Original Research Article

Mechanism of Spasmogenic Activity Stimulated by Aqueous Ethanolic Leaf Extract of Mucuna pruriens on Isolated Uterine Muscle of Albino Rats.

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Aims: To investigate the effect of aqueous ethanolic leaf extract of this medicinal plant on isolated uterine smooth muscle strips of the rat and to determine its mechanism of action.

Study design: Mention the design of the study here.
Place and Duration of Study: The study was carried out in the Department of Veterinary Physiology and Biochemistry of Michael Okpara University of Agriculture, Umudike, Nigeria, Department of Pharmacology and Toxicology of the Faculty of Pharmacy, University of Nigeria, Nsukka, and the Department of Veterinary Physiology and Pharmacology, University of Nigeria, Nsukka, Nigeria between June and October 2014.
Methodology: Fresh leaves of *Mucuna pruriens* were identified and collected by a taxonomist from Nsukka, Nigeria. The leaves were then air dried and pulverized into powder. This was then subjected to cold extraction using petroleum ether (70-90) and 70% aqueous ethanol, after which the extract was left todry at room temperature. Estrogenised uterine strips (12mm) were harvested from non-pregnant, sexully matured albino rats (180g -250g) and suspended in a 35ml organ bath containing Krebs''physiological salt solution. The organ bath was connected to an isometric electronic force displacement transducer and a physiograph. Drugs such as Salbutamol, Isoprenaline, Adrenaline, Propranolol, Atipamezole and Prazosin were used as either agonists or antagonists to determine the mechanism of action of the extract. Atropine sulphate and cyproheptadine were also used as test drugs. Concentrations of these drugs presented in the body of this work represent the final nutrient bath concentrations.
Results: *M. pruriens* caused a dose -dependent increase in uterine muscle contraction with a maximum peak in amplitude produced by 0.57 mg/ml of the extract, and an EC_{50} of 2.9 mg/ml. The contraction was unaffected by atropine sulphate (0.042 µmol), but abolished by salbutamol (0.012-0.4 µmol), isoprenaline (0.06-0.23 µmol), and adrenaline (16 nmol). The uterine muscle contractions were enhanced by propranolol (1 µmol) in a dose- dependent manner. Prazosin (0.069-0.14 µmol) and atipamezole (3.3-13.7 nmol) were unable to abolish contractions stimulated by the extract. However, 0.2 µmol of cyproheptadine caused 80% suppression of the extract –induced uterine contraction
Conclusion: It is concluded that aqueous ethanolic leaf extract of *M. pruriens*, has ability to cause uterine smooth muscle contraction hence, justifies its reported use traditionally as a uterine stimulant. This contraction is most likely exerted via the 5-HT receptor activation (activated by low concentrations of serotonin).

Keywords: Mucuna pruriens, myometrium, uterine contraction, spasmogenic, uterus, extract.

1. INTRODUCTION

Mucuna pruriens is of the family Fabaceae, sub- family Leguminosae consisting of various species of climbing vines and shrubs (Umberto, 2000). Different parts of the plant have been reported to be useful in traditional medicine in various parts of the world (Gill and Nyawuame, 1994; Vasudeva and Shanpru, 1991; Chikagwa-Malunga et al., 2009; Lieu et al., 2010; Warrier et al., 1996). In southeastern Nigeria, the leaves of *M. pruriens* are considered excellent natural herbal blood booster, used especially for acute blood loss and blood deficiency diseases (Obadoni and Ochuko, 2001). Since, in traditional medicine, this plant is also used as uterine stimulant (Amin et al., 1996), the present study was an attempt to investigate the effect of aqueous ethanolic leaf extract of this medicinal plant on isolated uterine smooth muscle strips of the rat and to determine its mechanism of action.

2. MATERIAL AND METHODS

2.1 Extraction studies

Fresh leaves of *Mucuna pruriens* were identified and collected by a taxonomist -Mr A.O. Ozioko of Bioresource Development and Conservation Center (BDCC), Aku road Nsukka, Enugu state of Nigeria with voucher No-INTERCEDD/1569. The leaves were air dried and pulverised into fine powder, using a conventional hammer mill. This was subjected to cold extraction initially using petroleum ether (70-90) for 72 hours and later with 70% aqueous ethanol for 48 hours with intermittent shaking at two (2) hours interval. The extracts were allowed to dry at room temperature and subsequently stored in the refrigerator at 4˚C.

2.2 Animals

Non-pregnant Albino rats of breeding age, weighing between 180 g and 250 g were used for the *in vitro* bioassay studies. The rats were supplied by a breeder and kept at the Laboratory Animal Unit of the Department of Veterinary Physiology, Micheal Okpara University of Agriculture, Umudike, Nigeria. Standard commercial pelleted feed (Vital feeds, Nigeria) and clean drinking water were given to the animals *ad libitum*. Each of the rats received 0.1mg/kg stilboestrol in paraffin oil administered subcutaneously 24 hours prior to the experiments.

2.3 Tissue Preparation and Isometric contraction studies

The animals were sacrificed by stunning and decapitation. About 12 mm segment of uterine horn was removed and attached by ligatures at one end to a specimen holder and at the other to an isometric force displacement transducer (Forte transducer Medicaid, India) connected to a physiogragh. This was suspended vertically in a 35 ml conventional organ bath containing physiological solution of the following composition (mmol): KCl (4.7); NaCl (118); $CaCl_2$ (2.5); KH_2PO_4 (1.2); $NaHCO_3$ (2.5); $MgSO_4$ (1.2); and glucose (11); and perfused continuously with a gas mixture of 95% O_2 and 5% CO_2, maintained at 37°C. All uterine strips were put under a little amount of tension and experiments started within 40 minutes following development of stable rhythmic, spontaneous uterine contractions. The extract and or experimental drugs at different concentrations were applied and a minimum of one (1) minute was allowed for tissue contact in each experiment following which the physiograph readings was paused and the preparation washed three (3) times with the physiological solution in preparation for subsequent experiment. Concentrations of the test substances presented in the body of this work represent the final nutrient bath concentrations unless otherwise stated.

2.4 Statistical Analysis of Data

Some datas generated were analyzed using student t-test and one-way Analysis of Variance (ANOVA) where necessary, and the results presented as mean ± standard error of the mean (SEM). Differences between the means of the control and experimental groups were considered significant where P=.05.

3. RESULTS

3.1 Concentration-Response Relationship and EC_{50} of *M. pruriens.*

Mucuna pruriens elicited uterine smooth muscle contractions in the physiological salt solution. These contractions were single, transient and concentration- dependent. The maximal uterine smooth muscle contraction (in terms of amplitude of contraction) was observed with 0.57 mg/ml. Thereafter, further increase in concentration did not elicit higher amplitude of contraction (Fig. 1). The EC_{50} was calculated as 2.9 mg/ml.

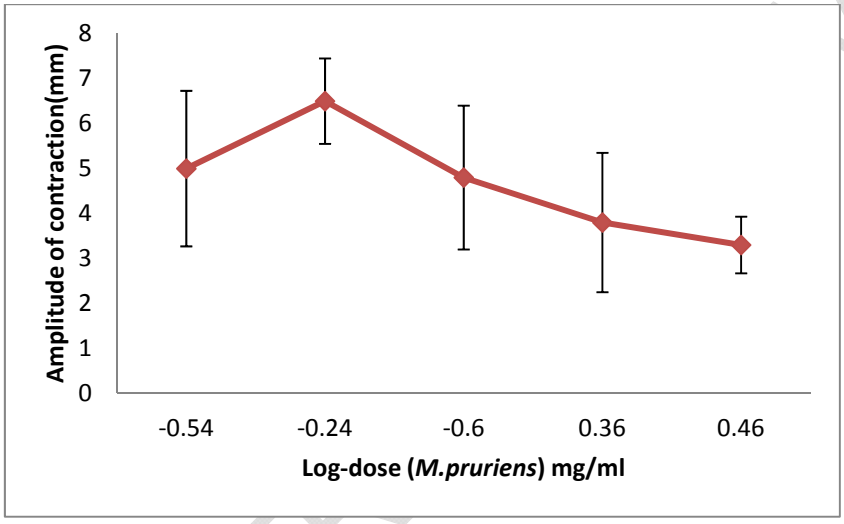


Fig. 1: Effective concentration (EC_{50}) of the extract.

3.2 Effects of Selective and Non- Selective β- Adrenergic Agonists on M. pruriens Contractile Activity.

Application of both salbutamol (0.012 - 0.4 µmol) and isoprenaline (0.06 - 0.23 µmol) as a selective and non- selective β- adrenergic agonist respectively, followed simultaneously by *M. pruriens* (0.86 mg/ml) resulted in complete inhibition (100% inhibition, P< 0.05) of *M. pruriens* mediated uterine smooth muscle contractions (Figs. 2a and 2b). Adrenaline (16 nmol) also caused a complete inhibition of *M. pruriens* (0.86 mg/ml) mediated contractions (Fig. 3). However, propranolol (1µmol), a β- adrenergic antagonist was able to reverse (56%) the inhibitory effect of isoprenaline (0.06 µmol) on the extract-stimulated contractions (Fig. 4a). Moreover, non-cumulative concentrations of propranolol (0.5 - 3 µmol) introduced before a fixed concentration of *M. pruriens* (0.86 mg/ml), potentiated the frequency of spikes in the burst of uterine smooth muscle contractions induced by the extract in a dose-related manner (Fig. 4b).

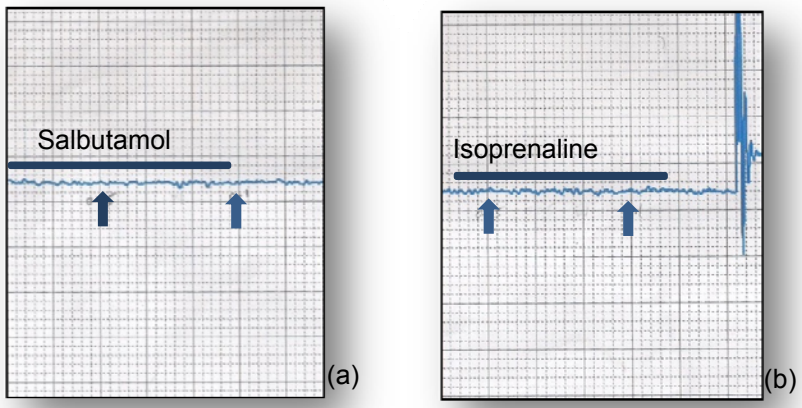


Fig. 2: Inhibitory effects of (a) salbutamol (0.012 µmol) and (b) isoprenaline (0.06 µmol) on uterine smooth muscle contractions stimulated by *M. pruriens*. Arrows indicate point of application of extract.

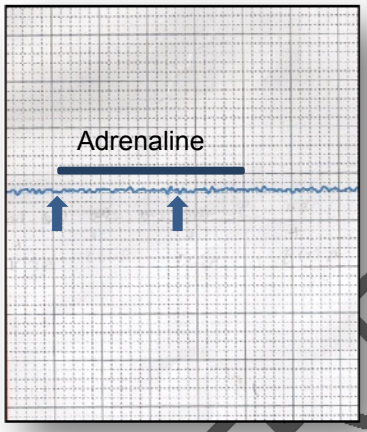
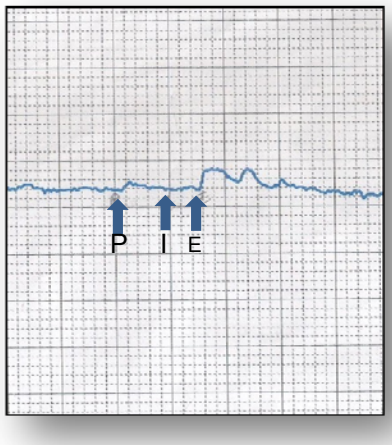
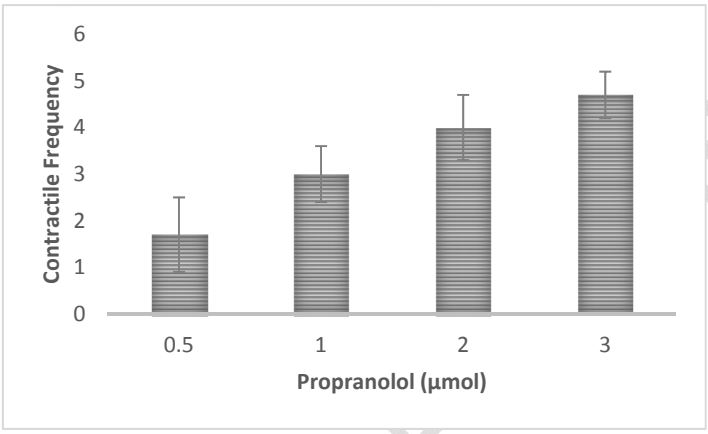


Fig. 3: Effect of adrenaline (16 nmol) on *M. pruriens* (0.86mg/ml) induced uterine smooth muscle contractions. Arrows indicate point of addition of extract.



(a)



(b)

Fig. 4: (a) Effect of propranolol (1µmol) on the inhibitory action of isoprenaline (0.06µmol) on *M. pruriens* (0.86mg/ml) induced uterine force. P = propranolol, I = isoprenaline and E = *M. pruriens*. (b) Effect of propranolol (0.5- 3 µmol) on the frequency of spikes in the burst of contraction stimulated by *M. pruriens* (0.86mg/ml); result represent the mean ± SE (n= 3).

3.3 Effects of α_1 and α_2- adrenoreceptor antagonists on *M. pruriens* mediated uterine smooth muscle contraction.

Prazosin (0.069 and 0.14 µmol) was incubated two minutes prior to introduction of *M. pruriens* (0.86 mg/ml). There was an immediate response of the uterine tissue characterised by a burst of contractions upon introduction of the extract (Fig. 5a). Contractile response of the uterine smooth muscle strips to the same concentration of *M. pruriens* (0.86 mg/ml) in the presence of atipamezole (3.3- 13.7 nmol), was not significantly different (P = 0.78) from that observed with prazosin (Fig. 5b). Moreover, phenylephrine (0.84 - 3.41 µmol) did not potentiate the contractile force induced by this extract (Fig. 6).

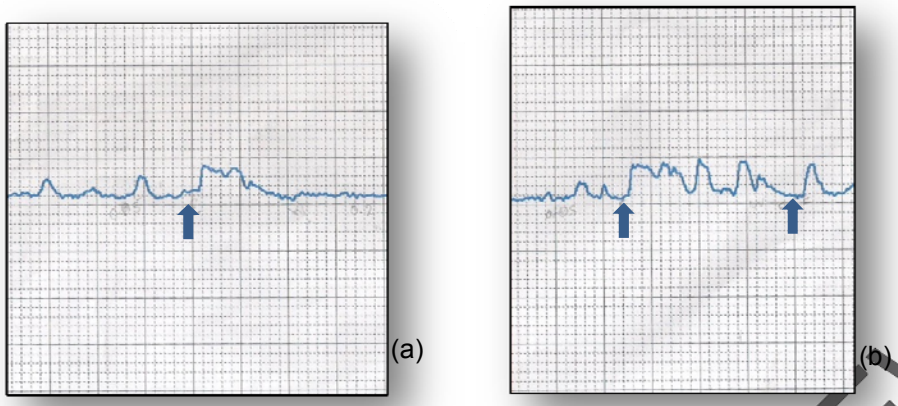


Fig. 5: Effects of (a) prazosin (0.14 µmol) and (b) atipamezole (13.7 nmol) on *M. pruriens* (0.86 mg/ml) induced uterine force. *M. pruriens* was applied 2min after incubation with prazosin and atipamezole at points indicated by arrows.

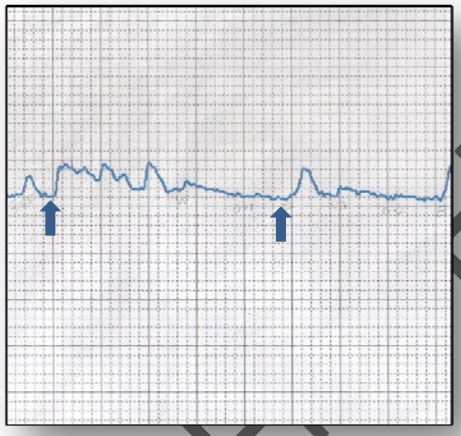


Fig. 6: Effect of phenylephrine (3.41 µmol) on *M. pruriens* (0.86) induced uterine force. Arrows indicate point where the extract was added 2 min after phenylephrine was incubated in the media.

3.4 Effect of Atropine Sulphate on M. pruriens and Carbachol Induced Uterine Smooth Muscle Contractions.

Uterine muscle contractions stimulated by *M. pruriens* (0.86 mg/ml) was not altered in the presence of atropine sulphate (0.042 µmol). The contractions were similar to those induced by *M. pruriens* alone. However, this same concentration of atropine caused a complete inhibition (100 %; P =.05) of carbachol (3 µmol) induced myometrial contractions (Fig. 7).

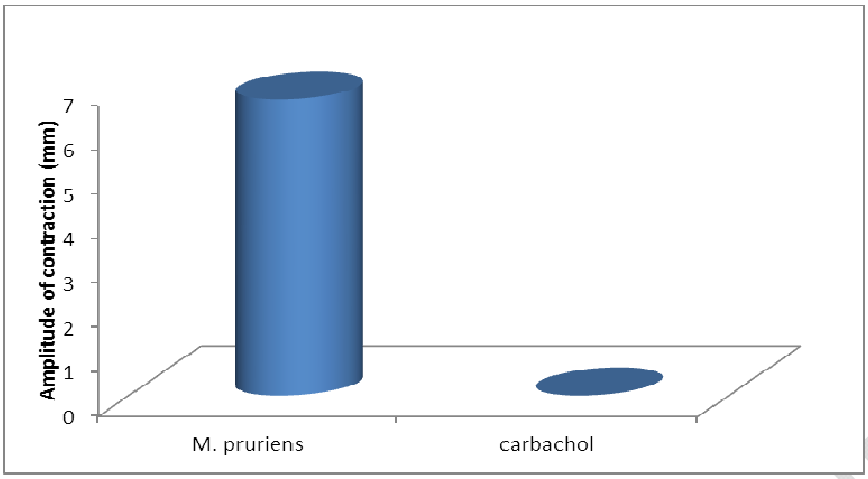


Fig. 7: Effect of atropine sulphate (0.042 µmol) on *M. pruriens* (0.86 mg/ml) and carbachol (3 µmol) induced uterine smooth muscle contraction.

3.5 Effects of 5-HT Receptor Antagonist on *M. pruriens* Mediated Uterine Smooth Muscle Contractions.

To test for possible involvement of 5-HT receptor, cyproheptadine was applied in graded concentrations (0.04 - 0.2µmol). Antagonism of *M. pruriens* (0.86 mg/ml) -induced uterine smooth muscle contractions by cyproheptadine was concentration dependent, with 0.2 µmol of the antagonist causing a significant (80%; P= .05) decrease in the amplitude of contractions (Fig. 8).

Drug/ Extract	Uterine responses to *M. pruriens* and Cypro (mm)
M. pruriens (0.86mg/ml) Control n=4	6.50 ± 0.96
Cypro (0.04 µmol) + *M. pruriens* n=3	4.67 ± 0.33
Cypro (0.08 µmol) + *M. pruriens* n=3	3.00 ± 0.56
Cypro (0.2 µmol) + *M. pruriens* n=3	1.30 ± 0.33

Table 1: Effect of Cyproheptadine on *M. pruriens* (0.86 mg/ml) stimulated uterine smooth muscle contractions. Cypro= cyproheptadine. Values are the mean ± SEM.

4. DISCUSSION

The present study demonstrates that the aqueous ethanolic leaf extract of *M. pruriens* induced uterine smooth muscle contraction. This contractile effect was concentration - dependent, and was found to be more effective at lower concentrations than at higher concentrations of the extract. The decrease in myometrial contraction seen at higher concentrations of the extract (1.14 – 2.86 mg/ml) could be due to receptor desensitization or increase in concentration of other substances present in the plant extract that may be antagonistic to its contractile effect. Similar effects were observed in isolated saphenous vein of the dog where serotonin caused contraction of this muscle at low concentrations, and was attributed to a direct action of serotonin on 5-HT1 -like receptors of the smooth muscle cells (Sumner et al., 1992).

Under normal physiological conditions, activation of α_1 -adrenergic receptors present in the plasma membrane of the uterine muscle cells elicits uterine smooth muscle contraction whereas stimulation of its β_2 adrenergic receptors inhibits uterine smooth muscle contraction. In the present study, both salbutamol and isoprenaline, well known β_2 adrenergic selective and non-selective agonists respectively abolished uterine responses to *M. pruriens* (Fig. 2a and 2b). Studies on the responsiveness of the uterus to β_2-adrenergic receptor agonists, as well as characterisation of myometrial β-adrenergic binding sites in several species (human, rat, and guinea pig), have indicated that β_2 is the dominant subtype of adrenergic receptors present in this tissue (Pennefather and Molenaar, 1986; Maltier and Legrand, 1988). Beta - adrenergic receptor agonists exert their relaxatory effects by activating G_s proteins, and their $G\alpha_s$ subunit stimulates adenyl cyclase leading to the elevation of cyclic adenosine monophosphate (cAMP) levels, leading to phosphorylation of the myosin light chain kinase (MLCK) via a cAMP -dependent protein kinase enzyme (Robert and Judit, 2013). The inability of the extract to stimulate uterine smooth muscle contraction in the presence of these β-adrenergic receptor agonists indicate that *M. pruriens* was unable to compete with these agonists for the β_2-adrenergic receptor sites and thus influence the ability of these agonists to cause elevation of intracellular cAMP levels which is necessary for uterine smooth muscle relaxation. Propranolol, a non- selective β- adrenergic receptor blocker (Young and Glennon, 2009), was able to reverse this inhibition and the associated rise in cAMP and hence promoted the stimulatory activity of the extract on the uterine muscle preparation in the present study.

We also found that the α-adrenergic receptor antagonists, prazosin and atipamezole were not able to inhibit uterine muscle responses to *M. pruriens* (Fig.5) most likely due to the fact that the contractile response to *M. pruriens* was mediated by mechanisms other than α-adrenergic receptor activation. Neither was the extract able to potentiate phenylephrine induced myometrial contraction. That is, no additive response was achieved. Phenylephrine a selective α_1-adrenergic receptor agonist has been shown to open receptor-operated channels (Fasolato et al. 1994), by involving a second messenger coupled to phospholipase-C activation (Fasolato et al., 1994; Barritt, 1999).

Smooth muscle relaxation has also been accomplished by atropine sulfate -a muscarinic receptor antagonist (Hiromasa et al., 1976) However, it did not inhibit contractile effect of the extract on the uterine smooth muscle preparation but completely inhibited the contractions caused by carbachol - a muscarinic cholinergic agonist. This suggests that the contractile effect of aqueous ethanolic leaf extract of *M. pruriens* is not mediated through muscarinic cholinergic receptor activation.

Cyproheptadine is a serotonin and histamine receptor antagonist. Administration of cyproheptadine (0.2 µmol) resulted in 86% suppression of uterine smooth muscle contractions stimulated by *M. pruriens* (Table 1). This observation is in agreement with the findings of other investigators where cyproheptadine was reported to cause powerful inhibition of serotonin induced contraction (Wahab et al., 2008; Katerere and Parry, 2000; Lin et al., 2014). Thus, the contractile activity of *M. pruriens* in the present study, may have been derived from its serotonin content and its direct stimulation of 5-HT receptors at a low concentration. It has been shown that the main 5-HT receptors responsible for modifying vascular tone are the 5-HT_1, 5-HT_2, and 5-HT_7 receptors. However, in many arterial beds such as that of the rat and mouse aorta, the 5-HT_{2A} receptor is the primary receptor mediating 5-HT-induced contraction (McKune and Watts, 2001; Russell et al., 2002). It may well be that stimulation of these 5-HT receptor subtypes is responsible for *M. pruriens* mediated contractions.

5. CONCLUSION

In conclusion, we have shown that aqueous ethanolic leaf extract of *M. pruriens* causes uterine smooth muscle contraction. This contraction is most likely exerted via the 5-HT-receptor activation (activated by low concentrations of serotonin) as indicated by cyproheptadine antagonism. Therefore, the use of *Mucuna pruriens* as a uterine stimulant by the natives has some scientific basis.

CONSENT

Not applicable.

ETHICAL APPROVAL (WHERE EVER APPLICABLE)

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 8523, revised 1985) were followed, as well as specific national laws where applicable.

REFERENCES

1. Umberto Q. CRC World Dictionary of Plant Names. 3 M-Q. CRC Press. 2000; 1738.
2. Gill LS, Nyawuame HGK. Leguminosae in Ethnomedicinal Practices of Nigeria. Ethnobotany, 1994; (6): 51 – 64.
3. Vasudeva RMK, Shanpru R. Some Plants in the Life of the Garos of Meghalaya. In: Jain S.K (Ed). Contributions to Ethnobotany of India. Scientific Publishers, Jodhpu. 1991; 183 – 190.
4. Chikagwa-Malunga SK, Adesogan AT, Sollenberger LE, Badinga LK, Szabo NJ, Littell RC. Nutritional Characterization of *Mucuna pruriens*: Effect of Maturity on the Nutritional Quality of Botanical Fractions and the Whole Plant. Anim. Feed Sci. Technol., New York. 2009; 148 (1):34-50.
5. Lieu CA, Kunselman AR, Manyan BV, Venkiteswaran K, Subramanian TA. Water Extract of *Mucuna pruriens* Provides Long-Term Amelioration of Parkinsonism with Reduced Risk for Dyskinesias. Parkinsonism Relat. Disord. 2010; 16 (7): 458-465
6. Warrier PK, Nambiar VPK, Ramankutty C. Indian medicinal plants. Orient Longman, Chennai, India. 1996; (4): 68-72.
7. Obadoni BO, Ochuko PO. Phytochemical Studies and Comparative Efficacy of the Crude Extracts of Some Haemostatic Plants in Edo and Delta States of Nigeria. Global J. Pure Appl. Sci., 2001; (8): 203 – 208.
8. Amin KMY, Khan MN, Zillur-Rehman S, Khan NA. Sexual Function Improving Effect of *Mucuna pruriens* in Sexually Normal Rats. Fitoterapia. 1996; (67):58 – 68.
9. Sumner MJ, Feniuk W, McCormick JD, Humphrey PP. Studies on the Mechanism of 5-HT1 Receptor-Induced Smooth Muscle Contraction in Dog Saphenous Vein. Br J Pharmaco. 1992; 105 (3): 603-8.
10. Pennefather JN, Molenaar P. Beta$_2$-Adrenoreceptor Binding Sites in Circular and Longitudinal Myometrial Layers of the Virgin Guinea-Pig: the Influence of Ovarian Steroids. Journal of Autonomic Pharmacology, 1986; 6 (3): 207–213.
11. Maltier JP, Legrand C. Characterization of β-adrenoceptors in myometrium of preparturient rats. Fundamental and Clinical Pharmacology. 1998; 2 (5): 369–383.
12. Robert G, Judit H. Calcium Channel Blockers as Tocolytics: Principles of Their Actions, Adverse Effects and Therapeutic Combinations. Pharmaceuticals. 2013; (6): 689-699.

13. Young R, Glennon RA. S(-)Propranolol as a Discriminative Stimulus and its Comparison to the Stimulus Effects of Cocaine in Rats. Psychopharmacology, 2009; 203 (2): 369–82.
14. Fasolato C, Innocenti B, Pozzan T. Receptor-Activated Ca^{2+} Influx: How Many Mechanisms For How Many Channels. Trends in Pharmacological Sciences. 1994; (15):77–83.
15. Barritt GJ. Receptor-Activated Ca^{2+} Inflow in Animal Cells: A Variety of Pathways Tailored to Meet Different Intracellular Ca^{2+} Signalling requirements. Biochemical Journal. 1999(337):153–169.
16. Hiromasa A, Juei-Tang C, Kouhei O, Kohtaro T, Hiroshi M. Irreversible Inhibitory Effect of Atropine on Contractile Responses to Drugs in Isolated Rabbit Ileum. Japan. J. Pharmacol, 1976; (26): 737-742.
17. Wahab SIA, Mohamed AWA, Mohamed OY, Taha MME, Abdul AB, Al-Zubairi AS. Serotonergic Properties of the Roots of *Clerodendron capitatum.* American Journal of Biochemistry and Biotechnology, 2008; 4 (4): 425- 430.
18. Katerere D, Parry O. Pharmacological actions of *Heteromorpha trifoliate* ("dombwe) on Isolated Muscle Preparations. Cent Afr J Med, 2000; 46 (1): 9-13.
19. Lin OA, Karim ZA, Vemana HP, Espinosa EVP, Khasawneh FT. The Antidepressant 5-HT_{2A} Receptor Antagonists Pizotifen and Cyproheptadine Inhibit Serotonin-Enhanced Platelet Function. PLOS ONE. 2014; 9(1): e87026. doi:10.1371/journal.pone.0087026.
20. McKune CM, Watts SW Characterization of the Serotonin Receptor Mediating Contraction in the Mouse Thoracic Aorta and Signal Pathway Coupling. J Pharmacol Exp Ther. 2001; 297 (1):88-95.
21. Russell A, Banes A, Berlin H, Fink GD, Watts SW. 5-Hydroxytryptamine (2B) Receptor Function is Enhanced in the N (omega)-Nitro-L-Arginine Hypertensive Rat. *J* Pharmacol Exp Ther. 2002; (303): 179–187.