

Full Length Research Paper

Phytochemical screening and haematological studies of *Parquetina nigrescens* ethanol and chloroform leaves extracts in normal albino rats

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The effects of crude ethanol-chloroform extract of *Parquetina nigrescens* leaves on the haematological parameters of normal albino rats were investigated at dose of 50 and 100 mg/kg body weight (*b.w*). Acute toxicity study (LD_{50}) and phytochemical constituents of the extracts were also evaluated. Thirty (30) male Wistar rats were grouped into five (5) of 6 rats each ($n = 6$). Group A: Control; administered 2 ml of normal saline. The other groups were administered extracts viz: Group B - 50 mg/kg *b.w* of ethanol extract, Group C - 100 mg/kg *b.w* of ethanol extract, Group D - 50 mg/kg *b.w* of chloroform extract and Group E - 100 mg/kg *b.w* of chloroform extract. After 21 days of administration, the blood samples were collected for the analysis. The results revealed that, the ethanol and chloroform extracts caused significant ($p < 0.05$) increase in packed cell volume (PCV), red blood cell (RBC), haemoglobin, lymphocyte, neutrophil and eosinophil concentration at the doses administered compared with the rats in the control group. The rats administered 100 mg/kg *b.w* of chloroform extract showed significant ($p < 0.05$) increase in white blood cell differential compared with the rats in the control group, while the animals in the other treated groups showed non-significant ($p > 0.05$) decrease in white blood cell differential compared with animals in the control group. The monocyte and platelet concentration of the rats administered both extracts were found to have non-significant ($p > 0.05$) decrease at 50 and 100 mg/kg *b.w* of administration compared with animals in the control group. The results of the qualitative phytochemical analysis showed that the ethanol-chloroform extract tested positively to flavonoids, alkaloids, tannins, saponnin and reducing sugars while, chloroform extract tested positive to fat and oil and steroids. Acute toxicity and lethality studies on ethanol-chloroform extracts revealed an oral LD_{50} equal or more than 5000 mg/kg body weight in mice. It can be concluded that, the plant *Parquetina nigrescens* leaf has beneficial haematological and immunological properties in Wistar albino rats. It also revealed that *P. nigrescens* possessed erythropoietic potentials at minimal dose which lends support to its use in the treatment of anaemia.

Key words: Acute toxicity, phytochemical, immunological, anaemia, haematological.

INTRODUCTION

Plants and their derivatives play key roles in world health and have long been known to possess biological activity (Omoboyowa et al., 2013). The use of these plants by man for the treatment of various diseases has been in practice and is very popular in many developing countries of the world for over a long period of time (Gill, 1990; Idowu et al., 2009). This practice has gradually gained popularity in some parts of Europe and North America (Leese and William, 1994; Odeigah et al., 1999). In Africa, especially in the tropical areas, several factors such as poverty and illiteracy still militate against availability and accessibility of Western medical services. The need to have a strong, healthy immune system cannot be overemphasized in our present day society. Most illnesses such as AIDS and cancer are believed to be immune-related disorders (Idowu et al., 2009).

Medicinal plants possess therapeutic properties and despite the widespread use of modern medicine, herbal products are still in use in most developing countries of Africa and Asia for the management of ailments, *Parquetina nigrescens* happens to be one of such medicinal plants (Owoyele et al., 2011). *P. nigrescens* (Apocynaceae), a shrub found in equatorial West Africa, has been in traditional medicine practice for centuries. The parts of the plants used for traditional medicine include the leaves, roots and latex (Agbor and Odetola, 2005). It is a perennial with twinning stem and woody base shortly tapering 10 to 15 cm long, 6 to 8 cm broad with a smooth long stem on the leaves. The leaves have been reputed for treatment of helminthiasis (intestinal worm), wound and have sympathomimetic effect (Agbor and Odetola, 2005), while the roots are used for the management of rheumatism (Adeyemi, 1994). Over the years, *P. nigrescens* has been used as an ingredient in the medications for insanity (Iwu, 1993), as well as an aphrodisiac in East Africa. Other uses include the decoction of the stem bark been given as cardiac tonic while the leaf and root decoction have been used for the treatment of gonorrhoea and menstrual disorders (Iwu, 1993; Odetola et al., 2006). Research has shown that oral ingestion of medicinal compounds or drugs can alter the normal range of haematological parameters, these alterations could either be positive or negative (Ajagbonna et al., 1999). It has therefore become necessary to investigate the effect of ethanol-chloroform leaf extract of *P. nigrescens* on haematological parameters of rats *in vivo*, screening for selected phytochemical constituents of the present bioactive determination.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade and supplied by sigma incorporated, US.

Plant

The leaves of *P. nigrescens* were collected from the metropolis of Ibadan, Nigeria. It was authenticated at the botany Department of University of Ibadan, Nigeria where a voucher specimen with voucher number V/No. 2002018 has been deposited. The leaves were air-dried at room temperature of 25 to 29°C after which it was ground. The pulverized leaves (1071 g) were macerated in 3 L mixture of chloroform and ethanol (2:1) for 48 h. The macerate was passed through Whatman No. 4 filter paper. The filtrate was shaken with 20% of distilled water to obtain two (2) layers. The upper layer (ethanol extract) was separated from the lower layer (chloroform extract) with a separating funnel. The two layers were concentrated with a rotatory evaporator and dried in a boiling water bath at 60°C. The weight of the extracts was taken after drying, the extract yields was obtained.

Phytochemical test

Basic qualitative phytochemical screening of the ethanol and chloroform extracts of the extract of the leaves sample was carried out by testing for the presence or absence of the following plant constituents: flavonoids, tannins, saponins, glycosides, terpenes, fat and oil, steroid, alkaloid, reducing sugar, phlabatannins and anthraquinone. The phytochemical analyses of the samples were carried out using the procedures outlined by Harborne (1989) and Trease and Evans (1989).

Experimental animal

Thirty male Wistar strain rats (200 to 240 g, initial weight) were used in the study. The animals were housed in wire mesh cages at the Department of Petroleum and Chemical Sciences, Tai Solarin University of Education, Ijagun, Ogun State, Nigeria. The animals were allowed to acclimatize for two weeks before commencement of the study. Food and water were provided *ad libitum*. Ethical approval was received from the College of Biological Sciences, Tai Solarin University of Education animal house committee.

Experimental design

The experiment lasted for 21 days and the animals were thus divided into five groups. Group A was the control and the rats were administered 2 ml of normal saline. The other groups (B, C, D and E) were the treatment groups. Groups B and C were administered 50 and 100 mg/kg *b.w* of ethanol extract, while groups D and E

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Table 1. Phytochemical constituents of the ethanol-chloroform extract of *P. nigrescens*.

Phytochemical constituents	Ethanol layer	Chloroform layer
Flavonoid	+	+
Alkaloid	+	+
Saponin	+	+
Tannins	+	+
Fat and oil	ND	+
Reducing sugar	+	+
Glycoside	ND	ND
Anthraquinone	+	+
Steroid	ND	+
Phlabatannin	ND	ND

Key: ND= Not Detected, + =Present.

were administered 50 and 100 mg/kg *b.w* of chloroform extract, respectively. All animals were sacrificed on day 21 after which blood samples were collected for the respective tests.

Acute toxicity and lethality (LD_{50}) test

The acute toxicity and lethality of chloroform-ethanol extract of the *P. nigrescens* leaves extract was determined using the modified method of Lorke (1983). The test was divided into two stages. In stage one, eighteen (18) randomly selected adult mice were divided into six groups, three per group ($n = 3$) and received 10, 100 and 1000 mg/kg body weights (orally) of the ethanol and chloroform extracts, respectively and the signs of toxicity and number of death was observed for a period of 24 h.

After 24 h observation, the doses for the second phase were determined based on the outcome of the results of the first phase. Since there was zero death, a fresh batch of animals was used following the same procedure in phase I but with higher dose ranges of 1900, 2600 and 5000 mg/kg body weights of the extracts. The animals were also observed for 24 h for signs of toxicity and possible number of death. The LD_{50} was calculated as the geometric mean of the high non-lethal dose and lowest lethal dose (Lorke, 1983).

Haematological Indices

After 21 days of oral administration of the extracts, the animals were sacrificed and 2 ml of blood samples were collected by cardiac puncture for haematological analysis. The haematological parameters (Packed cell volume, haemoglobin concentration, red blood cell count, white blood cell count, lymphocyte count, neutrophil count, eosinophil count, monocyte count count and platelet count) were evaluated with an automated hematological analyzer systemex KX-21 (Japan).

Statistical analysis

The data obtained from the laboratory result of the tests were subjected to one way analysis of variance (ANOVA). Significant differences were observed at $p < 0.05$. The results were expressed as mean $\pm$ standard error of mean (SEM). These analyses were done using computer software known as statistical package for social sciences (SPSS), version 16.

RESULTS

Yield of the extracts

The yields of the extracts were calculated as 5.68 g (0.53%) and 15.85 g (1.48%) for ethanol and chloroform extracts, respectively.

Phytochemical test

The qualitative phytochemical compositions as observed in Table 1 showed presence of bioactive compounds such as flavonoids, alkaloids, saponins, tannins and reducing sugar in the two extracts. The chloroform extract showed the presence of steroids and fat and oil. Anthraquinone was present in ethanol extract. The bioactive compounds found to be relatively absent in the extracts were glycosides and phlabatannins as shown in Table 1.

Acute toxicity and lethality (LD_{50}) test

Oral administration of up to 5000 mg/kg body weight of chloroform-ethanol extract of *P. nigrescens* leaves to mice caused no death in the two stages of the test. Thus, oral LD_{50} of the extract in mice was estimated to be greater than 5000 mg/kg body weight.

Effect of ethanol leaves extract of *Parquetina nigrescens* on haematological parameters in rats

Table 2 shows the results of the red blood cell count and some other haematological parameters in the experimental animals. The rats administered with 50 and 100 mg/kg *b.w* ethanol extract shows significant ($p < 0.05$) increase in packed cell volume, haemoglobin, red

Table 2. Effect of 21 days of oral administration of ethanol leaves extract of *Parquetina nigrescens* on haematological parameters in rats.

Parameter	Group A	Group B	Group C	Group D	Group E
PCV (%)	38.25 ± 0.63	41.75 ± 1.31*	42.50 ± 1.19*	41.00 ± 0.91*	40.50 ± 1.94*
Hb count (g/dl)	8.33 ± 0.31	9.20 ± 0.24*	9.30 ± 0.24*	8.43 ± 0.29*	8.65 ± 0.39*
RBC count ($\times 10^6 \text{ mm}^3$)	5.68 ± 0.35	7.64 ± 0.40*	8.52 ± 0.25*	7.49 ± 0.39*	8.32 ± 0.19*
WBC count ($\times 10^3 \text{ mm}^3$)	6.30 ± 0.41	6.28 ± 0.25	5.57 ± 0.50	5.35 ± 0.69	7.73 ± 0.14
Lymphocyte count (%)	64.25 ± 0.85	65.75 ± 4.35*	70.70 ± 2.66*	68.75 ± 1.79*	67.00 ± 2.42*
Neutrophil count (%)	27.25 ± 2.14	36.00 ± 3.37*	33.75 ± 4.53*	29.00 ± 2.71*	30.50 ± 1.94*
Eosinophil count (%)	1.0 ± 0.71	2.00 ± 0.71*	2.25 ± 0.48*	1.75 ± 0.25*	1.25 ± 0.25*
Monocyte count (%)	2.50 ± 0.29	2.25 ± 0.48	2.00 ± 0.71	1.75 ± 0.25	1.25 ± 0.25
Platelet count ($\times 10^4 \text{ mm}^3$)	9.80 ± 1.24	8.60 ± 1.50	8.63 ± 0.71	8.85 ± 1.47	8.63 ± 2.49

Key: Hb = Haemoglobin; PCV = Packed Cell Volume; WBC = White Blood Cells; RBC = Red Blood Cell *represents significant difference at $p < 0.05$. Group A: Control Group: 2ml of distilled water. Group B: Administered 50 mg/kg *b.w* of ethanol leave extract of *P. nigrescens*. Group C: Administered 100 mg/kg *b.w* of ethanol leave extract of *P. nigrescens*. Group D: Administered 50 mg/kg *b.w* of chloroform leave extract of *P. nigrescens*. Group E: Administered 100 mg/kg *b.w* of chloroform leave extract of *P. nigrescens*.

blood cell count, lymphocyte, neutrophil and eosinophil compared with the control group. The animals administered ethanol leave extract of *P. nigrescens* showed non-significant ($p > 0.05$) decrease in white blood cell differential compared with animals in the control group. The rats treated with 50 and 100 mg/kg *b.w* ethanol extract shows non-significant ($p > 0.05$) decrease in monocyte count and platelet count compared with the rats in the control group.

The results showed that, the red blood cell count and some other haematological parameters in the experimental animals treated with chloroform leave extract of *P. nigrescens*. The rats administered with 50 and 100 mg/kg *b.w* chloroform extract shows significant ($p < 0.05$) increase in packed cell volume, haemoglobin, red blood cell count, lymphocyte, neutrophil and eosinophil compared with the control group. The rats administered 100 mg/kg *b.w* of chloroform extract shows significant ($p < 0.05$) increase in white blood cell differential compared with the rats in the control group. The rats treated with 50 and 100 mg/kg *b.w* of chloroform extract shows non-significant ($p > 0.05$) decrease in monocyte count and platelet count compared with the rats in the control group.

DISCUSSION

Natural medicinal products have been used for the millennia for the treatment of multiple ailments although many have been superseded by conventional pharmaceutical approaches; there is currently a resurgence in interest in the use of natural products by the general public (Ghosh and Playford, 2003). The result of the haematological parameters observed in this study showed that the mean red blood cell count (RBC), haemoglobin, packed cell volume (PCV), lymphocyte,

neutrophil and eosinophil concentration increased significantly ($p < 0.05$) at 50 and 100 mg/kg body weight (*b.w*) of administration compared with the control group. The mean white blood cell differential increases significantly ($p < 0.05$) at 100 mg/kg *b.w* of administration while the monocyte and platelet concentration was observed to have non-significant ($p > 0.05$) decrease at 50 and 100 mg/kg *b.w* of administration compared with the control group. As observed in this study, the chloroform and ethanol extract of *P. nigrescens* leaves had some positive effect on the haemopoietic system of the tested rats; this was manifested by an increase in RBC, Hb, PCV, lymphocytes, neutrophil and eosinophil concentrations following the administration of the medicinal plant extracts to the rats at varying doses. The raised PCV is an indication of haemoglobin concentration which may be due to increase RBC mass (Nwinuka et al., 2008). The observed increase in these haematological parameters may be due to the presence of erythropoietin-like principles in the extract which probably stimulated erythropoietin synthesis or release at low dose.

The significant increase in the value of lymphocytes, neutrophil and eosinophil in rats treated with the varying doses of the extracts compared with the rats in the control group and the significant ($p < 0.05$) increase in the white blood cell differential in rats administered 100 mg/kg *b.w* of chloroform extract compared with the control and other treated groups may suggest that, the chloroform and ethanol extracts of *P. nigrescens* leaves must have influenced the defence mechanism and immunity of the tested rats. Therefore, continuous exposure of the body systems of animals to the medicinal products (herbs) may cause lymphocytosis, which may then account for the use of this plant for medicinal purposes (Keenwe and Bekalo, 1996).

These results agree with the findings of Agbo and Odetola (2005) who investigated the aqueous leaf extract

of *P. nigrescens* on the erythrocyte indices. RBC count, haemoglobin concentration, haematocrit, reticulocyte and erythrocyte osmofragility were used as erythrocyte indices. It was observed that the aqueous leaf extract of *P. nigrescens* significantly ($p < 0.05$) increased the erythrocyte indices which were attributed to erythropoietic potential of *P. nigrescens*. The results obtained in this study is also in agreement with the findings of Owoyele et al. (2011) who observed increase in the erythrocyte indices in the study of haematological and biochemical studies of *P. nigrescens* root extract in albino rats.

Monocytes are known to originate in the bone marrow from a common myeloid progenitor that is shared with neutrophil and they are then released into the peripheral blood where they are circulated for several days before entering the tissues and replenishing the tissue macrophage populations (Siamon and Philip, 2005). Non-significant ($p > 0.05$) decrease in the monocytes concentration observed in the rats administered 50 and 100 mg/kg *b.w* of both extracts when compared with the control rats is an indication that chloroform and ethanol extracts of *P. nigrescens* may not have any adverse effect on the bone-marrow metabolism (Young and Maciejewski, 1997).

The specific bioactive constituent responsible for the hematologic properties of *P. nigrescens* leaves is yet to be identified. None of the several phytochemical constituents identified from the extracts has been reported to possess hematologic properties. The result of the qualitative phytochemical analysis observed in this study showed the presence of such bioactive compounds as flavonoids, alkaloids, saponnins, tannins and reducing sugars in the two extracts; steroids and fats and oil in the chloroform extract only while anthraquinone is detected in the ethanol extract only. The bioactive compounds detected such as tannins and flavonoids have been implicated in the treatment of diarrhoea while flavonoids and phenolic compounds are known to have antioxidant properties (Agbor and Odetola, 2001). This is an indication of a possible application of *P. nigrescens* in the treatment of other disease condition. However, the experimental data from this study is insufficient to directly ascribe the hematological properties to any of phytochemicals present in the two extracts.

Acute toxicity test on the extract in mice estimated a high LD₅₀ value of more than 5000 mg/kg body weight which suggests that the leaf may be generally regarded as safe with a remote risk of acute intoxication. The high degree of safety is also consistent with the report of Owoyele et al. (2011) and its popular use as herbs in the western part of Nigeria.

Conclusion

The chloroform and ethanol leaves extract of *P. nigrescens* possesses erythropoietic potentials and

immunological properties at the varying doses used in this study and the overall results lend support to the folkloric use of the chloroform extract of *P. nigrescens* in the treatment of anaemia and in the enhancement of the immune system.

Conflict of Interests

The authors have not declared any conflict of interests.

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