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Aqueous extract of *Moringa oleifera* leaf used in Nigerian ethnomedicine alters conception and some pregnancy outcomes in Wistar rat

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ABSTRACT

The leaf of *Moringa oleifera* (Lam) Moringaceae, is globally accepted in ethnomedicine and particularly used to tackle malnutrition and enhance breast milk production in resource-limited settings. It is reported to be laden with a wide array of interesting bioactive plant metabolites. However, its safety at conception and during pregnancy has not been well reported notably in Nigeria. This work investigated the *in vitro* and *in vivo* uteroactive and contraceptive potentials of cold and hot aqueous extracts of *M. oleifera* leaves (MOL) commonly used in Nigerian Traditional Medicine. In addition, we determined the physicochemical characteristics of the freeze-dried aqueous extracts and powder.

Powdered MOL (100 g each, 1:10 *w/v*) was extracted in the cold (room temperature) and hot (boiling) distilled water. The *in vitro* protocol involved the dose-dependent effect of aqueous extracts of MOL on the tension produced by the isolated uteri of non-pregnant rats using organ bath. Uterine contractility and contraceptive properties of MOL were assessed *in vivo* using female Wistar rats (150–170 g). Particle morphology, size, polydispersity index and zeta potential of MOL were determined.

The *in vitro* administration of cold and hot aqueous extracts of MOL significantly ($p < .05$) produced uterine contractility with varying intensities. Contractile effect of cold MOL aqueous extract (89.7%) was higher than that of hot MOL (50.6%). *In vivo*, MOL cold aqueous extracts administered before and after mating, respectively produced 100% and 80% inhibition of conception, while 96.6% and 58% effect was comparatively produced by hot aqueous extracts of MOL.

This work has provided useful data on MOL, a commonly consumed medicinal preparation/food supplement and the possible implications of its intake before conception, at conception and during pregnancy.

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1. Introduction

M. oleifera (MO) Lam. (Millsp) (Moringaceae), of the order Brassicales, is a prolific drought resistant tropical tree cultivated in Africa, South and Central America as well as the Asian Continent. The genus *Moringa* comprises 13 known species among which *M. oleifera* is more widely used, accepted and most studied. Previously underutilized in Africa and other tropical regions, the nutritional and ethnomedicinal applications of *Moringa* is receiving wide acceptance (Leone et al., 2018; Leone et al., 2015; Khoza et al., 2014; Olugbemi et al., 2010; Anwar et al., 2007; Fuglie, 2001). Documented evidence support the age long and widespread use of the plant as food and medicine (Fahey et al., 2018; Chodur et al., 2018), making it one of the most relevant trees to man in the universe (Lea, 2010). The high nutritional content of *Moringa* leaves has attracted much interest due to the levels of vitamin A, vitamin C, calcium, iron, potassium and adequately high protein quality which contends with that of milk and eggs. (Fuglie, 2001). Several of the numerous ethnomedicinal applications and folkloric claims on *Moringa* have been supported by scientific evidence, thus promoting an evidence-based utilization of medicinal plants for primary healthcare needs (Leone et al., 2015). In fact, every morphological part of the plant has its unique and sometimes overlapping traditional

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medical uses, in an effort to achieve primary healthcare needs as well as general wellness (Anwar et al., 2007). Some of these claims have been scientifically validated and reviewed to include antioxidant, chemopreventive, anti-inflammatory, anti-ulcer, anti-diabetic, anti-mutagenic, anti-microbial, neuroprotective, cytotoxic, herbicidal, anti-trypanosomal, anti-cancer, anti-hypertensive, cardioprotective, enzyme inhibition, hormone modulation and uterotonic (Cirmi et al., 2019; Ajuogu et al., 2019; Rani et al., 2018; Michl et al., 2016).

In addition to the amazing mineral, vitamin and primary metabolite content of *M. oleifera leaf*, it is also known to accumulate specific secondary metabolites, such as flavonoids, phenolics and glucosinolates, which are of chemotaxonomic relevance (Cirmi et al., 2019; Molina-vargas, 2013; Brunelli et al., 2010; Singh et al., 2009; Benett et al., 2003). Based on accepted taxonomic hierarchy of the plant, one common chemotaxonomic marker of the Brassicales (order), Moringaceae (family) and *Moringa* (genus) is the pharmacologically active glucosinolates (Fahey et al., 2018). Furthermore, abundant phytochemical markers of the *Moringa* genus include flavonoids, phenolic glycosides, isothiocyanates and phenolic acids (Manguro and Lemmen, 2007). However, the bioactivities of *Moringa* glucosinolates and isothiocyantes is currently attracting more scientific attention (Cheng et al., 2019; Cirmi et al., 2019; Fahey et al., 2018; Michl et al., 2016). A cardioprotective indole alkaloid, vincosamide as well as some unusual glycosides of pyrrole alkaloids have been isolated from the leaves of *M. oleifera* (Panda et al., 2013; Sahakitpichan et al., 2011). The leaf of the plant has additionally been reported to be a rich source of bioactive mini proteins and peptides some of which have been reported to produce antioxidant, toxicity and antimicrobial activities (Shi et al., 2019; Liang et al., 2019; Lin et al., 2019; Shi et al., 2018; Lin et al., 2018; Kini et al., 2017; Mansurah et al., 2015). These peptides produce hormone-or drug-like activity that eventually modulate physiological functions *via* binding interactions to specific receptors on target cells leading to induction of physiological responses (Fitzgerald and Murray, 2006). Some new, biologically active and highly stable cysteine-rich peptides identified as morintides, have recently been identified in *Moringa* leaves (Kini et al., 2017). Cysteine-rich peptides (cyclotides) expressed in the uterotonic African plant, *Oldenlandia affinis* (Rubiaceae), have been reported to show oxytocin-like activities and responsible for the uteroactive properties of the plant (Koehbach et al., 2013). This peptide-rich plant has been used in the Central African Republic to induce uterine contraction and facilitate childbirth (Gran, 1973).

Many uteroactive secondary metabolites have been isolated from medicinal plants including diterpenes such as kaurenoic acid, grandifloric acid, kauradinoic acid (Campos et al., 1997; Lozoya et al., 1983; Perusquia et al., 1985), Z-venusol, heterocyclic aldehyde and fatty acids (Veale et al., 1989; Sewram et al., 2001), steroidal saponins (Guo et al., 2008), plant sterols such as β-sitosterol (Promprom et al., 2010) and indole alkaloids (Van Dongen and de Groot, 1995). Phenolic compounds, steroidal saponins and phytosterols have been implicated in female hormonal modulation and infertility (Guo et al., 2008; Swart et al., 2003; Tamura et al., 1997).

Conception occurs during ovulation when the egg becomes fertilized by the sperm cell within the fallopian tube. Contraception on the other hand refers to the interruption of pregnancy *via* the disruption of ovulation, fertilization or implantation (Moroole et al., 2019). Processes leading to conception and normal progression of pregnancy could be distrupted during the estrous cycle resulting in abnormal ovulation, lack of conception and poor pregnancy outcomes (Al-Qarawi et al., 2000). The imbalance in the levels of female reproductive hormones could lead to undesirable pregnancy outcomes and implantation failure (Sharanabasappa et al., 2002). For instance, compounds which prolong estrous and diestrous phases are reported to have antifertility properties (Jain et al., 2016). Several indigenous plant extracts have been described to possess bioactivities that alter the smooth release of reproductive hormones culminating in the inhibition of conception or the contraction of the uterus (Attah et al., 2012; Gruber and O'Brien, 2011; Keshri et al., 1998).

The leaves of *M. oleifera* Lam (*Moringa*ceae) is one of such plants commonly used in Nigerian homes and ethnomedicine for the management of several diseases including male fertility enhancement and in ethnogynaecology (Ajuogu et al., 2019). For instance, the leaves of this plant have a history of use as an ethnomedicinal vegetable meal in northern Nigeria where fresh leaves is a component of a local meal made up of powdered groundnut cake, chili, sweeteners, and local roasted beef called "Suya" (Ambi et al., 2011). Nigerian women, especially those from northern geopolitical extraction are known for the intake of *M. oleifera* leaf meal to increase breast milk. This may partly account for the reason why many of these lactating women do not get pregnant during this period of accelerated intake of the leaves despite unprotected sexual activity following reports on the abortifacient and fertility regulation claims on various morphological parts of *M. oleifera* in other geographical locations (Ajuogu et al., 2019; Nwamarah et al., 2015; Anwar et al., 2005). This has raised the question about its potential usefulness as nutritional *cum* child-spacing supplement among economically deprived women. But an elaborate scientific investigation on the impact of *M. oleifera* leaves on female reproduction is required across various geographical parts of the world, particularly regions like Nigeria where the plant is well cultivated and consumed. Until now, there is a narrow and limited scientific documentation regarding the probable contraceptive or abortifacient potentials of the leaf meal in wistar rats as applied in the Nigerian ethnomedicine. However, leaves of *Moringa* species cultivated in a remote village in India have been reported to show abortifacient activities *in vivo* at certain doses (Nath et al., 1992; Sethi et al., 1988), validating its folkloric claims as an abortifacient (Basak et al., 2016). It is not known if this remotely validated claim is limited by environment and geography or otherwise common to all *Moringa* leaves growing in any part of the World including Nigeria. This necessitated an investigative assessment of sub-saharan *M. oleifera* such as those grown in South-west Nigeria where the plant is cultivated and highly consumed by the largest African black population, including reproductive females. In this investigation, the effect of the intake of low amounts of *M. oleifera* leaves before conception attempts as well as its effect during conception was assessed using both *in vivo* and *in vitro* models. The *M. oleifera* leaves species cultivated in Nigeria and applied in Nigerian ethnomedicine were obtained and used for the study. In addition, some physical properties of the aqueous leaf extract and lyophilized leaf powder were determined.

2. Materials and methods

2.1. Preparation of M. oleifera leaf (MOL) aqueous extract

M. oleifera leaves (MOL) were obtained from Herbal Point, a local herbal medicinal store and the main distributor of *Moringa* products located in Zaria, Kaduna State, Nigeria in 2013. More MOL samples were further collected from the farm site of a Traditional Medicine Healer (TMH) located in Ajobo area of Ibadan, Oyo State, Nigeria. All collected MOL samples were authenticated at Forest Herbarium Ibadan (FHI) with voucher number FHI 109853. Plant materials were air-dried under shade and powdered samples were prepared for solvent extraction. Powdered *M. oleifera* leaves (100 g each; 1:10 *w*/*v*) was respectively extracted by maceration (24 h) and infusion (30 min) under room temperature (25–30 °C) and boiling water (100 °C), classified as cold and hot extraction, just as the plant is taken locally as a vegetable and tea. Aqueous extracts were freeze dried (Gallenkamp, BK-FD18S, UK) and percentage yields calculated. Extracts were kept at 4 °C in a refrigerator until required for further use.

2.2. Animals

The female Wistar rats used during the experiment weighed between 150 and 170 g and were obtained from the Laboratory for Reproductive Physiology and Developmental Programming, Department of Physiology, College of Medicine, University of Ibadan, Nigeria. Animals were kept in appropriate cages with room temperature maintained within the range of 22–27 °C with a half day light cycle. Wood shavings not previously treated were used as cage beddings which were replaced daily. Animals were fed with standard rat diet (Ladokun feeds, Ibadan, Nigeria) and water *ad libitum*. Test extracts were reconstituted in distilled water and orally administered to animals to mimick the common route of administration of the plant as used in Nigerian ethnomedicine. All animals were handled humanely in line with the University of Ibadan animal welfare and ethics committee guidelines with approval number FVM/UI/A.6/201301 and in line with the internationally accepted principles for laboratory animal use and care as found in the European Community guidelines (EEC Directive of 1986; 86/609/EEC). The study was carried out in line with the NIH standard of care for experimental animals and good research practice.

2.3. In vitro contractility experiment

Uterine horns were obtained from non-pregnant female animals and freed from the surrounding. Each horn was placed in a petri dish containing physiological salt solution (PSS) consisting of 119 mmol/L NaCl, 4.7 mmol/L KCl, 1.2 mmol/L KH_2PO_4,1.2 mmol/L $MgSO_4$, 15.0 mmol/L $NAHCO_3$, 1.6 mmol/L$CaCl_2$ and 11.5 mmol/L glucose. The uterine horn was cut into strips and mounted on 50 mL jacketed tissue baths containing PSS. This solution received constant aeration with 5% CO_2 and 95% O_2 with the pH maintained at 7.4 using a pH meter model 305L (N. Boyer, UK) at a constant temperature of 37 °C. Time allowed for equilibration was 90 min as previously described by Salahdeen et al. (2014). During this time, the tissue strip was stimulated three times with a stabilized dose 60 mmol/L potassium Chloride (KCl) to obtain a reproducible response. The isometric contractile force of the uterine muscle strip was recorded using Ugo Basil (Model 7004; Ugo Basile, Varese, Italy) coupled to Data capsule acquisition system (Model 17400, Ugo Basile, Varese, Italy). Concentration response curve was obtained. After the stabilization period, extracts were tested following cumulative addition of graded doses (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6 mg/mL) to the organ bath. A steady state is usually achieved before the addition of the next dose. In a parallel experiment, the effect of oxytocin (standard drug, Sigma–Aldrich Chem., St. Louise, MI, USA) on the contraction of the uterus was equally determined to serve as the positive control.

2.4. In vivo experiment

Female Wistar rats (n = 30; n = 6 per group) within the weight range of 150–170 g were used for the study. Animals with regular estrus cycle were used for this experiment. Animals were mated with proven male breeders and were randomly grouped into five with each group comprising six animals. The day sperm cells were detected in the vagina smear of a paired rat was designated as day one of pregnancy. Group 1 was administered with distilled water (dosing vehicle) to serve as the control group; groups 2 and 3 were administered with 58 mg each of *M. oleifera* cold and hot aqueous leaf extracts respectively per kilogram body weight and terminated at least 6 h before mating. Distilled water was subsequently given to the animals *ad libitum* from day one of pregnancy. Groups 4 and 5 were further administered with 58 mg/kg body weight of cold and hot MOL extracts beginning from day one of pregnancy and continued until day 14 of pregnancy. The chosen low dose was based on the previous report regarding the abortifacient potentials of some Indian ethnomedicinal plants by Nath et al. (1992). Dams were closely observed for signs of behavioral changes during the experiment. Pregnancy length, delayed labor, mortality and birth complications were monitored. All pregnant animals from the test and control groups were allowed to litter naturally.

2.5. Physicochemical characterization of M. oleifera (MOL) leaf particles

2.5.1. Particle morphology

The surface morphology of particles of MOL (aqueous cold/hot extract and powder) were observed by scanning electron microscopy (Hitachi S-4000 microscope) according to an earlier method (Nnamani et al., 2014). The particle microstructure of MOL was determined.

2.5.2. Particle size and polydispersity index

The mean diameter and polydispersity index of the MOL particles (aqueous cold/hot extract and powder) were measured using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire, UK) equipped with a 10 mW He–Ne laser employing the wavelength of 633 nm and a backscattering angle of 173° at 25 °C.

2.5.3. Zeta potential analysis

The zeta potential of the *M. oleifera* leaf (MOL) particles (aqueous cold/hot extracts and powder) was determined *via* electrophoretic mobility measurements using a Zetasizer Nano-ZS (Malvern Instruments, Worceshtire, UK). The zeta potential was calculated applying the Helmholtz–Smoluchowski equation (n = 3).

2.5.4. Statistical analysis

Data obtained from this experiment have been expressed as means (± standard error of the means, SEM) and as percentages. Data were analyzed using one way analysis of variance (ANOVA) and student's T test where applicable. Unless otherwise stated, $P \leq .05$ was taken as level of significance. Bonferroni's posthoc multiple comparison test at 95% confidence interval of difference was used. Data comparisms, transformaions and graph plotting were achieved using GraphPad Prism Software (version 5.0, San Diego, CA, USA) and SPSS version 16.0 Chicago, USA.

3. Results

In a more holistic approach, we investigated the antifertility potentials, abortifacient activity, *in vitro* contractility and the physicochemical characteristics of aqueous extracts (cold and hot) and dried leaf powder of *M. oleifera* growing in Africa and used in Nigerian ethnomedicine. To demonstrate or eliminate the possible effect of soil, environment and climatic conditions on phytochemical content and bioactivity, similar low *in vivo* doses were carefully chosen following previous scientific reports on some abortifacient plants, including *M. oleifera* growing in an Asian continent (Nath et al., 1992; Sethi et al., 1988).

The cold aqueous extract gave 23% yield, while 26% was obtained for the hot aqueous extract. Fig. 1A, B show that extract (0.1–0.6 mg) induced uterine smooth muscle contraction in a concentration-dependent manner in pre-contracted isolated rat uterine smooth muscle strip with KCl (60 mM), with an EC_{50} of 0.27 ± 0.01 and 0.41 ± 0.02 mg/mL for cold and hot MOL extract-induced contractions respectively, while Appendix 1 presents effects produced by a standard drug, oxytocin. The contractile effect of the cold extract on the uterine strip was 39.2% greater than hot MOL extract-induced contraction. The results suggest that the extract induced uterotonic activities on the uterine smooth muscle strip isolated from non-pregnant rat.

Table 1 presents a summary of the effect of administration of cold and hot aqueous extracts of MOL at a dose of 58 mg/kg body weight and 50 mg/kg body weight, respectively on conception, fertility and birth outcomes. The control group, which was given only distilled water all delivered their pups alive. Cold aqueous extracts of MOL administered before mating produced 100% antifertility activity. The

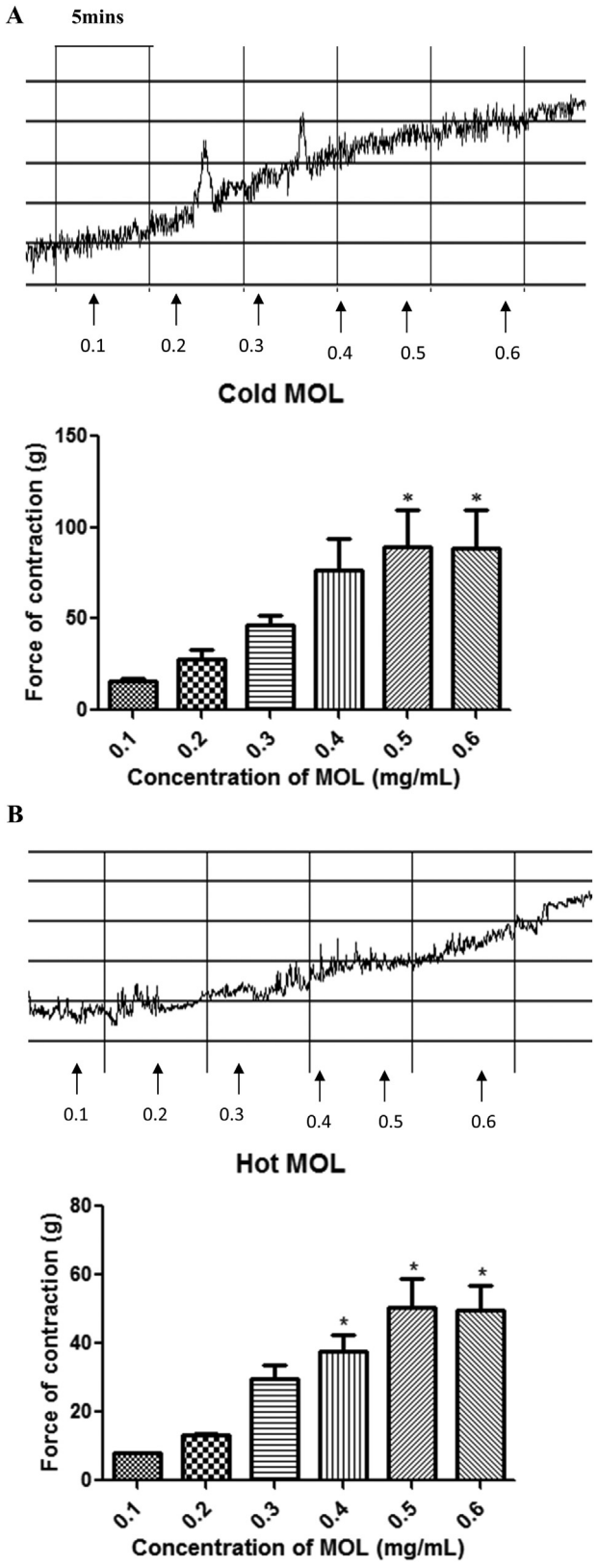


Fig. 1. The uterotonic Effect of *Moringa oleifera* Leaf (MOL) aqueous cold and hot extracts. (A) Typical tracings showing the contractile effects of graded concentrations of extract of cold aqueous extracts of MOL and the graph showing the mean tension obtained from six uterine tissue strips exposed to various doses of aqueous MOL ranging between 0.1 to 0.6 mg/mL. (B) Same as in A but hot aqueous MOL extracts was used here; uterine smooth muscle used were isolated from no pregnant rats. Values presented are mean ± SE (N = 6) (*p < .05).

Table 1
Summary of the effect of cold and hot aqueous extracts of MOL on fertility.

Groups	MI (%)	C I (%)	GSI (%)	PN
Control	100	100	100	33
HMBM	100	20	100	8
CMBM	100	0	–	–
HMAM	100	50	88.8	18
CMAM	100	20	100	9

CI Conception Index; Gestation; SI Survival Index;PN Pup number; MI Mating Index; HMBM hot MOL before mating; CMBM cold MOL before mating; HMAM hot MOL after mating; CMAM cold MOL after mating.
CMBM group has no data because the extract produced a 100% antifertility activity.

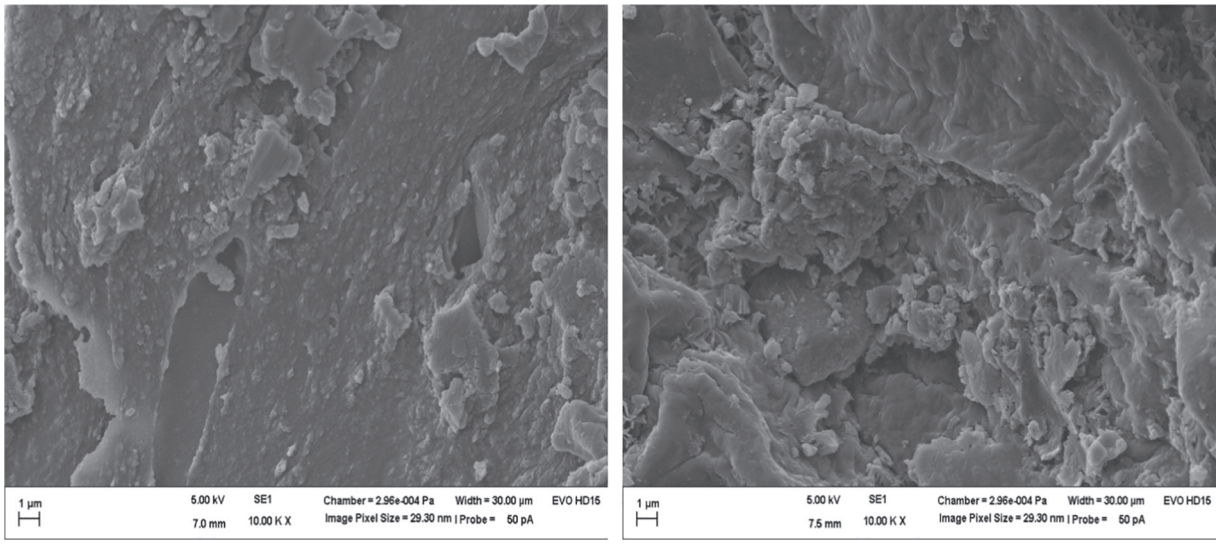
same extract was administered after mating. This led to 80% abortifacient activity. The hot aqueous extracts at a dose of 50 mg/kg body weight administered before and after mating produced antifertility and abortifacient activity of 83.3% and 50%, respectively. By extrapolation, an equivalent dose of 58 mg/kg body weight could respectively produce an activity of 96.6% and 58%. The weekly weight variation of dams taken after mating did not produce any significant difference ($p < .05$) in weight when compared with control at the end of the first week post mating period for all groups. They showed a significant difference ($p < .05$) in weight gain at the second and third week when compared with the control group. The changes in weight after mating for the groups showed that only cold MOL group indicated a significant variation in weight gain when compared with the control. At the third week post mating period, weight of both groups was significantly different from the control group. Appendix 2 and 3 represent a graphical summary of the weight variations obtained before and after mating. Table 2 shows some morphometric indices of pups from dams that received hot and cold MOL extracts before and after mating. Body weight, head diameter and crown tail length were all significantly ($p < .05$) higher when compared with the pups from control group. Since the cold MOL extract administered to dams before mating produced 100% anti-fertility activity, no pup resulted from this group. Pups from both cold and hot MOL groups did not show any significant difference in head diameter, abdominal distance, and crown tail length when compared with pups from the control group (Table 2).

Physicochemical characterization of *Moringa* leaf powder and freeze dried aqueous extracts (cold/hot), respectively yielded Z-Average (1917 nm ± 315.1), Polydispersity index (0.876), Zeta potential (−11.4 ± 1.69 mV) and Z-Average (3876 nm ± 100.8), Polydispersity index (0.915), Zeta potential (−8.23 ± 7.22 mV). Fig. 2 shows the scanning electron microscope images of MOL particles.

Table 2
Effect of cold and hot aqueous extracts of MOL on morphometric indices of female offspring.

Groups	BW(g)	ADI(mm/g)	HAC
Control[a]	5.2 ± 0.10	0.09 ± 0.00	0.87 ± 0.02
Control[b]	5.5 ± 0.56	0.19 ± 0.00	0.86 ± 0.02
HMBM[a]	5.8 ± 0.54	0.11 ± 0.02	0.82 ± 0.03
HMBM[b]	7.0 ± 0.55	0.16 ± 0.01	0.99 ± 0.02
CMBM[a]	-	-	-
CMBM[b]	-	-	-
HMAM[a]	4.8 ± 0.18*	0.10 ± 0.01	0.88 ± 0.05
HMAM[b]	5.6 ± 0.16	0.17 ± 0.00	0.94 ± 0.03
CMAM[a]	5.4 ± 0.20	0.10 ± 0.01	0.86 ± 0.03
CMAM[b]			

Data presented as the Mean ± SEM.
BW Birth Weight; ADI Anogenital Distance Index; HAC Head to Abdominal Circumference; HMBM Hot MOL Before Mating; CMBM Cold MOL Before Mating; HMAM Hot MOL After Mating; CMAM Cold MOL After Mating.
CMBM[a] and CMBM[b] has no data because no pup resulted from this group due to 100% antifertility activity of the extract.
* Significant difference based on 95% Confidence Interval
[a] Female offspring.
[b] Male offspring



A. MOL (Aqueous extract) B. MOL (powder)

Fig. 2. Images of *Moringa oleifera* leaf (MOL) particles observed by scanning electron microscopy (SEM) (Hitachi S-4000 microscope) showing the particle microstructures. (A) Depicts the representative image of the freeze-dried aqueous extract (cold or hot) of MOL. (B) Represents MOL dried fine powder.

4. Discussion

Available scientific evidence suggests that *M. oleifera* produces a wide array of bioactive compounds some of which have been reported for ethnomedicinally related activities such as abortificient and antifertility (Rani et al., 2018). These nature-derived phytochemical fingerprint occuring in *M. oleifera* leaves are thought to be associated with the various reported pharmacological activities including estrogenic activities and myometrial contractility (Ajuogu et al., 2019).

The yield from the hot aqueous extract was higher (26%) than that obtained from the cold aqueous extract (23%) as a result of the higher temperature used for the hot aqueous extract. This is consistent with previous reports regarding increased yield following higher temperatures (Wang and Weller, 2006). Since the aqueous hot extract yielded more than the cold aqueous extract, lower dose (50 mg/kg body weight) of the hot aqueous extract (translating to much lower quantity of an equivalent dried leaf powder) was used during the study. This was adopted because *M. oleifera* leaf is most commonly taken as hot tea extract. An equivalent dose of 58 mg/kg of the hot MOL aqueous extract produced over 96% anti-fertility activity before mating and abortifacient activity of 58% after mating. On the other hand, despite the lower solvent yield, the cold MOL aqueous extract was more active than the hot extract, producing 100% conception inhibition and 80% abortifacient activity. The dose (58 mg/kg body weight) of the cold MOL aqueous extract used in this study is equivalent to 250 mg dry plant material. Consistent with this study, Khoza et al. (2014) reported the effect of temperature on bioactive metabolites present in *M. oleifera* leaves such as chlorogenic acids and flavonoids. The findings of Khoza's team suggest that higher extraction temperature lowers the levels of heat sensitive metabolites of *M. oleifera* leaves possibly due to a significant change in their chemistry.

Studies in the recent past have shown that about 50% of human conception get aborted or do not result in successful delivery. Standard methods that have been frequently used to detect early gestational evidence have demonstrated that 32%–42% of pregnancies do not progress to parturition as a result of loss of the fetal embryo (Zinaman et al., 1996). Many factors may be tied to this including the consumption of unknown abortifacient botanicals. In this study, none of the extracts prevented the animals from mating and this suggests that the animals' cycles contained at least one estrus during the two weeks of mating although this does not supply any information about the regularity of the cycle. The cold extract seemed to have more adverse effect on maintenance of pregnancy possibly because of the presence of unfriendly metabolites which have not been inactivated partially or completely by heat. The pups born to the hot extract group had severely altered morphometry which suggests that the growth and development of these pups have been compromised during the gestation period. This is consistent with a previous report on the teratogenic activities of ethnomedicinal plants collected from India (Nath et al., 1992). The disparity in activity between the cold water and hot water extracts may be due to modification of metabolites resulting from higher extraction temperatures. The interference with the physiology and function of the reproductive system may have been triggered by an alteration in the synthesis of hormones, storage, liberation, transport or excretion. More so, any alteration in the interaction of the hormone to its specific receptor is capable of modifying the recognition of the hormone or the cellular response after interaction (Kavlock et al., 1996). Scientific literature indicates that phytochemicals synthesized in plants that are consumed as food or for health-care can interact and modulate the response of estrogenic receptors (Brzezinski and Debi, 1999) as well as induce uterine contraction (Gruber and O'Brien, 2011). Many of these bioactive metabolites have been isolated from the leaves of *M. oleifera* (Leone et al., 2015). The isoflavone sub-group of flavonoids have been implicated as superagonists of estrogenic receptors (Boonmuen et al., 2016). The abundance of these phenolic antioxidant metabolites in *M. oleifera* leaves (Leone et al., 2015; Ilyas et al., 2015) could result in significant disruption of endocrine physiology manifesting in the observed inhibition of conception and abortifacient activity. Moreso, the observed activity may be related to other constituents such as vincosamide, an indole alkaloid (with a nucleus similar to the ergot alkaloid group) as well as unusual glycosides of pyrrole alkaloids, which have all been isolated from the leaves of *M. oleifera* (Sahakitpichan et al., 2011; Panda et al., 2013). In Nigeria, many of these botanical materials are ingested as a result of their beneficial effects to boost immune system, for health and vitality, to fight malnutrition, to tone uterus muscle, induce labor or to remove unwanted pregnancy (Attah et al., 2016; Attah et al., 2012; Gruber and O'Brien, 2011). Infertility on the

other hand may result from several factors among which are nutrition, diseases and anatomical malformation of the uterus. Infertility has been described for over 186 million adult bisexual women in developing countries who are married (WHO, 2012). Asia and Sub-Saharan Africa bear the highest burden of female infertility (Mascarenhas et al., 2012). In other words, one in every four women of reproductive age in developing countries is infertile. This burden is unacceptably high and presents a serious concern as victims suffer from economic deprivation, social isolation and sometimes suicides. Food and medicinal plants regularly consumed may contribute to the nutritional factors responsible for infertility which should be scientifically investigated. *M. oleifera* leaf may be one such food and medicinal plant with abortifacient claims, which we have investigated for its anti-fertility properties. Although the concern of over population and limited resources tend to mask the growing need to tackle the problem of infertility in the developing world, yet the social, psychological, economic, political and health issues surrounding infertility far outweighs the other. Moreover, scientific investigation and validation of anti-fertility foods and medicinal plants can be helpful for informed child spacing purposes in economically deprived settings. *M. oleifera* leaves will be the most preferred candidate food/medicinal plant that will meet this desired population control need especially in sub-Saharan Africa and Asia where the plant is traditionally used, culturally embraced and naturally grows well. Coincidentally, these regions carry the highest burden of undesirable population growth as well as reported infertility cases among women of reproductive age. The preference for *Moringa* has been encouraged by the abundant data on low toxicity profiles in animal models as well as the nutritional and health promoting potentials of *Moringa* leaves (Paula et al., 2017; Al-Asmari et al., 2015; Asomugha et al., 2015; Asiedu-Gyekye et al., 2014; Ekundina et al., 2014).

Images of the shape and surface morphology of MOL particles in aqueous form (A) and powder (B) reveal that the former appeared smoother than the later. However, image B particles were more stable with zeta potential value of −11.4 mV than A (−8.23 mV) probably due to its dry form. This could suggest why most MOL preparations in the markets are packaged in the form of powdered leaf and not as aqueous extract. Meanwhile, both particles were polydispersed in the micrometer ranges. Image A particles were more amorphous in shape (than B) with openings for possible interaction, transport and/or entrapment of other moieties (Nnamani et al., 2010a). Amorphosity and/or less crystallinity of particles have been associated with better solubility and *in vivo* performances (such as bioavailability) (Nnamani et al., 2014; Nnamani et al., 2010a, 2010b). Therefore, this perhaps explains why despite having larger particle size (~3.9 μm) than image B (1.9 μm), MOL aqueous extract exhibited enhanced *in vivo* uterotonic activities in the uterine smooth muscle strip.

5. Conclusion

In conclusion, the ingestion of *M. oleifera* leaves just before conception, at conception and during pregnancy may be inimical to fetal development. Further research is ongoing to determine specific uterotonic metabolites, which may be of chemotaxonomic relevance and the mechanism of action of the extracts in its interference with female fertility in animal model. A retrospective survey as well as observational study is also encouraged among human female populations who regularly take *M. oleifera* leaves and its products in order to access the effect of *Moringa* on the fertility of women of reproductive age.

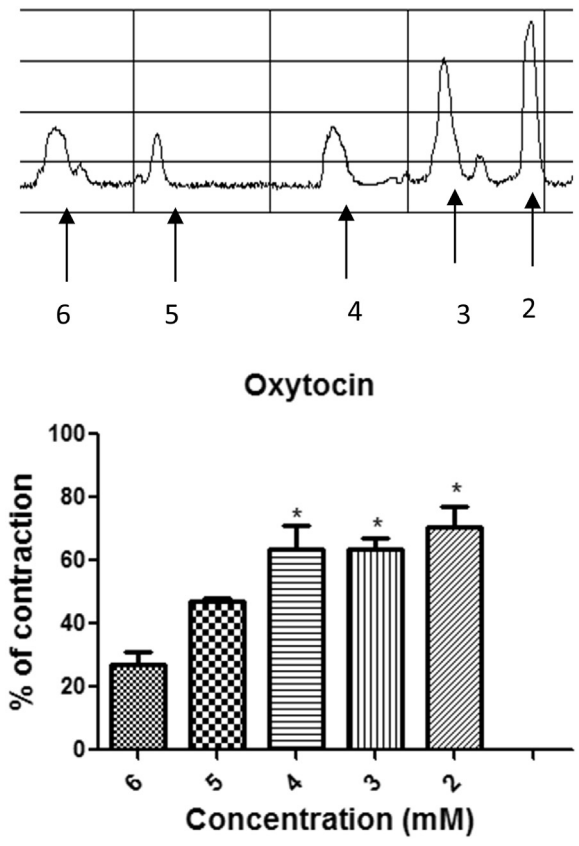
Declaration of Competing Interest

No conflict of interest associated with this work.

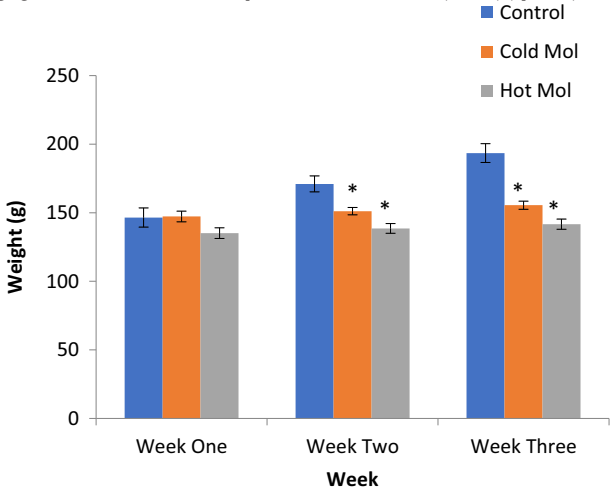
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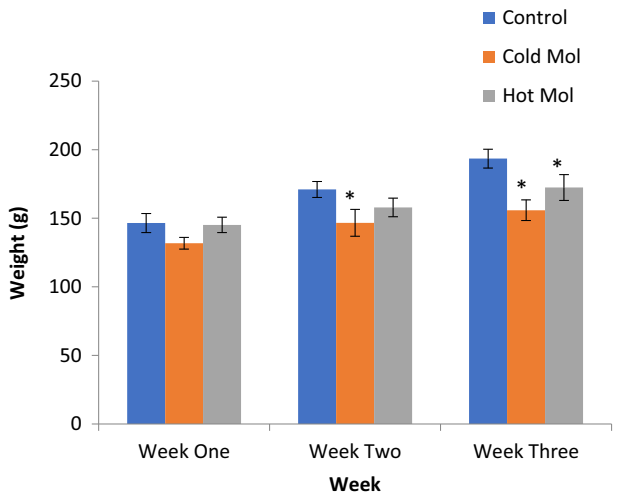
Appendix A. Appendix



Appendix 1. : The uterotonic effect of Oxytocin. (A) Typical tracings showing the contractile effects of graded concentrations of oxytocin and the graph showing the mean tension obtained from six uterine tissue strips exposed to various doses of oxytocin ranging between 6 to 2 Mm. Values presented are mean ± SE (N = 6) (*p < .05)



Appendix 2. : Weekly weight variations for groups that received MOL before mating of animals (*p < .05 when compared with control)



Appendix 3. : Weekly weight variations for groups that received MOL after mating of animals (*p < .05 when compared with control)

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