

Effects of methanolic extract of yohimbe bark (*Pausinystalia yohimbe*) on isolated rabbit aortic strip and rat uterus

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ABSTRACT

Background: Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments. **Objectives and Methods:** This study aimed to investigate the effects of the methanolic extract of *Pausinystalia yohimbe* bark (5 mg/ml) on isolated rabbit aortic strip and rat uterus. **Results:** methanolic extract of *P. yohimbe* bark (5 mg/ml) produced relaxation of the phenylephrine pre-contracted-rabbit aortic strip. This relaxation may be resulted through nitric oxide (NO), since the pretreatment of the isolated rabbit aortic strip with methylene blue inhibited the NO-mediated relaxation. Moreover, the extract exhibited relaxation of rat uterine muscles, which appeared to be not mediated by activation of β_2 -adrenoceptors and/or H₂ receptors, since the relaxant effect continued even after the pretreatment of the tissue with propranolol and ranitidine respectively. **Conclusion:** The obtained results revealed that methanolic extract of *P. yohimbe* bark caused relaxation of both isolated rat uterus and rabbit aortic strip through facilitating the role of endogenous compounds such as NO.

Keywords: *P. yohimbe* Bark; Isolated Tissues; Aortic Strip; Rat Uterus; Relaxation; Nitric Oxide

1. INTRODUCTION

Medicinal plants have played a key role in the world

health care with about 80% of Africans depending on phytomedicine, which has shown a wide range of uses in the treatment of diseases especially priority diseases of Africa such as malaria, sickle-cell anemia, diabetes and hypertension. The medicinal plants have more beneficial effects than their synthetic counterparts through being safer, acceptable, affordable, culturally compatible and suitable for chronic treatments. Some African phyto-medicines are well known in the international market and so supply economic benefit for producing countries [1]. *Pausinystalia yohimbe* (family Rubiaceae) is a valuable tree native to the Gulf of Guinea and distributed in ever-green closed-canopy forests from Southern Nigeria to Congolese Mayombe, Gabon, possibly the Democratic Republic of Congo and Cameroon [2-5]. The main active constituents of the bark are the indole alkaloids such as yohimbine and related compounds [6]. Yohimbine is considered a fairly selective α_2 -adrenoceptor antagonist (the α_2 : α_1 selectivity ratio varying between 10 and 100 depending on the model used) [7-11].

This study aimed to investigate the effects of the methanolic extract of *P. yohimbe* bark on isolated rabbit aortic strip and rat uterus. The experimental protocol was ethically approved by the ethical committee at Faculty of Pharmacy, University of Gezira.

2. MATERIALS AND METHODS

2.1. Materials

Plants Materials

The dried chopped small pieces of *P. yohimbe* barks had been collected from South West Cameroon in May 25th, 2008. The plant material was authenticated by the

Provincial Service for Forestry, Ministry of Forestry and Wildlife, Republic of Cameroon (Certificate No. 004/CO/MINFOF/PDFOF/PSF/SW/230).

2.2. Methods

2.2.1. Extraction of Plant Materials

The coarsely powdered barks (500 grams) of *P. yohimbe* were extracted by maceration using pure methanol in a conical flask for 72 hours with intermittent shaking, filtered and evaporated by rotary evaporator at 60°C. The dried extract powder was kept in an amber glass container in a refrigerator for the biological test.

2.2.2. Rabbit Aortic Strip Preparation

A rabbit of local strain (1.75 kg) was used in this experiment. The preparation was based on the method adopted by Furchgott and Bhadrakom (1953) [12]. The rabbit was killed by neck dislocation and exsanguination. The chest was opened, the internal viscera were pulled aside and the aorta had been exposed. The aorta was cut closed to the heart and dissected as far as possible. Then after, the tissue was transferred to a petri dish containing aerated Krebs solution. The aorta was located over a large plastic cannula, surrounding fats and connective tissues were removed, then the aorta was cut spirally by curved scissor to produce a continuous strip. Threads had been tied to each end of the strip and one end was attached to the tissue holder. The mounted tissue then was transferred to a 25 ml organ bath filled with oxygenated Krebs solution maintained at 37°C and the top thread was attached to Harvard isometric transducer connected to Harvard Universal Oscillograph recorder (Harvard Apparatus Limited UK). The preparation was allowed to stand for 45 minutes, under 2 g resting tension before addition of the reference drugs (acetylcholine 250 ng/ml, phenylephrine 12 µg/ml and 25 µg/ml methylene blue) and the *P. yohimbe* extract.

2.2.3. Rat Uterus Preparation

The preparation was based on the method of De Jalon *et al.*, (1945) [13] and Kitchen, (1984) [14]. A female Albino rat weighing 170 g was selected; it was brought to the estrus state by administration of 17β-estradiol (2 mg/kg) subcutaneously 72 hours prior to the experiment). The rat was killed by slaughtering and exsanguination. The abdomen was opened, and the two uterine horns (pink colorations) had been exposed by pulling aside the intestine. Each horn was freed carefully from the surrounding fats and mesenteric attachments then each of them was cut out separately, and transferred to a petri dish containing De Jalon solution. A longitudinal cut was made to form a sheet of muscle instead of a tube. Threads had been passed through one wall of the uterus

at both top and bottom, the bottom thread was attached to the tissue holder and transferred to a 50 ml organ bath containing aerated De Jalon solution maintained at 32°C and the top thread was attached to Harvard isotonic transducer connected to Harvard Student Oscillograph recorder. The preparation was allowed to adapt for 30 minutes, under 0.5 g tension before addition of the reference drugs (acetylcholine 50 ng/ml, adrenaline 100 ng/ml, atropine 100 ng/ml, histamine 500 µg/ml, ranitidine 1 mg/ml and propranolol 1 µg/ml) and *P. yohimbe* extract.

3. RESULTS

The effects of phenylephrine, acetylcholine and methanolic extract of *P. yohimbe* on rabbit aortic strip were shown in **Figures 1** and **2**. The tissue was contracted by phenylephrine (12 µg/ml); and an ostensible relaxation was obtained by acetylcholine (250 ng/ml), which indicated the integrity of the endothelial cells.

The *P. yohimbe* extract (dose 5 mg/ml) relaxed the contracted aortic strip to the normal baseline with maximal relaxation activity occurred after 1 minute. This relaxant effect of the extract was blocked by the pre-addition of methylene blue (25 µg/ml).

Figures 3 and **4** demonstrate the effects of methanolic extract of *P. yohimbe* bark on uterus taken from 72 hours estradiol-treated female Albino rat [13,14]. Treatment

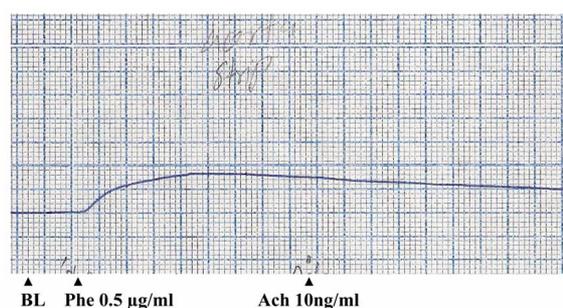


Figure 1. Effects of phenylephrine (Phe) and acetylcholine (Ach) on isolated rabbit aortic strip (BL: base line).

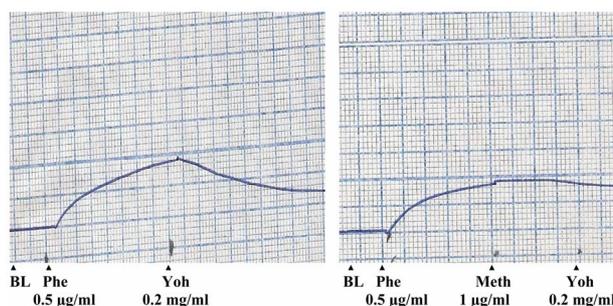


Figure 2. Effects of phenylephrine (Phe), methylene blue (Meth) and *P. yohimbe* extract (Yoh) on isolated rabbit aortic strip (BL: base line).

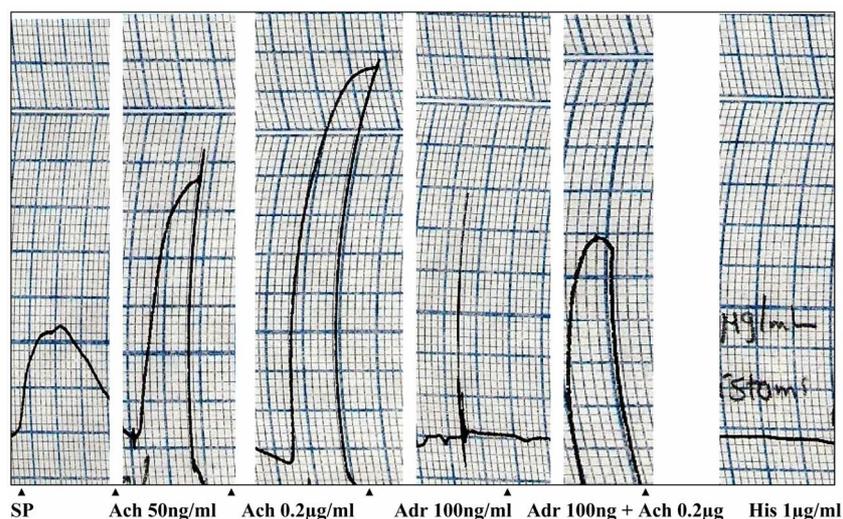


Figure 3. Effects of acetylcholine (Ach), adrenaline (Adr) and histamine (His) on isolated rat uterus (SP: spontaneous contractions).

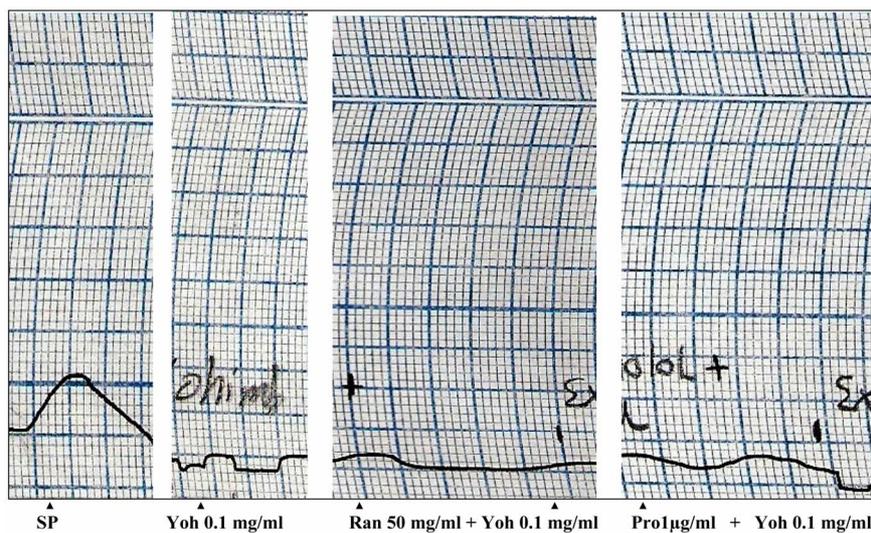


Figure 4. Effects of *P. yohimbe* extract (Yoh), ranitidine (Ran) and propranolol (Pro) on isolated rat uterus (SP: spontaneous contractions).

with estradiol (2 mg/kg subcutaneously) increased the sensitivity of uterine muscle towards the action of the tested drugs (acetylcholine 50 ng/ml, adrenaline 100 ng/ml, histamine 1 µg/ml and *P. yohimbe* extract 5 mg/ml). The isolated rat uterus was tested first by these drugs. Acetylcholine produced a dose-dependent contraction with a maximal contraction occurred by a dose of 0.2 µg/ml and this contraction was reduced by prior addition of adrenaline. Adrenaline and histamine showed a relaxant effect on the isolated uterus. When *P. yohimbe* (5 mg/ml) was tested on the uterus, it elicited a rapid relaxant activity which was attained in less than 1 minute. This inhibitory effect of the *P. yohimbe* extract was sustained and did not blocked, even after pre-treatment of the tissue with the histamine H₂-receptors antagonist

ranitidine (1 mg/ml) and the β-blocker propranolol (1 µg/ml).

4. DISCUSSION

These results indicated that, phenylephrine (α₁-adrenoceptors agonist) produced slow sustained contraction of the isolated aortic smooth muscle. It has been established that α₁-adrenergic agonists in vascular smooth muscle increase calcium influx via the slow-inward current. Thus they enhance the degree of contraction of blood vessels [15-18].

Contraction or force development by smooth muscle cells depends upon the elevation of intracellular calcium in the myoplasm. This is caused by either release of in-

tracellular calcium from the storage sites like mitochondria, or entry of calcium via voltage-operated calcium channels, or entry of calcium via receptor-operated channels [15,18,19].

On the other hand, acetylcholine caused relaxation to the contracted tissue; this effect indicated the presence of intact endothelial cells. This endothelial-dependent response is principally regulated by release of nitric oxide (NO) from the endothelium which is synthesized from the amino acid L-arginine by endothelial nitric oxide synthase (eNOS) [20]. Acetylcholine (ACh) produces dilation of essentially all vascular beds, which is primarily due to stimulation of muscarinic receptors (M_3) that activates the G protein-phospholipase C-inositol triphosphate (G_q -PLC-IP₃) pathway and mobilizes cell calcium. In endothelial cells, this leads to Ca^{2+} -calmodulin-dependent activation of eNOS and production of NO which diffuses to adjacent smooth muscle cells, where it stimulates the soluble guanylyl cyclase and causes relaxation [5,21,22].

Methanolic extract of *P. yohimbe* bark produced relaxation of the contracted aortic strip which was probably may be resulted through NO, since pretreatment of the isolated rabbit aortic strip with methylene blue (a soluble guanylyl cyclase inhibitor) inhibited the NO-mediated relaxation of smooth muscle cells which was produced in the absence of methylene blue [23-25].

These findings match with previous studies on the effects of yohimbine on vascular smooth muscles. Ikunobu *et al.*, (1990) [17] found that, yohimbine inhibited the blood vessels responses to adrenaline and phenylephrine. Moreover, yohimbine was found not significantly different than prazosin (a selective α_1 adrenoceptor antagonist) in inhibiting the effects of phenylephrine and noradrenaline on rat aortic strips [26].

It was reported that, the use of acetylcholine resulted in uterine smooth muscle contractions through stimulation of muscarinic receptors (M_3), while adrenaline caused relaxation by stimulating β_2 -adrenoceptors in the uterus [21].

On the other hand, histamine produced relaxation on the uterus, although it causes contractions in the other smooth muscles. Some investigators suggested that histamine-induced uterine relaxation is associated with activation of the histamine H_2 -receptors [27-30].

The methanolic extract of *P. yohimbe* bark produced relaxation of the uterine muscles, which appeared to be not mediated through activation of β_2 -adrenoceptors because the relaxant effect continued even after the pretreatment of the tissue with propranolol (a non-selective β -adrenergic blocker). Also it was not mediated through activation of H_2 receptors because the relaxation persisted after the prior addition of ranitidine, a selective H_2 receptors antagonist.

5. CONCLUSION

As experimentally evident, it could be concluded that, methanolic extract of *P. yohimbe* bark caused relaxation on both isolated rat uterus and rabbit aortic strip, which may be directly by releasing relaxing substances such as nitric oxide (NO).

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