International Journal of Health Sciences and Research
Vol.11; Issue: 2; February 2021
Website: www.ijhsr.org
Original Research Article *ISSN: 2249-9571*

Effects of *Citrus limon* Linn Juice on Reproductive Functions in Female Wistar Rats

T. T. Ahmed[1], O. T. Kunle-Alabi[2], O. O. Akindele[3], Y. Raji[4]

[1,2,3,4]Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan, Ibadan, Oyo, Nigeria.

Corresponding Author: T. T. Ahmed

ABSTRACT

Citrus limon Juice (CLJ) is used as a therapeutic agent due to its hypolipideamic property. It is commonly used by women as a vaginal douche. However, it is acclaimed to be used as a form of contraceptive in women. Thus, the effects of *Citrus limon* juice on reproductive functions in female Wistar rats were investigated. Twenty animals divided into four groups (n=5) were used for the study; Group I (control) received distilled water. Group II-IV received 1 ml/kg bodyweight of 50%, 75% and 100% CLJ respectively for 3 weeks. Oestrus cycle length and phase frequency, serum follicle stimulating hormone, luteinizing hormone and oestrogen levels, as well as histology of liver, ovary and uterus were assessed. Data were analysed using ANOVA and Student's t-test where appropriate at $P< 0.05$. Oestrus length reduced in 75% CLJ group. The frequency of Proestrus and oestrus reduced in all test groups. The frequency of metestrus increased in 75% CLJ group. Diestrus frequency increased in all test groups. Luteinizing hormone increased in 75% CLJ group. Serum level of oestrogen increased in 50% and 75% CLJ groups. Histology of the ovaries showed reduced number of follicles and degeneration of uterine glandular epithelia cells. It was therefore deduced from this study that administration of *Citrus limon* juice may be associated with reduced reproductive capability.

Keywords: *Citrus limon* juice, Oestrus cycle, Luteinizing hormone, Follicle stimulating hormone

INTRODUCTION

The use of plants in folkloric medicine for the treatment of illnesses and diseases is common [1]. This is due to the fact that they are relatively available, inexpensive and compatible with human body systems [2]. *Citrus limon* fruits, commonly known as "lemon" are widely cultivated and consumed globally [3]. *Citrus limon* juice (CLJ) is a rich source of vitamins and minerals such as vitamin A, vitamin C, potassium and calcium, folate, citric acid and essential oils [4]. The juice contains numerous phytochemicals, including flavonoids, polyphenols and terpenes [5].

Administration of CLJ has been shown to effectively prevent the development of urolithiasis in male rats [6]. Also, an *in vitro* study suggests the juice possesses spermicidal property by causing sperm immobilization [7]. The CLJ is used by women of reproductive age for vaginal douching [8, 9] and weight loss due to its hypolipideamic property [10]. In some Nigerian traditional societies, women of child bearing age are advised against the intake of CLJ if they want to conceive because it is believed to have contraceptive effect [11]. However, there is dearth of information on the actual effects of *Citrus limon* juice on female reproductive functions. Thus this study was aimed to evaluate the effects of *Citrus limon* juice on the reproductive functions in female Wistar rats.

MATERIALS AND METHOD

Ethical approval was obtained from the University of Ibadan Animal Care and Use Research Ethics Committee, with voucher number UI-ACUREC/19/0014 and the experimental protocols and procedures in the study were conducted according to the guidelines of animal protection and welfare of the University of Ibadan, Nigeria and according to International Guide for the Care and Use of Laboratory Animals [12].

Identification and preparation of *Citrus limon* juice

Fresh fruits of Citrus *limon* were obtained from a farm in Ibadan, the fruits were authenticated at Forestry Research Institute of Nigeria (FRIN), with voucher number 110938. Phytochemical screening was carried out on the juice using established protocols as described by Harborne [13].

The fruits were washed and the juice was expressed from the halved fruits into a glass beaker. The expressed juice was filtered using a clean sieve and the filtrate was collected into clean bottles. The undiluted (100%) CLJ was diluted with distilled water to obtain 50% and 75% *Citrus limon* juice The filtrate was administered to the animals in different groups at a dose of 1 mL/kg of respective concentrations orally [14].

Experimental Animals

Twenty female Wistar rats (120 -150 g) were used for the study. They were housed in the Postgraduate Animal House, Department of Physiology, College of Medicine, University of Ibadan, in aerated plastic cages and had access to rodent's pelletized feed (Ladokun feed, Ibadan) and water *ad libitum.* All animals were acclimatized to the environmental condition of the animal house for two weeks before commencement of the study.

Experimental protocol

The animals were divided into four groups of five animals each as follows:

Group I (Control) - received 1 mL/kg of distilled water
Group II (50% CLJ) - received 1 mL/kg of 50% *Citrus limon* juice
Group III (75% CLJ) -received 1 mL/kg of 75% *Citrus limon* juice
Group IV (100% CLJ) -received 1 mL/kg of 100% *Citrus limon* juice

The administration was done orally using an oral cannula for a period of three weeks. Prior to commencement of administration, oestrus cycle was monitored daily for a period of two weeks to determine the baseline cycle rhythm and also during the period of administration using the Marcondes technique [15].

At the end of three weeks of treatments, the rats were sacrificed at the proestrus phase of their oestrus cycle under sodium thiopentone anaesthesia (50 mg/kg, i.p.) [16]. Blood samples were collected from the heart into plain sample bottles and centrifuged at 3500 rpm for 10 minutes to obtain the serum. The ovary, uterus and liver of each animal were harvested, freed from adherent fat and weighed on a sensitive weighing balance (Lisay, China). Relative organ weights were calculated as percentage of body weight for each animal. The ovary, uterus and liver were then fixed in 10% formalin for histological examination. Serum level of follicle stimulating hormone, luteinizing hormone and oestrogen were determined using ELISA kits (Calbiotech Inc., Spring Valley, USA). All the tests were carried out according to manufacturer's instructions.

Statistical analysis

Data were expressed as mean ±Standard Error of Mean (SEM) and the differences in means were compared by analysis of variance (ANOVA) and Student's t-test where appropriate. $P<0.05$ was considered statistically significant. Graphpad prism 7.01 (Graphpad software, Inc, U.S.A.) was used to analyse the data.

RESULTS

Phytochemical screening of *Citrus limon* juice

Phytochemical screening revealed presence of saponins, flavonoids, anthraquinone, alkaloids, phenols and terpenes (Table 1).

Table 1: Phytochemical screening analysis of *Citrus limon* juice

S/N	Test	Observation	S/N	Test	Observation
1.	Saponins	+	7.	Tannin	-
2.	Flavonoids	+	8.	Anthraquinone	+
3.	Steroids	-	9.	Cardiac glycosides	-
4.	Alkaloids	+	10.	Terpene	+
5.	Resins	-	11.	Reducing sugars	-
6.	Glycosides	-	12.	Phenol	+

KEY: + = Present and - = Absent

Effects of *Citrus limon* juice on oestrous cycle

Oestrus cycle length was significantly reduced in 75% CLJ group after treatment with the juice when compared with before juice administration (figure 1).

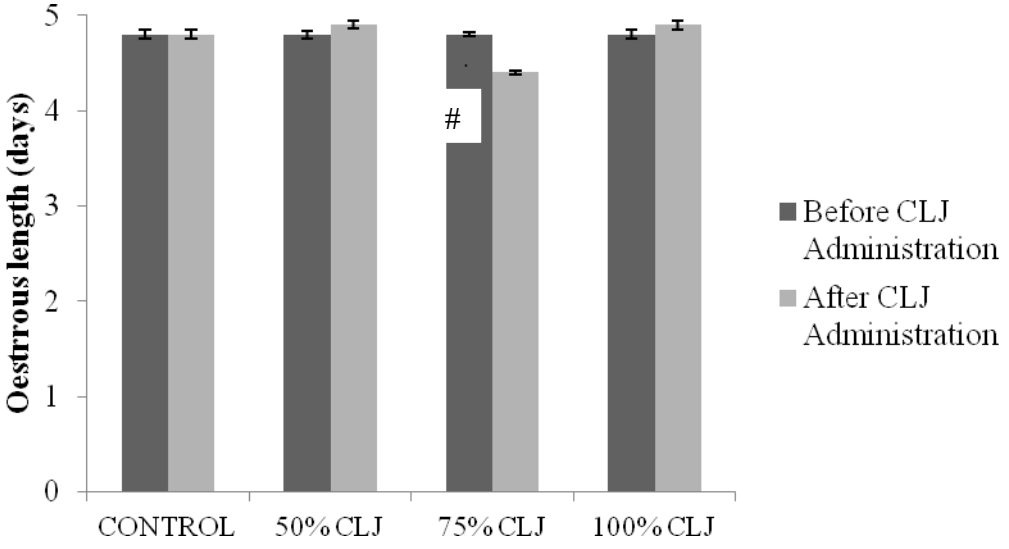


Figure 1: Effects of *Citrus limon* juice (CLJ) on oestrous cycle length in female Wistar rats.

Data are presented as mean ± SEM, n=5, #P<0.05 compared with before CLJ administration of the corresponding group.

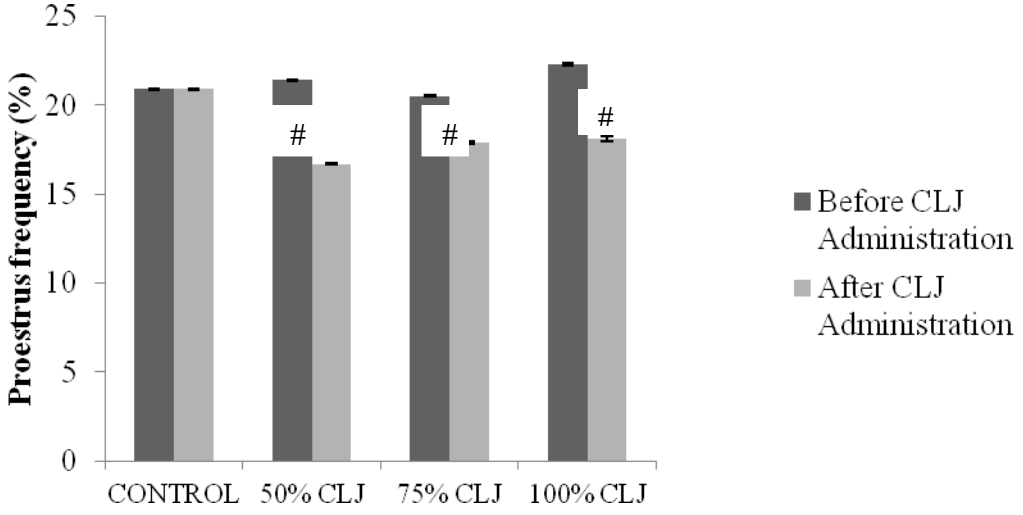


Figure 2: Effects of *Citrus limon* juice (CLJ) on frequency of the proestrus phase of the oestrus cycle in female Wistar rats.

The frequency of proestrus and oestrus phases was significantly reduced across the treated groups after juice administration when compared with before juice administration (figures 2&3).

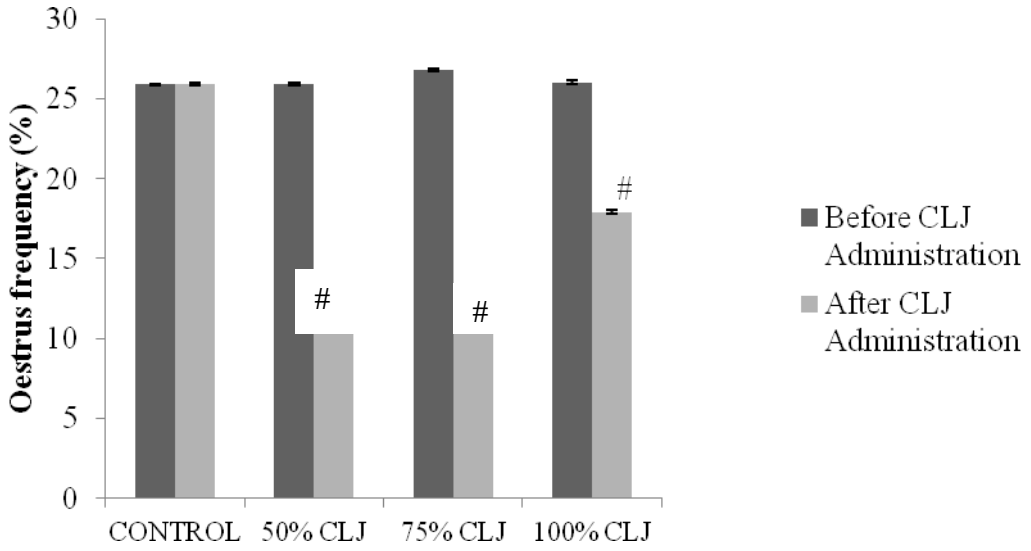


Figure 3: Effects of *Citrus limon* juice (CLJ) on frequency of the oestrus phase of the oestrous cycle in female Wistar rats.

Data are presented as mean± SEM, n=5, #P<0.05 compared with before CLJ administration of the corresponding group.

The frequency of metestrus phase was significantly increased in 75% CLJ and 100% CLJ groups after juice administration when compared with before juice administration (figure 4).

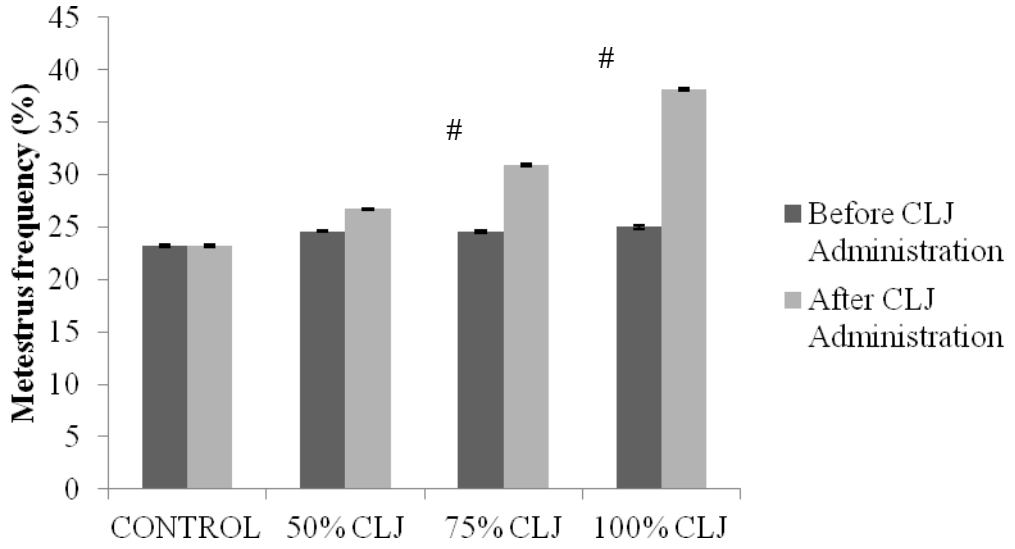


Figure 4: Effects of *Citrus limon* juice (CLJ) on frequency of the metestrus phase of the oestrous cycle in female Wistar rats.

Data are presented as mean± SEM, n=5, #P<0.05 compared with before CLJ administration of the corresponding group.

Diestrus phase frequency was significantly increased across the groups

after juice administration when compared with before juice administration (figure 5).

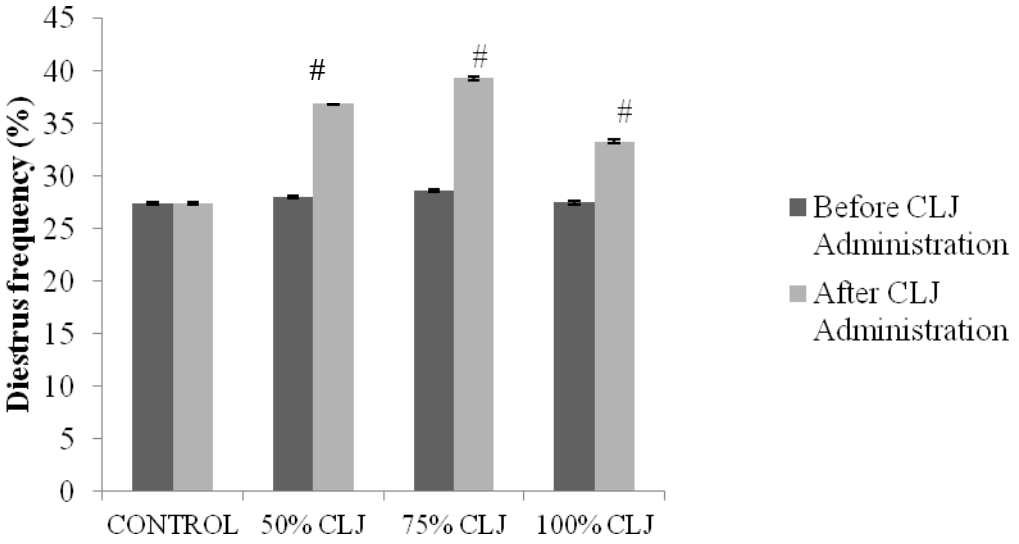


Figure 5: Effects of *Citrus limon* juice (CLJ) on frequency of the diestrus phase of the oestrous cycle in female Wistar rats.

Data are presented as mean± SEM, n=5, #P<0.05 compared with before CLJ administration of the corresponding group.

Effects of *Citrus limon* juice on serum hormone concentration

Serum level of oestrogen was significantly increased in 50% CLJ and 75% CLJ groups when compared with the control. Luteinizing hormone concentration was also significantly increased in 75% CLJ group when compared with the control group (Table 2).

Table 2: Effects of *Citrus limon* juice (CLJ) on serum hormone concentrations in female Wistar rats

GROUP	LH (mIU/mL)	FSH (mIU/mL)	Oestrogen(µg/mL)
CONTROL	2.70±0.02	2.28±0.01	20.24±0.01
50% CLJ	2.69±0.02	2.28±0.01	41.60±0.02*
75% CLJ	2.75±0.01*	2.29±0.02	34.68±0.02*
100% CLJ	2.69±0.02	2.28±0.01	26.33±0.01

*Data are presented as mean± SEM, n=5, *P<0.05 compared with control.*

Effects of *Citrus limon* juice on body and organ weights

There was significant reduction in mean progressive body weight of 50% CLJ and 100% CLJ treatment groups when compared with before CLJ administration (figure 6).

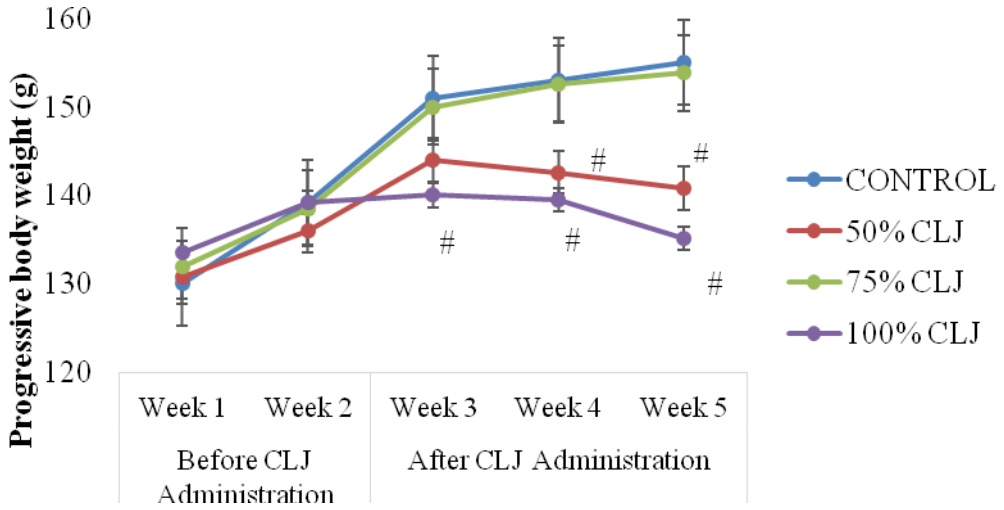


Figure 6: Effects of *Citrus limon* juice (CLJ) on mean progressive weight of female Wistar rats.

Data are presented as mean ± SEM, n=5, # = P<0.05 compared with the control group

Table 3: Effects of *Citrus limon* juice (CLJ) on relative organ weights in female Wistar rats

GROUP	Ovary (%)	Uterus (%)	Liver (%)
CONTROL	0.07±0.01	0.26±0.05	3.76±0.17
50% CLJ	0.10±0.01*	0.23±0.01	3.34±0.14
75% CLJ	0.09±0.00	0.17±0.01	3.23±0.02*
100% CLJ	0.11±0.01*	0.24±0.01	3.50±0.17

*Data are presented as mean± SEM, n=5, *P<0.05 compared with control*

Ovarian weight was significantly increased in 50% CLJ and 100% CLJ groups when compared with control, the weight of the liver was significantly reduced in 75% CLJ group when compared with the control (Table 3).

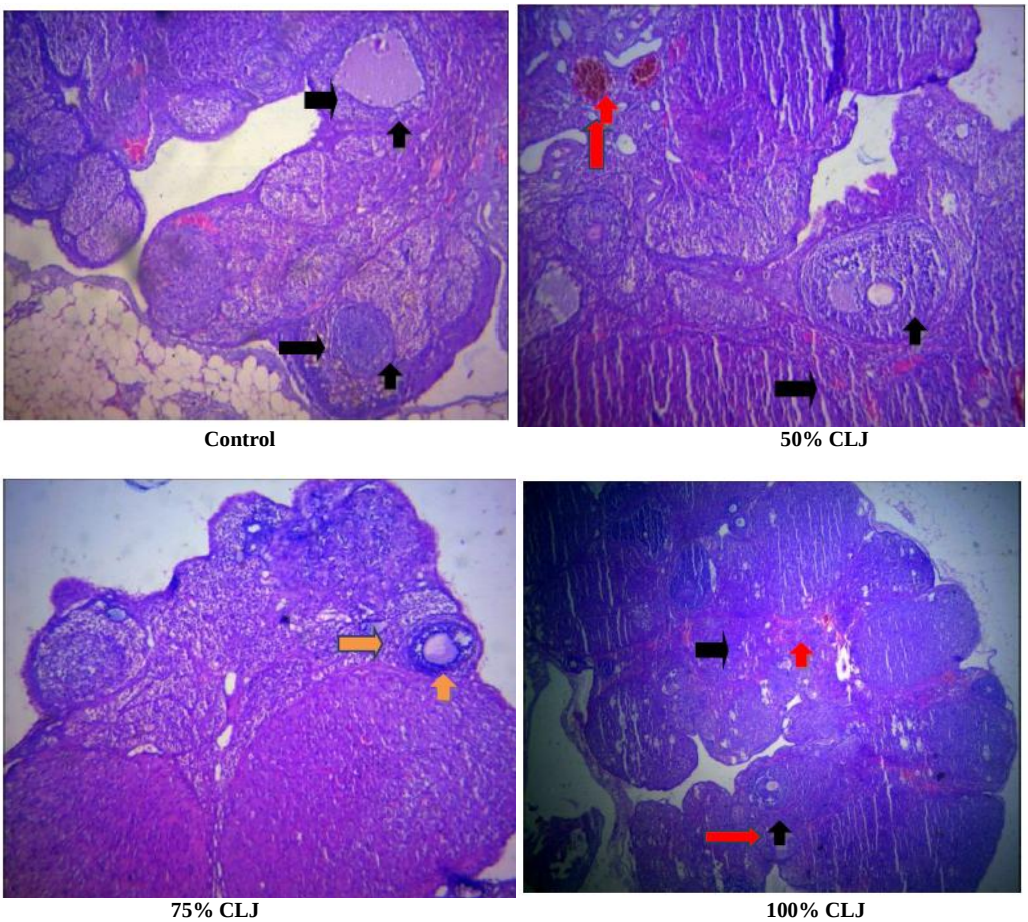
Effects of *Citrus limon* juice on lipid concentration

High density lipoprotein significantly reduced in 75% CLJ and 100% CLJ groups when compared with control (Table 4). Total cholesterol, low density lipoprotein and triglycerides were significantly reduced in all the treatment groups when compared with the control (Table 4).

Table 4: Effects of *Citrus limon* juice (CLJ) on serum lipid concentrations in female Wistar rats.

GROUP	TC (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	TG (mg/dL)
CONTROL	95.55±0.05	43.09±0.01	224.32±0.02	326.44±0.02
50% CLJ	82.49±0.02*	39.41±0.01	71.62±0.01*	269.84±0.05*
75% CLJ	79.12±0.02*	33.45±0.01*	60.58±0.01*	278.72±0.02*
100% CLJ	80.65±0.01*	35.05±0.01*	59.51±0.02*	290.49±0.02*

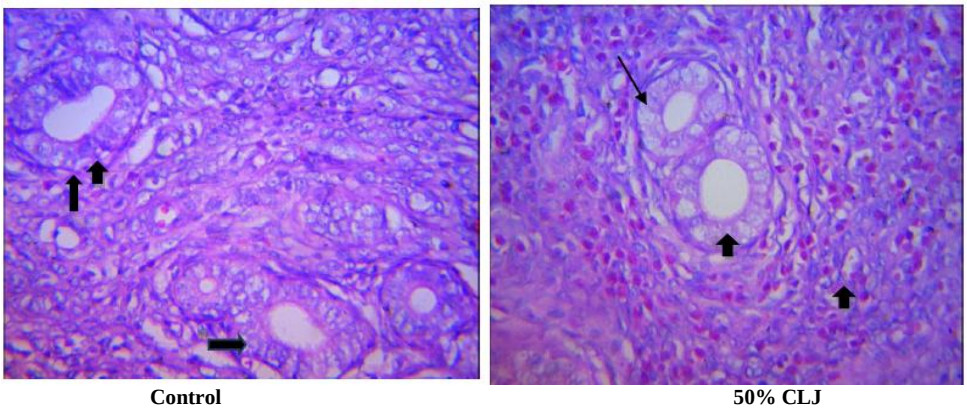
*Data are presented as mean ± SEM, n=5, *P<0.05 compared with control. TC= Total Cholesterol; HDL-C= High Density Lipoprotein-Cholesterol; LDL-C= Low Density Lipoprotein-Cholesterol; TG= Triglycerides.*



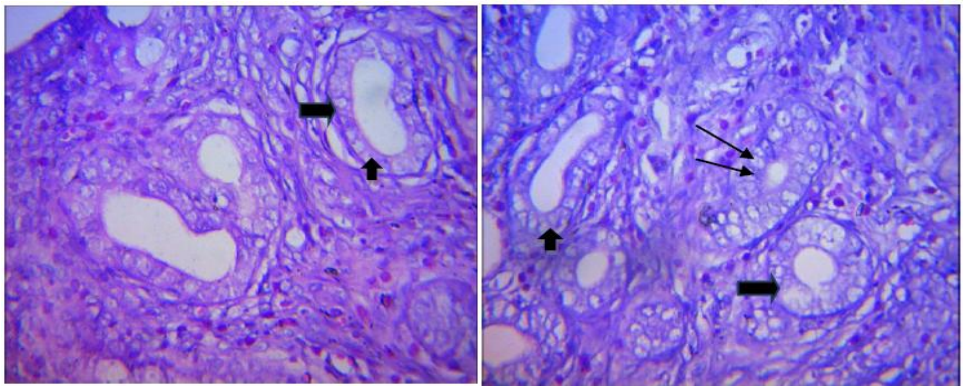
Control **50% CLJ**

75% CLJ **100% CLJ**

Figure 7: Photomicrographs of ovarian sections from control and *Citrus limon* juice treated non-pregnant rats showing the ovarian follicles at different stages of maturation (black arrow), mature follicle (orange arrow) and congestion in the medulla (red arrow) (mag. X 100, H&E stain). Sections from all groups show no visible signs of lesion.

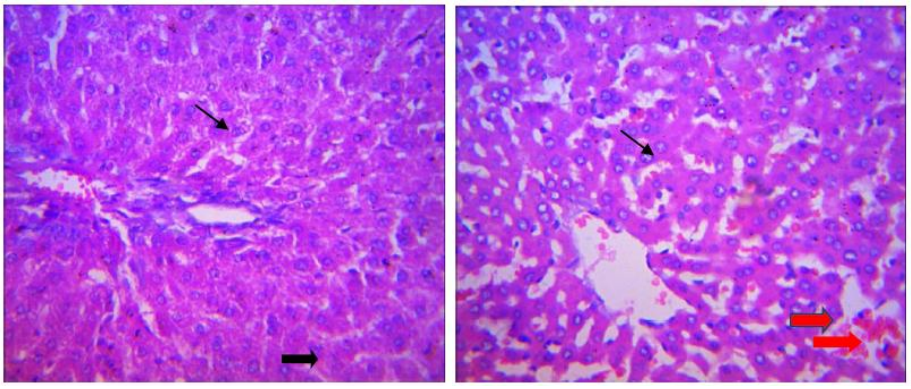


Control **50% CLJ**

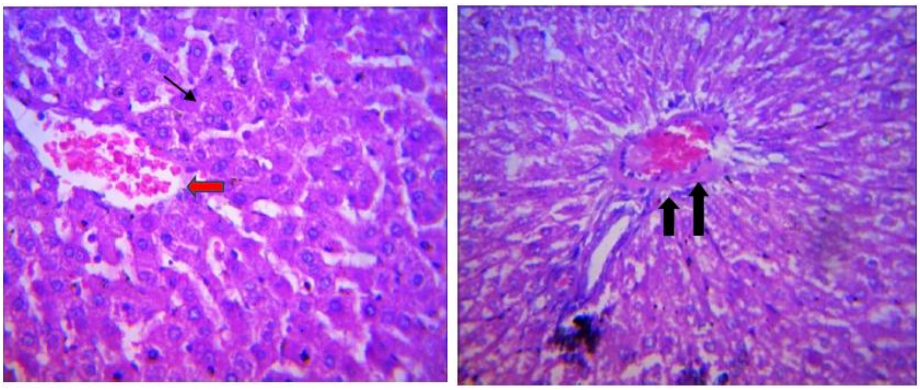


75% CLJ **100% CLJ**

Figure 8: Photomicrographs of uterine sections from control and *Citrus limon* juice treated non-pregnant rats showing uterine glands (black arrow), degenerating glandular epithelia cells (slender arrow) (mag. X 400, H&E stain). Sections from all groups show no visible signs of lesion.



Control **50% CLJ**



75% CLJ **100% CLJ**

Figure 9: Photomicrographs of liver sections from control and *Citrus limon* juice treated non-pregnant rats showing hepatocytes (thin black arrow), mild diffuse vascular degeneration of the hepatocytes (thick black arrow) and congestion of the portal vessels (red arrow) (mag. X 400, H&E stain). Sections from all groups show no visible signs of lesion.

DISCUSSION

Evaluation of the oestrus cycle in animals is a useful measure to determine the integrity of the hypothalamic-pituitary-ovarian axis and assessing reproductive status [17]; this is because oestrous cycle is regulated by oestrogen and gonadotropins [18]. The oestrous cycle comprises of four phases namely; proestrus, oestrus, metestrus and diestrus and lasts for 4-5 days on average [18]. The proestrus phase corresponds to the human follicular/ proliferative stage [19], during this phase ovarian follicle mature and uterine lining proliferates while ovulation occurs in the oestrus phase of oestrus cycle during which female animals are receptive to male animals [20]. The structural changes occurring in the ovary and uterus during proestrus and oestrus phases are regulated by FSH, LH and oestrogen, in this present study the increase in the concentration of LH in 75% CLJ group and oestrogen in 50% CLJ and 75% CLJ groups obtained, suggest that these hormones were adequate in the circulation but the reduction in the frequency of the phases and the number of ovarian follicles observed in the study may indicate sub-optimal tissue uptake of these hormones. Metestrus and diestrus phases are characterised by the activities of the corpus luteum which produces progesterone [21]. These phases correspond to human early and late secretory stages of the reproductive cycle [22]. During these phases, female animals are not receptive to males [20]; the observed increase in frequency of these phases reduces the chances of mating and reproduction [23]. Phytochemicals such as flavonoids, saponins, alkaloids, terpenes, phenols and anthraquinone in *Citrus limon* juice observed in this study has also been reported by Okwu [24]. Saponin, tannin and alkaloids have been shown to act as an estrous cycle disruptor [25].

Citrus limon juice although acidic, has an alkalinizing effect on the body thus helps in detoxification [26]. Also, consumption of low calorie diet such as *Citrus limon* juice has been reported to tend to be filling and induce satiety [27]. Thus in synergy with its hypolipidemic effect it therefore aids in weight loss. Hence, the reduction in mean progressive body weight observed in the treatment groups. Lipids such as cholesterol are involved in basic molecular processes for reproduction. It is the main substrate for steroid synthesis and has been shown to affect the hormonal *milieu* and steroidogenesis [28]. The observed reduction in body weight and lipid concentration in the study may also impact on reproductive capability. The results of the lipid concentration corroborate the findings from previous studies [14, 29, 30].

The results obtained from this study likely suggest that *Citrus limon* juice may be associated with reduced reproductive capability in Wistar rats.

CONCLUSION

The results observed in the present study suggest that administration of *Citrus limon* juice adversely affected reproductive functions in female Wistar rats, by increasing the frequency of metestrus and diestrus, reducing oestrus length, lipid profile and causing structural changes in the reproductive tissues as well as imbalance in the level of reproductive hormones.

Financial Supports

This research received no specific grant from any funding agency, commercial or not-for-profit sectors.

Conflicts of Interest: None

REFERENCES

1. Dias AD, Urban S, Roessner U. A historical overview of natural products in drug discovery. Metabolites. 2012; 2: 303-36.
2. Pal SK, Shukla Y. Herbal medicines: current status and the future. Asian Pac J Cancer Prev. 2003; 4: 281-8.
3. Owolabi MS, Avoseh ON, Ogunwande IA, et al. Chemical composition of Citrus limon (L.) Osbeck growing in southwestern Nigeria: Essential oil chemo

types of both peel and leaf of lemon. Am. J. Essent. Oil Nat. Prod. 2018; 6: 36-40
4. Mohannapriya M, Ramaswamy L, Rajendran R. Health and Medicinal properties of lemon. Int. J. Ayurvedic herb. Med. 2013; 3: 1095-100.
5. Rauf A, Uddin G, Ali J. "Phytochemical analysis and radical scavenging profile of juices of Citrus sinensis, Citrus anrantifolia, and Citrus limonum". Org Med Chem Lett. 2014; 4: 5.
6. Muhammed T, Amine L, Khadija E, et al. Lemon juice has protective activity in a rat urolithiasis model. BMC Urol. 2007; 7: 18.
7. Clarke GN, Scott GM, Roger VS. Sperm immobilizing properties of lemon juice. Fertil. Steril. 2006; 85: 1529-30.
8. Imade GE, Sagay AS, Onwuliri VA, et al. Use of lemon or lime juice douches in women in Jos, Nigeria. Sex Health. 2005; 2: 237-9.
9. Sagay AS, Imade GE, Onwuliri V, et al. Genital tract abnormalities among female sex workers who douche with lemon/lime in Nigeria. Afr J Reprod Health. 2009; 13: 37-45.
10. Fukuchi Y, Hiramitsu M, Okada M, et al. Lemon polyphenols suppress diet-induced obesity by up-regulation of mRNA levels of the Enzymes Involved in β-oxidation in mouse white adipose tissue. J Clin Biochem Nutr. 2008; 43: 201-9.
11. Chijoke-Nwauche IN, Dede EB. Effects of lemon juice on the reproductive hormones of female Wistar rats (Rattus rattus). J Pharm Allied Sci. 2010; 7: 5.
12. NIH. Guide for the Care and Use of Laboratory Animals. NIH Publication revised 1996: No. 85-23.
13. Harborne JB. Phytochemical methods: a guide to modern techniques of plant analysis. 3rd ed New York, NY: Chapman and Hall. 1998; p. 49–188
14. Khan Y, Rafeeq AK, Syeda A, et al. Evaluation of hypolipidemic effect of Citrus limon. J Basic Appl. Sci. 2010; 6: 39-43.
15. Marcondes FK, Bianchi FJ, Tanno AP. Determination of the oestrous cycle phases of rats: some helpful considerations. Braz J Biol. 2002; 62: 609-14.
16. Pereda J, Gomez-Cambronero L, Alberola A, et al. Co-administration of pentoxifylline and thiopental causes death by acute pulmonary oedema in rats. Br J Pharmacol. 2006; 149: 450-55.
17. Goldman JM, Murr AS, Cooper RL. The rodent oestrus cycle; characterisation of vaginal cytology and its utility in toxicological studies. Birth Defects Res (Part B): Dev Reprod Toxicol. 2007; 80: 84-97.
18. Long JA, Evans HM. The oestrous cycle in the rat and its associated phenomena. Memories of University of California. 1922; 6: 1-148.
19. Freeman ME. The neuroendocrine control of the ovarian cycle of the rat. In: Knobil, E., Neill, J.D., editors. The physiology of Reproduction. 2nd edition. New York: Raven Press. 1994; p 613-58.
20. Kim H, Son J, Yoo H, et al. Effects of the female oestrus cycle on the sexual behaviours and ultrasonic vocalisations of male c57b1/6 and autistic btb rt+ tf/j mice. Exp Neurobiol. 2016; 25: 156-62.
21. Hirshfield AN. Comparison of granulosa cells proliferation in small follicles of hypophysectomised, prepubertal and mature rats. Biol Reprod. 1985; 32: 979-87.
22. Heape W. "The 'sexual season' of mammals and the relation of the 'pro-oestrum' to menstruation'". Q J Microsc Sci. 1990; 44: 1-70.
23. Caligioni CS. Assessing reproductive status stages in mice. Curr Protoc Neurosci. 2009; Appendix 41.
24. Okwu DE. Citrus fruits: A rich source of phytochemicals and their roles in human health. Inter J Chem Sci, 2008; 6: 451-71.
25. Sharma RK, Goyal AK, Bhat RA. Antifertility activity of plants extracts on female reproduction. A review. Inter J Pharm Biol Sci. 2013; 3: 493-514.
26. Solomon IP, Oyebadejo SA, Ebenso IE, et al. Biochemical effects of citrus lemon juice on the liver of growing rabbits (Oryctologus cuniculus). Sch J Agric Vet Sci. 2015; 2: 356-61.

27. Hetherington MM, Cunningham K, Dye L, et al. Potential benefits of satiety to the consumer: scientific considerations. Nutr Res Rev. 2013; 26: 22–38.
28. Calonge RN, Kireev R, Guijarro A, et al. Lipid dysregulation in seminal and follicular fluids could be related with male and female infertility. Endo Metab Inter J. 2018; 6: 65-71.
29. Olukanni OD, Akande OT, Alagbe YO, et al. Lemon Juice Elevated Level of Reduced Glutathione and Improved Lipid Profile in Wistar Rats. American-Eurasian J Agric & Environ Sci. 2013; 3: 1246-51.
30. Kumbhakar S, Verma R, Biju, B. Effect of warm lemon water drink on selected physical parameters among the overweight female nursing students of RIMS and RUI. Inter J Nurs Res Pract. 2016; 3: 9-12.

How to cite this article: Ahmed TT, Kunle-Alabi OT, Akindele OO et.al. Effects of citrus limon linn juice on reproductive functions in female wistar rats. *Int J Health Sci Res.* 2021; 11(2): 262-271.

******