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UTEROTROPIC AND ANABOLIC EFFECT OF THE ROOT BARK OF *ADANSONIA DIGITATA* LINN IN WISTAR RATS

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ABSTRACT: An experiment was conducted to investigate the uterotropic and anabolic properties of the root bark of *Adansonia digitata* Linn in female Wistar rats. Mature normal cycling female rats between 80 to 90 days old and of average weight 158 ± 20g were blocked by weight and distributed into four groups A, B, C and D. Each group comprised of eight rats and groups A,B and C received 1000 mg/kg, 800 mg/kg and 500 mg/kg body weight of the ethanol extract per os for seven days, while group D which served as the control, received 0.5ml of distilled water in the same manner. All animals were weighed on the first and seventh day, while on the tenth day, all rats were sacrificed. The uteri and ovaries were harvested, weighed, and histopathologically examined.The effects of the extract on uterine and ovarian weights were not statistically significant (P>0.05).However, the groups given 1000mg/kg and 800mg/kg body weight of the extract produced significant increases (P < 0.01) in body weight [10.25 ± 2.18 g and 9.75 ± 2.18 g respectively] when compared to control [2.00 ± 2.18 g]. Histopathologic examination revealed stratified squamous metaplasia of the endometrial epithelial cells, empty spaces within epithelial layer and exfoliation of the epithelial layer.The ethanolic extract of the root bark of *Adansonia digitata* Linn has uterotropic effects and increased body weight in Wistar rats.

Keywords: *Adansonia digitata,* Anabolic, Uterotropic, rats

INTRODUCTION

The plant *Adansonia digitata* linn, belongs to the family Bombaceaea. It is known as 'kuka' in Hausa, where the leaves are often used as a soup vegetable. In northern Nigeria, among the Fulanis, the root bark exclusively or with the leaves is cooked and consumed for weight gain (Lockett and Grivetti, 2000). In Tanzania, a decoction of the barks and leaves are given to cattle and women for the expulsion of retained placenta and the stem bark is used for the treatment of menorrhagia (Minja, 1999; Marie, 2002). While in Zaria, a decoction of the root is given orally as an antifertility agent to women (by oral interview). This study is to preliminarily investigate the uterotropic effect of the root bark of *Adansonia digitata* and its anabolic property in female Wistar rats.

MATERIALS AND METHODS

Collection of Plant: The leaves of the plant were identified as those of *Adansonia digitata* (Linn) at the Department of Biological Sciences, Ahmadu Bello University, Zaria. The tree was also sighted growing in the premises of the Department of Biological Sciences with the herbarium voucher number 2512.

Fresh root barks from apparently healthy plants were collected within Zaria town between November and February (Zaria is about 650m above sea level. It lies between latitude 10° and11° North and longitude 7° and 8° East. It is situated in the northern boundary of the sub-humid zone and the natural vegetation is Northern Guinea Savanna. Mean annual rainfall in the area is1100mm, lasting from May to October. Mean daily temperature during the wet season is 25°C with relative humidity of 72%. The dry season lasts from November to April with mean daily temperatures ranging from 14 °C to 36 ° C, and mean relative humidity of 20% to 30%.).The barks were pooled and thoroughly bench dried at room temperature, pound to a coarse powder using wooden pestle and mortar, and stored in an air tight container.

Plant Extract: Initially, 500g of the coarse dried powdered sample was soxhletted with petroleum ether (60-80EC) at 75°C. Then, 400g of the marc was air dried and further soxhletted with 96% ethanol at 75-80°C for approximately 24 hours. The liquid extract was concentrated by evaporation and shaking to obtain a dark brown mass using GRANT WATER BATH with a shaker (Manufactured by Grant Instrument Ltd., Barrington Cambridge CB25QZ England). The dark brown was subsequently reconstituted with distilled water to appropriate concentrations suitable to achieve a 0.5ml dose by volume of extract.

Experimental animals: About fifty (50) mature virgin female Wistar rats (*Rattus norvegicus*) , between three to four months old were bought from the Animal Breeding Unit of the College of Education Kongo, Zaria. The rats were made to acclimatize to normal laboratory condition for two weeks and were provided with water and feed (Produced by Vital Feed Nigeria Ltd) at *ad-libitum*.

Experimental Design: Using the vaginal smear analysis method described by Maeda *et al.* (2000), the estrous cycles of the fifty (50) female rats were determined. Thirty-two (32) rats found to have a regular 5-day estrous cycle and of average weight 158 ± 20g were blocked by weight and randomly assigned into four different experimental groups comprising of eight animals each. The treatment consisted of oral administration of 500mg/kg; 800mg/kg and 1000mg/kg body weight of the ethanol extract for the treated group and 0.5ml of distilled water for the control group. Treatment commenced from the day rats were on proestrus to the seventh day. Body weights on the first and seventh day of treatment were observed. At the end of treatment, the difference in body weight between the first and seventh day was recorded, and all animals from each group were sacrificed using chloroform as light anesthesia. The ovaries and uteri were excised, blotted on paper, weighed on METTLER P 165 top loading balance (Produced by Greifence in zurich Switzerland) and fixed in 10% formalin for subsequent histopathologic examination.

Statistical analysis: Data collected were analyzed using SAS procedure of General Linear Model (SAS, 2000). P values < 0.05 were considered statistically significant. Where treatments were found to have significant effect on response variable, Duncan Multiple Range Test (DMRT) was used for mean separation.

RESULTS

The absolute and relative weight of the ovaries and uteri were not significantly affected (P>0.05) by the various dose level treatments [Table 1-2]. However, there was a highly significant increase (P<0.01) in body weight of rats treated with 800mg/kg and 1000mg/kg body weight of the extract [Table 3].

Only the uteri of treated rats had stratified squamous metaplasia of the endometrial epithelial layer. This was seen as transformation of the normal endometrial epithelial cells from simple cuboidal or columnar cells to stratified squamous epithelial cells [Fig. 1-4].In addition, the uteri of the group given 1000mg/kg body weight of the extract also revealed numerous empty spaces in the epithelial layer and areas of epithelial exfoliation [Fig. 4].

Table 1: Effect of the crude ethanol extract of the root bark of *Adansonia digitata* on body weight of female Wistar rats

Dose (mg/kg body weight)	Increase in body weight (g) (mean ± sem) n=8, P<0.01
1000	10.25 ± 2.18^{A}
800	9.75 ± 2.18^{A}
500	1.50 ± 2.18^{B}
Control	2.00 ± 2.18^{B}

P< 0.05 is statistically significant
P<0.01 is highly statistically significant

Keys

Sem => standard error of mean
N => number of animals in each group
P=> level of significance
Means with same alphabets => means are not statistically different

Table 2. Effect of crude ethanolic extract of *Adansonia digitata* Linn on absolute and relative ovarian weight of female wistar rats

Dose (mg/kg body weight)	Absolute weight of Ovaries (g) (mean ± sem) n=8, P>0.05	Relative weight of Ovaries (mg/100g) (mean ± sem)n=8, P>0.05
1000	0.1025 ± 0.0091^{A}	75.71 ± 4.601^{A}
800	0.1141 ± 0.0091^{A}	71.93 ± 4.601^{A}
500	0.1113 ± 0.0091^{A}	70.33 ± 4.601^{A}
Control	0.1111 ± 0.0091^{A}	68.54 ± 4.601^{A}

P> 0.05 is statistically insignificant
P<0.05 is statistically significant

Keys

sem => standard error of mean
n => number of animals in each group
P=> level of significance
Means with same alphabets => means are not statistically different

Table 3. Effect of crude ethanolic extract of *Adansonia digitata* Linn on absolute and relative uterine weight of female wistar rats

Dose (mg/kg body weight)	Absolute weight of uterus (g) (mean ± sem) n=8, P>0.05	Relative weight of uterus (mg/100g) (mean ± sem) n=8, P>0.05
1000	0.3978 ± 4.601^{A}	292.2 ± 30.91^{A}
800	0.4461 ± 4.601^{A}	288.0 ± 30.91^{A}
500	0.4875 ± 4.601^{A}	304.2 ± 30.91^{A}
Control	0.4298 ± 4.601^{A}	262.1 ± 30.91^{A}

P< 0.05 is statistically significant
P<0.01 is highly statistically significant

Keys

sem => standard error of mean
n => number of animals in each group
P=> level of significance
Means with same alphabets => means are not statistically different

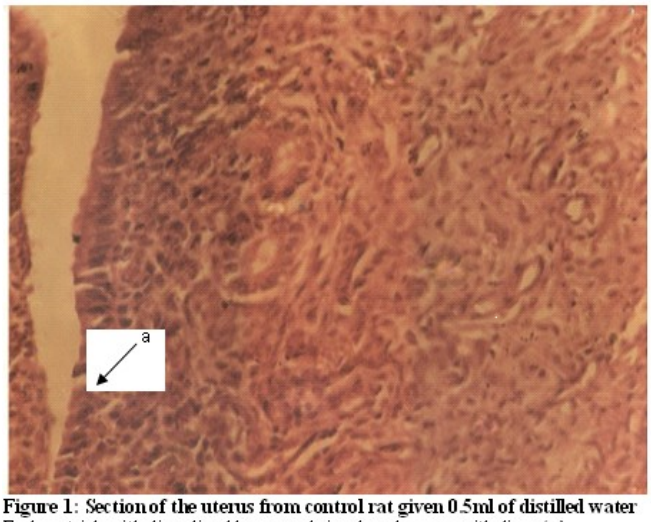

Figure 1: Section of the uterus from control rat given 0.5ml of distilled water Endometrial epithelium lined by normal simple columnar epithelium '**a**' (Magnification X312.5; H &E stain)

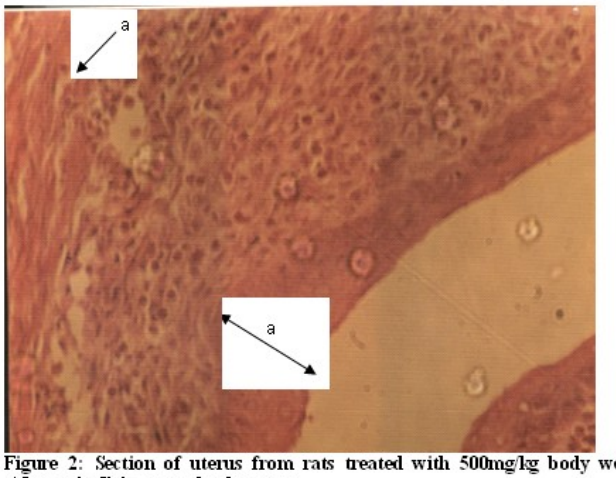

Figure 2: Section of uterus from rats treated with 500mg/kg body weight of *Adansonia digitata* root bark extract.
Squamous metaplasia of endometrial epithelium seen as squamous cells arranged in a stratum '**a**'. (Magnification X312.5, H & E stain)

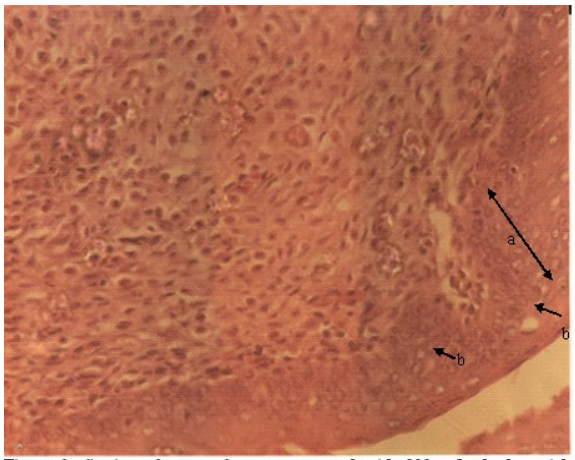

Figure 3: Section of uterus from rats treated with 800mg/kg body weight of *Adansonia digitata* root bark extract.
Squamous metaplasia of endometrial epithelium seen as squamous cells arranged in a stratum '**a**'. Few empty spaces within epithelial layer '**b**' (Magnification X312.5; H & E stain)

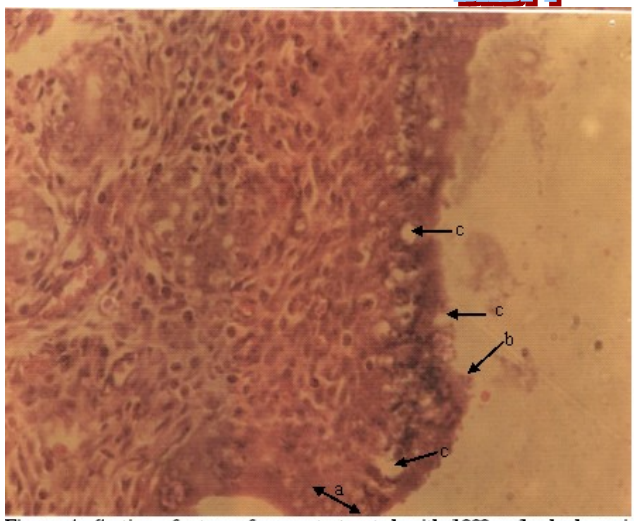

Figure 4: Section of uterus from rats treated with 1000mg/kg body weight of *Adansonia digitata* root bark extract.
Squamous metaplasia of endometrial epithelium seen as squamous cells arranged in a stratum '**a**'. Desquamation of epithelial layer seen as exfoliating epithelial layer '**b**' which resulted to thining of epithelial layer and numerous empty spaces within epithelium '**c**'. (Magnification X312.5; H & E stain)

DISCUSSION

Results showed that the extract produced significant increases in body weight of treated rats and may have anabolic properties. However only mean body weight increases produced by 800mg/kg and 1000mg/kg body weight of the extract was different from the control [Table 3]. This implies that at these doses, the ethanol extract of the root bark of *A. digitata* has body weight gaining effect and this may suggest a scientific basis for the consumption of the cooked bark for weight gain by the Fulanis (Lockett and Grivetti, 2000). It is important to note that both the absolute and relative weight of the ovaries and uteri were not significantly affected ($P>0.05$) by the various dose level treatments [Table 1-2].These results are similar to the effect of a well established androgenic anabolic steroid known as nandrolon decanoate which increased body weight of treated rats without affecting uterine weight (Gerez *et al*., 2005; Hamid *et al.* 2007 and Camargo *et al.*, 2009). Empty spaces within the epithelial layer [Fig. 1-4] of the treated rats, were also similar to the vacuolation of endometrial epithelial cells caused by nandrolon decanoate (Gerez *et al*., 2005).This may imply that the mechanism through which the extract produced weight gain and affected the uterus may be similar to that of androgenic anabolic steroids. Androgenic anabolic steroids affect androgen receptors on the uterus to cause physiological growth and development of the uterus. They also cause muscle tissue hypertrophy and the formation of new muscle fibres, and subsequently increase lean body mass (Hartgens and Kuipers, 2004 and Walters *et al.,* 2009). However, squamous metaplasia of endometrial epithelium is a lesion commonly associated with estrogen stimulation (Firoiu *et al*., 2009). Its occurrence in the midst of androgen related effects may be due to the possibility of aromatization of androgens to estrogen and the subsequent production of estrogen receptor mediated effects**.** Exfoliation or desquamation of the endometrial epithelial layer was seen only in the group given 1000mg/kg body weight of the extract [Fig. 4] and it may be dose dependent. Such uterotropic lesions may affect implantation and may encourage disengagement and expulsion of placenta. This may provide a scientific basis for the traditional use of the root bark in the expulsion of retained placenta in cattle and in women (Minja, 1999).

Conclusion

The crude ethanol extract of the rootbark of *Adansonia digitata* Linn when given *per os* to female Wistar rats at doses 1000mg/kg and 800mg/kg body weight produced a significant anabolic effect. While, the extract at dose levels 1000mg/kg, 800mg/kg and 500mg/kg body weight produced uterotropic effects. These findings may serve as a scientific basis for the traditional use of the root bark for weight gain and for expulsion of retained placenta.

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