

Effects of *Pterocarpus Erinaceus* Stem Bark Aqueous Extract on Anemic Rats.

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Abstract- The study was designed to evaluate the anti anemic effect of aqueous extract of *Pterocarpus erinaceus* stem bark in phenylhydrazine induced anemia in albino rats. The extract was also tested for safety. Acute toxicity studies revealed that values up to 5 g/kg body weight did not produced any visible effect. The dosages 250 and 500 mg/kg body weight fed the rats in this study were quite safe. Anemia was induced by administration of 80 mg/kg body weight of phenylhydrazine to the rats. The anemic rats were treated orally with 250 and 500 mg/kg body weight of aqueous extract of *Pterocarpus erinaceus* stem bark for fourteen days. Oral administration of 250 mg/kg body weight of aqueous extract to anemic rats significantly increased ($P < 0.05$) serum level of hematological parameters PCV from 40.15 to 54.15, Hb from 11.95 to 13.60, there were significant improvements in RBC, WBC, Neutrophil, Lymphocytes, Platelets, MCV, MCH, and MCHC of the treated rats when compared to the control. Also some serum biochemical parameters were tested. There were significantly reduced ($P < 0.05$) serum levels AST (13.75), ALT (6.67) when compared to control. Similarly, the serum levels of Albumin, bilirubin, Urea, Creatinine and lipid peroxidations significantly improve when compare with the control group. The anemic rats fed with 500 mg/kg body weight showed significant improvement in both hematological and biochemical parameters tested. But the difference in values between treated groups (250 and 500 mg/kg body weight) has no significant different. The results suggest that *Pterocarpus erinaceus* stem bark is safe for use and may use in managing anemia in rats.

IndexTerms—*Pterocarpus erinaceus*, anemia, phenylhydrazine, stem bark

I. INTRODUCTION

Medicinal plants are the nature's gift to human beings to make disease free healthy life. Several millions of Nigerian households have been using through the ages nearly 8,000 species of medicinal plants for their health care needs (National Drug Policy, 2003). Over a one million traditional healers use a wide range of medicinal plants for treating ailments of both humans and livestock across the length and breadth of the country.

Herbal and traditional medicines are widely used in the country. Many of the drugs used in modern medicine today originate from plants and there is no doubt that new drugs can still be discovered from plants, including those indigenous to Nigeria. There is, therefore, a need for the government to provide adequate funds in institutions and universities to promote research in drug development, especially herbal

medicine. Such research shall have demonstrable relevance to the needs of the society, with a high potential for immediate application (National Drug Policy, 2003).

Pterocarpus erinaceus is a deciduous legume tree of African savannas and dry forests famous for producing one of the finest woods in its native region. *Pterocarpus* means "winged fruit" from a Greek word "*Ptero*" (wing) and "*karpos*" (fruit). The seeds are kidney shaped to oblong. It is known under many common names including African rosewood, vene, madobia, l'amhi in English, French, Hausa (Nig.) and Fulani (Nig.) languages respectively. The trunk-bark and root-bark are use in malnutrition, debility, pregnancy, anemia etc (Bonkoungou, et al., 1998). The aim of this study is to evaluate the aqueous extract of *Pterocarpus erinaceus* stem bark on hematological and biochemical indices of anemic rats.

II. METHODOLOGY

Animals

Wistar albino rats (80-100g) of both sexes obtain from Animal Facility Centre, National Veterinary Research Institute Vom, Jos, Nigeria were used for the study. They were housed in polypropylene cages, and given standard laboratory diet and water *ad libitum* and maintained under laboratory conditions of temperature ($22 \pm 1^\circ\text{C}$) and 12 h light and 12 h dark cycle. The guides for the care and use of laboratory Animals, 1996 of the Institute of Laboratory Animal Research (ILAR) Commission on life Science, National Research Council were duly followed.

Collection of plant material

The stem bark of *Pterocarpus erinaceus* were collected in the month of January, 2013 at Vakuna village in Maiha local government area of Adamawa state, Nigeria. The plant was taxonomically identified and authenticated in Biological Science Department of Modibbo Adama University of Technology, Yola.

Extract preparation.

The stem bark was air-dried for 30 days. It was then reduced to powdered form by grinding in pestle and mortar. One hundred and sixty grams (160g) of the powdered stem bark was cold macerated in 1000ml of water for 24 hours with constant shaking and filtered using Whatmans filter paper No.1. It was then concentrated to dryness on a water bath. The crude extract will be kept in a desiccator. When required, a

known quantity of the extract was taken, dissolved in a known volume of distilled water to obtain the desired concentration.

Experimental design

The experiment in drug-induced anemia using phenylhydrazine was performed for 2 weeks. All the rats (except the ones in the first group) were injected with a single subcutaneous dose of 80 mg/kg of phenylhydrazine hydrochloride (Berger 1985). On day 0, before initiation of anemia, initial PCV was taken in all the groups. Anemia was allowed to establish in 24 hours (Nath and Prasanna, 1958). On the day of establishment of anemia, the PCV was taken in all the groups. Treatment with the extract (250 and 500 mg/kg), commenced 24 hours after the administration of phenylhydrazine hydrochloride and continues for 14 days once daily.

Groups	Description
I	Normal
II	Phenylhydrazine control
III	Anemia + 250 mg/kg bw of aqueous extract
IV	Anemia + 500 mg/kg bw of aqueous extract

Acute toxicity

The aqueous extract of *Pericarpus erinaceus* stem bark was carried out as described by Locke, 1983 and the rats were observed for the signs of toxicity, mortality and some biochemical parameters were determined after seventy two hours (Jayasekar et al., 1997).

Determination of haematological and biochemical parameters

After two weeks treatment with the aqueous extract, blood samples were obtained through cardiac puncture of the rats for the determination of the blood parameters: Red blood cells (RBC), packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and its differential counts using the method of Dacie and Lewis (1991). The red cell indices were also calculated. Also biochemical parameters were determined AST and ALT using Reitman and Frankel, 1957, Total and Direct Bilirubin as described by Maloy and Evelyn, 1937, Glucose using glucose oxidase method, Total proteins (Reinhold, 1953), Albumin (Spencer and Price, 1977), creatinine using Jaffe method, urea using Urease-Berthelot method.

Statistical Analysis

The mean and standard error of mean (SEM) was calculated for each parameter carried out. The results were statistically analyzed using Student's test. P values < 0.05 was considered significant.

III. RESULTS AND DISCUSSION

Anemia is the most common hematological disorder. Twenty percent of all hospital admission especially female or pregnant women and elderly are due to anemia (Djulgovic, 1992). Anemia is defined as decreased in red blood cells (RBC), hemoglobin (Hb) and pack cell volume (PCV) below the normal reference range which result in decreased in oxygen-carrying capacity of the blood. (Van Hove et al., 2000). According to World Health Organization (WHO) (1959) standard anemia is diagnosed in male when Hb < 130 g/l, PCV 39%; in female when Hb < 120 g/l and PCV 36%.

In an effort to assess the acute toxicity of a substance drugs, agricultural chemicals and industrial chemicals simply means of grading acute poisoning effect. Oral administration of *Pterocarpus erinaceus* stem bark aqueous extract was found to be greater than 5000 mg/kg bw suggest to be practically non toxic. Therefore the doses used in acute toxicity are safe in the rats because there were no mortality, weakness or any visible sign of toxicity and in its traditional use for the treatment of various diseases. This work tally with research carried out by Salawu et al., (2008), they also suggest that the plant is safe for use in treatment of diseases that required long term oral administration. The serum liver enzyme markers AST and ALT were insignificantly increased (P < 0.05) when compared to normal control (table 1).

The hematological indices were carried out during the anemic study in rats due to their role in providing reliable information on hematological changes caused by toxicants. The decreased in PCV, Hb and RBC values in the group administered on phenylhydrazine (group 2) (table) agree with the earlier report by Jain (1986) that xenobiotics can cause hemolytic anemia when sulphhydryl groups of the erythrocytes membrane is oxidized which inflicts injury to the erythrocytes membranes. Oral administration of 250 and 500 mg/kg bw aqueous extract have significantly increased (P < 0.05) PCV, Hb, RBC, MCV, neutrophils and significantly decreased in WBC, Lymphocytes, platelets, MCH, MCHC. Treatment with 250 mg/kg bw was more effective than 500 mg/kg bw.

The abnormal high concentration of glucose level found in the phenylhydrazine control group may be due to the reduction in PCV, RBC, and Hb content because erythrocytes depends on glucose (glycolysis) for its energy metabolism (Stamatoyannopoulos, et al., 2000). Oral administration of aqueous extract significantly decreased (P < 0.05) serum glucose level.

In treatment with aqueous extract of *Pterocarpus erinaceus* stem bark revealed significant decreased in the activities of serum liver enzyme markers AST and ALT as compared to control (table 2). Treatment with the extract found to regulate different antioxidant and detoxifying enzymes in the rats liver challenging with phenylhydrazine as compared to control.

In conclusion, the plant is safe for used and observed significant increased in Hb, RBC and PCV suggest that the aqueous extract of *Pterocarpus erinaceus* stem bark may have properties that stimulate the production of hemoglobin in the bone marrow when orally administered and may be useful in managing anemia. These give the credence to the traditional usage of the plant as anti anemic therapy.

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TABLE 1: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON SERUM LIVER ENZYMES MARKERS

Groups	AST (UL)	ALT (UL)
Normal	7.00 ± 0.00	4.67 ± 0.67
10 mg/kg bw	7.50 ± 0.50	6.00 ± 0.00
100 mg/kg bw	8.50 ± 0.00	6.67 ± 0.67
1000 mg/kg bw	9.00 ± 0.50	8.00 ± 0.00
1600 mg/kg bw	10.00 ± 0.00	8.67 ± 0.67
2900 mg/kg bw	11.00 ± 0.50	10.67 ± 0.67
5000 mg/kg bw	14.00 ± 1.00*	12.83 ± 0.83*

Values are mean of three determinant ± SEM

AST, ALT (Aspartate aminotransferase, alanine aminotransferase)

*Significantly different as compared phenylhydrazine control group at P<0.05

TABLE 2: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON SERUM LIVER ENZYMES MARKERS ON PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

Groups	AST (UL)	ALT (UL)
Normal	8.29±0.61	5.14±0.74
Phenylhydrazine control	24.71±1.41	21.57±1.29
Anaemia + 250 mg/kg bw A.E.	13.85±0.79*	11.51±1.18*
Anaemia + 500 mg/kg bw A.E.	13.85±0.79*	11.51±1.18*

Values are mean of five determinant ± SEM

AST, ALT (Aspartate aminotransferase, alanine aminotransferase)

*Significantly different as compared phenylhydrazine control group at P<0.05

TABLE 3: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON SERUM BIOCHEMICAL INDICES ON PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

Groups	T.proteins (g/dl)	Albumin (g/dl)	T.Bilirubin (mg/dl)	D.Bilirubin (mg/dl)	Glucose (mmol/l)	Lipid Peroxidation (nmol/l)
Normal	1.88±0.06	2.73±0.26	0.44±0.03	0.34±0.08	4.55±0.37	17.88±1.25
Phenylhydrazine control	5.81±0.04	18.24±0.17	1.25±0.90	1.04±0.04	12.85±0.27	33.87±1.08
Anaemia + 250 mg/kg bw A.E.	3.80±0.27*	4.19±0.19*	0.66±0.05*	0.60±0.02*	8.68±0.85*	25.44±1.06*
Anaemia + 500 mg/kg bw A.E.	3.25±0.15*	3.91±0.18*	0.50±0.03*	0.44±0.01*	8.83±0.60*	17.23±1.83*

Values are mean of five determinant ± SEM

*Significantly different as compared phenylhydrazine control group at P<0.05

TABLE 4: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON SERUM RENAL FUNCTION INDICES ON PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

Groups	Urea (mg/dl)	Creatinine (md/dl)
Normal	48.57±4.04	5.57±0.37
Phenylhydrazine control	127.14±4.74	21.00±0.31
Anaemia + 250 mg/kg bw A.E.	80.00±4.36*	14.29±1.44*
Anaemia + 500 mg/kg bw A.E.	65.71±5.71*	7.29±0.89*

Values are mean of five determinant ± SEM

*Significantly different as compared phenylhydrazine control group at P<0.05

TABLE 4: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON HEMATOLOGICAL INDICES ON PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

Groups	PCV (%)	RBC x10 ⁶ (μ ⁻¹)	Hb (g/dl)	WBC x10 ³ (μ ⁻¹)	Neutro	Lympho	Platelets (μ ⁻¹)
Normal	46.11±0.49	5.80±0.26	12.25±0.19	9.00±0.36	40.93±0.85	59.04±0.84	846.57±0.37
PHC	39.43±0.38	4.40±0.12	11.51±0.26	16.52±0.18	15.77±1.45	84.09±1.41	474.71±20.13
A E 250 mg/kg bw	54.66±0.16*	6.17±0.08*	13.57±0.13*	8.34±0.16*	23.00±1.77*	77.00±1.77*	976.43±9.02*
A E 500 mg/kg bw	41.33±1.60	5.57±0.21*	11.44±0.31	6.33±0.14*	33.43±1.27*	66.57±1.27*	487.00±17.92

Values are mean of five determinant ± SEM

***Significantly different as compared phenylhydrazine control group at P<0.05**

TABLE 5: EFFECT OF PTEROCARPUS ERINACEUS STEM BARK AQUEOUS EXTRACT ON RED BLOOD INDICES ON PHENYLHYDRAZINE INDUCED ANEMIA IN RATS

Groups	MCV (Fl)	MCH (pg)	MCHC (g/dl)
Normal	80.46±4.25	21.36±1.10	26.57±0.37
Phenylhydrazine control	89.87±2.36	26.24±0.81	29.17±0.41
Anaemia + 250 mg/kg bw A.E.	88.69±1.36	22.03±0.44*	24.94±0.29*
Anaemia + 500 mg/kg bw A.E.	75.33±3.11*	21.01±0.96*	27.86±0.45

Values are mean of five determinant ± SEM

*Significantly different as compared phenylhydrazine control group at P<0.05

MCV, NCH, MCHC (mean cell volume, mean cell hemoglobin, mean cell hemoglobin concentration)