

## Amelioration of carbon tetrachloride-induced hepatotoxicity and haemotoxicity by aqueous leaf extract of *Cnidoscolus aconitifolius* in rats

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**Summary:** This study was conducted to explore possible protective effect of *Cnidoscolus aconitifolius* (CA) leaf extract on carbon tetrachloride (CCl<sub>4</sub>)-induced hepatotoxicity and haemotoxicity in experimental animal models. Thirty six rats of six per group were used in this study. Group I received 10ml/kg normal saline as control. Group II-VI rats were administered with 1.25ml/kg body weight (bwt) of carbon tetrachloride intraperitoneally. Animals in groups III, IV, V and VI were however pre-treated with aqueous extract of *Cnidoscolus aconitifolius* at 100, 250, 500 and 750mg/kg body weight (bwt) respectively. Administration of CCl<sub>4</sub> in untreated rats led to microcytic hypochromic anaemia, thrombocytopenia, increased erythrocyte fragility and stress induced leucocytosis accompanied with significant (P<0.05) increase in neutrophils and decrease (P<0.01) in lymphocyte counts. CCl<sub>4</sub> also led to significant (P<0.05) increase in serum transaminases (ALT and AST) and phosphatase (ALP) respectively compared with control animals. Also, CCl<sub>4</sub> produced significant (P<0.05) increase in serum blood urea nitrogen (BUN) and creatinine compared with normal rats. Pre-treatment with *Cnidoscolus aconitifolius* leaf extract brought about significant restoration of the haematological parameters to values that were comparable to those of the control with concomitant decrease (P<0.05) in the activities of the marker of hepatic damage enzymes (ALT, AST and ALP), in a dose-dependent manner. Similarly, serum levels of blood urea nitrogen (BUN) and creatinine were also brought to near normal by the CA in a dose-dependent manner. From this study, we conclude that pre-exposure to *Cnidoscolus aconitifolius* leaf extract considerably reduced the effect of CCl<sub>4</sub> on the blood parameters and ameliorated hepatic damage by the haloalkane

**Keywords:** Hepatotoxicity, Haemotoxicity, Carbon tetrachloride, *Cnidoscolus aconitifolius*, Rats.

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### INTRODUCTION

The aetiology of liver injuries and the damaging effects on the subject have been well discussed. These include viral infections, autoimmune disorders, ischemia, and several xenobiotics, such as drugs, alcohol, or toxins (Kondo *et al.*, 1997). In elucidating the mechanism of the liver damage therefore, halogenated alkanes such as carbon tetrachloride (CCl<sub>4</sub>) are widely used as model compound to induce hepatotoxicity and elucidate its mechanisms of action following exposure to these compounds (Kim *et al.*, 2010). Effects such as fatty degeneration, fibrosis, hepatocellular apoptosis and carcinogenicity have

been associated with CCl<sub>4</sub> toxicity. Following administration, CCl<sub>4</sub> is activated by cytochrome CYP2E1, CYP2B1 or CYP2B2, and possibly CYP3A, to form trichloromethyl (CCl<sub>3</sub>\*) radical. This radical binds to cellular molecules (nucleic acid, protein, lipid) thereby impairing crucial cellular processes such as lipid metabolism, with the potential outcome of fatty degeneration while the reaction between CCl<sub>3</sub>\* and DNA is thought to function as initiator of hepatic cancer. This radical can also react with oxygen to form the trichloromethylperoxy (CCl<sub>3</sub>OO\*) radical, a highly reactive species. This compound initiates the chain reaction of lipid peroxidation, culminating in destruction of

polyunsaturated fatty acids, especially those associated with phospholipids (Gruebele *et al.*, 1996). This leads to alteration of permeabilities of mitochondrial, endoplasmic reticulum, and plasma membranes, resulting in the loss of cellular calcium, sequestration and disruption of calcium homeostasis with subsequent cell damage (Muriel *et al.*, 2001; Weber *et al.*, 2003).

CCl<sub>4</sub> have also been reported to activate tumour necrosis factor (TNF) $\alpha$ , nitric oxide (NO), and transforming growth factors (TGF)- $\alpha$  and - $\beta$  in the cell, processes that appear to direct the cell primarily toward self-destruction or fibrosis. TNF $\alpha$  pushes the cell toward apoptosis (Roberts *et al.*, 2001), whereas the TGFs appear to direct toward fibrosis (Tahashi *et al.*, 2002).

As a result of the reducing effect of cytochromes on CCl<sub>4</sub> and the subsequent formation of the active trichloromethyl radical as well as increased expression of NF $\kappa$ B, TNF $\alpha$  and TGF - $\alpha$  and - $\beta$ , the use of cytochrome P<sub>450</sub> antagonist, antioxidants and free radical scavengers, have proven to be useful in reversing the hepatotoxic effects of CCl<sub>4</sub>. Some plants have also been shown to ameliorate the hepatotoxic effects of CCl<sub>4</sub> through their antioxidant activities (Fadhel and Amran, 2002), inhibition of CYP2E1 (Jeong *et al.*, 2002), activation of NF $\kappa$ B and inhibition of inflammatory cytokines (Yoh *et al.*, 2002), while some act as mitogens.

The plant *Cnidocolus aconitifolius* is a perennial shrub belonging to the Family *Euphorbiaceae*. It is commonly found in the tropic and sub tropical regions worldwide, including Africa, south of Sahara, North and South America, India, etc. It is commonly eaten as vegetable in soup (Ganiyu, 2005) in South Western Nigeria where it is called 'IyanaIpaja'. It has been shown to possess haematinic effects and stabilized erythrocyte membrane in protein energy malnutrition in rats (Oyagbemi *et al.*, 2008). It also possesses antidiabetic (Oladeinde *et al.*, 2007) and antibacterial properties (Sarmiento-Franco *et al.*, 2002; Awoyinka *et al.*, 2007). Further characterizations have shown that it contains phenols, saponins, cardiac glycosides and Phlobatannin (Awoyinka *et al.*, 2007). The present study was conducted to investigate the ameliorative effects of *Cnidocolus aconitifolius* on the liver damage, anaemia and impairment of erythrocyte osmotic resistance associated with CCl<sub>4</sub> administration, using the Wistar rats as the model.

## MATERIALS AND METHODS

### Collection of Plant Materials

Fresh matured leaves of *Cnidocolus aconitifolius* were collected at the University Teaching Hospital, College of Medicine, Ibadan. The leaves were identified and authenticated at the Department of Botany and Microbiology, University of Ibadan, with samples deposited at the Forestry Research Institute of Nigeria, (FRIN), with voucher no: FHI 107727. It was then air-dried, blended into powdery form and kept separately in airtight containers until the time of use.

### Extraction of Plant Material

The extract was prepared according to Yakubu *et al.* (2008) with slight modification. The dried leaves (700 grams) were pulverized using blender (model MS-223, China) and the resulting powder was preserved in a plastic container. Air-dried powder (1kg) of fresh matured *Cnidocolus aconitifolius* leaves were extracted by percolation at room temperature with 70% ethanol. The leaf extract was then concentrated under reduced pressure (bath temp. 50°C) and finally defatted with n-hexane. The filtrate was evaporated to dryness. The mass yield of the extract was 65grams sequel to extraction and concentration at reduced temperature and pressure with rotatory evaporator.

### Drugs and Chemicals

Carbon tetrachloride purchased from British Drug Houses (Poole, Dorset, UK) was used in this experiment. All other chemicals were of analytical grade.

### Animal models

Thirty inbred male Wistar rats weighing 220-250g were used for the study. The animals were maintained on a 12-h light and dark cycle, at 30  $\pm$  5°C, fed *ad libitum* with standard rat chow (Ladokun feeds, Nigeria). They also had free access to water. The rats were acclimatized to laboratory condition for 2 weeks before commencement of the experiment. All the animals received humane care according to the criteria outlined in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy of Science and published by the National Institute of Health. The ethical regulations in accordance with National and Institutional guidelines for the protection of animals' welfare were strictly adhered to during the experiments (PHS, 1996)

### Study protocol

The rats were randomized into 6 groups comprising 5 animals each. Rats in Group I received 10 ml/kg body weight of 0.9% Physiological Saline orally once daily for 9 days. Groups III-VI rats were

pre-treated with the extract of *Cnidoscopus aconitifolius* at 100, 250, 500 and 750 mg/kg bwt for 7 days once daily by gastric intubation. Hepatic damage was induced in Groups II - VI rats as described by (Saraf and Dixit, 1991 and Mohideen et al., 2003) by administration of CCl<sub>4</sub> intraperitoneally at the dose of 1.25 ml/kg bwt CCl<sub>4</sub> in olive oil (at the ratio of 1:1), 30 min post-dose of *Cnidoscopus aconitifolius* on days 8 and 9 as described by Oyagbemi and Odetola (2010). The animals were fasted overnight and sacrificed on day 10 by cervical dislocation after collection of blood samples.

#### Blood Sample Collection and Analysis

Blood samples for haematological analysis were collected from all the rats through the retro-orbital venous plexus under ether-induced anaesthesia, into heparinized tubes while the sample for serum biochemistry was collected into plain tubes. From the blood samples collected, packed cell volume (PCV) was determined by micro haematocrit method, haemoglobin concentration (Hb) by cyanmethaemoglobin method while the red blood cells (RBC) and white blood cells (WBC) were counted using haemocytometer. Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated from the values of PCV, Hb and RBC count as described by Jain, (1986). Blood smear were then stained with Giemsa to obtain the differential leucocyte counts. Erythrocyte osmotic fragility was determined according to the method described by Oyewale, (1992) by diluting 0.02ml of blood in test tubes containing 0 – 0.9% NaCl in phosphate buffer at pH of 7.4. The tubes were gently mixed and incubated at room temperature (29°C) for 30 minutes, then centrifuged at 3500rev/min for 10 minutes. The supernatant were decanted and the optical density determined at 540nm using SM22PC Spectrophotometer (Surgienfield Instruments, England). Haemolysis in each tube was expressed as a percentage, taking the tube with the highest haemolysis (i.e. distilled water with 0.0% NaCl) as 100%.

**Table 1:**

Mean (±SD) haematological parameters of the wistar rats following concomitant administration of CCl<sub>4</sub> and ethanolic extract of *Cnidoscopus aconitifolius*.

Parameters	Control	CCl <sub>4</sub> only	CCl <sub>4</sub> + 100mg/kg Ca	CCl <sub>4</sub> + 250mg/kg Ca	CCl <sub>4</sub> + 500mg/kg Ca	CCl <sub>4</sub> + 750mg/kg Ca
PCV (%)	46.22±0.99	29.17±0.8 <sup>a</sup>	37.20±2.40 <sup>ab</sup>	35.67±2.6 <sup>ab</sup>	38.8±1.02 <sup>ab</sup>	41.2±1.35 <sup>ab</sup>
Hb (g/dl)	15.40±0.34	11.74±0.5 <sup>a</sup>	11.90±0.33 <sup>a</sup>	14.50±0.2 <sup>ab</sup>	15.20±0.5 <sup>ab</sup>	12.82±0.4 <sup>a</sup>
RBC (x10 <sup>6</sup> /μL)	7.81±0.19	6.49±0.19 <sup>a</sup>	7.91±0.10	8.26±0.22	7.68±0.06	7.93±0.19

*C. Aconitifolius* on hepatotoxicity and haemotoxicity

#### Serum Biochemistry

Whole blood was separated with high speed macro-centrifuge at 3,500 rev/minute for 10 minutes and serum was separated by Pasteur pipette for analysis of the following biochemical assays; Alkaline phosphatase (ALP) as described by Tietz and Shuey (1986), aspartate aminotransferase (AST), (Bergmeyer et al., 1985), alanine aminotransferase (ALT) (Klauke et al., 1983) albumin (Varely, 1994) and total protein (Keller, 1984).

#### Statistical Analysis

One-way ANOVA with Dunnett's post test was performed using GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego California USA, [www.graphpad.com](http://www.graphpad.com). Values of P < 0.05 were taken to be significant.

## RESULTS

#### Haematology

The effects of CCl<sub>4</sub> administration and the modulatory activities of *Cnidoscopus aconitifolius* on the haematological parameters of the wistar rats are shown in Tables 1 and 2. There was a significant reduction (P < 0.01) in the PCV, Hb concentration, RBC, platelet count, MCV and MCH values while MCHC was higher (P < 0.01) in the rats that were exposed to CCl<sub>4</sub> without pre-treatment with *Cnidoscopus aconitifolius* extract (Group II) when compared with those of the normal control. However there was a moderate increase on dose dependent basis in these parameters in the rats that were pre-treated with the extract, although these values except RBC were still lower (P < 0.01) than those of the control (Table 1). The RBC count obtained in the pre-treated groups on the other hand was comparable to that of the control. In fact, those rats in group IV pre-treated with 250mg/kg of the extract had significantly higher (P < 0.01) RBC count than were those of the control.

Plate( $\times 10^3/\mu\text{L}$ )	166.4 $\pm$ 37.1	118.4 $\pm$ 4.4 <sup>a</sup>	138.6 $\pm$ 8.3 <sup>b</sup>	123.2 $\pm$ 7.5 <sup>a</sup>	133.6 $\pm$ 7.7 <sup>a</sup>	128.2 $\pm$ 8.2 <sup>a</sup>
MCV (fl)	60.3 $\pm$ 1.12	42.7 $\pm$ 2.52 <sup>a</sup>	47.0 $\pm$ 3.39 <sup>a</sup>	41.8 $\pm$ 3.05 <sup>a</sup>	49.43 $\pm$ 1.6 <sup>a</sup>	53.48 $\pm$ 1.2 <sup>a</sup>
MCH (pg)	21.3 $\pm$ 1.23	17.7 $\pm$ 0.42 <sup>a</sup>	15.0 $\pm$ 0.61 <sup>ab</sup>	17.5 $\pm$ 0.48 <sup>a</sup>	19.3 $\pm$ 1.04 <sup>a</sup>	16.31 $\pm$ 0.8 <sup>a</sup>
MCHC (g/dl)	33.3 $\pm$ 0.01	41.5 $\pm$ 2.55 <sup>a</sup>	32.4 $\pm$ 1.86 <sup>b</sup>	42.0 $\pm$ 3.75 <sup>a</sup>	39.23 $\pm$ 2.3 <sup>ab</sup>	31.45 $\pm$ 1.5 <sup>a</sup>

<sup>a</sup> indicates value is significantly different from the control value at  $P < 0.01$  while <sup>b</sup> indicates significant difference between extract pre-treated and  $\text{CCl}_4$ -administered rat groups at  $P < 0.05$ .

**Table 2:**

Mean ( $\pm$ SD) haematological parameters of the Wistar rats following concomitant administration of  $\text{CCl}_4$  and ethanolic extract of *Cnidioscolus aconitifolius*.

Parameters	Control	$\text{CCl}_4$ only	$\text{CCl}_4$ + 100mg/kg Ca	$\text{CCl}_4$ + 250mg/kg Ca	$\text{CCl}_4$ + 500mg/kg Ca	$\text{CCl}_4$ + 750mg/kg Ca
WBC ( $\times 10^3$ )	7.27 $\pm$ 1.07	9.68 $\pm$ 0.72 <sup>a</sup>	8.84 $\pm$ 0.40 <sup>a</sup>	9.20 $\pm$ 0.30 <sup>a</sup>	9.32 $\pm$ 0.38 <sup>a</sup>	6.53 $\pm$ 0.40 <sup>b</sup>
Neutrophil ( $\times 10^3$ )	1.53 $\pm$ 0.04	3.68 $\pm$ 0.09 <sup>a</sup>	3.23 $\pm$ 0.28 <sup>a</sup>	3.44 $\pm$ 0.68 <sup>a</sup>	3.88 $\pm$ 0.58 <sup>a</sup>	2.56 $\pm$ 0.49 <sup>b</sup>
Lymphocy ( $\times 10^3$ )	5.55 $\pm$ 0.11	5.70 $\pm$ 0.30	5.22 $\pm$ 0.21	4.81 $\pm$ 0.59 <sup>ab</sup>	4.43 $\pm$ 0.64 <sup>ab</sup>	3.93 $\pm$ 0.50 <sup>ab</sup>
Monocyt ( $\times 10^3$ )	0.16 $\pm$ 0.05	0.19 $\pm$ 0.04	0.26 $\pm$ 0.03 <sup>a</sup>	0.92 $\pm$ 0.03 <sup>ab</sup>	0.93 $\pm$ 0.05 <sup>ab</sup>	0.02 $\pm$ 0.02 <sup>ab</sup>
Eosinophil ( $\times 10^3$ )	0.03 $\pm$ 0.02	0.11 $\pm$ 0.05 <sup>a</sup>	0.13 $\pm$ 0.05 <sup>a</sup>	0.03 $\pm$ 0.02 <sup>b</sup>	0.08 $\pm$ 0.04	0.02 $\pm$ 0.02 <sup>b</sup>

<sup>a</sup> indicates value is significantly different from the control value at  $P < 0.01$  while <sup>b</sup> indicates significant difference between extract-pretreated and  $\text{CCl}_4$ -administered rat groups at  $P < 0.05$ .

**Table 3.**

The effect of pre-treatment with *Cnidioscolus aconitifolius*(CA) on changes in liver function tests associated with  $\text{CCl}_4$  toxicity. Values are means  $\pm$  SD.

Parameters	Normal Group I	$\text{CCL}_4$ Only Group II	$\text{CCL}_4$ +CA (100mg/kg) Group III	$\text{CCL}_4$ +CA (250mg/kg) Group IV	$\text{CCL}_4$ +CA (500mg/kg) Group V	$\text{CCL}_4$ +CA (750mg/kg) Group VI
ALT (U/L)	70.00 $\pm$ 5.58	222.09 $\pm$ 5.00 <sup>a</sup>	217.00 $\pm$ 7.88 <sup>a</sup>	168.30 $\pm$ 9.55 <sup>ab</sup>	138.00 $\pm$ 4.33 <sup>ab</sup>	120.00 $\pm$ 7.93 <sup>ab</sup>
AST (U/L)	287.67 $\pm$ 8.89	460.30 $\pm$ 1.22 <sup>a</sup>	395.33 $\pm$ 5.00 <sup>ab</sup>	266.00 $\pm$ 8.46 <sup>ab</sup>	257.67 $\pm$ 5.93 <sup>ab</sup>	210.30 $\pm$ 0.80 <sup>ab</sup>
ALP (U/L)	164.70 $\pm$ 6.84	238.00 $\pm$ 8.52 <sup>a</sup>	200.80 $\pm$ 2.26 <sup>ab</sup>	193.00 $\pm$ 7.52 <sup>ab</sup>	173.30 $\pm$ 2.18 <sup>ab</sup>	156.00 $\pm$ 8.33 <sup>ab</sup>
TP (mg/dl)	6.50 $\pm$ 0.17	6.33 $\pm$ 0.39	6.63 $\pm$ .32	7.22 $\pm$ 0.30 <sup>b</sup>	7.27 $\pm$ 0.65 <sup>b</sup>	7.63 $\pm$ .63 <sup>ab</sup>
Alb (mg/dl)	9.00 $\pm$ 0.35	3.60 $\pm$ 0.17 <sup>a</sup>	3.67 $\pm$ 0.39 <sup>a</sup>	3.43 $\pm$ 0.38 <sup>a</sup>	3.60 $\pm$ 0.27 <sup>a</sup>	3.78 $\pm$ 0.16 <sup>a</sup>

<sup>a</sup> indicates value is significantly different from the control value at  $P < 0.05$  while <sup>b</sup> indicates significant difference between extract-pre-treated and  $\text{CCl}_4$ -administered rat groups at  $P < 0.05$ .

Comparing the pre-treated groups with those that were not treated i.e. group II, we observed that the PCV of all the pre-treated groups (100, 250, 500 and 750mg/kg extract), Hb concentrations of those that received 250,500 and 750mg/kg of the extract were higher ( $P < 0.01$ ) than those of the rats that were not pre-treated with the extract. Similarly, the RBC counts of all the pre-treated groups and the platelet counts of those treated with 100, 500 and 750mg/kg of the extract were significantly higher than those of the rats exposed to  $\text{CCl}_4$  only, whereas MCH values in those rats given 100mg/kg and MCHC values in those given 100 and 500mg/kg were lower ( $P < 0.01$ ) than those of the rats given  $\text{CCl}_4$  alone.

As shown in Table 2, with the exception of those pre-treated with 750mg/kg of the extract, the total

WBC count increased significantly ( $P < 0.01$ ) in all the groups exposed to  $\text{CCl}_4$  when compared to those of the control. This leucocytosis was observed to be as a result of higher ( $P < 0.01$ ) neutrophil counts while the lymphocyte counts was significantly lower ( $P < 0.01$ ) in the groups exposed to 250, 500 and 750mg/kg of the extract than those of either group I or II. The monocyte count was however higher ( $P < 0.01$ ) in those pre-treated with 100, 250 and 500mg/kg of the extract while those that received 750mg/kg of the extract had significantly lower monocytes than the control. The groups that received 250 and 500mg/kg extract also had higher monocyte counts than those in group II whereas, the monocyte count of the group that received 750mg/kg extract was lower than those of group II which did not receive the extract. The

eosinophil count was also higher in the rats in group II and III (treated with CCl<sub>4</sub> only and 100mg/kg extract respectively) than those of the control while those treated with 250 and 750mg/kg of the extract had significantly lower eosinophil count than were those of the negative control (CCl<sub>4</sub> only/group I)

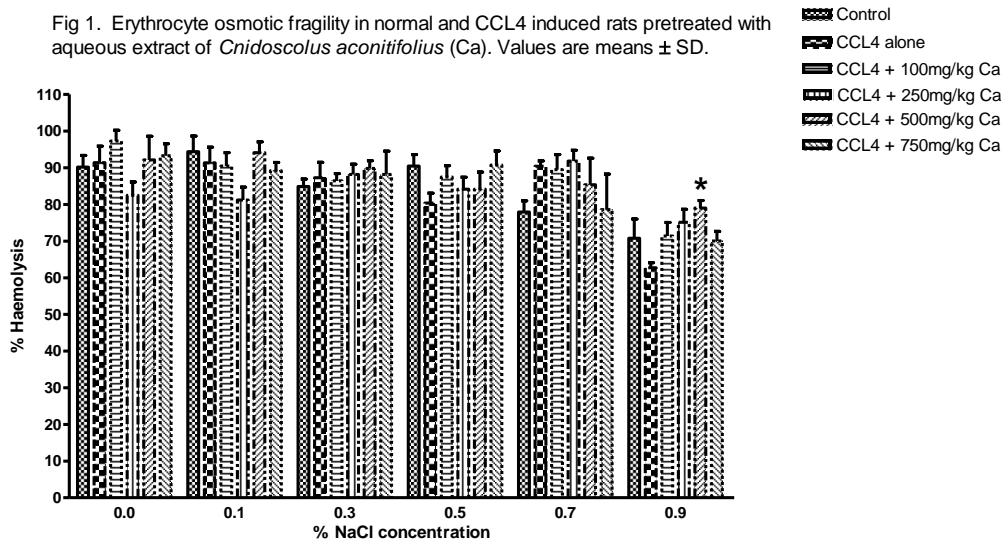
The erythrocyte osmotic fragility was observed to be higher in the rats treated with the extract at 0.7% and 0.9% NaCl concentrations. However, significant variation (P<0.05) was observed only in the rats treated with 500mg/kg of the extract at 0.9% NaCl, where the erythrocyte fragility was higher than those of the rats exposed to CCl<sub>4</sub> only.

### Serum Biochemistry

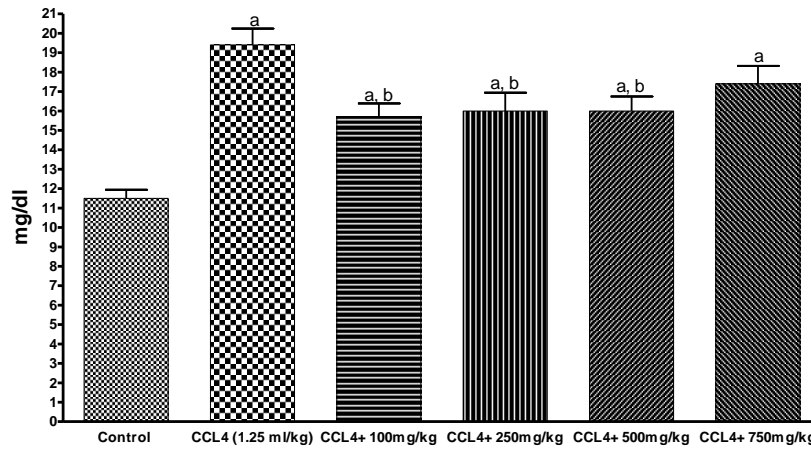
There was significant (p<0.05) increase in serum ALT levels of rats administered with CCl<sub>4</sub> compared with control (Table 3). Similarly, there were significant (p<0.05) reductions in serum ALT values of animals pre-treated with *Cnidoscopus aconitifolius* extract compared with CCl<sub>4</sub> treated group (Table 3). The activity of serum AST was significantly (p<0.05) higher in CCl<sub>4</sub> treated rats compared with control. In the same vein, CA extract significantly (p<0.05) and dose-dependently reduce serum AST values. The activity of Alkaline phosphatase (ALP) was also significantly (p<0.05) elevated by the administration of CCL4 compared with control rats.

Also, CA in the doses of 100, 250, 500 and 750mg/kg bwt significantly (p<0.05) reduced serum ALP ion dose-dependent in manner compared with CCl<sub>4</sub> treated rats. Together, the activities of serum ALT, AST and ALP were significantly (p<0.05) higher in animals pre-treated with CA compared with control. However, the values did not return to normal. There was no significant (p>0.05) difference in serum total protein of animals treated with CCL4 compared with control animals. But there was significant (<0.05) increase in total protein of rats that received 250, 500mg/kg bwt of CA compared with control or 750mg/kg bwt compared with control and CCL<sub>4</sub> respectively (Table 3). The result showed significant (<0.05) increase in blood urea nitrogen (BUN) in animal models treated with CCL<sub>4</sub> compared with control (Figure 1). Also, CA extract (100, 250, 500 and 750mg/kg bwt) significantly (<0.05) reduced serum BUN in dose-dependent manner (Figure 2). Administration of CCL<sub>4</sub> significantly (p<0.05) increased serum creatinine compared with control (Figure 3). But, those rats given CA extract at 100, 250 and 750mg/kg bwt had significantly lower (p<0.05) serum creatinine values than those given CCl<sub>4</sub> alone.

Fig 1. Erythrocyte osmotic fragility in normal and CCL4 induced rats pretreated with aqueous extract of *Cnidoscopus aconitifolius* (Ca). Values are means ± SD.



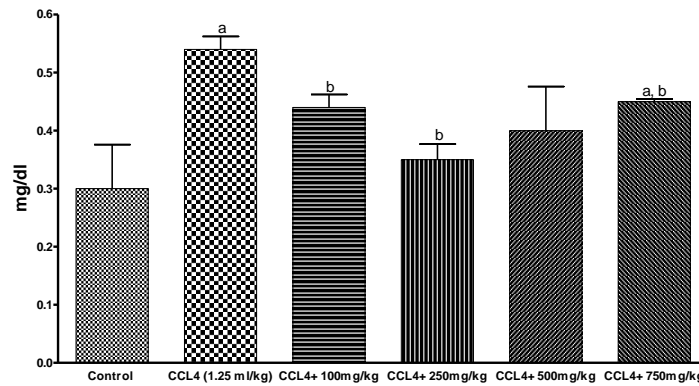
Asterisk indicates significant difference from those given CCL4 alone at P<0.05.



**Fig 2.**

The effect of pre-treatment with *Cnidioscolus aconitifolius* on CCl<sub>4</sub> induced changes in serum BUN of the Wistar rats. Values are means ± SD.

<sup>a</sup> indicates value is significantly different from the control value at P<0.05 while <sup>b</sup> indicates significant difference between extract-pretreated and CCl<sub>4</sub> -administered rat groups at P<0.05.



**Figure 3:**

The effect of pre-treatment with *Cnidioscolus aconitifolius* leaf extract on CCl<sub>4</sub> induced changes in serum creatinine of the wistar rats. Values are means ± SD.

<sup>a</sup> indicates value is significantly different from the control value at P<0.05 while <sup>b</sup> indicates significant difference between extract-pretreated and CCl<sub>4</sub> -administered rat groups at P<0.05.

## DISCUSSION

The present study demonstrated clearly that CCl<sub>4</sub> administration produced pancytopenia (a generalized reduction in the cellular elements in the blood) as shown by microcytic hypochromic anaemia, thrombocytopenia and lymphopenia in the blood as evidenced by the reduction in the PCV, RBC and platelets with the exception of total WBC counts, although, there was lymphopenia. We believe that this reduction in the formed elements in the blood is stress induced because of the leucocytosis (increased white blood cell counts) observed in these rats might not have been a result of significant increase in WBC production, but by the release of marginated neutrophils and other neutrophil pool into the circulation which produced the observed neutrophilia

in those rats that received CCl<sub>4</sub>, under the influence of the stress hormone cortisol and catecholamine (Swenson, 1993). Acute stress in animals including birds have been widely reported to be associated with increased white blood cell count occasioned by significant increase in the neutrophil count and the neutrophil/lymphocyte ratio (Larson et al., 1985 and Huff et al., 2005). The stress associated with CCl<sub>4</sub> administration in this study was further corroborated by the increased erythrocyte osmotic fragility which has also been reported to increase during stress (Droge, 2002). This is not farfetched because CCl<sub>4</sub> has been known to produce hepatic damage by generation of highly reactive trichloromethyl (CCl<sub>3</sub>\*) and trichloromethylperoxy (CCl<sub>3</sub>OO\*) radical when metabolized by cytochrome P<sub>450</sub> (Britton and Bacon, 1994, Weber et al., 2003). Previous study on the

effects of CCl<sub>4</sub> on haematological parameters showed that acute CCl<sub>4</sub> toxicity led to transient decrease in the Hb concentration and reticulocyte count as well as PCV and RBC counts by extension (Moritz and Pancow, 1989) which is similar to our observation in the present study.

Pretreatment with *Cnidoscolus aconitifolius* extract in this study was observed to increase the PCV, RBC, Hb, platelets, MCV and MCH values on dose dependent basis to values that were higher than values in those rats that did not receive the extract. Some of these values especially RBC count and haemoglobin concentration were even comparable to those of the normal control. This shows that *Cnidoscolus aconitifolius* extract significantly reduced the damaging effects of CCl<sub>4</sub> in the treated rats. Although the mechanism of the protection cannot be ascertained at the moment, it may be due to the antioxidant properties of the extract, because Vitamin E and other free radical scavengers such as black tea have been reported to reduce the toxic effects of CCl<sub>4</sub>, especially on the liver (Weber et al., 2003). On the contrary, the extract did not reduce erythrocyte fragility in the present study, which is expected to be reduced if it possesses any antioxidant property; but the plant was active at restoring the lymphocyte and neutrophil counts to their normal values especially at the dose of 750mg/kg.

CCl<sub>4</sub> has been demonstrated to cause acute hepatotoxicity with necrotic and apoptotic hepatocellular injury and impairment of liver function (Kovalovich et al., 2001). The mechanism of CCl<sub>4</sub> injury involves oxidative damage by metabolism of CCl<sub>4</sub> to CCl<sub>3</sub>• in hepatocytes which ultimately results in cell death with accumulation of lipid peroxidation and intracellular calcium ions and triggers secondary damage from the inflammatory process (Ishiyama et al., 1995).

Our data confirmed previous observations on the hepatocellular damage in CCl<sub>4</sub> toxicity, because serum AST, ALP and AST which are markers of hepatocellular damage increased significantly in the group exposed to CCl<sub>4</sub> alone. Whereas the extract markedly reduced the activities of these liver function enzymes in dose-dependent manner, of which 750mg/kg bwt was the most effective. The increased activities of liver marker enzymes such as ALT (ALT, EC: 2.6.1.2), AST (AST, EC: 2.6.1.1) and ALP in the serum of CCl<sub>4</sub> induced rats indicate damage to hepatic cells (Wolf, 1999). Damage to the cell integrity of the liver by CCl<sub>4</sub> is reflected by an increase in the activity of AST, which is released into circulation after cellular damage since ALP is an ectoenzyme of the hepatocytes' plasma membrane. CCl<sub>4</sub>-mediated acute toxicity increased permeability

of the hepatocytes membrane and cellular leakage (Paduraru et al., 1996). In the group treated with CCl<sub>4</sub> only, the ALT and AST activities were dramatically increased compared with the control group, indicating severe hepatocellular damage. In contrast, CA extract markedly decreased the release of ALT and AST. The decrease in total serum protein (TSP) observed in CCl<sub>4</sub> treated rats (Table I), might be due to the decrease in the number of hepatocytes which in turn, may result in decreased hepatic capacity to synthesize protein. But the restoration of the level of TSP after the administration of CA confirmed the hepatoprotective nature of CA. We also observed significantly lower serum albumin in those groups that received the plant extract when compared with control and CCl<sub>4</sub> alone. This may be due to short course duration of the experiment, post administration of CCl<sub>4</sub> which did not allow enough time for restoration. Significant decrease in serum albumin had been associated with active cirrhosis and biliary liver damages (Whicherand Spence, 1987 and Shukla and Bhatia, 2010). Conversely however, rise in the levels of serum bilirubin which was observed in the CCl<sub>4</sub> group must have been due to liver damage, because high serum bilirubin had been reported to be the most sensitive indicator for confirmation of and the intensity of liver damages (Shukla and Bhatia, 2010). Elevated serum levels of creatinine and blood urea nitrogen (BUN) in the CCl<sub>4</sub> group in this study was found to reduce significantly sequel to administration of CA in a dose-dependent manner. But the dosage of 750mg/kg bwt increased serum creatinine level significantly. This suggests that the high dose of this plant extract may precipitate kidney damage.

In conclusion, our study demonstrates that CA protects against microcytic hypochromic anaemia, thrombocytopenia and stress induced leucocytosis, neutrophilia and lymphocytopenia associated with CCl<sub>4</sub> administration in rats. It also antagonized CCl<sub>4</sub>-induced acute hepatotoxicity as evidenced by restoration of the liver function enzymes and indicators of acute liver damage. This study thus provides evidence that CA may be an alternative treatment for liver diseases caused by xenobiotics. Also, further studies are in progress for the isolation and characterization of the active principles of the plant extract to propose the fingerprint of the mechanism of action of CA.

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