

Cytological Effects of Alcoholic Extract *Cnidoscolus aconitifolius* on Bone Marrow Aspirate in Adult Male Wistar Rats

*E.I ODOKUMA, C.J OYEM,

Dept of Anatomy, Delta State University, Abraka, Nigeria

*Author for Correspondence

ABSTRACT

This study was designed at investigating the effect of alcoholic extract of *Cnidoscolus aconitifolius* on bone marrow aspirate of adult male wistar rats. The rats were acclimatized for two weeks, weighed and sorted into four groups (A - D) of three animals each with corresponding weights in the same group. Alcoholic extract of *Cnidoscolus aconitifolius* was administered orally to each animal in groups A - D at the rate of 0ml/kg, 200ml/kg, 300ml/kg and 500ml/kg respectively. Group A was the control group and was served with feed and water liberally. Subsequently, the animals were sacrificed and the bone marrow aspirate harvested from the medullary canal of the femur and fixed in 95% alcohol. Staining was with Papanicolaou stain. Results showed a dose dependent distortion in aspirate morphology with decrease in aspirate cellularity. Generally, the result showed a marked dose dependent distortion of bone marrow cytology with replacement of marrow with fibrous and reduced cellularity.

Key words; *Cnidoscolus aconitifolius*, bone marrow aspirate, rats.

Bone marrow is found in medullary canals of long bones and in the cavities of cancellous bones (Junqueira and Carneiro, 2005). Two types of bone marrow have been described based on their appearance on gross examination. Red or hematogenous bone marrow, whose colour is produced by the presence of blood and blood forming cells and yellow bone marrow whose colour is produced by the presence of great number of adipose cells (Junqueira and Carneiro, 2005).

Bone marrow produces blood cells by a process called haemopoiesis. The cells of blood are of three major functional classes: Red blood cells, White blood cells, Platelets (Barbara, 2006). Blood cell cellularity within the marrow has been known to alter with age ranging from 9: 1 with adipose tissue within the marrow in childhood and early adolescence, to 1: 1 in adults (Hoffbrand, 2006).

The marrow is a continuous tissue, being capable of replenishing itself continually and therefore serves as an excellent measure of haemopoietic function (Hoffbrand, 2006). Despite its properties, agents have been known to alter the normal morphology of the bone marrow to such an extent that it may result in its overstimulation or in failure.

Cnidoscolus aconitifolius commonly called chaya or tree spinach is a perennial shrub of the family *Euphorbiaceae* found in the tropics. It

is a green vegetable used for meals in south western Nigeria (Oyagbemi et. al., 2010) and south eastern Nigeria (Iwalewe et al., 2005). It is considered to have beneficial effects for several conditions including diabetes, obesity, kidney stones, hemorrhoids, acne etc (Diaz Bolio, 1975). Its shoots and leaves were previously ingested for their laxative and diuretic properties which suggested beneficial digestive properties (Rowe, 1994).

Florence et. al, (2009) demonstrated that the plant contained phenols, saponins, cardiac glycosides and phlobatanin. Its high fiber content and antibacterial activity have been reported (Oyagbemi et. al., 2008). In an earlier study, Temitope et. al., (2010), showed that the leaf extract of *C. aconitifolius* had no significant effect on several hematologic parameters including mean cell volume (MCV), neutrophil, lymphocyte, eosinophil and platelet counts following prolonged administration ($p > 0.05$).

It currently not clear if the extract has a toxic effect or stimulatory effect on the bone marrow despite its seemingly beneficial digestive properties. This study was therefore aimed at determining the effect of the crude plant extract on bone marrow function by examining the aspirate so as to either further advocate its usefulness or otherwise

MATERIALS AND METHODS

Approval for this study was obtained from the department of anatomy ethics committee Ref no. DELSU/BMS/ANA/ERC/10/11/02.

Twelve adult male wistar rats weighing 140g 185g bred in animal house of Delta State University, Abraka were used for this study. Animals were acclimatized for two weeks, weighed and sorted into four groups (A - D) with 3 rats per group. Alcoholic extract of *C. aconitifolius* was administered orally using a needle and a canula for a period of fourteen days.

Group A served as the control and was administered 0.15ml of extract, feed and water

ad libidum. Group B, C and D were administered 200ml/kg, 300ml/kg, and 500ml/kg of the extract respectively with feed and water freely,

At the end of administration, animals were euthanized with chloroform, sacrificed and the femur dissected out of the thigh and the bone marrow aspirate was then aspirated from the femur through the nutrient foramen. Samples were fixed in 95% alcohol, and stained with Papanicolaou stain. Pictures of bone marrow stained slides were captured using digital microscopic eyepiece ?? SCOPETEK?? DCM 500, 5.0 mega pixel connected to USB 2.0 computer (Barker et al., 1998).

RESULT



Figure 1; An illustration of *C. aconitifolius* plant (*Euphorbiaceae*)

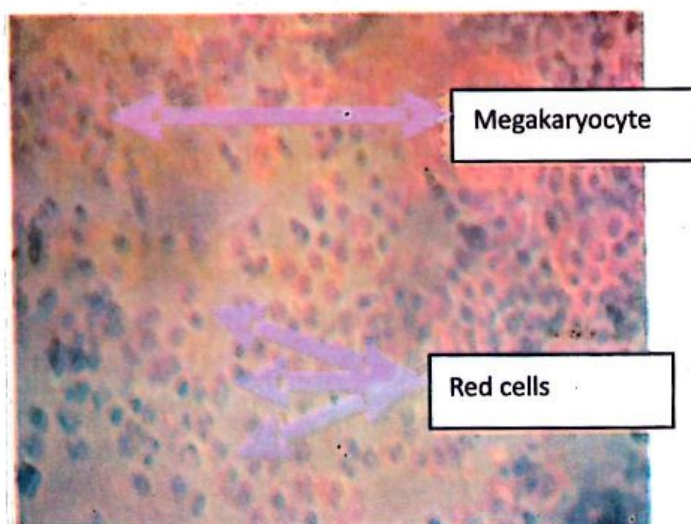


Figure 3; GROUP B (200ml/kg) Sections of bone marrow aspirate show a megakaryocytes disposed within a mixed infiltrate of red/white cells at various stages of development. Some fibrous strands are also seen. Bone marrow aspirate (papanicolaou) $\times 400$

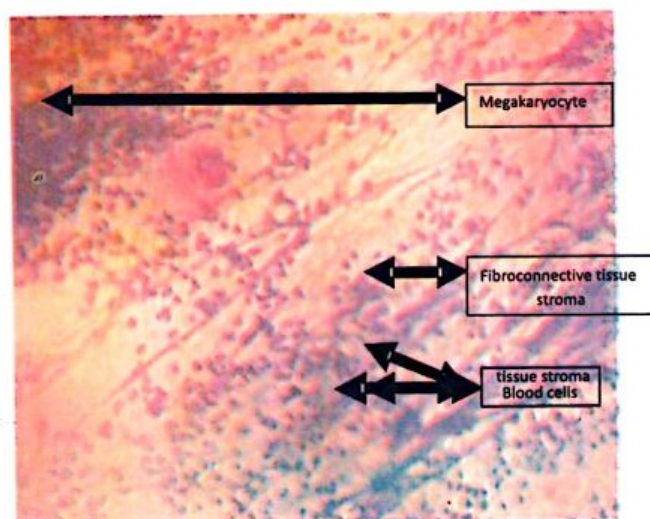
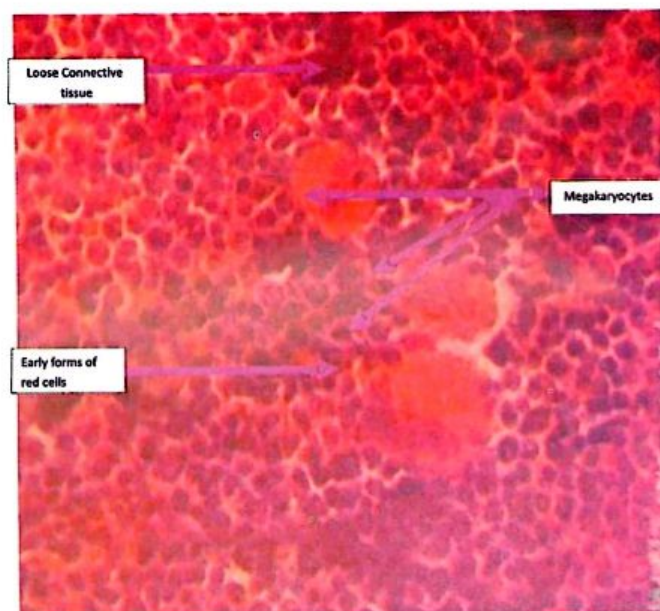


Figure 4 ; GROUP C (300ml/kg) Bone marrow aspirate show sparse megakaryocytes dispersed within sparse fibro connective tissue stroma in which are few late forms of red/white blood cells. Bone marrow aspirate (papanicolaou stain) $\times 40$

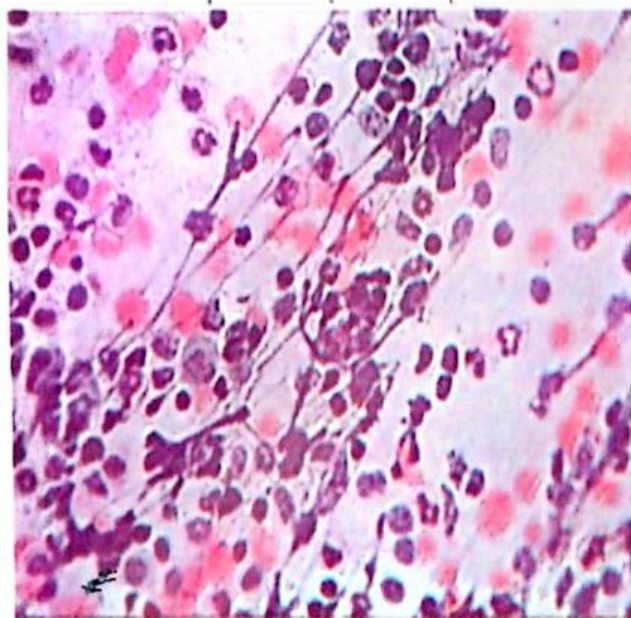


Figure 5; GROUP D (500ml/kg)- Bone marrow aspirate shows few islands of cells with distorted cell membrane and vesicular nuclei. No megakaryocytes and scant connective tissue fibers are seen. Bone marrow aspirate (Papanicolaou) × 400

DISCUSSION

The cytological features of the bone marrow aspirate showed a dose dependent decrease in cellularity especially as regards megakaryocytes. This was further emphasized when after two hours after sacrificing, the blood in the group exposed to the highest concentration of the extract showed no features of coagulation. Platelets produced from megakaryocytes are essentially for blood coagulation homeostasis and relative or total absence may result in the observed findings.

This resultant pattern indicated that most of the phytochemical constituents of the plant may likely have destructive effect on bone marrow especially in high concentrations. These effects may be a result of tannin and phenol content as noted previously (Okwu, 2004). Other phytochemical substances which may affect or cause bone marrow toxicity include flavonoid which has been stated to contain Kaempferol and quercetin glycosides (Florence et al., 2009).

CONCLUSION

Consumption of *C. aconitifolius* causes bone marrow destruction in a dose dependent pattern.

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