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Uterine Relaxation Potential of Ethanol Leaf Extract of *Moringa oleifera Lam*. VIA the Muscarinic Receptor Pathway

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Authors' contributions

This work was carried out in collaboration between all authors. Author NSI designed the study. Author KGM performed the statistical analysis. Author ONN wrote the protocol. Authors CUE and NSI wrote the first draft of the manuscript. Authors CUE and CON managed the analyses of the study. Author ONN managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: The effect of Ethanol Leaf Extract of *Moringa oleifera* (ELMO) on uterine smooth muscles of non- pregnant female rats was studied *in vitro* with a view to finding out the mechanism(s) for observed effects.

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Experiential Design: *In vitro* studies using isolated rat's uteri.
Methods: Female albino rats (140-180g) pretreated 24 hours before experiments with diethylstilbestrol were sacrificed and the uterine horns carefully harvested into a beaker of De Jalon solution bubbled with 95% oxygen and 5% carbon dioxide. Each horn was mounted in an organ bath and allowed to equilibrate for 30 minutes, then the effects of graded doses of Acetylcholine, oxytocin and ELMO were established, using a physiograph and its accessories. The drugs were re-administered in the presence of their respective antagonists (Atropine for Acetylcholine and Atosiban for Oxytocin) and also in the presence of ELMO.
Results: Results obtained showed that while Acetylcholine and oxytocin induced uterine contractions, ELMO caused relaxation. ELMO significantly ($P<0.05$) blocked the uterine contractile effect of Acetylcholine but had no effect on Oxytocin induced uterine contractions. The experiments therefore indicate that ELMO contain active ingredients capable of inducing uterine relaxation via the muscarinic receptor pathway.
Conclusion: The extract therefore, may not be a valuable tocolytic agent in cases of Oxytocin induced uterine contractions, particularly in pregnancy but its observed strong anticholinergic activity may be exploited in the treatment of diseases associated with hyper activity of the parasympathetic arm of the autonomic nervous system.

Keywords: Acetylcholine; Moringa oleifera; muscarinic receptors; oxytocin; uterine contractions.

1. INTRODUCTION

Herbal medicine in Nigeria is today enjoying a boost. This may be because the nation is host to over 10,000 plants species, of which many are of medicinal value [1]. Many of these plants have been exploited while a host of others remain latent. Researchers have continued to explore the systemic effects of these plant preparations with the intention to discover new drugs and/increase the potency of existing ones. *Moringa oleifera* Lam is one of such medicinal plants that are being use for the treatment of various ailments [2].

Moringa oleifera Lam. popularly called horse radish tree, drumstick tree or ben oil tree in English [2], is a plant belonging to family Moringaceae is a fast growing evergreen deciduous, perennial tree which grows to a height of 10-12 meters and has slender stem, drooping and brittle branches and feathery leaves [2]. The plant in reported to be used in phytomedicine as an antioxidant, antimicrobial, anti-inflammatory, antipyretic, antiulcer, anti-diabetic, antiturmor and as a hypocholesteromic agent [3].

The widespread use of the leaf extract of the plant for the management of diseases in ethnomedicine coupled with its acclaimed anti-pregnancy potential were the catalyst for this study designed to evaluate the effect of the ethanol leaf extract of *Moringa oleifera* (ELMO) on isolated rat uteri.

2. MATERIALS AND METHODS

2.1 Collection of Plant Materials and Preparation of Plant Extract

Fresh leaves of *Moringa oleifera* were collected from a farm settlement in Umuakwela, Obodo Ahiara in Ahiazu Mbaise Local Government Area of Imo State, Nigeria.

The extract of *Moringa oleifera* was prepared using a modified method of Akah et al. [4]. The collected leaves were dried under shade in the laboratory at room temperature for 7 days, after which they were ground to powder using a manual blender. The powdered material weighing 35g was introduced into the extraction chamber of the soxhlet extractor and extraction was done using ethanol as solvent. Extraction temperature was maintained at 70℃ for 48 hours. At the end of the period, the ethanol was evaporated at low temperature in an electric oven to obtain a crude extract which weighed 10.97g and represented a yield of 31.34%.

2.2 Animals

Female rats (140-180g) obtained from the animal production unit of the College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike were used for the study. These animals were housed in an aluminum cage and were fed with standard pelleted feed (vital feed, Nigeria), with water *ad libitum*. Each animal was pretreated with a subcutaneous injection of diethylstilbestrol (0.1mg/kg body weight) 24 hours and starved for 12 hours before commencement of experiment. The administration of diethylstilbestrol was to bring the animal to oestrous and thereby enhance spontaneous contractions. Animal experiments were conducted in compliance with NIH guideline for care and use of Laboratory Animals (Pub. No. 85-23, Revised, 1985) as expressed by Akah et al. [4]. The study was carried out in the Physiology Laboratory of the Department of Physiology, Pharmacology, Biochemistry and Animal Health, Michael Okpara University of Agriculture, Umudike, Nigeria.

2.3 Effect of ELMO on Isolated Rat Uterus

2.3.1 Preparation of uterine of uterine muscle tissue and isometric contraction studies

The method of Uchendu, (1998) [5] was employed. In this method, the animals primed with diethylstilbestrol (0.1mg/kg body weight) 24hrs earlier were killed by stunning and decapitation. The uterine horns were carefully isolated, trimmed of fat and transferred to De Jalon solution that was continuously bubbled with oxygen (95%) and carbondioxide (5%) mixture, maintained at 37℃ with pH value 7.4. The physiological salt solution (De jalon) had the following salts composition per liter of water: NaCl- 9g, KCl- 0.42g, $CaCl_2$-0.06g, $NaHC0_3$-0.5g and Glucose-0.5g. Uterine strip, about 2cm in length was cut out and suspended vertically in a 35ml organ bath by means of ligatures attached at one end to a tissue holder and at the other end to an isometric force displacement transducer attached to a physiograph. The physiograph was connected to a computer screen for displaying isometric contractions. Resting tension on the muscle strip was readjusted, just sufficient to remove slack and the preparation was allowed to equilibrate within 40 minutes of mounting. In all the experiments, a 2 minutes time was allowed for individual tissue responses before being washed 2-3 times with de jalons solution.

All concentrations of test substances given in the text are final bath concentrations (FBC), except otherwise stated.

Serial dilutions were made for reference drugs and ELMO and administered in the following order:

1. Graded doses of Acetylcholine (Sigma, USA)(1.43, 2.86, 5.70, 8.5 and 11.40µg/ml)
2. Graded doses of Oxytocin (Shanxi Co., China)(1.43, 2.86, 5.70, 8.5 and 11.40µg/ml)

3. Graded doses of ELMO(1.43, 2.86, 5.70, 8.5 and 11.40µg/ml)
4. Graded doses of Acetylcholine in the presence of Atropine(Sigma, USA)
5. Graded doses of Oxytocin in the presence of Atosiban.
6. Graded doses of Acetylcholine in the presence of ELMO
7. Graded doses of Oxytocin in the presence of ELMO

2.4 Statistical Analysis

Results were expressed as Means ± Standard error of Mean (SEM) and analyzed by one way analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS for windows, version 15.0). P. values less than 0.05 were adjudged as being significant.

3. RESULTS

3.1 Effects of Acetylcholine, Oxytocin and ELMO on the Rat Uterus

All doses of Acetylcholine and Oxytocin significantly ($P<0.05$) increased contractions of the rat uterus when compared to basal values. All doses of ELMO significantly lowered the amplitude of spontaneous contractions of the rat uterus in a dose dependent manner with a final bath concentration of 11.40 µg/ml lowering the amplitude of spontaneous contractions from 8.63±0.98 in basal to 1.40±0.26 (Table 1, Figs. 1-3).

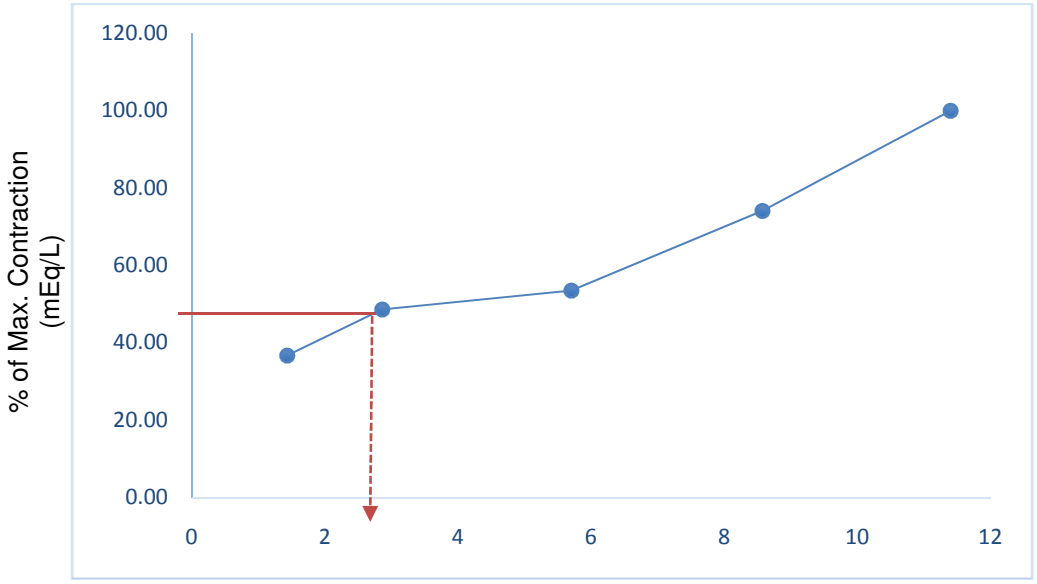
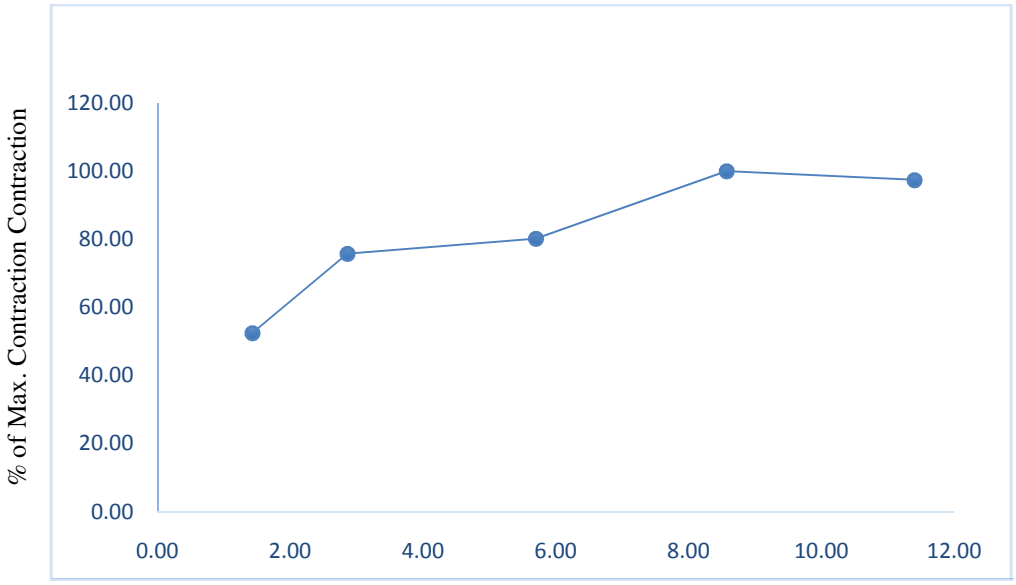
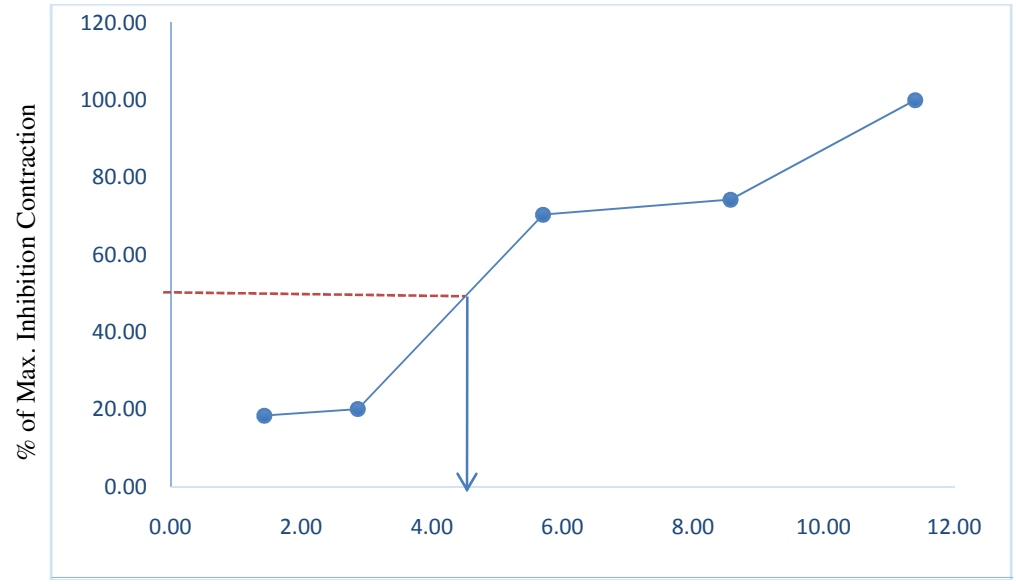


Fig. 1. Dose response curve for acetycholine



EC_{50} = 1.80µg/ml FBC (µg/ml)

Fig. 2. Dose response curve for Oxytocin



IC_{50} = 4.21µg/ml FBC (µg/ml)

Fig. 3. Dose response curve for ELMO

Table 1. Effects of acetylcholine, oxytocin and ELMO on the rat uterus

FBC (µg/ml)	Basal values (mm)	Height of contraction in response to acetylcholine (mm)	Height of contraction in response to oxytocin (mm)	Height of contraction in response to ELMO (mm)
1.43	9.34±0.97	14.80±0.76*	13.20±0.54*	8.00.±0.20*
2.86	9.00±1.05	16.24±0.22*	14.58±0.70*	7.54±1.05*
5.70	10.20±0.43	18.16±0.71*	16.10±0.91*	5.11±0.96*
8.57	8.87±0.75	19.90±0.43*	16.23±0.83*	3.50±0.60*
11.40	8.63±0.98	23.52±1.25*	15.80±0.79*	1.40±0.26*

**P<0.05 versus basal value; FBC = Final Bath Concentration*

3.2 Effects of Antagonists and ELMO on Acetylcholine and Oxytocin Induced Uterine Contractions

A final bath concentration of 8.57µg/ml of ELMO significantly (P<0.05) inhibited the effect of Acetylcholine on the rat uterus but had no effect on Oxytocin induced uterine contractions (Table 3). The inhibitory effect of ELMO compared favorably with that of Atropine (0.029µg), a standard muscarinic receptor blocker (Table 2).

Table 2. Effects of atropine and atosiban on acetylcholine and oxytocin induced uterine contractions respectively

Acetylcholine + Atropine (0.029µg/ml)			Oxytocin + Atosiban (20µg/ml)	
FBC(µg/ml)	Height of contraction (mm)	% of inhibition	Height of contraction in (mm)	% inhibition
1.43	0.00+ 0.00	100.00	0.00 + 0.00	100.00
2.86	0.00 + 0.00	100.00	0.00 + 0.00	100.00
5.70	2.00 + 0.20	75.00	0.00+0.00	100.00
8.57	1.00 + 0.15	85.30	2.00 + 0.16	60.00
11.40	1.00 + 0.18	71.43	1.00 + 0.09	82.14

Table 3. Effect of ELMO (5.70µg/ml) on acetylcholine and oxytocin induced uterine contractions

Response to acetylcholine + ELMO			Response to oxytocin + ELMO	
FBC(µg/ml)	Height of contraction (mm)	% Inhibition	Height of contraction in (mm)	% inhibition
1.43	0.00±0.00	100.00	4.00±0.15	0.00±0.00
2.86	0.00±0.00	100.00	5.00±0.13	8.70±0.31
5.70	0.00±0.00	100.00	5.80±0.15	0.00±0.00
8.57	1.10±0.10	83.80	5.60±0.20	0.00±0.00
11.40	1.40±0.10	80.00	5.00±0.22	10. 71±0.15

4. DISCUSSION

Acetylcholine and oxytocin induced contractions of the isolated rat uterine muscles by their activities on muscarinic and oxytocin receptors respectively. The presence of numerous muscarinic receptors, particularly the M_2 and M_3 types in the uterus have been reported

[6,7,8]. A muscarinic agonist like Acetylcholine binds to these receptors resulting to the activation of the inositol triphosphate (IP_3) and diacylglycerol (DAG) cascade. Both IP3 and DAG have been implicated in the opening of the smooth muscle calcium channels and release of calcium from the endoplasmic and sarcoplasmic reticulum [9]. These chain of physiological processes mediates Acetylcholine induced uterine contractions. Oxytocin on the otherhand induced uterine contractions by binding to the oxytocin receptors which are present in the uterine smooth muscles. The drug-receptor complex so formed causes myometrial contractions by increasing intracellular calcium ion and production of prostaglandins [10]. Any agent capable of blocking the contractile effects of Acetylcholine and Oxytocin by binding to their respective receptors will cause uterine relaxation. The effect of such an agent on the uterus mimics a Betamimetic drug. Betamimetic drugs usually bind to Beta receptors in the uterus to cause relaxation of the uterus [11].

The results of this study has shown that ELMO relaxed the rat's uterine smooth muscles by its action on the uterine muscarinic receptors evidenced by significant ($P<0.05$) inhibition of the effect of Acetylcholine and had no significant effect on Oxytocin induced uterine contractions. These results suggest that ELMO may contain active anticholinergic principles capable of relaxing uterine smooth muscles via the muscarinic receptor pathway. By blocking the parasympathetic arm innervation of the uterine muscles and allowing the sympathetic arm (Beta pathway) to have upper hand, ELMO caused relaxation of the uterine smooth muscles. This result tends to agree with [12] who reported that fractions of *Moringa oleifera* inhibited the spontaneous contractions of the rat uterus.

5. CONCLUSION

In conclusion, results obtained indicate that ethanol leaf extract of *Moringa oleifera* is a potent agent with uterine muscle relaxing potentials which acts by the anticholinergic mechanism via muscarinic receptors. The extract may not be valuable in managing oxytocin induced uterine contractions, especially in pregnancy, as most tocolytic agents do but its anticholinergic potentials may be exploited and employed in the management of disorders associated with hyperactivity of the parasympathetic arm of the autonomic nervous system.

6. LIMITATIONS OF STUDY

This work was not without some limitations due to financial constraint and unavailability of some drugs which could be used to explore in totality the mechanisms of ELMO actions.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee".

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee as practiced in Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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