on the liver, which was presented as alterations of liver synthetic functions and derangement of hepatocyte membranes (as evidenced by increased serum activities and decreased liver activities of AST and ALT) corroborate the interpretation of liver sections.

Based on available results, we can conclude that ASECP possesses the ability to elicit toxic effects on the liver and also induce alterations of hematological parameters in albino rats. It is therefore recommended that caution should be applied in its use in traditional medicine.

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