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BIOCHEMICAL IMPLICATION OF ADMINISTRATION OFMETHANOL EXTRACT OF*OCIMUM GRATISSIMUM* LEAF ON HAEMATOLOGICAL PROFILE OF WISTAR RATS.

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ABSTRACT

In the search for medicinal plants that will provide ameliorative measure to patients with anaemic disorders. The methanol extract of *O. gratissimum* leaf was administered to Wistar rats for its effect on haematological profile. Twenty-eight (28) male Wistar rats ranging from 180-200g was randomly picked and placed into plastic cages labeled A-D. Group A served as the control group while groups B-D was the test groups. The animals in group A was administered with distilled water orally by gavage. Group B were administered 50mg/ kg body weight of methanol extract of *O. gratissimum*, group C were administered with 100mg/kg bodyweight while group D were administered with 200mg/body of methanol extract of *O. gratissimum* for 14 days. Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle then dispensed into tubes containing EDTA. The extract displayed a significant increase (p<0.05) in RBCs, Hb, PCV and platelet counts. More so, the extract produced no significant (p>0.05)difference in MCV, MCH, MCHC and RDW WBC counts when compared with the normal control .Therefore, it will be logical to conclude that the extract of *O. gratissimum* might be a panacea in the management of anaemic conditions when properly harnessed due to its erythropoietic, haematopoietic and thrombopoietic effect.

KEYWORDS: Anaemia, erythropoietic, haematopoietic, thrombopoietic and phytochemicals.

INTRODUCTION

Plants such as *Ocimum gratissmium* are richest resources of drugs in the traditional and modern systems of medicine, nutraceuticals, food supplements, pharmaceutical intermediates and chemical entities for synthetic drugs (Mbata and Saikia, 2006). The use of *O. gratissimum* can be used as therapeutics regimes such as epilepsy, diarrhoea, and can be use as food supplement, and medicinal effect. Plants products such as *O. gratissimum* can be used in medicines, which can be traced to its Ayuvidic origin. In developing countries like Nigeria in particular, were several plant of folkloric medicine are used in treatment of diseases like malaria, diabetes, obesity, atherosclerosis, anaemia, opportunistic infections such as HIV/ AIDS as well as microbial and anti-inflammatory management etc (Adeyemi *et al.,* 2002).

Medicinal plants are plant which when administered to man or animal (mammal), exert a sort of pharmacological action on them. Medicinal remedies are seen to have various advantage of traditional medicine namely, low cost, affordability, acceptability and perhaps, low toxicity (reduced site effect). Herbs make up most the plant sources for the productions of useful drugs that are being utilized by people worldwide (Agbo *et al.,* 2000).

The phytochemical evaluation of *Ocimum gratissimum* shows that it is rich in alkaloid, tannins, phytates, flavonoids and oligosaccharides (Ijeh, *et al.,* 2004). In the coastal area of Nigeria, the plant *Ocimumgratissimum* is used in the treatment of epilepsy, high fever and diarrhoea (Ladipo *et al*., 2010). The plant *O. gratissimum* is one of those plants widely known and used for both medicinal and nutritional

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purposes. It is a perennial plant that is widely distributed in the tropics of Africa and Asia. It belongs to the Family *Labiatae* and it is the most abundant of the genus *Ocimum.* The common names of the plant are Basil Fever plant or Tea bush and vernacular names include *Daidoya tagida* (Hausa), Nichonwu (Igbo), Tanmotswangiwawagi (Nupe), Ntong (Efik) and Efinrin (Yoruba) (Idris *et al*., 2011).

It is woody at the base and has an average height of 1-3 meters. The leaves are broad and narrowly ovate, usually 5- 13cm long and 3-9cm wide. It is a scented shrub with lime green leaves. The plant is consumed by the Igbos as a leafy vegetables and the nutritional importance of this plant is based on its usefulness as a seasoning due to aromatic flavour. It is also used by the Igbos in the management of the baby's cord. It is believed to keep the baby's cord and wound surface sterile, as well as treatment of both viral and microbial infections. In the coastal area of Nigeria, the plant *O. gratissimum* is used in the treatment of epilepsy, high fever and diarrhoea (Ladipo *et al*., 2010). This research was aimed at determining the effect of extract of methanol *O. gratissimum* on haematological profile.

MATERIALS AND METHODS

Materials

Plant materials

Fresh leaves of *O. gratissimum* were collected from the Federal Secretariat Farms, Calabar, Cross River State, Nigeria. The leaves were taken to the University of Calabar, Department of Botany for identification and authentication. The voucher number of 201 was been deposited for future reference at the Department's Herbarium.

EXPERIMENTAL ANIMALS

The Wistar rats were obtained from the animal holding unit of the Department of Medical Biochemistry, Cross River University of Technology. The animals were allowed to acclimatize for a period of 7 days, in a well-ventilated room at room temperature and relative humidity of 29°C and 70% respectively with 12 hours natural light-dark cycle. They were allowed feed and water *ad libitum*. Good hygiene was maintained by daily cleaning and removal of faeces and spills from their cages.

METHODS

Preparation of methanol extract of *O. gratissimum* leaves

The leaves of *O. gratissimum* were collected and dried at room temperature for a period of 21days until constant weight was obtained. The dried leaves were then pulverized to powder form by a machine blender and sieved. Thereafter, 400g of the pulverized plant material (*O. gratissimum*) was dissolved in 1200ml of 70% methanol for 72 hours. This was followed with vacuum filtration and extracts was concentrated using rotary evaporator and water bath at 40°C to obtain a solvent free extract, and stored in a refrigerator at 4°C.

Animal grouping and administration of extract

Twenty-eight (28) male Wistar rats were randomly picked and placed into plastic cages labeled A-D. Group A served as the control group while groups B-D were the test groups. The animals in group A was administered with distilled water orally. Group B was administered 50mg/ kg body weight of methanol extract of *O. gratissimum*, group C was administered with 100mg/kg bodyweight while group D were administered with 200mg/body of methanol extract of *O. gratissimum.*

Blood Sample Collection

Blood was collected from all the test rats and control by cardiac puncture using disposable syringe and needle then into tubes containing the anticoagulant ethylene diamine tetra acetic acid (EDTA). The specimens were labeled with the identification alphabets/ number. The EDTA samples were kept at room temperature until processing, which occurred within 30 minutes of collection.

Laboratory analysis

Full blood count was performed using a KN-21N Haematology analyzer (Sysmex, Kobe, Japan), a three-part auto analyzer able to test 7 parameters per sample including Hb concentration, PCV, RBC concentration, MCH, MCV, MCHC, WBC count, and PLT count. Standardization, calibration of the instrument, and processing of the samples were done according to the manufacturer's instructions.

Procedures

Each blood sample was mixed well and then approximately 20 µL was aspirated by allowing the analyzer's sampling probe into the blood serum sample and depressing the start button. Results of the analysis were displayed after about 30 seconds, after which the analyzer generated hard copy of the results on thermal printing paper.

Statistical analysis

The data obtained were analyzed using One Way Analysis of Variance(ANOVA) followed by post hoc test at $P<0.05$. The Statistical Package for Scientific solutions (SPSS) Software version 20.0 was used for the analysis.

RESULTS

The result below indicates the effect of administration of methanol extract of *Ocimum gratissimum* on haematological profile of Wistar albino rats. The effect administration of the extract on red blood cells (RBC), haemoglobin (Hb) and haematocrit (PCV) concentration showed a significant increase ($P>0.05$) following the administration of the extract at 50, 100 and 200mg/kg body weight respectively when compared with the normal control respectively (figure1).

From the results, it was also observed that the extract produced a significant ($P<0.05$) increases in platelet count at 50mg/kg body weight when compared to the normal control (figure 2). The extracts also produced no significant different on White blood cells count (WBC) at 50, 100 and 200mg/kg body weight respectively when compared to the normal control (fig.2).

The effect of administration of methanol leaf extract of *O. gratissimum* on MCV indicate a significant ($p<0.05$) increase at 100mg body weight when compared with the normal control but the extract produced no significant difference in serum MCV at 200 mg/kg body weight when compared with the normal control (Table 1).

Likewise, the extract produce a significant ($P<0.05$) decrease at 50mg/kg body weight from MCH when compared with the normal control while the extract produced no significant ($p<0.05$) difference at 100mg/kg body weight, but produced a significant ($p<o.05$)

increase at 200mg/kg body weight when compared to the normal control. More so, the extracts produced no significant difference for serum MCHC at 50 and 200mg/kg body weight when compared with the normal control (table 1).

The extract produced no significant (*P>0.05*) different in serum RDW at 50,100 and 200mg/kg body weight when compared with the normal control.

It was also observed the effect of administration of *O. gratissimum* on serum MPV and PDW showed no significant (P>0.05) difference at 50, 100 and 200mg/kg body weight when compared with the normal control (fig.3).

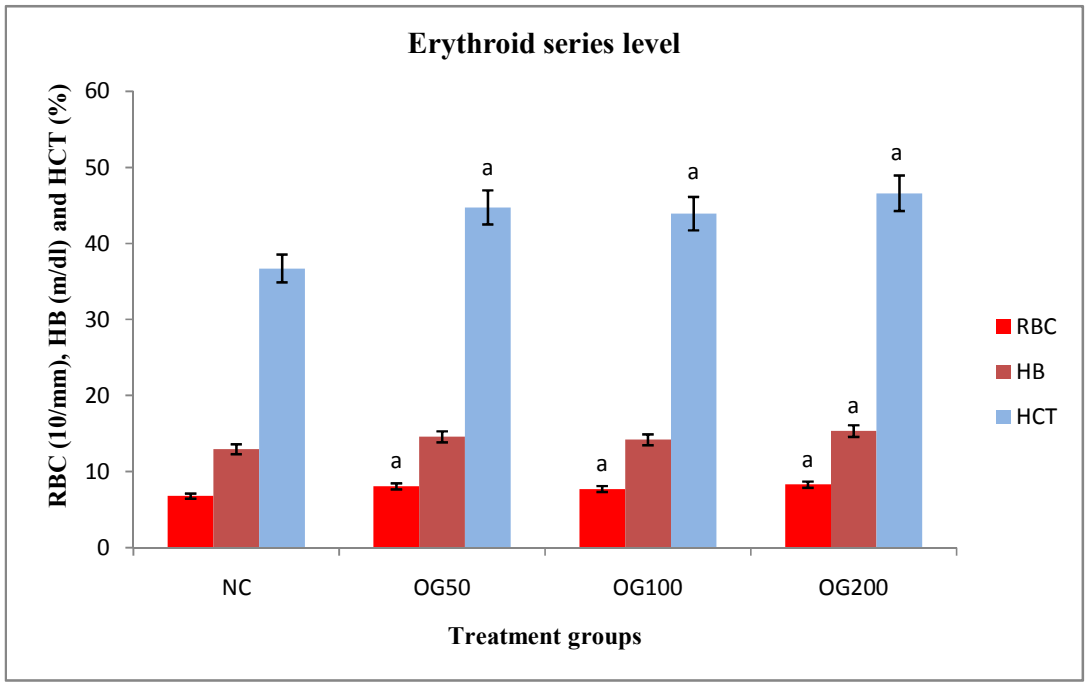

Figure 1: Effect of administration of methanol extract of *O. gratissimum* on red cells, haemoglobin and heamatocrit (packed cell volume) of Wistar rats.

Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*). Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

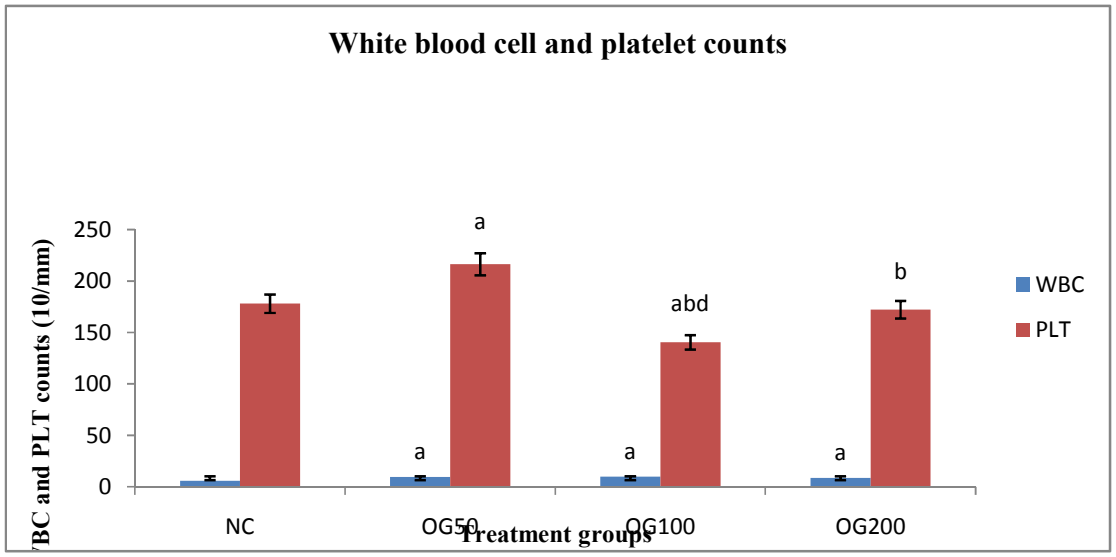

Figure 2: Effect of administration of methanol extract of *O. gratissimum* on white blood cell and platelet counts (10/mm) of Wistar rats

Values are expressed as mean ± STD; n = 5 rats per group. The same colour bars; a = significantly different from NC (*P<0.05*), b = significantly different from OG_{50} (*P<0.05*) and d = significantly different from OG_{200} (*P<0.05*) Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

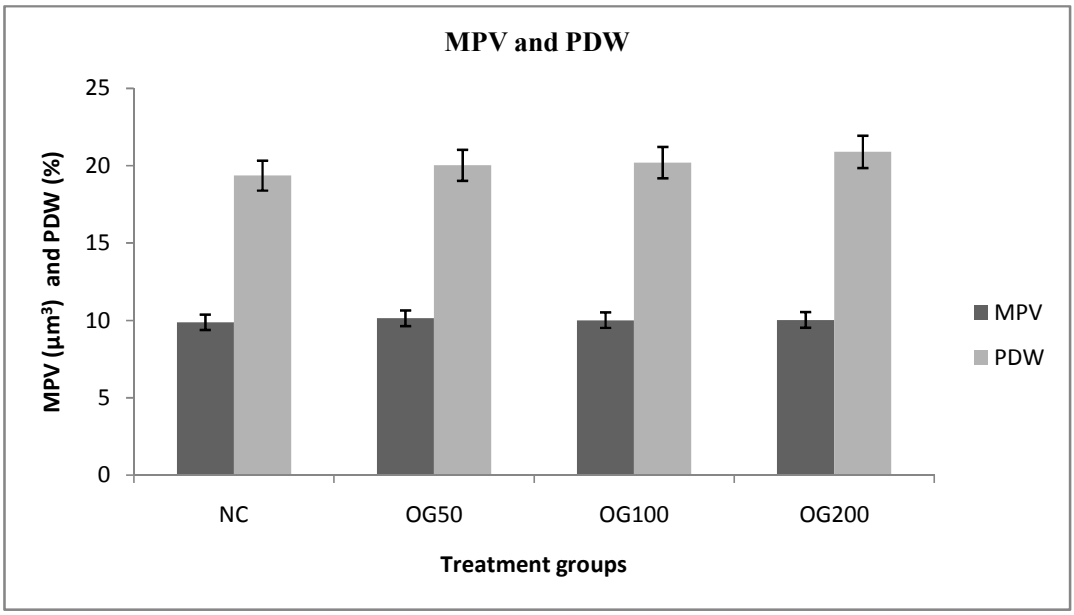

Figure 3: Effect of administration of methanol extract of *O. gratissimum* on MPV (µm^3) and PDW (%).
Values are expressed as mean ± STD; n = 5 rats per group. No significant difference among the groups. Legend: NC = normal control; OG_{50} = dose I group; received 50mg/kg body weight of methanol extract, OG_{100} = dose II group; received 100mg/kg body weight of methanol extract and OG_{200} = dose III group; received 200mg/kg body weight of methanol extract.

DISCUSSION

Haematological parameters are useful markers used to ascertain the adverse effect of plant extracts or drugs on blood constituents (Ashafa *et al*., 2010). Haematological parameters are determined in order to assess the degree of the well-being of an animal (Ajayi and Raji, 2012). Thus, haematological parameters are good indicators of the physiological and biochemical status of animals (Khan and Zafar, 2005).

The major functions of the white blood cell and its differentials are to fight infections, defend the body by phagocytosis against invasion by foreign organisms and to transport and distribute antibodies by immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high WBCs counts are capable of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases (Soetan *et al.,* 2013) and enhance adaptability to local environmental and disease prevalent conditions (NseAbasi *et al.,* 2014). Decreased count of WBCs also indicates the suppression of deleterious effect of the extracts on leucocytes and their production from bone marrow (Odesanmi *et al.,* 2010).From this present research, the non-significance difference in serum WBCs following the administration of methanol extract of *O. gratissimum* at 50,100 and 200 mg/kg body weight suggests that the extract might contain no bioactive ingredient to fight against infection or defend the body against invasion by foreign organisms.

Packed cell volume (PCV) which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in blood (Purves *et al*., 2003). It measures the percentage volume of red blood cells in the blood; anaemic condition is associated with low production of red blood cells (Guenter & Lawrence, 2005). Packed cell volume is also involved in the transportation of oxygen and absorbed nutrients. Increased PCV concentration shows a better transportation and thus results in an increased primary and secondary polycythemia (Isaac *et al*., 2013).The observed marked increase in PCV from this work suggests that the plant extract at varying concentrations may positively interfere with osmoregulatory and haematopoietic system of the blood that can enhance management of anaemia. This was contrary to the report by (Audu *et al*., 2014). Whose report acknowledged that significant reduction in PCV could be indication of severe anaemia caused by destruction of erythrocytes or haemodilution, resulting from impaired osmoregulation across the epithelium (Audu *et al.,* 2014). In this study, treatment with the plant extract led to a significant increase in PLTs in rats in administered with 50,100 and 200 mg/kgbwt of the methanol extract. According to McLellan *et al*., (2003), increase in PLT in experimental rats indicates good action on the blood's oxygen transporting ability as well as thrombopoietin. The observed increase in the PLTs in this study indicates that the extract may improve the blood oxygen transporting ability.

Increases in red blood cells (RBCs) and Hb were also observed. The observed increase in haematological indices could indicate erythrocyte synthensis (Dede *et al.,* 2002) .Therefore, the increase observed in RBC count and Hb may connotes that the extract enhances haematopoeisis and or erythropoiesis. Likewise, the oxygen-transporting ability of the blood and the oxygen supplied to the tissues may be improved following the administration of the extract

The other haematological parameters like MCH, MCHC and MCV display no significant difference following the administration of the extract of *O. gratissimum* at varying concentrations. Therefore, since MCH, MCHC and MCV are not affected by the treatment at 50,100 and 200 mg/kgbwt. It suggests that there is neither the incorporation of extract of *O. gratissimum* into Hb or RBC, nor an alteration in the morphology and fragility of RBCs.

CONCLUSION

From the data obtained we therefore, conclude that the extract of *O gratissimum* might be a panacea in the management of anaemic conditions due to its erythropoietic, haematopoietic and thrombopoietic effects.

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