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Full Length Research Paper

Effects of ethanolic leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters of albino Wistar rats

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The effect of oral administration of the leaf extract of *Chrysophyllum albidum* G. on biochemical and haematological parameters were investigated in albino rats for 16 days. The extract did not show any significant effect ($p > 0.05$) on the plasma concentrations of total bilirubin, albumin and alkaline phosphatase (ALP) as well as the packed cell volume (PCV), haemoglobin (Hb), red blood cell (RBC), reticulocytes, neutrophils, lymphocytes, eosinophils, basophils, mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH). The concentration of the platelets was significantly decreased ($p < 0.05$) at 1000 mg/kg body weight, while white blood cell (WBC) was significantly increased at 500 mg/kg body weight. The doses significantly reduced ($p < 0.05$) plasma levels of AST, ALT, total protein, glucose and creatinine while urea was significantly increased. While the extract significantly increased the lung, brain and liver-body weights, the kidney, heart, testis, spleen and epididymis-body weights were not significantly affected. The result suggests that the leaf extract of *C. albidum* contains antiplatelet and hypoglycemic properties and exhibited selective organ toxicity to the rats.

Key words: *Chrysophyllum albidum*, Sapotaceae, haematological parameters, biochemical indices.

INTRODUCTION

Medicinal plants are plants which contain substances that could be used for therapeutic purposes or which are precursors for the synthesis of useful drugs (Abolaji et al., 2007). Medicinal plants, since time immemorial have been used in virtually all cultures as a source of medicine. Over 5000 plants are known to be used for medicinal purposes in Africa, but only a few have been described or studied (Taylor et al., 2001). Natural products from plants can be another potent source for the discovery of excellent biological activities, that is: anticancer and antioxidant activities (Adebayo et al., 2010). *Chrysophyllum albidum*, from the Sapotaceae family, is commonly found in the Central, Eastern and Western Africa (Amusan et al., 2003). They are distributed in Nigeria, Uganda, Niger, Cameroun and Cote d' Ivoire (Adewusi, 1997). It is often called the white star apple and distributed throughout the southern part of Nigeria (Idowu et al., 2006). In South-western Nigeria, the fruit is called "agbalumo" and popularly referred to as "udara" in South-eastern Nigeria. *C. albidum* is a popular tropical fruit tree and widely distributed in the low land rain forest zones and frequently found in villages (Madubuike and Ogbonnaya, 2003). The roots, barks and leaves of *C. albidum* have been employed in folk medicine for the treatment of diseases. The bark is used for the treatment of yellow fever and malaria, while the leaf is used as an emollient and for the treatment of skin eruption, stomachache and diarrhea (Adisa, 2000; Idowu et al., 2006). The cotyledons from the seeds of *C. albidum* are used as ointments in the treatment of vaginal and dermatological infections in Western Nigeria. The fruit pulp is rich in vitamin C and iron and an excellent source of raw material for industries (Adisa, 2000; Akubugwo and Ugbogu, 2007). Tannins, flavonoids, terpenoids, proteins, carbohydates and resins are the phytochemicals that have been reported in *C. albidum* (Akaneme, 2008). Bioassay-guided fractionation of the

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methanolic extract of the cotyledons from the seeds of *C. albidum* led to the isolation of eleagnine, tetrahydro- 2 - methylharman and skatole. Eleagnine was found to be the main compound responsible for its antimicrobial activity (Idowu et al., 2003). Eleagnine was further shown to exhibit anti-nociceptive, anti-inflammatory and anti-oxidant activities (Idowu et al., 2006). The seed cotyledon has been reported to possess anti-hyperglycemic and hypolipidemic effects (Olorunnisola et al., 2008). Information on the safety potential of this plant is lacking, thus there will be need to evaluate the toxicity potential of this popularly used medicinal plant. The study therefore is aimed at providing information on the effects of the ethanolic leaf extract on biochemical and haematological parameters in albino Wistar rats.

MATERIALS AND METHODS

Plant material

The leaves of *C. albidum* G. were collected on February, 2009, in Abule-Egun of Lagos State, Nigeria and a voucher specimen (PCG 435) was deposited in the Department of Pharmacognosy, University of Lagos, Lagos, Nigeria. The leaves of the plant collected were air-dried for two weeks and then blended into powder.

Plant extraction

The method used was as described by Adebayo et al. (2006). The powdered leaves of *C. albidum* (778 g) were soaked in 7 L of 70% ethanol for four days, after which the extract was filtered using a Whatman no. 1 filter paper and a cotton wool. It was further concentrated at 50°C using a rotary evaporator and further concentrated using water bath at 48°C. The weight of the extract obtained was 67 g giving a percentage yield of 8.6%.

Experimental animals

Male albino rats (25) of Wistar strain obtained from Nigerian Institute of Medical Research (NIMR), weighing between 100 - 160 g were used and maintained under laboratory conditions: humidity, temperature (23 - 25°C) and light 12 h light/dark cycle in the Animal House of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria and were allowed free accesses to grower mash and water *ad libitum*. The animals were acclimatized for four weeks. All animals were treated in a manner that complied with the National Institutes of Health (NIH) Guidelines for the Care and Use of Laboratory Animals (NIH publication, 1985).

Experimental design

Twenty-eight male albino Wistar rats were used for the study. The rats were divided into four groups of seven rats per group. The crude extract was dissolved in normal saline (0.9% (w/v) NaCl) before the treatment. Groups 1, 2, 3 and 4 were used for the sub-chronic experiment. Groups 2, 3 and 4 were, respectively, administered with 250, 500 and 1000 mg/kg body weight of ethanolic leaf extract of *C. albidum* for 16 days via gastric intubation. Furthermore, animals in group 1 (control group) did not receive the extract, but was treated with normal saline. Animals were weighed within a three day interval during the period of administration. After this period, the rats were subjected to overnight fast. The rats were subsequently anaesthetized with diethyl-ether and blood sample was collected by cardiac puncture into EDTA and lithium heparin bottles. The heparinized samples were centrifuged at 3,000 x g for 10 min to obtain the plasma and stored at - 20°C until ready for analysis; while the whole blood samples were maintained at 4°C, the plasma glucose level was determined immediately.

Biochemical assays

Commercial test kits obtained from Roche Diagnostics, GmbH, Mannhein, Germany were used for all biochemical parameters measured using Uniscope 23D spectrophotometer, England. The following biochemical parameters were carried out in plasma: alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using Reitman and Frankel's (1957) method; alkaline phosphatase (ALP) was carried out using the phenolphthalein monophosphate method (Babson et al., 1966); total bilirubin was calculated using Doumas et al. (1973) method; total protein was determined using biuret method (Peters, 1968); glucose level was obtained using the enzymatic GOD-PAP method (Trinder, 1969); urea was analysed using the Bethlot Searcy's method (Searcy, 1967); creatinine was determined by the method described by Larsen (1971), and albumin was estimated by bromo cresol green (BCG) method (Doumas et al., 1971).

Haematological estimation

The haematological parameters (red blood cell count (RBC), haemoglobin (Hb), packed cell volume (PCV), white blood cell count (WBC), mean cell volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet (PLT) count, reticulocytes and WBC differentials (neutrophils, monocytes, lymphocytes, basophils and eosinophil) were analyzed according to the standard techniques described by Baker et al. (1998) and Cheesbrough (2000).

Statistical analyses

The differences among experimental and control groups were determined using SPSS for Window XP Software Programme (version 13.0). Group comparisons were done using the analysis of variance (ANOVA) test. Significant differences between control and experimental were assessed by least significant difference (LSD) and student's t-test. All data were expressed as mean ± SEM; p-values less than 0.05 were considered to be significant.

RESULTS

No deaths occurred in the period of treatment. No change in locomotor activity was observed. There was no significant change in body weights of the treated groups when compared to the control group (Figure 1). There was also no significant difference ($p > 0.05$) in the relative and absolute weights of kidney, testis, heart, spleen and epididymis during the treatment period. The relative weights (weights of organ with respect to the body weight) of the liver and lungs were significantly increased ($p < 0.05$) in the group treated with 1000 mg/kg body weight of the extract of *C. albidum*. The weight of brain was also significantly elevated ($p < 0.05$) in the groups

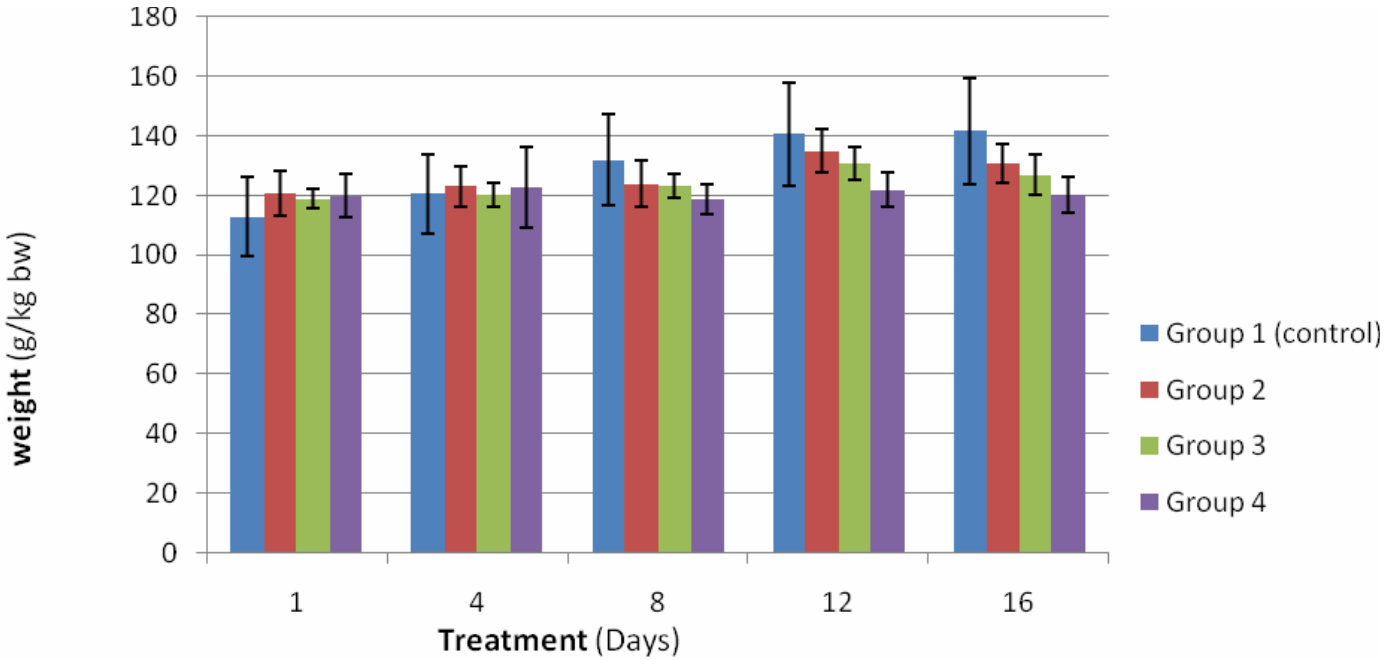

Figure 1. Effect of ethanolic leaf extract of *Chrysophyllum albidum* on weight of rats. Values represent mean ± SEM of 7 replicates.

treated with 500 and 1000 mg/kg body weight of the extract (Table 1). The ethanolic extract of *C. albidum* did not have any significant effect on RBC, Hb, MCHC, MCH, reticulocytes, neutrophils, basophils, monocytes, lymphocytes and eosinophils. While WBC was significantly elevated ($p < 0.05$) in the group treated with 500 mg/kg body weight, the platelet was significantly reduced ($p < 0.05$) in rats treated with 1000 mg/kg body weight (Table 2). Plasma glucose level was significantly reduced ($p < 0.05$) in the group administered with 500 mg/kg body weight. The extract did not exact any significant effect ($p > 0.05$) on alkaline phosphatase (ALP), total bilirubin and albumin. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) and creatinine were significantly decreased ($p < 0.05$) in all the treated groups. There was a significant reduction ($p < 0.05$) in total protein in the group treated with 1000 mg/kg body weight of the extract. Similarly, there was a significant increase ($p < 0.05$) in urea in all the groups treated as compared to the control group (Table 3).

DISCUSSION

The various biochemical and haematological parameters investigated in this study are useful indices of evaluating the toxicity of plant extract in animals (Yakubu et al., 2008). Assessment of haematological parameters can not only be used to determine the extent of deleterious effect of extracts on the blood of an animal, but it can also be used to explain blood relating functions of a plant extract or its products (Yakubu et al., 2007). Analysis of blood parameters is relevant in risk evaluation as changes in the haematological system have higher predictive value for human toxicity when the data are translated from animal studies (Olson et al., 2000). From the observed significant values of WBC, it is clear that an increase in the number of WBC is a normal reaction of rats to foreign substances, which alter their normal physiological processes. The leucocytosis observed in the present study indicates a stimulation of the immune system which protects the rats against infection that might have been caused by chemical and secondary infections. Leucocytosis, which may be directly proportional to the severity of the causative stress condition, may be attributed to an increase in leukocyte mobilization (Celik and Suzek, 2008).

The non-significant effect of the extract on the RBC may be an indication that the balance between the rate of production (erythropoiesis) and destruction of the blood corpuscles was not altered. MCHC and MCH relate to individual red blood cells while Hb, RBC and PCV are associated with the total population of red blood cells. Therefore, the absence of significant effect of the extract on RBC, Hb, PCV, MCH and MCHC could mean that neither the incorporation of haemoglobin into red blood cells nor the morphology and osmotic fragility of the red blood cells was altered (Adebayo et al., 2005). *C. albidum* extract exerts a significant reduction in the platelets which indicates thrombocytopenia. Platelet aggregation plays a pivotal role in the physiopathology of thrombotic diseases. Moreover, platelet activity may play a major role in the development as well as in the stability of atherosclerotic plaques and as a consequence, anti-platelet agents have been used clinically in patients at

Table 1. Effect of ethanolic leaf extract of C*hrysophyllum albidum* on the weight of organs.

Organs	Group 1 (Control)	Group 2 (250 mg/kg bw)	Group 3 (500 mg/kg bw)	Group 4 (1000 mg/kg bw)
Kidney	0.94 ± 0.09[a]	0.93 ± 0.07 [a]	0.97 ± 0.06 [a]	0.84 ± 0.05 [a]
	[0.68 ± 0.03][a]	[0.71± 0.03] [a]	[0.77± 0.05] [a]	[0.70 ± 0.03] [a]
Spleen	0.74 ± 0.10[a]	0.69 ± 0.07 [a]	0.62 ± 0.09 [a]	0.66 ± 0.12 [a]
	[0.53 ± 0.06][a]	[0.52 ± 0.04] [a]	[0.49 ± 0.07] [a]	[0.54 ± 0.08] [a]
Liver	7.76 ± 0.88[a]	7.80 ± 0.27 [a]	7.67 ± 0.61 [a]	7.60 ± 0.40 [a]
	[5.50 ± 0.20][a]	[6.01 ± 0.20] [a]	[6.01± 0.22] [a]	[6.33 ± 0.21] [b]
Heart	0.56 ± 0.04[a]	0.54 ± 0.02 [a]	0.50 ± 0.04 [a]	0.47 ± 0.02 [a]
	[0.41± 0.03][a]	[0.42± 0.01] [a]	[0.39 ± 0.01] [a]	[0.40± 0.02] [a]
Epididymis	0.38 ± 0.09 [a]	0.52 ± 0.05 [a]	0.28 ± 0.06 [a]	0.47 ± 0.09 [a]
	[0.26± 0.09] [a]	[0.39 ± 0.10] [a]	[0.22 ± 0.10] [a]	[0.38 ± 0.15] [a]
Testis	1.68 ± 0.29 [a]	1.72 ± 0.16 [a]	1.75 ± 0.21 [a]	1.50 ± 0.11 [a]
	[1.17 ± 0.03] [a]	[0.24± 0.03] [a]	[1.41± 0.03] [a]	[1.27± 0.03] [a]
Brain	1.26 ± 0.12 [a]	1.43 ± 0.09 [a]	1.53 ± 0.05 [a]	1.64 ± 0.04 [a]
	[0.96 ± 0.17] [a]	[1.10 ± 0.07] [a]	[1.22 ± 0.07] [b]	[1.38 ± 0.07] [b]
Lung	1.04 ± 0.16 [a]	0.96 ± 0.09 [a]	1.00 ± 0.09 [a]	1.14 ± 0.07 [a]
	[0.75 ± 0.10] [a]	[0.75 ± 0.09] [a]	[0.79 ± 0.04] [a]	[0.95 ± 0.04] [b]

Values represent mean ± SEM of 7 replicates; values in parenthesis are % weight of organ with respect to the body weight of rats.

Values on the same row followed by different superscript letters differ significantly ([a] $p > 0.005$, [b] $p < 0.05$, versus control).

risk for myocardial ischemia, unstable angina and acute myocardial infarction (Albers, 1995; George, 2000). Flavonoid has been shown to act at the blood platelet level by preventing platelet activity-related thrombosis (Harnafi and Amrani, 2007). *C. albidum* has been reported to contain flavonoids as one of its active compounds (Akaneme, 2008). Some studies suggested various mechanisms by which flavonoid exert its antiplatelet property that is, by lowering intracellular Ca^{2+} levels; alteration in the metabolism of cAMP, and thromboxane A2 (Roy et al., 1999; Kang et al., 2001). Thus, it may be inferred that the antiplatelet property of the extract of *C. albidum* might be mediated by lowering the intracellular Ca^{2+} levels or by inhibiting key aspects of cAMP and thromboxane metabolism. The extract therefore might be useful in the management of cardiovascular diseases. Tissue damage is usually associated with the release of enzymes specific to the affected tissue or organ in circulation. The consequence is an increase in the activity of such enzymes in body fluids (Aliyu et al., 2006). The significant reductions observed in the activity of ALT and AST indicate that the extract of *C. albidum* was not harmful to the liver. ALT is a cytoplasmic enzyme found in very high concentration in the liver and an increase of this specific enzyme indicates hepatocellular damage, while AST is less specific than ALT as an indicator of liver function (Aliyu et al., 2006). Enhancement in tissue serum total protein is an indication of tissue damage while a significant decrease in total protein of the liver contents is a reflection of hepatic toxicity (Gatsing et al., 2005). The significant reductions of protein in group administered with 1500 mg/kg body weight indicate depletion in the protein reserve and thus suggest hepatic toxicity. Serum glucose level was significantly lowered in the groups treated with 500 and 1000 mg/kg body weight suggesting that the leaf extract possess hypoglycemic activity. The result corroborates with the study conducted on the seed cotyledon of *C. albidum* which significantly decreased blood glucose level in diabetic rats (Olorunnisola et al., 2008). The serum creatinine level was decreased significantly suggesting that the leaf extract was not toxic to the kidney. Creatinine is the major catabolic products of the muscle and it is excreted in the kidneys. Creatinine levels are used as indicator of renal failure (Aliyu et al., 2006). The increased level of urea observed is an indication of azotaemia. High blood urea is associated with increased tissue protein catabolism, excess breakdown of blood protein and diminished excretion of urea (Nduka, 1999). An increase in organ-body weight ratio is an indication of inflammation while a decrease may be due to cell constriction (Moore and Dalley, 1999). The increase in the brain, lung and liver-body weight ratio observed with the extract may be due to increase in the functional ability of the organ (Ashafa et al., 2009). The absence of significant effect on the kidney, spleen, testis, epididymis and heart-body weight ratios of the animals is an indication that the extract did not adversely affect the size of these organs in relation to the weight of the animals.

In conclusion, the study has demonstrated that the

Table 2. Effect of ethanolic leaf extract of *Chrysophyllum albidum* on haematological parameters in albino Wistar rats.

Parameters	Group 1 (control group)	Group 2 (250 mg/kg bw)	Group 3 (500 mg/kg bw)	Group 4 (1000 mg/kg bw)
PCV (%)	38.80 ± 2.78[a]	38.43 ± 1.11[a]	38.00 ± 1.84[a]	35.14 ± 1.28[a]
HB (g/dl)	12.85 ± 0.92[a]	12.70 ± 0.37[a]	12.21 ± 0.44[a]	11.78 ± 0.38[a]
RBC ($\times10^{12}$/L)	4.16 ± 0.30[a]	4.07 ± 0.13[a]	4.02 ± 0.16[a]	3.83 ± 0.14[a]
Platelet ($\times10^{9}$/L)	247.60 ± 27.01[a]	212.43 ± 12.43[a]	224.00 ± 27.95[a]	184.38 ± 4.33[b]
WBC ($x10^{14}$/L)	63.76 ± 3.55[a]	87.07 ± 96.07[a]	134.11 ± 7.49[b]	84.93 ± 9.97[a]
Reticulocyte (%)	3.40 ± 0.44[a]	2.86 ± 0.46[a]	2.77 ± 0.30[a]	3.23 ± 0.38[a]
Neutrophil (%)	25.80 ± 5.17[a]	26.00 ± 4.70[a]	29.17 ± 4.38[a]	26.29 ± 4.37[a]
Lymphocyte (%)	73.40 ± 5.57[a]	73.57 ± 4.78[a]	70.83 ± 4.38[a]	73.57 ± 4.37[a]
Monocyte (%)	0.40 ± 0.19[a]	0.43 ± 0.17[a]	0.42 ± 0.18[a]	0.41 ± 0.14[a]
Eosinophil (%)	0.40 ± 0.09[a]	0.41 ± 0.12[a]	0.41 ± 0.13[a]	0.42 ± 0.12[a]
Basophil (%)	9.33 ± 0.12[a]	9.76 ± 0.38[a]	9.33 ± 0.56[a]	9.18 ± 0.10[a]
MCH (pg)	3.09 ± 0.04[a]	3.12 ± 0.03[a]	3.02 ± 0.06[a]	3.09 ± 0.06[a]
MCHC (g/L)	0.33 ± 0.01[a]	0.33 ± 0.01[a]	0.32 ± 0.02[a]	0.34 ± 0.02[a]

Values represent mean ± SEM of 7 replicates.
Values on the same row followed by different superscript letters differ significantly ([a] $p > 0.005$, [b] $p < 0.05$, versus control).
WBC = White blood cell count, RBC = red blood cell count, PVC = packed cell volume, Hb = haemoglobin, MCH = mean corpuscular haemoglobin, and MCHC = mean corpuscular haemoglobin concentration.

Table 3. Effect of ethanolic leaf extract of *Chrysophyllum albidum* on biochemical parameters of albino Wistar rats after 16 days of treatment.

Parameters	Group 1 (Control)	Group 2 (250 mg/kg bw)	Group 3 (500 mg/kg bw)	Group 4 (1000 mg/kg bw)
AST (U/L)	124.6 2 ± 16.73[a]	79.57 ± 6.80[b]	62.5 ± 5.65[b]	66.71 ± 9.79[b]
ALT (U/L)	119.13 ± 15.34[a]	73.44 ± 7.31[b]	68.23 ± 4.11[b]	66.17 ± 5.71[b]
Total bilirubin (mg/dl)	0.22 ± 0.08[a]	0.16 ± 0.01[a]	0.18 ± 0.08[a]	0.19 ± 0.07[a]
ALP (U/L)	314.61 ± 3.10[a]	316.20 ± 2.48[a]	303.70 ± 6.34[a]	314.43 ± 1.68[a]
Total protein (mg/dl)	6.62 ± 0.21[a]	6.34 ± 0.37[a]	6.37 ± 0.12[a]	5.17 ± 0.19[b]
Albumin (mg/dl)	3.90 ± 0.19[a]	4.45 ± 0.29[a]	4.11 ± 0.32[a]	5.16 ± 0.40[a]
Glucose (mg/dl)	187.04 ± 19.41[a]	177.11 ± 24.53[a]	119.20 ± 5.54[b]	126.80 ± 10.24[b]
Urea (mg/dl)	43.43 ± 1.24[a]	48.50 ± 1.44[b]	57.70 ± 0.84[b]	50.85 ± 1.56[b]
Creatinine (mg/dl)	0.83 ± 0.04[a]	0.98 ± 0.04[b]	0.65 ± 0.03[b]	0.62 ± 0.02[b]

Values represent mean **±** SEM of 7 replicates.
Values on the same row followed by different superscript letters differ significantly ([a] $p > 0.005$, [b] $p < 0.05$, versus control).
ALP = alkaline phosphatase, ALT = alanine aminotransferase, and AST = aspartate aminotransferase.

leaf extract of *C. albidum* may not cause any adverse effect on the biochemical and haematological indices of toxicity. Moreover, the extract was found to possess anti-platelet and hypoglycemic properties and might be employed in the management of myocardial infarction and diabetes mellitus, respectively. Further investigation is needed to establish the antiplatelet property of the extract.

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