

Effect of *Cnidoscolus aconitifolius* Leaf Extract on Selected Renal Parameters and Hematological Indices of Carbon Tetrachloride Induced Toxic Rats

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Abstract Effect of *Cnidoscolus aconitifolius* leaf extract on selected renal parameters and hematological indices of carbon tetrachloride (CCl₄) induced toxic rats was investigated using standard analytical methods. Forty-two (42) wistar rats weighing 91-185g was used for this study. The rats were divided into 7 groups of 6 rats each and allowed to acclimatize for one week with food and water *ad libitum*. Carbon tetrachloride was prepared in the ratio of 1:5 (v:v) in olive oil and administered subcutaneously at 0.17 mL/kg body weight on the first day after acclimatization. Group I received normal feed and water only, group II received CCl₄ only, group III received olive oil only, groups IV, V and VI received 50 mg/kgbw, 75 mg/kgbw and 100 mg/kgbw oral dose of *C. aconitifolius* respectively in addition to CCl₄ while group VII received vitamin C plus CCl₄. After oral administration for twenty-one days, the rats were painlessly sacrificed; plasma and blood were collected for renal and hematological analyses respectively. Results showed that CCl₄ increased Ca, Na, K and decreased RBC, PCV, Hb and platelet number of rats induced, however oral administration of *C. aconitifolius* significantly decreased; Ca and Na at 50 mg/kgbw, K in a dose-dependent pattern and significantly increased RBC, PCV, Hb and platelet number at all doses. This study has shown that the leaf extract of *Cnidoscolus aconitifolius* had a dose-dependent positive effect on the hematological indices as well as the potential to ameliorate kidney parameters of CCl₄ treated wistar rats at the given concentrations.

Keywords *Cnidoscolus aconitifolius*, Renal Parameters, Hematological Indices, Carbon Tetrachloride

1. Introduction

Cnidoscolus aconitifolius is a native of Central America

but seen in tropical and subtropical regions like India and Africa [1]. It belongs to the family of *Euphorbiaceae* and commonly referred to as “hospital too far” or “Catholic vegetable” in southern Nigeria [2, 3]. In southern Nigeria, *C. aconitifolius* is majorly utilized as fencing material [4]. Traditionally, the leaves of *C. aconitifolius* is utilized as tonic by some herbalist, as it is presumed to treat anaemia, malaria, diabetes, dermal infections and cardiovascular diseases [5, 6]. According to [7], *C. aconitifolius* leaf has high concentration of potassium, phosphorus, calcium, sodium, zinc, iron and magnesium minerals. The high composition of minerals in *C. aconitifolius* makes this vegetable relevant in health maintenance [8, 9, 10].

Extract from *Cnidoscolus aconitifolius* aids red blood cell formation and maintains stability especially protein-energy malnourished patients [11]. This is very essential for managing sickle cell disease and malaria parasite infection as both diseases are rooted to the red cells. According to studies, the ability of *Cnidoscolus aconitifolius* extract to boost erythrocytes may be due to its vitamin and mineral contents as it was established that zinc, vitamin A, ascorbic acid and metal ions regulated damaged growth and immune functions that occurred from malaria illness [12, 13, 14, 15, 16, 17].

2. Methodology

2.1. Extraction of the Leaves

Cnidoscolus aconitifolius leaves were collected fresh, washed to get rid of dirt and dried. The leaves were crushed with a mortar and pestle and aqueous extraction done according to [18]. The crude extract was subjected to evaporation using a water bath before freeze-drying.

2.2. Experimental Design

The animals (42 wistar rats) weighing 91-185g, used in

this work were purchased from the animal house of the Department of Physiology, University of Port Harcourt. The rats were divided into 7 groups of 6 rats each and allowed to acclimatize for one week. Water and food were given to the rats *ad libitum*. The groupings and dosage of administration was as shown in Table 1. Carbon tetrachloride was prepared in the ratio of 1:5 (v:v) in olive oil and administered subcutaneously at 0.17 mL/kg body weight on the first day after acclimatization. The method of [19] was adopted for dosage administration of CCl₄. Twenty-four hours after CCl₄ administration, treatment with the leaf extract commenced. Administration of the extract and antioxidant (Vitamin C) was done orally on daily basis for twenty-one days as adopted from [20] and [21]. The rats were grouped as Table 1.

After oral administration for twenty-one days, the six rats per group were fasted over night, weighed and

anaesthetized by exposure to chloroform. The rats were sacrificed painlessly and blood was collected through cardiac puncture from each rat into EDTA and heparin sample bottles. The anti-coagulated blood in EDTA sample bottles was centrifuged at 1000 rpm for 10 minutes to obtain plasma that was used for the analysis on renal parameters while the anti-coagulated blood in heparin sample bottles was used for hematological analysis.

2.3. Determination of Kidney Function Parameters

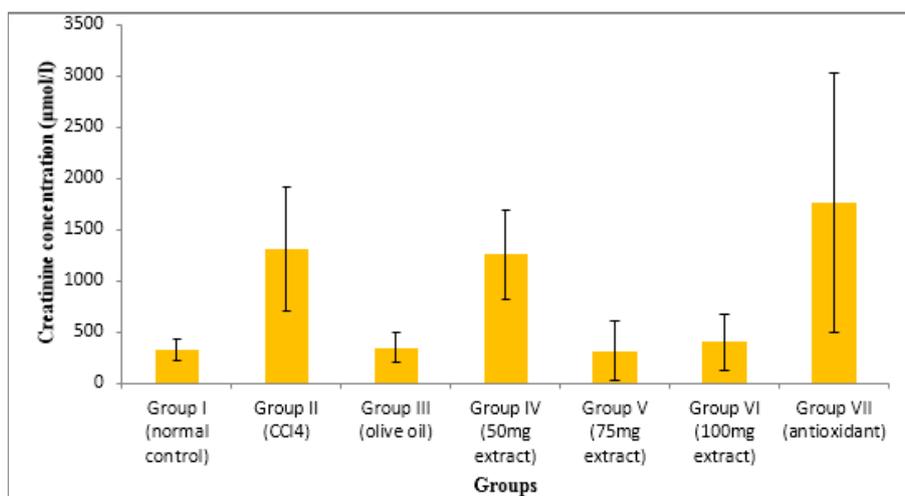
Creatinine was determined using the method of [22], calcium ion, sodium ion and potassium ion were assayed according to the method of [23], Chloride ion was carried out using the method of [24] while magnesium ion was determined using the method of [25].

Table 1. Procedure for Dosage administration

Group	Treatment
I (normal control)	Received normal feed and water daily
II (CCl ₄)	Received subcutaneous dose of CCl ₄ (0.17mL/kgbw) on the first day only
III (olive oil)	Received olive oil on the first day only
IV (50 mg/kgbw)	Received subcutaneous dose of CCl ₄ (0.17mL/kgbw) on the first day + oral dose of <i>C. aconitifolius</i> extract (50 mg/kgbw) daily for 21 days
V (75 mg/kgbw)	Received subcutaneous dose of CCl ₄ (0.17mL/kgbw) on the first day + oral dose of <i>C. aconitifolius</i> extract (75 mg/kgbw) daily for 21 days
VI (100 mg/kgbw)	Received subcutaneous dose of CCl ₄ (0.17mL/kgbw) on the first day + oral dose of <i>C. aconitifolius</i> extract (100 mg/kgbw) daily for 21 days
VII (Vitamin C)	Received subcutaneous dose of CCl ₄ (0.17mL/kgbw) on the first day + oral dose of Vit. C daily for 21 days

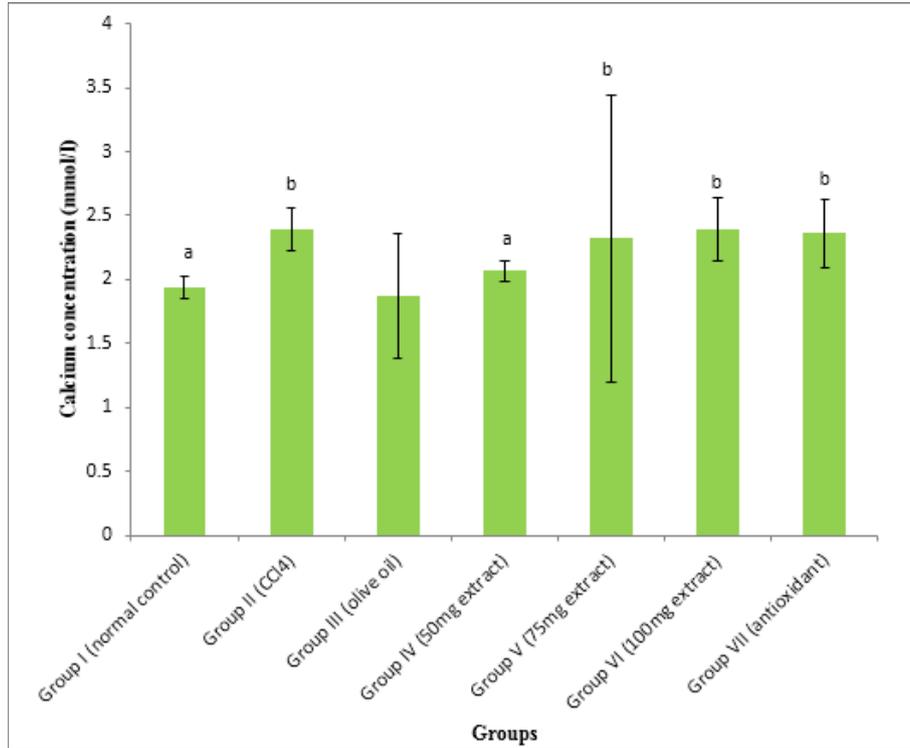
3. Results

3.1. Effect of *Cnidioscolus aconitifolius* Leaf Extract on the Kidney Function Parameters



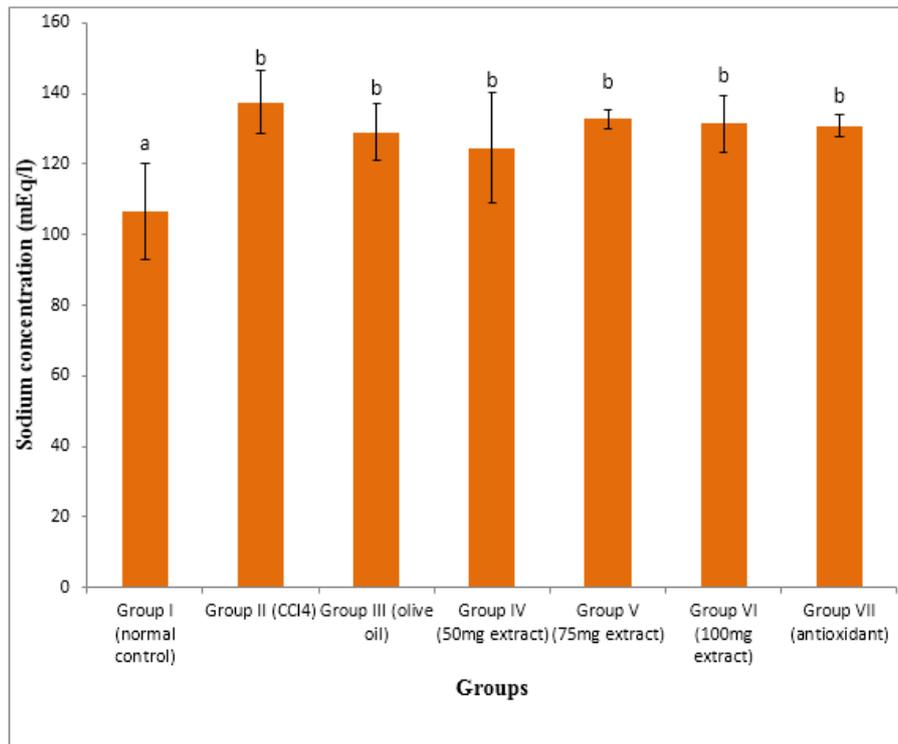
Values are mean±S.D, n=6 per group. There was no significant difference at $P < 0.05$ across all the groups.

Figure 1. Ameliorative effect of the leaf extract of *C. aconitifolius* on creatinine concentration of CCl₄ treated wistar rats



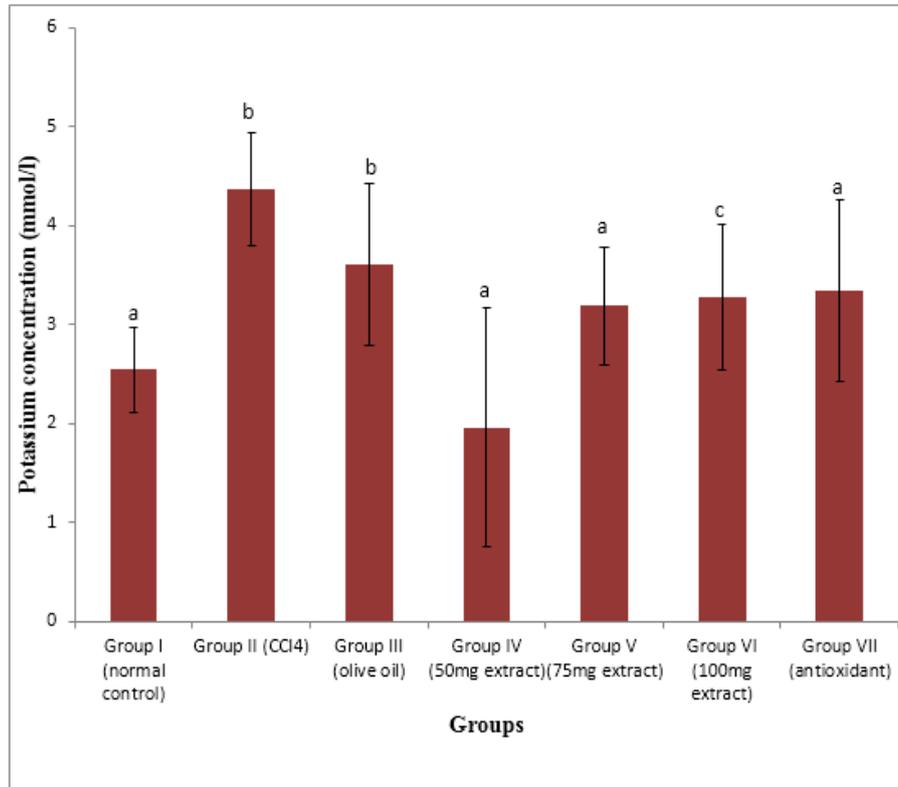
Values are mean±S.D, n=6 per group. Bars bearing different superscript letters (a, b) are significantly different at $P<0.05$ when compared to groups I and II respectively.

Figure 2. Ameliorative effect of the leaf extract of *C. aconitifolius* on calcium concentration of CCl_4 treated wistar rats



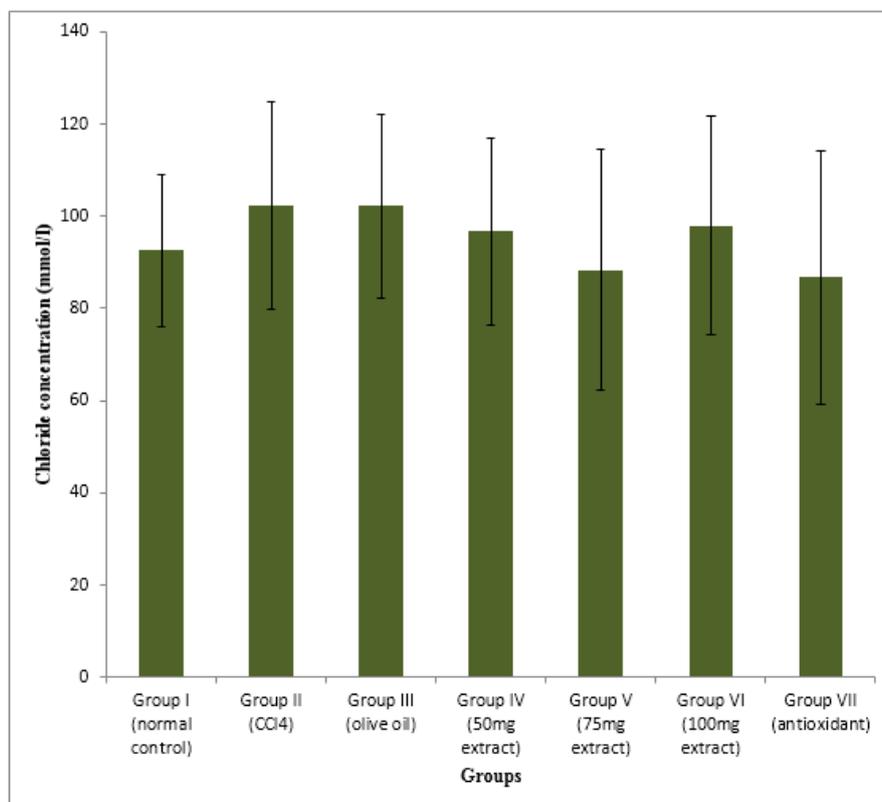
Values are mean±S.D, n=6 per group. Bars bearing different superscript letters (a, b) are significantly different at $P<0.05$ when compared to groups I and II respectively.

Figure 3. Ameliorative effect of the leaf extract of *C. aconitifolius* on sodium concentration of CCl_4 treated wistar rats



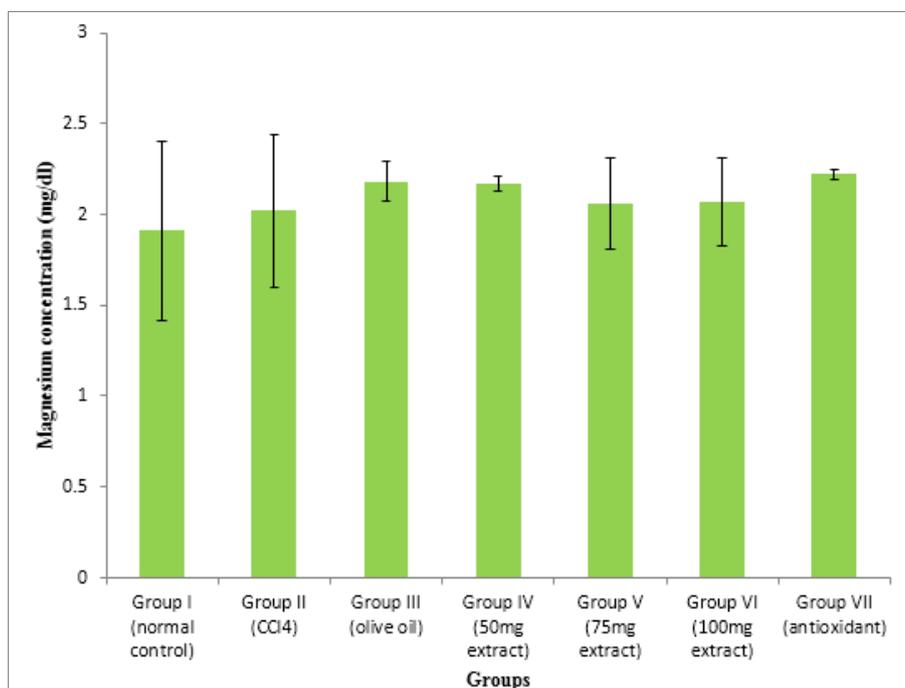
Values are mean±S.D, n=6 per group. Bars bearing different superscript letters (a, b, c) are significantly different at P<0.05 when compared to groups I and II respectively.

Figure 4. Ameliorative effect of the leaf extract of *C. aconitifolius* on potassium concentration of CCl₄ treated wistar rats



Values are mean±S.D, n=6 per group. There was no significant difference at P<0.05 across all the groups.

Figure 5. Ameliorative effect of the leaf extract of *C. aconitifolius* on chloride concentration of CCl₄ treated wistar rats



Values are mean±S.D, n=6 per group. There was no significant difference at $P<0.05$ across all the groups.

Figure 6. Ameliorative effect of the leaf extract of *C. aconitifolius* on magnesium concentration of CCl₄ treated wistar rats

Table 2. Effect of the Leaf Extract of *Cnidocolus aconitifolius* on Hematological Parameters

Parameters	Group I (normal control)	Group II (CCl ₄)	Group III (olive oil)	Group IV (50mg/kgbw extract)	Group V (75mg/kgbw extract)	Group VI (100mg/kgbw extract)	Group VII (vitamin C)
RBC (10 ¹² /L)	7.15±0.54 ^a	5.54±1.13 ^b	6.87±1.24	7.83±0.57 ^a	7.14±0.93 ^a	7.63±0.49 ^a	7.65±1.11 ^a
WBC (10 ⁹ /L)	5.14±1.71	4.05±1.42	4.53±1.74	4.30±0.99	4.27±0.88	3.83±1.09	6.15±2.04
Hb (g/dl)	13.40±0.97 ^a	10.78±0.85 ^b	13.02±0.88 ^a	14.43±0.92 ^c	13.47±1.43 ^a	14.25±1.12 ^c	14.07±1.91
PCV (%)	39.10±3.28 ^a	30.83±6.31 ^b	37.58±6.91	40.95±2.29 ^c	37.87±4.32 ^a	41.88±3.45 ^c	39.73±5.11
MCV (fl)	54.68±2.62 ^a	54.83±1.78 ^a	54.70±0.98	52.48±1.35 ^b	53.18±1.95 ^a	55.68±1.95 ^a	52.10±1.99 ^b
MCH (pg)	18.80±0.79 ^a	18.68±0.42 ^a	19.02±0.89	18.47±0.30 ^a	18.95±0.70 ^a	19.50±0.89 ^b	18.43±0.50 ^a
MCHC (g/dl)	34.40±0.67 ^a	34.08±0.53 ^a	34.80±1.25	34.85±0.56 ^a	35.63±0.54 ^b	35.07±1.38 ^a	34.93±2.26 ^a
Lym (10 ⁹ /L)	3.44±2.04	2.60±1.23	2.73±1.73	3.35±1.85	2.43±1.58	3.13±1.08	4.27±1.77
MPV (fL)	6.08±0.38 ^a	6.10±0.25 ^a	6.10±0.17	6.02±0.21 ^a	6.17±0.30 ^a	6.50±0.24 ^b	6.28±0.19 ^a
RDW (%)	16.90±0.66 ^a	17.68±1.23 ^a	16.30±1.45 ^b	17.68±1.01 ^a	17.12±1.14 ^a	15.45±0.58 ^b	16.90±1.12 ^a
PDW (%)	9.46±0.55 ^a	9.37±0.32 ^a	9.37±0.23	9.31±0.31 ^a	9.53±0.49 ^a	9.95±0.37 ^b	9.62±0.26 ^a
Platelets (10 ⁹ /L)	289.60±55.76 ^a	229.33±49.23 ^b	362.00±70.84	508.83±62.04 ^c	387.50±89.28 ^c	404.17±69.44 ^c	385.33±100.98 ^c
PCT (%)	0.17±0.09 ^a	0.24±0.10 ^a	0.22±0.10 ^a	0.30±0.09 ^b	0.24±0.14 ^a	0.15±0.08 ^a	0.24±0.15 ^a

Values are mean±S.D, n=6 per group. Values bearing different superscript letters (a, b, c) are significantly different at $P<0.05$ when compared to groups I and II respectively.

KEY: RBC- red blood cell, WBC- white blood cell, Hb- haemoglobin, PCV- packed cell volume, MCV- mean cell volume, MCH- mean cell haemoglobin, MCHC- mean cell haemoglobin concentration, Lym- lymphocytes, MPV- mean platelet volume, RDW- red cell distribution width, PDW- platelet distribution width, PCT- plateletcrit

4. Discussion

Figure 1-6 shows the effect of the leaf extract of *C. aconitifolius* on kidney parameters of CCl₄ treated wistar rats. Creatinine is a renal function index that helps determine the functional status of the nephron [26]. This study showed that plasma creatinine concentration increased slightly in CCl₄ treated rats but were ameliorated with the extract of *C. aconitifolius* leaf at 75 mg/kg body weight and 100 mg/kg body weight.

There was no significant difference in the chloride and magnesium electrolyte levels in all the compared groups (Figure 5 and 6). There was a significant increase ($P < 0.05$) in the calcium levels of CCl₄ treated rats and rats administered with the leaf extract at 75 mg/kg and 100 mg/kg when compared with the normal. This was however, reduced significantly ($P < 0.05$) by the extract at 50 mg/kg body weight (Figure 2). There was a significant increase ($P < 0.05$) in the sodium level of CCl₄ treated rats (Figure 3) but were decreased by administration of the leaf extract of *C. aconitifolius*.

Also, there was a significant increase ($P < 0.05$) in the potassium levels of CCl₄ treated rats (Figure 4) but this was decreased by the administration of the leaf extract of *C. aconitifolius* in a dose dependent manner. The increase in calcium, sodium and potassium levels might be from olive oil as shown by their increase ($P < 0.05$) in the rats administered with olive oil when compared to the normal control (Figures 2, 3 and 4). Olive oil served as a vehicle to convey CCl₄ into the blood stream of the rats. This study further agrees with earlier studies by [27], [28] and [29], on hepatoprotective activity, anti-hepatotoxic and antioxidant defense potential and alteration of plasma biochemical, haematological and ocular oxidative indices of *Tridax procumbens* plant extracts respectively. According to [30] and [31], the concentration of calcium ion in body fluids and its handling by cellular proteins are disturbed. The extract reversed the effect of CCl₄ toxicity by lowering the plasma calcium, sodium and potassium levels. It significantly ($P < 0.05$) lowered the calcium levels at 50 mg/kgbw and potassium levels all extract dose administered. Similar observations were made by [32] in their research on plasma electrolytes in sub-chronic salt-loaded rats by aqueous leaf extract of *Tridax procumbens* linn. Calcium fluxes helps in cushioning hormonal effect on target organs via numerous intracellular pathways and neuromuscular activities [33, 34]. *C. aconitifolius* leaf extract might have exerted this effect through its action on the secretion of parathyroid hormone. This hormone facilitates intestinal calcium absorption by encouraging the renal production of 1, 2, 5 dihydroxy vitamin D and in the process, increasing re-absorption of calcium in the renal tubule [32]. Invariably, results obtained in this study showed that *C. aconitifolius* leaf extract at various doses independently enhanced kidney function especially at lower doses.

Anti-hypertensive drugs especially diuretics act by decreasing the plasma chloride and sodium electrolytes through slowing their reabsorption at different points in the nephrons [35, 34]. Results of this study showed that there was no significant change in the chloride concentration while sodium; there was a significant decrease in the rats administered with the leaf extract. This implies that *C. aconitifolius* leaf extract may be used as anti-hypertensive drug.

The effect of CCl₄ treated rats administered with the leaf extract of *C. aconitifolius* on hematological indices is represented in Table 2. There were no significant differences ($P < 0.05$) in the total white blood cell (WBC) count and lymphocytes count of treated rats when compared to CCl₄ and normal control groups. There was a significant increase in the red blood cell (RBC) count, packed cell volume (PCV), haemoglobin (Hb) and platelet number of rats administered with *C. aconitifolius* leaf extract at all doses when compared to CCl₄ group. The plant extract enhanced the RBC count better at 50 mg/kg, followed by 100 mg/kg and then 75 mg/kg body weights. The mean cell volume of rats administered with 50 mg/kg body weight leaf extract was significantly lower ($P < 0.05$) than CCl₄ and normal control groups. The mean cell haemoglobin (MCH) of rats administered with 100 mg/kg body weight extract was significantly higher ($P < 0.05$) than CCl₄ treated rats while the mean cell haemoglobin concentration (MCHC) of rats administered with *C. aconitifolius* at all extract doses were higher but significantly higher ($P < 0.05$) at 75 mg/kg body weight than CCl₄ treated group. Also, the mean platelet volume (MPV) of rats administered with 100 mg/kg body weight extract was significantly higher ($P < 0.05$) than CCl₄ and normal control groups. The red cell distribution width (RDW) of rats administered with 50 mg/kg body weight leaf extract was the same as CCl₄, but lower with the extract at 75 mg/kg body weight, while the group administered with 100 mg/kg body weight extract was significantly lower ($P < 0.05$) than CCl₄ and normal control groups. The platelet distribution width (PDW) of rats administered with 100 mg/kg body weight extract was significantly higher ($P < 0.05$) than CCl₄ and normal control groups. The platelet crit (PCT) level of rats administered with 75 mg/kg body weight extract was the same as CCl₄ group.

The result of the study showed that the extract had a positive effect that was dose dependent on the haemopoietic system of the test rats. It increased significantly the red blood cell count, haemoglobin, packed cell volume and platelet count at all extract doses, mean cell haemoglobin at 100 mg/kg body weight extract dose and mean cell haemoglobin concentrations at 75 mg/kg body weight extract dose. According to some researchers [36, 37, 38, 39], WBC help in destabilizing plaques in the coronary artery during the early stages of acute coronary syndrome. Nevertheless, increased white blood cell count is a sign of coronary artery disease in the peripheral blood

[39]. Results obtained here showed that there was no significant change in the WBC and lymphocytes count which indicates that the immune systems of the rats were not compromised by the extract. Results of this study is similar to findings of [40] and [29] on his works on “moderation of hematological and plasma biochemical indices of subchronic salt-loaded rats, by an aqueous extract of the leaves of *Acalypha wilkesiana*” and “alteration of plasma biochemical, haematological and ocular oxidative indices of alloxan induced diabetic rats by aqueous extract of *Tridax procumbens* linn” respectively.

5. Conclusions

The present study has shown that the leaf extract of *Cnidoscopus aconitifolius* had a dose-dependent positive effect on the hematological indices as well as the potential to ameliorate kidney parameters of CCl₄ treated wistar rats at the given concentrations. It significantly decreased the calcium levels at 50 mg/kg body weight and potassium levels at all extract doses administered thereby enhancing the cushioning effect and increasing their re-absorption.

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