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University of Eldoret, Kenya, Mount Kenya University, Kenya, Chukwuemeka Odumegwu Ojukwu University, Nigeria, Kyambogo University, Uganda and University of Makeni, Sierra Leone

Effect of Ethanol Leaf – Extract of *Justicia carnea* on Body Weight and Hematology of Cadmium Chloride Induced Toxicity in Wistar Rats

***Angelista Ojiugo Amadi[1], Andrew Chibuzo Nwaka[2], Collins Bambode Lukong,[2] Emeka Sabastine Asiwe[3], Ugochi Vivian Chukwuemeka[1], Vivian Nneka Soronnadi[1], Valentine Chukwuebuka Ezeanyanwu[1], Angela Mma Ochia[3,] Nneka Clare Onuoha[1], Theodore Ogochukwu Akunna[1], Sixtus Ikenna Nwachukwu[1]**

[1]*Science laboratory technology, Imo state polytechnic Omuma, Imo state.*
[2]*Department of Biochemistry, Chukwuemeka Odimegwu Ojukwu University Uli, Anambra state.*
[3]*Department of Biochemistry, University of Agriculture and environmental sciences Umuagwo, Imo state.*

***Correspondence:** angelistaamadi@gmail.com*

Abstract

Medicinal plants are good sources of antioxidants that prevent body cells against oxidative assaults. They can also occasionally act as pro-oxidants to alter biochemical pathways and organ functions. The present study was carried out to determine the effects of ethanol leaf- extract of *Justicia carnea* on body weight and some hematological parameters of cadmium chloride-induced toxicity in Wistar rats. A total of forty seven adult Wistar rats (males) were used. While twelve rats were used for acute toxicity study of the extract, Thirty five divided into seven groups of five animals each were used for the body weight and hematological study. The groups were group A (Normal control/normal animals), group B (Negative control/untreated animals), group C (Positive control/group treated with standard drug (100mg/kg Silymarin)), group D (animals treated with 100mg/kgbwt extract(pre and post treatment)), group E (animal treated 300 mg/kgbwt (pre and post treatment)), group F (animals treated with 100mg/kgbwt extract (post treatment)) and group G (animals treated with 300mg/kgbwt extract (post treatment)). Both Pre treatment (treatment before induction) and post treatment (treatment after induction) of the extract were carried out on groups D and E at doses of 100mg/kgbwt and 300 mg/kgbwt respectively and in groups F and G only post treatment of the extract were carried out at doses of 100mg/kgbwt and 300mg/kgbwt respectively. While the treatment of groups D and E was effective for four weeks, treatment of groups F and G lasted for two weeks and treatment effects on body weight, full blood count, differential blood cells were compared with the three controls (normal, Negative and Positive Controls). In the acute toxicity test, the results of the two phases indicated that LD_{50} of the extract could be higher than 5000mg/kg bwt as no death or observed toxicity was recorded after doses of 10 to 5000 mg/kg bwt. The results indicate progressive and significant ($p<0.05$) increase in body weight in all the groups. The result of HGB, PCV and RBC showed significant ($p<0.05$) increase while WBC showed significant ($p<0.05$) decrease in groups D, E, F and G when compared to negative control, the result of neutrophil and lymphocyte showed significant ($p<0.05$) increase in all the groups compared to normal control. The results of esinophil and monocytes counts showed no significant difference ($p>0.05$) in ethanol extract treated groups when compared with normal control. The same result was obtained in basophils except group E that showed significant ($p<0.05$) increase when compared with the normal control. The results of this study suggest that ethanol leaf extract of J. carnea is relatively safe and could be beneficial in alleviating hematology related abnormalities.

Keywords: *hematology, body weight, wistar rats, Justicia carnea, cadmium chloride.*

Introduction

In recent time, profuse attention has been focused on the use of medicinal plants in the management and treatment of ailments such as anaemia, diabetes, malaria, etc. Due to the local availability, easy access and relatively low cost, medicinal plants are gaining attentions in health care programmes. Based on estimation by World Health Organization (WHO), larger percentage (between 80 and 90%) of the world's population especially in developing countries depends on traditional system of medicine, (Kone *et al.* 2012) and (Van *et al.* 2013). Despite the therapeutic importance of medicinal plants, toxic substances have been shown to be present in large numbers of plants investigated, (Mounanga *et al.* 2015). Contamination of medicinal plant could be as a result of contaminants (such as heavy metals, aflatoxin and pathogenic microbes) from soil and ways of herbal preparations, (Olaniyan *et al.* 2016). Consumption of medicinal plants without scrutinizing its efficacy and safety can result in unexpected

toxic effects resulting in physiology changes of different organs in the body such as Liver and kidney which are the main targets of toxicants due to their involvement in biotransformation and excretion of xenobiotics respectively. Hepatic and renal damage has been recently linked with the use of medicinal plants in the treatment of various diseases, (Paes-Leme *et al.* 2005) and (Mapanga & Musabayane , 2010). *Justicia carnea* is a flowering plant, widely distributed in various parts of Africa. It is an edible and medicinal plant used in therapy of some diseases, of which majority of its species have been reported to contain vital chemical compounds of medicinal importance. Despite the immense use of medicinal plant, preliminary toxicity studies remain essential tools to ensure safe consumption and prevent unexpected toxicity that could arise from long term exposure.

Methodology

Collection of Blood

At the end of 7 days of treatment with respective doses of the extract and standard drug for ameloriation, animals were fasted overnight and after being lightly anaesthetized were sacrificed by cervical dislocation and blood collected by cardiac puncture

Body Weight*:* This was determined daily using electronic Weighing balance and weight recorded

Acute Toxicity (LD_{50}) Study

Oral acute toxicity study on *J. Carnea* ethanol extract was carried out according to Lorke (1983) method using a total of twelve (12) wistar rats weighing between 130g and 160g for the study. The animals were randomly grouped for the study according to body weight. They were fasted overnight, but with access to water *ad libitum* and then treated orally with *Plant* extract. The study was carried out in two Phases.

Phase (1): This phase requires nine rats subdivided into three (3) groups of three animals each. The groups in this phase of study were administered doses of 10, 100 and 1000mg/kg.b.wt. of the extract and then observed for 24 hours for paw licking, salivation, stretching of the entire body, weakness, respiratory distress, coma and possible death in the first 24hrs and subsequently observed daily for 7 days.

Phase (2): The procedure above was repeated using another set of fresh wistar rats divided into three groups of one rat each, and were given 1600mg/kg, 2900mg/kg and 5000mg/kg body weight, respectively. The doses were chosen based on deduction from the first phase. These were observed for signs of toxicity and mortality for the first critical 24hrs and thereafter daily for 7 days. Thereafter, the LD_{50} value was calculated as the square root of the product of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

$LD_{50} = \sqrt{HNLD \times LLD}$

Determination of Packed Cell Volume

Principle; When whole blood sample is subjected to a centrifugal force for maximum RBC packing, the space occupied by the RBCs is measured and expressed as percentage of the whole blood volume.

***Proedure*;** Using micro haematocrit method, a well-mixed anticoagulated whole blood was allowed to enter capillary haematocrit tubes until appropriately 2/3 filled with blood. Blood filling was done for each tube. One end of each tube was sealed with Bunsen flame and placed in the medial grooves of the haematocrit centrifuge head exactly opposite each other, with the open end towards the center. The lid was replaced and centrifuged for

five minutes at 11,000rpm. The tubes were removed as soon as the centrifuge had stopped spinning. And the value of the packed cells was read off using the microhaematocrit reader.

Determination of Haemoglobin Concentration

Principle; When whole blood is added to Drabkin's reagent: a solution containing KCN (Potassium cyanide) and (Potassium ferricyanide) K3Fe(CN)6, KCN converts Hb-Fe2+ (ferrous) to Hb-fe3+ (ferric) state to form methaemoglobin which then reacts with KCN to form a stable pigment, cyanmethaemoglobin complex. The colour intensity of this mixture is measured in a spectrophotometer at a wavelength of 540nm (or using a yellow-green filter). The optical density (OD) of the solution is proportional to the haemoglobin concentration.

Prcedure*;* Using Cyanmethaemoglobin method, exactly 5.0ml of Drabkin's reagent was pipetted into two test tubes 1 and 2. A well-mixed sample of EDTA blood (0.02ml) was pipetted into the tubes, rinsing the pipette five times with the reagent, until all the blood was removed from the pipette. The solutions were well mixed and allowed to stand at 25℃ for 10 minutes in order to allow the formation of Cyan – met- haemoglobin. The mixtures were transferred into cuvettes and read in a spectrophotometer at a wavelength of 540nm. The Drabkin 's reagent in tube 1 was used as the blank (setting the percentage transmittance at 100%). The readings from each tube was recorded and the actual Hb values in g/dl were determined from a pre- calibrated chart.

Determination of White Blood Cells (WBCS)

Principle; When whole blood is mixed with weak acid solution such as glacial acectic acid solution, it dilutes the blood and haemolyses the RBCs, enabling the WBCs to be counted.

Procedure;

The blood specimen was mixed approximately for one minute, using the white blood cell pipette, blood will be drawn to the 0.5mark in the pipette. Blood was removed from the outside of the pipette with clean gauze. The tip of the pipette was placed into the counting diluting fluid to draw it slowly until it reached the 11 mark. The counting chamber and the cover glass were cleaned with a cloth. The counting chamber was filled with diluted blood. The four corners of the chamber was visualised under a low power (10X) objective and the cells were counted in all the four marked corner squares.

Determination of Red Blood Cells (RBCs)

Principle; To facilitate counting, whole blood is diluted with Gower's solution which hemolyze white blood cell and prevent red blood cell lysis.

Procedure;

A 1:200 dilution was made by diluting 20µl of EDTA anticoagulated blood in 3.98ml of Gower's solution and mixed for 3minutes. The counting chamber and cover glass was cleaned appropriately. 10µl of the diluted fluid was used to both chambers of the haemocytometer avoiding air bubble, it was allowed to stand for 3minutes prior to counting. The haemocytometer was carefully placed on the microscope stage, the condenser on the microscope

was lowered and the chamber was scanned using 10X objective lens. The cells were counted using the 40X objective lens.

NOTE: Gower's solution contains sodium sulphate 12.5g, glacial acetic acid 33.3ml and distilled water 100ml

Determination of Differential Cells

Principle; A drop of blood is smeared on a slide, stained and examined under the microscope, to establish the morphology of red blood cells, leucocytes and platelets and the relative frequency of different leucocytes. The slide is stained with one of the Romanowsky stains (Leishman stain).

Procedure;

A drop of well mixed anticoagulated blood was placed on a clean, grease free slide, using a spreader the blood was smeared on the slide and allowed to air dry. The slide was flooded with Leishman stain and allowed to stand for 2minutes. The stain was diluted with twice its volume of buffered distilled water. It was mixed by blowing air gently on the stain to ensure uniform mixing. The stain was allowed to stand for 8minutes. Excess stain were rinsed off with buffered distilled water, the back of the slide was wiped to remove all traces of the stain. The slide was drained and stood upright in a draining rack to dry. The slide was examined microscopically with 100X oil immersion objective lens.

Results And Discussion

Acute Toxicity Study

***Table 1*:** Acute Toxicity of Ethanol Extract of *J. Carnea* Leaves.

Groups of rats	**Extract Dosage** (mg/kg body weight)	Mortality recorded
PHASE 1		
GP 1	10	0/3
GP 2	100	0/3
GP 3	1000	0/3
PHASE 2		
GP 1	1600	0/3
GP 2	2900	0/3
GP 3	5000	0/3

Table 1presented the result of the acute toxicity studies (LD_{50}) of *J. carnea* leaves where the extract in the two phases of the studies at the doses of 10 – 5000 mg/kg body weight proved no mortality and no adverse effects was observed from the animals using Lorke *et al*. (1984).

Body Weight

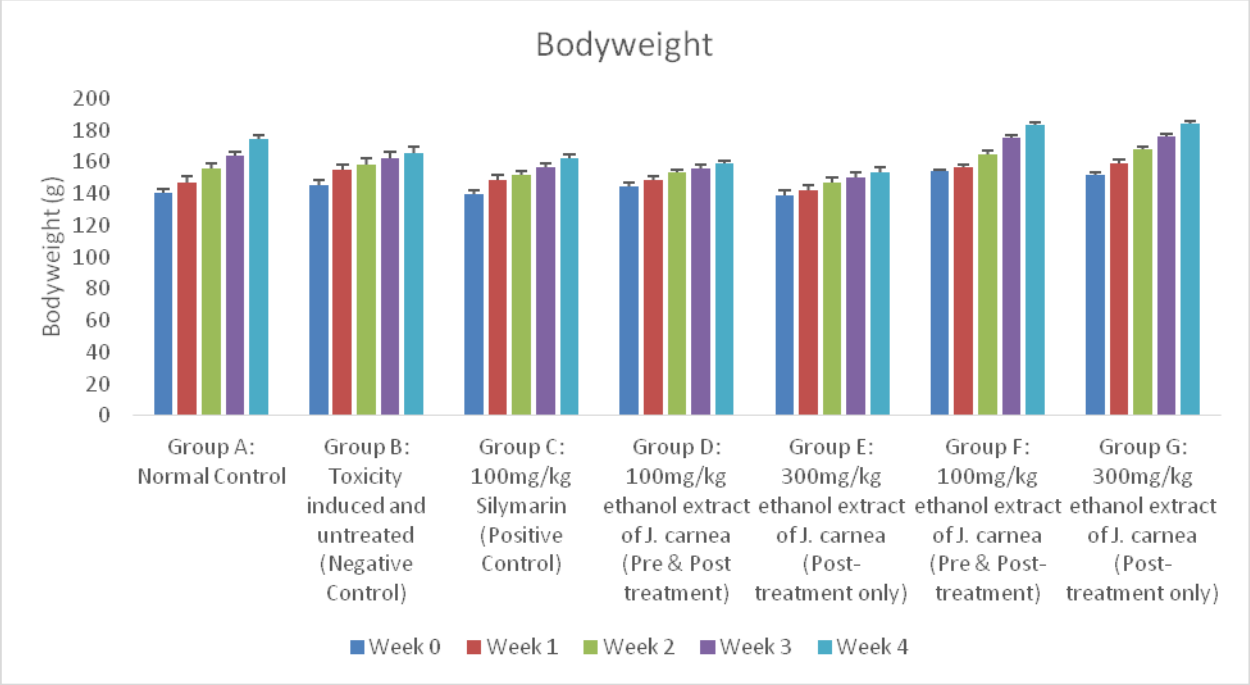

***Figure 1*:** Effect of Ethanol Extract of *J. Carnea* on Body Weight of Cadmium Chloride-Induced Toxicity in Wistar Rats.

Full Blood Count

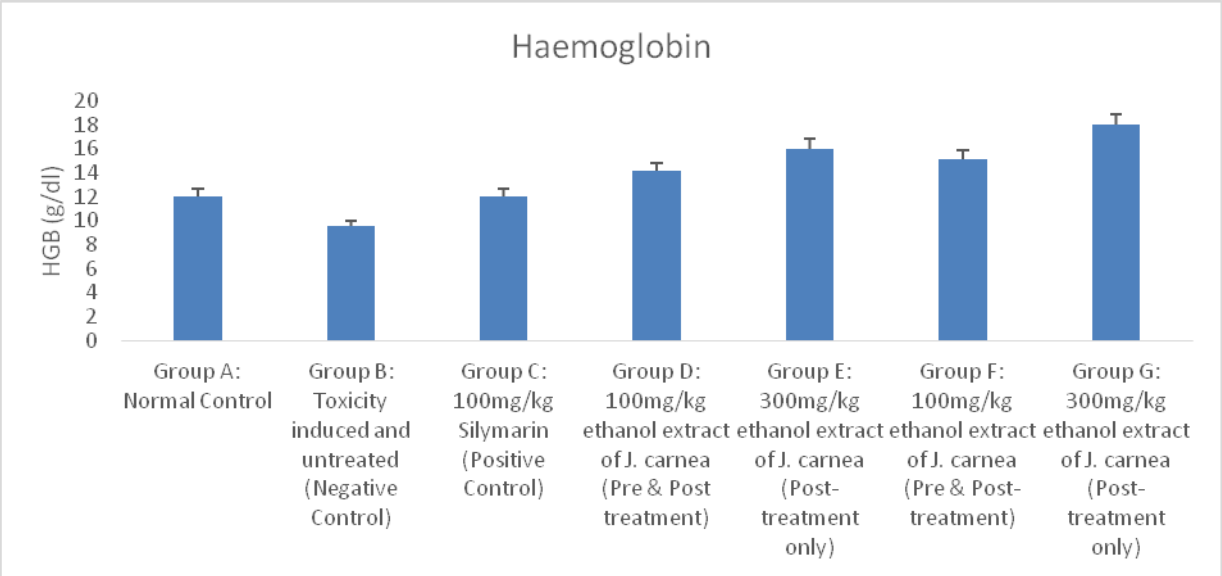

Figure 2: Effect of ethanol extract of *J. carnea* on haematological parameters of cadmium chloride-induced toxicity in rats.

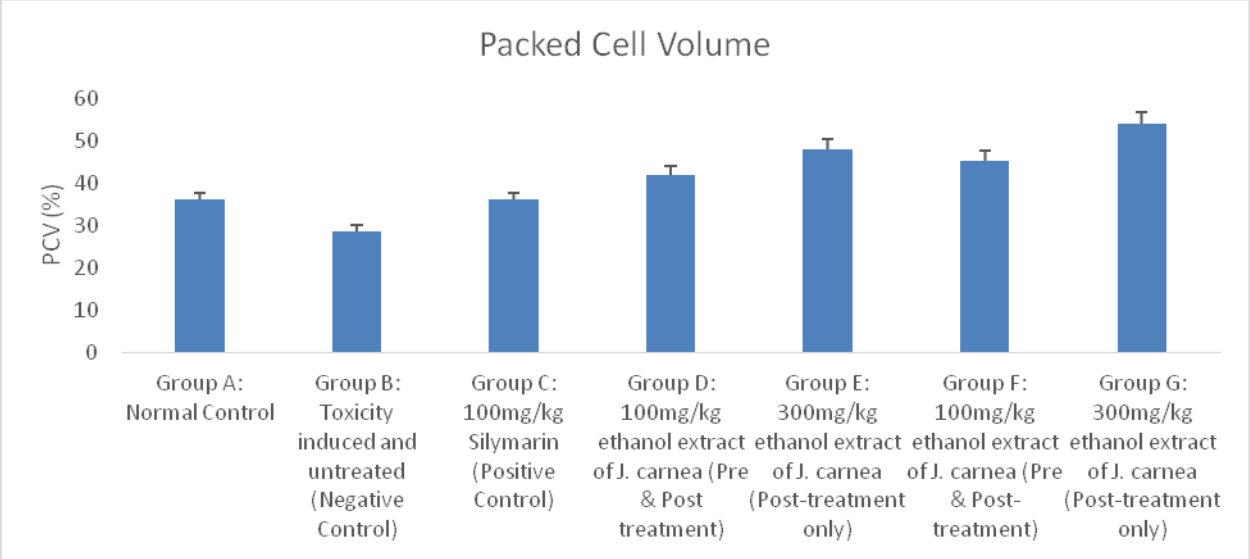

Figure 3: Effect of ethanol extract of *J. carnea* on packed cell volume of cadmium chloride-induced toxicity in rats.

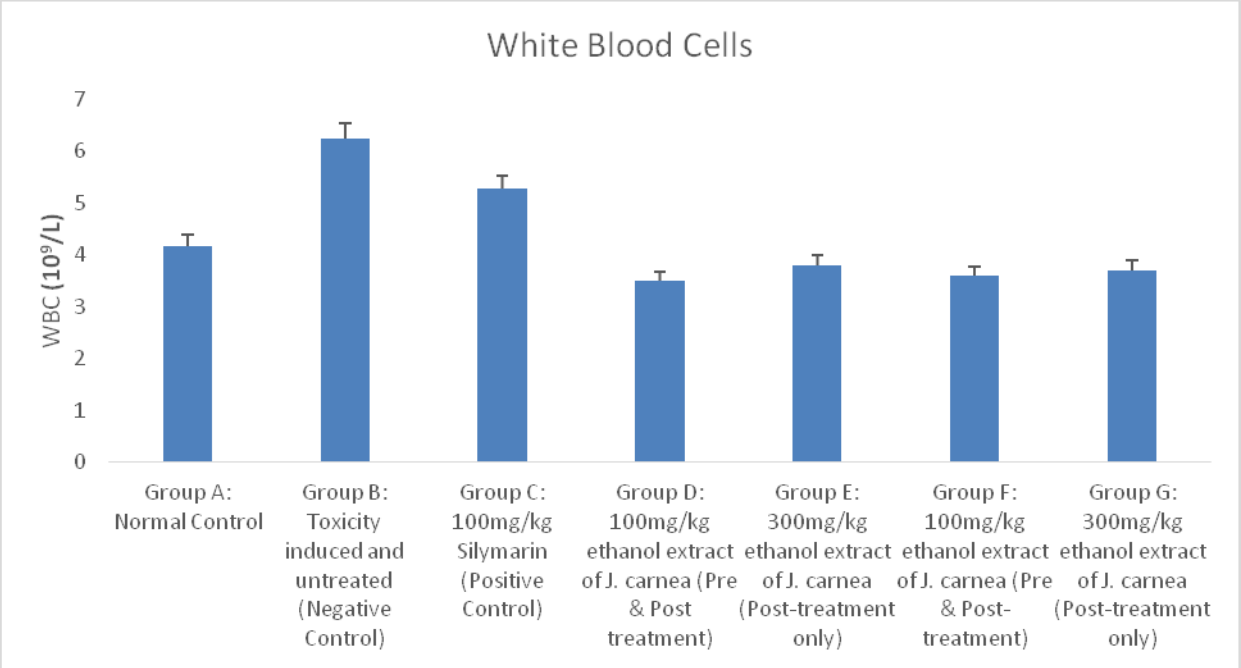

Figure 4: Effect of ethanol extract of *J. carnea* on white blood cells of cadmium chloride-induced toxicity in rats.

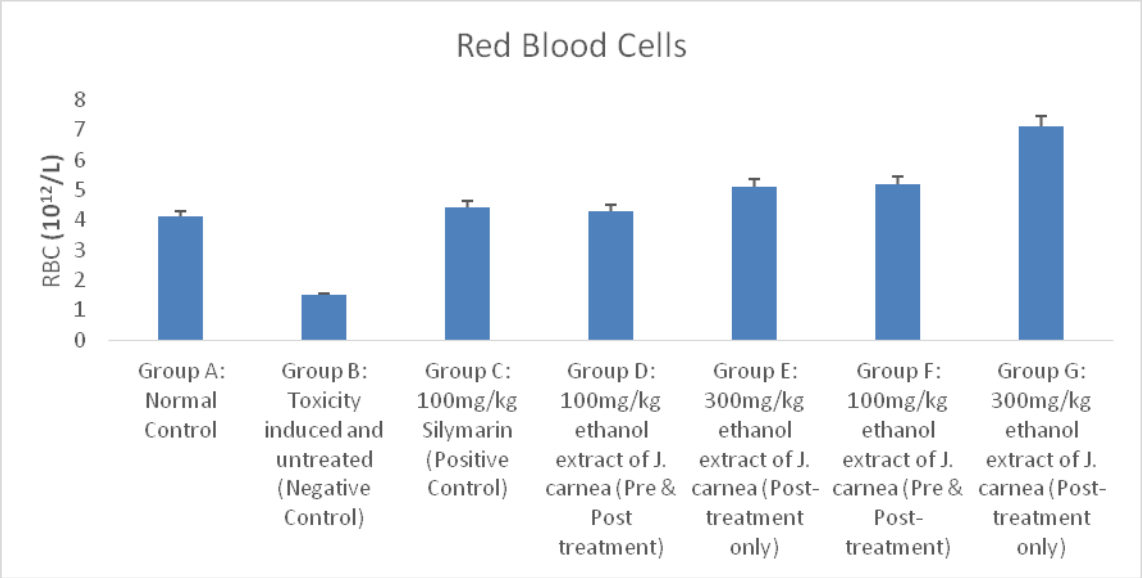

Figure 5: Effect of ethanol extract of *J. carnea* on red blood cells of cadmium chloride-induced toxicity in rats.

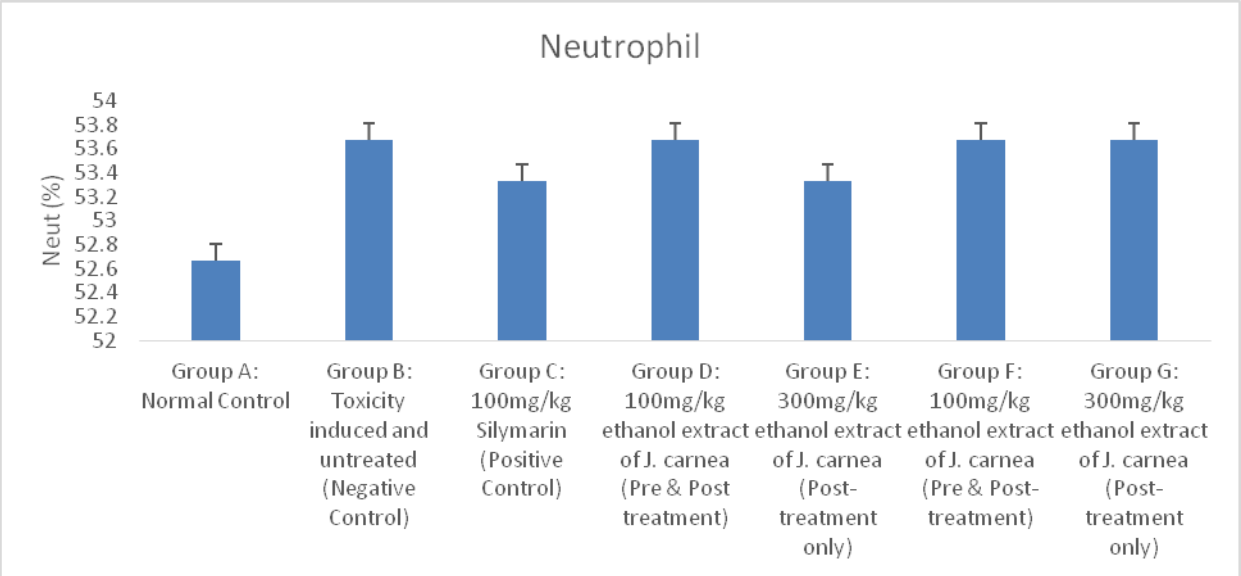

Figure 6: Effect of ethanol extract of *J. carnea* on neutrophil level of cadmium chloride-induced toxicity in rats.

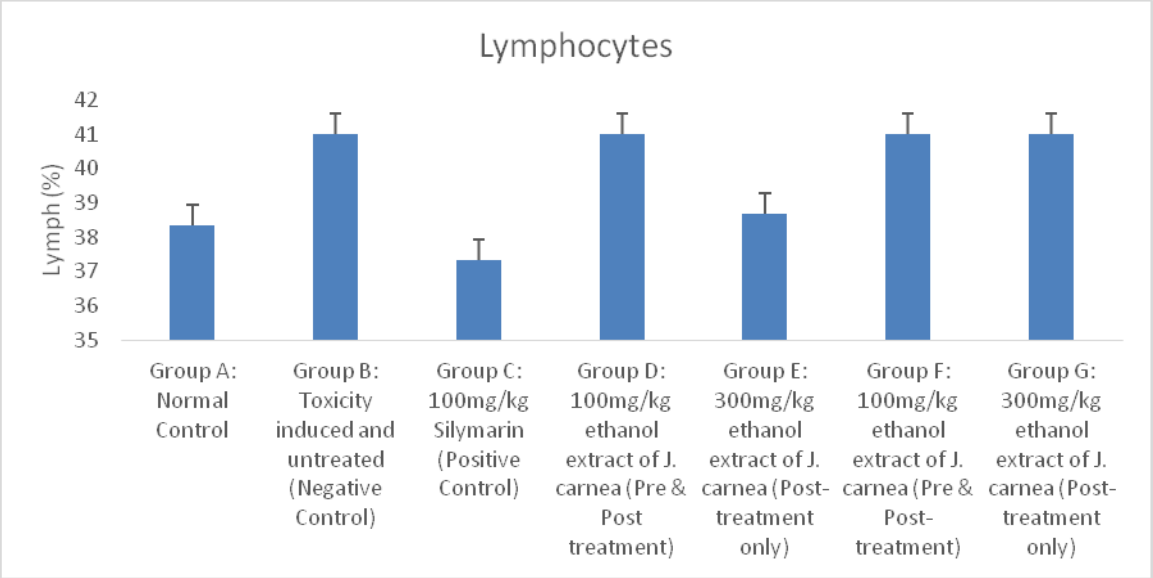

Figure 7: Effect of ethanol extract of *J. carnea* on lymphocytes of cadmium chloride-induced toxicity in rats.

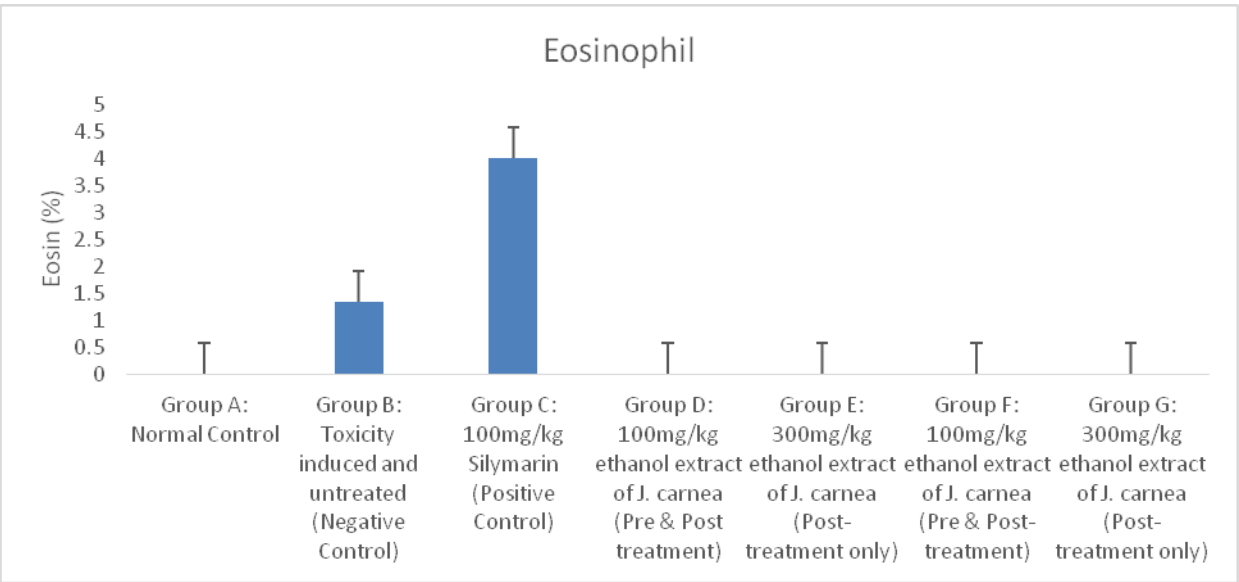

Figure 8: Effect of ethanol extract of *J. carnea* on eosinophil of cadmium chloride-induced toxicity in rats.

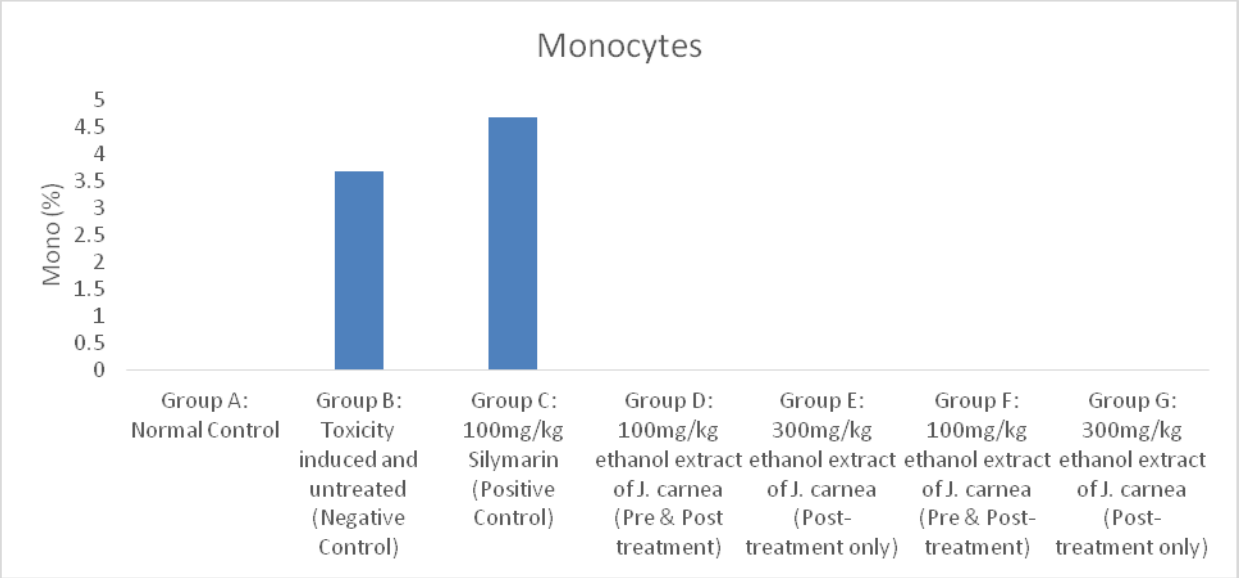

Figure 9: Effect of ethanol extract of *J. carnea* on monocytes of cadmium chloride-induced toxicity in rats.

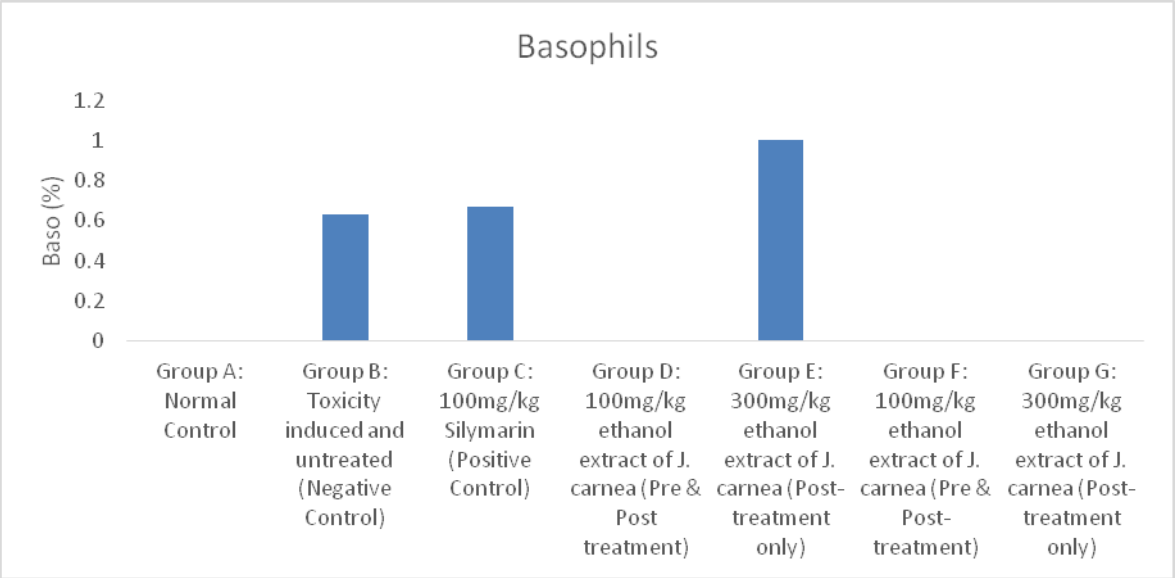

Figure 10: Effect of ethanol extract of *J. carnea* on basophils of cadmium chloride-induced toxicity in rats.

Conclusion

The result of this study agrees in greater percentage with previous works on the plant and therefore suggests that ethanol extract of *J. carnea* leaf is relatively safe, could not cause inflammation related abnormalities in the body system.

Recommendation

From the result of this study, the leaves of this plant (*justicia carnea*) is recommended for its use as Anti-inflammatory therapy but with caution on its dosage as very high dosage could cause some damages to some of the tissues in the body.

Findings

The study showed that;

1. The leaf of the plant (*Justicia Carnea*) is safe for consumption as no mortality was recorded in the acute toxicity study even at 5000mg/kgb.wt
2. The plant possesses anti-inflammatory properties as shown in arrays of heamatological tests (Full Blood Count).
3. The plant is a blood boaster and could remedy aneamia and other heamatology related issues.

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