The Pharmacodynamic Profile of Hydroethanolic Seed Extract of *Moringa Oleifera* on Intestinal and Uterine Contractility

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A B S T R A C T

Moringa oleifera is extensively distributed and used in many countries all over the world. The seed of *Moringa oleifera* is a high value part and has an amazing medicinal and therapeutic uses with high nutritional value. This study, therefore, was designed to clarify the pharmacological effects of hydroethanolic seed extract of *Moringa oleifera* (HSMO) on isolated intestinal and uterine motility with a view to finding out the site of actions of *Moringa oleifera* (MO). Tension recording technique was used for studying the effect of *Moringa oleifera* seeds extract on isolated rabbit's duodenum, guinea pig's ileum, rat's colon and uterus. Results obtained showed that HSMO produced a concentrations dependent relaxation of intestinal muscle. The minimal effect of MO was observed at concentration of 4 µg/ml bath that produced 6.3% inhibition of duodenum contractility. Complete relaxation of duodenum smooth muscle was established after the addition of MO at concentration of 512 µg/ml bath. While in isolated guinea pig's ileum and rat's colon, complete inhibition was established after the addition of MO at concentration of 256 µg/ml bath. HSMO inhibited the contractility of rat's uterus during nonpregnant and pregnant stages. Complete relaxation was attained by addition of 256 µg of MO extract /ml bath. The inhibitory action of MO could be attributed to, at least partly, its action on muscarinic receptors, indicated by decreasing stimulant effect of Ach. The phytochemical screening of *Moringa oleifera* seeds in our investigation indicated the presence of flavonoids and tannins and these components play a key role in spasmolytic activity by blocking muscarinic receptors. It could be concluded that, MO may have a depressant effect on intestinal and uterine muscles via muscarinic cholinergic pathway. These data may support the effective and safe use of MO in gastrointestinal disorders and pregnancy and these finding may give evidence to its traditional use on the management of intestinal ailments such as pains, diarrhea and dysentery.

Key words: *Moringa oleifera*, Smooth muscle, Spasmolytic activity, Autonomic pharmacology.

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1. INTRODUCTION

Moringa oleifera (Family: *Moringaceae*) is a fast growing tree. *Moringa oleifera* has been adapted in many tropic and sub tropic regions of the world. The plant is referred to number

of names such as miracle tree, ben oil tree (Coppin, 2008). It is a small or medium sized tree about 10 to 12 m in its height, open crown of drooping fragile branches (Roloff *et al.*, 2009). Fruits contain 20 seeds which are globular, 1 cm in diameter. Seeds are three-angled and on average weigh 0.3 g, with the kernel responsible for 70-75% of the weight (Sengupta and Gupta, 1970). *Moringa* are a good source of protein, amino acids, vitamins, minerals, phytochemical compound (alkaloids, glycosides, sterols, flavonoids, saponin, tannins and various phenolics (Anwar et al., 2007 and Mehta *et al.*, 2011). All parts of the plant are edible; the leaves can be eaten raw. The seeds can be removed and pressed to extract high quality oil (Ben oil) rich in oleic acid. The seeds of *Moringa oleifera* when compared to other parts of the plant, were found that it is high value part and has amazing range of medicinal and therapeutic uses. The seeds contain 38-40% oil can be used for cooking, in soaps and perfumes (Ogbunugafor et al., 2011). A significant amount of thiamin, riboflavin, nicotinic acid, folic acid, pyridoxine, ascorbic acid, beta -carotene and alpha -tocopherol were detected in seeds (Dahot, 1988). Seeds are extensively used for liver diseases, hematological disorders, treating inflammation, cardiovascular action, used as antidiabetic (Caceres *et al.*, 1992), antimicrobial activity against gastrointestinal pathogens (Fowoyo and Oladoja, 2015) and having efficacy in purification by flocculation of contaminants in drinking water (Fahey, 2005).

Moringa oleifera plant is widely used in management of gastrointestinal disorders and safely consumed by pregnant women in traditional medicine and there is no clear scientific basis for such use.

Thus, the aim of the present study is to investigate the pharmacodynamic profile of hydroethanolic seeds extract of *Moringa oleifera* on isolated intestinal and uterine smooth muscle to verify its use in folk medicine.

2. MATERIALS AND METHODS

2.1. Materials.

2.1.1. Moringa oleifera

The seeds of *Moringa oleifera* were purchased from Haraz - Company of agricultural seeds, spices and medicinal plants, Cairo, Cairo Governorate, Egypt. The seeds were collected in March 2017.

2.1.2. Chemicals

1. Acetylcholine chloride (Hoffman- La Roche, France), propranolol (Ram mandir, Goregaon, Mumbai) and nicotine sulphate (Hopkin and Williams, England).

2.1.3. Perfusion fluids for pharmacological experiments:

The isolated rabbit's duodenum, guinea pig's ileum and rat's colon were suspended in the organ bath containing warm oxygenated Tyrod's solution at 37°C with the following composition (sodium chloride 8.00 g, potassium chloride 0.20 g, calcium chloride 0.20 g, magnesium chloride 0.10 g, sodium dihydrogen phosphate 0.05 g, sodium bicarbonate 1.0 g, glucose 1.0 g and distilled water to 1000 ml). While uterine muscles were suspended in Dale's solution at 37°C with the following composition (sodium chloride 9.00 g, potassium chloride 0.42 g, calcium chloride 0.24 g, sodium bicarbonate 0.50 g, glucose 0.50 g).

The above mentioned physiological salt solutions were prepared as indicated by Department of Pharmacology, University of Edinburgh.

2.1.4. Laboratory animals:

Rats, rabbits, guinea pigs, were used for studying the pharmacological effects of hydro ethanolic extract of *Moringa oleifera* seeds. Guidelines for Care and Use of Laboratory Animals in Biomedical Research. All animals used in this study were housed at the Animal House at 23-25°C and were given a standard

diet and tap water ad libitum. The animals were submitted to a 12h light/dark cycle with free access to food and water. Twelve hours before experimentation, the food was withdrawn, but water remained available *ad libitum.*

Rabbits

Male rabbits weighing (1.5 – 2 kg) were employed for studying the effect of hydro ethanolic extract of *Moringa oleifera* seeds on isolated duodenum motility.

Rats

Rats of both sex weighing (150 – 200 g) were used for studying the effect of *Moringa oleifera* on isolated colon and uterine muscle.

Guinea pigs

Male guinea pigs of different weights (400 – 500 gm) were used for studying the pharmacological action of hydro ethanolic extract of *Moringa oleifera* seeds on isolated ileum.

2.1.5. Instruments

1-Shaking water bath (model YCW-012, Gemmy industrial corp., Taiwan) was used for concentration of hydroethanolic extract of *Moringa oleifera* seeds.

2-Glass jar bath

The device composed of a glass water bath of about 750 ml capacity fitted onto a metal stand. A movable electric heater was located into the stand to maintain the temperature as required. An inner glass tube (organ bath) of 50 ml capacity passed through the bottom of the stand. The organ bath was connected by a T- shaped glass tube, one of its branches was connected to a rubber tubing with a clamp which was used for draining the solution. The other branch was connected to a bottle at a high level containing fresh physiological solution. In the organ bath, an oxygen tube (glass cannula) was firmly held by means of a clamp fitted to one of the two stand uprights, on the other stand upright there was an ink writing lever attached to a kymograph.

3-Kymograph

A kymograph, (Griffin George Ltd., London, England) with drum was used for studying the effect of hydro ethanolic extract of *Moringa oleifera* seeds on isolated organ. Drum was covered by divided paper from Harvard apparatus Ltd. Chart no 50-5362 for recording tracing of other isolated organs experiments.

2.2. Methods:

2.2.1. Preparation of hydro-ethanolic extract of *Moringa oleifera* seeds:

Moringa oleifera seeds were refluxed with bi-distilled water, shade dried at room temperature. Extract were prepared by macerating (100 g) of crushed seeds in a known volume (1 Litre) of water/organic solvent (bi-distilled water: absolute ethanol, 70:30, v/v). Maceration continued for 72 hours in refrigerator with intermittent shaking. The hydro-ethanolic extract was then strained through muslin mesh, filtered through Whatman paper 1. The obtained filtrate was then concentrated using a shaking water bath at 70 °C and the obtained residue was semi solid extract (brownish colour). it was then weighed. It re-constituted by dissolving in measured amount of iso saline (0.85%, w/v) or hydro ethanol (30%). The extract was stored in air tight container in refrigerator below 10 °C. For *in vitro* studies, a stock solution of 1 mg/mL was prepared and then serially diluted to get 256, 128, 64, 32, 16, 8, 4, 2 & 1 µg/ml. The method was modified after Harborne, (1973).

Percentage yield was determined using the formula:

$$\text{Yield \%} = \frac{\text{weight of extract}}{\text{weight of plant material}} \times 100$$

2.2.2. Tension recording technique

The method described by the Staff member of Department of Pharmacology, University of Edinburg (19٧٠) and Valeri et al., (1990) was used for studying the effect of *Moringa oleifera* seeds extract on isolated rabbit's duodenum, guinea pig's ileum and rat's colon.

Animal were slaughtered, viscera were exposed and the parts of the intestine (duodenum, ileum and colon) was dissected out and kept in Tyrod's solution warmed at 37 °C. An intestinal strip of about 2 centimeters. Length was taken and suspended in the organ bath, where one end was fixed to the glass cannula, while the other end was tied by a thread to a lever with ink writer. Staff member of Department of Pharmacology, University of Edinburg (١٩٧٠) described the method of studying the effect of *Moringa oleifera* seeds hydro ethanolic extract on uterine smooth muscle of rats at various stages of sex cycle (non oestrus, oestrus, early pregnant and late pregnant). Female rats uteri were dissected out and one uterine horn was fixed in an organ bath containing warm oxygenated Dal's solution at 37 °C. The tissue was subjected to a resting tension of 1 g and allowed to equilibrate for 30 min and then the effect of graded increased concentrations of *Moringa oleifera* was demonstrated on the normal rhythmic motility of the isolated intestinal and uterine smooth muscle. The drum of the kymograph was covered by divided paper from Harvard apparatus Ltd. Chart no 50-5362. The speed of the kymograph was 0.25 millimeters /second. The site of action of the *Moringa oleifera* was located after studying the effect of *Moringa oleifera* on intestinal and uterine motility.

3. RESULTS

The percentage yield of the extract was found to be 12.5 %.

The effects of graded increased concentrations of HSMO on the contractility of intestinal preparations, including rabbit's duodenum, guinea pig's ileum and rat's colon are recorded in Table 1 and shown in figure (1 and 2). HSMO at concentrations up to 2 µg/ml bath had no effect on the duodenal contractility. The minimal effect of MO was observed at concentration of 4 µg/ml bath that produced 6.3% inhibition of duodenum contractility. Complete relaxation of duodenum smooth muscle was established after the addition of MO at concentration of 512 µg/ml bath. While in isolated guinea pig's ileum and rat's colon, minimum relaxation was achieved by 2 µg of *Moringa oleifera* /ml bath. Complete relaxation was established after the addition of MO at concentration of 256 µg/ml bath.

Trials were performed to locate the site of action of HSMO on the rabbit's duodenum. Concentrations of the drugs that produced maximal inhibitory effects were used in such experiments. To investigate the hypothesis that MO produce it's inhibitory effect on rabbit's duodenum, nicotine at a small concentration (1 µg/ml bath) was added and able to evoke its stimulatory effect in spite of the presence of *Moringa oleifera* Extract onto the duodenal preparations (Figure 3).

To study the probability of involvement of adrenergic pathway in the inhibitory effect of HSMO on rabbit duodenal preparations. the MO extract was added after pre-addition of propranolol (non-selective beta blocker, 1 µg/ml bath). The MO extract produced its inhibitory effect despite blocking the inhibitory β2 adrenergic receptor (Figure 4).To examine depressant effect of MO extract *via* blocking of muscarinic receptors, Ach (0.25 µg/ml bath) was added to the duodenal preparation in presence of MO extract and it's effect was compared to the effect of Ach alone. Extract of *Moringa oleifera* produced a decrease in the contractile effect of Ach on the duodenal motility (Figure 5).

The effects of graded increased concentrations of HSMO on rat uterine contractility at various stage of sex cycle were almost similar, therefore, we presented only those of the oestrus stage, which are recorded in Table 2 and shown in figure 6 and 7). HSMO at concentrations up to 1 µg/ml bath had no effect on the uterine contractility. The

minimal effect of MO was observed at concentration of 2 µg/ml bath that produced 13.4 % inhibition of uterine contractility. Complete relaxation of uterine smooth muscle was produced after the addition of MO at concentration of 256 µg/ml bath.

Trials were applied to locate the site of action of HSMO on the rat's uterus. Concentrations of the drugs that produced maximal inhibitory effects were used in such experiments. To investigate the hypothesis that MO produce it's inhibitory effect on rat's uterus via adrenergic pathway in the inhibitory effect of HSMO on rabbit duodenal preparations. the MO extract was added after pre-addition of propranolol (non-selective beta blocker, 1 µg/ml bath). The MO extract produced its inhibitory effect despite blocking the inhibitory β2 adrenergic receptor (Figure 8). To study depressant effect of MO extract via blocking of muscarinic receptors, Ach (0.25 µg/ml bath) was added to the uterine preparation in presence of MO extract and its effect was compared to the effect of Ach alone. Extract of *Moringa oleifera* produced a decrease in the contractile effect of Ach on the uterine motility (Figure 9).

Table 1: Effects of graded concentrations (0.5~512 µg/ml bath) of HSMO on isolated rabbit's duodenum. (mean±SEM; n=3).

Conc. (µg/ml bath)	Response of rabbit's duodenum motility.	
	HSMO amplitude (g)	HSMO inhibition (%)
Pretreated	0.400±0.289	0.000±0.000
0.5	0.400±0.289	0.000±0.000
1	0.400±0.289	0.000±0.000
2	0.400±0.289	0.000±0.000
4	0.375±0.029	6.300±0.448*
8	0.350±0.029	12.632±0.919*
16	0.323±0.027*	19.320±1.091*
32	0.280±0.019*	31.440±2.194*
64	0.227±0.016*	43.312±0.258*
128	0.175±0.014*	56.314±0.461*
256	0.083±0.022*	79.728±3.969*
512	0.000±0.000*	100.000±0.000*

*Significantly different from pretreated ($p \leq 0.05$; ANOVA followed by LSD test).

Table 2: Effects of graded concentrations (0.5~256 µg/ml bath) of HSMO on isolated rat's uterus (oestrus) (mean±SEM; n=3).

Conc. (µg/ml bath)	Response of uterine motility Rat's uterus.	
	HSMO amplitude (g)	HSMO inhibition (%)
Pretreated	0.430±0.017	0.000±0.000
0.5	0.430±0.017	0.000±0.000
1	0.430±0.017	0.000±0.000
2	0.371±0.018*	13.415±3.959*
4	0.336±0.019*	21.712±2.685*
8	0.315±0.017*	26.496±3.602*
16	0.263±0.013*	38.820±0.963*
32	0.191±0.017*	55.658±2.555*
64	0.129±0.014*	70.078±2.513*
128	0.057±0.008*	86.746±1.445*
256	0.000±0.000*	100.000±0.000*

*Significantly different from pretreated ($p \leq 0.05$; ANOVA followed by LSD test).

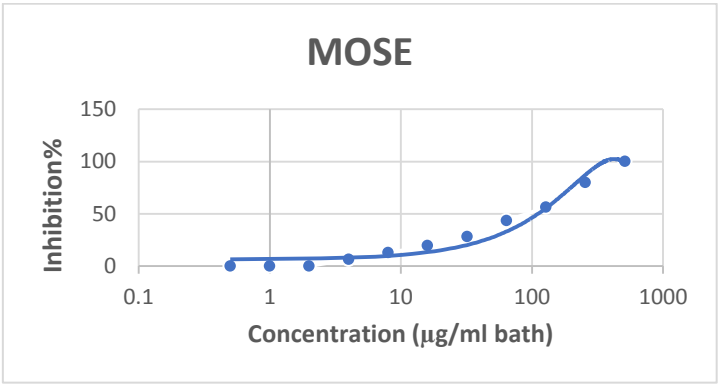

Figure 1: Inhibition % produced by graded concentrations (0.5~512 µg/ml bath) of HSMO on isolated rabbit's duodenum (mean±SEM; n=3).

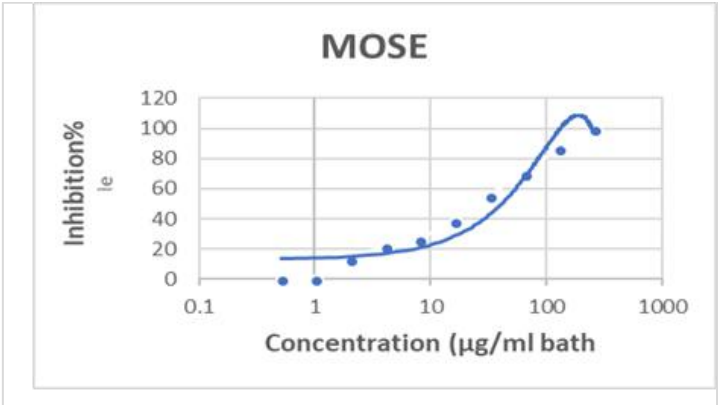

Figure 2: Inhibition % produced by graded concentrations (0.5~512 µg/ml bath) of HSMO on isolated rat's uterus (mean±SEM; n=3).

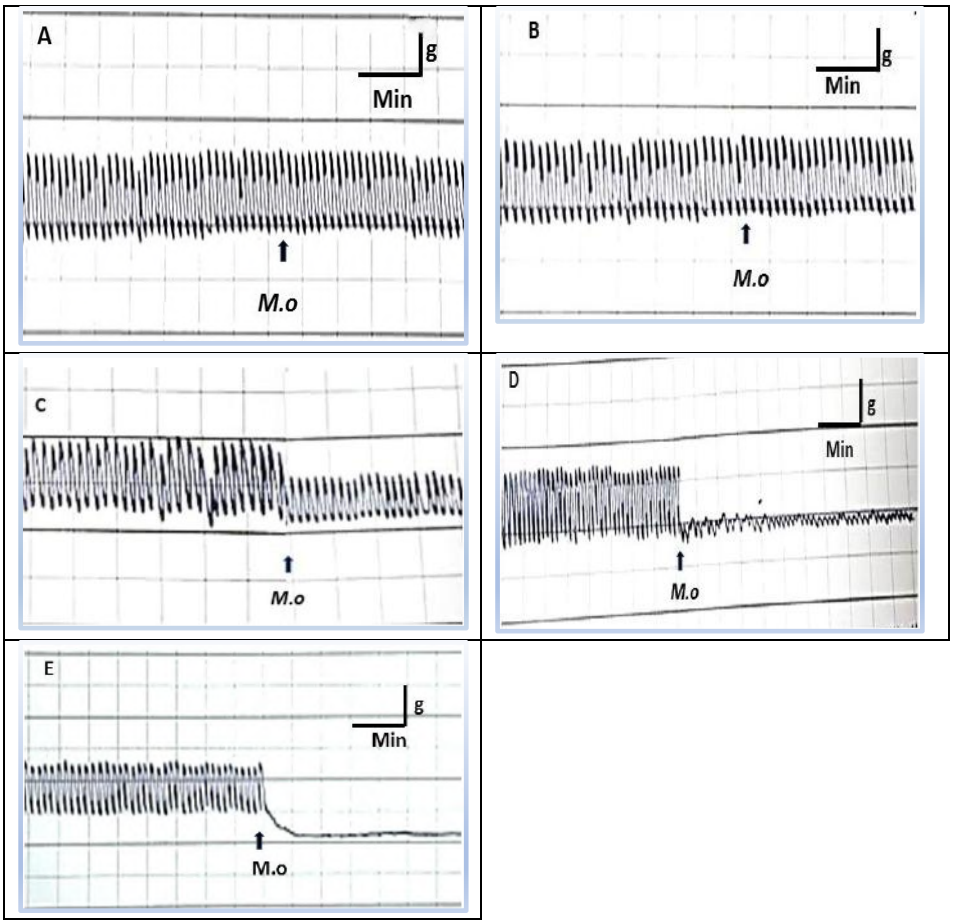

Figure 3: Effect of hydro ethanolic seeds extract of *Moringa oleifera* on rabbit's duodenum (A) Effect of 2 µg/ml bath HSMO, (B) 4 µg/ml bath HSMO, (C) 32 µg/ml bath HSMO,(D) 128 µg/ml bath HSMO and (E) 512 µg/ml bath HSMO.

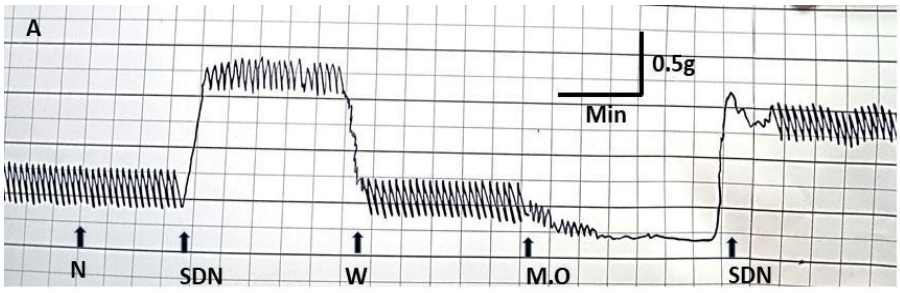

Figure 4: Effect of 0.25 µg/ml bath of nicotine (NSD) alone and after HSMO 512 µg/ml bath on rabbit's duodenum.

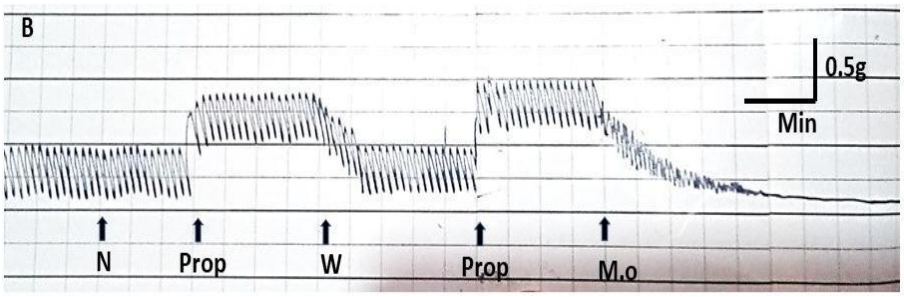

Figure 5: Effect of 512 µg/ml bath HSMO after propranolol (prop., non selective β-blocker; 1 µg/ml bath) on rabbit's duodenum.

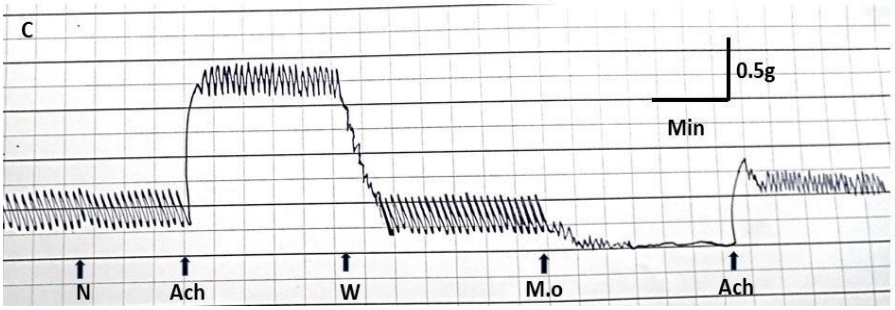

Figure 6: Effect of 0.25 µg/ml bath acetylcholine (Ach) alone and after 512 µg/ml bath HSMO on rabbit's duodenum.

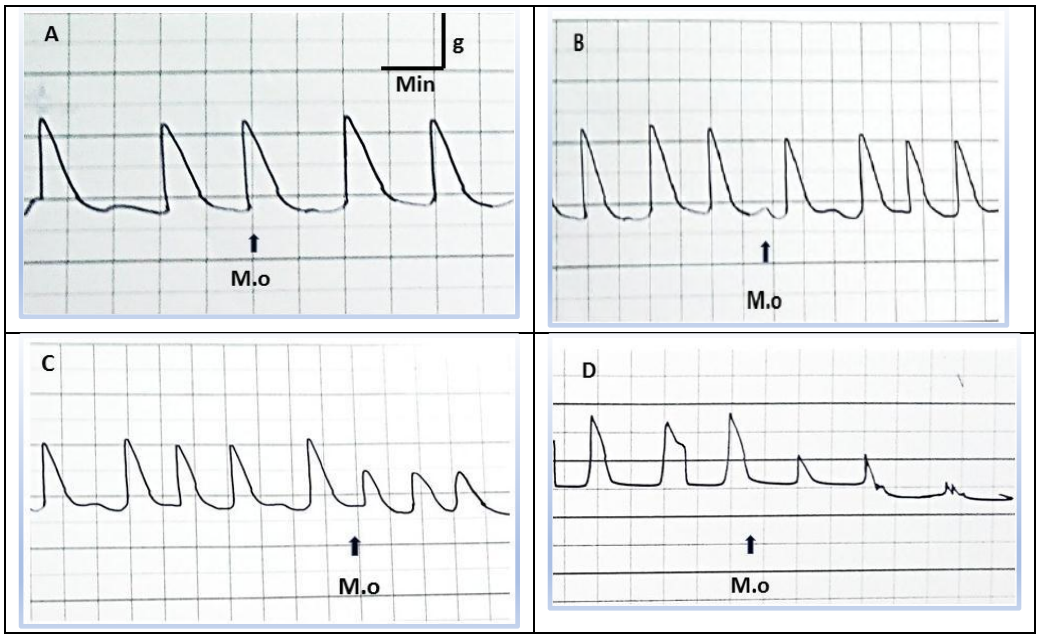

Figure 7: Effect of hydro ethanolic seeds extract of *Moringa oleifera* on isolated rat's uterus (oestrus): (A) Effect of ١ µg/ml bath HSMO, (B) 8 µg/ml bath HSMO, (C) ٦٤ µg/ml bath HSMO and (D) 128 µg/ml bath HSMO.

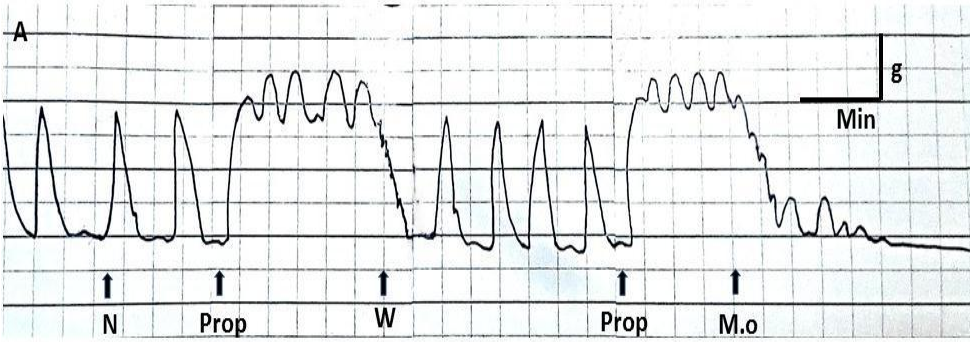

Figure 8: Effect of 512 µg/ml bath HSMO after propranolol (prop., non-selective β-blocker; 1 µg/ml bath) on rat's uterus.

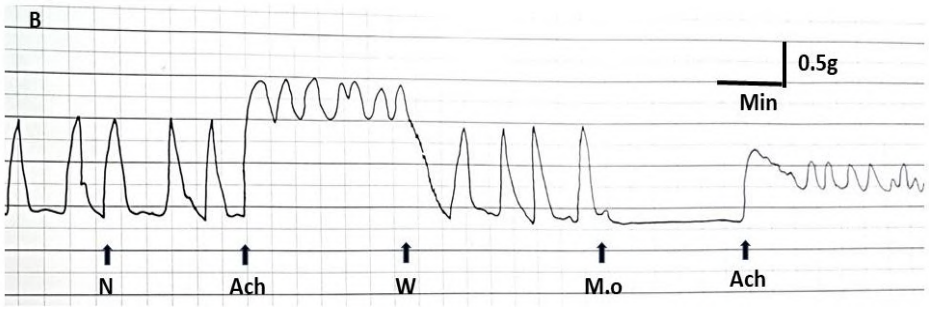

Figure 9: Effect of 0.25 µg/ml bath acetylcholine (Ach) alone and after 512 µg/ml bath HSMO on rat's uterus.

The obtained results revealed that *Moringa oleifera* produced its inhibitory effect via muscarinic receptor pathway by decrease the stimulatory effect of Ach.

4. DISCUSSION

Despite *Moringa oleifera* seeds are extensively used as hepatoprotective, antidiabetic, anti-inflammatory, antioxidant, antihypertensive, antibacterial and antifungal effects, however, there is no a clear scientific evidence have been reported about the pharmacodynamic effect of MO on intestinal and uterine muscle. The present work, therefore, was performed to investigate some pharmacodynamic effects of HSMO on isolated rabbits' duodenum, guinea pig's ileum, rat's colon and rat's uterus as a farm animal model in vitro.The present investigation showed that, HSMO, *in vitro*, inhibited the contractility of rabbits' duodenum, guinea pig's ileum and rat's colon. The inhibitory effect of HSMO was proportional to the graded tested concentrations. The maximal inhibitory responses were recorded at 512 µg/ml bath for rabbits' duodenum and 256 µg/ml bath for guinea pig's ileum and rat's colon. Data of the present study may be partially inconsistent with (Somé *et al.*, 2015) who reported that MO extract failed to display significant activity on rat duodenum basal tone when applied to the organ bath. These disparities among the results of the present study and those of other studies may be attributed to different parts of plant used, concentrations, methodologies and different environmental

conditions as well as different experimental animals that have been used.

Addition of small dose of nicotine (0.25 µg/ml bath) as a nicotinic (ganglionic) blocker in the presence of HSMO produced its characteristic stimulant action indicating that the inhibitory effect of Mo at the tested concentrations on duodenal motility did not involve ganglia. Similarly, application of propranolol (1 µg/ml bath) as adrenergic antagonist for β receptor did not affect the inhibitory responses of duodenal contractility to HSMO, indicating that involvement of adrenergic pathway in the action of HSMO is unlikely. Addition of Ach (0.25 µg/ml bath) as a muscarinic cholinergic agonist in the presence of HSMO produced a decrease of its stimulant action indicating that the inhibitory effect of HSMO at the tested concentrations involved muscarinic receptor pathway. From the abovementioned trial to locate site of action of Mo, the inhibitory action of Mo on rabbit's duodenum, therefore, may be attributed to its action on the duodenal muscarinic receptors indicated by decrease contractile effect of Ach. Ach is the major neurotransmitter in gastrointestinal tract, produce contractions by activating muscarinic receptors. Numerous muscarinic receptors were identified in smooth muscle layer and five subtypes of muscarinic receptors, namely M1, M2, M3, M4 and M5 have been identified (Tobin *et al.,* 2009). Specifically, M2 and M3 receptors played a key role in mediating their activity in intestinal smooth muscle (Unno *et al.,* 2005). The muscarinic receptors are coupled to G proteins, resulting in the activation of phospholipase C (PLC) and the formation of inositol trisphosphate (InsP3) and diacylglycerol (DAG). These two substances released in the cytoplasm will interact with the receptor on intracellular calcium store sites and causes calcium release from intracellular stores or calcium influx into the cell, resulting in contraction of smooth muscle (Kirschstein *et al.,* 2009). Therefore, the extract of MO can act in this way by blocking chain of physiological processes which lead to calcium release and then inhibited the contractions induced by Ach. The phytochemical analysis of *Moringa oleifera* seeds in our experimentation indicated the presence of flavonoids and tannins and this is in harmony with the reported by Akineye *et al.*, (2013) that these components exhibit spasmolytic activity. Flavonoids isolated from other medicinal plants showed spasmolytic activities on smooth muscles preparations. Mehmood *et al.,* (2011) showed that the flavonoids exhibited antispasmodic activity by blocking muscarinic receptor and calcium channels. On the other hand, George et al., (2002) stated that tannin-containing plant extracts are used as astringents and against diarrhea. Certainly, tannin exhibit valuable role in smooth muscle relaxation.

These findings may be in accordance with Gilani *et al.*, (1994) who reported that fractions of *Moringa oleifera* depressed the spontaneous contractions of gastrointestinal motility and antispasmodic effect exhibited by the ingredients of the plant provides a logical basis for the popular uses of the plant in gastrointestinal motility disorders.

Also, these results tend to agree with Somé *et al.*, (2015) who concluded that the aqueous leaf extract of *Moringa oleifera* decreased the contractile effects of Ach on duodenal motility and produced a dose dependent relaxation of these muscles. These results indicate that aqueous leaf extract of *Moringa oleifera* possesses antispasmodic effects on isolated duodenum smooth muscle.

HSMO *in vitro* inhibited the contractility of rat's uterus during nonpregnant stages (estrus and non estrus) and during pregnant stages (early and late pregnancy). The inhibitory effect of HSMO was dose dependant. The

maximal inhibitory responses were recorded at 256 μg/ml bath.

Application of propranolol (1 μg/ml bath) as adrenergic antagonist for β receptor did not affect the inhibitory responses of uterine contractility to Mo, indicating that involvement of adrenergic pathway in the action of Mo is unlikely. Addition of Ach (0.25 μg/ml bath) as a muscarinic cholinergic agonist in the presence of Mo produced a decrease of it's stimulant action indicating that the inhibitory effect of Mo at the tested concentrations involved muscarinic receptor pathway. From the aforementioned trial to locate site of action of Mo, the inhibitory action of Mo on rat's uterus, therefore, may be attributed to its action on the uterine muscarinic receptors via decrease stimulant effect of Ach on uterine contractility. Ach produced uterine contractions by it's activity on muscarinic receptor. The muscarinic receptor is bind to G proteins, resulting in the activation of (PLC) and the formation (InsP3) and diacylglycerol (DAG) (Prestwich and Bolton, 1995) InsP3 causes release of calcium from its cellular stores (Morel et al., 1997). The obtained results were consistent with those recorded by (Ijioma *et al.*, 2014) who stated that, ELMO relaxed the rat's uterine smooth muscles by its action on the uterine muscarinic receptors. ELMO may contain active anticholinergic principles capable of relaxing uterine motility via the muscarinic receptor pathway. It has been reported that some components isolated from the leaves of Mo inhibited the spontaneous contractions of the rat uterus (Gilani *et al.*, 1994) and this result is in a good agreement with our experimentation on rat uterus and phytochemical analysis of seed of Mo. Flavonoids and tannin were detected from the seeds of MO of our experimentation could play an important role on the relaxation of uterine smooth muscle.

5. CONCLUSION

Data of the present study indicated that HSMO possesses inhibitory effect on intestinal and uterine muscles and the spasmolytic activity on isolated intestinal and uterine contractility could be attributed to it's anticholinergic mechanism via muscarinic receptors. These results can explain the traditional use of *Moringa oleifera* seed extracts in the treatment of intestinal disorders such as pain and diarrhea and diseases associated with hyperactivity of the parasympathetic branch of the autonomic nervous system.

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