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Original Research Article

Anti-implantation and uterotonic properties of *Mentha pulegium* L. in female Sprague-Dawley rats

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ABSTRACT

Background: Some traditional herbs disrupt endocrine-endometrial synchrony, affecting embryo-endometrium communication during fertility. Hormonal imbalances cause non-receptive conditions, leading to anti-implantation or abortion. Historically, *Mentha pulegium* has served for contraceptive and abortive purposes. Its effects on the post-coital contraceptive and hormonal activities were evaluated.
Methods: Thirty-six Sprague-Dawley female rats were selected based on the presence of copulation plugs and received 200, 500, and 1000 mg/kg of Hydroethanolic leaf extract of *Mentha pulegium L*. (MPE) for seven days. Reproductive organ weights and serum estrogen and progesterone levels were measured. The resorption index, anti-implantation activity, and pre-implantation loss were also calculated using the number of implantation sites and resorptions in all treatments. Data were presented as mean±standard error mean (SEM), and significance was defined as $p<0.05$ using one-way ANOVA.
Results: Post-coital administration of MPE resulted in resorptive, pre-implantation loss, and anti-implantation activity. A dose of 200 mg/kg reduced the number of implantations and exhibited a high resorption index, percentage pre-implantation loss, and anti-implantation activity. A marked decline in serum progesterone levels and a significant reduction in serum estrogen and progesterone ratio was observed at 200 mg/kg MPE. A significant increase in uterine weight was observed in the 500 mg/kg treatment. Doses of 500 and 1000 mg/kg resulted in a significant reduction in anti-implantation activity.
Conclusions: The observed anti-implantation activity and pre-implantation loss suggest the abortifacient properties of MPE. However, its effects were seen to be dose-dependent.

Keywords: Post-coital contraceptive, Anti-implantation, Pre-implantation loss, Estrogen, Progesterone, Dose-dependent

INTRODUCTION

Fertility control finds great significance, as its importance on the check of the extraordinarily rapid growth of population has become an issue of global public health concern.[1] In Ghana, population growth is alarming, with a current growth rate of 2.15% per year; its population is expected to move from about 13 million (2020) to 78.71 million in 2099.[2]

Fertility regulation with plant preparations has been reported in ancient literature as the indigenous systems of medicine. Medicinal plants with anti-fertility agents have been topical in the search for new anti-fertility drugs, out of which quite a number have been tested and subsequently fortified by national and international agencies. This is in line with the renewed interest in plant-based therapeutic agents for their efficacy and better tolerability compared to most orthodox medicines, which pose numerous side effects.[3] Fertility regulation in females and modern research show that these have various modes of activity, with some modifying the function of the endocrine and reproductive systems. Certain herbs used traditionally to cut back fertility may have the ability to act as implantation inhibitors and potentially interfere with implantation. [3,4]

Like other *Mentha* species, *Mentha pulegium L. (Lamiaceae)* is one of the world's oldest herbs, traditionally used for decades for various purposes.[5] Its dried aerial parts and extracts, mostly as essential oils, have been used to address multiple health issues, including some digestion problems, and respiratory, urinary, and cardiovascular disorders. They are used as analgesics, diuretics, carminative and antispasmodic agents and also possess antioxidant effects, muscle relaxant effects, and antimicrobial effects. It is also known for its antiemetic, insecticidal, antibacterial, anticandidal, and anticholinergic properties.[5-7]

Mentha pulegium L. and its essential oils are currently still in use, as it was acclaimed in folk medicine to exhibit some contraceptive and abortive effects. However, its reputed use as a uterine stimulant, either as an emmenagogue (menstrual flow stimulant**)** or an abortifacient, was reported as far back as in ancient Greek medicine use. The whole plant is said to be a rich reservoir of phytochemicals and its component phytoestrogens may have the ability to alter reproductive function. With limited evidence, some form of a mechanism by which *Mentha pulegium* carries out its anti-fertility properties was purported by Riddle and some medical writings.[8-10] Because *M. pulegium L* is widely used in folk medicine, limited information on the mechanism underlying its abortifacient property has recently sparked some interest. Nonetheless, studying its effects and actions on the different stages of the reproductive processes evaluates its purported effects on fertility.

For further scientific validation of the perceived efficacy, this study aims to establish *Mentha pulegium L.'s* anti-fertility activity through a comprehensive analysis of its effects on various reproductive processes. This was ascertained by evaluating its effect on post-coital contraceptive activity, anti- and pre-implantation events in pregnant rats, and hormonal levels.

METHODS

Duration of study

The research study was carried between October 2023 and December 2023.

Materials

Plant collection and extraction

Leaves of *Mentha pulegium L.* were obtained from Relish, an authorized herbal shop located in Accra, Ghana, and authenticated at the Department of Pharmacognosy, Kwame Nkrumah university of science and technology, with herbarium specimen voucher number (KNUST/HM1/2019/L011). Extraction was done in 70% ethanol for 72 hours. The extract obtained was decanted and concentrated using a SolventVap rotary evaporator (Across International SE05.110) into a dry semi-solid extract (130.6g), with a percentage yield of 10.9%. The dried hydroethanolic leaf extract of *M. puleguim L.* (MPE) was stored at 4^{o} C until the experimental testing. A solution of the extract was made by mixing it with distilled water to obtain the respective dosages to be administered.

Animals

Adult non-pregnant female Sprague-Dawley (200-220 g) rats were kept under standard conditions in the animal house, department of pharmacology, KNUST. They were fed with standard rat pellets and had free access to water. The animal house was well-ventilated, and the animals had a 12-hour light/dark schedule. Experimental procedures and animal handling were performed with approval by the institutional animal research ethics committee (AREC), KNUST (KNUST 0045), and following the national institute of health guidelines for care and use of laboratory animals (NIH publication No. 85-23, revised 1985). The study adhered to the ARRIVE guidelines (Animal research: reporting of *in vivo* experiments) in carrying out the work and reporting the findings.

Methods used

Preliminary safety assessment

A modified Irwin observation protocol was used. Healthy rats (25) were put into five groups of 5 each and treated with 0, 50, 100, 500, and 1000 mg/kg MPE daily by oral

gavage, based on a modified existing acute toxicity assay protocol.[7] Animals were observed for changes in their behavioral, neurological, and autonomic profiles and lethality within (0-15, 30, 60, 180) minutes, (24, 48) hours, and (7, 14) days after administration, in simultaneous comparison with the control group.[11] The study assessed 18 parameters in various cages, employing open-field handling where necessary. Physical factors and gross appearances, such as body weight and skin color changes, were observed. Behavioral modifications (sleep, locomotor activity, bizarre behavior, stereotypy, vocalization, lethargy, and aggressive behavior), as well as neurological parameters (pelvic elevation, tail elevation, tremors, twitches, convulsions, coma, Straub tail,) and autonomic profiles (diarrhea and piloerection), were observed. Deaths were also reported. Some observations were also confirmed from video-recorded footage using a Jovision NVR ND6008-H2-8 channel network IP video recorder system 9 (Jovision, USA). JWatcher 1.0 software was used to track observed behaviors.

Post-coital anti-implantation activity of MPE

Experimental study and observations were done using procedures described by Shah and Jhade.[12] Implantation studies were carried out in animals only with proven fertility through the assessment of their vaginal opening status and regular estrous cycle.[13] Only female rats that completed at least two consecutive estrous cycles were used. The selected rats were left overnight with males, also of proven fertility in the ratio of 1:1. Prior to mating, the females were isolated for one month to rule out pre-existing pregnancies. The 36 rats showing thick clumps of spermatozoa in their vaginal smears and or copulation plugs were considered pregnant and separated. This day was subsequently designated as day one of pregnancy.

These rats were randomly distributed into six groups: three control groups and three experimental groups (n=5). Group I (naïve control) received distilled water, whereas groups II (positive control) and III (negative control) received 17β-estradiol (E_2, 0.45 mg/kg; Sigma-Aldrich Corp, St Louis, MO, USA) subcutaneously and Mifepristone (8 mg/kg, Mylan Laboratories Ltd, Gujarat, India) respectively. Experimental groups IV, V, and VI were administered orally with 200 mg/kg, 500 mg/kg, and 1000 mg/kg of MPE, respectively. All the treatments were given once daily for seven days. This study was terminated almost mid-term (days 10-11) of the rat's gestational period of 23 days, which is comparable to days 28-40, and about 4-6 weeks in human embryonic development.[14] On 11th day of pregnancy, animals were weighed and euthanized under light ether anesthesia.

Body and reproductive organ weights

Pregnant rats were weighed daily as an assessment of pregnancy and the percentage of maternal body weight gain was calculated according to the following equation: mean final weight-mean initial weight×100/ mean initial weight

The uterus and ovaries were harvested, surrounding tissues removed and the ovaries detached. Absolute weights were determined and expressed as percentages of body weights.

Pre-implantation assessment and anti-implantation activity

The excised uteri were photographed and examined. Total implantation sites, number of embryos, corpus lutea and resorptions were determined for animals in each group. The percentage resorption index was calculated and statistically analyzed.[15]

Percentage resorption index=number of resorptions/total implantation sites × 100

Anti-implantation activity was expressed as the percentage of animals showing an absence of implantations in uteri when sacrificed on day 11 of pregnancy.[16]

Anti-implantation activity=No. of implants in control-no. of implants in test/no. of implants in control×100

Pre-implantation loss is used to establish a correlation between the number of released ova which after fertilization manage to implant in the uterus.[17]

Pre-implantation loss=number of Corpora lutea-number of implantations/number of Corpora lutea ×100

Serum reproductive hormones

Blood was drawn by cardiac puncture, collected into serum separator tubes (Cland medical instruments Co. Ltd, China), and coagulated for 30 minutes for hormonal analysis. Using ACNA 90-1 centrifuge (Jiangsu Zhengji, China), the serum was extracted by centrifuging at 1308 RCF for 15 minutes. The resulting serum pipetted into Eppendorf tubes for estrogen and progesterone hormone evaluation.[18] The analysis was done using rat-specific enzyme-linked immunosorbent assay (ELISA) kits (Melson medical corporation Ltd, Melson, China) according to the manufacturer's instructions. With the sensitivity of hormone detection being 1.0 pg/ml (estrogen) and 0.1ng/ml (progesterone), all the samples were analyzed in a single assay to avoid inter-assay errors for each of the hormones. All samples were run in triplicates.

In-vitro studies on uterotonic properties

Non-pregnant uteri were removed and placed in a pulsating bowl containing Rat DeJalons of composition NaCl, 153.9 g; KC1, 5.6 g; $NaHC0_3$, 6.0 g; $CaCl_2$, 0.25 g and glucose, 2.8 g. From the isolated uterus, muscle strips

of 2 cm in length, were prepared as tubes, which will then be suspended in a 30 ml organ bath containing Rat DeJalons solution continuously supplied with 95% oxygen and 5% carbon dioxide to provide pH 7.4 at 37°C. The lower ends of the strips were fixed to a metal hook and the upper end was attached to an isometric force-displacement kymograph (MAY 10BS99; Commat, Ankara, Turkey). The uteri strips were allowed to equilibrate under a passive resting tension of 1 g for 30 minutes. After manifestation of graded contractions during this equilibration period, contractions of myometrial strips were recorded either spontaneously or evoked by their respective dose levels 50, 100, 200, 500 mg/kg MPE and 0.01M, $1x10^{-4}$M, $1x10^{-6}$M oxytocin (Syntocinon® (oxytocin)-batch number 477650: Novartis, Basel, Switzerland**)** respectively in 0.2, 0.4, 0.6, 0.8 and 1ml volume of MPE and standard drug.

The effects of cumulative concentrations of MPE on the spontaneous and induced contractions (isometric tension) were quantified by fluctuations in mean amplitude, area under the contractile curve (AUC), and frequency. These values were analyzed and given as percentages.

Statistical analysis

Anti-implantation activity, pre-implantation loss and resorption index were calculated using Microsoft excel. Data were analyzed using one-way analysis of variance (ANOVA) using GraphPad InStat19 statistical software to analyze the degree of variance among groups followed by Dunnett's test. Data were presented as mean ± standard error mean (SEM), and significance was defined as $p<0.05$. Values were considered statistically significant if $p<0.05$ (Dunnett's test).

For the *in-vitro* studies contractions were recorded as mean percentage maximal response ± SEM for all the treatment groups.

RESULTS

Preliminary safety assessment

Relative to controls, there were no observable signs of physical, neurotoxicity, autonomic or central nervous system (CNS) related toxic symptoms in the rats treated with 50, 100, 500, and 1000 mg/kg MPE. No deaths were recorded. However, a decrease in locomotor activity (spontaneous movements from one place to another) was observed in all treated rats during the first 15-30 minutes (Figure 1).

Movement time and speed of progression markedly decreased as the dose of intake increased. Subsequently, normal movement in these rats was regained after 30 minutes.

Effect of the different doses of hydroethanolic leaf extract of *M. pulegium* L on locomotor activity.

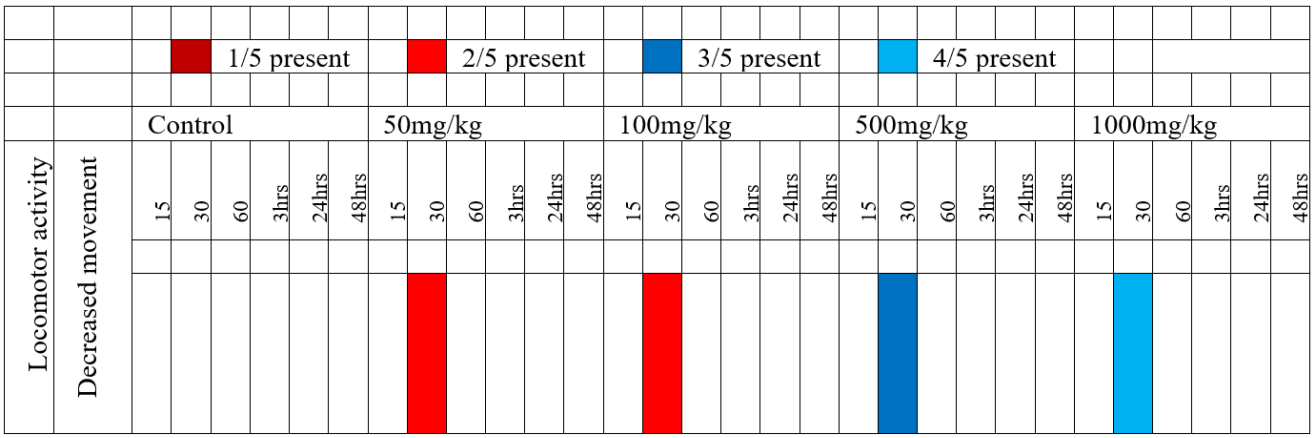

Figure 1: Data, as presented, quantifies rats that showed some behavioral changes in movement.
Dosage groups and timing of observations are in columns. Colors indicate the number of rats showing symptoms of decreased movement. Each color indicates how many rats out of five were affected at each dose and time.

Post-coital anti-implantation activity of MPE

Effect on body and organ weights

Percentage (%) weight change for the 200 and 1000 mg/kg were increased while that of 500 mg/kg and mifepristone were decreased. Percentage changes in body weight of rats in 200 and 1000 mg/kg treatment showed a significant statistical decrease ($p<0.0001$, $p=0.0105$; $p<0.05$) compared to the control. Also, the 500 mg/kg and mifepristone treatment group showed a significant statistical decrease ($p<0.0001$) when compared to the control (Figure 2).

Doses of 500 mg/kg MPE and 8 mg/kg mifepristone showed significant uterine weight increases ($p=0.0018$ $p<0.01$, $p=0.0009$ $p<0.001$) and decreases ($p=0.0037$, $p<0.01$) respectively. Both relative and absolute ovarian weights were not significantly affected by all treatments of MPE and the reference drug control groups (Table 1).

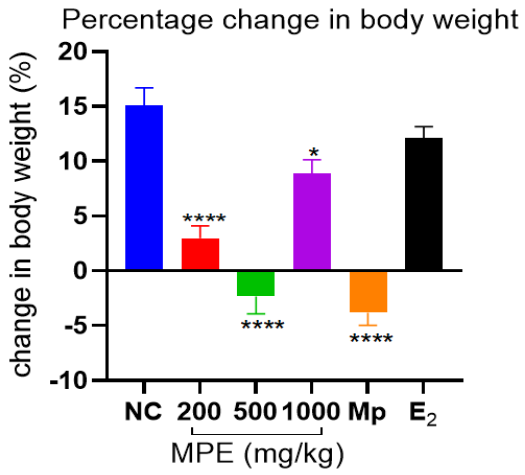

Figure 2: Percentages change in body weight after 11 days of study.

Statistical analysis was carried out using one-way ANOVA followed by Dunnett's post hoc multiple comparison test. As compared with control group values were significantly different at (*p<0.05, ****p<0.0001).

Effect on pre-implantation and anti-implantation activity

For evaluation of interceptive activity, 200 mg/kg MPE (p=0.0424, p<0.05) and mifepristone significantly (p=0.0054, p<0.01) reduced no. of implantations (embryos) (Table 2).

Findings showed an insignificant increase in total implantation sites and decrease in percentage resorption index (11.76±11.76) in 1000 mg/kg treated rats compared to untreated controls, 200 and 1000 mg/kg treatment group showed significantly higher pre-implantation loss (p=0.0043, p=0.0047, p<0.01). Anti-implantation activity was dose-dependent with 200 mg/kg recording the most significant increase (p=0.0002, p<0.001) in all MPE dose-treated groups. Increased anti-implantation activity, percentage pre-implantation loss for estradiol (p=0.0001; p<0.001, p=0.0002; p<0.001) and mifepristone (p=0.0009; p<0.001, p=0.00034; p<0.001) with significance as expected (Table 2).

Table 1: Effect of hydroethanolic leaf extract of *M. puleguim L.* on absolute and relative organ (uterus and ovary) weights.

Treatment	Body weight	Uterine weight		Left ovary weight		Right ovary weight	
		Absolute	Relative	Absolute	Relative	Absolute	Relative
Control	189.1±13.50	0.846±0.324	0.437±0.152	0.048±0.009	0.026±0.004	0.039±0.006	0.021±0.004
200 mg/kg	147.0±6.78	0.859±0.289	0.298±0.104	0.335±0.289	0.030±0.005	0.041±0.004	0.028±0.003
500 mg/kg	168.1±21.26	1.534±0.928**	0.982±0.553***	0.128±0.092	0.021±0.003	0.044±0.009	0.026±0.004
1000 mg/kg	172.0±14.74	0.503±0.196	0.543±0.208	0.106±0.054	0.030±0.006	0.048±0.007	0.030±0.006
Mifepristone	172.9±16.46	0.245±0.364**	0.140±0.016	0.362±0.327	0.027±0.002	0.044±0.007	0.025±0.002
17β-estradiol	175.5±11.17	0.551±0.062	0.317±0.044	0.112±0.061	0.033±0.005	0.046±0.006	0.027±0.003

Values are expressed as mean (n=5) ± SEM of the final body weight, absolute and relative uterus and ovary weight after 11 days period of study. Statistical analysis was carried out using one-way ANOVA followed by Dunnett's post hoc multiple comparison test. As compared with control group values were significantly different at **p<0.01, ***p<0.001.

Table 2: Effect of hydroethanolic leaf extract of *M. puleguim L* on implantation and pre-implantation loss at gestational day 11.

Treatments	Total implantation[a] sites	No. of embryos	Resorptions	Resorption index[b]	Anti-implantation activity[c]	% pre-implantation loss[d]
Control	8.80±0.860	6.60±1.720	3.00±1.897	30.00±20.00	-24.20±13.00	3.80±8.80
200 mg/kg	5.20±1.463	1.60±1.60*	3.60±1.568	75.00±25.00	63.63±11.65***	50.90±9.53**
500 mg/kg	8.00±3.332	2.80±1.828	5.20±3.878	50.00±28.87	29.76±9.276**	29.76±6.71
1000 mg/kg	11.00±1.703	9.00±0.949	2.00±2.00	11.76±11.76	23.54±8.961*	50.42±6.34**
Mifepristone	6.60±1.860	0.00±0.00**	6.60±1.860	100.0±0.00	53.33±12.02**	51.95±11.30**
17β-estradiol	4.00±2.345	0.00±0.00**	3.20±1.985	100.0±0.00	73.33±17.64***	66.67±8.82***

Values expressed as mean (n=5)±SEM of implantation and post-implantation loss after 11 days of study. [a]Total implantation sites=no. of embryos + resorptions; [b]resorption/total implantation sites* 100, [c]No. of implant in control-no. of implant in test/no. of implant in control* 100, [d]no. of Corpora lutea-number of implantations/no. of Corpora lutea *100. Statistical analysis was carried out using one-way ANOVA followed by Dunnett's post hoc multiple comparison test. As compared with control group values were significantly different at (*p<0.05, **p<0.01, ***p<0.001).

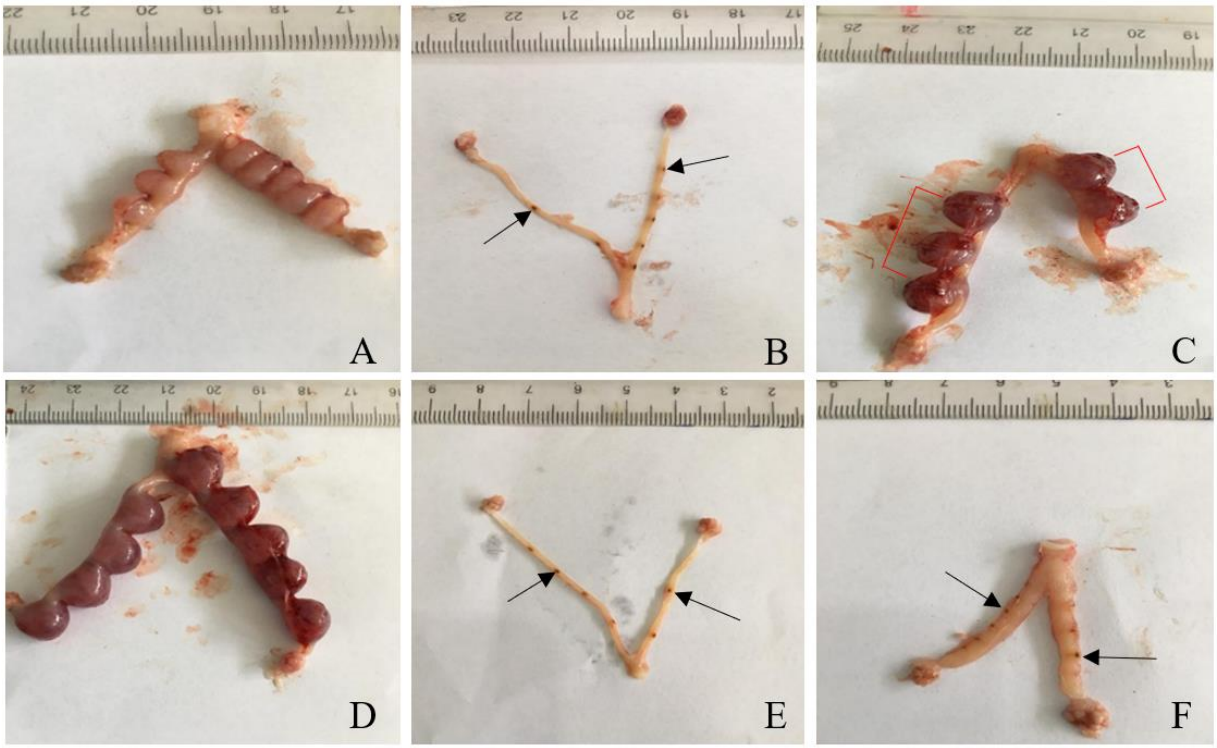

Figure 3 (A-F): Female rat uterus at gestational day 11 of control, MPE (200, 500, 1000 mg/kg respectively), mifepristone and 17β estradiol.

A and D-uteri with normal physiological appearance of pregnancy and symmetrical distribution of implanted fetuses. C-No complete resorptions but asymmetric and unequal distribution of fetuses: red brackets. B, E and F- uterine horns showing pinpoint hemorrhagic implantation sites (early resorptions): black arrows. Embryonic resorptions in F are symmetrically distributed.

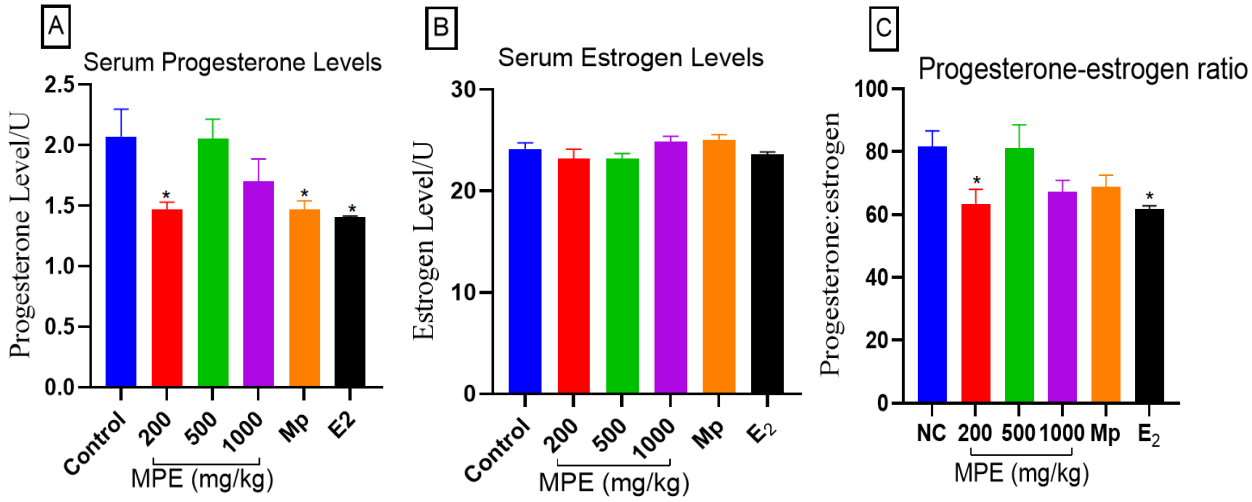

Figure 4 (A-C): Serum levels of progesterone, estrogen and progesterone to estrogen ratio after 11 days of study.

Values are presented as mean (n=5) ± SEM and statistical analysis was carried out using one-way ANOVA followed by Dunnet's post hoc multiple comparison test. As compared with control group values were significantly different at (*p<0.05).

Effects of MPE on serum estrogen and progesterone

Rats treated with mifepristone, E_2 and 200 mg/kg of MPE all had a marked decline in serum progesterone levels when compared to the naïve control (p=0.042, p=0.0231, p=0.0403; p<0.05). A statistical reduction (p<0.05) in serum estrogen and progesterone ratio (P:E) was seen in the 200 mg/kg MPE (p=0.0438) and estradiol (p=0.0247) treated groups when compared to the control (Figure 4 A-C).

Serum levels of progesterone, estrogen and progesterone to estrogen ratio after 11 days of MPE administration.

Uterotonic properties of MPE

Administration of oxytocin yielded graded responses as shown in Figure 5. Administration of 50 and 500 mg/kg MPE yielded no response in the uterine tissue however, administration of 100 and 200 mg/kg MPE yielded a graded response similar to that of the oxytocin.

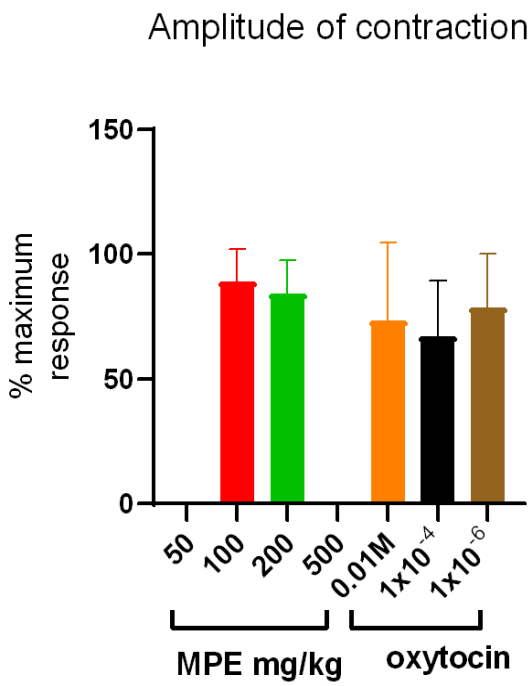

Figure 5: Percentage maximum response of uterine myometrium to oxytocin and MPE at different doses.
Values are represented by mean and SEM.

DISCUSSION

In assessing the anti-fertility potential of MPE, administered doses caused no changes in some parameter's indicative of toxicity nor physical discomfort and maternal deaths, regardless of the variations in percentage body weight change seen. El-Gazar and others also using *M. pulegium* made similar observations.[9] However, the decrease in locomotor activity observed is similar to one of Gerenutti's studies; their highest dose of *M. pulegium* treatments showed a decrease in general activity.[7] Many plants and extracts rich in glycosides and flavonoids possess sedative properties, and the decreased movement could be considered a sedative effect attributed to flavonoids and glycosides in MPE. Such changes are excellent preclinical indicators of neurobehavioral effects in observational studies such as this. Reduced movement indicates some level of CNS depressive effect at higher doses within a short time of intake.[19,20] However, a no-observable-adverse-effect-level (NOAEL) ≤1000 mg/kg and an oral medium lethal dose (LD_{50}) greater than 1000 mg/kg b. w. indicated that MPE was relatively safe.

The dialog between the embryo, the endometrium, and its receptivity is hormonally regulated and must be executed within a limited period of the window of fertility.[21] For implantation to take place, equilibrium levels of estrogen and progesterone are essential and any disturbance in levels may cause infertility.[12]An altered equilibrium could cause some endometrial changes resulting in non-receptive uterine conditions. Tubal transport of the embryo and its apposition, adhesion and implantation may be affected, and the blastocysts either fail to implant or are not maintained if implanted.[21] Absolute levels of estrogen and progesterone also regulate endometrial cell development, proliferation, and uterine remodeling to create an optimal environment for pre-implantation embryo development, blastocyst implantation, and pregnancy maintenance.[22]

The anti-implantation effects of *M. pulegium L.* observed in this study may be due to a disturbance in endocrine-endometrial synchrony. The estrogenic activity of some phytoestrogens can disrupt the functional equilibrium needed between endogenous estrogen and progesterone for sustainable implantation. The extensively studied metabolic profile of *Mentha pulegium L.* and its alcoholic extracts are reported to possess the ability to fluctuate estrogen/progesterone levels.[23] Amentoflavones, caffeic and rosmarinic acids reported to be estrogen receptor agonists and inhibitors of aromatase enzymes, modulators of estrogen receptors and increase serum estradiol are components of *M. pulegium L.*[9] The antiandrogenic properties of some alkaloids and flavonoids may have the ability to modulate these hormones.[10,24] Saponins, also found in MPE, are known for their anti-fertility properties through their effects of creating imbalances in hormonal levels and disrupting progesterone secretion by the corpora lutea, leading to uterine unreceptiveness.[25,26] Therefore, the imbalance in levels caused by MPE in this study could be responsible for the anti-implantation activity and increased fetal resorption, which was evident in the 200 mg/kg MPE-treated rats.[27] However, the P:E ratio is an efficient reflector of the endometrium receptivity status and a good predictor for either implantation success or failure compared to the absolute level of either estradiol or progesterone. Low P: E ratio contributes to implantation failure and high estrogen levels cause an absence of implantation sites.[28] Effects of 200 mg/kg MPE as an abortifacient dose with an anti-implantation potential are comparable to the standard mifepristone and E_2 in this study where low P:E matched implantation failure or resorptions. These doses' effect could be similar in blocking the activity of progesterone, explaining the fetal resorption and anti-implantation activity observed.

In a study using 250 mg/kg MPE, serum progesterone was also decreased but with an increase in estradiol level.[9] Similarly, the study found that treatments with the extract and mifepristone affected progesterone levels, potentially leading to fetal resorption. These decreased levels in this study may be due to the phytoestrogenic

effect of MPE on steroidal hormone trajectories, including the production, transport, and receptors of serum sex hormones.[29] Alkaloids inhibit the synthesis of cellular progesterone.[30] Subsequently, progesterone decreases by MPE suggest impaired endometrial function, consequently hampering the secretion of some proteins that nourish fertilized and implanted eggs, affecting the luteal phase in which the uterine lining will not thicken to support pregnancy and also facilitate the onset of labor.

Decreased resorption index observed for increasing doses further indicates abortifacient properties of MPE. The resorption index measured tells the number of blastocysts implanted and those that did not develop.[17] An increase in resorption index for 200 mg/kg MPE could mean inhibition of embryo development, indicating the pregnancy-terminating potential of the extract. High incidences of percentage fetal resorption suggest that interruption of pregnancy could also occur after implantation with the use of MPE. Modifying the endometrium to create a non-conducive environment for fetal development could also account for embryonic resorption.[17,31]

The similar reduction in the number of implantation sites and the anti-implantation activity observed for 200 mg/kg MPE, as was for E_2 and mifepristone also confirms some anti-implantation properties at this dose. The % pre-implantation loss increase and anti-implantation activity in this study imply an effect of MPE before and after implantation.[17,31] The observed reductions in implantation sites could also be attributed to its estrogenic effects caused by its component phenolics, steroids, alkaloids and glycosides, steroidal terpenoids, and flavonoids present, and gestational exposure at 200 mg/kg may increase the risk of pre-implantation loss.[9] Several studies using other plants with common phytoconstituents have also reported similar effects to the anti-implantation activity of MPE. Observed reductions in implantation sites and risk of implantation loss were attributed to the anti-fertility and abortifacient activities of phenolics, phytosteroids, and saponins that are common to all these plants.[32,33]

By disrupting some endocrine functions, MPE may act on the ovary inhibiting the release of estrogen and ultimately reducing the chances of implantation and conception.[34] Nevertheless, an increase in estrogen from hyperstimulated ovaries could also affect endometrial receptivity negatively by preventing blood flow in the uterus and trophoblast invasion.[35] Additionally, some compounds isolated in *M. pulegium L.* may have a direct anti-estrogenic effect, which impedes uterine and endometrium growth.[9] The significant decreases in the P:E ratio at 200 mg/kg MPE may also affect ovum transport creating an unfavorable uterine environment for successful implantation.

Variations in the percentage body weight change, are comparable to some research in which anti-fertility properties were determined.[15] The reduction in weight gain for MPE-treated rats was consistent with one of the novel studies on the reprotoxicity of *M. pulegium*. Nevertheless, even with a reduction, reproductive capacity was not affected when daily doses were administered.[7] However, the change in percentage body weight may be due to either a direct effect of MPE or one that affected the appetite of the rats. The reduction in maternal weight seen may also be due to fetal resorptions and growth retardation caused by MPE.[37] Uterine weight changes may affect implantation due to a diminished count of viable fetuses, heightened rates of resorption, and pre-implantation loss compared to the naïve control group.

Oxytocin, a well-known α_1-adrenergic agonist causes uterine contractions through the influx of calcium ions via the L-type calcium channels or the release of calcium ions from the sarcoplasmic reticulum.[30]These contractions in the uterine muscle can be attributed to depolarization and, thus the opening of the L-type Ca^{2+} channels thereby increasing intracellular Ca^{2+} or stimulating inositol-tri-phosphate.[38] *In vitro* studies confirmed induced contractions in the uterine myometrium of non-pregnant rats at MPE doses of 100 and 200 mg/kg. This could indicate that MPE may have acted on the same receptors as oxytocin, thereby accelerating the influx of extracellular Ca^{2+}. The study shows that agonist-induced increase in intracellular free Ca^{2+} concentration plays a major role in signaling pathways, raising the cytosolic free-Ca^{2+} in cells by the influx of Ca^{2+} from the extracellular environment into the cell. The absence of contraction in the higher dose (500 mg/kg) could be due to receptor desensitization or an increase in the concentration of other substances present in the plant extract that may be antagonistic to its contractile effect.[39]

CONCLUSION

This study agrees with El-Gazar and others in purporting *M. pulegiums'* abortifacient effects on the phytoestrogenic potential encompassed in its constituent metabolites.[9] The pre-implantation loss and anti-implantation activity seen in this study support the claims for the traditional usage of *M. pulegium L.* as an abortifacient. It confirms its potential to be an effective and safe alternative for terminating unplanned pregnancies.

As a limitation, the findings from our study cannot be directly extrapolated to the level of the human person, they provide the possibility of objectively and ethically investigating the effects and putative consequences of pregnancy termination at the biological and physiological levels.

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