

Effects of Pausinystalia Yohimbe Methanol Root Extract on Some Cardiovascular and Haematological Variables in Wistar Rats

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Abstract - Plants have been a major source of medicine and have been largely considered to alter the range of blood pressure and parameters. The present study was undertaken to investigate the effect of *Pausinystalia yohimbe* on blood pressure and haematological factors in normotensive male wistar rats. The rats were randomly divided into four groups of nine rats each. The control group received 1 mL/kg distilled water while the three treatment groups received 25 mg/kg, 50 mg/kg and 100 mg/kg of the extract respectively, and the blood pressure was measured three hours thereafter. After the study period, blood samples were taken from experimental rats, and haematological factors were determined using digital cell counter. Administration of the extract, caused a dose-dependent fall in blood pressure and heart rate, and appeared to increase leucocyte (WBC and lymphocytes) and erythrocyte parameters (RBC, HCT and Hgb) when compared with the control group. While thrombocyte parameters (PLT and Pct) were decreased. The findings demonstrated that acute hypotensive and antitachycardiac effect of the extract may be due to direct effect of the metabolites on cardiac-vascular smooth muscle, and that *Pausinystalia yohimbe* does not cause any significant undesirable alterations in haematological factors in wistar rats. It also enhances white blood cell concentration and lymphocytes which probably stimulate the immune defence mechanism.

Keywords - Medicinal Plants, Hypertension, Immune System, *Pausinystalia yohimbe*, Wistar Rats.

I. INTRODUCTION

Plants from time immemorial have been resourceful to mankind in every aspect of life, medicine in particular, and exhibit a vital role in the sustainability of health globally [1]. Medicinal plants are considered to be the backbone of traditional medicine and are widely used in the treatment of various diseases, acute or chronic, before and after the introduction of the orthodox medicine. Traditional medicine is still being practiced despite advances in modern medicine because the source of the plant is cheap and local [2]; and have always played a prominent role as important alternatives to conventional medicines in developing countries. However, the rationale for the utilization of medicinal plants has rested largely on long-term clinical

experience with little or no scientific data on their efficacy and safety. Medicinal herbs have their use as medicament based simply on a traditional folk use that has been perpetuated along several generations; and a great number of them have been used throughout the world from time immemorial for the prevention and cure of sickness [3]. Therefore, research on medicinal plants always remained a potential area of investigation that necessitate continuous search for novel agents which are more effective, cheaper, come with less side-effects, and preferably used alone.

High blood pressure also called hypertension is a condition that afflicts almost one billion people world-wide, and is a leading cause of morbidity and mortality [4]. It also represents a major risk factor for developing other diseases

such as endothelial dysfunction, metabolic syndrome, diabetes, stroke and renal dysfunction [5]. More than both 50% of men and 60% of women over 60 years of age have hypertension and epidemiological studies have demonstrated that cardiovascular diseases are the principal cause of death in human society. Modern hypertension treatment is costly for many people in developing countries and such medical expenses represent an additional burden [6]. Meanwhile, the desire of everyone is to live as many years as possible; and this can be achieved through maximum dependency on natural product e.g. herbs, which have been reported to have contributed to revolutionary breakthrough in the management of diseases. Hitherto, this population often relies on the alternative therapy of medicinal plants for the treatment of various disorders.

Hypertension treatment has been shown to prevent cardiovascular diseases, extending and enhancing life, but its inadequate management is wide-spread. The World Health Organization estimates that more than 80% of people use traditional medicine, mainly plants to treat the primary diseases, and about 85% of traditional medicine involves the use of plants extracts [7]. Thus, it is often said that every plant is a potential medicine for one disease or the other [8]. Traditional healers have put forward many claims about the healing power of the plant world. For example, along the edge of a garden, field or residential building in Nigeria, and beyond, hedges of the bitter leaf shrub (*Vernonia amygdalina*) are closely grown because this plant is highly esteemed as antioxidant that helps in the functioning of the heart, reduces the viscosity of the blood making it flow freely. Also, it lowers blood sugar levels and the LDL cholesterol levels in the blood.

A large number of reports have highlighted that consumption of medicines or herbal drugs can alter the normal range of haematological parameters. These changes could be either positive or negative [9]. In recent years, most automated blood cell counters determine the number of white blood cell, red blood cell, platelet etc. These haematology analyzers are a boon in the improvement of cellular haematology, as the results obtained are quicker and accurate. Also, since the assessment of haematological parameters can be used to determine the extent of deleterious effect of foreign compound including plant extract on the blood, it can therefore be used to explain blood relating functions of chemical compound/plant extract.

Pausinystalia yohimbe (K.Schum.) Pierre ex Beille is a medicinal plant that belongs to the Rubiaceae family and is

used in Nigerian traditional medicine for the treatment of various disorder, including hypertension. This family comprises *Pausinystalia*, an important genus in medicine that has species with significant biological activities such as *Pausinystalia yohimbe* K.Schum, *Pausinystalia zenkeri*, W. Brandt, and *Pausinystalia trillesii* Beille. All of these species synthesize types of bioactive principles, yohimbine and have aphrodisiac and xerostomia activity [10]. Yohimbe is a native of South-Western Nigeria, Gabon and Zaire. It is an evergreen tree which grows to a height of 30-35m tall and 1.2m in girth with a spread of 8m, and 30-60 cm wide with alternate vegetative and generative ramification. It is abundant in the forest. It exhibits tap root system, and the root can be erect, bend, folded and branching. The bark is grey, smooth; slash creamy-white. It has fibrous leaves that are 7-20cm long by 3.5-7.5 cm broad, the shape of the leaves varies from ellipsoid to oblong, sharply acuminate, narrowly cuneate, and rather thin; with 5-10 pairs of lateral veins. Its flowers (May to September) are white, scented with axillaries and terminal panicles. The fruit (January to March) is spindle-shaped, measuring up to 2 cm long with narrowly elongated winged seeds. Yoruba call it "Idagbon", Ibo call it "Likiba" while Hausa call it "Burantashi" [10].

An extract of the stem bark have also been reported to have a broad range of molecules that modify cell functions, and some have been reported on its hypotensive and aphrodisiac activities [11]. The cardiovascular effect of extract of the plant's roots have however not yet been reported. But traditionally, many herbal doctors and local healers claim, some plants, *Pausinystalia yohimbe* root inclusive are known for their anti-hypertensive effects. The high patronage of sellers of such herbs may be an indication of the plants efficacy. However, the metabolites that could be responsible of antihypertensive effects as well as their mechanisms of actions may not have been documented. Meanwhile, Akinniyet *al.* [2] has reported the presence of yohimbine and some other alkaloids in the stem bark of the plant. Its popular use by the local people of Ilaje, Ondo State and some migrant workers in the hinterland as aphrodisiac motivated further investigation conduct on the plant's metabolites with particular interest in its effect on blood pressure and parameters to bring into limelight the better understanding of cellular implications in rats.

In this research, I studied to evaluate the claimed hypotensive effect of the methanol extract of *Pausinystalia yohimbe* root in normotensive rats; and as well, address whether or not the extract had pronounced effect of alteration on blood parameters.

II. MATERIALS AND METHODS

A. Plant Material

The roots of *Pausinystalia yohimbe* (K. Schum.) Pierre ex Beille, were collected from village area of Okomu National park, Udo, Edo State, Nigeria and identified by Dr. Odaro Timothy and authenticated by Prof. MacDonald Idu, both of Phytomedicine unit of the Department of Plant Biology and Biotechnology, University of Benin, Benin City, Nigeria. A herbarium specimen (UPBHx1066) has been deposited at the herbarium of Plant Biology and Biotechnology Department, University of Benin, Benin City, Nigeria.

B. Preparation of Plant Extract

The fresh roots were washed free of debris and air dried under shade for fourteen days, kept in the oven at 40°C for five days and later pulverized to powder using mechanical grinder. One thousand two hundred grams of the powdered roots was subjected to methanol extraction using Soxhlet apparatus. The extract was concentrated to dryness and it gave a yield of 50%. The yield was stored in a refrigerator (4°C) till when needed.

C. Chemicals and Reagents

All chemicals were of an analytical grade and were supplied from Sigma chemical co. USA. Distilled water was used in haematological assays

D. Experimental Animals

Adult male albino rats (Wistar strain) weighing between 200-250g, purchased from the central animal holding unit of the Department of Anatomy, University of Benin, Benin City, Nigeria were used for the study.

D. 1 Acclimatization: 15 days prior to dosing.

D.2 Identification of Animals: By cage number.

D.3 Diet: Pelleted feed

D.4 Water: Portable drinking water

D.5 Housing and Environment: 9 animals each in a group

D.6 Animal Ethics: All of the animals received humane care according to the criteria outline in the Guide for the Care and the Use of Laboratory Animals prepared by the National Academy Science and published by the National Institute of Health (USA). The ethic regulation have been followed in accordance with national and institutional

guidelines for the protection of animals' welfare during experiments.

E. Experimental Design

Group A: Normal control (1 mL/kg distilled water)

Group B: 25 mg/kg *Pausinystalia yohimbe*

Group C: 50 mg/kg *Pausinystalia yohimbe*

Group D: 100 mg/kg *Pausinystalia yohimbe*

E.1 Method of Administration

Oral administration of the extract through the use of oral gavage.

E.2 Duration of Treatment: 21 days

F. Chemical and Reagents Preparation

All chemicals were of an analytical grade and prepared according to standard analytical procedures/methods. Distilled water was used in all haematological assays.

G. Cardiovascular Evaluation

The blood pressure was measured three hours after extract administration. The experimental rats were introduced into rat restrainer (5 minutes each) for acclimatization. Thereafter, rat's tails were connected, and the carotid artery was cannulated and connected via a Bentley pressure transducer to a twin-channel UgoBasile recorder (Gemini 7070) for recording blood pressure and heart rate.

H. Collection of Blood

H.1 Haematological Parameters: Blood samples were collected from the abdominal aorta of experimental rats into heparinized sample bottles containing EDTA as anticoagulant, using sterile surgical blade. Blood so collected was used for the various estimations.

H.2 Blood Analysis: The haematological examinations performed were according to standard methods. The haematological components including white blood cell (WBC), lymphocyte (LYM), red blood cell (RBC), haematocrit (HCT), haemoglobin (Hb), platelet (PLT) and plateletcrit (PCT) were determined by injecting 50 ml of blood samples into an automated blood analyzer SYSMEX KX21, a product of SYSMEX Corporation Japan, employing the method described by Dacie and Lewis [12]. Also, the principle of an automated multi-parameter blood cell counter machine involves the employment of the

differences in characteristics possessed by each of the blood components to distinguish them and estimate their numbers.

I. Statistical Analysis

The experimental results/data were analyzed using statistical package for social science (SPSS) version 20 and presented as mean $\pm$ SEM. Comparison between different groups was done using Analysis of Variance (ANOVA) and Duncan's Multiple Range Test (DMRT). Values of $p < 0.05$ were considered as statistically significant difference.

III. RESULTS AND DISCUSSIONS

A. Effect of *Pausinystalia yohimbe* on the Blood Pressure (BP) and Heart Rate (HR)

The results obtained in this study demonstrate that the methanol extract of *Pausinystalia yohimbe* decreases the

blood pressure and heart rate in a dose dependent manner in extract treated wistar rat when compared with the control. The Mean Arterial Pressure (MAP) of the normotensive control rats was 86.43 ± 2.89 mmHg. This was progressively reduced by the extract to 80.2 ± 1.33 mmHg by 25 mg/kg and to 70 ± 3.95 mmHg by 50 mg/kg, while the maximum dose used, 100 mg/kg, reduced the MAP to 68.86 ± 5.66 mmHg. The heart rate was also correspondingly reduced from the normotensive rate of 400 ± 11.29 beats/min. 25 mg/kg of the extract reduced the heart rate to 387.17 ± 3.23 beats/min and the 50 mg/kg lowered it to 386.44 ± 10.84 beats/min, while the maximum dose of 100 mg/kg lowered it to 379.56 ± 6.16 beats/min. (Table 1).

Table 1: The effect of the methanol extract of the root of *Pausinystalia yohimbe* on rat blood pressure and heart rate

Dose of extract (mg/kg)	Mean Arterial Pressure (MAP) (mmHg)	Heart rate (beats/min)
Control (1 mL/kg)	$86.43^a \pm 2.89$	400 ± 11.29
25	$80.2^a \pm 1.33$	387.17 ± 3.23
50	$70^b \pm 3.95$	386.44 ± 10.84
100	$68.86^c \pm 5.66$	379.56 ± 6.16

abc Means in the same column with different superscripts differ significantly ($p < 0.05$).

The results show that the methanol extract has a significant dose- dependent hypotensive effect. The healing properties of plants are known to be associated with the production of secondary metabolites, highly active compounds that give them the ability to interact with their environment. Different mechanisms regulate blood pressure and the secondary metabolites from plants have the potential to modify cell biology [13]; for example plants components like alkaloids, flavonoids etc. are capable of producing hypotension in whole animals by releasing histamine from most cells, and may stimulate muscarinic receptors causing endothelium-derived relaxing factor-mediated

vasodilatation, and hence hypotension. Equally importantly, some components may lower blood pressure by acting directly on vascular smooth muscle while some others have myocardial effects [14].

The observations of a hypotensive effect of *Pausinystalia yohimbe* root provide evidences for potential therapeutic application of this plants extract, and that the antihypertensive effect could be due to its secondary metabolites.

B. Effect of *Pausinystalia yohimbe* on the Haematological Parameters

Equally importantly, the effect of various doses of methanol extract of *Pausinystalia yohimbe* on mean haematological parameters when compared with the control are shown in Table 2. There was progressive increase in the leukocyte parameters of the rats in all dose levels of the extract used when compared with the control. Rats that received 25 mg/kg body weight of the extract had elevated white blood cell (WBC) count values of 14.73 ± 1.18 than the control (9.37 ± 2.27). At a dose of 50 mg/kg and 100 mg/kg body weight of the extract, WBC count values were 11.93 ± 2.38 and 10.47 ± 1.68 . Extract treated rats also showed increase in lymphocytes count at all dose levels of the extract used compared with the control (Table 2).

The erythrocyte parameters namely, red blood cell, haematocrit and haemoglobin of the extract treated rats increased at all dose levels ($p > 0.05$). At the dose of 25 mg/kg, 50 mg/kg and 100 mg/kg body weight of the extract, red blood cell count values were 8.9 ± 0.04 , 8.8 ± 0.44 and 8.5 ± 0.38 compared with the control (5.9 ± 0.82). Also, there is increase in haematocrit and haemoglobin concentrations in treated group of rats when compared to control group of rats at all dose levels of the extract used.

However, the results of the thrombocytes indices (platelets and plateletcrit) as shown in Table 2 were found to

markedly decrease in extract treated group of rats when compared to control. The above findings report that extract treated rats has elevated leukocyte counts, and are in accordance with the results observed by Kashinah [15] and Abdulrahman *et al.* [9] who observed increased WBC production in hyperlipidemic rats which may lead to possible stimulation of the immune defense system.

It was also observed that the methanol extract used in this study could stimulate and thus increase the level of erythrocyte counts, and this agrees with the results observed by Effraim *et al.* [16] Who stated that the aqueous extract of *Corynantheyohimbe* significantly increased the Hb, erythrocyte count and packed cell volume, with the increasing doses of the extract. And that improvement in these parameters is likely to improve upon the well-being of the patients thereby justify its empirical use in conditions like loss of libido.

The present study described that there is a decrease in thrombocytes indices in extract treated group. The primary physiological function of platelets is to mediate the haemostatic response. An elevation in platelet count is considered as an indicator of vascular disease like microangiopathy and macroangiopathy [17]. It is therefore possible that administration of *Pausinystalia yohimbe* root extract may not contribute to the risk for cardiovascular disease.

Table 2: Effect of various doses of *Pausinystalia yohimbe* methanol root extract on some blood parameters in wistar rats

Methanol extract dose in mg/kg body weight

Parameters	control	25	50	100
White blood cell (m mm ⁻³)	9.37±2.27	14.73±1.18	11.93±2.38	10.47±1.68
Lymphocyte (m mm ⁻³)	3.16±1.17	5.9±0.1	5.5±1.47	5.8±1.06
Red blood cell (m mm ⁻³)	5.9 ^b ±0.82	8.9 ^a ±0.04	8.8 ^a ±0.44	8.5 ^a ±0.38
Haematocrit (%)	33.50±4.21	41.90±1.70	39.90±2.01	37.80±1.51
Haemoglobin (g dL ⁻¹)	13.0±1.58	15.3±0.6	14.4±0.87	13.7±0.82
Platelet (m mm ⁻³)	892.10±193.71	767.0±121.23	635.0±75.55	595.70±24.06
Plateletcrit (%)	0.6±0.12	0.5±0.09	0.4±0.05	0.40±0.2

ab Means in the same row with different superscripts differ significantly ($p < 0.05$).

IV. CONCLUSIONS

This is the first time the effect of *Pausinystalia yohimbe* root on blood pressure, a characteristic of hypertension, has been demonstrated. The methanol extract had a potential antihypertensive effect due to the presence of a yet to be identified bioactive principles, metabolites with antihypertensive properties, justifying the traditional use of this plant in hypertension treatment. From the foregoing, *yohimbe* have no adverse effects on haematological parameters. Furthermore, it improved the levels of lymphocytes and white blood cell count, indicating the stimulation of immune defense mechanism. *P. yohimbe* treated groups also exhibited low count of blood platelets which improves blood haemostasis by alleviating thrombosis-inducing platelets.

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ETHICAL APPROVAL

This study was approved by the University of Benin Research Ethics and Intellectual Development Committee with reference number (UNIBEN/REC./PHYMED./017)

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