HISTOLOGICAL EFFECTS OF CITRUS AURANTIFOLIA ON GRAVID AND POST-PARTUM UTERUS

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Abstract

Diseases have afflicted man for ages but humans always make effort to remedy the situation in order to regain a life disturbed by these ailments. The earliest form of healing substances had been Traditional (herbal) medicines, but with the advent of civilization which had led to better scientific understanding of diseases and medications, orthodox medicines have become the main and well recognized products for the management of diseases in modern health systems. The aim of this study was to investigate the effects of citrus aurantifolia (lime plant) on gravid and non-gravid uterus of female wistar rats and their pups. A total of 50 Wistar rats were used in carrying out the experiment and classified into two Models. Each group in Model 1 contained 8 rats each, while in the Model 2, each group comprised 5 rats. The body weight of the rats were measured and recorded weekly. Group I-III in model 1 received 1ml/kg, 1.5 ml/kg and 2 ml/kg respectively, two rats from each group were euthanized at 7, 14 and 21 days to observe acute, subacute and chronic effect, group IV (control) received food and distilled water only. While in model 2, group I rats commenced 1 ml/kg of aqueous lime juice from the first day of gestation, group II received 1 ml/kg of aqueous lime 1week after gestation and group III were administered food and water. The histomorphological study of the uterus revealed deleterious effect of lime juice in a dose dependent pattern and Hormonal assay carried out showed a reduction in gonadotrophin level. In conclusion, high consumption of Lime juice compromises the outcome of pregnancy in the treated rats by the reduction in the crown rump length and weight of pups.

INTRODUCTION

Diseases have afflicted man for ages but humans always make effort to remedy the situation in order to regain a life disturbed by these ailments. The earliest form of healing substances had been Traditional (herbal) medicines, but with the advent of civilization which had led to better scientific understanding of diseases and medications, orthodox medicines have become the main and well recognized products for the management of diseases in modern health systems (Kuhn; 2002: Osemene et al., 2011).

Plants have been use as sources of drugs in traditional and orthodox medicine. Most indigenous plants have been proven to be valuable in both prevention and treatment of various ailments. With the indiscriminate use of synthetic drugs and antibiotics and the realization of the health hazards and toxicities associated with it, there has been shift in the increase use of herbal medicine globally. Also affordability and perceived safety of the herbal drugs have increased their acceptability as alternates to orthodox drugs, most especially in the developing and underdeveloped countries (Enejoh et al; 2015).

The use of Lime (Citrus aurantifolia) juice has shown to have both medicinal and cosmetic values. Lime juice as a rich source of vitamin C, is very effective in boosting the immune system when its juice is mixed with warm water, it promotes biliary secretion from the liver, resulting in an easier release of faeces, thus making it a natural recipe for constipation (Adegoke and Oyelami, 2011). Lime juice has been perceived by women as an abortificient agent and contraceptive, there is a long known history of women in African douching with lime juice, vinegar and oral consumption of lemon juice or acidic soft drinks with lime, believing that it may prevent or terminate pregnancy and/ or sexually transmitted diseases (De Castillo et al.; 2000). According to a research carried out by Bakare et al (2012), lime juice showed no abortifacient effect but had some teratogenic outcome and there were some irregularities in the estrous cycle. In the rats administered lime juice, the uterus showed areas of inflammation and vascular congestion; the ovaries also showed cortical area with lining degenerated, marked cellular degeneration, follicular degeneration, atrophy and inflammation while the vagina showed marked areas of cellular degeneration, vascular degeneration and congestion.

In another study, reduction in body weight was noted when overweight adults were given lime juice (Gharagozloo et al.; 2002). Noxynol9(N-9), krest bitter lemon (soft drink) and lime juice have been demonstrated to have antimicrobial and spermicidal properties and have been considered as biological agents for the prevention of pregnancy and sexual transmitted infections (Roger and Short, 2000).

In recent times, the consumption of lime juice among pregnant women has increased daily as it is believed to stimulate the liver, improve digestion and control diarrhoea during pregnancy (Adegoke and Oyelami, 2011). There is also an indiscriminate use of lime juice as an abortificient agent among women. Hence there is a need to investigate the effect of lime juice on the uterus and ovaries. The aim of this study was to investigate the effects of citrus aurantifolia (lime juice) on the uterus of female wistar rats and their pups.

MATERIALS AND METHODS

Materials

Ethical clearance was obtained from the Research and Ethics Committee in Faculty of Basic Medical Sciences, Delta State University prior to the commencement of this research. Forty seven (47) female and six (6) male Wistar rats weighing 150 - 200g were adopted as experimental model. They were obtained from the Animal House unit of college of Health Sciences in Delta State University Abraka, Delta State, Nigeria. Fresh lime fruits were harvested in Abraka, Delta State, Nigeria. The fruits were then authenticated at the Department of Botany, University of Benin. They were properly washed and sliced into two halves each, which were squeezed gently. The resulting lime juice was filtered through a Whatman number 1 paper (Arat et al., 2012), and residual pulp and seeds were discarded. The lime juice processed in this manner, pooled and was collected into a clean plastic container, covered and stored. The following materials were used in this study; standard rat chow, clean water, syringes and needles, sample collection materials, wired mash cage, hand gloves, digital weighing balance, measuring tape, laboratory coat, dissecting kit, computer and printer, digital vernier calliper. Chloroform (use in sedating the rats), Formal saline (use for preserving the harvested organs). H & E stain.

Methods

The animals had free access to clean tap water and pellets. The cages were kept at the rat control room of Anatomy Department, Delta State University at room temperatures. Lighting in the room was by sun rays reflecting through the glass windows such that the rats were exposed to approximately 12 hours of day and about 12 hours of night cycle to maintain standard photoperiodicity of nature in our locality. The rats were labelled by tail marking using permanent marker and kept in well ventilated metal cages (5 animals in each cage) and they were allowed to acclimatize for a period of 14 days. The experiment was carried out for a period of 3 weeks.

A total of fifty three (53) Wistar rats were used in carrying out the experiment and classified into two Models.

Each group in Model 1 contained 8 rats each. While in Model 2, each group comprised 5 rats each.

MODEL 1

Group I: Rats received 1mL/kg (Yibala et al., 2020) of aqueous lime juice for a period of 21 days. Two rats from the group were euthanized at 7, 14 and 21 days.

Group II: Rats received 1.5 mL/kg (Yibala et al., 2020) of aqueous lime juice for a period of 21 days. Two rats from the group were euthanized at 7, 14 and 21 days.
Group III: Rats received 2 mL/kg (Yibala et al., 2020) of aqueous lime juice for a period of 21 days. Two rats from the group were euthanized at 7, 14 and 21 to observe the prolong effect of lime juice.
Group IV: Served as control and were administered distilled water and rat chow adlibitum.

MODEL 2
Female rats in model 2 were mated with the male Wistar rats on the oestrous day (heat period) of oestrous cycle. Two male rats were kept in each of the groups. The presence of copulatory vaginal plug the next morning was used to ascertain successful mating and this day was taken as the first day of gestation.

Each group received aqueous lime juice for a period of 21 days after successful mating. They were then euthanized at the 21 day of gestation
Group I: Rats started receiving 1 mL/kg of aqueous lime juice from the first day of gestation.
Group II: Rats received 1 mL/kg of aqueous lime 1week after gestation
Control Group III: Rats were administered distilled water.

Methods
Animal care and grouping
The animals had free access to clean tap water and pellets. The cages were kept at the rat control room of Anatomy Department, Delta State University at room temperatures. Lighting in the room was by sun rays reflecting through the glass windows such that the rats were exposed to approximately 12 hours of day and about 12 hours of night cycle to maintain standard photoperiodicity of nature in our locality. The rats were labelled by tail marking using permanent marker and kept in well ventilated metal cages (5 animals in each cage) and they were allowed to acclimatize for a period of 14 days. The experiment was carried out for a period of 3 weeks.

Experimental Design
A total of fifty three (53) Wistar rats were used in carrying out the experiment and classified into two Models.
Each group in Model 1 contained 8 rats each. While in Model 2, each group comprised 5 rats each.

3.2.2.1 Oestrous cycle and gestational period of wistar rat
The mean duration of oestrous cycle is 4 days for 60% of female wistar rats. However, some rats have longer regular and irregular cycles (Marcondes et al., 2002).
The normal period of gestation for a female Wistar rat ranges from 21-23 days (Marcondes et al., 2002).

Oestrous cycle and gestational period of wistar rat
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Determination of Oestrous Cycle
The rats underwent a recruitment phase of 16 days during which rats that met the inclusion criteria were selected. Inclusion criteria included cyclicity of vagina smear, at least three consecutive regular oestrous cycles, typically having a sequence of proestrus, estrus, metestrus, and diestrus (P- E-M-D) phases were considered having regular oestrous cycle (Goldman et al., 2014) and then selected for this study. Animals that showed deviation from the P-E-M-D pattern, and or absence of four day cycle were considered to have irregular oestrous cycles (Goldman et al., 2014).

The cycle phases were determined from the cytology of vaginal smears obtained daily between 7.00 a.m. and 9.00 a.m. Normal saline was drawn into the tip of the pipette, which was inserted 2 mm deep into the vaginal canal and 2 drops emptied. The mixture of vaginal fluid and normal saline was suctioned into the tip of the pipette. The smear was placed on glass slide and examined under the light microscope immediately before drying up. The proestrus phase was characterized by presence of rounded and nucleated epithelial cells. The estrus phase showed presence of cornified cells. The metestrus phase was characterized by presence of a combination of leucocyte, cornified and rounded epithelial cells. The diestrus phase was characterized mainly by presence of leucocytes.

Determination of dose
According to Deshmukh et. al. (2017) the LD50 of Citrus aurantifolia is found to be greater than 1000 mg/kg in rats. A dose of 1ml/kg, 1.5mL/kg and 2ml/kg (group I, II, III respectively) and 1ml/kg (all groups in model two) was used. (Yibala et al., 2020).

Animal Euthanizing and sample collection
The experimental rats were euthanized using chloroform and the uterus and ovaries were carefully dissected out from the experimental rats in model 1 and 2 following abdominal incision The following samples were collected: Uterus, Ovaries and blood sample for biochemical analysis.

Histological analysis
The samples were fixed in 10% forma-saline for tissue processing using Hematoxylin and Eosin Stain (H / E) according to the method described by Akpantah et al. (2003) and sections were observed microscopically.

Location of the Study
The experiment was conducted in the Animal holding facility of the Faculty of Basic Medical Sciences of the College of Health Sciences in Delta State University of Abraka.

Data Analysis
Data obtained from hormonal assay, the crown-rump length and weight of the pups were subjected to statistical analysis using one-way analysis of variance (ANOVA) and Scheffe's post-hoc test. Results were expressed as Mean ± Standard Deviation (SD). p value lesser than 0.05 was considered to be statistically significant.

RESULT

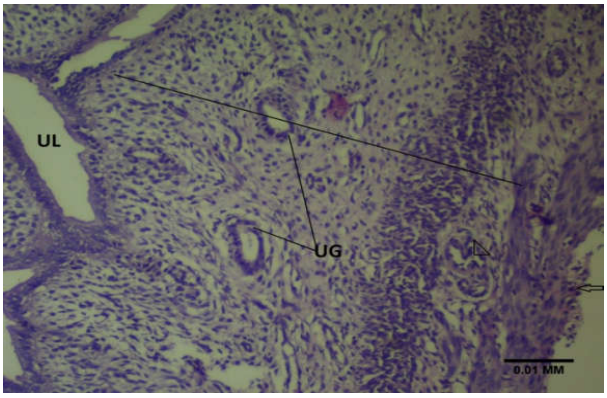

Plate 1: A1 Photomicrograph of group I rats after 7days of treatment (H & E x 100)

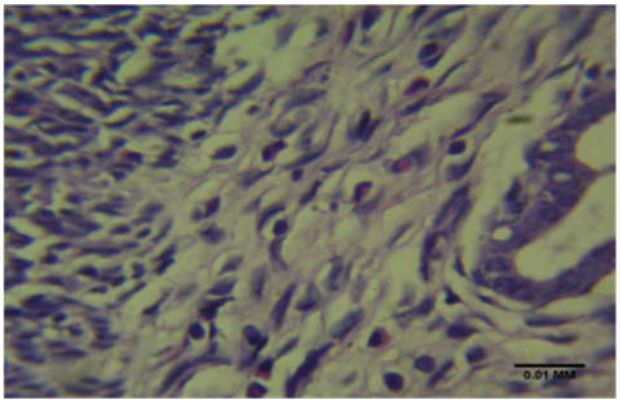

Plate 2: A1 Photomicrograph of group I rats after 7days of treatment (H & E x 400)

Histological observations in the uterus of group I experimental rats admiminitered 1ml/kg of lime juice after 7days
A1 Photomicrographs shows uterine tissues consisting of the essential layers: the endometrium, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head) while the perimetrium consist of the visceral peritoneum (arrow) (plate 1).

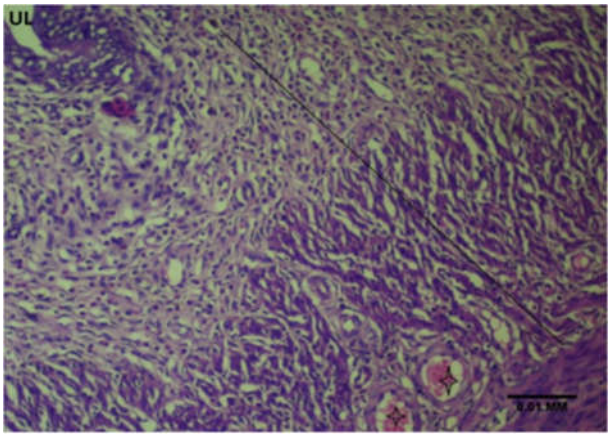

Plate 3: A2 Photomicrograph of group I rats after 14days of treatment (H & E x 100)

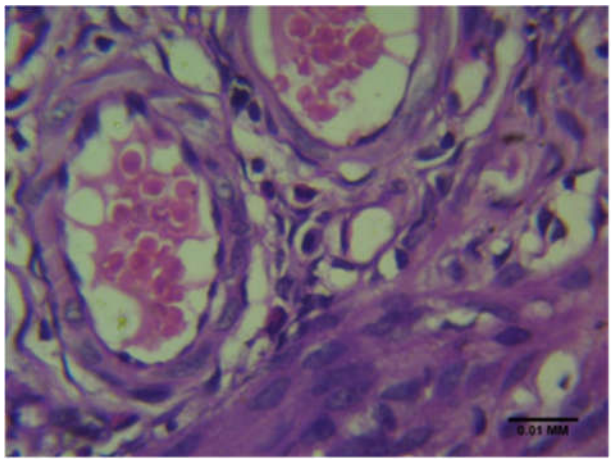

Plate 4: A2 Photomicrograph of group I rats after 14days of treatment (H & E x 400)

Histological observations in the uterus of group I experimental rats admiminitered 1ml/kg of lime juice after 14days

B2 Photomicrographs shows uterine tissues consisting of the essential layers: the endometrium and myometrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels. The vessels are dilated (Stars) (plate 9).

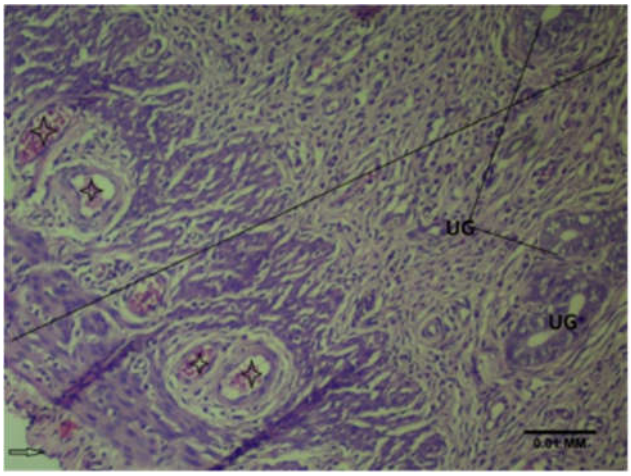

Plate 11: B3 Photomicrograph of group II rats after 21days of treatment (H & E x 100)

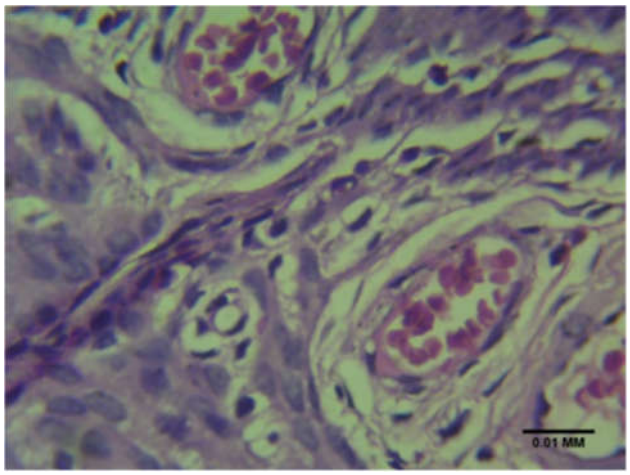

Plate 12: B3 Photomicrograph of group II rats after 21days of treatment (H & E x 400)

Histological observations in the uterus of group II experimental rats admiminitered 1.5ml/kg of lime juice after 21days

B3 Photomicrographs shows uterine tissues consisting of the essential layers: the endometrium, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels while the perimetrium consist of the visceral peritoneum (arrow). The vessels are dilayed (Stasr) (plate 11).

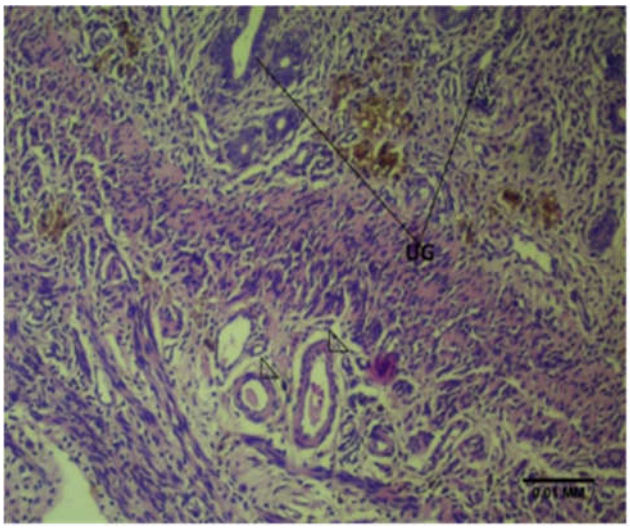

Plate 13: C1 Photomicrograph of group III rats after 7days of treatment (H & E x 100)

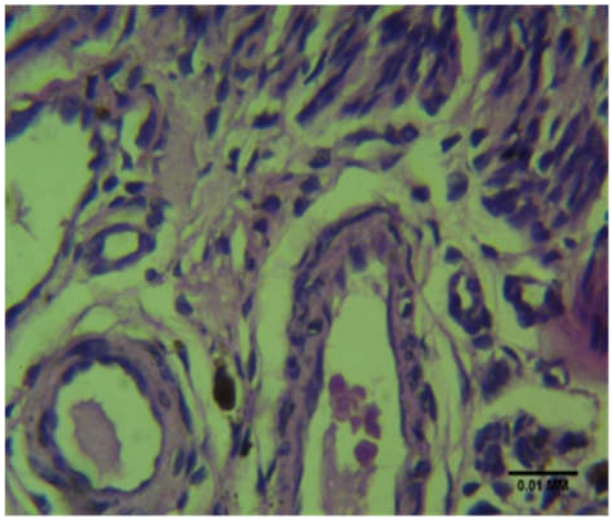

Plate 14: C1 Photomicrograph of group III rats after 7days of treatment (H & E x 400)

Histological observations in the uterus of group III experimental rats admiminitered 2ml/kg of lime juice after 7days

C1 Photomicrographs shows uterine tissues consisting of the essential layers: the endometrium and myometrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head) while the perimetrium consist of the visceral peritoneum (arrow) (plate 13).

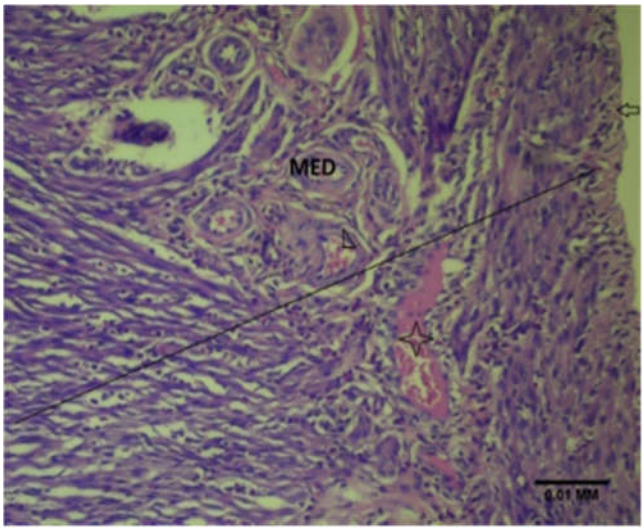

Plate 15: C2 Photomicrograph of group III rats after 14days of treatment (H & E x 100)

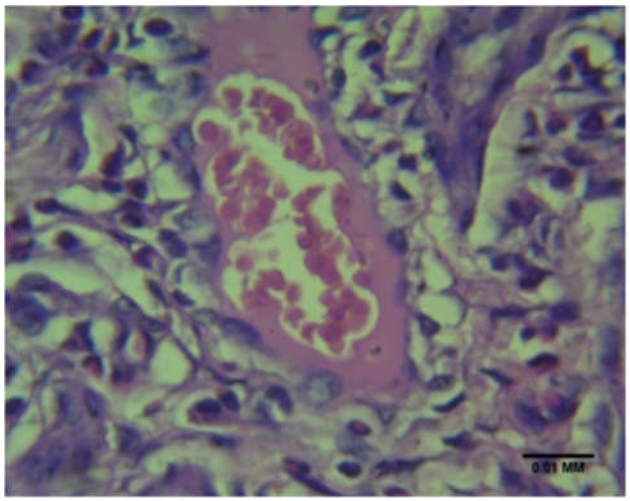

Plate 16: C2 Photomicrograph of group III rats after 14days of treatment (H & E x 400)

Histological observations in the uterus of group III experimental rats admiminitered 2ml/kg of lime juice after 14days

C2 Photomicrographs shows uterine tissues consisting of the essential layers: the endometriu, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels and while the perimetrium consist of the visceral peritoneum (arrow). Some vessels appear dilated (Star) (plate 15).

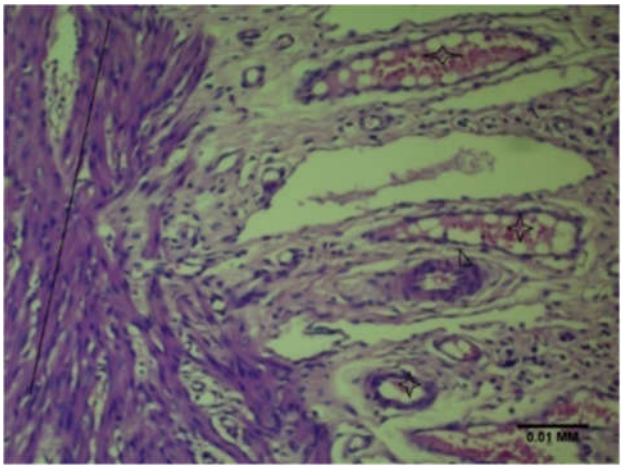

Plate 17: C3 Photomicrograph of group III rats after 21days of treatment (H & E x 100)

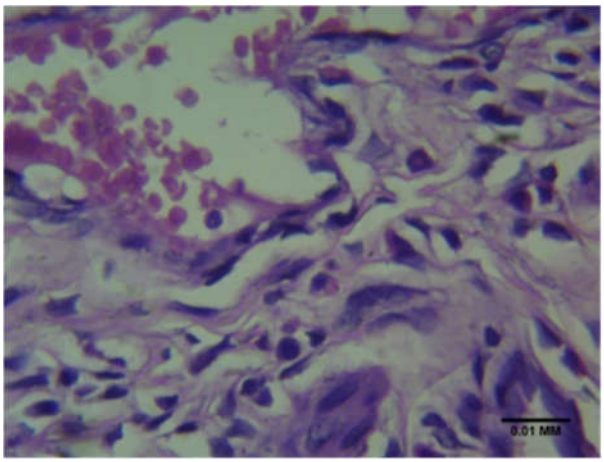

Plate 18: C3 Photomicrograph of group III rats after 21days of treatment (H & E x 400)

Histological observations in the uterus of group III experimental rats admiminitered 2ml/kg of lime juice after 21days

C3 Photomicrographs shows uterine tissues consisting of the essential layers: the endometrium and myometrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels. The vessels appear dilated (Star) (plate 17).

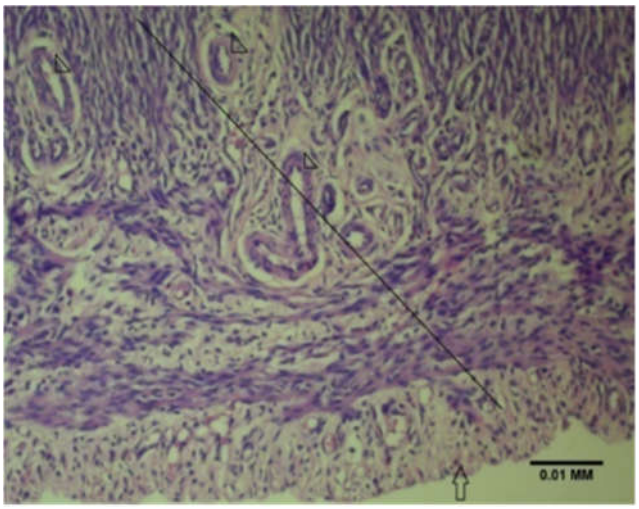

Plate 19: C Photomicrograph of rats in the control group after 21days of treatment (H & E x 100)

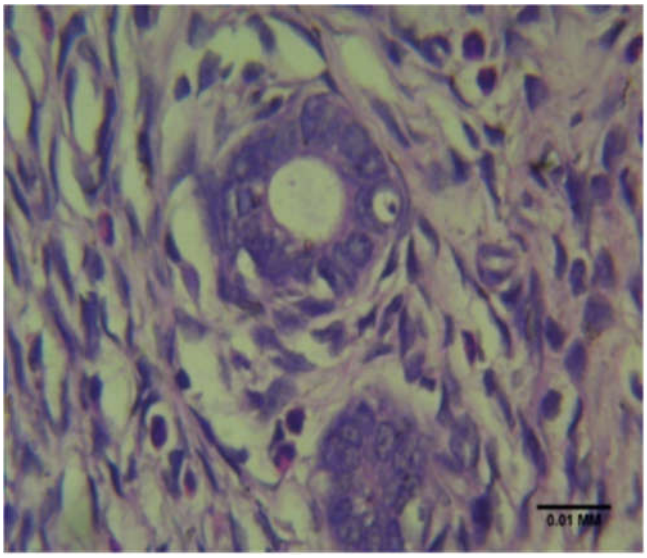

Plate 20: C Photomicrograph of rats in the control group after 21days of treatment (H & E x 400)

Histological observations in the uterus of control experimental rats

C Photomicrographs shows the uterine tissues consisting of the essential layers: the endometrium, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head) while the perimetrium consist of the visceral peritoneum (arrow) (plate 19).

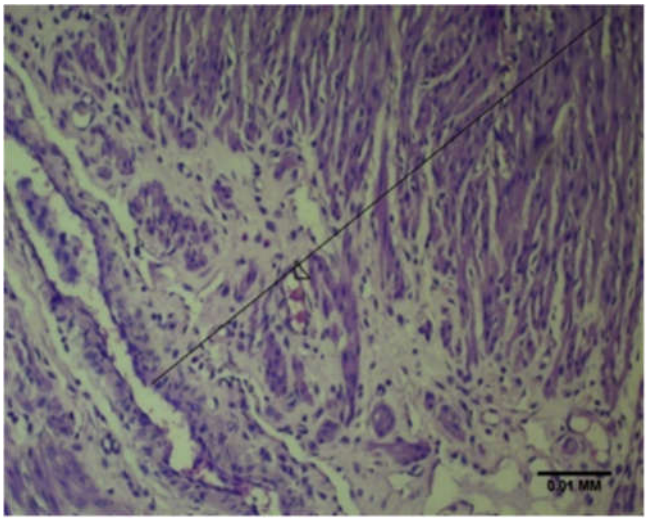

Plate 21: E Photomicrograph of rats in the control group after 14days of treatment (H & E x 100)

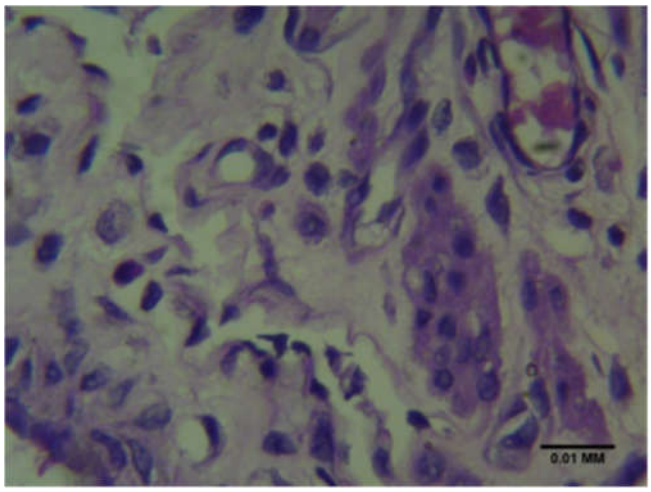

Plate 22: E Photomicrograph of rats in the control group after 14days of treatment (H & E x 400)

Histological observations in the uterus of group I experimental rats admiminitered 1ml/kg of lime juice after 21days

E Photomicrographs shows the uterine tissues consisting of the essential layers: the endometrium, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head) while the perimetrium consist of the visceral peritoneum (arrow) (plate 21).

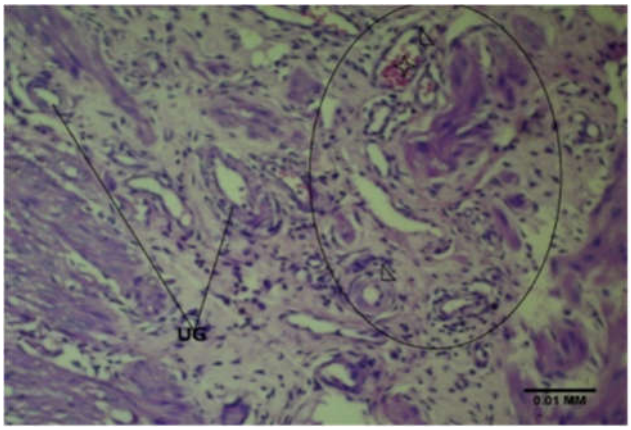

Plate 23: F Photomicrograph of rats in the control group after 21days of treatment (H & E x 100)

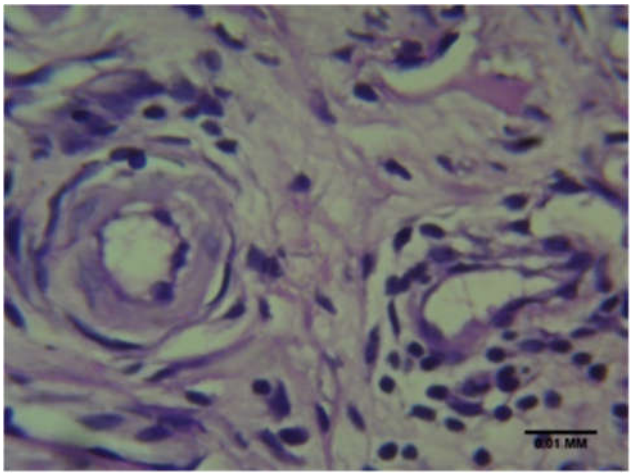

Plate 24: F Photomicrograph of rats in the control group after 21days of treatment (H & E x 400)

Histological observations in the uterus of group II experimental rats admiminitered 1ml/kg of lime juice after 14days

F Photomicrographs shows the uterine tissues consisting of the layers: the endometrium and myometrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head). The vessels showed dilation and inflammatory cells infiltration (circle) (plate 23).

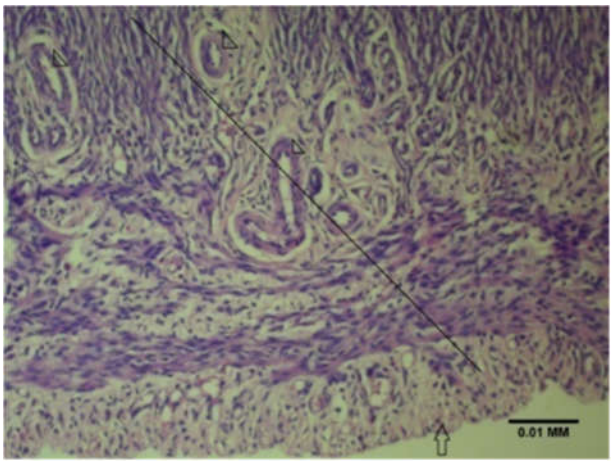

Plate 25: G Photomicrograph of rats in the control group after 21days of treatment (H & E x 100)

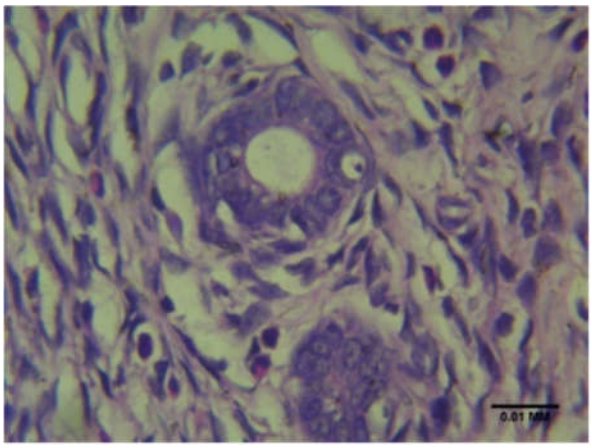

Plate 26: G Photomicrograph of rats in the control group after 21days of treatment (H & E x 400)

Histological observations in the uterus of control experimental rats

G Photomicrographs shows the uterine tissues consisting of the essential layers: the endometrium, myometrium and perimetrium, the endometrium is lined by regular epithelium, the uterine gland, all opening into the uterine lumen, the myometrium contained smooth muscle dispersed within the connectives, blood vessels (arrow head) while the perimetrium consist of the visceral peritoneum (arrow) (plate 25)

Discussion

Result from this study confirms that lime juice leads to endometrial inflammation and endometritits in the uterus of experimented animals. The endometrial inflammation and endometritits is characterised by increased amount of neutrophil and large luminal content which was largely exacerbated in rats treated with the highest dosage for the period of 21 days. The outcome of the result was found to be dose dependent with the rats treated with the highest dosage of aqueous lime juice having the most severe effects. Endometritis is referred to as the inflammation of the endometrial lining of the uterus. In addition to the endometrium, inflammation may involve the myometrium and occasionally, the parametrium. Endometritis can be divided into pregnancy and non pregnancy related endometritis. Embryo attachment to the luminal epithelium of the uterus initiates a process called decidualization, followed by angiogenic and inflammation reaction (Fonseca et al., 2012). Decidualization which is characterised by functional and morphological changes in the uterus is initiated during implantation, on the antimesometrial (AM) pole of the endometrium, and gives rise to the AM decidua, and on the opposite side, cells differentiate to the mesometrial (M) deicudua (Fonseca et al., 2012).

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