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Original Article

Haematological evaluation of *Cryptolepis sanguinolenta* stem ethanolic extract in rats

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ABSTRACT

Background: The use of *Cryptolepis sanguinolenta* extract in medicare has gained attention since its discovery. **Aim:** This study sought to evaluate the haematological effect of ethanolic extract of *Cryptolepis sanguinolenta* stem in rat model. **Materials and Methods:** Control rats received 0.5ml of distilled water. Treated rats were administered oral doses of the extract at different concentrations (50, 150, and 250mg/kg body weight). All rats were maintained on a control diet. Treatment lasted for 21 days. **Results:** Red blood cells and haematological parameters were comparable in all groups. Similarly, total white blood cells, neutrophils and lymphocytes counts were not significantly altered except in rats treated with 250mg/kg/bw of the extract. However, administration of the extract led to a dose-dependent rise in platelet counts. **Conclusion:** This study showed that *C. sanguinolenta* stem ethanolic extract presents haematological challenges on white blood cells and platelets. It showed localized systemic toxicity by selectively stimulating the bone marrow.

Key words: *Cryptolepis sanguinolenta,* red blood cell, white blood cell, platelet, toxicity

INTRODUCTION

Complementary and alternative medicine has been from time immemorial. It involves the use of plants and their products for treatment of ailment. Herbal medicine plays a major role in the health of millions of people worldwide.[1] Though, it is not considered as an integral part of orthodox medicine,[2] which has grown to be sophisticated over years,[3] a chunk of the drugs used in orthodox medicine are from plants and their bioactive principles extracted from them. Though the desire for a more Western lifestyle and other factors promoting globalization has influenced the use of herbal remedies,[1] they remain easily and cheaply available options. An elaborate plant knowledge-base is found in Africa[4] and other parts of the globe, which has been transferred within the family unit or community[5] from one generation to another, however, the search for more plants of medicinal value should be encouraged to provide cheaper and safer alternatives.

Cryptolepis sanguinolenta is one of the plants that have gained more attention since its discovery and isolation of the major active alkaloid in Nigeria and subsequently Ghana.[6,7] It is a thin-stemmed twining and scrambling shrub.[8] The leaves are

petiolate, glabrous or elliptic and are about 7cm long and 3cm wide. The roots are yellowish brown on the outer surface with a faint odour and a bitter taste.[8] *C. sanguinolenta* has been reported to have various therapeutic values including antimalarial,[9-13] antidiabetic,[14-17] anticancer,[18] antithrombotic,[19] antimicrobial,[20-26] and anti inflammatory.[27,28] The biological activities of *C. sanguinolenta* have been ascribed to its alkaloid constituents, mainly cryptolepine, indoquinoline alkaloids.[29-34]

Although the medicinal values of *C. sanguinolenta* extract in the management of various pathologic conditions have been well documented, it is necessary to evaluate its toxic effect. The present study thus sought to investigate the potential haematoxicity of *C. sanguinolenta* stem ethanolic extract.

METHODS AND MATERIALS

Plant material

C*ryptolepis sanguinolenta* stem were obtained from Ojurin Akobo- Olorunda road, Oyo, Oyo state, Nigeria. The plant was authenticated by Ugbogu A, Chukwuma E.C, and Shasanya O.S of the Forest Herbarium, Ibadan, Nigeria, where a voucher specimen (FHI. 108847) has been deposited. The dried stem was broken into pieces and beaten into smaller sizes using mortar and pestle before being pulverized. The powder formed after pulverization was weighed and stored until required.[14]

Preparation of C*ryptolepis sanguinolenta* stems ethanolic extract

Extract was prepared as described by previous studies.[14,35] Briefly, 1,226g of the powdery stem was dissolved in 4.8 litres of 65% ethanol. The mixture was dissolved to stand for 48 hours. The extract was thereafter filtered using clothen sieve and was evaporated at 40^0C. From the viscous solution obtained, a 0.1M solution of extract was prepared by dissolving 5ml of viscous solution of extract in 45ml of distilled water.

Acclimatization

Albino rats (*Rattus norvegicus*) of both sexes and comparable weights were obtained from the Animal Holding Unit of the department. The rats were housed in clean standard plastic cages in a well-ventilated laboratory condition (12:12h light/dark cycle at 25°C ± 2). The animals were allowed free access to standard rat chow and water.[36]

Animal grouping and extract administration

After two weeks of acclimatization, the rats (24) were randomized completely into four groups of 6 each and were orally administered as follows using an oropharyngeal cannula. Doses are as used in our previous study.[14] The control was administered 0.5ml of distilled water while groups 1, 2 and 3 were given equal doses of the extract corresponding to 50, 150 and 250mg/kg body weight respectively. Rats were sacrificed 24hr after the 21st day of treatment. Animals received humane care as recommended by the institution's guideline and criteria for humane care as stated in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.

Serum preparation and determination of haematological parameters

Serum was prepared accordingly to previously established procedure of Yakubu *et al*.[37,38] Briefly, the cervical region of the rats was shaved to reveal the jugular veins under ether anaesthesia. The veins were slightly displaced to prevent blood contamination by interstitial fluid, and sharply cut with sterile surgical blade. Blood samples were collected in lithium-heparinized sample bottles for haematological analysis.

Haematological parameters were evaluated as described in previous studies[39-43] using standard laboratory assay kits in accordance to manufacturer's instruction.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) and unpaired t-test. Data were expressed as mean±standard error of mean. Significant levels were tested at $p<0.05$.

RESULTS

Though ethanolic extract of *C. sanguinolenta* stem caused marginal fluctuations of red blood cells (RBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), these alterations were not statistically significant. In contrast, the extract led to a dose-dependent increase in platelet count.

Total white blood cell (TWBC), neutrophils and lymphocytes were comparable in the control and groups treated with 50 and 150mg/kgBW of the extract. However, these white cell variables were

significantly increased in rats treated with 250mg/kg/bw of the extract.

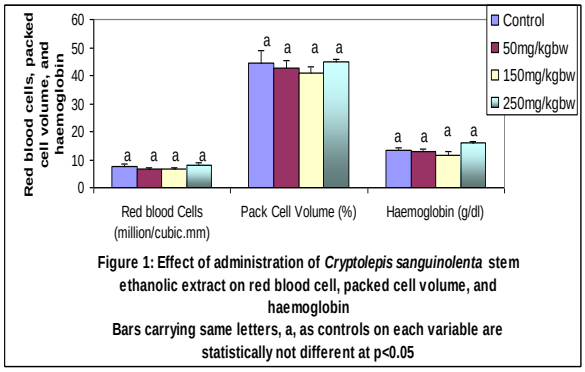

Figure 1: Effect of administration of *Cryptolepis sanguinolenta* stem ethanolic extract on red blood cell, packed cell volume, and haemoglobin
Bars carrying same letters, a, as controls on each variable are statistically not different at p<0.05

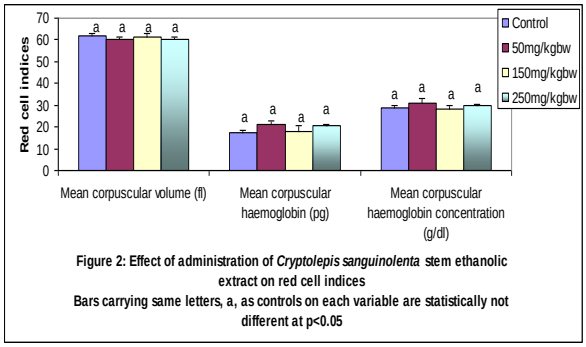

Figure 2: Effect of administration of *Cryptolepis sanguinolenta* stem ethanolic extract on red cell indices
Bars carrying same letters, a, as controls on each variable are statistically not different at p<0.05

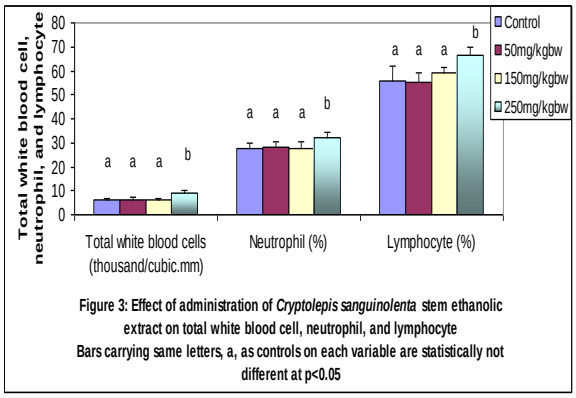

Figure 3: Effect of administration of *Cryptolepis sanguinolenta* stem ethanolic extract on total white blood cell, neutrophil, and lymphocyte
Bars carrying same letters, a, as controls on each variable are statistically not different at p<0.05

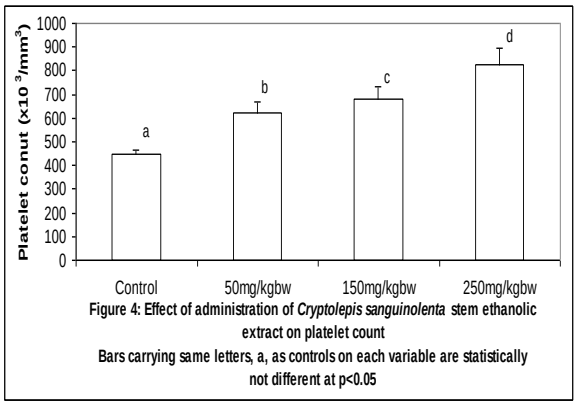

Figure 4: Effect of administration of *Cryptolepis sanguinolenta* stem ethanolic extract on platelet count
Bars carrying same letters, a, as controls on each variable are statistically not different at p<0.05

DISCUSSION

Haematological indices provide physiological information on the blood picture and the reticuloendothelial system,[44] thus are used in the diagnosis of diseases[45] and evaluation of the toxic effects of exogenous compounds including plant extracts and drugs. Haematological analysis is consequently relevant to toxicity evaluation as haematological changes have higher predictive value for human toxicity when the data are interpreted from animal studies.[46]

The results from this study revealed that *C. sanguinolenta* did not alter red blood cells and its related indices. This may also show that the extract did not stimulate the humoral regulator of RBC production, erythropoietin.[38,47] RBC, PCV and Hb are associated with the total population of the red cells, while MCV, MCH, and MCHC are related to individual RBC. The non-toxic effect of the extract on these parameters suggests that it does not modulate the incorporation of haemoglobin into the red cells nor alter the morphology and osmotic fragility of the red blood cells.[48] These observations also imply that the extract does not affect the oxygen-carrying capacity of the red cells, implying that the botanical does not possess anaemic potential.

It is striking that though *C. sanguinolenta* did not alter red cells and its related indices, white cells and related indices were significantly increased in rats treated with 250mg/kgBW. The rise in TWBC may be due to enhancement of white cell production, increase in its entrance into the blood, and a reduction in its removal from the circulation.[38] This may indicate that the extract led to hyperstimulation of haematopoietic regulatory elements by the macrophages and stromal cells in the bone marrow such as colony stimulating factors, interleukins (IL-2, IL-4 and IL-5) which regulate the proliferation, differentiation and maturation of committed stem cells necessary for the production of white blood cells.

White cells and its indices play a vital immune function. Neutrophils have phagocytic activities.[42] They attack and destroy foreign particles, cell waste materials and bacteria. The lymphocytes help to specifically recognize a diverse range of antigens, differentiate and mature to functional capacity, respond to the antigens and establish immunologic memory.[49-52] The increased neutrophils and lymphocytes seen in the study showed that the

extract at 250mg/kg/bw boosted the immune system.

Results from this study also showed that *C. sanguinolenta* caused an increase in platelets (thrombocytes) in a dose-dependent fashion. Platelets are responsible for coagulation.[53] The rise in platelets count seen in the present study may suggest that the extract has a stimulatory effect on thrombopoietin,[54] an important factor in thrombopoiesis.

The blood cells are produced mainly in the bone marrow.[55,56] The rise in white cells and platelets accompanied with normal red cell counts suggest a selective stimulatory effect of *C. sanguinolenta* on the bone marrow.[38] This may infer a localized systemic toxicity of the botanical. This is in consonance with previous findings[48] that documented selective stimulatory effects of different botanicals on the bone marrow.

CONCLUSION

This study showed that *C. sanguinolenta* stem ethanolic extract poses haematological challenges on white blood cells and platelets. It showed localized systemic toxicity by selectively stimulating the bone marrow.

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