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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 13 (3): 126-131, 2010
ISSN 1028-8880

Effects of Aqueous Extract of *Aspilia Africana* on Reproductive Functions of Female Wistar Rats

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Abstract: Effects of *Aspilia africana* leaf extract on oestrous cycle and ovulation were studied in adult female Wistar strain rats. Cyclic female rats weighing 150 to 200 g were divided into two study groups: the oestrous study and ovulation study group. For the oestrous study, the experimental group received 500 mg kg^{-1} b. wt. of the extract for 14 days while the control group received distilled water for the same period. In both groups, vaginal lavage was taken daily from the 5th day to monitor the oestrous cycle. For the ovulation study, there was a control group and two experimental groups. The control group received distilled water while group 1 and 2 received 500 and 1000 mg kg^{-1} b.wt. of *Aspilia africana* leaf extract for 16 days, respectively. The animals were sacrificed on the estrous following the treatment. The results showed a significant decrease in the body weight of the treated rats ($p = 0.01$) and the oestrous cycle was altered after the commencement of extract. This was indicated by the prolonged proestrous and a reduced dioestrus and estrus. There was a dose-dependent reduction in the ovulation s shown by the reduced number of ova observed in the oviduct from the treated rats compared with control ($p<0.05$). The extract caused inflammation of the fallopian tube, degeneration in the ovarian cortex in the stroma cells of the ovary and disruption of the endometrium of the uterus. Results suggest that aqueous extract of *Aspilia africana* leaf has antifertility effect by altering oestrous cycle and causing a dose dependent adverse effect on ovulation in Wistar strain rats.

Key words: *Aspilia africana*, antifertility, proestrous, diestrous, estrus, ovulation

INTRODUCTION

The populations of developing countries worldwide continue to rely heavily on the use of traditional medicines as their primary source of healthcare. Ethnobotanical studies carried out throughout Africa confirm that native plants are the main constituent of traditional African medicines (Sandhu and Heinrich, 2005; Gupta *et al*., 2005). With 70-80% of Africa's population relying on traditional medicines, the importance of the role of medicinal plants in the healthcare system is enormous (Mander amd Breton, 2006). Medicinal plants are now being given serious attention, as is evidenced by the recommendation given by the World Health Organization in 1970 (Wondergem *et al*., 1989) that proven traditional remedies should be incorporated within national drug policies, by recent moves towards a greater professionalism within African medicine (Last and Chavunduka, 1986) and also by the increased commercialization of pharmaceutical production using traditional medicinal plants with known efficacy (Sofowora, 1981).

Natural products have been and have remained, the cornerstone of health care. Present estimates show that, 80% of the world's population still rely on traditional medicine for their health care needs (Duraipandiyan *et al*., 2006). In order to have standard natural plant products, preliminary studies have to be done in order to evaluate possible risks such as, undesirable effects, overdose or poisoning.

In Nigeria, many indigenous plants are used in herbal medicine to cure diseases and heal injuries. *Aspilia africana* (Pers.) C.D. Adams is not an exception. It is a perennial herb which belongs to the Asteraceae family. It has been classified among substances with low toxicity, with an LD_{50} averaging 6.6 g kg^{-1} b.wt. (Taziebou *et al*., 2007). Traditional African medicinal uses include its ability to stop bleeding from fresh wounds (Okoli *et al*., 2007a). It has anti-malaria (Waako *et al*., 2005) and anti viral (Chepkwony *et al*., 2007) properties. The juice of the leaves is reported to be haemostatic and vascoconstrictive (Achonye, 1976). The decoction of the root is a remedy for lumbago and sciatica neuralgia (Watt and Breyer-Brandwijk, 1962). The antibacterial

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(Macfoy and Cline, 1990; Adeniyi and Odufowora, 2000) membrane stabilization (Oyedapo *et al*., 1998) and anti-inflammatory activities (Okoli *et al*, 2007b) of *Aspilia africana* have been reported.

The plant *Aspilia africana* enjoys a folk reputation in Africa for its ability to stop bleeding even from severed artery as well as promote rapid healing of wounds and sores and for the management of problems related to cardiovascular diseases. The leaf extract has also been shown to cause extracellular Ca^{2+} dependent increase in vascular tone (Dimo *et al*., 2002). The decoction of the leaves is used to wash face to relieve febrile headaches. The infusion of the leaves is given as tonic to women after delivery. The leaf juice with little salt and lime juice is applied to eyes for corneal opacities and other foreign bodies in the eyes. The leaf decoction with native chalk is used to cure stomach troubles. A root decoction is taken for tuberculosis and in Ghana, the leaves are made into cough medicine for children.

Phytochemical studies revealed the presence of saponins and tannins as the most abundant compounds in the plant while flavonoids were the least (Obadoni and Ochuko, 1998). Other studies showed that essential oils from the leaves of *Aspilia africana* were rich in sesquiterpenes and monoterpenes. Germacrene D was reported as the major sesquiterpene while α-pinene and limonene were the major monoterpenes (Kuiate *et al*., 1999). The medicinal plant contained ascorbic acid, riboflavin, thiamine and niacin. These herbs are good sources of minerals such as Ca, P, K, Mg, Na, Fe and Zn (Okwu and Josiah, 2006).

This study was carried out to investigate the effect of the aqueous leaf extract of the plant on ovulation and oestrous cycle in female albino rats to assess its contraceptive effects.

MATERIALS AND METHODS

Plant source: Fresh leaves of *Aspilia africana* were handpicked from Okada village in Ovia North-East L.G.A of Edo state in June, 2009. The plant was identified and authenticated at the Department of Botany the University of Benin, Benin City, Nigeria.

Preparation of the extract: The harvested leaves were dried at room temperature (22-25°C) and ground into fine powder. Five hundred grams of the powder was dissolved in distilled water for 48 h at room temperature and filtered through a mesh. The filtrate obtained was transferred to a rotary evaporator until a semi-solid paste was obtained. This was allowed to dry further at room temperature.

Experimental animal: Twenty five adult female Wistar rats weighing between 150-200 g were used for this project. The animals were obtained from the University of Benin Small Animal House, Edo state, Nigeria. They were acclimatized for 2 weeks prior to the commencement of the experiment under standard conditions of light and illumination (12 h dark: 12 h light) cycle. They were fed commercially available rat's pellets and had access to drinking water *ad libitum*.

Experimental design

Pre-treatment phase: The animals were acclimatized in the laboratory for 2 weeks. Four pre-treatment oestrous cycles were established by vaginal smears as described by Marcondes *et al*. (2002). The female rats that have undergone four successive 4 days cycle were selected for this study.

Treatment phase: The experiment was carried out in 2 parts:

Part I: Oestrous cycle study: Oestrous cycles of 10 rats were studied using vaginal smear.

The rats were separated into 2 groups of 5 rats each and treated as follows:

- **Group 1:** Animals were treated with distilled water. They were called the control group
- **Group 2:** Animals were treated with 500 mg kg^{-1} b.wt. of the aqueous extract of *Aspilia africana*. Administration of the extract was done orally. The extract administration was done for one 4 day cycle before the commencement of the oestrous cycle study while treatment continued. The oestrous cycle was monitored for two and half (2½) cycles before they were sacrificed

Part II: Ovulation study: The rats were separated into 3 groups of 5 rats each and treated as follows:

- **Group 1:** Animals were treated with distilled water. They were called the control group
- **Group 2:** Animals were treated with 500 mg kg^{-1} b.wt. of the aqueous extract of *Aspilia africana*. Administration of the extract was done orally. The extract administration was done for 4 day cycle and the animals were sacrificed on the day of oestrous after the treatment
- **Group 3:** Animals were treated with 1000 mg kg^{-1} b.wt. of the aqueous extract of *Aspilia africana*. Administration of the extract was done orally. The extract administration was done for 4 day cycle and the animals were sacrificed on the oestrous after the treatment

Examination of vaginal smear: The vaginal smear was made as described by Marcondes *et al.* (2002) as follows: Vaginal smear samples were collected by the aspiration technique. During the experimental period, on each morning between 8:00 and 9:00 am each animal cage was carried to the experimental room. Vaginal secretion was collected with a plastic pipette filled with 10 μL of normal saline (NaCl 0.9%) by inserting the tip into the rat vagina, but not deeply. Vaginal fluid was placed on glass slides. A different glass slide was used for each cage of animals. One drop was collected with a clean tip from each rat. Unstained material was observed under a light microscope, without the use of the condenser lens, with 10 and 40x objective lenses.

Ovulation count: At the end of the 4 cycles, the animals in the ovulation study were sacrificed on the morning of the oestrous by cervical dislocation. The finbriated part of the oviduct was dissected out, suspended in normal saline and placed on a microscope slide to count the number of ova in the oviduct.

Histological study: The fallopian tube, ovary and the uterus were removed from each animal and processed for histological study. The tissue were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 7 μ thick were obtained using a rotatory microtome. The deparaffinised sections were stained routinely with haematoxylin and eosin. Photomicrographs of the desired results were obtained using digital research photographic microscope.

Duration of study: This study was carried out between February and July 2009 in the Department of Physiology, Igbinedion University, Okada, Edo State, Nigeria.

Statistical analysis: Results are expressed as Mean±SEM. The statistical analysis was carried out using student's t-test. The p-values of 0.05 or less were considered significant for all experiment. Simple percentages were used to present the degree of alteration in the different phases of oestrous cycle.

RESULTS

Body weight: The treated rats exhibited a significant decrease ($p \leq 0.05$) in the body weight when compared with the control group and this decrease in weight also was evident across the cycle as shown in Table 1 and 2.

Table 1: Effect of aqueous extract of *Aspilia africana* (500 mg kg^{-1} b.wt.) on body weight

Treatment given	Weight (g)
Control	130±0.24
Treated with *A. africana*	178±2.95*

Values are Mean±SEM. *Significance at $p<0.05$ when compared with control group

Table 2: Changes of body weight per oestrous cycle of rats treated with aqueous extract of *Aspilia africana* (500 mg kg^{-1} b.wt.)

	Weight (g)		
Oestrous phase	1st cycle	2nd cycle	3rd cycle
Control	128.8±0.26	131.2±0.21	132.4±0.16
Treated	180.1±4.85*	178.1±4.68*	175.0±6.55*

Values are Mean±SEM. *Significance at $p<0.05$ when compared with control group

Table 3: Effect of administration of 500 mg kg^{-1} of *Aspilia africana* leaf extract on the normal phases of oestrous cycle in rats

Oestrous phase	Control (%)	Treated (%)
Proestrous	25	52.5
Estrous	25	12.5
Metestrous	25	25.0
Diestrous	25	7.5

Table 4: The effect of aqueous extract of *Aspilia africana* (500 and 1000 mg kg^{-1} b.wt) on ovulation

Treatments	No. of ova
Control	16±0.54
Treated with 500 mg	10±0.24*
Treated with 1000 mg	5±0.37 *

Values are Mean±SEM. *Significance at $p<0.05$ when compared with control group

Oestrous cycle: The control rats had regular days oestrous with normal alternatively diestrous, proestrous, estrus and metestrus phases. The normal pattern of oestrous cycle was significantly altered in the treated rats after extract administration (Table 3). It was observed that the duration of the diestrous phase was significantly reduced ($p \leq 0.05$) from 25 to 7.5% in the treated rats after 10 days. The proestrous phase was significantly increased (at $p \leq 0.05$) from 25 to 52% in the treated rats while the duration of the estrus phase was significantly reduced (at $p \leq 0.05$) from 25 to 12.5%. There was no significant difference in the metestrus phase.

Ovulation: The number of ova in the oviduct of treated rats was significantly reduced after commencement of the administration of the extract ($p \leq 0.05$). Two doses of *Aspilia africana* were used for this study: 500 and 100 mg kg^{-1} b.wt. It was discovered that the reduction in the number of ova was dose dependent. There was a significant dose-dependent reduction in the number of ova released in the treated rats compared with the control (Table 4).

Histological studies: The histological section through the fallopian tube of the *Aspilia africana* extract treated rats

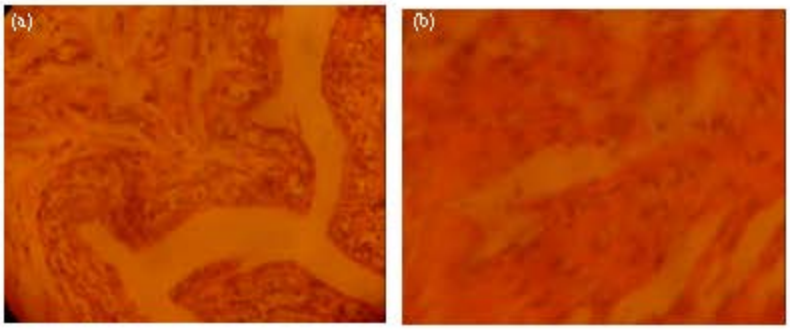

Fig. 1: (a) Control Group Fallopian tube (x 40)-Haematoxylin and Eosin stain and (b) Treatment Group Fallopian tube (x40)-Haematoxylin and Eosin stain

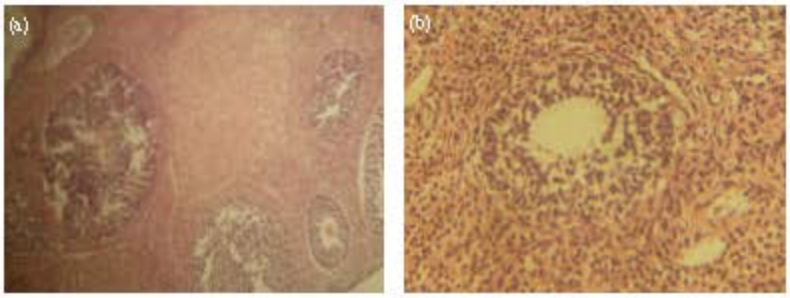

Fig. 2: (a) Control Group Ovary (x 40)-Haematoxylin and Eosin stain and (b) Treatment group ovary (x40)- Haematoxylin and Eosin stain

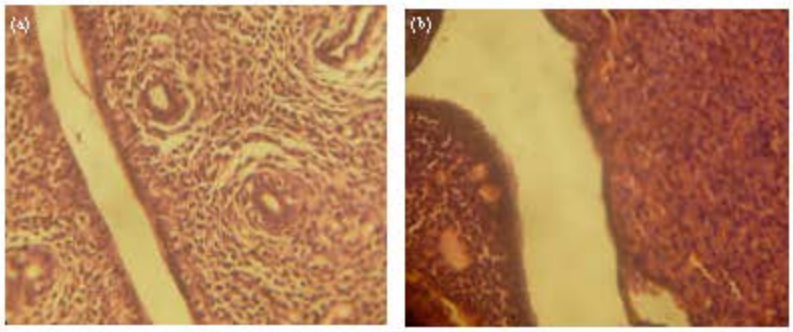

Fig. 3: (a) Control Group Uterus (x 40)-Haematoxylin and Eosin stain and (b) Treatment Group Uterus (x40)- Haematoxylin and Eosin stain

showed inflammation while the control rats appeared normal with no visible signs of lesions and disorganization (Fig. 1a, b). The histological section of the ovary of the treated rats showed lesion and there was degeneration in the ovarian cortex and vacuolations in the stroma cells while the ovary of the control rats showed numerous stages of development (Fig. 2a, b). The uterus of the treated rats showed degeneration with disruption of the endometrium while the control rats showed no visible signs of lesion or disorganization in the histological section of the uterus (Fig. 3a, b).

DISCUSSION

Aqueous extract of *Aspilia africana* had a significant decrease on the weight and growth rate of the rats similar results was reported by Taziebou *et al.* (2007). But this is contrary to the results of Okwuonu *et al.* (2008), who reported that there was no significant difference in weight of rats fed with methanolic extract of *Aspilia africana* for 30 days. This contradiction may be due to the method of extraction of the extract. The histological sections of the ovary in treated rats have structural changes

different from the normal group which is in line with the study of Eweka (2007). The extract of *Aspilia africana* has been reported to have an anti-inflammatory activity (Okoli *et al*., 2007b). Studies have revealed that the process of ovulation is comparable to an inflammatory process (Epsey, 1994). Anti-inflammatory drugs have been employed in blocking ovulation (Gaytan *et al*., 2002). The anti-inflammatory property of *Aspilia africana* may be responsible for its observed effect in blocking ovulation. The anti-inflammatory property of flavonoids is believed to result from inhibition of cyclo-oxygenase enzyme (Liang *et al*., 1999). It was revealed that all traditional non-steroidal anti-inflammatory drugs produce most of their effects by blocking COX-2. The COX-2 deficient mice suffer from defect in reproductive functions such as ovulation and fertilization (Lim *et al*., 1997) since, COX- 2 is important in ovulation through its role as an essential enzyme for follicular rupture. The results of our study suggest that *Aspilia africana* may block ovulation by inhibiting cyclooxygenase activity (perhaps COX-2) and inhibition of Prostaglandin synthesis.

The concentration we administered may have been enough to inhibit cyclooxygenase in relation to ovulation.

In conclusion, these studies suggest that administration of *Aspilia Africana* leaf extract alters oestrous cycle with a prolonged proestrous and a reduced diestrus and estrus phases and may cause a dose dependent adverse effect on ovulation in Wistar strain rats.

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