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Full Length Research Paper

Effects of *Ruellia praetermissa* extract on ovulation, implantation, and the uterine endometrium of female rats

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The effects of extracts of *Ruellia praetermissa (Acanthaceae)* on ovulation, implantation, uterine weight and foetal development were studied in adult rats. Female rats weighing 180 to 220 g were divided into three experimental groups. Group 1 was administered 200 mg/kg body weight of the extract at 10, 14 and 18 h respectively on proestrous rats. Vaginal smears were taken daily to monitor the oestrous cycle and ovulation. Group 2 consisted of pregnant rats, which received the same dose of the extract on days 1 - 5, 7 - 9, 9 - 17 and 1 - 17 of gestation. Gestational parameters (number of corpora Lutea, implantation sites, resorption sites and dead foetuses) were monitored. In group 3, non-pregnant mature female rats were treated with the same dose of the extracts from 1 - 17. The uterine muscle weight was recorded on days 4, 9, 15, and 18. Ovulation was partially blocked resulting in reduced number of ova in the oviduct of the treated rats (3 ± 1, 5 ± 1 and 10 ± 3 treated at 10: 00, 14: 00 and 18: 00 h respectively) compared with control (12 ± 5: $p < 0.05$). There was 20% increase in implantation rate in rats which received treatment for the first five days of gestation ($p < 0.05$). There is an increase (0.05 ± 0.002 - 0.35 ± 0.001) of the uterine weight comparable to that produced by using 3 µM 17β-estradiol (0.03 ± 0.001 - 0.35 ± 0.005). The decrease in effects on ovulation and the increase in the uterine walls are possibly due to flavaonoids (luteolin, quercetin, and apigenin). Increase in Implantation and the uterine weight are due in part by plant sterols (β-sistosterol and stigmasterol) identified in the EtOAc extracts of this plant drug. These findings provide the pharmacological basis for the traditional use of this plant for prenatal care in the North West Region of Cameroon.

Key words: *Ruellia praetermissa*, ovulation, implantation, uterine endometrium, flavonoids, plant sterols.

INTRODUCTION

Reproductive health is even in the 21st century still a potential source of mortality for both gestation mothers and their developing foetuses in the underdeveloped world in general and Africa in particular. There is thus an urgent need to get effective safe prenatal drugs from natural sources. Medicinal Plants are readily available to women in these regions with limited access to modern medicines. *Ruellia praetermissa* is a wild herb and indigenous to central and south eastern Asia and also widespread in tropical and subtropical Africa. In Cameroon, it enjoys a folk reputation as blood and pregnancy medicine. In other south Eastern African countries; it is widely applied to relief pain (Gelfand et al., 1985). In a previous work, we communicated the estrogenic and cholinergic properties of the methanol extracts of *R. praetermissa* in female rats (Salah et al., 2002). This plant drug regularizes pregnancies threatened with miscarriages in early stages. This is due to its ability to mimic 17β-estradiol. It stimulates the growth of the uterine endometrium. This is by the proliferation and the development of the cells of the uterine endometrium as it up-regulates estrogen, LH and progesterone receptors on the uterine muscles at the beginning of gestation and to excite the uterine myometrium at term (Salah et al., 2002). In addition, the extracts of this plant drug has a stimulatory effects on the

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motility of the gastrointestinal tract (Salah et al., 2000) and antihypertensive effect by the Inhibition of Angiotensin-Converting Enzyme activity (Salah et al., 2001). These biological effects are of particular interest since high blood pressure and indigestion frequently characterise gestation period. This herbal drug is rich in flavonoids such as luteolin, quercetin and apigenin. The favonoids also have an antispasmodic effect on uterine smooth muscle (Salah and Martins, 2001). The plant extract has 5-Lipoxygenase inhibition and antisplasmodic effects of uterine having Angiotensin-Converting Enzyme (ACE) inhibitory ability (Salah, 1999). The anti-inflammatory properties of the flavonoid components of *R. praetormissa* led us to investigate the effect of the extract on ovulation and the gestation to assess its physiological action on the Reproductive parameters. The importance of the anti-inflammatory property of *R. praetermissa* is emphasized by evidence that ovulation is an inflamma-tory process (Epsey, 1980, 1994). Ovulation in rat as in humans is brought about by a luteinising hormone (LH) surge. The circulating levels of LH begin to rise in the afternoon of prooestrus, about 2 pm to 3 pm and reaches peak at about 5 - 7 pm. This rapid surge induces follicular rupture and ovulation. Ovulation can be blocked experimentally by high doses of anti-inflammatory drugs administered before the LH surge because once the levels start to rise it may not be brought down by any drug (Gaytan et al., 2002). Once ovulation is followed by fertilization, effective gestation and parturition has to be ensured. The present study aims at finding out the scientific basis of the use of *R. praetermissa* as a prenatal herbal drug in the North-Western region of Cameroon.

MATERIALS AND METHODS

Plant material

The plant material was collected in Belo, North West Province of Cameroon in September 2004. The specimen was verified and authenticated by Dr. Tina Fongot as the one earlier identified by Kofany of the Cameroon National Herbarium Obili Yaounde under the voucher specimen number 43596 deposited in 1996.

Extraction and sample preparation

Sun dried leaves of the plant were pulverized and 250 g was extracted using the sohxlet for 12 h in each case progressively with 2 l of n-hexane, chloroform, ethyl acetate, and methanol. The extracts (160, 140, 250 and 300 mg, respectively) were recovered by rotavaporization. The chaffs were boiled in water at 85°C for 6 h and 225 mg of extract was recovered by lyophilization.

Plant drug analysis

TLC: The ethyl acetate extract from bio-guided preliminary screening was used exclusive in the study. The extract (5 mg) was co-chromatographed with flavontest samples (rutin, chlorogenic acid, hyperoside and isochlorogenic acid), luteolin and luteolin-7-glucoside, quercetin, apiginin, isoquercetin, delphinidin and caffeic acid using ethyl acetate-formic acid –glacial acetic acid-water (100:11:11:26) as the mobile phase and precoated silica gel 60 F_{254} (20 x 20 thickness 0.25 mm Merck, Darmstadt, Germany) as the stationary phase. The plate was first observed at UV_{254} nm then sprayed with natural products-polyethylene glycol reagent (NP/PEG) and evaluated at UV_{366} nm.

HPLC: This was carried out with an HP 1090 A liquid chromatography and an HP 1040 photodiode array with a Hewlett Packard detector as a LiChrospher 100 RP 18 (5 µm) column 125 x 4 mm (Merck, Darmstadt, Germany), and a precolumn LiChrospher RP-18 4-4 mm (5 µm) (Merck, Darmstadt, Germany) with detection at wavelength 210, 254, 280 and 366 nm. The mobile phase used for the separation was distilled water (Solvent A) and acetonitrile (Solvent B), all acidified with 33 µL of phosphoric acid (85%). It was started at 10% of solvent B and a linear gradient of 10 - 30% MeCN during 20 min for a total run of 30 min, at a flow of 1 mL/min, and a volume of 12.5 µL of 1 mg/mL of Ethyl Acetate extract of *R. praetermissa* was injected.

Experimental animals

Sixty adult female Swiss rats were bred in cages in the Animal House of the University of Yaounde I. Before the study, they were acclimatized for 2 weeks in the laboratory under standard conditions of temperature and illumination (12 h dark: 12 h light) cycle. They were fed with commercially available rat's pellets and had access to drinking water *ad libitum.* Rats that underwent 2 successive 4 or 5 day cycles, weighing between 180 - 220 g were used. They were divided into subgroups of twelve each.

The study of the extracts on the animals

Ovulation experiment: In the ovulation experiment, group I (n = 20), the animals were replicated into control and three experimental subgroups a, b, c; of 5 rats each which received the extract by oral administration using a curved needle and a tuberculin syringe, 200 mg/kg body weight at 10, 14 and 18 h respectively for three estrus cycles. The control group received distilled water. Vaginal smears were obtained daily by vaginal lavage to monitor ovulation and oestrous cycle. At the end of group 1 experiment, the animals were sacrificed using ether anesthesia and the fimbriated part of the oviduct was dissected out from the rats, suspended in normal saline and placed on a microscopic slide with a cover slip to count the number of ova in the oviduct.

Gestation and foetal development experiment

In the gestation and foetal development experiment, group II (n = 22) the animals were mated during the proestrous to oestrus night and the presence of spermatozoa was determined by microscopic examination of the vaginal smear the next morning. The presence of spermatozoa indicated conception and represented day 1 of pregnancy (Oderinde et al., 2002). These pregnant rats were subdivided into groups a, b, and c which received orally 200 mg/kg body weight of R. *praetermissa* on days 1 to 5 of gestation (implantation studies) for group 2, 7 - 9 days of gestation for 3 (beginning of organogenesis), 9 - 17 for group 4 and 1 - 17 days of gestation for group 5. The control group 'd' received 3 ml of distilled water. Body weight, food consumption, gross appearance and behaviour were monitored daily. On day 21 of gestation, foetuses were removed from pregnant rats by ventral laparatomy and examined. The number of total implants, resorption, live and dead foetuses recorded. Live foetuses were removed from the uterus and weighed, and examined for gross malformations.

Table 1. The effect of single oral dose of 200 mg/kg of ethyl acetate extract of Ruellia praetermissa given at 10: 00, 14: 00 and 18: 00 h on the number of ova.

Time of administration of the extract	Number of Ova (Impact on Ovulation	
	Control Group (administered 3 ml of distilled water)	Experimental group (treated with 200 mg/kg of extract)
10: 00 h	12 ± 5	3 ± 1
14: 00 h	12 ± 5	5 ± 1
18: 00 h	12 ± 5	10 ± 3

Uterine muscle weight experiment

Daily (1 - 17) dose (200 mg/kg/day) was administered to 3 groups of virgin female rats (n = 5). Control group was treated with 3 µM 17β-estradiol. The animals were sacrificed successively on day 4, 9, 15 and 18. The uteri were removed and weights recorded.

Statistics

Results were expressed as Mean ± SEM. ANOVA with one degree of classification was used for comparison of more than two means followed by Scheffe's post hoc test. P values of 0.05 or less were considered significant.

RESULTS

Active principles

Chemical analysis of the four extracts revealed that the EtOAc extract of *R. praetermissa* had flavonoids aglycons (luteolin and apigenin) and their respective glycosides. The extract also contains a high concentration of triterpens (campesterol, stigmasterol, β-sitosterol, lupeol) and iridoid glycosides

Ovulation: The number of ova in the oviduct of treated rats was significantly reduced after commencement of treatment ($p < 0.05$) when compared with the control, (Table 1), meaning a partial inhibition of ovulation.

Gestational parameters and morphologic defects: All dams on study survived to their scheduled termination day. There were no abortions, no early deliveries and no death of animal during the study (Table 2).

The numbers of corpora Lutea, the number of implantation sites and the percentage of implantation recorded in pregnant rats, which received EtOAc extract of *P. praetermissa* were significantly increased ($p < 0.05$). Among the experimental groups, there was an increase of approximately 20% in the implantation rate for animals treated in the first 5 days of gestation as compared to the control.

Uterine muscle weight: Daily oral administration of the plant extract (200 mg/kg/day) and 3 µM of Estradiol 17β significantly increased the weight of the uterus (0.05 ± 0.002 - 0.35 ± 0.001) and (0.03 ± 0.001 - 0.35 ± 0.005 respectively, $p < 0.05$) in a time dependent manner (Table 3).

DISCUSSION

R. praetermissa has anti-inflammatory effect by inhibiting both 5-lipoxygenase and cyclooxygenase activity (Salah, 1999). Ovulation has been shown to be an inflammatory process (Epsey, 1980). The anti-inflammatory property of flavonoid-rich EtOAc fraction of the extract of *R. praetermissa* is in part responsible for the observed effect in inhibiting ovulation. This is when administered in rats before luteinising hormone (LH) surge (which causes follicular rupture and release of ova) reach its peak (Freeman, 1988). COX-2 is an essential enzyme that causes follicular rupture (Katori and Majima, 2000). Flavonoids such as apigenin, luteolin and quercetin are highly concentrated in the extract of *R. praetermissa*. These flavonoids inhibit the activity of cyclooxygenase and consequently ovulation (Akpantah et al., 2005). The extract promotes implantation as revealed by the increase in the number of implantation sites in the animals treated in the first 5 days of gestation and steady increase in uterine endometrium. The gain in the uterine weight of the non-pregnant rats treated with the extracts comparable to animals treated with 3 µM estradiol 17β confirms an estrogenic effect, (Salah et al., 2002). Plant sterols (β -sitosterol and stigmasterol) identified in this plant drug (Wagner and Bladt, 1998) are the active principles promoting growth in the uterine endometrium and hence implantation and gestation (Salah et al., 2002). These plant sterols stimulate the synthesis of endogenous estradiol in the animal system which promotes the growth of uterine endometrium. This endogenous extradiol up-regulates estrogen, LH and progesterone receptors on the uterine muscle cells hence potentiating implantation process and maintaining gestation. *R. praetermissa* has antisplasmodic effect on the uterine smooth muscle (Salah et al., 2001) by increasing the threshold to the contractile stimuli during gestation. Endogenous estradiol activates oxytocin receptors in the uterine myometrium at term by modifying the binding sites of Ca^{2+} and increases the responsiveness to oxytocin thus facilitating parturition by initiating the onset of labour (Berne and Levy, 1988).

Table 2. Reproductive and foetal parameters of the pregnant rats receiving 200 mg/kg/day of R. praetermissa EtOAc extracts by forced oral administration in different stages of gestation.

Parameters of Investigation	Group 1: Control (3 ml) of distilled water	EtOAc extract of *Ruellia praetermissa* (200 mg/kg/day)			
		Group 2: Day 1 - 5	Group 3: Day 7 - 9	Group 4: Day 9 - 17	Group 5: Day 1 - 17
Pregnant female rats	5	5	5	5	5
Average weight (g)	210 ± 10	220	210 ± 10	210 ± 10	210 ± 10
Day 18 weight(g)	366 ± 13	353	340 ± 12	336 ± 10	350 ± 10
Net Weight Gain (g)	156 ± 12	133 ± 15	130 ± 10	126 ± 10	140 ± 10
Number of Corpora Lutea	83	91	84	82	87
Mean of Corpora Lutea/female	24 ± 1.5	14 ±1.5	16 ± 1.6	15 ± 1.5	14 ± 1.5
Number of death foetuses	56	57	56	54	55
Number of implantation sites	57	64	58	56	66
Number of foetus/female	11.0 ± 0.7	12 ± 1.0	11.0 ± 1.5	10 ± 1.6	12 ± 1.5
Mean Weight of the foetuses	1.8 ± 0.2	1.6 ± 0.2	1.5 ± 0.3	1.6 ± 0.4	1.5 ± 0.5
Number of resorption sites	2	3	3	2	3
Mean number of resorption sites	0.4 ± 0.2	0.6 ± 0.4	0.4 ± 0.2	0.3 ± 0.2	0.5 ± 0.3
Percentage of implantation (5)	71 ± 5.5	89 ± 5.9	70 ± 0.5	73 ± 5.4	91 ± 0.5

Table 3. Effect of EtOAc extract (200 mg/kg/) of R. praetermissa pre-treatment on the uterine muscle weight in rats (n = 5).

Duration of treatment (days)	Uterine muscle weight (g)		
	Control (0.9 M NaCl)	Estradiol 17β (3 µM)	EtOAc extract (200 mg/kg/day)
4	0.018 ± 0.001	0.3 ± 0.001	0.05 ± 0.002
9	0.020 ± 0.008	0.29 ± 0.003	0.30 ± 0.004
15	0.022 ± 0.009	0.36 ± 0.001	0.34 ± 0.001
18	0.021 ± 0.005	0.35 ± 0.005	0.35 ± 0.001

In conclusion, these studies suggest that administration of *R. praetermissa* extract partially inhibits ovulation, stimulates in a dose dependent manner the growth of the uterine endometrium and hence potentiates implantation at the early stages of gestation. In combination with our previous studies on the estrogenic effects, these findings provide the pharmacological basis for the traditional use of this plant for prenatal care in the North West Region of Cameroon.

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