

## Toxic Effects of Methanolic Extract of *Aspilia africana* Leaf on the Estrous Cycle and Uterine Tissues of Wistar Rats

Efectos Tóxicos del Extracto Metanólico de la hoja de *Aspilia africana*  
Sobre el Ciclo Estral y Tejidos Uterinos de Ratas Wistar

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**SUMMARY:** Effects of graded dosages of methanolic extract of *Aspilia Africana* were examined on the estrous cycle, uterine weights and histology to determine its effects on the reproductive functions of 25 cyclic female rats. The rats were randomized into five groups A, B, C, D, and E. They were given 0mg/kg body weight, 150 mg/kg body weight, 200 mg/kg body weight, 250 mg/kg body weight, and 300 mg/kg body weight, respectively. The effect on the estrous cycle was determined by vaginal lavage while routine histological preparations were done with haematoxylin-eosin stains. All values were statistically compared at appropriate confidence intervals. Estrous cycles were significantly reduced in a dose-dependent manner and histology revealed a dose-dependent toxicity.

**KEY WORDS:** *Aspilia africana*; Estrous; Uterine weight; Histology; Reproductive functions.

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### INTRODUCTION

Plant materials continue to play an important role in the maintenance of human health since antiquity. Over 50% of all modern chemical drugs originated from natural plant sources. These plant products are the major source of drug development in pharmaceutical industry (Burton *et al.*, 1983). Several plants are now being used in part or as a whole to treat many diseases. Active components of these plants are now being investigated, extracted and developed into drugs with little or no negative effects or contra-indication (Oluyemi *et al.*, 2007). Rural dwellers in most parts of the world do not depend on the orthodox medicine for the cure of diseases and ailments. This is because most of the modern equipments are expensive and service delivery too expensive to afford. As a result of this, a larger section has resulted to the use of traditional medicines, which are believed to be less expensive, and of little or no side effects. The initial identification of more than 20,000 species of medicinal plants of tropical forests origins by the World Health Organization, in 1978 has contributed immensely to our knowledge of different uses of plants. Most of these plants have their present uses rooted in traditional medicines, which plays a major part in maintaining the health and welfare of both rural and urban dwellers in developing countries.

One of such plants considered of great importance is *Aspilia africana* (Compositae). It is a semi-woody herb from a perennial woody root stock up to 2 meters high. It is very polymorphic and occurs throughout the region on wasteland of the savannah forest. It is also widely distributed across tropical Africa (Dalziel, 1973).

Phytochemical analysis of the plant reveals that it has high crude oil protein content (Burkill, 1985). It is also rich in saponin, tannins, glycoside and alkaloids (Adeniyi & Odufowora, 2000; Iwu, 1993). Kuate *et al.* (1999) reported the presence of four essential oils obtained by hydro-distillation from the leaves of *Aspilia africana*. These oil samples include Sesquiterpenes, monoterpenes, Germacrene-d and alpha-pinenene.

Historically, *Aspilia africana* has been used in Mbaise and most Igbo speaking parts of Nigeria to prevent conception suggesting its contraceptive and anti-fertility potencies. The safety usage of this plant in this manner has not been validated. The Okpameri people of Bendel North in Nigeria use the decoction of the leaves to wash face to

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relieve febrile headache (Gill, 1992). The methanol/chloride extract of this plant re-enforced the vascular smooth muscle contraction induced by norepinephrine and relaxed pre-contracted tension at a low and high concentrations, respectively (Dimo *et al.*, 2002). *Aspilia mossambicensis* has been found to possess anti-malarial activity against *plasmodium falciparum* and galactogogne activity. It has also been used to alleviate menstrual cramps (Page *et al.*, 1992).

There is a dearth of publications on the effects of this plant on the uterine tissues and estrus cycles. The present study aims at finding out the effects of *Aspilia africana* on the uterine structures and estrus cycles of female Wistar rats.

## MATERIAL AND METHOD

25 cyclic female rats were sorted randomly from the animal house of the Igbinedion University, Okada, Edo State. The rats were kept in the animal control room and acclimatized for two weeks. The rats were fed on standard rat pellet produced by Bendel Feed and Flour Mills Limited. They were allowed access to water *ad libitum* and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25 - 27°C under day/night 12-12 hour photoperiodicity. The rats were randomly grouped into five groups of 5 rats each A (0mg/kg body weight), B (150mg/kg body weight), C (200mg/kg body weight), D (250mg/Kg body weight) and E (300mg/Kg body weight).

The plant material, *Aspilia africana* leaves, were obtained from Okada village in Ovia North-East L.G.A of Edo State and authenticated by the Botany Department, University of Benin. The leaves were sun-dried and grounded to fine powder. Crude methanolic extraction was done using 90% methanol. The solution was filtered after 72 hours while the filtrate was concentrated to a semi solid form using the rotary evaporator, weighed and the solutions were prepared as 100mg/ml and 200mg/ml, respectively.

The administration of the extract was totally by gavage. Proper concentrations were administered by the use of metal oropharyngeal canula and calibrated hypodermic syringe. The administration of *Aspilia africana* extract was done once in a day, everyday of the week for 30 days. The control group A received no extract but 1ml of distilled water, while groups B, C, D and E received 150mg/kg body weight, 200mg/Kg body weight, 250mg/Kg body weight and 300mg/kg body weight. of the extracts respectively. The animals were sacrificed a day after the administration of extracts stopped using the humane method.

The uteri were dissected out by laparotomy immediately after sacrificing the animals. They were perfused in normal saline, blotted dry and weighed in electronic weighing balance. The uteri were then fixed in 10% buffered formalin for histological assessment.

The histology of the uteri was done by modification of method described by Oluyemi *et al.* (In press). The organs were fixed in 10% buffered formalin for a day after which it was transferred to ascending grades of alcohol for dehydration. The tissues were passed through 50%, 70%, 90% and two changes of absolute alcohol and xylene for different durations, before they were transferred into two changes of molten paraffin wax, for 1 hour each, in an oven at 65 °C for infiltration. They were subsequently embedded and serial sections cut using rotary microtome at 6 microns. The tissues were fixed into albumenised slides and allowed to dry on hot plate for 2 minutes. The slides were dewaxed with xylene and passed through absolute alcohol (2 changes); 70 % alcohol, 50% alcohol and then to water for 5 minutes. The slides were then stained with haematoxylin-eosin. The slides were mounted in DPX.

The estrous cycles were monitored by a method described by Marcondes *et al.* (2002). Vaginal smear was collected with a glass pipette filled with 10ml of normal saline (NaCl 0.9%). The vaginal fluid was placed on clean glass slide. The unstained slide was observed under a light microscope at x100 and x400 magnification. The three types of cells recognized were epithelial, cornified and leucocytes cells. The proportion among these cells were used to determine the estrous phases according to Long & Evans (1922).

All statistical data were compared at appropriate confidence intervals. Values are recorded as mean  $\pm$  S.E.M.

## RESULTS



Fig. 1. The photomicrograph of the uterus of the control group A. It presents a normal structure of the uterus with endometrium having large epithelia cells with nuclei. Numerous irregular and tortuous glands are present.

Table I. Effect of *Aspilia africana* leaf extract on estrous cycle of Wistar rats.

Group	Treatment (mg/Kg)	Number of cycles	Duration in days (Mean± SEM)				
			Proestrous	Estrous	Metestrus	Diestrus	Diestrus
A	Control	5.72±0.18	5.00±0.22	7.71±0.29	4.86±0.26	12.29±0.42	40.93
B	150	3.57±0.20*	2.43±0.20*	4.71±0.18*	3.29±0.81*	19.86±0.34 <sup>a</sup>	66.19
C	200	3.57±0.30*	1.86±0.26*	4.71±0.36*	3.14±0.26*	20.43±0.57 <sup>a</sup>	68.09
D	250	2.86±0.34*	1.43±0.20*	3.86±0.26*	2.57±0.20*	22.42±0.48 <sup>a</sup>	74.73
E	300	2.00±0.31*	1.14±0.14*	3.43±0.37*	2.29±0.18*	23.29±0.18 <sup>a</sup>	77.62

\* Significantly lower when compared to the Control. <sup>a</sup> Significantly higher when compared with the control (p>0.05).

Diestrus index=Number of days with clear diestrus smear X 100 / total duration of treatment (days).

Table II. Weight of uteri after the experiment.

Group E (300mg/kg)	Group D (250mg/kg)	Group C (200mg/kg)	Group B (150mg/kg)	Group A (00mg/kg)
0.020±0.007*	0.030±0.002*	0.019±0.007*	0.034±0.001*	0.048±0.001

n=5, \* Values significantly lower when compared with the control (p>0.001).



Fig. 2. The photomicrograph of the uterus of Group B that received 150mg/Kg B.W of extract. The tortuous endometrial glands are still intact. The thick myometrium is unaffected and the epithelia are intact. Cystically dilated glands are seen.

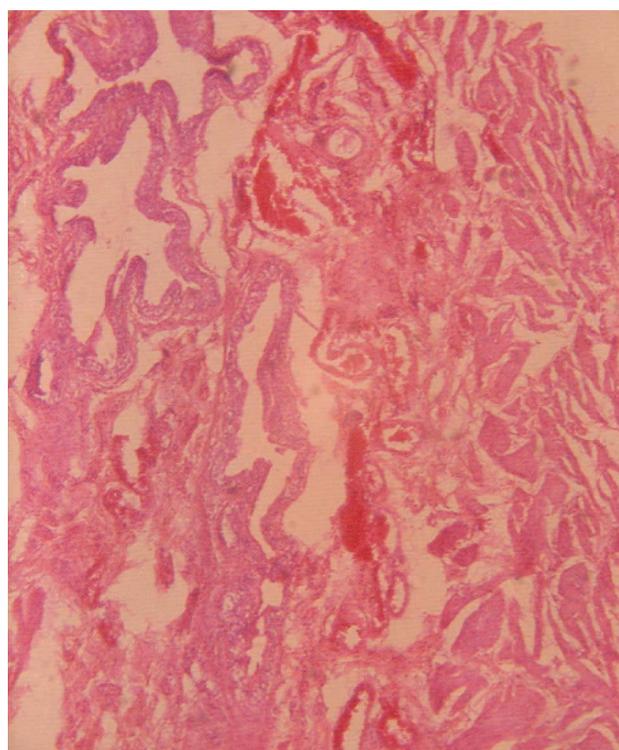


Fig. 3. The photomicrograph of the uterus of Group C that received 200mg/Kg B.W of extract. Irregular endometrial glands are present. The blood vessels are thickened and the endometrial stroma slightly disaggregated.

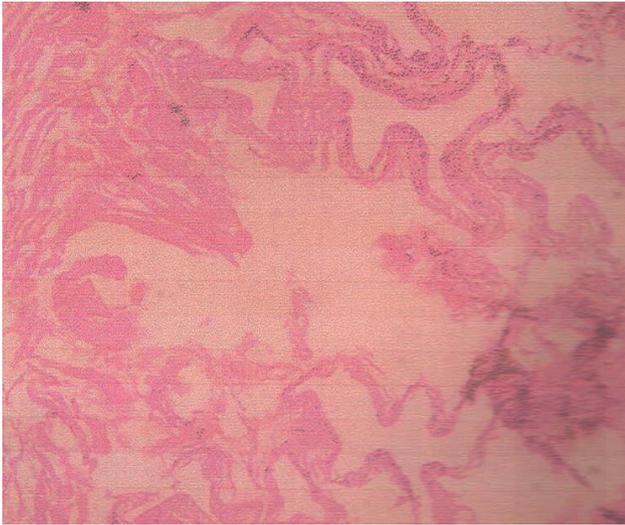


Fig. 4. The photomicrograph of the uterus of group D, that received 250mg/kg body weight of extract. The uterine lumen slightly distended. High deletion of endometrial glands and cells observable.

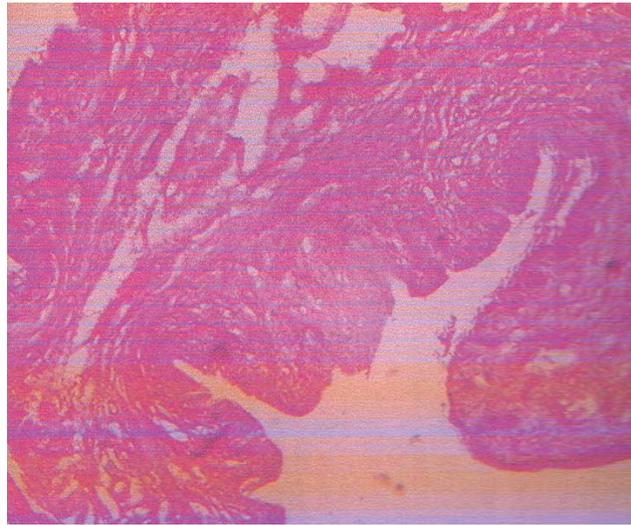


Fig. 5. The photomicrograph of the uterus of group E that received 300mg/kg body weight of extract. The tissues of this group show signs of alteration in the endometrial stroma cells and high loss of epithelial lining.

## DISCUSSION

Table I shows the effects of methanolic extract of *Aspilia africana* on the oestrous cycle of female Wistar rats. It shows a dose-dependent decrease in the duration of proestrous, estrous and metestrus, while it increases the duration of diestrus. This is suggestive of negative influences on the estrous cycle as this reduces the number of days/ova ovulated during the proestrus and estrus phases. The reason for these could be due to the presence of high level of phytoestrogens like saponins and essential oils. The inhibitory effect of steroidal saponin on the oestrus cycle has been reported by Tamura *et al.* (1997). They have been found to reduce fertility in animals upon continuous administration. Phytoestrogenic plants have both estrogenic and anti-estrogenic effects on mammalian systems. They prevent implantations and other estrogen-dependent activities in the reproductive system. They do this by causing hormonal imbalances in the systems of the subject concern. Hormonal balance is required for implantation and proper development of conceptus (William, 1999; Guyton, 1991). Some plants that possess oxytocic effects have been found to also have anti-fertility effects. Falodun *et al.*, (2006) reported the oxytocic effects of a plant that has saponin, saponin glycosides, steroid, tannins, volatile oils, and alkaloids on the function of isolated uterus from female rats. Most of these compounds are also present in *Aspilia africana*.

The decrease in the weights of the uteri (Table II) at the end of the experiment, could be due to the ability of the *Aspilia africana* extract to contract smooth muscle fibers as

reported by Dimo *et al.* They reported that methylene chloride/methanol extract of *Aspilia africana* increases in vitro vascular smooth muscle contraction in rats' aortic ring preparations. The myometrium of the uterus is made of smooth muscle fasciculi mingled with loose connective tissue, blood vessels, lymphatic vessels and nerves (Williams *et al.*). These structures, especially, the smooth muscles of the blood vessels and myometrium must have been acted upon by the extract in a dose-dependent manner leading to their atrophy.

Figures 2-5 show the photomicrographs of the uterus of the rats treated with different doses of the extract of *Aspilia africana*. When compared with Fig. 1., they showed dose-dependent deletion and derangement of the endometrium, endometrial stroma and thickening of the endometrial blood vessels. These effects are suggestive of toxic potency of *Aspilia africana*.

These effects are most probably due to imbalances in hormonal level caused by high level of saponins and other phytoestrogens. The thickness of endometrium varies considerably according to the individual's hormonal state (Spornitz, 1992).

The results of this research showed that methanolic extract of *Aspilia africana* leaves possess negative influences on the estrous cycle and histo-architecture of the uterus of female rats suggesting negative influences on the reproductive health of the animals.

**OLUYEMI, K. A.; OKWUONU, U. C.; BAXTER, D. G. & OYESOLA, T. O.** Efectos tóxicos del extracto metanólico de la hoja de *Aspilia africana* sobre el ciclo estral y tejidos uterinos de ratas Wistar. *Int. J. Morphol.*, 25(3):609-614, 2007.

**RESUMEN:** Se examinaron los efectos de las distintas dosificaciones de extracto de metanol de *Aspilia africana*, en los ciclos estrales, peso uterino y en la histología para determinar sus efectos en las funciones reproductivas de 25 ratas hembra. Las ratas fueron randomizadas en cinco grupos: A, B, C, D y E y recibieron 0 mg/kg de peso corporal, 150 mg/kg de peso corporal, 200 mg/kg de peso corporal, 250 mg/kg de peso corporal y 300 mg/kg de peso corporal respectivamente. El efecto en el ciclo estral fue determinado por frotis vaginal, preparado con técnicas histológicas de rutina y teñido con hematoxilina-eosina. Todos los valores fueron estadísticamente comparados con intervalos de confianza adecuados. Los ciclos estrales fueron significativamente reducidos en forma dosis dependiente y la histología reveló una toxicidad dosis dependiente.

**PALABRAS CLAVE:** *Aspilia africana*; Estro; Peso uterino; Histología; Funciones reproductivas.

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