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Analysis of haematological effects of *Picralima nitida*

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Abstract: The protective effects of the leaf powder of *Picralima nitida* in male rats were evaluated to establish its haematopoietic potential. To achieve this, albino rats (n = 30), weighing 120 - 160g were grouped into 5, labelled A to E. Groups C and D were intraperitoneally induced for anaemia with 0.1mg/kg body weight (b.wt) of phenyl hydrazine for 7 day. Groups A and B and C and D orally received 200 and 400 mg/kg b.wt of *Picralima nitida* leaf extract respectively for 14 days. Group E served as the control. Blood sample (5.0ml) was collected from each rat on days 8 and 15 and dispensed into ethylene diamine tetra acetic acid containers for haemogram using haematology auto analyser. The result showed that on day 8, *Picralima nitida* leaf extract produced a significant ($P<0.05$) increase in haemoglobin (Hb) and haematocrit (Hct) when compared with the control. On day 15, *Picralima nitida* leaf extract produced a significant ($P<0.05$) increase in the red blood cell (RBC), Hb and Hct when compared with the experimental control. The results indicate time-dependent haematopoiesis.

Keywords: Anaemia, haemopoiesis, haematinics, time-dependent, *Picralima nitida*

INTRODUCTION

Blood is a specialized body fluid that has four main components namely plasma, red blood cells, white blood cells and platelets. The blood has many different functions which include: regulation of the body temperature, transport of nutrients, waste products, oxygen and formation of blood clots to prevent excessive blood loss. Blood also performs immune function by carrying cells and antibodies that fight infection (American Society of Hematology, 2020).

Anaemia is one of the most common disorders of the blood which is characterised by shortage of red blood cells (RBCs) or hemoglobin in the blood and as a result of this; the cells do not perform the above mentioned functions (*Janz et al., 2013; Smith, 2010)*. The presence of anaemia indicates a nutritional deficiency and/or some pathological condition. Iron is an essential nutrient required by every human cell. Most of the body's iron is in haemoglobin and is absorbed by the intestines, transported in the blood by a carrier protein called transferrin and stored as ferritin. Iron deficiency is by far the commonest cause of anaemia worldwide.

It is the main, but not the only cause of anaemia. Nutritional deficiency (deficiency of haematinics) is the commonest cause of iron deficiency. Other causes are menstrual losses and gastro-intestinal blood loss which hookworm is the most frequent specific cause. *Schistosoma mansoni* and *schistosoma japonicum* (bilharziasis) are other worm infections associated with blood loss (Ezeh *et al.,* 2019). Gastrointestinal lesions such as peptic ulcers, malignant disease and haemorrhoids can also cause iron deficiency due to blood loss (World Health Organization, 2001).

The incidence of anaemia is higher in the developing countries than in developed countries with children and pregnant women being the most affected (Henri and Djakalia, 2010; Ogbe *et al.,* 2010). A study in a rural population of Nigeria reported that 19.7% of the children were anaemic. Such prevalence has been attributed to various aggravating factors such as poor nutrition due to poverty and poverty has forced a lot of people to embrace herbal therapy which is cheap and readily available (Ughasoro, 2019; World Health Organization, 2008).

The herb *Picralima nitida* have been claimed to possess numerous medicinal properties including haematinic properties**.** *Picralima nitida* has widely varied applications in Africa folk medicine. Various parts of the plant; the leaves, seeds, stem bark and roots are used by herbalists for the treatment of fever, hypertension, jaundice, gastro-intestinal disorders and for malaria (Iwu, 1993; Etukudo, 2003; Kouitcheu, 2008). The previous pharmacological studies of *Picralima nitida* extract showed that it possess sympathomimetic, antimalarial, antipsychotic and anesthetic activities. It also has antimicrobial, hypoglycemic and anti-diarrheal properties (Fakeye *et al*., 2004; Nkere *et al*., 2005; Inya-Anya *et al*., 2006; Kouitcheu *et al*., 2013; Salihu *et al*., 2009).

In search of cheap natural therapeutic agent for various

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illnesses, this study was initiated to investigate the haematological effects and haematopoietic potential of methanol extract of *Picralima nitida* leaves in albino rats.

MATERIALS AND METHODS

Collection and Identification of plant

The fresh leaves of the plant were collected from its tree in a rural community in Orumba-North Local Government area of Anambra state in Nigeria and were authenticated as *Picralima nitida* by a taxonomist at the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka. A specimen was deposited in the herbarium for future reference with a voucher number of UNH 483.

Extraction of plant materials

The leaves were washed, shade-dried and pulverized into fine powder with a domestic hand mill. Extraction of plant material was done using standard method as described by Jovanović et al., 2017 with little modification. Five hundred grams of the powdered plant material was immersed in 2500mls of 99% methanol for 72 hours with intermittent shaking every one hour. It was filtered through Whatman no.1 filter paper and was allowed to evaporate. The filtrate was weighed and preserved in a refrigerator at 4^{o}C until its use.

Phytochemical analysis

The standard methods of Trease and Evans (1989) were used in the analysis of the phytochemical components. The constituents analysed for were alkaloids, saponins, tannins, flavonoids, carotenoids, phenol and glycoside.

Experimental Animals

Albino rats (n = 30), aged 10-12 weeks old and weighing 120-160g were randomly allotted into 5 groups of 6 rats each. They were obtained from the laboratory animal unit of the Department of Veterinary Obstetrics and Reproductive Diseases, University of Nigeria, Nsukka and housed under standard environmental conditions of temperature of between 20-26°C, humidity range of between 30% to 70% and 12 hours light 12 hours dark cycle (Zeitler *et al.,* 2019; Institute of Laboratory Animal Resources, 1996). They were fed *ad libitum* with pelletized growers mash containing 18% crude protein with free access to drinking water and were allowed to acclimatize for a period of two weeks prior to the experiment.

Acute toxicity testing

Acute toxicity [evaluation/determination of median lethal dose (LD_{50})] was done according to the method of Lorke (1983) with little modification.

Research Design

The study adopted experimental research design. Albino rats (n = 30), weighing 120 - 160g, were grouped into 5, labelled A to E. Groups C and D were intraperitoneally induced for anaemia with 0.1mg/kg bodyweight (b.wt) of phenyl hydrazine for 7 day. Groups A and B; and groups C and D orally received 200 and 400mg/kg b.wt of *Picralima nitida* leaf extract respectively for 14 days. Group E served as the control.

Blood sample collection

Blood sample collection was done as described by Parasuraman *et al*., 2010. Blood sample (5.0ml) was collected from each rat on day 8 (protective phase) and day 15 (recovery phase) and dispensed into ethylene diamine tetra acetic acid containers for haemogram.

Determination of hematological parameters

The haematological parameters were determined using hematological auto-analyzer system following manufacturer's instruction (Sysmex, 2015).

Ethical approval

The procedures followed in this study were in accordance with the ethical standards of Ethics Committee of University of Nigeria on animal experimentation.

STATISTICAL ANALYSIS

Data were subjected to inferential statistics and analysed with student t-test and analysis of variance. Probability value of less than 0.05 was considered statistically significant. IBM SPSS 20.0 for windows was used for statistical analysis with data expressed as mean ± SD. One-way analysis of variance (ANOVA) was used to compare group data. Probability value of less than 0.05 was considered statistically significant.

RESULTS

The acute toxicity test of the extract revealed an oral LD_{50} of 4000mg/kg b.wt. The phytochemical analysis of the methanol extract of *Picralima nitida* leaves revealed carotenoids, alkaloids, flavonoids, tannin, phenol, saponin and glycoside as shown in table 1.

Table 1: The phytochemical analysis of the methanol extract of *Picralima nitida* leaves

TEST	RESULT
Carotenoids	+
Alkaloids	+++
Flavonoids	++
Tannin	+
Phenol	+
Saponin	++
Glycoside	+

Key: +++ = present in high concentrations;
++ = present in moderate concentrations;
+ = present in low concentrations; - = absent.

Table 2: The mean ± standard deviation of haematological parameters of albino rats administered with graded-doses of the extract during the protective phase (day 8)

Parameter	A Normal 200mg/kg b.wt extract	B Normal 400mg/kg b.wt extract	C Anaemic 200mg/kg b.wt Extract	D Anaemic 400mg/kg b.wt Extract	E Control
Lymphocytes (%)	67 ± 2.5	69 ± 1.2	63 ±1.5	66 ± 2.3	41 ± 1.4
Monocytes (%)	3 ± 0.5	3 ±0 .3	2 ± 0.6	3 ± 0.5	3 ± 1.4
Neutrophils (%)	29 ± 0.6	26 ± 3.1	34 ± 1.7	30 ± 1.2	54 ± 1.2
Eosinophils (%)	1 ± 0.7	2 ± 1.2	1 ± 0.5	1 ± 0.8	2 ± 0.5
RBC ($x10^{12}$/L)	10 ± 0.8	13 ± 0.1	8 ± 0.4	9 ± 0.1	9 ± 0.1
Haemoglobin (g/dL)	15 ± 0.1*	17 ± 1.0*	13 ± 1.3	13 ± 1.6	13 ± 0.3
Haematocrit (%)	50 ± 0.5*	54 ± 0.4*	35 ± 2.0	41 ± 1.2	46 ± 0.5
MCV (FL)	56 ± 2.0	60 ± 2.1*	44 ± 0.0*	48 ± 2.9	54 ± 5.4
MCH (Pg)	23 ± 4.1	28 ± 2.1*	18 ± 0.6	25 ± 1.9*	20 ± 2.6
MCHC (g/dL)	44 ± 1.0*	49 ± 2.4*	28 ± 0.7*	31 ± 2.1*	36 ± 0.1
Platelet ($x10^{9}$/L)	704 ± 4.8	707 ± 2.7	684 ± 2.1	686 ± 0.8	712 ± 2.4

Table 3: The mean ± standard deviation of haematological parameters of albino rats administered with graded-doses of the extract during the recovery phase (day 15)

Parameter	A Normal 200mg/kg b.wt extract	B Normal 400mg/kg b.wt extract	C Anaemic 200mg/kg b.wt Extract	D Anaemic 400mg/kg b.wt Extract	E Control
Lymphocytes (%)	70 ± 0.9*	71 ± 2.7*	67 ± 1.5*	70 ± 1.0*	41 ± 1.4
Monocytes (%)	3 ± 3.9	3 ± 2.4	3 ± 2.1	3 ± 1.8	3 ± 1.4
Neutrophils (%)	26 ± 4.1*	24 ± 4.8*	28 ± 1.5*	25 ± 1.0*	54 ± 1.2
Eosinophils (%)	1 ± 0.9	2 ± 1.5	2 ± 0.8	2 ± 0.7	2 ± 0.5
RBC ($x10^{12}$/L)	18 ± 0.7*	20 ± 0.2*	14 ± 0.9*	18 ± 0.3*	9 ± 0.1
Haemoglobin (g/dL)	21 ± 0.9*	27 ± 2.2*	18 ± 2.8*	20 ± 4.1*	13 ± 0.3
Haematocrit (%)	56 ± 0.7*	60 ± 2.4*	48 ± 4.1	52 ± 1.7*	46 ± 0.5
MCV (FL)	60 ± 2.0	67 ± 1.0*	56 ± 1.0	58 ± 2.1	54 ± 5.4
MCH (Pg)	31 ± 4.9*	32 ± 9.6*	26 ± 1.2*	27 ± 5.6*	20 ± 2.6
MCHC (g/dL)	46 ± 0.8*	48 ± 1.5*	34 ± 4.2	40 ± 2.4	36 ± 0.1
Platelet ($x10^{9}$/L)	709 ± 2.3	709 ± 0.9	695 ± 3.8	699 ± 1.9	712 ± 2.4

Key: $p < 0.05$ (Significant)

During protective phase (day 8), group A Haemoglobin (Hb) (15±0.1g/dL) and Haematocrit (Hct) (50±0.5%) and group B Hb (17±1.0g/dL) and Hct (54±0.4%) increased significantly ($p<0.05$) when compared with controls, Hb (13±0.3g/dL) and Hct (46±0.5%) as shown in table 2.

During recovery phase, there were significant increases ($p <0.05$)in the red blood cell (RBC), Hb and Hct in groups A, B, C and D when compared with control as shown in table 3.

DISCUSSION

Administration of medicinal compounds or drugs can alter the normal range of hematological parameters positively or negatively (Adeneye, 2008). Assessment of these parameters can be used to determine the extent of deleterious effect of foreign compounds including plant extracts in living systems (Ashafa *et al.,* 2009). The various haematological constituents investigated in the study are useful indices that can be employed to assess the toxic potentials of plant extracts in living systems (Sunmonu and Oloyede, 2010). Such toxicity testing is relevant to risk evaluation as changes in the haematological system have higher predictive value for human toxicity, when data are translated from animal studies (Olson *et al.,* 2000).

The observed high acute toxicity (LD_{50}) of 4000 mg/kg of the methanol extract of *Picralima nitida* leaves indicate that the extract is non toxic and is relatively safe for consumption and for herbal therapy. Olufunsho *et al.* (2019) obtained an LD_{50} of 707.107 mg/kg. Erharuyi *et al.* (2014) got an LD_{50} of 900 mg/kg in a methanolic seed extract study while Koffi *et al.* (2014) in Cote d'Ivoire found an LD_{50} that was greater than 3000 mg/kg.

The observed phytochemical constituents in the methanol extract of *Picralima nitida* leaves such as carotenoids, alkaloids, flavonoids, tannin, phenol, saponin and glycoside indicate its pharmacological strength as an herbal plant. These phytochemicals have been reported to have some health benefits (Shibata *et al.,* 1995). The results were partly confirmed by Olufunsho *et al*. (2019) in which they found the same constituents except carotenoids, tannin, phenol. But in addition to our similar constituents, they found steroids, anthraquinones and terpenoid. The findings of Aghedo *et al.* (2021) on n-Hexane and chloroform stem bark extracts of *P. nitida* also confirm the phytochemical screening result of this study.

During protective phase (day 8), the observed dose-dependent increases in hemoglobin and hematocrit of the normal groups (A and B) indicates hematopoietic potential of the extract at this stage, though anemia was not corrected in the anemic groups (C and D). During recovery phase (day 15), the observed dose- and time-dependent increases in red blood cells, hemoglobin and hematocrit of the normal groups (A and B) and anemic groups (C and D) indicates hematopoietic potential of the extract and was able to demonstrate hematinic action to correct anemia in the anemic groups. These findings were partly confirmed by Olufunsho *et al*. (2019) in which they found insignif-icant changes in RBC count, haematocrit and red cell indices like MCV and MCH suggesting that *picralima nitida* is unlikely to cause anaemia. This study is also in agreement with the study of Otoo *et al.* (2015) that failed to show anaemic effect of Picralima nitida

The observed slight reduction in red blood cells of anaemic rats compared to normal rats may be an indication of an imbalance between the rate of erythropoiesis and destruction of the blood cells in favour of the haemolysis caused by phenyl hydrazine. The reduction differences in haematocrit, haemoglobin and red blood cells of normal and anaemic groups confirm that anaemia was achieved using phenyl hydrazine in the anaemic groups. This suggested that the extracts have the potential to stimulate erythropoietin release in the kidney which is the humoral regulators of red blood cells production (Sanchez-elsner *et al*., 2004).

CONCLUSION

In conclusion, the study clearly demonstrated that methanol extract of *Picralima nitida* leaves possess some useful phytochemicals that stimulates haematopoiesis and demonstrated haematinic action. From the results of this experimental study, *P. nitida* methanolic extract's ability to slightly increase WBC shows that it may be useful in boosting the immune system, fighting immunity dependent infections and leucopenia but its ability to increase PCV and RBC shows that it may be useful in the management of anaemia.

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