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Effect of Rutex®, a Nigerian Polyherbal Formula on Chloramphenicol-induced Anaemic Wistar Rats

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Abstract

This study evaluated the haematinic potentials of a polyherbal formula- Rutex® (RHF). Anaemia was induced by oral administration of chloramphenicol (50 mg/kg. Treatment was carried out once daily for 7 days after which the rats were bled for determination of PCV, Hb and WBC count. Chloramphenicol induced a significant ($p< 0.05$) decrease PVC and Hb indicating anaemia and also resulted to a significant increase in WBC count. RHF produced significant ($p< 0.05$) increase in PCV and Hb with a corresponding decrease in WBC after 7 days of oral administration to anaemic rats. Conclusively, RHF exhibits haematinic effect and could be useful in the management of anaemia.

Keywords: Polyherbal formula, Haematinic, Anaemic, Wistar rats

Introduction

Anaemia is a disease characterised by a reduction in the concentration of haemoglobin, circulating red blood cell and pack cell volume per unit of the peripheral blood below the normal for the age and sex of the patient (Aguwa, 1996). The prevalence of anaemia is high in children with a high risk of placental malaria infection (Muriel and Jean-Yves, 1998). Anaemia impairs normal development in children and it constitutes a major public health problem in young children in the developing countries with wide social and economic implications (Montalemberk and Girot, 1996).

Through the ages man has learnt to take advantage of the many resources placed at his disposal by nature to meet his essential needs in all fields. As important reserves and sources of abundance, natural resources are indispensable for socio-economic development. According to Gbile (1986), the diversity of the flora in Africa partly explains the strength of traditional medicine. This refers to the use of plants in the treatment or amelioration of diseases within an organized system. A good number of medicinal plants are traditionally employed to alleviate anaemia. Some of these plants include *Telfeira occidentalis*, *Psorospermum ferbrifugum*, *Jatropha curcas*, and *Brillantasia nitens* and *Ficus exasperata* (Dina *et al.*, 2006).

Polyherbal formulations are mixtures of many plant parts (which could be roots, leaves, stem, flowers, pods and seeds) obtained from various plant species and families. These plants/ their combinations usually contain an array of bioactive compounds making them suitable for the treatment and management of a variety of disease conditions (Pieme, 2016). By using herbal combinations, nature provides a balance of ingredients that may act as buffers, synergists or counterbalances, which work in harmony to rid the body of diseases and infirmities (Montrale, 1998). Some polyherbal extracts have been scientifically proven for efficacy in the treatment of diseases while many others are yet to be investigated (Idakwoji *et al.*, 2016).

Rutex® is a polyherbal formulation consisting of *Borreria stachydea* (Leaves) *Magnifera indica* (stem bark), *Allium sativum* (seed), *Moringa oleifera* (seed), *Morinda lucida* (seed) and A*zadirachta indica* (Bark). It is usually indicated for a wide range of diseases which include malaria fever, typhoid fever, diorrhoea, dysentery, hypertension, pile and diabetes. This study was aimed at profiling the polyherbal formulation for its anti-anaemic effect as it has been speculated to be useful in the treatment of anaemia.

Materials and Methods

Materials

Chemicals and drugs

All chemicals used in this study were of analytical grade and were purchased from Sigma Chemical Co. Ltd (USA) through a local vendor. Ferrous gluconate was purchased from a local pharmacy shop. Rutex ® Herbal Formula (RHF) was purchased from the company vendors around 'Garage market' area of Anyigba, Kogi State, Nigeria.

Animals

Adult male Wistar rats weighing 150–220g were used for this study. They were kept in stainless steel cages under standard laboratory conditions. They were maintained on clean water and standard rodent feed.

Methods

Drying and reconstitution of the polyherbal formulation

Several bottles of RHF were emptied into a beaker and dried in an oven at 45ºC to a constant weight. Subsequently, the extract was reconstituted using normal saline to the required concentration.

Acute Toxicity Study

The oral median lethal dose (LD_{50}) of the extracts was determined in rats according to the method of Lorke, 1983.

Induction of Anaemia

Haematological parameters were initially determined for untreated rats according to established method (Baker *et al.,* 1998). Anaemia was induced by orally administration of chloramphenicol (50 mg/kg) for 2 weeks in four groups and each contains five rats. The Anaemia was confirmed by the markedly low PCV compared to untreated rats.

Anti- anaemic Screening

Four (4) non- anaemic rats and twenty (20) anaemic rats were used in this study. The non – anaemic rats served as normal control and received 5 ml/kg distilled water (Group 1). The 20 anaemic rats were divided into 5 groups of 4 rats each (groups 2- 6). Group 2 served as anaemic control and received 5 ml/kg distilled water, group 3 served as positive control (reference drug) and received ferrous gluconate (900 mg/kg) while groups 4- 6 received 200, 400 and 800 mg/kg RHF respectively. Treatment was carried out once daily for 7 days. All the treated animals were fed with water and pellets for a week. Animals were bled for determination of PCV, Hb, WBC parameters (Baker *et al.,* 1998) before and after administration of the formula and reference drug.

Statistical Analysis

Data were expressed as mean standard error of mean (SEM). Statistical comparisons were performed by one-way ANOVA, followed by Tukey- Kramer multiple comparisons test and student-Newman-Keuls multiple comparisons test and the values were considered statistically significant when p-value is less than 0.05 ($p<0.05$)

Results

Effect of Rutex ® Herbal Formula (RHF) on Haematological Parameters

The effect of the administration of RHF on haematological parameters of chloramphenicol-induced anaemia in Wistar rats is shown in tables 1-3. Chloramphenicol produced a significant ($p< 0.05$) decrease in PCV and Hb concentration with a significant ($p< 0.05$) increase in WBC count compared to non-anaemic control. Treatment with RHF at doses of 200, 400 and 800 mg/kg produced significant ($p< 0.05$) increase in PCV and Hb concentration in a dose- dependent fashion compared to the control. All the doses of RHF also produced a corresponding significant ($p< 0.05$) decrease in WBC count after 7 days of oral administration to anaemic rats compared to the control. The observed effect of RHF at 400 mg/kg was comparable to that of the reference drug- ferrous gluconate on the haematological parameters.

Table 1: Effect of the Administration of Rutex ® Herbal Formula (RHF) on Packed Cell Volume (%) of Chloramphenicol- induced Anaemia in Wistar Rats

Treatment	Pre-treatment	Post- treatment
Non anaemic control (5ml/kg dist. H_2O)	-	48.02 ± 2.56^{b}
Anaemic control (5 ml/kg dist. H_2O)	19.58±2.58	13.43 ± 1.67^{a}
Ferrous gluconate (900 mg/kg)	18.22±2.77	40.78 ± 2.26^{b}
RHF (200 mg/kg)	17.15±1.67	18.89 ± 1.98^{a}
RHF (400 mg/kg)	18.36±2.01	32.02 ± 3.46^{ab}
RHF (800 mg/kg)	19.51±2.36	45.29 ± 3.89^{b}

Data are presented as mean ± SD, n=5), (-) not treated. Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Table 2: Effect of the Administration of Rutex ® Herbal Formula (RHF) on Haemoglobin (g/dl) of Chloramphenicol- induced Anaemia in Wistar Rats

Treatment	Pre- treatment	Post- treatment
Non anaemic control (5ml/kg dist. H_2O)	-	12.58 ± 2.56^b
Anaemic control (5 ml/kg dist. H_2O)	7.45±0.45	7.28 ± 1.52^a
Ferrous gluconate (900 mg/kg)	7.26±0.78	12.49 ± 2.92^b
RHF (200 mg/kg)	6.95±0.48	7.11 ± 1.16^a
RHF (400 mg/kg)	7.47±0.98	7.90 ± 1.45^a
RHF (800 mg/kg)	6.37±0.55	12.01 ± 2.49^b

Data are presented as mean ± SD, n=5), (-) not treated. Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Table 3: Effect of the Administration of Rutex ® Herbal Formula (RHF) on White Blood Count ($x10^3/mm^3$) of Chloramphenicol- induced Anaemia in Wistar Rats

Treatment	Pre- treatment	Post- treatment
Non anaemic control (5ml/kg dist. H_2O)	-	4.56 ± 0.45^a
Anaemic control (5 ml/kg dist. H_2O)	7.34±0.89	6.89 ± 0.46^b
Ferrous gluconate (900 mg/kg)	7.11±0.45	4.45 ± 0.56^a
RHF (200 mg/kg)	7.88±0.45	4.54 ± 0.67^a
RHF (400 mg/kg)	6.99±0.38	4.68 ± 0.14^a
RHF (800 mg/kg)	7.30±0.37	4.50 ± 0.77^a

Data are presented as mean ± SD, n=5), (-) not treated. Mean values with different alphabets as superscripts down the column are significantly different at $P<0.05$

Discussion

Anaemia constitutes a serious health problem in many tropical countries because of the prevalence of malaria and other parasitic infections (Dacie and Lewis, 1994). In anaemia there is decreased level of circulating haemoglobin, less than 13 g/dl in male and 12 g/dl in females (Okochi *et al.*, 2003). In the tropics, due to endemicity of malaria, between 10 to 20% of the population presents less than 10 g/dl of Hb (Diallo *et al*, 2008). Children are more vulnerable. Blood parasites, bacterial infections, viral infections, drugs/chemical agents and metabolic diseases may result in destruction of red blood cells leading to haemolytic anaemia (Ramzi *et al.*, 1994). Though there are a large number of medicinal plants with haematinic effects, polyherbal formulas are thought to have more beneficial effects than single herbs. Therefore, this study was undertaken to validate the haematinic potentials of a polyherbal formula-Rutex® in chloramphenicol- induced anaemic rats.

The main function of the RBC is the transportation of oxygen in to the tissues of the body. At such, any pathological or physiological condition that affects the RBC alters its function and this may be detrimental to the body. In this study, chloramphenicol affected RBC, consequently, decreased PCV and hemoglobin concentration. However, our results indicated that RHF markedly increased the PCV and concentration of haemoglobin. . It was also observed that the recovery of the treated groups was dose related with the highest dose of 800 mg/kg effecting the highest change. The chemical constituents (such as minerals, Sterols, Proteins and other vitamins) of the component plants of the formulation might be responsible for the haematinic activity.

The formula also resulted in fall in the WBC values which might be expected recognizing that chloramphenicol which was used to induce anaemia is noted as a high risk drug which obviously affects the immune system of the rats leading to a fall in WBC. This is in agreement with the report that full recovery of experimental animals from biochemical effects of plant extracts could be achieved by chronic administration of the extracts (Adebajo *et al.*, 2006; Bumah *et al.*, 2005). The speedy and progressive recovery of anaemic rats responding to treatment of RHF may be due to increased erythropoiesis. Conclusively, as shown in the results, KHF possess haematinic effects and hence could be useful in the management of anaemia.

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