Original Research Article

Lime juice induces ovarian follicle degeneration and reduces serum gonadotrophin level in Rats Model

Dare .B.J*, Beremako .O, Omotoso O.D., Olaniyan O.T[a]., Akinrinade I.D., Shafe M.O[a]. Ifeonu .S.C[b]

Department of Anatomy, Bingham University, P.M.B 005 Karu, Nigeria
Department of Physiology, Bingham University, P.M.B 005 Karu, Nigeria[a]
Department of Biochemistry, Bingham University, P.M.B 005, Karu, Nigeria[b]

Corresponding author:
Email addresses:
DBJ: jdare_tolu@yahoo.com,

ABSTRACT

Background: Lime juice possesses antimicrobial and anti-proliferative property and caused reduced sperm motility in animals. This study aimed at investigating the anti-proliferative effect of lime juice on the ovarian cortex of adult Wistar rats.

Methods: Twenty (20) adult Female Wistar rats weighing between 160 - 190 g were divided into 4 groups (n= 5). Group 1, 2 and 3 received 1ml/kg, 1.5 ml/kg and 2.23 ml/kg body weight of undiluted lime juice respectively; Group 4 received 0.5 ml of distilled water 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 sample was collected for hormonal (reproductive hormones) assay.

Results: Plasma concentration of FSH and LH significantly ($p < 0.05$) lowered in the lime – treated rats compared with control rats, histological observation revealed degeneration in the follicular cells, stroma hyperplasia and immature follicles in the animals treated with the undiluted lime juice compared with the control group; that revealed follicular cells at different stages of development.

Conclusions: Low plasma concentration of FSH and LH observed with consequent degeneration of follicular cells expressed in the ovarian cortex demonstrate anti fertility potential of lime juice.

Key Words: Lime juice, Ovary, Wistar rats, Follicular cells, Reproductive hormones (Follicle stimulating and Luteinizing hormones)

Background: Lime juice 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 *(1, 2).* Lime juice 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 have thin yellowish skin *(1)*. Key limes are fragrant and extremely juicy; possess 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; *''aurantifolia''* is regarded as the key limes *(3)*

Lime juice 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 caused reduction in sperm motility and has also been shown to alter estrus cycle by significantly prolonging the diestrus and estrus phases, thus exhibiting anti-fertility potential on animals. Lime juice can result in mild and transient side effects; including vaginal dryness, itching and burning (6).

Women that were douched in vaginal with lime juice expressed significantly high levels of pro-inflammatory cytokines (IL-1, IL-6, and IL-8) and increased numbers of ($CD45^{+}$) leukocytes, an indication of a mucosal inflammatory response (7). Furthermore, study revealed statistically significant association between use of lime juice and lemon juice and the presence of cervicovaginal intraepithelial neoplasia (8).

In another study, reduction in body weight was noted when overweight adults were given lime juice (9). Lime juice is being used by women as a barrier contraceptive relative to lemon juice, vinegar or acidic soft drinks; in the aim to prevent pregnancy and sexually transmitted diseases (9). Irregular pattern was observed 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 confer anti-fertility effect by altering the histology of the endometrial lining, prolonging one or more of the phases, reducing the number of ova shed and partially obstruction of ovulation (9). The present study aimed at investigating the effects of lime juice extract on the plasma reproductive hormones (FSH and LH) and on histological architecture in the ovary of adult ''Wistar'' rats.

Materials and Methods

Extract preparation; Fresh fruits of *Citrus aurantifolia* (lime fruit) were obtained from Nyanya Market in Karu, Nasarawa State, Nigeria. Authentication was done in 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; filtered through a sieve and the residual pulp and seeds were discarded. Lime juice of fifty lime fruits was processed, pooled and collected into a clean plastic bottle, covered and refrigerated (-4°C) throughout the course of the experiment to prevent fermentation.

Experimental animals; Twenty (20) Adult Female rats weighing between 160 – 190 g were procured from the Nigerian Institute for Trypanosomiasis and Onchocerciasis, Kaduna Nigeria. Principles of Laboratory Animal Care (NIH Publication No. 85-23, Revised 1985) Were Followed, As Well As Specific National Laws Where Applicable. All Experiment methods were Examined

and Approved by Institutional Animal Care Committee of Bingham University, Karu, Nigeria. Animals were kept in laboratory for two weeks of acclimatization and fed on standard diet (Vital Feeds and Grand Cereals Ltd); food and water were 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.

Experimental procedure; A Total of twenty (20) rats were used in this experiment and were subdivided into 4 Groups (n=5). The animals in Group 1 received 1 ml/kg body weight of undiluted lime juice; Group 2 received 1.5ml/kg body weight of undiluted lime juice, Group 3 received 2.23 ml/kg body weight of undiluted lime juice, Group 4 received 0.5 ml of distilled water only. Administration was done by gavages oro-gastrically daily using metal canula at 0900 hours for period of ten days respectively (15)

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 samples were collected from descending aorta for Hormonal assay.

Analytical Procedure:

Histological analysis: Ovaries were carefully dissected out following abdominal incision; fixed in 10% formo-saline for tissue processing using Hematoxylin and Eosin Stalin (H / E) according to the method described by Akpantah *et al*., 2003 (10) and sections were observed microscopically.

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

Statistical Analysis; SPSS-V11 statistical software package 13 for analysis of the data was used and statistical analyses was carried out using the Student's t-test and a value of ''P= .05''was taken as significant.

Result:

Histological Observations

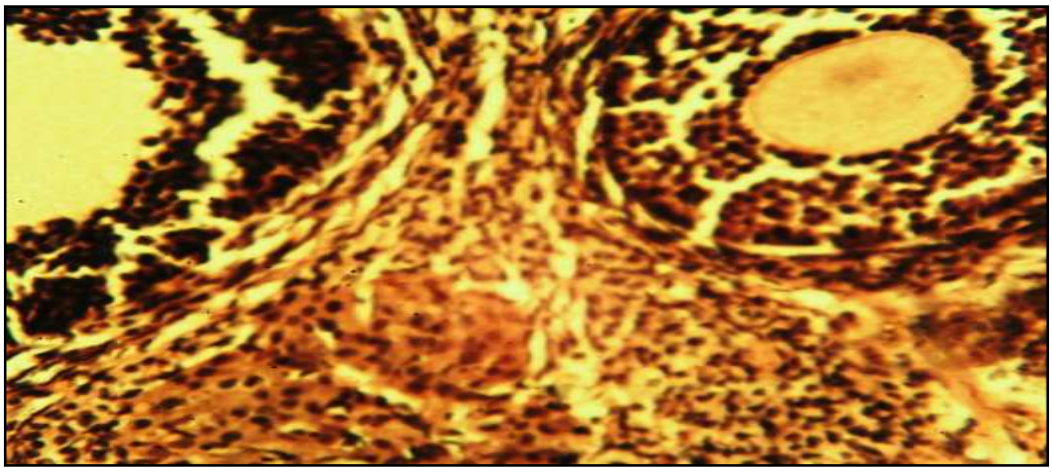

Figure 1; Micrograph of the ovary of the control rats showing the basic architecture of ovarian follicles at mature stage with numerous follicular and stroma cells x100 (H&E stain).

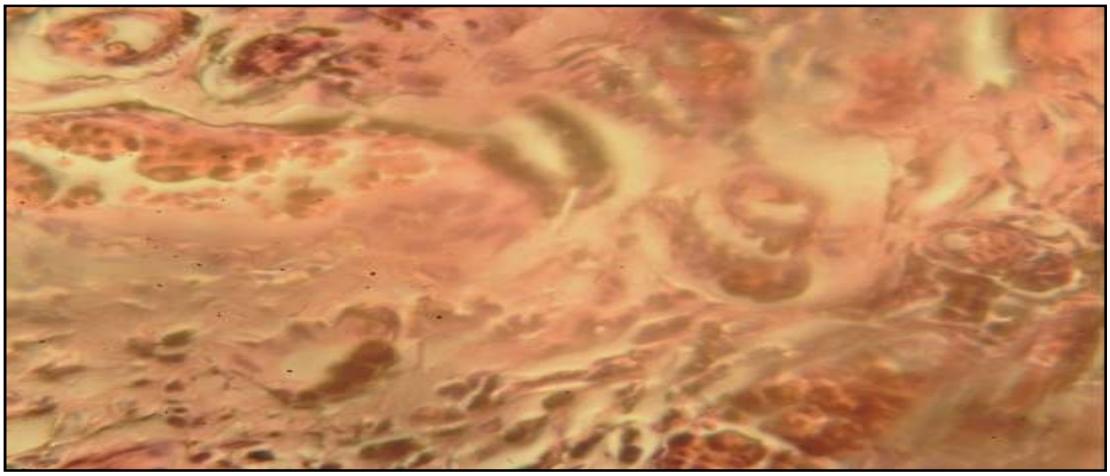

Figure 2 Micrograph of the Ovary of rats treated with 1ml/kg of lime showing degeneration of the follicular cells and stroma hyperplasia, absent of mature follicle was noted in the cortex x100 (H&E).

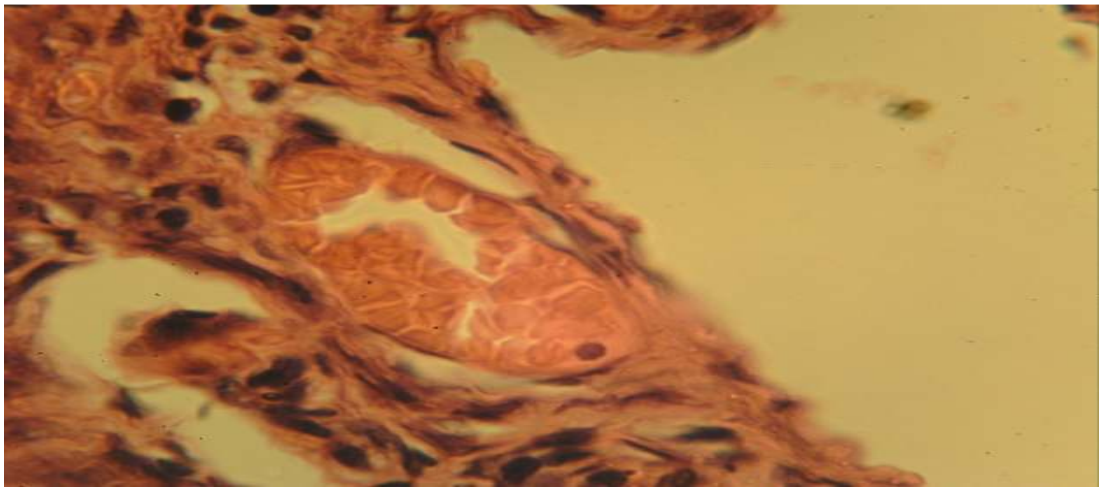

Figure 3; Micrograph of the ovary treated with 1.5 ml of lime juice showing mass degeneration of the follicular cells and abnormal spaces were observed in the ovarian cortex (X100) H/E stain

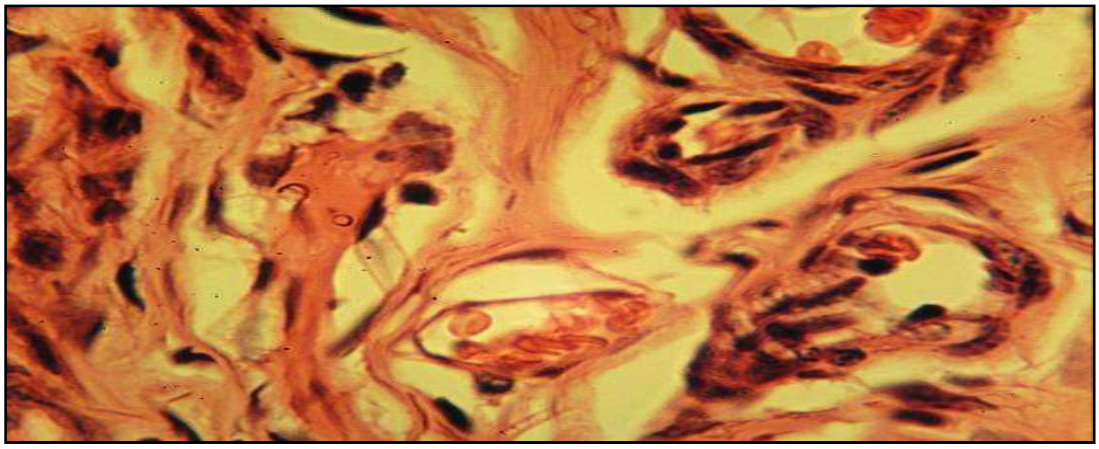

Figure 4; Micrograph showing ovary of rats treated with 2.0 mg/kg of lime juice, indicating follicles at immature stage with degenerated follicular cells and stroma hyperplasia.

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 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 significant reduction across the group treated with the lime juice extract in dose dependent manner.

Discussion

Lime juice has contraceptive property, therefore women douched in lemon juice, vinegar or acidic soft to 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 has been described as a natural spermicide; a contraceptive substance that reduces sperm concentration to prevent pregnancy *(14)*, lime juice also alters oestrus cycle by significantly prolonging the diestrus and oestrus phases, thus exert 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* caused irregular changes in the phases of the estrous cycles and blocked ovulation partially as observed by *Bakare et al*, 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,* who worked on anti-inflammatory property of lime *(16)* whereas; ovulation has been likened to an inflammatory process 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

171 before the expected upsurge of luteinizing hormone (which causes follicular rupture and
172 release of ova) *(17)*. *Liang et al, 1999* stated that anti-inflammatory property is present in
173 abundance in lime juice *(18)* and can result from inhibition of cyclooxygenase enzyme *(19)*.
174 Cyclo-oxygenase, an enzyme that converts arachidonic acid to prostaglandins, has two isomers,
175 cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2) (20). The COX-1 is the endogenous
176 form of the enzyme necessary for production of prostaglandins while the COX-2 is thought of as
177 being an inducible enzyme associated with inflammation *(21)*. COX-2 is considered to be
178 essential for the ovulatory mechanism. COX-2 deficient-mice suffer from defect in reproductive
179 functions such as ovulation and fertilization (*21)*, underscoring the role in ovulation of COX- 2,
180 the enzyme being suggested to be blocked by flavonoids in lime juice *(21)*.
181 Concerning reproductive hormonal changes in the present study, a significant decrease in the
182 concentrations of LH and FSH were recorded in lime juice treated group compared to control
183 group. The FSH is produced from the anterior pituitary gland and is critical for follicular
184 formation and maturation in the ovarian cortex.
185 The ovulatory process is initiated at the moment when follicular tissue is stimulated by a surge
186 of pituitary gonadotropins (FSH/LH) *(15).* The pituitary surge can result in as much as a
187 hundred-fold increase in the circulating level of luteinizing hormone. Follicle-stimulating
188 hormone is best known for its role in follicular development and both are the principal
189 hormones that are responsible for initiating ovulation *(15)*. These hormones significantly reduce
190 in the treated rats; this reduction was more significant in the group treated with lime juice as
191 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.

In conclusion, alteration in the follicular differentiation and development in the ovarian cortex as a result of the reduction in serum level of gonadotrophin following the administration of Lime juice extract compromise fertility in animals

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