Original Research Article

Anti-proliferative Effect of Lime Juice on the Ovarian Cortex of Adult Wistar Rats (Rattus Novergicus)

ABSTRACT

Background: Lime juices has been reported to exhibit antimicrobial activity, cause anti-proliferative effects on tumors cell lines and has been shown to immobilize sperm. This study is Therefore, aimed at investigating the anti-proliferative effects of Lime juice on the Ovarian Cortex of Adult Wistar Rats.

Methods: A total of Twenty (20) adult wistar rats weighing between 160 - 190 g were randomly selected into groups 1, 2, 3 and 4 (n= 5), The animals in Group 1 received 1ml/kg body weight of undiluted lime juice; Group 2 received 1.5 ml/kg body weight of undiluted lime juice; Group 3 received 2.23 ml/kg body weight of undiluted lime juice while, Group 4 received 0.5ml of distilled water respectively at 0900 hours for period of Ten (10) days. Administration was done by gavages oro-gastrically daily using metal oral canula. Animals were sacrificed by cervical dislocation 24 hours after the last administration of Lime juice; ovary was dissected out following abdominal incision, fixed in 10 % Formo-saline for histological observation using H/E stains and blood samples were collected for hormonal (reproductive hormones) assay.

Results: Plasma concentration of FSH and LH were significantly ($p < 0.05$) lowered in the Lime – treated rats and histological observation revealed degeneration in the follicular cells, stroma hyperplasia and immature follicles in the animals treated with the undiluted Lime juice when

compare with the control animal; that revealed follicular cells at different stages of development.

Conclusions: Reduction in the plasma concentration of FSH and LH with consequent degeneration of follicular cells expressed in the ovarian cortex demonstrate anti proliferating effect of Lime juice.

Key Words: Lime juice, Ovary, Wistar Rats, Follicular cells, Reproductive Hormones

Background: Lime juice is one of the Citrus fruits that are widely planted for direct human consumption in the world; its juice has been shown to have both medicinal and cosmetic values (1). It has several health benefits, and one could make use of it from its skin, to its juice, down to its pulp. Its juice can do wonders to the body and it can relieve a person from numerous diseases (2). Lime as a rich source of vitamin C, is very effective in boosting the immune system (3). 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 (3).

Lime juices have been reported to exhibit antimicrobial activity against Vibrio strains (4). The in vitro effects of concentrated lime juice extract reveal its anti-proliferative effects on tumour cell lines (5). Lime juice under laboratory conditions had been shown to immobilize sperm as well as advocated as a 'natural' spermicide. Spermicide is a contraceptive substance that eradicates sperm, inserted vaginally prior to intercourse to prevent pregnancy. Lime juice has also been shown to alter estrus cycle by significantly prolonging the diestrus and estrus phases, thus having an anti-fertility effect on animals. Both lime juice and water were found to cause mild and transient side effects in 70% of women, including vaginal dryness, itching and burning, but burning and dryness occurred more frequently in women using 20% lime juice (6).

Cervicovaginal lavages of women using lime juice for seven days showed high levels of pro-inflammatory cytokines such as IL-1, IL-6, and IL-8 and increased numbers of CD45-positive

leukocytes, indicating the presence of a mucosal inflammatory response (7). Furthermore, a recent cross-sectional observational study of 374 Female sex workers in Nigeria found a statistically significant association between use of lime juice and lemon juice (n = 81) and the presence of cervicovaginal intraepithelial neoplasia (CIN) (8).

In another study, a reduction in body weight was noticed and somewhat in agreement with previous studies, which also found a reduction in body weight when overweight adults were given lime juice (9). Lime juice is being used by women as a barrier contraceptive, and there is a long reported history of African women douching with lime juice, lemon juice, vinegar or acidic soft drinks in the belief that it may prevent pregnancy and/ or sexually transmitted diseases (9). Previous studies have also that there was an irregular pattern in all phases of the estrous cycle of 100% of the rats given undiluted lime juice and in 80% of those given 50% diluted lime juice indicating that lime juice has an anti-fertility effect by altering the histology of the walls of the uterus, prolonging one or more of the phases, reduction in the number of ova shed and blocking of ovulation partially (9).

~~This study is~~ aimed at investigating the effects of Lime juices extract on the plasma reproductive hormones (FSH and LH) and on Histological architecture in the ovary of adult wistar rats.

Materials and Methods

Experimental animals; Twenty (20) Adult male rats weighing between 160 – 190 g were procured from the animal house of the Nigerian Institute for Trypanosomiasis and Onchocerciasis, Kaduna Nigeria. The ethical approval on animal act right was obtained from the Institutional Animal Care Committee of Bingham University, Karu, Nigeria. They were kept in laboratory for two weeks of acclimatization and were fed on standard diet (Vital Feeds and

Grand Cereals Ltd); food and water was given *ad libitum* and maintained under standard conditions. The animal room was well ventilated with a temperature range of 25-27^{o}C under day/night 12-12 h photoperiodicity.

Extract preparation; Fresh fruits of Citrus aurantifolia (lime fruit) were obtained from Nyanya Market in Karu, Nassarawa State, Nigeria. Authentication was done in the Biology Department, Faculty of Science, Bingham University, Karu, Nigeria. The fruits were properly washed and sliced into two halves each. The juice was extracted using a juice extractor. The resulting lime juice was filtered through a sieve and residual pulp and seeds were discarded. Lime juice of fifty lime fruits was processed in this manner, pooled and collected into a clean plastic bottle, covered and refrigerated (-4°C) throughout the course of the experiment to prevent fermentation.

Experimental Procedure; A Total of twenty rats were used in this experiment and were subdivided into 4 Groups 1, 2, 3 and 4, (n=5). The animals in Group 1 received 1ml/kg body weight of undiluted lime juice; Group 2 received 1.5/kg body weight of undiluted lime juice, Group 3 received 2.23ml/kg body weight of undiluted lime juice, Group 4 received distilled water only. Administration was done by gavages oro-gastrically daily using metal canula at 0900 hours for period of Ten days respectively.

Animal Sacrifice; Animals were sacrificed by cervical dislocation 24 hours after the last administration of undiluted Lime juice; Ovary was excised following abdominal incision, fixed in

10 % Formo-saline for histological observation using H/E stain while blood sample was collected from descending aorta for hormonal assay.

Analytical procedure:

Histological analysis: Testes were carefully dissected out following abdominal incision and fixed in 10% formo-saline and processed routinely for paraffin embedding. 5 µ sections were obtained with rotary microtome and processed for Hematoxylin and Eosin Stalin (H / E) according to the method described by Akpantah *et al*., 2003 (10) and Sections were observed.

Hormonal Assay; Blood samples were collected in a reagent bottle for determining plasma level of Follicles Stimulating Hormones (FSH) and Luteinizing Hormone (LH) micro-well enzyme Radio-immuno-assay method produced by Syntron Bioresearch Inc. of United State of America (USA) as described by (11,12,13)

Statistical Analysis; Results were expressed as Mean ± Standard error of mean (SEM) and subjected to statistical analysis using the SPSS-V11 statistical software package 13 for analysis of the data. Statistical analyses carried out using the Student's t-test. Differences were considered to be of statistical significance at an error probability of less than 0.05 ($P<0.05$).

Result:

Histological Observations

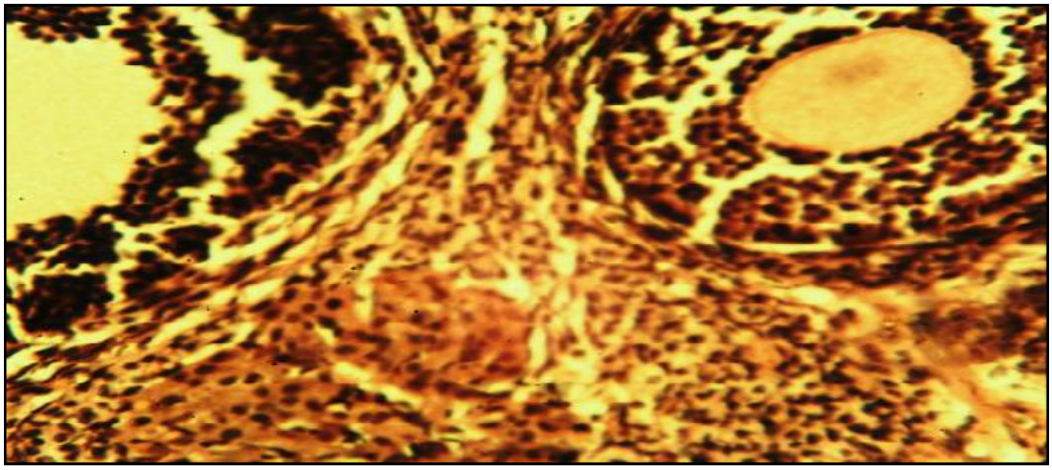

Figure 1; Micrograph of the Ovary of control rats showing the basic architecture of ovarian follicles at mature stage with numerous follicular and stroma cells x100 (H&E stain).

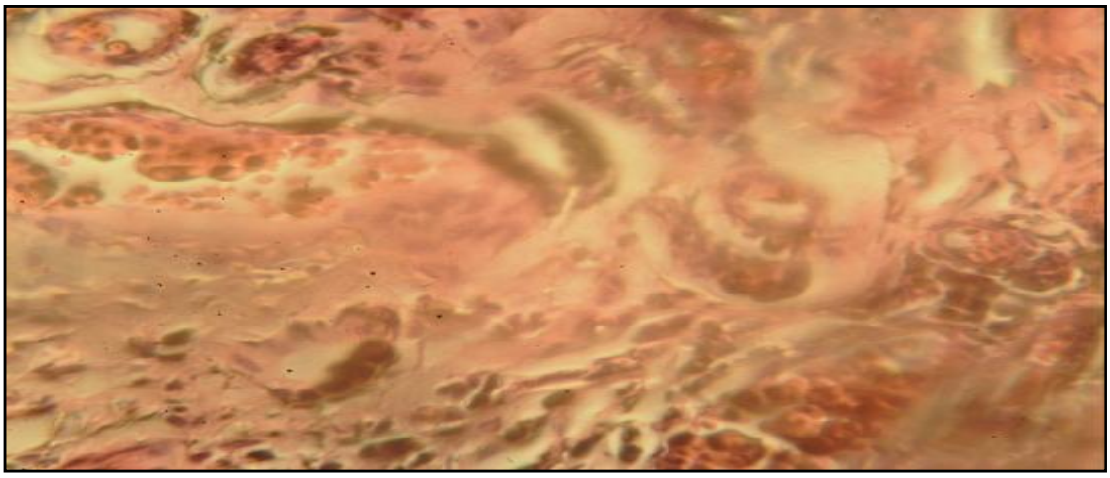

Figure 2 Micrograph of the Ovary of rats treated with 1ml/kg of lime showing degeneration of the follicular cells and stroma hyperplasia Absent of mature follicle was notice in the cortex x100 (H&E).

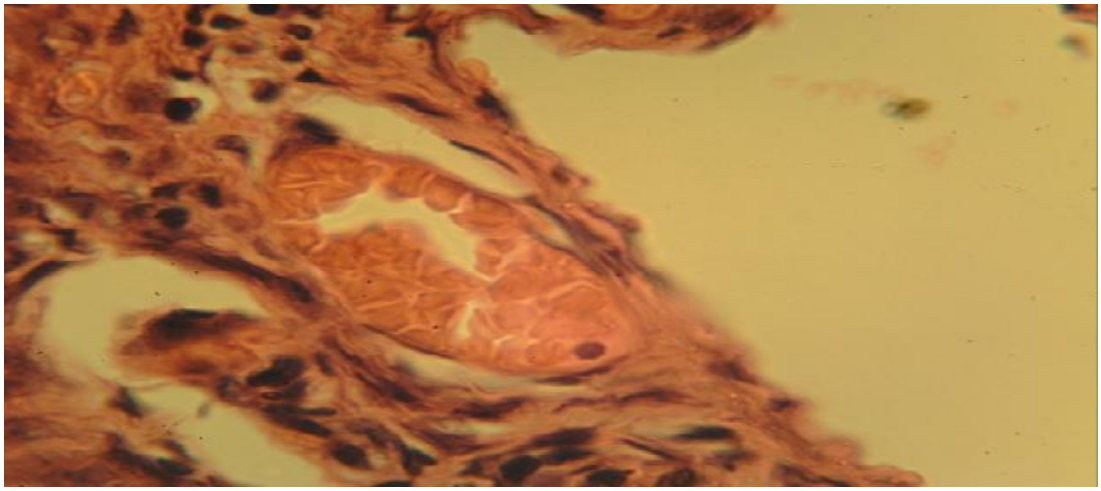

Figure 3; Micrograph of the ovary treated with 1.5 ml of Lime juice showing mass degeneration of the follicular cells and abnormal spaces were observed in the ovarian cortex (X100) H/E stain

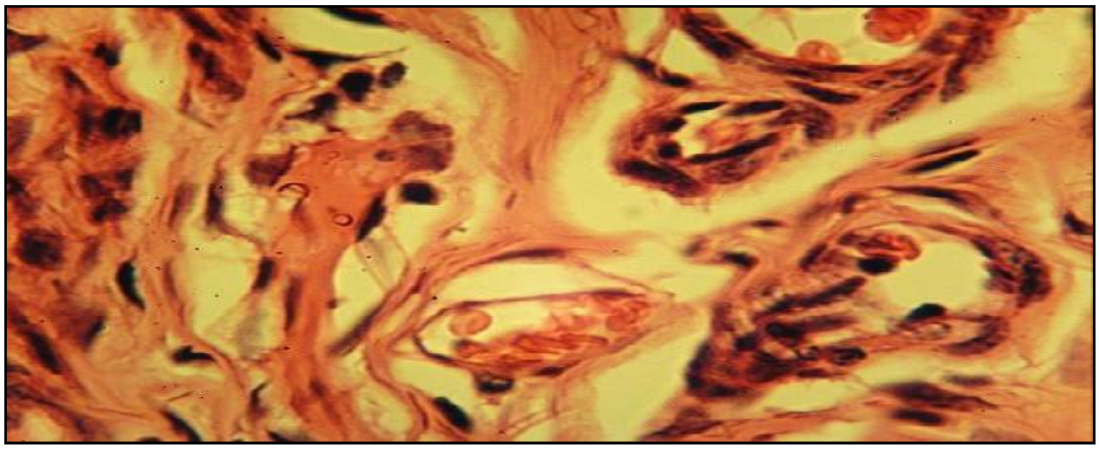

Figure 4; Micrograph showing ovary of rats treated with 2.0 mg/kg of Lime juice, indicating follicles at immature stage with degenerated follicular cells and stroma hyperplasia.

Hormone Assay

Table 1: Showing the Effect of lime juice on Plasma concentration of Reproductive Hormones

Hormone	GROUP 1 (Mean ± SEM	GROUP 2 (Mean ± SEM)	GROUP 3 (Mean ± SEM)	GROUP 4 (Mean ± SEM)
FSH	9.25±0.75	11.8±0.75	13±1.O	20.5±1.5
LH	6.75±0.8	6.25±0.25	7.5±0.5	11±1.0

$P < 0.05$ level of significant

The plasma level of FSH shows a significant reduction in the treated rats, this reduction in the level of FSH was more significant in the group 1 treated with higher dosage as shown in the Table 1. Serum level of LH also revealed a significant reduction across the group treated with the Lime juice extract in dose dependent manner as indicated in the Table above.

Discursion

Lime juice is being used by women as a barrier contraceptive, and there is a long reported history of African women douching with lime juice, lemon juice, vinegar or acidic soft drinks in the belief that it may prevent pregnancy and/ or sexually transmitted diseases (9).

This study demonstrated that undiluted lime juice alters histological architecture of ovary. The histology of the ovary of rats observed from the control group shows numerous primordial cells and mature follicles indicating a normal architecture of the ovary. All the rats in group 1 (which received 1ml/kg weight of aqueous lime juice) showed a smaller dimension in their histological sections; the ovarian follicles were not seen at different stages of maturation and the mature

(secondary) follicles were essentially absent at the periphery and also no prominent ova when compared with the rats in group 4(control group). Lime juice, under laboratory conditions, has been shown to immobilise sperm as well as advocated as a 'natural' spermicide *(14).* Spermicide is a contraceptive substance that eradicates sperm, inserted vaginally prior to intercourse to prevent pregnancy. Lime juice has also been shown to alter oestrus cycle by significantly prolonging the diestrus and oestrus phases, thus having an anti-fertility effect *(15).* Its action as natural spermicide is mainly due to high acids, this is also reflected in the degeneration of the follicular cells observed in the cortex of the ovary of the treated rats as shown in Fig.2,3 and 4 above. The undiluted lime juice of Citrus aurantifolia administered between 8.00 and 10.00 a.m. on the morning of proestrus caused irregular changes in the phases of the estrous cycles studied and similarly blocked ovulation partially as observed by *Bakare et al, 2012*, suggesting a similar mechanism of blocking the rise in luteinizing hormone during early proestrus *(15).* This is also in agreement with the work of Noronha et al, 2001 who suggested a possible mechanism of the anti-ovulatory effect of lime juice as through its anti-inflammatory property *(16)*. Ovulation has been likened to an inflammatory process *(16)*, and is therefore blocked by anti-inflammatory agents *(17)*. The anti-inflammatory property of lime juice may be responsible for its observed effect in partially blocking ovulation when administered to the rats before the expected upsurge of lutenising hormone (which causes follicular rupture and release of ova) *(17)*. *Liang et al, 1999* stated that anti-inflammatory property of flavonoids present in abundance in lime juice *(18)* can result from inhibition of cyclooxygenase enzyme *(19)*. Cyclo-oxygenase, which converts arachidonic acid to prostaglandins, has two isomers, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (20).

COX-1 is the endogenous form of the enzyme necessary for production of 9 prostaglandins while COX-2 is thought of as being an inducible enzyme associated with inflammation *(21)*. The latter is considered to be essential for the ovulatory mechanism. COX-2 deficient-mice suffer from defect in reproductive functions such as ovulation and fertilization (*21)*, underscoring the role in ovulation of COX- 2, the enzyme being suggested to be blocked by flavonoids in lime juice *(Lim et al, 1997)*.

Concerning reproductive hormonal changes in this study, a significant decrease in the concentrations of LH and FSH were recorded in Lime juice treated group compared to control group. FSH is produce from the posterior pituitary gland and is critical for follicular formation and maturation in the ovarian cortex.

The ovulatory process is initiated at the moment when follicular tissue is stimulated by a surge of pituitary gonadotropins (FSH/LH) *(15).* The pituitary surge can result in as much as a hundred-fold increase in the circulating level of luteinizing hormone. Follicle-stimulating hormone is best known for its role in follicular development and both are the principal hormones that are responsible for initiating ovulation *(15)*. These hormones significantly reduce in the treated rats; this reduction was more significant in the group treated with Lime juice as shown in the Table 1 above.

Reduction in the serum level of these reproductive hormones is implicated in the degeneration of the follicular cells observed in the histology of the ovary as shown above and consequently leading to anovulation, promoting infertility in animal following administration of undiluted Lime juice.

LH an hormone require for proliferation of the functional stratum of the endometrial layer for the receipt of fertilized ovum and also LH surge require in the ovulation of mature ovum from the cortex is significantly lowered in the rats treated with the Lime juice. This reduction in the reproductive hormones implicated in the degeneration of ovarian follicles and glandular hyperplasia of the uterus consequently promote infertility.

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