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The Effect of Khaya senegalensis on the Endometrial and Vaginal Epithelium: A Histological and Morphometrical Study

Research Article

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Abstract

Introduction: *Khaya senegalensis* is a plant with many medicinal uses and application which could serve as an available and alternative source of medication. It has been used traditionally as an abortifacient agent following folkloric information handed down by word mouth. The aim of this study is to validate this claim by investigating the effect of the extract on the endometrial and vaginal epithelium. Methodology: Four groups of albino rats were administered different dosages of the extract orally and after 28 days, the rats were sacrificed. The micrographs of the uterus and vagina were studied for histological observations. Morphometric analysis was also carried out to determine the thickness of the epithelial lining in all groups. Measurements were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism. Results: Histological observations showed that the extract at a concentration of 50mg/kg and 200mg/kg disrupted the epithelial lining and as the concentrations administered increased, surface and stromal endometrial glands became smaller and sparse and the endometrial stroma became less compact. Morphometric analysis revealed a significant increase of endometrial epithelium in the group administered the least concentration of the extract. Vaginal epithelial thickness was also significantly reduced in all groups. Conclusion: Results obtained suggests that the extract has an anti-fertility effect on the lining of the uterus and vagina. Further studies are recommended.

Key Words: *Khaya senegalensis*, Endometrial glands, Endometrium, Morphometric, Uterus, Vagina.

Introduction

Medicinal plants have a broad basis of application in therapeutic management of diseases in many parts of the world as they serve as a source of available and affordable alternative to orthodox medication. *Khaya senegalensis* (Desr.) *A. Juss*. is one of the numerous plants with varied medicinal applications and uses. The plant belongs to the family *Meliaceae* is locally known as Mahogany tree (Senegal Mahogany) (1). *Khaya senegalensis* has been used in treatment of malaria, hepatitis, dysentery and sinusitis, its leaves of plants have been used in treatment of dermatological disorders, abdominal diseases, trachoma, wound healing and malaria (1,2). Other applications of the plant as documented are: anti-malarial and antibacterial effects (3). The stem bark extract has been shown previously to be toxic to *Plasmodium falciparum* [4]. anti-hyperglycemic (5), antimicrobial (6,7) antifungal (8), antiprotozoal (9), anthelmintic effects

(10) and anti-cancer and free radical scavenging activities (11,12). Its hepatoprotective (13,14) and hepatotoxic (5) activities have been also investigated. The plant is rumored to have abortifacient applications and it has been used in this regard in Guinea and Ivory Coast (15). Hot water extract of the bark of *Khaya senegalensis* has been used as an abortifacient and for menstrual troubles after administration through the oral route (15,16).

Despite the folkloric use as an abortifacient agent, there is paucity of literature supporting the scientific basis for this use. In the present study, the authors seek to investigate the effect of the extract on the histology of the female reproductive organs especially the uterus and vagina as a first step to establishing the pharmacological basis for the use of *Khaya senegalensis* as an abortifacient agent.

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Plant Collection of Plant Authentication

Khaya senegalensis bark was obtained from the botanical garden located in the University of Maiduguri, Borno State, Nigeria. The plant was authenticated by a Botanist in the Department of Biological Sciences, Faculty of Sciences in the same University.

Experimental Animals and Animal Husbandry

Twenty-four female albino rats were purchased from the Department of Veterinary Medicine, Ahmadu Bello University, Kaduna state and transported to Maiduguri in padded plastic crates. The animals were kept in the animal house of the Department of Human Anatomy, University of Maiduguri and were given one week to acclimatize. They were housed in well ventilated plastic cages at room temperature in hygienic conditions under natural light (13 hours) and dark (11 hours) schedules and were fed with standard rat chow which were given *ad libitum*.

Preparation of Plant Extract

Khaya senegalensis stem bark was air dried for a period of 14 days after which it was pounded and sieved to obtain the powdered form. A solution of the powdered stem bark in distilled water was made and placed in an electrical oven to dehydrate. The resultant composite was then collected for use.

Experimental Design

The experimental grouping and dosage is as indicated in the table below:

Group	**Treatment**
Control	0mg/kg of *Khaya senegalensis*
Group I	50mg/kg of *Khaya senegalensis*
Group II	100mg/kg of *Khaya senegalensis*
Group III	200mg/kg of *Khaya senegalensis*

Administration of Extract

The extract was administered via the oral route using an orogastric tube and the extract was given in concentrations as indicated by the table above. The extract was administered for a period of 28 days and at the end of this period, the rats in all groups were sacrificed to obtain the tissue of interest.

Animal Sacrifice

The rats were sacrificed at the end of the treatment period by inducing sleep using ketamine hydrochloride (Ralingtonpharma LLP, India). The injection was given to the right thigh of the rats to induced sleep. A laparoscopic procedure was performed on the rats with a horizontal midline incision performed to expose the abdominal and pelvic organs. The uterus was harvested from the perineum and the vagina was also collected and this was subjected to routine histological processing to observe the effect of the extract on these tissues.

Tissue Processing

The uterus and vagina were fixed in 10% formalin (Balaji Formalin Private Limited, India), and then, the tissue was washed under running water before it was dehydrated using alcohol (Sigma- Aldrich, USA). This process was carried out by immersing the specimen in a graded series of ethanol (alcohol) solutions of increasing concentration until pure, water-free alcohol was reached. Xylene (Veckridge Chemicals, New Jersey) was used to remove the alcohol found in the tissue and prepare it for the next stage of tissue preparation. The tissue was then infiltrated with wax after which it was embedded to form a tissue block which was clamped into a microtome for sectioning using a microtome (Leica RM2125 Rotary Microtome) and stained with Hematoxylin and Eosin (Abbey Colour, Philadelphia). The embedding process was reversed to dewax the tissue and allow dyes to penetrate the sections by running them through xylene to clear and alcohol to rehydrate. The tissue was stained with Hematoxylin and Eosin (Abbey Colour, Philadelphia). The sections were then photographed using an Amscope light microscope (MBJX- ISCOPE, Los Angeles) with a digital camera (M500, X 64, version 3.7) under X40 and X100 magnifications. Images of the histological sections were photographed using 10X objective lens and these images are presented as results.

The thickness of the epithelial layers of both uterus and vagina were measured using image J application (National Institutes of Health, USA, Version 1.35k) tracing tool to trace a line from stratum basale to stratum corneum at several regions along the thickness of the micrograph. These measurements were analyzed by one-way ANOVA followed by Dunnett's multiple comparisons test using GraphPad Prism (version 8.0.2). The results were represented as a bar graph with the X axis representing the groups and the Y axis showing the thickness of the epithelia in μm.

Results

Morphological Analysis of the Epithelial Linings of the Uterus and Vagina in Control and Treated Groups

The measurement of the thickness of the epithelial lining of the uterus of all groups are indicated in Figure 1. In the control group, the thickness was approximately 102.6μm. Group I had a significantly increased ($P< 0.05$) uterine epithelial lining at 110.6 μm when compared to the control group, and group II was significantly decreased ($p<0.01$) when compared to the control group at 91.3 μm. The epithelial layer in the uterus of rats in group III was non-significant at 106.9 μm.

Measurement of the vaginal thickness in the control and treated groups are indicated in figure 2. The epithelial wall of the control group was thickest (120.6μm) its thickness was significantly increased ($P<0.001$) when compared to the treatment groups: I (95.7 μm), II (95.2 μm) and III (95.7 μm). The extract seemed to decrease the thickness of the epithelium of the vagina in all groups.

Figure 1 showing uterine epithelial thickness in all groups

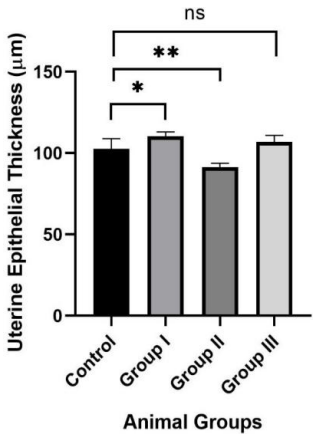
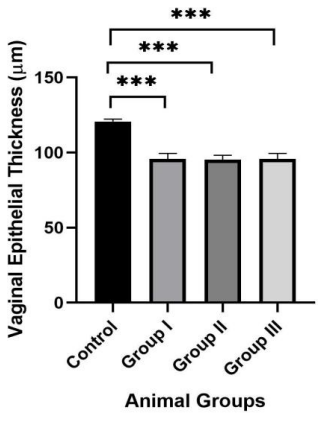


Figure 2 showing thickness of the vaginal epithelium in all groups



Morphological Observations in the Epithelial Linings of the Uterus and Vagina in Control and Treated Groups

The micrograph representing the uterus of the rats in the control group is labelled as Figure 3A. The epithelial lining of the uterus was intact and continuous and was simple columnar ciliated epithelium. The lamina propria was glandular exhibiting numerous uterine glands which were concentrated towards the papillary region. There lamina was also rich vascular supply in the dermis as evidenced by the presence of numerous vasculature. The dermis of the rats in group I was more glandular as noted by the presence of numerous glands, several of which had more secretory units when compared with the uterine glands in other groups (Figure 3B). The dermis also had a denser arrangement than in the dermis of the uterine endothelium in group II and III (Figures 3C and D). The epithelial layer in group I showed signs of disorganization and the regular arrangement was altered as many degenerated cells were observed. Numerous basal cells were arranged at the base of the epithelial lining in groups I and III (Figures 4A-D). Inflammatory cells were also present in the papillary region of the lamina propria of the rats in group I and III (Figures 4B and D).

The epithelial lining of the vagina is depicted in figures 5A-D. In the control group, there was a regular arrangement with the basal layer of the stratified squamous epithelium consisting of low columnar epithelium and this rested on dermal papillae. The cells in stratum spinosum were polygonal with rounded nuclei and these cells flattened as they advanced to the apical region and the cornified layer consisted of flattened sheets which were eosinophilic in the micrograph (Figure 6A). The lamina propria was loosely organized and consisted of connective tissue cells. The epithelial lining in group I and III showed similar characteristics as the apical cells remained rounded and were squamous (Figure 6B and D) as observed in the control group and group II (Figure 6C). The lamina propria of the vagina of rats in groups I and II were denser in arrangement when compared to the control group.

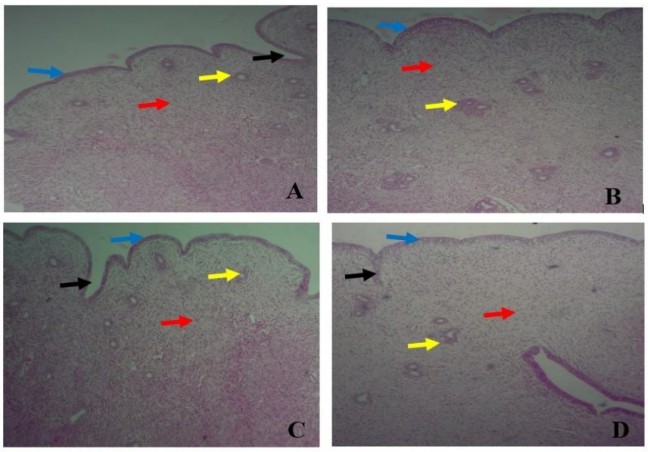


Figure 3 showing the histological layers of the uterus in control and treatment groups (I – III) showing the endometrial lining (blue arrow) lamina propria (red arrow) and surface (black arrow) and stromal endometrial glands (yellow arrow) which were more numerous in group I and sparse but clustered in group III. H and E X40

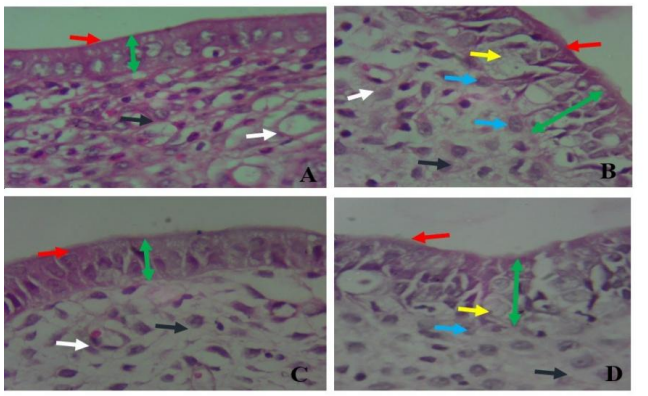
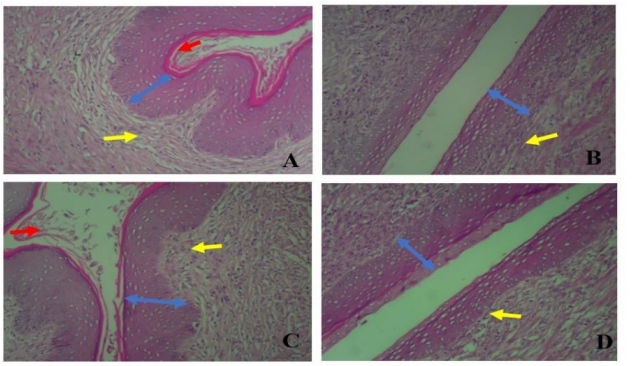


Figure 4 showing the endometrium and lamina propria of the uterus at a higher magnification in the control group and groups I, II and III showing a disrupted epithelial layer (green arrow) with basal cells (blue arrow) replacing them in groups I and III, the epithelial lining (red arrow) is present on the apical part of the cells and the lamina propria is denser in the control group and had blood vessels (white arrow) present. H and E X 400



Figures 5 A – D showing the epithelial and connective tissue layers of the vagina in all groups with a blue arrow on showing the thickness of the epithelium, yellow arrow on the lamina propria and red arrow showing stratum corneum on the surface of the epithelium in the control group and group II. H and E X100

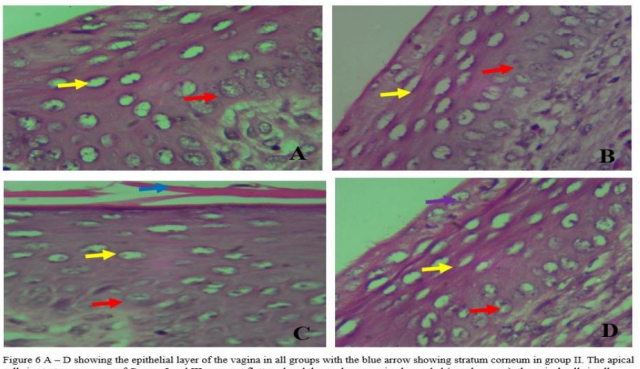


Figure 6 A – D showing the epithelial layer of the vagina in all groups with the blue arrow showing stratum corneum in group II. The apical cells in stratum corneum of Groups I and III were not flattened and the nucleus remained rounded (purple arrow), the apical cells in all groups remained rounded and is represented with a red arrow. The lamina propria was dense in groups I and III (green arrow) butthis layer was not so densely arranged in the control group. H and E X 400

Discussion

Many women across several cultures; including women of Spanish and Mexican decent have used plants as emmenagogues and abortifacients to bring on their periods if pregnancy is suspected. The absence of menses in a woman in such cultures are treated as a disease because menses is believed to be the removal of bad blood (17). The use of abortifacient plants is also commonly practiced among the natives in Nigeria as a means of terminating pregnancy. Abortifacient substances used are either chemical or physical and capable of inducing abortion (18). In Mexico, these plants are usually administered by curanderos (healers), herbalists, who use many of the materials used in traditional medicine; and *brujos*, who are sorcerers and witches. Plants used for these purposes include osha, chuchupate-lovage; ponso or tanse-tansy; poleo-spearmint or pennyroyal mint; amolillo-wild licorice; dormilon-tall cone flower; malva, lanten-plantain. Gossypium, Ruta, Ligusticum, Asclepias, and Rudbeckia (17).

Khaya senegalensis is used as an abortifacient agent based on folkloric beliefs without a scientific basis for its use, the plant extract is taken based on self-medication with no determined dosage, hence, it is essential to determine what dosage of the extract is effective. The current research seeks to investigate the histological and morphometric effect of this extract on the epithelial lining of the vagina and uterus of albino rats following oral administration.

The extract at concentrations of 50mg/kg and 200mg/kg caused disorganization of the arrangement of the uterine epithelium which is observed as one of the mechanisms described in abortifacient activities of a plant on the uterus. Other mechanisms of abortion could possibly be observed through changes in the uterine mileu, altered hormone levels, luteolysis and estrogenicity (19,20).

There was an observed increase in the number and size of endothelial glands in the rats treated with the extract at a concentration of 50mg/kg and it could be deduced that at this concentration, the extract promoted growth and proliferation of uterine glands. The glands became fewer in number and smaller in size as the concentration increased and at a concentration of 20mg/kg, these glands were few and sparsely distributed in the endometrial stroma, suggesting that the extract had an inhibitory effect on the proliferation and growth of endometrial glands. Histological studies by (21) showed a significant reduction in myometrial thickness, uterine gland diameter, luminal diameter of uterine glands and luminal epithelial cell height in rats treated with the seed extract.

The epithelial luminal glands in the luminal surface were present and the luminal surface showed numerous folds which were reduced to scalloped ridges in the group treated with the highest concentration of the extract, showing that the extract reduced the number of uterine glands at the highest magnification which is consistent with studies undertaken by (21) who reported an absence in uterine pits and folds in luminal epithelial as well as mitotic activity in the luminal and glandular epithelial cells of uterus.

The endometrial stroma showed a reduction in its composition and robustness and there was also less vascularity in the connective tissue as the concentration of extract administered increased which supported the fact that administration of *K. senegalensis* did not support the preparation of the uterus for implantation. In other studies carried out, researchers have reported that the administration of other plant extracts have similarly reduced endometrial height. One of these plants being *Sesbania sesban* seed powder whose administration caused great reduction in endometrial height, shrunk uterine glands, caused poor vascularity to the compact stroma which prevented implantation in female albino rats.

The epithelium of the vagina also had similar features in the control group and the group treated with 100mg/kg of extract. There was a layer of cornified tissue overlying stratum spongiosum and papillary dermal lines. These were absent in the groups treated with 50mg/kg of extract and 200mg/kg and a layer of non-flattened cells with rounded epithelia were observed on the luminal surface. There is no known explanation at present why there was a difference in surface epithelial cells in the treatment groups, and literature search revealed that several antifertility extracts, even when administered via the vaginal route caused no significant changes in the vaginal epithelium (24). (25) however, reported the presence of predominantly cornified and nucleated epithelial cells in the vagina after administration of *Balanites roxburghii* extract which was consisted with the result obtained in the current study in some treated groups.

The morphometric study carried out supported the results obtained by observation of the histology of the uterus and vagina. Uterine epithelial cell thickness was significantly increased in the group administered 50mg/kg and insignificantly in the group that received 200mg/kg and this could be attributed to a disorganization of the epithelial lining followed by a replacement at the basal layer of these groups. The extract and significantly reduced the epithelial lining in the group that received 100mg/kg of the extract as observed by shorter columnar cells in this group compared to the control group.

Morphometric analysis of the vagina in all treated groups revealed that the extract significantly decreased the thickness of the vaginal epithelium in all groups when compared to the vaginal epithelial thickness in other groups, this suggests that although it was not apparent in the micrographs, the extract reduced the thickness in the epithelial lining of the vagina in all treated groups.

Conclusion

The current study investigated the effect of oral administration of *Khaya senegalensis* as an abortifacient agent following its folkloric use to that effect. Different dosages were administered to determine its effect on the histology of the uterine and vaginal lining. Histological observations of these layers

were carried out as well and the results obtained showed that the extract at a concentration of 50mg/kg and 200mg/kg disrupted the epithelial lining and as the concentrations administered increased, surface and stromal endometrial glands became smaller and sparse and the endometrial stroma became less compact. Morphometric analysis revealed a significant increase of endometrial epithelium in the group administered the least concentration of the extract. Vaginal epithelial thickness was also significantly reduced in all groups suggesting that the extract has antifertility effect on the lining of the uterus and vagina. Further experimental studies however are required to validate the results obtained in the present study.

Ethical Considerations

The experimental procedures were conducted in accordance with the University of Maiduguri.

Research and Ethical Committee guidelines, the ARRIVE guidelines (reporting of in vivo experiment), and the National Institutes of Health (NIH) guide for the CARE and use of laboratory animals (NIH Publications No. 8023, revised 1978). The research was also conducted in accordance with the Helsinki Declaration of 1975, as revised in 2000.

Conflict of Interest

The Authors have no conflict of interest to declare

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