British Journal of Medicine & Medical Research
13(12): 1-10, 2016, Article no.BJMMR.23414
ISSN: 2231-0614, NLM ID: 101570965

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Blood Parameters and Spleen Histology Following Chronic Consumption of Ethanolic Extract of *Costus afer* Stem and Juice on Albino *Wistar* Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Author AOA designed the experiment and interpreted the result. Author UA procured the experimental animals and was involved in extract administration. Author MAE did the statistical analysis. Author TEI handled photomicrography. Author MU did literature search and took care of the extraction process while author TBE was the supervisor and adviser on scientific procedures and methodology. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2016/23414
Editor(s):
(1) Claudia Borza, "Victor Babes" University of Medicine and Pharmacy, Department of Pathophysiology, România.
Reviewers:
(1) Ahmed M. S. Hegazy, Benha University, Egypt.
(2) Mehlika Benli, Ankara University, Turkey.
Complete Peer review History: http://sciencedomain.org/review-history/13445

Original Research Article

Received 30th November 2015
Accepted 6th February 2016
Published 26th February 2016

ABSTRACT

Aim: *Costus afer* is a medicinal plant used as a therapy for diabetes and hypertension. This study investigated the effect of crude ethanolic extract of *Costus afer* (Monkey sugar cane) stem and its juice on the histology of the spleen and some blood parameters of albino *wistar* rats.
Place and Duration of Study: This study was carried out in the Department of Human Anatomy, University of Calabar, Nigeria for four weeks.
Methodology: Twenty four (24) rats were divided into four groups of six (6) animals each. Group one (I) served as control, Group two (II) served as experimental group and received 200 mg /kg

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body weight (low dose) of the crude ethanolic extract. Group three (III) also served as experimental group, tested with 500 mg/kg body weight (high dose) of the crude ethanolic extract and Group four (IV) was tested with 5 mls /kg body weight of *Costus afer* stem juice. Treatment was orally using orogastric tube for twenty eight (28) days after which the animals were sacrificed within twenty four hours after the last dose.

Results and Conclusion: Results showed no morphological changes. Histological sections of the spleen from experimental animals in the control group showed normal histology of the spleen with well distinct lymphatic tissue. Sections of the spleen from animals tested with 200 mg /kg ethanolic extract showed no pathological changes compared with the control group. Sections of the spleen from animals tested with 500 mg/kg body weight of the crude ethanolic stem extract were observed to have more red pulp than the white pulp. Animals tested with 5mls /kg body weight of *Costus afer* stem juice showed no pathological observations. Hematological observations showed decreased Packed Cell Volume (PCV) and increased Platelet count (P =.05), but had no significant effect on total white blood cell count and differential count. In conclusion, *Costus afer* at the administered dose had no pathological effect on the spleen histology, but increased platelet count and decreased packed cell volume.

Keywords: Costus afer extract; spleen histology; blood parameters.

1. INTRODUCTION

Man has used various parts of plant as food and for the prevention and treatment of many ailments [1]. Today a substantial number of drugs are developed from plants which are active against a number of diseases [2]. In the developed countries 2.5 percent of the medicines are based on plants and their derivatives [3]. The use of medicinal plant is common among the indigenous people in rural areas of many developing countries. In Nigeria, traditional medicine has become a part of the people's culture; the ministry of health in many African countries including Nigeria now has departmental agencies which oversee the affairs of traditional health care delivery system. In addition, formal training of traditional health care attendants is done in many African countries [4].

Costus afer (C. afer) is a perennial rhizomatous herb, found in the forest belt of Senegal, South Africa, Guinea, Sierra Leone and Nigeria Ghana etc. [5]. It belongs to the family, *Zingerberacea,* a monocot and relatively tall herbaceous, tropical plant with creeping rhizome. It is commonly called bush cane, *Irekeomode* in Yoruba, *Kakizawa* in Hausa, *Opete* in Igbo, *Mbritem* in Efik [5]. The seed and rhizome are harvested from the wild plant and they contain several bio-active compounds capable of preventing and treating most oxidative related disease [6]. *C. afer* is useful medicinal plant that is valued for its antidiabetic [7] and hypolipidermic [8]. It has anti-inflammatory effect [9]. The methanol stem extract has antioxidant effect [10]. The pounded fruit is used to relief cough [11]. The leaves are reported to be an effective remedy for fever and malaria when boiled with the leaves of *Carica papaya* [4]. The leaf and stem extracts have hypoglycemic/antihyperglycemic activity [7,12,13], antinociceptive [14], hepatotoxic effect [11] while hepatoprotective effect against alcohol induced liver cirrhosis [15] and against carbon tetrachloride induced hepatotoxicity has also been reported [5].

The young and tender leaves when chewed are believed to give strength to the weak dehydrating patient. An infusion of the inflorescence is taken to treat stomach complaints [4] while the dried aerial part is used to treat hypertension. The stem is used as enema to worms and hemorrhoid. The pulp stem taken in Water is strongly diuretic. In traditional medicine, the juice from the stem is applied to fresh wound to stop bleeding. A cold water extract of the stem is used to treat small epileptic attack. Pulp is also applied to teeth to cure tooth ache. The decoction is taken to treat leprosy and viral disease [4]. Phytochemical screening of ethanolic extract of *C. afer* shows the presence of alkaloid, Saponin, Triterpennoids, Tannis, Protein, Glycosides, Flavonoids and Carbohydrates. The presence of these phytochemical compounds is the basis for its beneficial role in agriculture and folk medicine [16]. Evaluation on bacterial strain which included *streptococcin*, pyogene, *Staphylococcus aereus* and *Staphylococcus pneumonia* was effective. This justifies the traditional use of plant (*C. afer)* as a remedy for stomach ache, cough and rheumatic pains in rural areas [17].

2. MATERIALS AND METHODS

2.1 Plant Collection and Preparation of Extract

Fresh stem of *C. afer* were obtained from animal farm of the University of Calabar, and air dried for two weeks thereafter followed by oven drying for one day. The dried stems were homogenized into powder form using an electric blender. The powdered form, 7 kg was obtained. The powder was then socked in an equal volume of ethanol, dichloromethane (1:1). The solvent was put in large quqntity (15 Litres) and kept for 72 hours for thorough extraction of active components. The mixture was filtered first using a chess cloth followed by the filtrate being filtered through watt man No. 1 filter paper of pore size 0.45 micrometer. The filtrate was placed in beakers and allowed to concentrate in a water bath by evaporation at 40℃ to complete dryness yielding the extract. The obtained extract was in the form of a paste.

2.2 Extraction of *Costus afer* Stem Juice (CASJ)

The collected stems were debarked after that the foliage leaves covering them were removed. The debarked stems were then introduced into a clean manual blender and were crushed to squeeze out their juice. The resulting juice was filtered to obtain the CASJ each day.

2.3 Animals and Experimental Procedures

Twenty four male and female albino *Wistar* rats were used weighing between 127 – 210 grammes for the work; they were purchased from The Zoology Animal House, University of Calabar. They were kept in the animal house in the department of Human Anatomy College of Basic Medical Sciences, University of Calabar, Calabar. The animals were kept in a stable standard environmental condition with appropriate room temperature of about 25-27℃ throughout the duration of the experiment. The weights of the rats were taken before commencement of the experiment. The twenty four adult male and female albino *Wistar* rats were divided into four groups (Control, Low dose, High dose and Juice) of six animals each. The extract given to the experimental groups was reconstituted to obtain an appropriate concentration administered to the rats according to their body weights. It was administered through oral route using orogastric intubation at a dose of 200 mg/kg body weight for low dose, 500 mg/kg body weight for high dose and 5 mls/kg stem juice. The control group received distilled water of equivalent amount in volume as the extract. The administration was done once a day for twenty eight days.

2.4 Collection of Samples for Analysis

At the end of 28th day of administration, animals were sacrificed the day after the last dose using chloroform as the anesthesia. The thoracic cage was incised using a pair of scissors and blood was collected from the left ventricle into an EDTA bottle. It was spun and serum extracted for biochemical assay. The spleen was cut out and immediately fixed in buffered formaldehyde, prepared for paraffin sections at 5microns and later processed for histological staining with haematoxylin and eosin stains as reported by Fischer et al. [18].

2.5 Statistical Analysis

One way Anova and the LSB post hoc test were used for the statistical analysis by using the SPSS package.

3. RESULTS

Morphologically, it was observed that there was no observable morphological anomaly in the body of the tested groups compared to the control group.

3.1 Histological Observations

3.1.1 Control

Section of the spleen from animals in this group showed normal histology of the spleen with well distinct lymphatic tissue seen as aggregation (white pulp) and a diffused part (red pulp)- (Fig. 1).

3.1.2 Low dose, 200 mg /kgw

Animals in this group tested with 200 mg/kg body weight (low dose) of the ethanolic crude extract for twenty eight days. The histological section of the spleen showed no pathological changes compared with the control group (Fig. 2).

3.1.3 High dose, 500 mg /kgw

Animals in the third group were tested with 500 mg/kg body weight (high dose) of *C. afer*

ethanolic crude extract for twenty eight days. The histological section of the spleen from animals in this group were observed to have lymphatic tissue seen as the red pulp which appears to be more in section than the white pulp (Fig. 3).

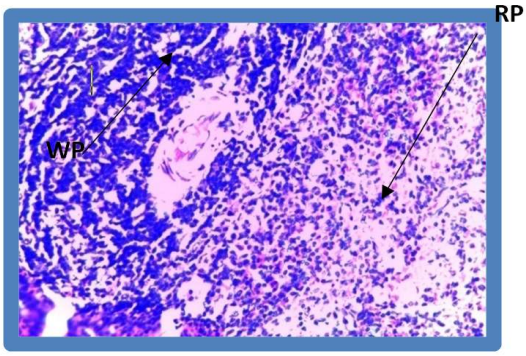

Fig. 1A

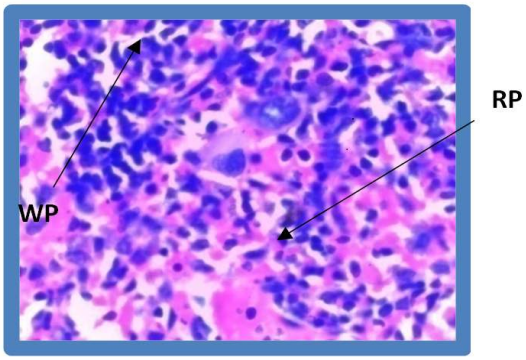

Fig. 1B

Fig. 1. (A): The histology of the spleen from animal in the control group,showing normal histological features with lymphatic nodules, white pulp(WP) and diffused lymphatic tissue, Red pulp (RP). Haematoxylin and eosin stains (H and E) Magnification (mag)., X100 (B): Mag., X160, has similar features as A

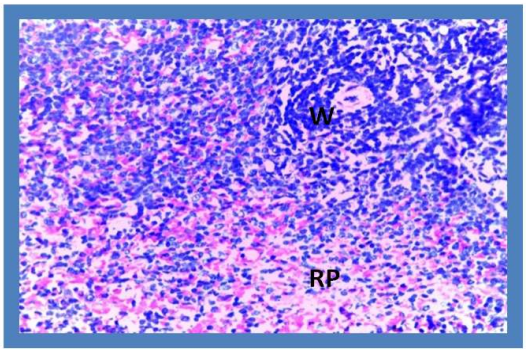

Fig. 2A

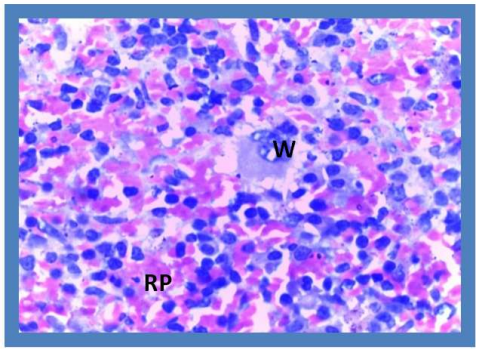

Fig. 2B

Fig. 2. (A): The histology of the spleen from animal treated with 200mg/kg body weight of *Costus afer* Crude ethanolic extract. (Haematoxylin and Eosin), mag:X100 (B): Mag., X160, has similar features as A. WP-white pulp; RP-red pulp

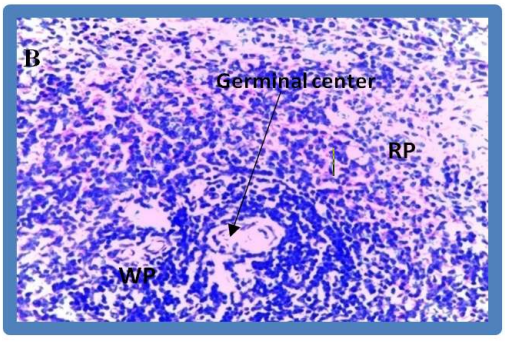

Fig. 3A

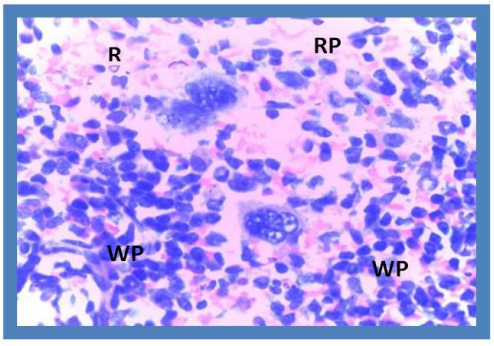

Fig. 3B

Fig. 3. (A): The histology of the spleen from animal treated with 500mg/kg body weight of *Costus afer* crude ethanolic stem extract. (Haematoxylin and Eosin stains) mag., X 100 B: Mag., X160, has similar features as A. WP- white pulp; RP- red pulp

3.1.4 Juice dose, 5 mls/kgw

Animals in this group were treated with 5 mls/kg body weight of the stem juice for twenty eight days. The histological section of the spleen was observed to show lymphatic tissue intact and there was no observable pathological feature- (Fig. 4).

3.2 Haematological Observations

3.2.1 Packed cell volume (pcv)

The packed cell volume result were as follows: control- (42.00±0.91); (low dose, 200 mg/kgw)- (38.50±2.10); high dose, 500 mg/kgw, (36.00±0.82); and 5 mls juice dose (40.0±1.62) respectively. The packed cell volume in low and high doses were significantly lowered (*P* =0.01) and (*P* =.05) compared to control. The value in the group that received juice was statistically not significant (Fig. 5).

3.2.2 Platelets

The platelets count in control and extract treated group were as follows: control (220±11.84), low dose (228.05±13.27) high dose (301.78±11.41) and juice dose (190.35±13.27) respectively (Fig. 6). The platelets count in the animals treated with high dose was significantly higher (P<0.001) than that of the control while the low dose and juice treated groups showed no statistical differences compared with the control.

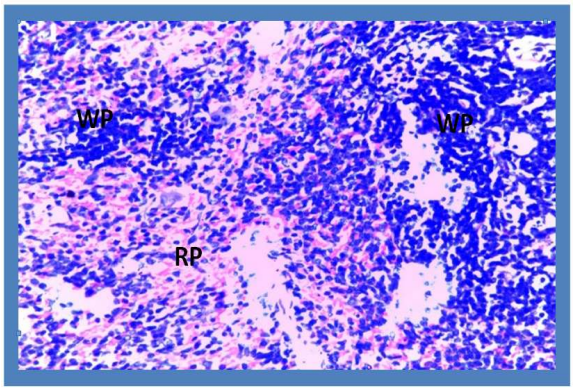

Fig. 4A

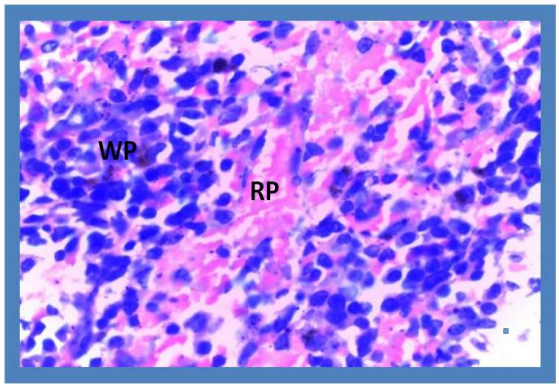

Fig. 4B

Fig. 4. (A): The histology of the spleen from animal treated with 5mls /kg body weight of *Costus afer* stem juice (Haematoxylin and Eosin),mag., X100
(B): Mag., X160 has similar features as A. WP- white pulp; RP- red pulp

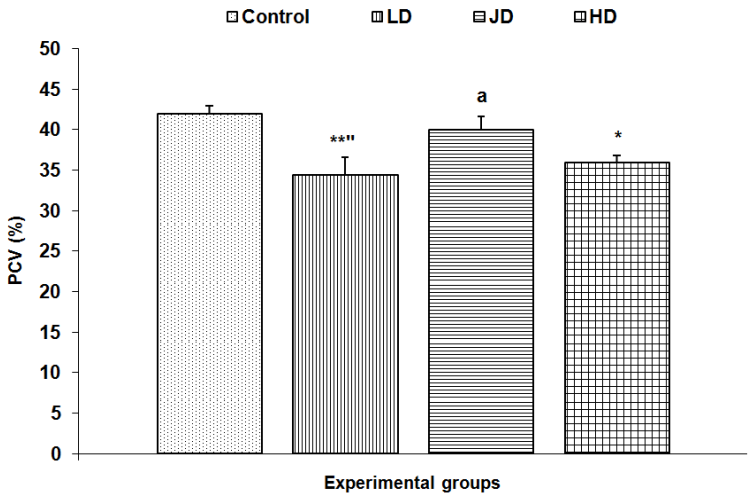

Fig. 5. Comparison of packed cell volume in control and extract treated group
*Values are expressed as mean ± SEM, n =6; * = P =.05, ** = P =.01 vs control; a = P = .05 vs LD*

3.2.3 Total white blood cell (Twbc)

The Twbc in control and extract tested groups were as follows: control (6.43±1.15), low dose (5.48±0.91), high dose (5.55±0.83) and juice dose (5.80±1.12) respectively (Fig. 7).

The Twbc in all the tested groups showed no significant differences when compared with the control group.

3.2.4 Differential counts

The neutrophils value was as follows: control (26.85±1.59), low dose (24.03±2.39), high dose (34.24±3.71) and juice dose (28.06±4.34) respectively. The difference between the control and the extract treated groups was not statistically significant, although the count in animals tested with higher dose was higher than the control animals (Fig. 8).

The result for lymphocytes count was as follows: control (70.32±0.20), low dose (73.66±1.73), high dose (63.39±3.38) and juice dose (69.66±5.12) respectively.

The lymphocytes count for the control and extract tested groups were not statistically significant (Fig. 9).

The total eosinophil count in control and extract tested groups were as follows: control (2.64±1.25), low dose (2.37±1.28), high dose (2.26±1.31) and juice dose (2.32±1.28) respectively. The counts for eosinophils in all treated groups were of no statistical significance when compared with the control group (Fig. 10).

The monocytes result was as follows: control (0.63±0.16), low dose (0.53±0.16), high dose (0.00±0.00) and juice dose (0.41±0.16) respectively. The monocytes in all treated groups were not significantly different when compared to the control group (Fig. 11).

4. DISSCUSION

The knowledge that compounds derived from plants have potential as therapeutic weapons against disease in humans and animals in addition to their food and nutritional values have made plant valuable and indispensable to human and animal lives [19].

Our study revealed no significant reduction in body weight of the treated group compared to the control group. This is different from the study carried out by Samuel et al. [20] in which they observed a significant change in weight of rats after treatment for the period of 56 days.

The extract of *C. afer* has been reported to have hypolipidemic property [8]. One would have thought that the animals would lose weight during the period of the treatment but this was not the case in this study.

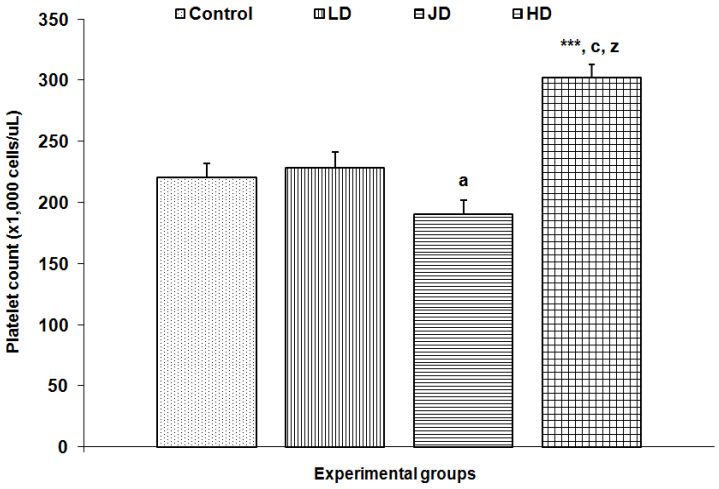

Fig. 6. Comparison of platelet count in control and extract treated group

*Values are expressed as mean ± SEM, n = 6; *** = P < .001 vs control; a = P =.05, c = P.001 vs LD; z = P<.001 vs MD*

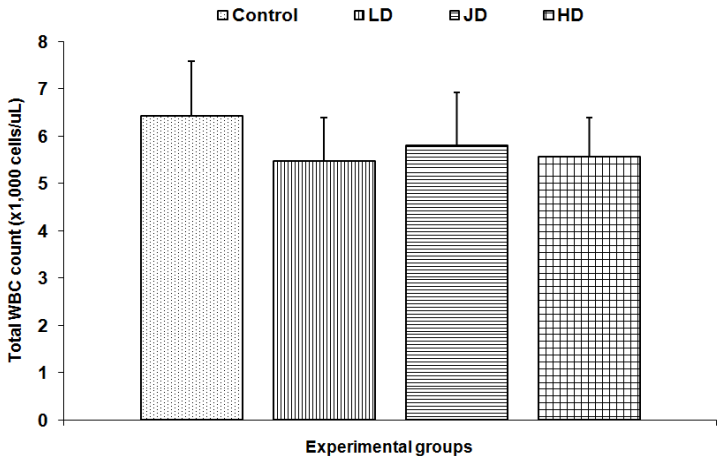

Fig. 7. Comparison of total white blood cell count in control and extract treated group
Values are expressed as mean ± SEM, n = 6; P =.05; No significant differences among groups

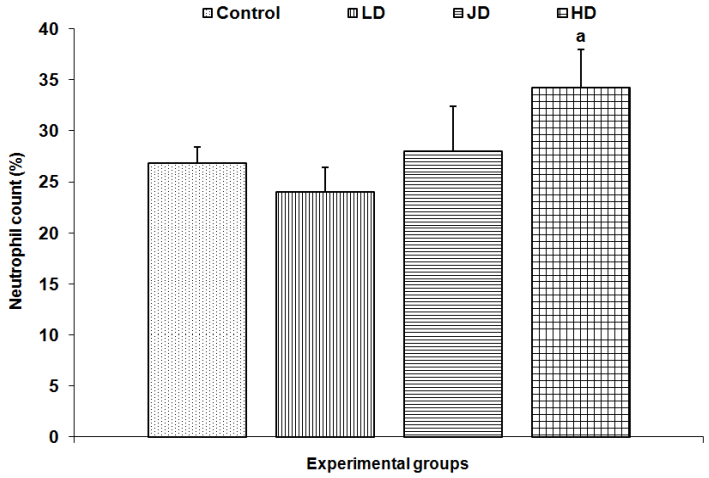

Fig. 8. Comparison of neutrophil count in control and treated group
Values are expressed as mean ± SEM, n = 6; a = P =.05 vs LD

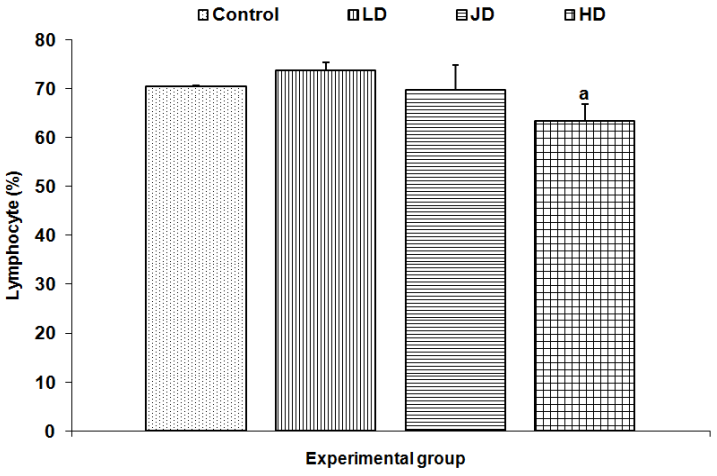

Fig. 9. Comparison of lymphocyte count in control and extract treated group
Values are expressed as mean ± SEM, n = 6. a = P =.05 vs LD

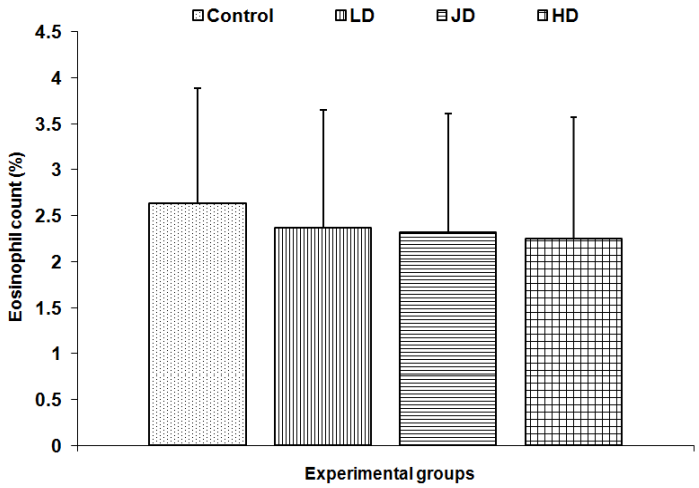

Fig. 10. Comparison of eosinophil count in control and extract treated group
Values are expressed as mean ± SEM, n = 6; No significant differences among groups

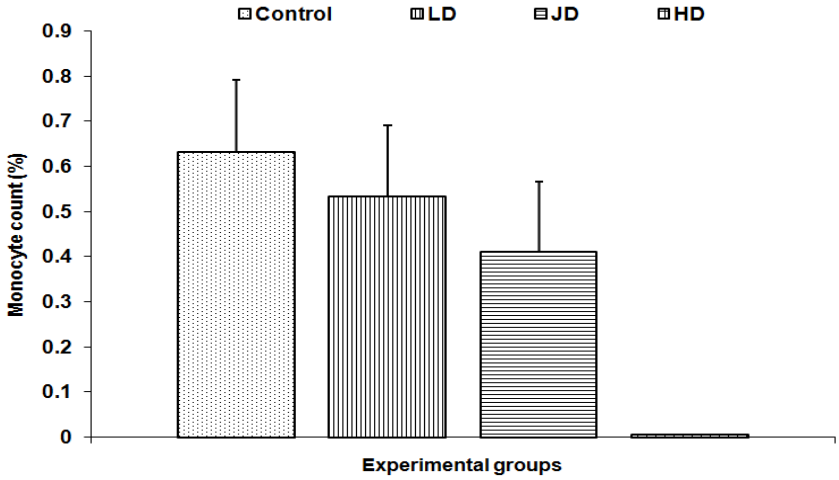

Fig. 11. Comparison of monocyte count in control and extract treated groups
Values are expressed as mean ± SEM, n = 6; P =.05. No significant differences among groups

This research showed that *C. afer* administration to albino *Wistar* rats for 28 days had no pathological effect on the histology of the spleen in control and extract tested groups. This may be due to the constituents of *C. afer* such as flavonoids, alkaloids, phenols with anti-inflammatory and antioxidant properties. These may have protected the spleen cytoarhitecture from damage as reported by Ayakeme et al. [15]. Flavonoids, which are known to exhibit anti-cancer, anti-inflammatory and anti-viral roles may prevent tissues from damage [10].

Hematological observations in this study showed that *C. afer* crude stem extract / juice administration decreased Packed Cell Volume (PCV) and increased platelets. Increase in platelets counts (Thrombocytosis) usually occurs in allergic conditions, hemorrhage, bone fracture, hypersplenism and splenectomy [21].

C. afer contains Saponins [10], Saponins have been indicated as one of the compounds that cause increase in platelets, and therefore the increased level of platelet in this study may be induced by Saponin. This potential effect of *C. afer* to increase platelets may support the traditional uses of *C. afer* in treatment of cut wounds to stop bleeding. This aspect may be developed phamaceutically and employed in the

management of fresh wounds especially in the rural areas.

Decreased PCV usually accompanies anemic condition which suggests that *C. afer* may have the potential to induce anemia in the body of chronic users. This serves as caution to the users although there is the existence of such beliefs already as folklore. The Report of investigation on the constituents of *C. afer* [10] showed that *C. afer* contain phenols which have the possible tendency to cause a decrease in PCV. This suggests that the decrease in PCV may be due to the presence of phenol which is one of the constituents of *C. afer*. This also agrees with the traditional belief that persistent consumption of *C. afer* 'dries up the blood'.

5. CONCLUSION

The results of this study suggest that *C. afer* crude Ethanolic stem extract and its stem juice at the administered dose had no pathological effect on the spleen histology but decreased Packed Cell Volume (PCV) and increased the total number of platelet counts in the serum of *Wistar* rats.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the ethical committee on the use of laboratory animal in the Department of Human Anatomy in the Faculty of Basic Medical Sciences of the University of Calabar, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
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