



Dietary Consumption of *Citrullus lanatus* can Ameliorate Infertility Potential of *Carica papaya* Seeds Extract in Male Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Author NOA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NOA and UPI managed the analyses of the study. Author UPI managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study examined dietary consumption of *Citrullus lanatus* as an ameliorative agent against infertility potential of seeds extract of *Carica papaya* in male wistar rats.

Study Design: Eighteen adult male Wistar rats were randomly assigned into three groups of six (n=6): A, B, and C. Group A served as the control group while the other groups served as the treated groups.

Place and Duration of Study: This study was carried out in the Animal Holdings of the Department of Anatomy, University of Ilorin, Ilorin, between July, 2014 and October, 2014.

Methodology: The Group A animals were given feed and water liberally throughout the study. Group B received 150 mgkg/bwt/d of *Carica papaya* extract intraperitoneally for 8 weeks, Group C received 150 mgkg/bwt/d of *Carica papaya* extract intraperitoneally plus *Citrullus lanatus* orally for 8 weeks. The rats were sacrificed at the end of administration, Testosterone, FSH and LH levels were assayed. Also, semen analysis was carried out to ascertain the level of other parameters.

Results: Generally there was significant reduction in the level of Testosterone, FSH and LH in Group B when compared with the Control and Group C. Also, there was significant reduction in the

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level of sperm count, motility, concentration and morphology in Group B when compared with the Control and Group C. However, there was no significant difference when almost all these parameters were directly compared between Control and Group C.

Conclusion: This study has demonstrated that dietary consumption of *Citrullus lanatus* can ameliorate infertility potential of *Carica papaya* seeds extract in adult male Wistar rats.

Keywords: Infertility; *Carica papaya*; *Citrullus lanatus*; male rats.

1. INTRODUCTION

Citrullus lanatus is a tropical plant which belongs to the family of Cucurbitacea. It is grown all over South East Asia and most part of Africa [1]. It is commonly referred to as Egusi melon, West African watermelon, dessert watermelon, cooking melon [2,3]. *Citrullus lanatus* has inner parts that are red, orange, yellow or white in colour depending on the variety and green outer part [4,5]. The inner red part of *Citrullus lanatus* contains large amounts of beta carotene and is a significant source of lycopene [2,1]. Lycopene is of great interest because of its rich antioxidant and potential health benefits. *Citrullus Lanatus* plant has bioactive compounds such as cucurbitacin, triterpenes, sterols and alkaloids [6,7].

The extracts of *Citrullus lanatus* are used for various purposes such as; protection against prostate cancer [8,9], treatment of type 2 diabetes [10], improve blood vessel function [11], reduction of blood pressure in hypertensive individuals [12]. Others are: antimicrobial activities, anti-giardial activity, anti-inflammatory activity [13,14].

Carica papaya is a tasty fruit that is rapidly propagated in the tropics, it is believed to be a native of southern Mexico and neighbouring Central America [15]. It belong to the family of *Caricaceae* [16]. It is commonly referred to as pawpaw (UK), papaya (France), papita (India), fruta bomba (Cuba) and mamao (Brazil) [17].

Carica papaya has active compounds like Papain, Alkaloids, Carpaine, chymopapain, Nicotine, Caoutchone, Oleic acid [18]. The extracts of *Carica papaya* are known for medicinal uses and activities such as nephroprotective activity [19], anti-sickling activity [20], anti-tumor activity [21], wound healing effects [22], anti-hypertensive activity [23]. The seeds are effective in treating hypercholesterolemia, diabetes mellitus and hypertension [24,25,26].

Despite the important medicinal uses of *Carica papaya*, there exist some unpleasant side effects of this plant such as; anti-implantation, abortifacient [27] and antifertility effects [28]. High doses of aqueous extract *Carica papaya*, reduces sperm motility, decreases sperm count and viability in wistar rats [29].

Male infertility is referred to as the inability of a male to achieve pregnancy in a fertile female for at least 1-2 years after constant mating. Male infertility account for 20-30% of infertility cases [30]. The common causes of male infertility are low sperm cell counts, abnormal sperm morphology as well as hormonal imbalance [31,32].

It is as a result of reports of infertility potential of *Carica papaya* seeds extract, that this study was carried out to examine; dietary consumption of *Citrullus lanatus* as an ameliorative agent against infertility potential of methanol seeds extract of *Carica papaya* in male wistar rats.

2. MATERIALS AND METHODS

Eighteen adult male Wistar rats (with an average weight of 240 g) were procured from department of anatomy, Ladoke Akintola University of Technology. The animals were acclimatized for two weeks at the animal holdings of Anatomy department, University of Ilorin, Ilorin before the commencement of the experiment. They were exposed to normal laboratory conditions of temperature, light and humidity. The rats were fed with pelletized feeds (produced by Vital feeds, Nigeria) and water *ad libitum*. The animals were given adequate care in accordance with the Principle of Laboratory and Animal Care prepared by the National Academy of Sciences and published by the National Institute of Health [33]. All the rats were carefully assessed and screened at the end of the acclimatization period. The investigation was conducted in accordance with the principles and guidelines for animal research.

2.1 Plant Materials and Extraction

Ripe *Carica papaya* was procured from Oja Oba market, Ilorin and it was authenticated by Pharmacology department of university of Ilorin. The seeds were removed and air dried for 7 days. There were later pounded into powdery form using mortar and pestle. 80.65 g of the sample was immersed in 300 mL of methanol for 48 hours after which the solution was sieved and evaporated to dryness at 30°C. It was then weighed and refrigerated until use.

The *Citrullus lanatus* used was procured from Oja Oba market, Ilorin and it was authenticated by Pharmacology department of university of Ilorin. The rind was separated from its inner part. The inner part was chopped into small pieces then blended properly with an electronic blender. The juice was later concentrated using Rotary evaporator and then stored in a refrigerator at 4°C until use.

2.2 Experimental Design

Eighteen adult male rats were randomly divided into three (3) groups of six (6) rats each;

Group A (control): Received only distilled water

Group B: Received extract of *Carica papaya* (intraperitoneally) 150 mg/kg/ bwt/d for 8 weeks.

Group C: Received extract of *Carica papaya* (intraperitoneally) 150 mg/kg/ bwt/d for 8 weeks + *Citrullus lanatus* mix in their drinking water throughout the experiment.

2.3 Termination of Treatment

At the end of 8 weeks, the rats were sacrificed by cervical dislocation. 5 ml of blood samples were collected using syringe by cardiac puncture into EDTA bottles. The blood was centrifuge at 2000 rpm for 10 minutes then the serum was decanted and store at -10°C in a refrigerator. Laparotomy was performed, testes were excised and cut open so that the semen flowed from it into a beaker containing normal saline.

Serum testosterone assay was carried out using the Enzyme linked immunoassay (ELISA) method. Kit used was from Biotec laboratories Ltd, UK. Absorbance was measured with a spectrophotometer at 450 nm LH and FSH were assayed from blood serum using ELISA kit protocol. LH level was measured spectrophotometrically at 450 nm using microtiter plate reader within 15 minutes.

2.4 Sperm Concentration

A modified method of Yokoi and Mayi [34] was adopted in counting the spermatozoa from the right epididymis. In this method, epididymis was minced with anatomic scissors in 5 ml physiologic saline, placed in a rocker for 10 min. This was allowed to incubate at room temperature for 2 min, after which the supernatant fluid was diluted 1:100 with a solution containing 5 g sodium bicarbonate (NaHCO₃) in 1 ml formalin (35%). Total sperm number was determined by using a haemocytometer. Approximately 10 µl of the diluted sperm suspension was transferred to each counting chamber of the haemocytometer and was allowed to stand for 5 min. This was viewed under a light microscope then sperm concentration was calculated [26].

2.5 Sperm Motility

The method of Sonmez [35] was use to evaluate the sperm motility. Motility estimates were performed from 5 different fields in each sample. The mean of the 3 different estimates was used as the final motility score. Samples for motility evaluation were stored at 25°C.

2.6 Sperm Count

Assessment of sperm count was done. Twenty microlitres of the liquefied semen was diluted with 20 ml of sodium bicarbonate formalin. A small amount of the diluted semen was put in both chambers of the Neubauer counting chamber using a Pasteur pipette and allowed to spread and settle for 3-5 minutes. Using 10x objective lens, the sperm cells in an area of 2 sqmm were counted and multiplied by 1000,000.

2.7 Sperm Morphology

Under a light microscope at x400 magnification, Sperm cell morphology was evaluated using the method of Saalu [36]. It was determined using sperm taken from the original dilution for motility. Diluted 1:20 with 10% neutral buffered formalin (Sigma-Aldrich, Oakville, ON, Canada). The sperm cells were categorised based on the presence of one or more abnormal features such as tail defects (short, irregular, coiled or multiple tails); neck and middle piece defects (distended, irregular, bent, middle piece, abnormally thin middle piece); and head defects (roundhead, small or large size, double or detached head).

Findings were expressed as percentage of morphologically normal sperm.

2.8 Statistical Analysis

Data was analysed using Analysis of Variance (ANOVA) and students t-test (Tukey's test) with the statistical software SPSS version 20.0 at 95% confidence interval. Values were reported as mean \pm S.E.M and $p < 0.05$ was considered statistically significant.

3. RESULTS

3.1 Testosterone Assay

The result shown (Fig. 1) indicates significant reduction ($p < 0.05$) in the level of Testosterone in Group B when compared with Control and Group C. However, there was no significant difference ($p > 0.05$) in the level Testosterone when Control and Group C were directly compared (Fig. 1).

3.2 Luteinizing Hormone (LH)

There was significant reduction ($p < 0.05$) in the level of LH in Group B when compared with Control and Group C (Fig. 2). However, there was no significant difference ($p > 0.05$) in the level LH when Control and Group C were directly compared (Fig. 2).

3.3 Follicle Stimulating Hormone (FSH)

The result of FSH as shown (Fig. 3) indicates significant reduction ($p < 0.05$) in the level of FSH in Group B when compared with Control and Group C. However, there was no significant difference ($p > 0.05$) in the level FSH when Control and Group C were directly compared (Fig. 3).

3.4 Analysis of Semen

There was significant reduction ($p < 0.05$) in sperm concentration for Group B when compared with Control and Group C (Table 1). Though there was difference in sperm concentration comparatively between Control and Group C the difference is not significant (Table 1).

Sperm motility was significantly decreased ($p < 0.05$) in Group B when compared with Control and Group C (Table 1); but sperm motility between Control and Group C when compared appeared the same.

The result of sperm count as shown in Table 1 indicates significant reduction ($p < 0.05$) in Group B when compared with Control and Group C. There was also significant difference ($p < 0.05$) in sperm count when Control and Group C were directly compared (Table 1).

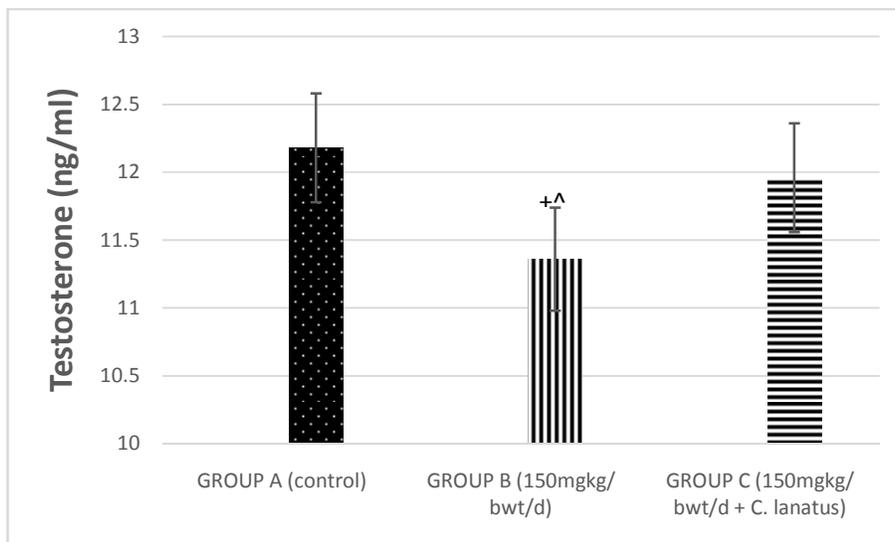


Fig. 1. Level of Testosterone following the administration of *Carica papaya* extract and *Citrullus lanatus* in adult male wistar rats

(n = 6). +, indicates significant difference between control and Group B; ^ indicates significant difference between Group B and Group C

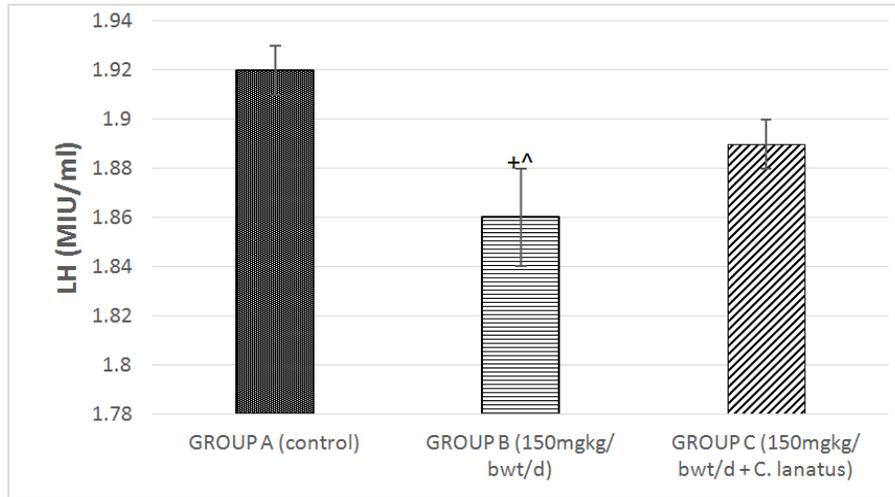


Fig. 2. Level of Luteinizing Hormone (LH) following the administration of *Carica papaya* extract and *Citrullus lanatus* in adult male wistar rats
 (n = 6). +, indicates significant difference between control and Group B; ^ indicates significant difference between Group B and Group C

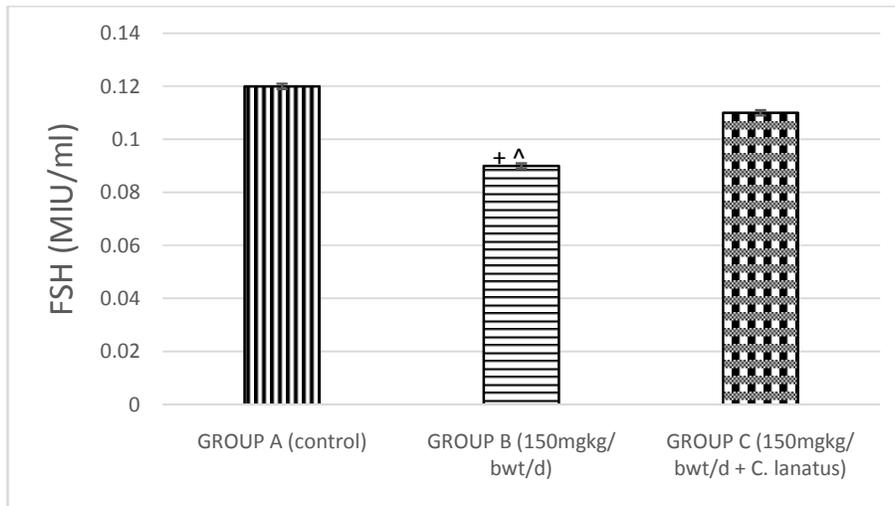


Fig. 3. Level of Follicle Stimulating Hormone (FSH) following the administration of *Carica papaya* extract and *Citrullus lanatus* in adult male wistar rats
 (n = 6). +, indicates significant difference between control and group B;
 ^ Indicates significant difference between group B and group C

Table 1. Analysis of semen

Groups	Sperm concentration (million/ml)	Sperm motility %	Total sperm count ($\times 10^6$)
Group A (control)	9.24 \pm 0.25	53.85 \pm 4.54	405 \pm 6.25
Group B (150 mgkg/bwt/d)	6.68 \pm 0.30*	28.88 \pm 7.96*	215 \pm 2.43*
Group C (150 mgkg/bwt/d + <i>C. lanatus</i>)	7.98 \pm 0.01	48.88 \pm 10.60	320 \pm 1.19^

Values presented as mean \pm SEM; n=6; * Significant difference between control and group B as well as group C.
 ^ Significant difference between control and group C

Table 2 shows that Group B has lower percentage of normal sperm morphology and higher percentage of defects when compared with other groups especially Group C.

4. DISCUSSION

It is a known fact that seed extract of *Carica papaya* causes infertility in male albino rats [28,37]. However, lycopene is hypothesized to be a treatment for idiopathic infertility [31]. *Citrullus lanatus* is one of many plants that has lycopene [1]. Lycopene is of great interest because of its rich antioxidant and potential health benefits.

Male fertility in mammals is regulated by the two anterior pituitary hormones, Luteinizing hormone (LH) and Follicle stimulating hormone (FSH) through synthesis of testosterone.

The regulated release of the hypothalamic gonadotropin-releasing hormone (GnRH) ensures that hypothalamo-hypophysio-gonadal axis function normally, through secretion of gonadotropins and testosterone in systemic circulation [38,39].

Testosterone is a steroid hormone that belong to the androgen group. It is secreted primarily in the Leydig cells of the testis through stimulation of luteinizing hormone (LH). Testosterone promotes male sexual differentiation, pubertal androgenization, and fertility [38].

The present study (in Fig. 1) revealed a decrease in the serum level of testosterone in Group B when compared with Control and Group C. This observation is supported by earlier findings [40, 41, 38]. Reduction of testosterone level may impair spermatogenesis and cause male infertility. However, the effect of

Carica papaya extract on Testosterone level was ameliorated in Group C. This could be the result of lycopene present in *Citrullus lanatus* administered to this Group. Earlier findings supported this observation [31,32].

Follicle stimulating hormone (FSH) released by the anterior pituitary binds with receptors in the sertoli cells and stimulates spermatogenesis [42,43]. The present study (in Fig. 3) revealed a decrease in the serum level of FSH in Group B when compared with Control and Group C. These observation is supported by earlier finding [44]. The effect of *Carica papaya* extract on Testosterone level was ameliorated in Group C. This could be the result of lycopene present in *Citrullus lanatus* administered to this Group. It could also be due to suppression of negative feed-back inhibition of anterior Pituitary [38,45].

Result of this study (in Fig. 2) revealed a decrease in the serum level of LH in Group B when compared with Control and Group C. These observation is supported by earlier finding [44]. LH stimulates the production of testosterone in Leydig cells, which in turn stimulates spermatogenesis [42]. With decreased level of LH in Group B there is reduced activity of spermatogenesis which will lead to infertility. However, the effect of *Carica papaya* extract on LH level was ameliorated in Group C. This could be the result of antioxidant activity of lycopene present in *Citrullus lanatus* administered to this Group.

The increase in the sperm count of rats in Group C when compared with Group B suggests that antioxidant (lycopene) capability of *Citrullus lanatus* could be responsible for this increase. This is possible as a result of reduction in the number of abnormal sperm produced. This observation is supported by earlier finding [31].

Table 2. Sperm morphology

Groups	Normal morphology %	Tail defect %	Head defect %	Neck defect %
Group A (control)	54.25±1.70	14.25±5.14	15.50±1.26	9.50±1.26
Group B (150 mgkg/ bwt/d)	46.00±1.58*	26.00±0.41*	22.75±1.32*	7.25±0.91
Group C (150 mgkg/ bwt/d + C. lanatus)	52.50±1.44	22.00±2.38	19.50±2.72	7.25±3.22

Values presented as mean ±SEM; n=6;

* Significant difference between parameters in control and experimental groups

The result of normal sperm morphology, sperm concentration, sperm volume and sperm motility show appreciable increase in the various parameters in Group C than what was obtain in Group B. This is probably due to the antioxidant activity of lycopene present in *Citrullus lanatus* taken by rats in Group C. This observation is supported by earlier findings [31,29,32].

5. CONCLUSION

This present investigation has demonstrated that dietary consumption of *Citrullus lanatus* can indeed ameliorate infertility potentials of seed extract *Carica papaya* in adult male Wistar rats. However, further research is recommended to establish the findings of this study.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee. All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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