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Influences of Ethyl Acetate and Water Bulb Extracts of *Crinum zeylanicum* (Linn.) on Haematological Constituents of Rabbit Mediated Through Osteotomy

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Abstract:- *Crinum zeylanicum* bulb is a traditional folk's herb that has been widely accepted by most rural communities for the treatment of bone fracture and other illnesses. This study assessed the influences of *C. zeylanicum* bulb extracts on blood pictures of rabbits through close tibia osteotomy over the period of three months. Assay of the whole blood collected through cardiac puncture was done by determining the Packed Cell Volume (PCV%) (Micro-hematocrit method), White Blood Cell (WBC%), Red Blood Cell (RBC%) (Hemo-cytometer method), Haemoglobin (HB g/dl) (Cyanomoglobin method), Neutrophil (N%), Lymphocyte (L%), Monocyte (M %), Eosinophil (E%), and Basophil (B %) (Thin smear and Leishman's Methods). The Mean Cell Volume (MCV%), Mean Corpuscular Hemoglobin (MCH %), and MCH-Concentration (MCHC %) were calculated according to standard methods. There was significant difference ($p<0.05$) recorded for MCV, MCH, N and L for treated-groups as compared with WBC, MCV and N for control group while the resultant increase of WBC by ethyl acetate extract (EAE) treated group than water extract (WE) and control groups was an indication that the plant has influence on the blood constituents. M, B and E were not dictated during the pre- and post-examinations. Hence, the use of *C. zeylanicum* can be monitored, modified, and/or regulated for medicinal uses as the characterization of the chemical components of the plant could be a better focus for further investigation.

***Keywords*:-** *Haematological Constituents, Crinum Zeylanicum, Ethyl Acetate Extract, Osteotomy, Rabbit, Water Extract.*

I. INTRODUCTION

Natural components of plants have given mankind access to sources of pharmaceutical agents long before now, which have been essential in the treatment of many diseases (Agrahari et al., 2010; Sofowora et al., 2013; Babu et al., 2018). These are sometime known from the ancient practices of native people's herbal knowledge to find new foods that led to invention of the pharmacopoeia of scientific medicine today (Fabricant & Farnsworth, 2001; Shi et al., 2010). The availability and effectiveness of medicinal plants has been widely gained, particularly in poorer nations, as an effective therapy and method of illness prevention (Shale et al., 2002; Naveed et al., 2012; Rathore et al., 2012). Through experimentations, observations or error testing of several well-known remedies of the traditional medical system that have treated health issues with certain plants has been brought into the recent century (Cooper, 2004; Bais et al., 2014). For the development of modern medicine, ethnobotanical information had offered crucial preliminary data that had helped chose natural medicines with prospective health benefits (Haffner-Luntzer et al., 2017; Gupta & Kumar, 2018; Tseng et al., 2018; Mäkitaipale et al., 2020). Therefore, the safety of drugs and plant products for human use can be determined using various experimental animals (Diallo et al., 2010; Miranda et al., 2014; Chuohan & Sudip, 2017; Tizhe et al., 2018).

Haematological system of animals have been established and is important in risk evaluation that gives higher predictive value for study in human (Olson et al., 2000; Ozkan et al., 2012; Nakyinsige et al., 2013; Unung et al., 2019). It also facilitated the investigation of pathogens in blood, through repeated preclinical testing of new substances (Arens et al., 2015). Rabbit model has been used in haematological studies due to its haversian system that is similar to man, which allows possibilities of several experimental protocols and the merit of extrapolation (Nunamaker, 1998; Matos et al., 2008). Rabbits are .likewise used for experimental studies of various toxic agents which Hewitt et al., (1989) have establihed a long-term studies of exposure to trace metals on organ systems while Nakyinsige et al., 2013 have observed changes in blood constituents of rabbits. Other investigations include the physiological values of some blood indicators in selected dwarf rabbits' breeds (Simek et al., 2017) and effects of varying protein levels on the haematology and serum biochemical components of non-descript rabbits (Unung et al., (2019). Rabbits have also been used in other fields of researches on biochemical parameters (Prasad, 2008; Dontas et al., 2011; Ozkan et al., 2012; Ozkan & Pekkaya, 2019); histopathology (Melillo, 2007; Matos et al., 2008); pathogens investigations (Orhue et al., 2005; Arens et al., 2015), bone fracture healing (Matos et al., 2008; You et al., 2016), bone substituents (Liu et al., 2013; Faot et al., 2017), and wound healing (Tijani et al., 2012; Piotrowski et al., 2019) .

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The popularity of *C. zeylanicum* in traditional medicine practices and its usages among several tribes in Plateau State (Dashak & Ano, 2007) has called for concern to investigation it's uses. The plant is locally called *Dau* and *Leḣ zipir* in Doemak and Mushere respectively (Daben & Dashak, 2016). Other languages on the Plateau and other parts of Nigeria, identified *C. zeylanicum* as *Juéébót* (Berom); *Ndan-murenang* (Gumai) *Gadali-Albassar kwaadi* (Hausa), *Isumeri* (Yoruba) (Burkil, 1985). The ethno-pharmacological and ethno-medicinal uses include bone fracture healing (Daben & Dashak, 2016), wound healing (Tijani et al., 2012), reformed rickets in children (Agrahari et al., 2010), anti-inflammatory (Mukherjee, 2000) and other skin diseases (Doepke et al., 1986; Refaat et al., 2013; Daben et al., 2017; Okhale et al., 2018). The ethno-medicinal usages are largely based on experiences, without due scientific evidence especially its effects on blood parameters. Therefore, in this study, the influence of ethyl acetate and water bulb extracts of *C. zeylanicum* on haematological profiles mediated through close tibiae osteotomy within a period of 3 months were explored.

II. MATERIALS AND METHODS

➢ *Sample Collection and Preparation*

C. zeylanicum bulb were obtained from Kopmabar – Doemak, Qua'an Pan Local Government Area (LGA) of Plateau State-Nigeria and transported to the laboratory in a clean sack. Thereafter, it was cleaned, sliced into pieces, oven-dried at 60 ^{0}C and subsequently pulverized into smaller fibres stored in an airtight vial for further analysis (Harborne, 1991).

➢ *Preparation of Extracts*

The extracts were obtained from a successive extraction with absolute ethyl acetate and water by Soxhlet extractor for 8 hours. The extracts were concentrated to about 20 ml using rotary evaporator (RE -52A) at 50 ^{0}C. It was further allowed open to drain in a fume cupboard before being stored in a desiccator till analysis (Harborne, 1991).

➢ *Preparation of Animal for Surgical Procedures.*

Rabbits (4-5 months old) of an average weight of 1.1 kg, having acclimatized to laboratory environment for two weeks (OECD, 2002; Ozkan and Pekkaya, 2019) were observed for vital and blood parameters prior to experimental study (Hassan & Hassan, 2003; Duncan & Prasse, 2011), in order to understand their fitness before commencement of experiment (Ozkan *et al.*, 2012; Nakyinsige et al., 2013; Simek et al., 2017; Unung et al., 2019). Fifteen (15) rabbits were randomly assigned to three groups of 5 rabbits each for ethyl acetate, water and control.

The Surgical Procedures was according to Alford et al., (2012) and Faot et al., (2017), with slight modifications as stated: Both the experimental and control groups underwent surgical procedures with the equipment (Plate 1) of close tibia osteotomy after an overnight fasting. The hind limb was used to locate the tibia, which was used as landmark.

At the distal half (½) of the shaft; a 2 ml syringe was used to draw 2 ml of 2% Lidocaine to anesthetise the animals by infiltration injection that was used to achieve a ring block at the distal ½ of the hind limb. After 5 minutes, the fur around the mid-hind limb to the distal portion of the anaesthetised area was shaved (Plate 2) and a blunt force was applied to the well-padded shaved limb, using a blunt osteotome and mallet (Plate 3) to create a tibia fracture as shown by the x-ray (Plate 4).

Plate 1: Surgical Equipment

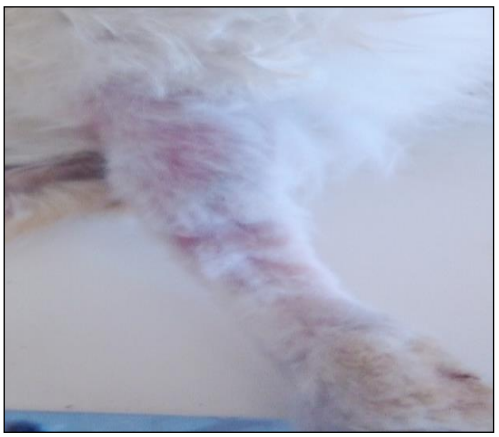

Plate 2: Shaved Limb

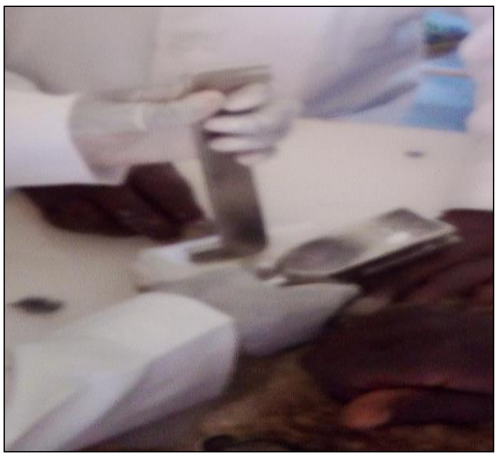

Plate 3: Acquiring Tibia Osteotomy.

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Plate 4: X-Ray of Close Osteotomy

➢ *Application of Plant Extracts and Bone Reduction*

This was according to previous osteotomy reports of (Matos et al., 2008; Liu et al., 2013; Arens et al., 2015; You et al., 2016); where 0.5 g portion of each extracts was topically applied around the close fracture (Plate 5), before close reduction and immobilized (Plate 6). This was achieved by cotton gauge bandages 10 cm/4.5 m (Huanggang Huangzhou Xianghui Textiles Co. Ltd, China), Agary Plaster of Paris (POP) 2.5 cm/5 m, cotton crepe elastic bands (7.5 by 4.5 cm), cotton wool and 10 cm wooden splints (Plate 5) to mimic the traditional bone fracture healers. Provisions were made for easy application of the plant extracts on the 3rd and 7th day of the fracture after which it was allowed for self-healing. No antibiotic or any form of medication was given after the reduction.

Plate 5: Application of Plant Extract

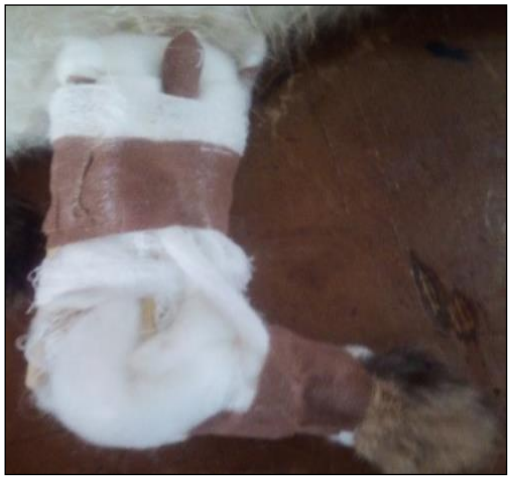

Plate 6: Immobilized Fracture

➢ *Collection of Blood Sample and Haematological Analysis*

This was done by cardiac puncture on a supine position using needle mounted on a 5 ml syringe into tubes with anticoagulant ethylene diamine tetra acetic acid (EDTA). Samples were collected before and after the experimental period of 3 months, for haematological assessment (Matos et al., 2008; Liu et al., 2013; You et al., 2015; Debebe et al., 2017). All experimental rabbits were allowed to fast overnight before a 2 ml blood samples were collected; in order to assess the effects of plant extracts administered through the close tibia osteotomy.

The blood samples were delivered to the laboratory within 2 hours of collection and promptly assayed at the Central Diagnostic laboratory, National Veterinary Research Institute, Vom-Plateau Sate-Nigeria. Assay of the whole blood was done by determining the PCV(%) (Micro-hematocrit method), WBC x 10/L and RBC x 10/L (Hemo-cytometer method), HB (g/dl) (Cyanomoglobin method) (Lassen and Weiser, 2004), N(%), L(%), Monocyte (M %), Eosinophil (E %), and Basophil (B %) (Thin smear and Leishman's Methods). While the Mean Cell Volume (MCV%), Mean Corpuscular Hemoglobin (MCH %), and MCH-Concentration (MCHC %) were calculated according to Jain, (1986) and Duncan & Prasse, (2011).

➢ *Statistical Analysis*

Statistical analysis was performed by SPSS (Windows program Inc. Chicago, IL, USA) version 23.0. Haematological parameters were expressed as Mean ± Standard deviation (SD). Statistical significance was determined at $P = 0.05$; where $P < 0.05$, was considered significant.

III. RESULTS AND DISCUSSION

The changes observed in haematological parameters of the rabbits after treatment with *C. zeylanicum* bulb extracts (Table 1) was an indication that the plant has influence on the blood constituents. The wide acceptability of traditional herbs for health remedy by most rural communities brings an attempt to establish the toxicity profile on the blood parameters. The significant changes recorded in MCV, MCH,

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N and L for treated-groups were limited to WBC, MCV and N for control while M, B and E were not dictated during the pre- and post-examinations (Table 1). It is worth noting that the resultant increase of WBC by EAE treated group than water and control groups could be attributed to the low-level flavonoid and alkaloids chemical constituents of EAE of the plant as earlier reported in the study by Daben et al., (2021) on quantitative evaluation of alkaloids, flavonoids, saponins, steroids and tannins contents from a successive solvent extracts of *C. zeylanicum* bulb.

Furthermore, the assertion of WBC increasing immune boosting properties in relation to *C. zeylanicum* agrees with the earlier result of Tijani et al., (2012) on wound healing activity of 70% methanolic bulb extract of *C. zeylanicum*, healing an excision wound measuring to about 200 mm^2 on Wister rat. This property of defense mechanisms of WBC had also been reported in other plant species such as garlic – *Allum sativa* (Linn) which presented the same response in relation to its effect on haematological parameters. It is as well confirmed with other earlier findings by Adebayo et al., (2010) using *A. conyzoides* on rats and *S. incunum* in mice (Muriithi et al., (2016). In addition, the emphasis made by Debebe et al.,(2017); and Chinenye et al., (2019) on *Albizia gummifera* seed and *Portulace oleracea* leaf extracts respectively were in support. Likewise, the outcome of this research on aqueous extract producing less increase of WBC than ethyl acetate extract are the work undertaken by Ofem & Eno, (2012), on aqueous leaves extract of *Ocimum gratissimum* in rat. Again, previous studies have shown that aqueous extract has less influence on WBC by the study conducted earlier by Effraim et al., (2000) on the effect of aqueous leaf extract of *O. gratissium* in rabbits that brought about decrease in WBC; was also later repeated on the same plant on Wister rat and it gave the same decrease (Jimoh et al., (2008).

Contrary to our findings, Mohebbati et al., (2019) on the effect of ethyl acetate and aqueous fractions of *Ziziphus jujube* extract on biochemical and haematological parameters in rat revealed that aqueous fraction produced higher WBC than ethyl acetate fraction. In another research by Asanga et al., (2013) on *Nauclea lafiloia* leaf for blood parameters using rat showed that ethyl acetate fraction showed significant reduction in WBC level.

Other blood parameters are RBC, MCV, MCH and MCHC which are considered useful indicators in the diagnosis of anaemia (Melillo, 2007; Simek et al., 2017; Ozkan et al., 2019). Though none of the treated animals was anaemic, since the PCV level was not less than 30% within the period of investigation but RBC slightly reduced for water extract treated and control groups while ethyl acetate treated group RBC remain unchanged. This is similar to the findings of Mohammed et al., (2009) on ethyl acetate *Ganoderma lucidum* extract of *A. conyzoides* on rats and Geidam et al., (2013) considering prolong administration of ethyl acetate fraction of *Psidium guajava* on some haematological values in chickens; and Sam et al., (2019) on protective effects of *Zingiber zerumbet* ethyl acetate extract hydrogen peroxide-induced damage of RBC. Other earlier researches that supported low-level PCV and RBC in other plant species are likewise reported by Effraim et al., (2000); and (Jimoh et al., (2008) on the effect of aqueous leaf extract of *O. gratissium* in rabbits and Wister rat respectively. Contrary to this view is the work of Lohdip *et al.*, (2013) on aqueous extract of *Chenopodium ambrosioides* (Linn) on Balb/C strain mice; and Iyare and Obaji, 2014 on aqueous extract of *A. indica* in rat where the extracts were known to increase the level of RBC.

Nothwithstanding, the absence of monocyte M, basophils B and eosinophils E so to add also are the absence of chronic inflammation and bone infection from this study, might indicate that the extracts were involved in disease control; similar to the earlier work by Melillo (2007) on clinical pathology of rabbit. The presence of neutrophils and an increase in lymphocytes for the treated groups than the control group might also be an indication that the plant extracts had better response to infections than the control. Meanwhile, the finding was also similar to Melillo,(2007); who equally reported no increase in neutrophils but contrary to no increase in lymphocytes in the blood and urine as a result of injection with bacteria and yeast cells. Hence, the treated groups did not produce any significant effect on these blood parameters. The indication that animals normally respond to changes with behavioral pattern such as, production of immune mediators or cytokines, natural immune and metabolic changes are designed to restore homeostasis in order to be adaptive or to promote survival (Knowles & Warriss, 2000; Muir, 2004). Consequently, these physiological and behavioral alterations can be used to measure and monitor animal welfare (Mokry *et al.*, 2006; and Nakyinsige *et al.*, 2013) under experimental condition.

Table 1: Effect of *C. Zeylanicum* Bulb Extracts in Rabbit Hematological Parameters

Extract	Ethyl acetate		Water		Control	
Examination	Pre	Post	Pre	Post	Pre	Post
Parameters						
PCV	41.00±1.73[a]	31.00±2.31[a]	37.00±1.73[a]	35.00±2.89[a]	41.00±2.31[a]	39.67±5.55[a]
HB	13.70±1.09[a]	10.00±1.73[a]	12.90±0.64[a]	11.70±0.64[a]	13.80±2.19[a]	12.70±0.23[a]
RBC	4.80±0.29[a]	4.80±0.17[a]	4.80±0.52[a]	3.50±0.06[a]	4.50±0.75[a]	4.30±0.23[a]
WBC	7.00±1.16[a]	8.80±1.21[a]	11.80±1.04[a]	12.00±0.98[a]	7.60 ± 0.35[a]	8.20±0.40[b]
MCV	85.00±5.19[a]	65.00±2.31[b]	92.00±1.16[b]	100.00±5.77[a]	90.00±6.35[a]	78.00±4.04[b]
MCH	29.00±5.19[a]	21.00±1.16[b]	29.00±0.58[b]	32.00±1.16[a]	31.00±1.16[a]	30.00±1.73[a]
MCHC	33.00±0.00[a]	33.00±0.00[a]	33.00±0.00[a]	33.00±0.00[a]	33.00±0.00[a]	33.00±0.00[a]

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N	32.00±0.58[a]	19.00±2.02[b]	19.00±2.31[b]	25.00±1.16[a]	87.00±2.89[a]	77.00±5.77[b]
L	66.00±10.39[b]	79.00±1.73[a]	68.00±0.58[b]	75.33±2.89[a]	17.00±1.79[b]	23.00±2.19[a]
M	Abs	Abs	Abs	Abs	Abs	Abs
B	Abs	Abs	Abs	Abs	Abs	Abs
E	Abs	Abs	Abs	Abs	Abs	Abs
LSD	9.64					
p-value	p<0.001 ****					

Data are presented as Mean ± SEM. Values with the same superscript are not significant (p>0.05). Packed Cell Volume (PCV %), White Blood Cell count (WBC x 10/L), Red Blood Cell count (RBC x 10/L) Hemoglobin concentration (HB g/dl), Heterophil-Neutrophil (H %), Lymphocyte (L %), Monocyte (M %), Eosinophil (E %), Basophil (B %), Mean Cell Volume (MCV%), Mean Corpuscular Hemoglobin (MCH %). Mean Corpuscular Hemoglobin Concentration (MCHC %), Absent (Abs).

IV. CONCLUSION

The outcome of this investigation supported the widely used *C. zeylanicum* bulb by most rural communities as a traditional folk's herb for the treatment of several ailments. To this study, it could be attributed to the extracts' influences that resulted in relative increase of WBC and other blood parameters which are considered useful indicators in the diagnosis of anaemia likely to be associated with the bone fracture healing. Notwithstanding, the traditional healers should note that long-term application, may result in some form of negative effects on the host's system; evident in mild reduction of the PCV. However, further investigation on *C. zeylanicum* bulb can be used as a drug supplement that can help boost the immune system.

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