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Effect of *Plumbago zeylanica* Linn Root Extract on Haematological Parameters in Laboratory Animals

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ABSTRACT

Plumbago zeylanica Linn enjoys considerable folkloric used in the management of many medical conditions and literature sources contain reports of its many pharmacological effects. Despite this widespread use, its haematological effects and toxicity remain largely unknown. This study investigates the haematological effects of *Plumbago zeylanica* Linn root extract in laboratory animals. Oral acute toxicity (LD_{50}) was assessed by Lorke's method and haematological effects by the 14 day repeated oral dose procedure. Sixteen albino Wistar rats divided into four groups were used for the haematological investigations. Group one was the control and received distilled water only by the oral route. Groups two to four received 500, 1000 and 1500 mg/kg body weight respectively of the root extract daily. After 14 days animals were sacrificed and blood collected for haematogical investigations. We found oral acute toxicity of the root extract to be more than 5000 mg/kg body weight. There were no statistically significant differences in haematological parameters - red blood cells, haemoglobin, total white blood cell counts and differentials and; haematological indices (parked cell volume, mean corpuscular volume, mean corpuscular haemoglobin and mean corpuscular haemoglobin concentration) – in treated animals relative to the control group at the $P < 0.05$ level. The root extract of *Plumbago zeylanica* Linn displays no haematological toxicity as it showed no effect (adverse or otherwise) on haematological parameters in laboratory animals. This justifies the extensive folkloric use to which the plant has been deployed.

Keywords: Haematological indices; medicinal herb; *Plumbago zeylanica*; repeated dose oral toxicity

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INTRODUCTION

The plant kingdom is the main source of or provides inspiration for the synthesis of many orthodox medicines and interest in the scientific scrutiny of herbal drugs continues to grow. This mounting interest is premised on many factors amongst which is the perceived relative safety and affordability of plant based drugs[1, 2]. This is in contrast to orthodox medicines which may be prohibitive in cost, largely unavailable in resource constrained countries or their use may be associated with considerable inconvenience and time consuming professional care[1]. This leads a large and increasing number of patients to use medicinal herbs or consult with medical personnel regarding their use. As a result of this scenario, the World Health Organization estimates that close to 80% of populations in developing countries especially in Africa use herbal remedies[3]. *Plumbago zeylanica* Linn is one medicinal plant that enjoys such considerable usage. It is an evergreen shrub belonging to the Plumbaginaceae family, can reach a height of two to three meters and has wide distribution in the tropical and subtropical areas of the world[4]. It is known as Leadworth or Ceylon Leadworth in English. The Yorubas of south western Nigeria call it *inabiri*[5]. The plant has been referred to as nature's gift to man[6] because of its widespread use. Almost all parts of it - leaves, flowers, stem and most especially the roots - have been found to be of medicinal importance. Literature sources credit the plant with many folkoric uses such as in the treatment of dyspepsia, piles, diarrhoea, skin diseases and rheumatism[7]. Scientific scrutiny has revealed that the roots have antibacterial, antifungal and abortifacient properties[8], analgesic and anti inflammatory[9] as well as antispasmodial activity[10]. In addition the plant has been shown to possess anti-cancer, hepatoprotective, anti-diabetic, anti-fertility, and immunosuppressive properties[4] and also anti-ulcer effects[11]. Recent reviews[6, 12] give an account of so many other pharmacological properties that can be attributed to the drug. Despite this extensive use and its many pharmacological activities, very little is known about its toxicological profile on various organs and systems in the body and in particular on the haematological system. Our objective is to assess the haematological effect of *Plumbago zeylanica* Linn in laboratory animals and provide information about its haematological toxicity in light of the widespread use of the plant.

MATERIALS AND METHOD

Collection, preparation and extraction of plant material

Fresh *Plumbago zeylanica* Linn roots were collected from gardens within the grounds of Jos University Teaching Hospital in Jos Plateau State, North central Nigeria and authenticated at the Federal college of Forestry, Jos then dried under shade. Voucher specimens were kept at the

college herbarium (Number FHJ 153) and at the Department of Pharmacognosy, University of Jos (Number PCG/HSP/12PO2). Extraneous materials were removed from the roots which were then reduced to a coarse powder with the aid of a mortar and pestle followed by grinding with the aid of an electric blending machine (National®, Model MX391N, Matsushita Electric Co. Japan). The coarse powder was then subjected to cold maceration by soaking in 70% ethanol and allowed to stand for 24 hours followed by shaking for three hours on a mechanical shaker, then filtered through Whatman Number 1 filter paper in several parts of rinsing. The solvent was recovered under reduced pressure in a rotary vacuum evaporator and the extract was concentrated to dryness by placing in a water bath maintained at 70 °C. A reddish brown coloured solid of yield 15% was obtained. This was stored in an airtight container and kept in a refrigerator until needed for use.

Phytochemical analysis and Thin layer chromatographic (TLC) profiling

Phytochemical screening was carried out according to standard methods described in literature sources[13, 14, 15]. Capillary tubes were used to apply the reconstituted extract on silica gel pre-coated TLC plates and developed in a TLC chamber with the following solvent systems: chloroform: water (8:2) and chloroform. The developed TLC plates were air dried then visualized under day light and ultra violet light (UV) at a wavelength of 254 nm. The plates were then sprayed with different spraying reagents (iodine vapour and 10% sulphuric acid) for the development of colour in separated bands. Movement of the various spots from the point of application on the TLC plates was expressed as the retention factor ($R_{f)}$. The R_f values were calculated for the different spots using the following formula (Sharma and Paliwal 2013):

R_f value = Distance travelled by the solute/Distance travelled by the solvent front of the TLC plate

Laboratory animals

Twenty eight albino Wistar rats of either sex weighing between 150-200 gm obtained from the National Veterinary Research Institute, Vom, Plateau State, Nigeria were used for the study. The animals were allowed to acclimatize for two weeks at the animal house unit of the Faculty of Pharmaceutical Sciences before being used. They were kept under controlled temperature conditions (25 ± 2°C), in a 12 hour dark/light cycle with free access to food and water.

Acute toxicity study

Oral acute toxicity was determined according to Lorke's method[16]. Twelve animals were used for the investigation which was carried out in two phases. In the first phase, there were three groups of three animals each. The groups received 10, 100 and 1000 mg/kg body weight

respectively of extract freshly reconstituted in distilled water by the oral route with the aid of an oral cannula. In the second phase, there were three groups with one animal per group. The groups received 1500, 2900 and 5000 mg/kg body weight of freshly reconstituted extract respectively. In both phases the animals were closely observed for any signs of toxicity such as rubbing of nose on the floor or walls of the cage, food refusal, lachrimation, salivation, micturation, defecation, aggressiveness or weakness, sleepiness, laboured breathing, coma and death. The numbers of any animals that died in each phase within 24 hours were noted and recorded. The acute toxic effects of the extract were assessed on the basis of mortality, which was expressed as LD_{50}. Lethal dose (LD_{50}) was calculated as the geometric mean of the highest dose that did not cause any deaths and the lowest dose where deaths occurred.

Haematological investigations

Sixteen animals (four groups of four animals each) were used for this study. Animals in group one served as the control group and received 2ml of distilled water. Animals in groups two, three and four received 500, 1000 and 1500 mg/kg body weight of freshly prepared extract respectively by the oral route everyday for 14 days. All animals were sacrificed on day 15 with the aid of chloroform anaesthesia. Blood was collected through cardiac puncture into ethylenediaminetetraacetic acid (EDTA) specimen bottles for the analysis of haematological parameters on Sysmex KX – 21 haematology autoanalyzer (Sysmex Corporation, Japan).

Data analysis

Data was analyzed using One way analysis of variance (ANOVA) and student *t* test followed by Newman-keuls Post Hoc Multiple comparison test on GraphPad Prism Version 5.01 (GraphPad Software Inc, California, USA). Figures for haematological parameters were expressed as mean ± standard error of mean (SEM). Differences of the means between groups were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

Oral LD_{50} was found to be more than 5000 mg/kg body weight and results of phytochemical screening show that flavonoids and carbohydrates were present in substantial amounts; alkaloids, tannins and steroids in moderate amounts while anthraquinones were present in trace amounts. Thin layer chromatographic profiling (table 1) shows the presence of peaks with distinct R_f values and colours especially with the chloroform:methanol:water solvent system. This gives an indication of the dominant phytochemical constituents identified by screening tests and mirrors other investigations showing similar results[8]. No mortality was observed in all dose ranges up to

the dose of 5000 mg/kg body weight indicating that *Plumbago zeylanica* root extract can be considered to be relatively safe. According to the Hodge and Sterner scale[17], a test substance/drug/chemical can be said to be extremely toxic at doses below 1 mg/kg, highly toxic at doses between 1 and 50 mg/kg, moderately toxic at doses between 50 – 500 mg/kg, slightly toxic at 500 – 5000 mg/kg and practically non toxic at doses between 5000 and 15000 mg/kg. Thus the observed reduction in water and food intake; and movement activities (table 2) at doses between 1500 and 5000 mg/kg body weight could be a pointer to slight toxicity, similar to other reported findings[12]. This observation may need to be carefully noted and considered in light of the extensive use of this plant and may call for more extensive investigations of the effects on organ systems in either a sub acute or chronic fashion. Our study represents a sub acute investigation of the effects of the root extract on the haematological system. This is important because assessment of haematological parameters can be used to determine the toxic effects of xenobiotics including plant extracts on the blood constituents of an animal. Such analysis is relevant to risk evaluation because changes in the haematological system are highly predictive for human toxicity, when data are translated from animal studies[14].

Table 1: R_f values of peaks from *Plumbago Zeylanica* Linn root extract obtained with different solvent and visualization systems

Solvent System	Visualization System, R_f value and colours of peaks					
Chloroform: Methanol (8:2)	UV (254 nm)		Iodine Vapour		10% Sulphuric acid	
	R_f values	Colour	R_f values	Colour	R_f values	Colour
	0.97	Yellow	0.93	Yellow	0.5	Yellowish ash
					0.97	Violet blue
Chloroform: Methanol: Water (7:3:1)	0.29	Brown	0.29	Yellowish brown	0.29	Yellowish ash
	0.79	Pink	0.68	Light Yellow	0.79	Purple
	0.89	Pinkish	0.89	Deep yellow	0.89	Dark ash
	0.97	Yellow			0.97	Purple blue

Table 2: Oral LD_{50} of ethanolic root extract of *Plumbago zeylanica* Linn.

Phase/ Groups	N	Dose (mg/Kg body weight)	Mortality	Percentage Mortality	Other Symptoms
Phase I					
1	3	10	0	0 %	-
2	3	100	0	0 %	-
3	3	1000	0	0 %	-
Phase II					
1	1	1500	0	0 %	Reduced food intake, reduced movement.
2	1	3000	0	0 %	Reduced food intake, reduced movement.
3	1	5000	0	0 %	Reduced food and water intake,

reduced movement

N = number of animals per group

Table 3: Effect of ethanolic root extract of *Plumbago zeylanica* Linn on WBC, platelets and differentials.

	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
WBC (10^{12}/L)	9.35 ± 6.14	7.20 ± 2.73	10.43 ± 7.23	9.30 ± 5.10
Monocyte (%)	3.90 ± 0.77	3.34 ± 0.25	3.70 ± 0.22	3.20 ± 0.72
Lymphocyte (%)	68.05 ± 1.39	71.15 ± 3.26	67.75 ± 3.25	77.50 ± 4.76
Granulocyte (%)	28.10 ±4.14	25.48 ± 4.61	28.5 ±1.5	19.38 ± 3.20
Platelet(10^5/L)	444.80 ± 64.12	414 ± 87.80	417 ± 49	301.50 ± 66.58

WBC = White blood cells, Number of animals per group = 4, Values are mean ± SEM

Table 4: Effect of ethanolic root extract of *Plumbago zeylanica* Linn on red blood cells and hematological indices.

	Control	500 mg/kg	1000 mg/kg	1500 mg/kg
PCV (%)	39 ± 2.0	39 ± 1.51	38.60 ± 2.86	37.03 ± 2.04
RBC(10^{12}/L)	7.24 ± 0.46	7.60 ± 0.89	7.0 ± 0.23	7.0 ± 0.46
Haemoglobin(g/L)	116.80 ± 4.46	116.50 ± 7.09	112 ± 6.07	111.25 ± 6.25
MCHC (g/dL)	300.25 ± 11.25	294 ± 4.72	296.50 ± 9.98	301.50 ± 2.24
MCH (pg)	16.15 ± 0.64	15.58 ± 0.35	16.40 ± 1.02	15.33 ± 0.64
MCV (fL)	53.90 ± 0.80	53.98 ± 2.03	55.40 ± 2.40	52.68 ± 2.04
PDW (%)	15.10 ± 0.20	15.10 ± 0.14	15.30 ± 0.10	15.10 ± 0.14
MPV(fL)	6.10 ± 0.14	6.20 ± 0.05	6.0 ± 0.18	6.0 ± 0.14
PCT	0.27 ± 0.04	0.25 ± 0.06	0.25 ± 0.01	0.17 ± 0.04
RDW (%)	16.30 ± 0.27	11.60 ± 2.47	15.0 ± 0.65	14.50 ± 0.48

RBC= red blood cells, PCT = Plateletcrit, MCHC = Mean Corpuscular Hemoglobin concentration, MCH = Mean Corpuscular Hemoglobin, PDW = Platelet distribution width, MCV = Mean corpuscular volume, RDW = Red blood cell distribution width, MPV = Mean Platelet volume, Number of animals per group = 4, Values are mean ± SEM

These effects may range from decreased red blood cells, packed cell volume and heamoglobin leading to anemia, decrease in white blood cells and white blood cell differentials with potential effects on the immune system. There may also be effects on the platelets potentially affecting the blood coagulation and clotting system. There were no statistically significant differences in the platelet parameters - total platelet count, plateletcrit, platelet distribution width and mean platelet volume – between the control and treatment groups as shown in tables 3 and 4. The lack of differences in platelet count between treated animals and controls has also been noted in earlier reports[18, 19]. But these reports also point to a significant altering (reduction) of platelet adhesiveness leading to prolongation of the bleeding time and delayed coagulation. These anti-platelet effects have been attributed to napthoquinone derivatives, particularly plumbagin and

coumarins which are reportedly present in the plant[6, 12, 20, 21]. Therefore even as there may not be any statistically significant changes in these haematological parameters, great caution needs to be exercised especially for patients on concomitant therapy with anticoagulants, hypocholesterolemic or other cardiovascular drugs. Other haematological effects may occur through alteration of indices such as mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, mean corpuscular volume and red blood cell distribution width, all with potentially deletrious consequencies. However our results show that there were no statistically significant differences (at $P < 0.05$) between the treatment and control groups in the various haematological parameters investigated. Literature sources indicate that *Plumbago zeylanica* Linn possess significant antimicrobial, antiviral and antifungal activities. [6, 10, 12] These are effects which may partly be expressed through boosting of the immune system especially cellular immunity represented by the white blood cells and differentials.

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Patient Consent

Not applicable.

Ethical Approval

We declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985)[22] were followed. All experiments and procedures have been examined and approved by the Animal Care and Use Committee, Faculty of Pharmaceutical Sciences, University of Jos.

Competing Interests

We declare that no conflicts of competing interests exist.

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