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Phytochemical, aphrodisiac and anti-hypertensive properties of *Pausinystalia yohimbe*

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ABSTRACT

In order to investigate the folkloric and traditional use of *Pausinystalia yohimbe* (PY) *in vivo* experiments were conducted. Male rats weighing between 170 and 260gm were randomly chosen for the experiment for the *in vivo* study. Rats were lightly anesthetized with 20mg/kg im of ketamine and 30mg/kg of phenobarbitone intra peritonially. The anaesthesia was maintained with ketamine intravenously. The rats breathed spontaneously. The left femoral artery was catheterized using a PP50 polyethylene catheter and connected to a strain-gauge blood pressure transducer (connected to a Grass polygraph, model 7D) for BP measurement. A second catheter in the right femoral vein (PP50) was used for the injection of drugs or extract. A 24G needle placed in the corpus carvanosum tissue and connected to a strain-gauge pressure transducer was used to measure the intra-cavernous pressure. Normal saline was slowly injected through the femoral vein and recordings made for a period of 30 to 45 mins to serve as control. Eight rats were used for each set or group. The result from the *in vivo* study was a significant increase intra-cavernous pressure (ICP) indicating erectile potential of the PY crude extract. The mean arterial blood pressure was lowered significantly to below 80mmHg which latter rouse to 90mmHg for the PY treated group while that of the control remained above 110 mmHg throughout the experiment. Since PY extract could lower blood pressure and also enhance erectile functions it is likely to become novel drug in management of hypertensive patients with erectile dysfunction.

Keywords: *Pausinystalia yohimbe, rats, Aphrodisiac, Erectile Dysfunction, Extract, and in vitro*

INTRODUCTION

In the typical traditional African setting, satisfactory sexual performance is most often equated to masculine prowess. A man who fails to have a satisfactory sexual performance is said to have lost his manhood. The cultural secrecy attached to sexual problems, the desire to restore lost manhood, and the high cost of modern drugs which enhances sexual performance, drive sufferers to resort to the cheap local herbs doled out in the secrecy of herbal homes of traditional healers or hawked at motor parks or luxurious buses.

It suffices to note that sexual dysfunction is currently considered to be of vascular origin in the vast majority of the patients, due to atherosclerotic lesions of the penile arteries [1]. The evidence that increased blood pressure is responsible for stenotic lesions secondary to atherosclerosis, smooth muscle hypertrophy of the cavernous arteries, and implicit blood flow impairment in the penile vasculature, provides a link between hypertension

and erectile dysfunction [2]. The expected number of hypertensive adults will reach 60% by 2025, amounting to1.5 billion patients worldwide [3].

One of the common locally used potent herbal aphrodisiacs in Sokoto metropolis is *Pausinystalia yohimbe (Dan Kamaru).* Pausinystalia yohimbe (PY), (Syn: *Corynanthe yohimbe*), family *Rubiaceae*, an evergreen rapidly growing tree, native to the tropical rain forest of West and Central Africa, where it is used as medicinal plant [4]. Its significance as a medicinal plant lies in its use as an aphrodisiac.

Phytochemistry has developed in recent years as a distinct discipline, somewhere in between natural products, organic chemistry and plant biochemistry and is closely related to all. It is concerned with enormous variety of organic substances that are elaborated and accumulated by plant and deals with the chemical structures of these substances, their biosynthesis, metabolism and distribution in plants [5]. Many of the plants used in herbal medicine contain ingredients whose effects are pharmacological and the action can usually be related to that of the isolated constituents [6]. The aphrodisiac property of *Pausinystalia yohimbe* has been attributed to yohimbine, an alkaloid believed to be contained in the plant. Yohimbine is an alpha 2 adrenergic receptor blocker [7] [8] and has had a long use as an aphrodisiac [9] [10] [11]. Various scholars have documented physiological and pharmacological effects of yohimbine [12] [13] [14] [15]. Yohimbine has been used in the treatment of erectile dysfunction (ED) [8] [14] [16], although with conflicting results [17]. Research has concentrated more on the effects of the alkaloid, yohimbine with the result that studies on the administration of stem bark of *Pausinystalia yohimbe* are few and doubtful[18].

MATERIALS AND METHODS

Collection and processing of plant materials

About 5kg of the stem bark of *Pausinystalia yohimbe* (*PY*) (*Dan Kamaru*), consisting of all tissue outside the woody part of the stem were purchased identified by the Taxonomist(Dr. Mohammed Abubakar) in the Department of Botany Usmanu Danfodiyo University, Sokoto with a voucher specimen deposited in the herbarium with the specimen number UDUH/ANS/0008. The stem bark was cleaned, air dried and grounded into powder and stored in air tight water proof containers until required for extraction.

Preparation of aqueous crude extract

Extraction was done on 20gm of the powdered stem bark with 200cm^{3}distilled water in a soxhlet extraction assembly for six hours. The extract obtained was filtered with whatman filter paper no 1 and concentrated to dryness using digital aeration oven set at 50 degrees centigrade. The extract was stored in tightly covered specimen bottles, in a refrigerator at 4 degrees Celsius until use.

Phytochemical Analysis

The presence of saponnins, tannins, anthraquinones, alkaloids, flavenoids, glycosides, reducing sugars and phlobatannins were determined by qualitative and quantitative methods of Trease and Evans [5].

Preparation of Animals

The experiment was conducted in accordance with the Guide for the care and use of laboratory animals, [19]. Male albino rats were obtained from the laboratory animal house of the department of biological Sciences Usmanu Danfodiyo University, Sokoto, for the entire study. Male rats weighing between 170 and 260 gm were randomly chosen for the experiment. The rats were kept in metal cages in the metabolic laboratory with uniform temperature of 22-25 degrees centigrade, 12 hours light and 12 hours dark periodicity. The rats were fed standard rat chow (Vital Feeds, Nig. Ltd) and water ad libitum and allowed to acclimatize for 14 days before the procedures.

Measurement of Blood Pressure (Bp) and Intra Cavernous Pressure (ICP)

The method used for this experiment followed that described by Hiroya *et al* [20] and as modified by Chen *et al*, [21]. On the day of the experiment, the rats were lightly anesthetized with 20mg/kg im of ketamine and 30mg/kg intraperitonially, of phenobarbitone. The anaesthesia was maintained with ketamine intravenously. The rats breathed spontaneously. The left femoral artery was catheterized using a PP50 polyethylene catheter and connected to a strain-gauge blood pressure transducer (connected to a Grass polygraph, model 7D) for BP measurement. A second catheter in the

right femoral vein (PP50) was used for the injection of drugs or extract. The urinary bladder was catheterized using a self-retaining PP100 polyethylene catheter to guide against urinary bladder distension. A 24G needle placed in the corpus carvanosum tissue and connected to a strain-gauge pressure transducer was used to measure the intra-cavernous pressure. The transducer was calibrated in mm of water. The experiment was carried out observing aseptic procedure and with consent of ethical committee of the College of Health Sciences, Usmanu Danfodiyo University, Sokoto.

Normal saline was slowly injected through the femoral vein and recordings made for a period of 30 to 45 minutes to serve as control. Then PY ($0.5cm^3$ of $50mg/cm^3$) was injected as slow intravenous injection through the femoral vein to a set of rats and recordings made for a period of 41 minutes. Eight rats were used for each set or group.

Statistical Analysis

Results are presented as mean ± SEM. Graphs were drawn using the Excel package for drawing graphs. Statistical analysis was done using one way analysis of variance (ANOVA) followed by a post-hoc students Newman-Keuls test. A P value of less than 0.05 was taken as statistically significant. The student's paired t test was used to analyze the Bp and ICP results. These were done using a computer assisted statistical package SYSTAT.

RESULTS

Phytochemical analysis

Phytochemical analysis of PY showed it contains alkaloids, tannins, flavenoids, cadiac glucosides, anthraquinones, saponins and redusing sugars. (Table 1)

Table 1: Phytochemical constituents of aqueous stem bark of *Pausinystalia yohimbe*. (PY)

Tests	Results
Alkaloids	
i. Draggendoff's test	+++
ii. Mayer's test	++
iii. Wagner's test	+++
Tannins	
i. Bromine water test	++
ii. Ferric chloride test	++
Phlobatannins	+
Flavonoids	
i. Lead acetate test	+++
ii. Ferric chloride test	+++
iii. Sodium chloride test	+++
Cardiac glycosides	
i. Keller–Kelliani test	+++
ii. Salkowski's test	+++
iii. Legal's test	+++
Anthraquinones	
i. Free anthraquinones	+
ii. Bound anthraquinones	++
Saponins	
i. Benedict's test	+
ii. Emulsion test	+
iii. Frothing test	+
Reducing sugars	
Hexose sugar	+
Ketosugar	+
Pentosugar	+

Monosaccharide	+
Anthocyanides	-

+++: present in high concentration. ++: present in moderate concentration. +: present in low concentration. −: not detected.

Mean Arterial Blood Pressure (MABP)

Figure 1 showed the curves of mean arterial blood pressure following intra venous administration of aqueous stem bark extract of *pausinystalia yohimbe* to anaesthesized albino rats. By the third minute the mean arterial blood pressure had been lowered significantly to below 80mmHg. This mean arterial pressure rose to about 90mmHg by the fifth minute with slight variations for the subsequent ten minutes. While that of the control remained above 110 mmHg.

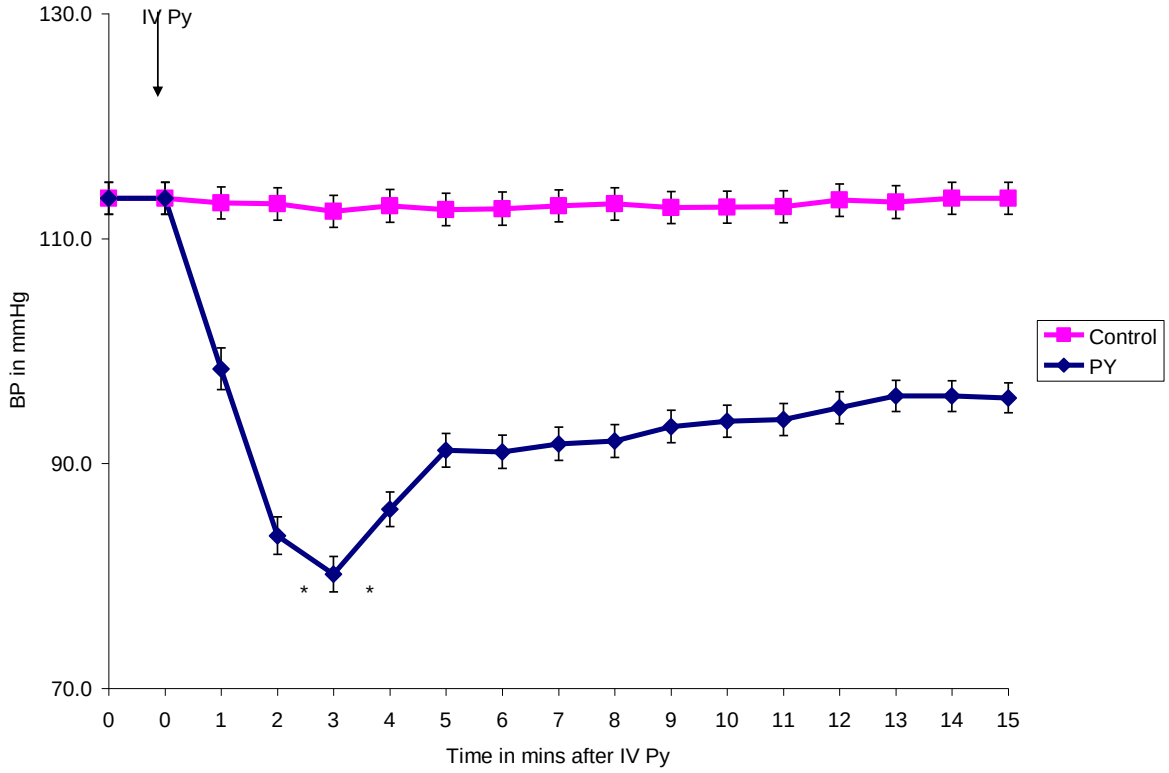

Figure 1: Changes in Blood Pressure Following iv injection of aqueous Stem Bark Extract of *Pausinystalia yohimbe*

Each point on the graph represents mean ±SEM. n=8. *P<0.05. Student t

The Effect of Aqueous Extracts of *Pausinystalia yohimbe* on Intra Cavernous Pressure (ICP) of Albino Rats

Figure 2 showed the curves of intracavernous pressure changes in albino rats following intravenous administration of aqueous stem bark extract of *pausingstalia yohimbe (PY*) to anaesthesized albino rats. After 14 minutes of administration of the extract, the ICP began to rise and peaked significantly (13mmH_2O) after 27 minutes and dropped to about 5mmH_2O above the basal ICP in the 42nd minute. This gave a total period of over 25 minutes of sustained ICP rise. The effect of the extract on the intra-cavernous pressure was significant P<0.05.

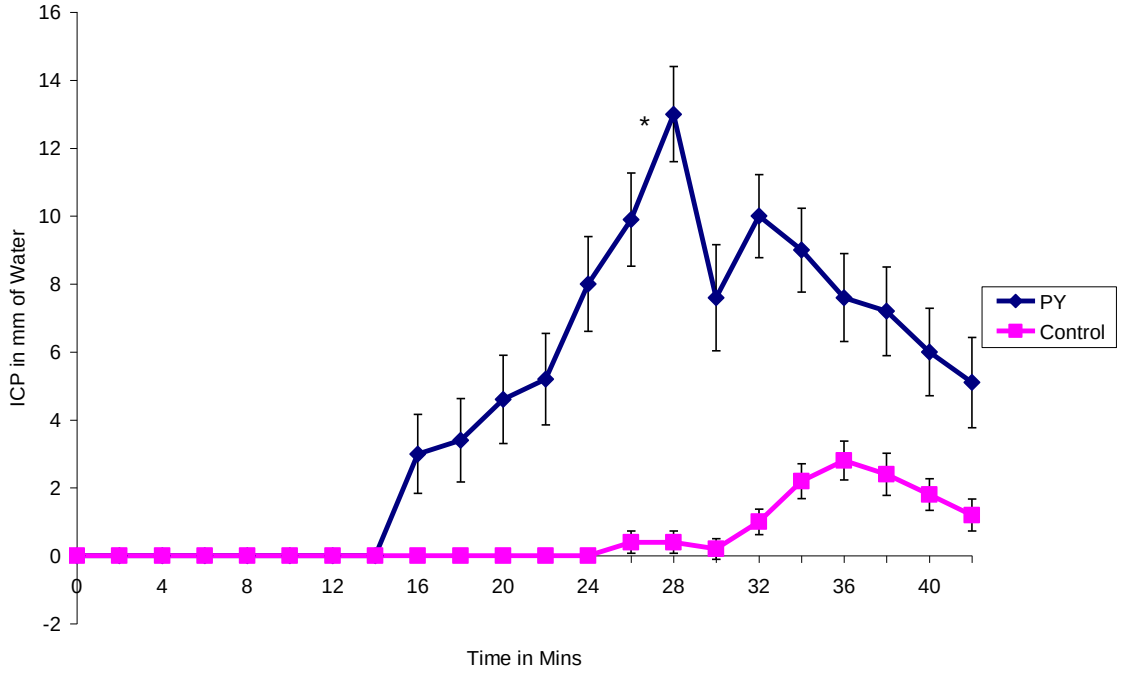

Fig. 2: Intra Cavernous Pressure Changes Following IV Administration of *Pausinystalia yohimbe* (PY) to Albino Rats.

Each point on the graph represents mean ±SEM. n=8. *P<0.05. Student t

DISCUSSION

Alkaloids, Tannins, Phlobatannins, Flavonoids, Cardiac glycosides, Anthraquinones, Saponins and Reducing sugars (Hexose sugar, Ketosugar, Pentosugar, Monosaccharide) were present in the phytochemical analysis of PY. This supports studies that say the alkaloid; Yohimbine is to be attributed for an aphrodisiac effect [9] [10] [11].

In order to validate the aphrodisiac properties of PY, whole laboratory animals (albino rats) were used in this study to assess erectile potential of the crude extract. Although there are various in-vivo animal models for study of penile erection, in this study the method described by Chen, *et al*. [21], for assessing the erectile potentials of the extracts was adopted due to its convenience in choice of animal, ease of technique and recording of results. This study is an improvement of the rat model for monitoring ICP upon electro stimulation on lumbosacral roots as reported by Martinez-Pineiro, [22]. The procedures in this study and that by Martinez-Pineiro [22] were carried out under general anaesthesia. From the results in the present study and that upon electro stimulation of lumbosacral roots by Martinez-Pineiro, [22], it is clear that both electrical stimulation and intracavernous drug administration produced reproducible ICP increases in anaesthetized animal. The intracavernosal pressure (ICP) measurement represents a direct investigation of erectile function [20]. It is a definitive in-vivo predictor of the ability of a substance to affect erection. The ICP in PY treated rats started to rise at 13 minutes, and peaked at 27 minutes after administration. At 41 minutes after administration, the ICP was still about 5mm H_2O above the baseline. These (results of this study) are in line with the work of other researchers who have reported PY usefulness in the treatment of erectile dysfunction [23] [24] and bringing about nocturnal tumescence and rigidity in sexually naïve male rats [25]. The effect of the extract was evident 13 minutes after intravenous administration and continued beyond 40 minutes. One would want to find out in subsequent studies, the onset and duration of action if administered

orally. Negative studies have also been reported [24].

In this study, while the control group had their blood pressure stationary above 110mmHg, the PY extract treated group averaged 90mmHg. But ironically, it is known that most anti-hypertensive drugs cause erectile dysfunction [26]. Since PY extract could lower blood pressure and also enhance erectile functions it is likely to become novel drug in management of hypertensive patients with erectile dysfunction. The implication is that in the use of these plant materials for aphrodisiac purposes, the blood pressure must be closely monitored.

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