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Original Article

ETHANOL ROOT EXTRACT OF THE AFRICAN APHRODISIAC, *MONDIA WHITIE* (PERIPLOCACEAE), POSSESES ESTROGENIC ACTIVITY

KWESI BOADU MENSAH, CHARLES BENNEH, ERIC BOAKYE-GYASI

Department of Pharmacology, Faculty of Pharmacy and Pharmaceutical Sciences, College of Health Science, Kwame Nkrumah University of Science and Technology
Email: qwesimensah@gmail.com

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ABSTRACT

Objective: To determine the effects of ethanol extract of the dried root *Mondia whitie* (mondia) *on female* reproductive system using the chick uterotrophic assay and the rodent estrous cycle studies.

Methods: Phytochemical screening was done to detect the presence of secondary metabolites. Using the Chick Oviduct Uterotrophic assay, estrous cyclicity assay in Sprague Dawley rats and serum biochemical analysis, the effects of ethanol root extract of mondia on female reproduction was assessed.

Results: Mondia (30-300) mg/kg or estradiol benzoate (0.1-0.8 ug/kg) caused dose dependent increases in chick oviduct of white leghorns. Treatment of rats with Mondia (30-300) mg/kg increased duration of estrous and altered the repeatability of the next cycles. The estrus index ranged between 41.67-49.21 at the doses of mondia used compared with 25 for controls. There were alterations in the lipid profile with reductions in HDL, but increases in VLDL, LDL and triglycerides.

Conclusion: Ethanol extract of the dried root *Mondia whitie* possess estrogen-like activity on the female reproductive system

Keywords: *Mondia whittie*, Estrous cycle, Uterotrophic, Female reproduction.

INTRODUCTION

The sweet fragrance African plant, *Mondia whitie*, has been used for many purposes in traditional medicine. It has been reported to be anti-asthmatic, purgative, antiemetic, carminative, expectorant, orexigenic, pain and stress reliever, diuretic, antepileptic and anticonvulsant by several workers [1-5]. It is also commonly used for culinary purposes both as a spice and a vegetable [6].

Most importantly, however, it is used traditionally to boost fertility in males and females [7]. In males it functions primarily by improving erectile dysfunction, sexual weakness, preventing premature ejaculation and to increase sperm production [8, 9]. For this purpose, the fresh or dried parts, the root bark or the leaves are chewed. The mechanisms leading to these effects have only partly been elucidated but may involve increased tissue nitric oxide (NO) and cyclic guanosine monon Phosphate (cGMP) and reduced α-adrenergic stimulation [9-12].

The male and female reproductive system share similar neuro-endocrine pathways from the hypothalamus through to the pituitary and several hormones and neurotransmitters. In fact with the exception of Sexual Dimorphic Nucleus of Medial Pre Optic Area (SDN-MPOA) where there appears to be sexual dimorphism, several brain regions are similar. Subsequently substances that affect male fertility and reproduction most invariably affect female fertility. It is therefore not too surprising that mondia use is also popular amongst women. Besides is classical uses as an aphrodisiac, some women also use it as uterine stimulant to induce labour, for postpartum haemorrhage, stimulation of lactation etc [7, 13]. There is limited scientific data to support this usage. The aim of this study therefore was to determine the effects of mondia on female reproduction.

MATERIALS AND METHODS

Plant material

Mondia whitei, (Periplocaceae) was a kind donation from Professor Eric woode of the Department of Pharmacology. It was then authenticated at the department of Herbal Medicine, by Kwame Nkrumah University of Science and Technology by Dr Kofi Anan and a voucher specimen has been deposited in the herbarium.

Preparation of Mondia extract and phytochemical screening

One kilogramme of the root back were powdered and cold macerated in 3L of 70% ethanol for 72 hours. The macerate was filtered and the solvent evaporated at 70^{o}C under reduced pressure. The yield was 9.62% w/w. Routinely, mondia was reconstituted by dissolving in distilled water.

Preliminary phytochemical test for the detection of secondary metabolites were carried out according methods adopted from Figel, (1960) and Gibbs (1974) [14, 15]. Briefly 500 mg of ethanolic extract of Mondia was dissolved in 100 ml of ethanol and filtered through Whatmann filter paper No 1. The filtrate obtained was subjected to preliminary phytochemical screening tests for alkaloids, glucose, glycosides, lignin, phenols, saponins, sterols and tannins as described elsewhere [14, 15].

Determination of the effects of Mondia on estrous cycle

Adult female Sprague-Dawley rats (N=50, 12wks old, wt 130-155) were obtained from the Animal House of the Faculty of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana. The animals were housed in standard aluminum cages, five per cage, in a controlled temperature room (28 °C), with a 12 hour light/dark cycle, in the Animal room. They were fed on standard laboratory pelleted feed (from GAFCO, Limited, Tema) and water *ad libitum*. All animal experiment was approved by Departmental Ethics committee.

Screening of the rats

Vaginal smears were taken daily, and only animals displaying at least two consecutive 4-5 day estrous cycles were used. All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, and refusal of feeds, diarrhea, weight loss, hair-loss, coma and death) throughout the duration of the experiment. All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

Every morning between 8:00 and 10:00 a. m. vaginal secretions from each rat were collected with a plastic pipette filled with 0.2 ml of

normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Drowned vaginal fluid was placed on glass slides. Unstained material was observed under a light microscope, with 10 and 40x objective lenses. The types of cells were noted as either round or nucleated (epithelial) or irregular non-nucleated (cornified) cells; and leukocytes. The phase estrous was determined according to methods described elsewhere [16, 17]. Rats exhibiting a 4-stage and 4-5 day estrous cycle of proestrous-estrous-metestrous-diestrous were classified as normal while any deviation from this pattern in terms of duration and sequence was categorized as abnormal. Sprague Dawley rats with normal estrous were then selected for the subsequent experiment.

Effects of mondia on estrus cycle of sprague-dawley rats

Adult female Sprague-Dawley rats exhibiting regular cycle were assigned to one of four groups (n=6). They were treated according to the schedule below;

Group A rats received only distilled water throughout the experiment.

Group B received 30 mg/kg mondia orally for 21 days.

Group C received 100 mg/kg mondia orally for 21 days.

Group D received 300 mg/kg mondia orally for 21 days.

The estrous phase was determined daily as by the method described by Long & Evans, (1922) and Mandl (1951). Sprague dawley rats were sacrificed by cervical dislocation at the end of this period and the ovaries, uteri, spleens, livers, and kidney harvested and blood samples analyzed.

Effect of mondia on the chick oviduct uterotrophic assay

Fifty (50) one-day old White leghorn chicks were purchased from Topman farms, Kumasi Ghana and maintained in the animal house of the Department of Pharmacology, College of Health Sciences, KNUST, Kumasi, Ghana and fed twice daily which chick starter mash. They were housed in stainless steel cages with soft wood shavings as bedding. The chicks were handled humanely throughout the experimental period. On day six chicks were grouped into seven (n=6) and treated according to the schedule below.

Group 1: received only distilled water for 6 days

Group 2-4: received (30, 100, 300) mg/kg *p. o* of *Mondia*, respectively for 6 days

Group 5-8: received (0.1, 0.2, 0.4, 0.8)µg/kg of 17-*ß* oestradiol benzoate *Sc* for 6 days

Group 9: 0.2 ml of coconut oil (oestradiol solvent) *Sc* for 6 days

Based on methods described elsewhere [18, 19], chicks were euthanized after the 6th day with *Ip* sodium pentobarbital. Oviducts were dissected out at the juncture with the cloaca and the wet weight determine.

Statistical analysis

Data were reported as the mean and standard error of mean (SEM). Significance was calculated by one way ANOVA using Neuman-Keuls Test using graph pad prism version 5.

RESULTS

Phytochemical screening

Phytochemical screening of Mondia showed the presence of reducing sugars, glycosides, and triterpenes. However, Alkaloids, lignin, tanins, sterols, phenols were all absent in the ethanol extract.

Effect of the ethanol root extract of mondia on estrous cycle

Treatment of rats for 21 days with extract showed significant changes in duration and sequence of the estrous cycle and repeatability of the next cycles. Doses of mondia (30-100) mg/kg showed increases in the duration of the estrus phase but a decrease in proestrus, metestrus and diestrus phases of the estrous cycle. At 300 mg/kg there was an increase in estrus and diestrus but decrease in proestrus and metestrous phases. The estrus index was also higher at all doses than that of the control as indicated in table 1.

Table 1: Effects of mondia on the duration of phase estrus cycle of Sprague dawley rats

Duration of Estrous cycle Phase (days)	Dose of *Mondia whitie* extract (mg/kg)			
	030100300			
Proestrus	5.25±0.250	3.333±0.453	2.667±1.202*	3.750±0.173
Estrus	5.25±0.250	8.667±0.333**	10.333±0.882***	8.750±0.946**
Metestrus	5.25±0.250	4.333±0.333	4.667±0.333	3.000±0.946*
Diestrus	5.25±0.250	4.500±0.882	3.333±0.755	5.500±1.440
Estrus Index	25.00	42.28	49.21	41.67

Results presented as mean±SEM. Statistical analysis is by One way ANOVA using Newman-Keuls Post hoc test. * means P<0.05, ** p<0.01, *** p<0.001 when compared with control

$$\text{Estrus Index} = \frac{\textit{Number} \text{ of days with clear estrus}}{\text{Total duration of treatment}} x100$$

Effect of the ethanol root extract of mondia on haematology and serum biochemistry

The hematological analysis revealed no significant changes in red blood cells (RBC), haemoglobin (HGB), haematocrit (HCT), platelets aggregation (PLT-AG), mean cell volume (MCV), mean cell haemoglobin concentration MCHC, and mean cell haemoglobin (MCH), but there were significant increase white blood cell count (table 2).

In the serum biochemistry, there were no significant changes in albumin, globulin, total protein and urea. However there were alterations in the lipid profile. There were reductions in HDL, but increases in VLDL, LDL and triglycerides when compared to control. There were however no significant difference between organs (liver, spleen, kidney, ovary, uterus) to body ratio of treated animals and control.

Table 2: Effects of Mondia on haematology of female Sprague dawley rats

Hematological parameters	Dose of *Mondia whitie* extract (mg/kg)			
	0	30	100	300
WBC	7.35±2.46	11.68±1.60	19.38±1.30**	22.95±2.09**
RBC	7.99±0.45	8.63±0.30	7.95±0.13	7.14±0.18
HGB	13.03±0.37	11.83±0.38	11.95±0.25	11.78±0.48
HCT	45.66±2.34	48.10±1.14	42.80±0.97	39.93±1.53
MCV	58.03±0.32	55.93±0.25	55.63±0.28	52.20±4.08
MCH	17.10±0.59	15.70±0.17	16.10±0.44	13.30±0.39***
MCHC	29.30±0.84	25.18±1.59	27.27±0.39	29.45±0.41
PLT	645±117	547±890	586±260	412±507

Results presented as mean±SEM. Statistical analysis is by One way ANOVA using Newman-Keuls Post hoc test.** p<0.01, *** p<0.001 when compared with control

Table 3: Effects of Mondia on serum biochemistry of female Sprague dawley rats

Parameter	Dose of *Mondia whitie* extract (mg/kg)			
	030100300			
Albumin	73.04±1.97	69.20±0.80	73.31±0.09	76.3±1.02
Globulins	271.23±20.12	262.16±1.04	305.48±9.98	277.17±28.90
Total protein	345.62±21.40	331.81±1.94	371.500±8.876	357.982±28.689
Cholesterol	8.31±0.34	7.84±0.15	9.11±0.15	9.14±0.48
HDL	1.28±0.02	0.39±0.01***	0.17±0.04***	0.53±0.18***
VLDL	2.5±0.02	2.58±0.04	2.96±0.08***	2.850±0.11**
LDL	5.4±0.038	5.13±0.28	6.18±0.06	6.403±0.129*
Triglycerides	5.363±0.186	5.520±0.134	6.403±0.129*	6.368±0.239**
Urea (mmol/l)	21.92±1.18	21.79±1.44	38.10±3.61	28.67±11.27

Results presented as mean±SEM. Statistical analysis is by One way ANOVA using Newman-Keuls Post hoc test. * means P<0.05, ** p<0.01, *** p<0.001 when compared with control

Effect of Mondia on chick oviduct uterotrophic assay

Treatments of 6 day old white leghorn chicks with Mondia (30-300) mg/kg or estradiol benzoate (0.1-0.8 ug/kg) led to dose dependent increases in oviduct. Mondia had the similar effect to oestradiol but oestradiol was more potent at the doses used.

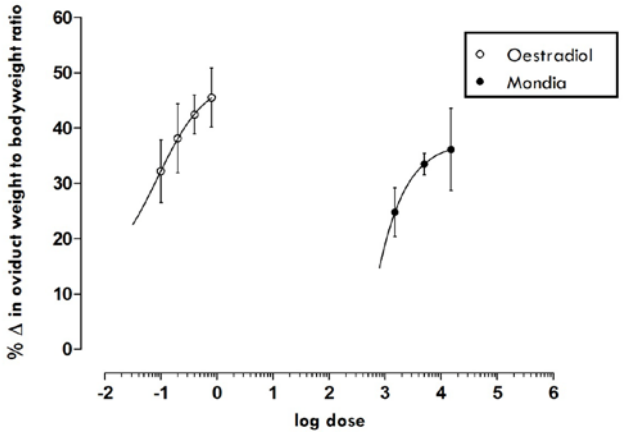

Fig. 1: Effects of *Mondia whitie* on the chick oviduct (uterotrophic assay)

DISCUSSION

Reproduction in mammals is regulated by Hypothalamus Pituitary Gonad axis. Examining the effects of xenoboitics on the duration of estrous cycle gives a reasonable idea on their potential effects on reproduction. In this study, monda increased the estrous phase of the estrous cycle whilst depressing the proestrous, metestrous and diestrous phases. The cornification in the vaginal epithelial cells during estrous is mainly due to high levels of estrogens secreted by the ovarian matured follicles. Exogenous estrogens and substances with estrogenic activity produce similar effects. This observation of mondia in the rat was further strengthened by the uterotrophic assay in the chick. The underlying principle of the chick oviduct uterotrophic assay stems from the fact that elevated levels of estrogens and estrogenic compounds at the early developmental phase of female animals; rodents, birds etc. Increases the uterine weight dose dependently [19]. These studies show clearly that mondia effects in the female were similar to estrogen.

The levels of estrogen are regulated by pituitary gonadotrophins, LH and FSH. Gonadotrophins stimulate developing follicles as the principal source of estrogen. Substances that affect gonadotrophins may indirectly possess estrogenic activity especially in studies where female animals were on ovariectomized as in the case of the estrous study [20-22]. However, this will be unlikely for mondia because the hypothalamus of the chick is in a quiescent state, presupposing that mondia effects on the uterus are not mediated through the hypothalamus.

Estrogens are known to alter serum biochemical parameters particularly clotting factors and body lipids [20]. They slightly elevate serum triglycerides and slightly reduce total serum cholesterol levels. They increase HDL levels and decrease the levels of LDL partly explaining why premenopausal women have lower cardiovascular risk [23, 24]. Mondia caused an elevation in triglyceride especially at high doses (100-300) mg/kg consist with literature on estrogens. However, unlike estrogens, mondia had in significant effect on total cholesterol but increased the plasma VLDL, LDL significantly and reduced HDL as well. This may have cardiovascular consequencies if such effects were to occur during longterm human usage. The difference between mondia and estrogens in terms of cholesterol could be due to estrogen receptor sub s types (ERa and ERb) selectivity and the likelihood of multiple competing constituents in ethanol extract of mondia.

Traditionally mondia has been used as uterine stimulant for augemting labour and for postpartum haemorrage. These effects are largely under the influence of endogenous oxytocin. However uterine responsiveness to oxytocin is highly estrogen dependent. Furthermore estrogen increases the expression of the oxytocin receptors. Proliferation of mammary tissues are aslo highly regulated by circulation estrogen, progesterone and prolactin. Oestrogens increase both prolactin secretion and the proliferation of lactotrophs through release. Mondia's estrogenic activity may explain its traditional usage as an ecbolic and lactation stimulant [7, 13].

In conclusion ethanol extract of the dried root *Mondia whitie* possess estrogen-like activity on the female reproductive system

CONFLICT OF INTERESTS

Declared None

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