

Full Length Research Paper

Lime Juice (*Citrus aurantifolia*): Effect on fetal parameters of pregnant Sprague-Dawley rats

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Objective: The aim of this study was to determine the effect of lime juice on the fetal parameters of Sprague-Dawley rats

Materials and Methods: Forty adult female Sprague-Dawley (SD) rats were used. They were randomly divided into 2 groups (I and II) of 10 rats each. The estrous cycles of the rats were studied for the first 16 days to establish cyclicity. The rats were mated with male SD rats of proven fertility on the estrous day (heat period) of estrous cycle. Rats in group I received 1ml of undiluted lime juice while rats in group II received distilled water by gastric gavage. The rats were sacrificed on the 20th day of gestation using chloroform anesthesia and fetal parameters were evaluated.

Results: There was a reduction in the number of fetus of treated pregnant rats when compared to the control which has between eight to eleven litters. There was a significant reduction in the crown-rump length, weight and umbilical cord length of the fetus when compared with the control which was normal.

Conclusion: Lime juice showed abortifacient effect but no obvious teratogenic effect was observed.

Key Words: Lime juice, Ovulation, Estrous cycle

INTRODUCTION

Lime (*Citrus aurantifolia*) juice has been shown to have both medicinal and cosmetic values.

Studies have shown that lime juice destroys both human immunodeficiency virus (HIV) and sperm cells (1-3). Ten percent of lime juice produced a 1000-fold reduction in HIV activity in a laboratory sample, while fifty percent of the lime juice wiped out 2000 of sperm cells in 30 seconds. The high acidity of lime juice is probably responsible for this destruction (1-3).

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 (4). In fact, lemon and lime juice are widely used for douches among women at high risk of HIV transmission

in Central Nigeria (5). Potentially, to be effective against HIV *in vivo*, women would need to apply a volume of neat lime juice equal to that of an ejaculate, and maintain this ratio vaginally for 5 to 30 minutes after ejaculation. However, data have suggested that this would have significant adverse effects on the genital mucosa, raising serious questions about the plausibility and safety of such a preventive approach (6).

Lemon and lime juices have been reported to exhibit antimicrobial activity against *Vibrio* strains (7,8). The *in vitro* effects of concentrated lime juice extract on the spontaneous proliferation of a human breast carcinoma cell line (MDA-MB-453) and a human lymphoblastoid B cell line (RPMI-8866) have been investigated and the results suggested that the juice may have anti-proliferative effects on tumor cell lines (9).

Citrus is a genus of flowering plants in the family Rutaceae (orange family) and a common name for edible fruits of this genus and sometimes related genera. Citrus fruits are a distinctive berry with the internal parts divided into segments and include oranges, lemons, limes,

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citrons, grapefruit, pomelos, and mandarins (tangerines). Citrus is likely the most widely planted fruit for direct human consumption in the world. The juice contains a high quantity of citric acid giving them their characteristic sharp (tart) flavor. Citrus fruits are notable for their fragrance, partly due to flavonoids and limonoids contained in the rind, and most are juice-laden.

Lime tree is a small shrub ranging from 3.5 to 9 m in height and 2.5 to 7.5 m in width. The fruit is typically round, green to yellow in color and about 3-6 cm in diameter (10).

This present study was carried out to investigate the effects of lime juice on fetal parameters of Sprague-Dawley rats, being a plant (*Citrus aurantifolia*) that is widely available in all most every part of the world.

MATERIALS AND METHODS

Plant material

Fresh lime fruits were harvested in Mushin area of Lagos, Nigeria in July. The fruits were authenticated at the Department of Botany of the University of Lagos, Nigeria. The fruits were properly washed and sliced into two halves each, which was then squeezed gently. The resulting lime juice was filtered through a Whatman number 1 paper, and residual pulp and seeds were discarded. The lime juice of twelve lime fruits was processed in this manner, pooled and collected into a clean plastic container, covered and stored in the refrigerator (-4°C) for later use. The pH of the pooled limed juice was 2.4.

Animal Material

Adult female Sprague-Dawley rats, 6-8 weeks old, weighing 150 - 170 g were obtained from the Animal House of the Department of Anatomy, Ladoke Akintola University of Technology, Ogbomosho, Oyo state, Nigeria, and were authenticated at the Department of Zoology, University of Lagos, Nigeria. A total of 25 rats were used for this research work.

The rats were labeled by ear puncture, kept in well ventilated metal cages (5 animals in each cage) and were allowed to acclimatize for a period of 14 days. The rats then went through a recruitment phase of 16 days during which rats that met our inclusion criteria were selected.

Inclusion criteria include cyclicity of vagina smear, at least three consecutive regular estrous cycles and apparent good state of health. The animals had free access to clean tap water and pellets. The cages were kept in the rat control room of Anatomy Department, University of Lagos, where the ambient temperatures ranged between 28 and 31°C. 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.

Experimental Procedure

This Experiment was designed to determine the effect of lime juice on the fetal parameters of pregnant Sprague-Dawley rats. The cycle phases was determined from the cytology of vaginal smears obtained daily between 8.00 a.m. and 10.00 a.m. Briefly, 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 then suctioned into the tip of the pipette. The smear was placed on glass slide and examined under the light microscope immediately before drying up. A total of 40 rats were used in this experiment and were randomly subdivided into 2 groups (I and II) with 10 rats in each of another repeated experiment. The rats in each group had a 4-day estrous cycle. The first day of the estrous cycle designated as the diestrus phase, showed predominance of leukocytes and a few large nucleated cells. The second day showed large nucleated cells with the leukocytes, designated as the proestrous phase. The third day designated the estrous phase showed large flakes of squamous cells with small pyknotic nuclei. The metestrous, the fourth day of the estrous cycle, showed leukocytes amidst remnants of large squamous cells with pyknotic nuclei. The body weight of the rats were measured and recorded daily. The rats were mated with male Sprague-Dawley rats of proven fertility on the estrous day (heat period) of estrous cycle. The presence of copulatory vaginal plug on the floor of the cage and/or the presence of sperm cells in fresh vaginal smear the next morning was use to ascertain successful mating and this day was taken as the first day of gestation. Group I rats received 1 ml of aqueous lime juice and Group II rats were administered distilled water (control). The rats in both groups were sacrificed on the 20th day of gestation using chloroform anesthesia. The rats were dissected and the number of fetuses was counted, their weight, crown-rump length and umbilical cord length were measured and recorded. External features of the rats were also examined for possible abnormalities.

Both the aqueous juice and distilled water were administered by gastric gavage to groups I and II respectively. All animals were observed for clinical signs of drug toxicity (such as tremors, weakness, refusal of feeds, diarrhea, weight loss, hair-loss, coma and death) throughout the duration of the experiment. Food and water intake were determined by measuring the differences between the amount supplied daily and the daily leftovers in the cages.

All procedures involving animals in this study conformed to the guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (11)

Table 1: Effect of Aqueous Lime Juice on Number of Fetuses, Crown-Rump Length, Fetal Weight and the Umbilical Cord Length of Pregnant Sprague-Dawley rats

| GROUPS | NUMBER OF FETUSES | CROWN-RUMP LENGTH (CM) | FETAL WEIGHT (CM) | UMBILICAL CORD LENGTH (CM) |
|--------|-------------------|------------------------|-------------------|----------------------------|
| I | 5.12±0.5 | 2.97±0.10 | 2.89±0.33 | 2.89±0.35 |
| II | 8.00± 2.23 | 3.37±0.11 | 3.45±0.34 | 3.22±0.43 |

Significant difference from value of control ($p < 0.05$)

Table 2: Effect of Aqueous Lime Juice on Body Weight of Female Sprague-Dawley rats

| BODY WEIGHT (G) | EXPERIMENTAL (I) | CONTROL (II) |
|-------------------|------------------|----------------|
| BEFORE EXPERIMENT | 174± 15.21 | 164.22 ± 18.39 |
| AFTER EXPERIMENT | 150 ± 21.64 | 177.59 ± 18.74 |

Significant difference from value of control ($p < 0.05$)

and were approved by the Departmental Committee on the Use and Care of Animals.

Statistics

Results were expressed as Mean ± Standard Deviation (SD) and subjected to statistical analysis using one-way analysis of variance (ANOVA) and Scheffe's post-hoc test. The significance level considered was $p < 0.05$.

RESULTS

The number of fetuses of the rats in group I (which received aqueous lime juice) were reduced when compared to the control, group II (Figure 1 and 2). There was also a significant reduction in the crown-rump length, fetal weight and umbilical cord length of the fetus when compared with the control (Table 1). There was vaginal bleeding at the later period of extract administration in the treated group. It was also observed that all the fetuses were alive and had no external gross abnormalities.

All the rats used in this study were alive throughout the duration of the experiment. The rats treated with lime juice, showed a statistically significant difference in their mean body weight at the end of the experiments when

compared to the control (Table 2), though there were no significant differences in food and water consumption of the treated and control rats.



Figure 1: Photograph of control fetuses (20th day of gestation)

DISCUSSION

This study demonstrated that aqueous lime juice has abortifacient effect by significantly reducing the number of

fetuses of treated rats. The experimented rats were pregnant with four fetuses as shown on the labeled photograph and the control have eight fetuses which confirmed that other implanted embryo were aborted in the treated rats, thereby confirming its abortifacient effects (12). There was a statistical significant reduction in body weight of experimented rats treated with lime extract when compared with the control which showed a significant increase in body weight probably due to normal feeding habit. The weight and size of the fetuses of the experimented rats also confirms the result of lime juice effect on body weight. The exact reason for this observation is not too clear. However, this is somewhat in agreement with previous studies that showed a reduction in body weight when overweight adults were given lime juice (13, 14). In addition, in the present study, there was a tendency to regain weight when the juice was discontinued. Though this study was carried out in rat, it can be applied to the human since the mechanism of

pregnancy (the central theme of gestation) is quite similar in the two. A cascade of similar events (in both rat and human) drives pregnancy, initiated upon by upsurge of progesterone for the maintenance of normal pregnancy.



Figure 2: Photograph of experimental fetuses (20th day of gestation)

CONCLUSION

The results of this investigation have demonstrated that lime juice has abortifacient effects in animals which can be further explored. We therefore recommend that lime should not be consumed during pregnancy.

REFERENCES

- American Physiological Society. Guiding principles for research involving animals and human beings. *Am. J Physiol Regul Integr Comp Physiol.* 2002;283:R281-R283.
- Brown D (1995). The herb society of America- New Encyclopedia of Herbs and their uses. Darling, Kingsley Limited, London,.
- Colker CM, Kalman DS (1999). Effect of Citrus aurantifolia extract, caffeine and St John's wort on body fat loss, lipid levels and mood states in overweight healthy adults. *Current Therap Res.*,60:145-153.
- De Castillo MC, De Allori CG, De Gutierrez RC, De Saab OA, De Fernandez NP, De Ruiz CS, Holgado AP, De Nader OM. Bactericidal activity of lemon juice and lemon derivatives against *Vibrio cholerae*. *Biol Pharm Bull.* 2000; 23(10): 1235-1238.
- Fletcher PS, Harman SJ, Boothe AR, Doncel GF, Shattock RJ (2008). Preclinical evaluation of lime juice as a topical microbicide candidate. *Retrovirol.*;5:3.
- Gharagozloo M, Doroudchi M, Ghaderi A (2002). Effects of Citrus aurantifolia concentrated extract on the spontaneous proliferation of MDA-MB-453 and RPMI-8866 tumor cell lines. *Phytomedicine.*9:475-477.
- Imade GE, Sagay AS, Onwuliri VA, Egah DZ, Potts M, Short RV (2005). Use of lemon or lime juice douches in women in Jos, Nigeria. *Sex Health.*,2:237-239.

- Michaud JP (1999). Aggregation by alatae of *Toxoptera citricida* (Homoptera: Aphididae) *Environ Entomol.*,28: 205-211.
- Moro C, Basile G (2000). Obesity and medicinal plants. *Fitoterapia.* (Supplement 1); s73-s78
- Preuss HG, DiFerdinando D, Bagchi M, Bagchi D (2002). *Citrus aurantifolia* as a thermogenic, weight-reduction replacement for ephedra: an overview. *J Med.*;33:247-264.
- Roger A, Short A (2000). Protection from cholera by adding lime juice to food- results from community and laboratory studies in Guinea-Bissau, West Africa. *Trop Med Int Health.*, 5:418-422.
- Roger A, Short A (2000). Protection from cholera by adding lime juice to food- result from community and laboratory studies in Guinea-Bissau, West Africa. *Int. Health*; 5(6):418-419.
- Siddiqi RP, Tricker AR, Preussmann R (1988). Formation of N-nitrous compounds under simulated gastric condition from Kashmir foodstuffs. *Concert Lett.*,39:259-265.
- Tomotake H, Koga T, Yamato M, Kassu A, Ota F (2006). Antibacterial Activity of Citrus Fruit Juices Against *Vibrio* Species. *J NutrSci Vitaminol.*;52:157-160.