

Abortifacient Potential Effect of Aqueous Extract of *Millittiaaboensis* on Reproductive Health of Matured Wistar Rats

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Abstract: The present study evaluated the abortifacient potential of aqueous extract of *Millittiaaboensis* on reproductive health of matured Wistar rats by assessing some fertility indices, hormonal profile and litter size of pregnant wistar rats. A total of thirty (30) matured female wistar rats were used for this study. Each matured female rat was mated with two male rats (2:1) until pregnancy was established using vaginal smear method and cage side observations. The pregnant rats were randomized into six (6) groups with five (5) animals in each group. Group 1 animals served as control group and were not treated with the extract but provided with food and water ad libitum. Groups 2,3,4,5 and 6 received concentrations of 1000,2000,3000,4000 and 5000mg/kg body weight of the extract accordingly. All animals were allowed to go full term and sacrificed thereafter. The foetuses were observed for signs of clinical toxicity, counted and weighed. Blood was collected for hormonal assay. Clinical toxicity such as respiratory distress, salivation, changes in appearance of hair were not observed during the exposure period in the extract treated animals. However, maternal mortality was observed upon administration of 4000 and 5000mg/kg body weight of the extract. Diarrhoea (evident from watery stool) and fatigue was also observed. The final weights of the rats increased significantly ($p < 0.05$) when compared with the initial weights. None of the rats showed signs of vaginal bleeding or expulsion of the products of conception. With these notwithstanding, the weights of the foetuses decreased significantly ($p < 0.05$) in the groups administered 4000 and 5000mg/kg body weight of the extract. The litter size and number of pups at birth also showed a decrease when compared with the control group. The duration of pregnancy was shortened to 20 days in animals that received the extracts at 3000-5000mg/kg body weight. No morphological abnormality was observed in the pups. The crown-rump length also decreased in the treated groups. The Results of the female reproductive hormones of the treated animals revealed that FSH, LH and prolactin were significantly reduced ($p < 0.05$) in all the treated groups by the extract. Progesterone and oestrogen were also reduced significantly at 4000 and 5000mg/kg body weight of the extract.

Keywords: Abortifaciation, *Millittiaaboensis*, Fertility Indices, Reproductive Health

I. Introduction

Traditionally, *M. aboensis* is one of such plants that have been widely used in the southern part of Nigeria for the treatment of several diseases. The plant is widely distributed in tropic Africa and found abundantly in Cameroon and Equatorial Guinea. Its leaves have been used for general healing and as a laxative while its roots have been used for the treatment of venereal infections. Over the years, there have been claims that the leaves of *M. aboensis* have the potency to induce abortion and also serve as a laxative. Although there is dearth of organized scientific information about the beneficial or harmful effect of the plant as well as its measure of safety in use. In light of these, the present study attempts to provide some information on its abortifacient and hormonal effect. Plants which are the source of large proportion of medicines have been used for the treatment of several ailments for thousands of years [1]. These plants also have derived chemicals that influence endocrine functions in both humans and animals and have received a great deal of attention due to their possible beneficial as well as adverse effects. The importance of these plants as a source of anti-fertility drugs have been emphasized by many researchers [2]. Some of these plants are known to possess anti-fertility effect through their action on hypothalamo-pituitary gonadal axis or direct hormonal effects on reproductive organs. Throughout history, women have tried to control or enhance their fertility with various levels of societal support. Many herbal remedies were used traditionally as contraceptives, abortifacients, emmenagogues or oxytocics [3]. Some of these plants are *momordica cymbalaria* and *Goniothalamus spp.* which were used traditionally to control fertility as well as abortifacients during early months of pregnancy. Most of the plants have estrogenic property that can directly influence pituitary action through peripheral modulation of reproductive hormones by decreasing the secretion of these hormones as well as blocking ovulation. [4]. Also, these plants may intercept the synchronized development of the ovum and endometrium, while others may have abortifacient or anti-progestational effects [5, 6]. This, however, has now led to the increased quest for oral contraceptive agent which can be employed to control human fertility. Although a variety of synthetic contraceptive agents are available, their use cannot be continued because of their possible side effects. The

present study aims to evaluate the potential abortifacient effects of *M. aboensis* on the reproductive health of matured pregnant Wister albino rats.

II. Methods

2.1 Collection and identification of plant materials

Fresh leaves of *M. aboensis* were collected from the botanical garden of department of Pharmacy, University of Port Harcourt, Rivers state, Nigeria. The plant was identified and authenticated by a Biotechnologist Dr Edwin Nwosu of the department of plant science and biotechnology, University of Port Harcourt. A specimen was deposited at the department of Plant and Science Biotechnology for reference purposes with accession number UPH 587 [7].

2.2 Animals

The experimental animals used for this study were healthy pure breed female Wister albino rats with weights between 150-220grams. All animals used in the course of this study were obtained from the animal house of the department of Biochemistry, faculty of science, University of Port Harcourt. The animals were kept in cages in a well ventilated room and provided with food and water *ad libitum*.

2.3 Reagents/Materials

All chemicals used in this study were of analytical grade and products of May and Baker, England; BDH, England and Merck, Darmstadt, Germany. Reagents used for the assays were products of Accu-Bind Commercial Kits.

2.4 Experimental Design

2.5 Animal Grouping and Extract Administration

A total of 30 matured female Wister albino rats were used for this study. The animals were kept in cages under standard conditions of temperature and ventilation and had free access to water and food. Each mature female rat was mated overnight with two male rats (2:1). Pregnancy was established using the vaginal smear method, weight gain as well as other behavioural patterns observed in the animals. The day a positive smear of spermatozoa was observed was designated day 1 of pregnancy for that particular animal. The pregnant animals were then randomized into 6 groups: 5 animals each for the treated and control groups. Group 1 animals served as pregnant control animals and were not treated with the extract but provided with adequate conditions and access to food and water *ad libitum*, while groups 2, 3, 4, 5 and 6 received concentrations of 1000, 2000, 3000, 4000 and 5000mg/kg body weight respectively of aqueous extract of *M. aboensis*. All animals were allowed to go full term (litter) and thereafter sacrificed by chloroform inhalation. The foetuses were counted, weighed and observed for signs of clinical toxicity. Blood was collected via cardiac puncture for hormonal assay.

2.6 Preparation of Vaginal smear

The method by [8] was followed. The animals were held with the ventral side up. A drop of 0.9% w/v normal saline was inserted carefully into the vagina with a dropper without damaging the vagina to avoid false positive smears. A drop of normal saline was aspirated and introduced twice before withdrawing from the vagina. The withdrawn fluid was transferred to a microscopic glass slide. A cover slip was placed carefully on the smear to avoid entry of air bubbles. The slide was then observed under an optical microscope.

2.7 Preparation of Plant Extract

The method described by [2] was adopted for the preparation of aqueous leaf extract of *M. aboensis*. Fresh leaves were harvested from the botanical garden of the department of pharmacy. They were plucked from the stem and shade dried for a period of 14 days. The dried leaves were then ground to fine powder. The powdered sample was stocked in a plastic container from which 680g of *M. aboensis* was extracted in 1000ml of distilled water for 24 hours at room temperature. This was then filtered with Whatmann No 1 filter paper. The filtrate was then concentrated in a steam bath to give 150g of dark brown residue. The residue was then reconstituted in distilled water to give the required doses and administered in mg/kg body weight of animals that was used for the study.

2.8 Abortifacient Activity

Possible abortifacient property of the plant extract was tested using the method of [9]. Male rats were mated with female rats (2:1) and the presence of clumps of spermatozoa was designated as day 1 of pregnancy. Rats were randomly distributed into six (6) groups: 1 control group and 5 experimental groups. Aqueous extracts of *M. aboensis* leaves were then fed to the pregnant rats (experimental groups) at doses of

1000,2000,3000,4000 and 5000mg/kg body weight of animal once daily throughout the period of pregnancy. All animals were allowed to go full term (litter) and the pups were counted and weighed. All animals (maternal) were then sacrificed by chloroform inhalation and thereafter blood samples were collected for hormonal investigation. The following parameters were computed to determine possible abortifacient property of the plant:

- Number of pups at birth
- Maternal weight
- Foetal weight
- Litter/foetal quality(appearance)
- Crown rump length(CRL)
- Gestation period
- Pregnancy index
- Number of rats with vaginal bleeding
- Malformed pup

2.9 Hormonal Assay Procedure

Each of the micro plate wells was first formatted i.e. the desired number of wells required for the investigation was collected. The micropipette was then set to desired volume by turning the knob. 50ul of standards, specimen and control was then added into the appropriate well of the antibody pre-coated micro-titre plate. This was then followed by addition of 100ul of the enzyme conjugate reagent (Biotin) into each well with thorough mixing for 30 seconds. The mixture was then incubated at room temperature for 37°C for 60 minutes. The incubation mixture was then removed by flicking each plate content into a sink. A washing buffer was then used to remove and flick the micro-titre wells. This was done several times. After the final washing, the plate was then inverted and blot dried by striking plate onto absorbent paper to remove all residual water droplets. Each well was then incubated again at room temperatures for another 20 minutes and this was followed by addition of 100ul of stop solution to stop the reaction process. Each well was gently mixed by rocking plates for 30 seconds to ensure complete discoloration from blue to yellow. The optical densities at 450nm of the mixture in each well were then read using a micro-titre well reader within 30 minutes. The procedure described in the hormone assay kits was used according to the principle highlighted by [10].

III. Statistical Analysis

The arithmetic mean and standard mean error (MEAN±SEM) was calculated for each value. The results were subjected to statistical analysis using student t- test method. Statistical significance was read at p<0.05. Values with the same superscript were considered not statistically significant when compared to their respective controls.

IV. Results

Table 1: Effect of aqueous leaf extracts of *M. aboensis* on maternal weights of pregnant Wister rats

Group (N=5)	Initial body weights	Final body weights
1	204.0±2.57	234±0.15
2	158.9±1.78	172.1±0.01
3	181.4±0.96	200.4±1.58
4	168.8±1.89	185.3±0.54
5	217.4±1.90	242.0±1.15
6	196.8±3.33	215.6±1.86

The result from table 1 above shows the effect of *M. aboensis* on maternal body weights of pregnant Wister albino rats. There was significant increase in body weights of all the experimental groups when compared with the control group.

Table 2: Effect of aqueous leaf extract of *M. aboensis* on fertility indices in pregnant Wister rats for 21 days of pregnancy

Parameters	Pregnant non-treated group(Control)	Pregnant treated group (Experimental) (mg/kg body weight)				
	Control	1000mg	2000mg	3000mg	4000mg	5000mg
No of rats delivered (Litter size)	5(10,11,9,10,10)	5(8,10,11,10,9)	5(9,10,10,11,10)	5(8,6,9,8,9)	5(8,6,8,8,7)	5(6,7,7,8,7)
No of live foetus	50	48	45	31	22	20

Foetal body weight(g)	6.20±0.56	6.00±0.44	5.48±0.24	3.41±0.31	3.15±0.20	2.30±0.18
Gestation period(days)	22	22	21	20	20	20
Crown rump length(mm)	6.45±0.05	6.20±0.32	6.00±0.55	5.87±0.13	5.17±0.67	5.00±0.06
Pregnancy index (%)	100	100	100	80	60	60
Rats with vaginal bleeding	Nil	Nil	Nil	Nil	Nil	Nil
Malformed pups	Nil	Nil	Nil	Nil	Nil	Nil
Survival ratio (%)	100	96	90	77.5	59.4	57.1

Survival ratio=no of live foetus/no of live + no of dead foetus × 100/1; Pregnancy index= Values in bracket were computed figures;

The results from table 3 above shows varying effects of aqueous leave extracts of *M. aboensis* on fertility index in pregnant wistar rats. The number of live foetus and weight of the foetus decreased significantly with increasing concentrations of the extract. This was most evident at 4000 and 5000mg body weight of the extract. The survival ratio decreased to 57.1% at highest concentration (5000mg) of the extract when compared with the control (100%).None of the rats showed signs of vaginal bleeding as well as malformations of pups in the treatment groups. The gestation length was shortened to 20 in the treatment groups that received 3000, 4000 and 5000mg of the extract. The crown rump length (CRL) when computed also decreased significantly (p<0.05) with increasing concentrations of the extract.

Table3: Effect of Aqueous Extract *M. aboensis* on serum levels on Follicle Stimulating Hormone (FSH) and Luteinizing hormone (LH) in pregnant Wister rats.

Groups (N=5)	Follicle Stimulating Hormone (ng/dl)	Luteinizing Hormone (ng/dl)
1	5.24±0.10 ^a	9.38±0.17 ^a
2	0.82±0.03 ^b	0.98±0.03 ^b
3	0.52±0.00 ^c	0.80±0.02 ^c
4	0.46±0.05 ^d	0.56±0.03 ^d
5	0.15±0.01 ^e	0.53±0.01 ^e
6	0.13±0.01 ^f	0.48±0.03 ^f

Values with different superscript are statistically significant (p<0.05) when compared to the control; N=number of rats in each group.

Table4: Effect of *M. aboensis* on serum levels of Prolactin, Progesterone and Oestrogen in pregnant Wister rats.

Groups (N=5)	Prolactin ng/dl	Progesterone ng/dl	Oestrogen (ng/dl)
1	3.04±0.01 ^a	18.49±0.21 ^a	51.75±0.44 ^a
2	0.81±0.01 ^b	18.20±0.01 ^a	50.05±0.88 ^a
3	0.32±0.01 ^c	18.09±0.01 ^a	50.55±0.64 ^a
4	0.23±0.13 ^d	17.39±0.16 ^a	49.05±0.52 ^a
5	0.16±0.04 ^e	15.46±0.21 ^b	46.58±0.73 ^b
6	0.12±0.01 ^f	14.08±0.21 ^c	31.60±0.67 ^c

Values with different superscript are statistically significant (p<0.05) when compared to the control; N=number of rats in each group

The effects of administration of aqueous extract of *M. aboensis* leaves at 1000, 2000, 3000, 4000 and 5000 mg/kg body weight for 21/22 days on the concentration of serum reproductive hormones in matured pregnant female rats are shown in tables 3-4. When compared with the controls, administration of the extract produced significant decrease (p<0.05) in serum follicle stimulating hormone(FSH), Luteinizing hormone(LH) and prolactin concentrations in the experimental groups (Table 3).Progesterone and oestrogen were also significantly reduced (p<0.05) at concentrations of 4000 and 5000mg/kg body weight of the extract(Tables 4).However, a decrease was also observed in serum oestrogen and progesterone at concentrations of 1000, 2000 and 3000mg/kg body weight of the extract but values obtained were not statistically significant (p>0.05).

V. Discussion/Conclusion

The quest for naturally occurring compounds of plant origin that could provide some help benefits as contraceptives and fertility control agents prompted the interest in *M.aboensis* leaves. Several research involving plants have made significant rewarding progress in many vital areas such as antibody development [11] (Adebayo *et al.*, 2001), Cancer [12] (Harvey, 1999) and reproductive medicine including foetal delivery, pre-natal development, pre and post coital contraceptives. For e.g., seeds of *Carica papaya*, *Garcinia kola* and leaves of *bambusa vulgaris* have been authenticated by several scientific studies to serve as abortifacients in rats [13][14] [15]. Some of these agents can be used as post coital contraceptives. Preliminary phyto-chemical screening of the leaf extract of *M. aboensis* revealed the presence of alkaloids, flavonoids, tannins, cardiac glycosides and phlobatanins[7].These plant chemicals can serve as precursors in the manufacture of drugs. For example, ergot alkaloids which have been proven to have adverse effect on foetal development is being used by physicians, either alone or synergistically with oxytocics to induce abortion[14]. Furthermore, anti-fertility and abortifacient activities of phenolics, saponins and phytoosteroids have been confirmed with sufficient data on animal models [16].This may suggest a plausible reason for alkaloids and saponins being partly responsible for the observations in this study. There can be disruption of pregnancy by interference with mitotic division of the foetus as in the case with cytotoxic agents with the implantation process which may eventually result in pre and post implantation embryonic loss[17], destruction of the endometrial lining of the uterus or disturbances in the hormone profile of the animals. Changes in body weight and vaginal bleeding can also be used to evaluate the integrity of maternal homeostasis [18]. Clinical toxicity signs such as respiratory distress, salivation, appearance of hair colour were not observed from the cage side at any time during the exposure period in the extract treated animals. However, diarrhoea (evident from their watery stool) and fatigue were observed in the animals treated with the extract. This episode of fatigue and diarrhoea in the extract treated animals corroborates with the earlier perceived property of the plant as a laxative. The marked increase in the maternal weights of the treated animals when compared with their initial weights may suggest that growth was not impaired by the extracts in all experimental groups. This finding is in agreement with previous works by [19] but contrary to reports by [20] who reported retarded growth in rats fed with *Garcinia kola* for six weeks. None of the experimental rats showed signs of vaginal bleeding or expulsion of products of conception. With these notwithstanding, the extract still produced varying effects on indices used to evaluate abortifacient property. For e.g., the weights of the foetuses decreased significantly with increasing concentration of the extract and this was most pronounced at 4000 and 5000mg/kg body weight of the extract. This may suggest that there was insufficient nutrient transfer from mother to the foetus.The chemicals present in the plant(tannins) may contribute to the decrease in foetal weight as they may interfere with the absorption of nutrients into the maternal blood stream, thus reducing the availability of nutrients for placental transfer to the foetus [21]. The litter size and number of pups at birth showed a decrease when compared to the control group. This observation agrees with [22] following administration of methanolic extract of *Achyranthesaspera* leaves to pregnant rats. Also in agreement with this finding is work by [23] who reported the reduction of litter size and foetal weight by intra-peritoneal administration of *Phyllanthusamarus* extract. The result, however, contrast with reports by [24] who reported that *Ficusplatyphylla* increased litter size in female *Rattusnorvegicus*, thus promoting fertility in the species. The calculated gestation period (in days) was shortened to 20 in animals which received 3000, 4000 and 5000mg/kg body weight of the extract. There was no evidence of malformation of pups in all the treated groups. All of these are pointers to possible abortifacient activity of the extract during the post implantation period.

Quantitative determination of female reproductive hormone in the serum of animals including humans could be used to diagnose an ectopic pregnancy or falling pregnancy, ovulation characterizing luteal phase defect, conception and fertility. Follicle stimulating hormone is a hormone which is secreted by the pituitary gland in the brain and it functions to promote the production and maturation of ova in females[25].The significant reduction ($p<0.05$) in the concentration of FSH by the extract in this study suggests possible dysfunction of the gonads which may stop folliculogenesis and delay maturation of the follicles in the pre-ovulatory phase [25]. It is also possible that the extract might have exerted its effect on the anterior pituitary or the hypothalamus since the secretion of FSH is regulated by the gonadotropic releasing hormone. This reduction may also adversely affect subsequent conception in female rats.

Luteinizing hormone (LH) is required for continued development and functioning of the *coporalutea* as well as ovulation during the pro-oestrous stage. The significant reduction ($p<0.05$) in the serum concentration of LH in the extract treated animals could be explained with the physiological process of luteolysis preceding parturition [26], or luteal phase that is not being maintained. Furthermore, reduction in LH concentrations may also hinder luteinisation of ovarian follicles[27].Any substance capable of inhibiting the release of LH could provoke disruption of ovulation. This may result in impairment of the oestrous cycle, hamper conception and normal reproduction in female.

Again, alkaloids and flavonoids have been implicated in reducing plasma concentration of LH and FSH[28][29].Therefore, it could be stated that *M.aboensis* leaves contain anti-gonadotropic substances which may affect the oestrous cycle and reproduction in females.

Measurement of progesterone could be used to determine ovulation as well as characterize luteal phase defect [30].The feedback inhibition effect of gonadotrophic releasing hormone(GnRH) by oestrogen and progesterone provides the basis for the most widely used form of contraception. The feedback inhibition by component of the extract might have decreased the pulse frequency of GnRH by the hypothalamus, hence the decreased secretion of FSH and LH observed in this study. The significant reduction ($p<0.05$) in serum progesterone at concentrations of 4000 and 5000mg/kg body weight in this study may hamper the secretion of normal proteins required to nourish an implanted fertilized egg. It is possible that if the concentration is raised above this level, it may in turn have an adverse effect on prenatal development and also encourage menstruation or toxemia[31].Such reductions in progesterone as observed in this study could bring about shortened gestational period as seen upon administration of 3000, 4000 and 5000mg/kg body weight of extract. The findings on progesterone in this study agree with works by [32] following administration of seeds of *Coriandumsativum* to rats. Alkaloids have equally been reported to inhibit the synthesis of cellular progesterone [33], therefore the significant reduction ($p<0.05$) in serum progesterone at 4000 and 5000mg/kg body weight of extract may be due to presence of alkaloids in the extract.

In females, the measurement of estradiol is useful in evaluating the status of ovarian function since it stimulates the growth of the uterine lining, causing it to thicken during the pre-ovulatory phase of the cycle. In synergy with FSH, estradiol stimulates granulosa cells of proliferation during follicular development[34].Plants with estrogenic property can directly influence pituitary action by peripheral modulation of LH and FSH, decreasing secretion of these hormones and blocking ovulation. The serum concentration of estradiol was significantly reduced($p<0.05$) at 4000mg and 5000mg.This reduction suggests amongst others, possible decreased aromatase activity or substrate supplementation during oestrogen synthesis[35] and this may hamper ovulation, preparation of the reproductive tract for implantation and subsequent maintenance of pregnancy[36]. [37]also reported that plant alkaloids inhibited aromatase activity, thus altering the potential for steroid production and reproductive performance. Therefore, the alkaloid in the extract may be responsible for the reduced level of estradiol probably inhibiting aromatase activity.

Prolactin helps to initiate breast development by inducing the lobuloaveolar growth of the mammary gland. It also stimulates lactogenesis. Dopamine serves as the major inhibiting factor or break on prolactin secretion [38].It is a hormone produced by the pituitary gland in the brain. The significant reduction ($p<0.05$) in serum prolactin at all concentrations of the extract observed in this study can be explained by the plant acting on dopamine receptors found on pituitary gland. The result from the study may provide useful information in literature of the plant having anti-fertility effects and also as a laxative which has been an age-long claim. The toxic effect of the plant was most pronounced at 4000 and 5000mg/kg body weight of the extract. The decrease in foetal weight, litter size, live foetus and increase in dead foetusetc. could serve as useful indices for its abortifacient claim. Also, significant alterations in the levels of reproductive hormones may in part, account for the mechanism of action of the extract as an abortifacient. Further studies may lend credence to this proposition.

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