Original Research Article

Lime juice induces ovarian follicle degeneration and reduces serum gonadotrophin level in Rats (*Rattus novergicus*)

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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 Female wistar rats weighing between 160 - 190 g were randomly divided to 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 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 0.5 ml of distilled water respectively 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 as compared with the control group; 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 fertility effect of lime juice.

Key Words: Lime juice, Ovary, Wistar rats, Follicular cells, Reproductive hormones

Background: Lime juice has both medicinal and cosmetic values (1). It health benefits have been reported ranging from its skin, to its juice, and its pulp and contains various bio functional

nutrients such as flavonoids, carotenoids and ascorbic acid but the major component is the citric acid (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). There are 2 major varieties of limes, Key (Mexican) and Bears (Persian), Key Limes are small, slightly bigger than a walnut; oval and having a thin yellowish skin *(1)*. Key Limes are fragrant and extremely juicy, having a stronger and more acidic taste compared to Persian Limes *(2)*. Both Key and Persian Limes contain a higher citric acid and sugar level than lemons, with Key limes higher in acid level compared to Persian limes. *Citrus Aurantifolia* is key limes *(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 immobilized 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

92 statistically significant association between use of lime juice and lemon juice (n = 81) and the
93 presence of cervicovaginal intraepithelial neoplasia (CIN) (8).
94 In another study, reduction in body weight was noted and somewhat in agreement with
95 previous studies, which also found a reduction in body weight when overweight adults were
96 given lime juice (9). Lime juice is being used by women as a barrier contraceptive, and there is a
97 long reported history of African women douching with lime juice, lemon juice, vinegar or acidic
98 soft drinks in the belief that it may prevent pregnancy and/ or sexually transmitted diseases (9).
99 Previous studies have also that there was an irregular pattern in all phases of the estrous cycle
100 of 100% of the rats given undiluted lime juice and in 80% of those given 50% diluted lime juice
101 indicating that lime juice has an anti-fertility effect by altering the histology of the walls of the
102 uterus, prolonging one or more of the phases, reduction in the number of ova shed and
103 blocking of ovulation partially (9).
104 The present study aimed at investigating the effects of lime juice extract on the plasma
105 reproductive hormones (FSH and LH) and on histological architecture in the ovary of adult
106 wistar rats.
107 **Materials and Methods**
108 **Extract preparation;** Fresh fruits of *Citrus aurantifolia* (lime fruit) were obtained from Nyanya
109 Market in Karu, Nasarawa State, Nigeria. Authentication was done in the Biology Department,
110 Faculty of Science, Bingham University, Karu, Nigeria. The fruits were properly washed and
111 sliced into two halves each. The juice was extracted using a juice extractor. The resulting lime
112 juice was filtered through a sieve and residual pulp and seeds were discarded. Lime juice of fifty
113 lime fruits was processed in this manner, pooled and collected into a clean plastic bottle,

114 covered and refrigerated (-4°C) throughout the course of the experiment to prevent
115 fermentation.

116 **Experimental animals**; Twenty (20) Adult Female rats weighing between 160 – 190 g were
117 procured from the animal house of the Nigerian Institute for Trypanosomiasis and
118 Onchocerciasis, Kaduna Nigeria. The ethical approval on animal act right was obtained from the
119 Institutional Animal Care Committee of Bingham University, Karu, Nigeria. They were kept in
120 laboratory for two weeks of acclimatization and were fed on standard diet (Vital Feeds and
121 Grand Cereals Ltd); food and water were given *ad libitum* and maintained under standard
122 conditions. The animal room was well ventilated with a temperature range of 25-27^{o}C under
123 day/night 12-12 h photoperiodicity.

124 **Experimental procedure;** A Total of twenty rats were used in this experiment and were
125 subdivided into 4 Groups 1, 2, 3 and 4, (n=5). The animals in Group 1 received 1ml/kg body
126 weight of undiluted lime juice; Group 2 received 1.5/kg body weight of undiluted lime juice,
127 Group 3 received 2.23ml/kg body weight of undiluted lime juice, Group 4 received distilled
128 water only. Administration was done by gavages oro-gastrically daily using metal canula at 0900
129 hours for period of ten days respectively (15)

130 **Animal sacrifice;** Animals were sacrificed by cervical dislocation 24 hours after the last
131 administration of undiluted lime juice; ovary was excised following abdominal incision, fixed in
132 10 % Formo-saline for histological observation using H/E stain while blood samples were
133 collected from descending aorta for hormonal assay.

134 **Analytical Procedure:**

Histological analysis: Ovaries 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

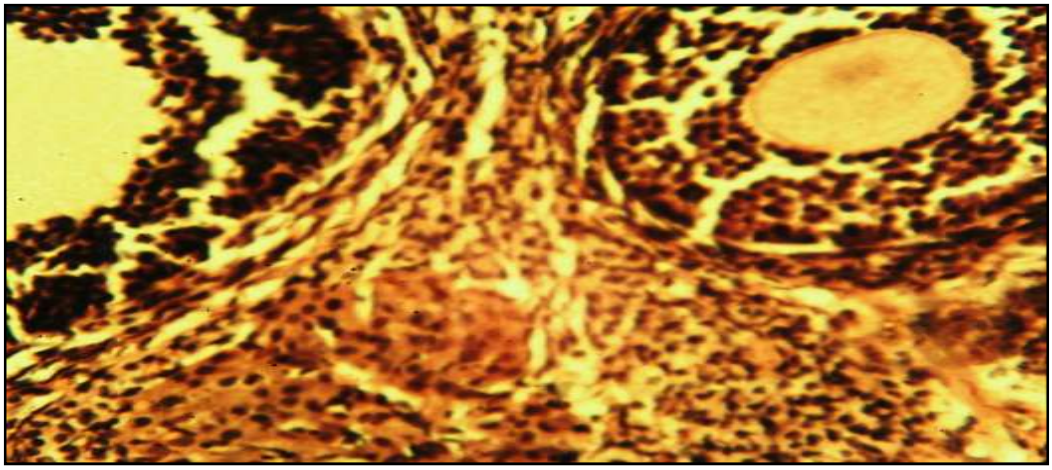

Figure 1; Micrograph of the ovary of the control rats showing the basic architecture of ovarian follicles at mature stage with numerous follicular and stroma cells x100 (H&E stain).

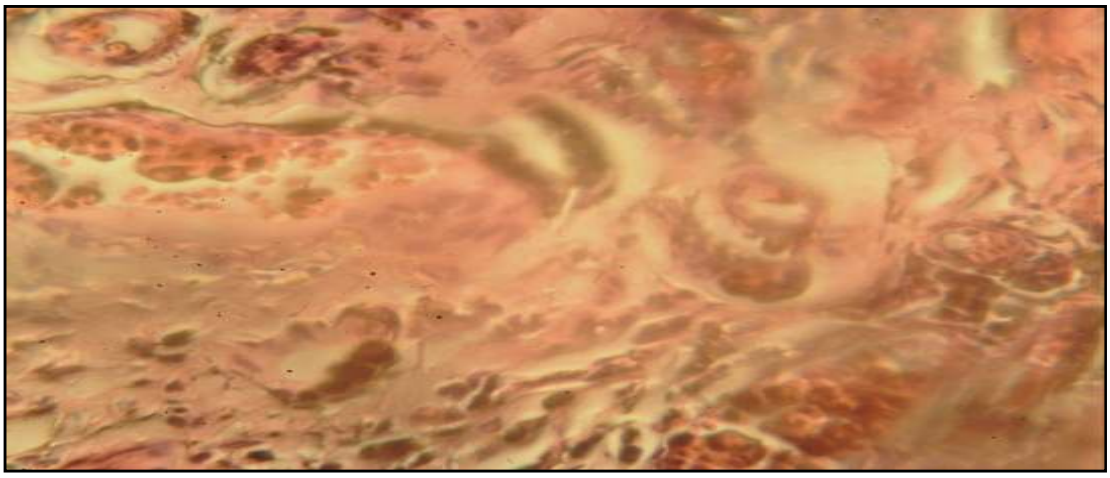

Figure 2 Micrograph of the Ovary of rats treated with 1ml/kg of lime showing degeneration of the follicular cells and stroma hyperplasia absent of mature follicle was noted in the cortex x100 (H&E).

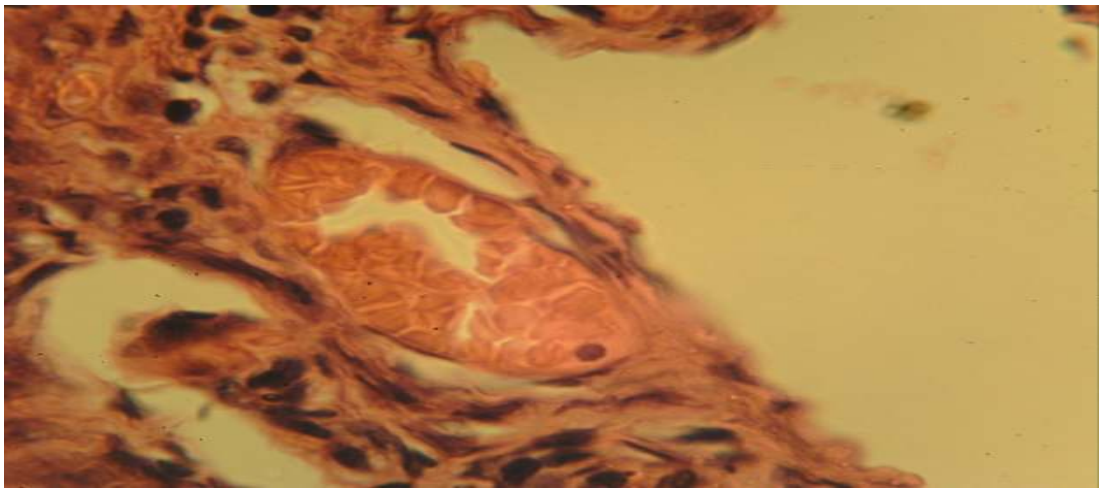

Figure 3; Micrograph of the ovary treated with 1.5 ml of lime juice showing mass degeneration of the follicular cells and abnormal spaces were observed in the ovarian cortex (X100) H/E stain

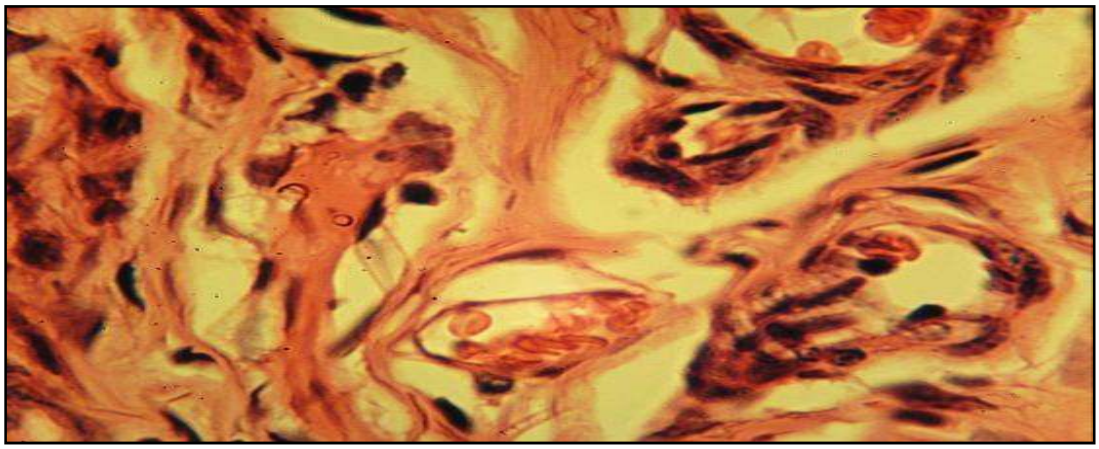

Figure 4; Micrograph showing ovary of rats treated with 2.0 mg/kg of lime juice, indicating follicles at immature stage with degenerated follicular cells and stroma hyperplasia.

Hormone Assay

Table 1: 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 (ng/mol)	9.25±0.75	11.8±0.75	13±1.0*	20.5±1.5*
LH (ng/mol)	6.75±0.8	6.25±0.25	7.5±0.5	11±1.0*

$P < 0.05$ level of significant,
* Significant difference

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 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 Table 1.

Discussion

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).

The present 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 Figs.2,3 and 4. 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 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).

The COX-1 is the endogenous form of the enzyme necessary for production of 9 prostaglandins while the 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 *(21)*.

Concerning reproductive hormonal changes in the present study, a significant decrease in the concentrations of LH and FSH were recorded in lime juice treated group compared to control group. The FSH is produced from the anterior 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.

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 previously and consequently leading to anovulation, promoting infertility in animal following administration of undiluted lime juice.

The LH hormone is required for proliferation of the functional stratum of the endometrial layer for the receipt of fertilized ovum and also LH surge is required in the ovulation of mature ovum from the cortex is significantly lowered in the rats treated with the lime juice than the control. 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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