

Contraceptive Potential of Graded Doses of Carica Papaya Root Methanol Extract on Male Wistar Rats

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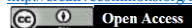
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ABSTRACT

This study investigated the contraceptive potential of Carica papaya methanol root extract in male Wistar rats. A total of 24 rats were divided into four groups: control, dexamethasone treated (0.06 mg), and extract treated groups at 200 mg/kg and 400 mg/kg. Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, proteins, sugars, terpenoids, quinones, and phenols. Toxicity studies showed that the extract was safe at doses up to 5000 mg/kg. Hormonal analysis indicated a dose-dependent effect, with the 200 mg/kg extract suppressing testosterone while the 400 mg/kg extract elevated FSH and LH levels. Despite these hormonal changes, sperm count and motility were not significantly improved compared to controls. The study concludes that Carica papaya root extract possesses antifertility potential, likely mediated through hormonal modulation and changes in sperm physiology, with limited spermatogenic benefits. Further studies are recommended to isolate bioactive compounds, test higher doses, and explore cross-species contraceptive efficacy.

Introduction

Contraception is the deliberate prevention of pregnancy, achieved either by impeding ovulation (the release of eggs from the ovaries), preventing the fertilization of an egg by sperm, or interfering with the process by which a fertilized egg gets implanted in the uterine lining (Casey, 2023). A study by Nita *et al.* (2020) indicates that most contraceptive options are designed for women, while male contraception remains uncommon. Although men have relied on methods such as withdrawal and vasectomy, these approaches have proven either ineffective or financially inaccessible (Kretschmer, 2023). Consequently, there is an increasing interest in developing safe and effective male contraceptive options (John Hopkins Bloomberg School of Public Health, 2025). The disparity in contraceptive options is evident, with women having access to nine different methods, whereas men are limited to just two vasectomy and condoms (Dela Cruz *et al.*, 2023).

Expanding male contraceptive options could significantly shift reproductive responsibility, promoting a more balanced approach to contraception between partners, regardless of gender (WHO, 2018).

However, the synthetic contraceptive remedies contain steroids which can cause several adverse effects (Ebrahim *et al.*, 2018). Studies reported that side effects associated with synthetic contraceptives include menstrual irregularities (such as heavy bleeding, absence of menstruation, or infrequent periods), fluctuations in body weight, headaches, dizziness, nausea, and cardiovascular issues. Additionally, some women fear long-term consequences, including infertility and complications during childbirth (Benson *et al.*, 2018; Rademacher *et al.*, 2018).

Birth control plays a crucial role in managing both domestic and wild animal populations.

Orchiectomy in male cattle is a standard practice in farm management aimed at minimizing aggressive and mounting behaviors. It also helps prevent reproduction by males that are physically or genetically undesirable, as well as unintended pregnancies in mixed groups of sexually mature animals (Marquette *et al.*, 2023). Surgical contraceptive measures have been shown to trigger physiological, hormonal, and behavioral responses that reflect pain and discomfort (Coetzee *et al.*, 2010). According

to (Ansari *et al.*, 2017; Nyeko *et al.*, 2016;) herbal contraceptive options are considered more affordable and are associated with fewer adverse effects compared to chemical-based contraceptives. Among these Plant based contraceptive options is the *Carica papaya*. The *C. papaya* is commonly known as pawpaw or papaya. *Carica papaya L.* is a medicinal plant known for its biomedical properties, which enable it to treat various health conditions, including inflammation, diabetes, hypertension, and the regulation of blood pressure and cholesterol levels, among others (Dotto and Abihudi, 2021). It is also recognized to possess some contraceptive properties and is also used as an antiseptic and antimicrobial agent in regions such as Central America, South Asia, Africa etc (Elgadir *et al.*, 2014; Daniel *et al.*, 2023).

Extracts from different parts of *Carica papaya*, including seeds and roots, have demonstrated contraceptive potential in both male and female rodents (Nwaehujor *et al.*, 2019). Studies has shown that *Carica papaya* exhibits antifertility properties in males, leading scientists to investigate various parts of the plant as a potential cost-effective and safer alternative for fertility regulation in male animal models (Chinoy and Padman, 1996). Some steroids such as the synthetic glucocorticoid (Dexamethasone) have been reported to have anti-inflammatory and immunosuppressive properties. Dexamethasone is used in this study to serve as a control due to studies that show its ability to affect testicular function (Sadeghzadeh *et al.*, 2018).

In adult mice, dexamethasone administration caused Leydig cell apoptosis and a decline in the average numbers of spermatocytes along with round and elongated spermatids. Furthermore, DEX promotes the production of reactive oxygen species (ROS), leading to oxidative stress and an imbalance in cellular redox homeostasis. This oxidative stress results in lipid peroxidation, which disrupts cell membranes and impairs cell-cell interactions within the testes, ultimately causing cellular damage (Sadeghzadeh *et al.*, 2018).

Notwithstanding the adverse effects of *Carica papaya*, studies have shown that various extracts of *Carica papaya* exhibit antifertility effects in both male and female rats (Chinoy *et al.*, 1997). The plant has also been noted for its ability to induce abortion and stimulate milk production (Burkill, 1985). Studies by (Nwaehujor *et al.*, 2014) found that administering aqueous extracts of *C. papaya* leaves led to a decline in male reproductive parameters as a result of damage to the seminiferous tubule epithelium. Extracts from different parts of *Carica papaya*, including seeds and roots, have demonstrated contraceptive effects in both male and female rodents (Nwaehujor *et al.*, 2019). To counter the side effects associated with conventional contraceptives and the need for more accessible options, many individuals have been seeking to utilize herbal medicine as an alternative to synthetic contraceptives, as it is more affordable and has minimal adverse effects (Ansari *et al.*, 2017; Nyeko *et al.*, 2016).

Materials and Methods

Study location

The study was conducted at the Department of Theriogenology, Faculty of Veterinary Medicine, Yakubu Gowon University (formerly University of Abuja). The University is geographically located in the Federal Capital Territory, Abuja, Nigeria at approximately latitude 8°58'N of the equator and longitude 7°10'E of the Greenwich meridian (Amarachukwu *et al.*, 2020).

Materials Used

The materials used during this study were Whatman® Qualitative Filter Paper Size 1, Conical flask, Beaker, Fridge, Water bath (DK-420 Digital water bath), Distilled water, Sieve, SF-400 Electronic Digital scale, 80 % Methanol, Measuring cylinders, Funnel, Aluminium foil, Knife, Test tubes, Spatula, Microscopic slides, Cover slips, Mortar and Pestle.

Drugs and Plant Material

Dexamethasone Phosphate tablet, *Carica papaya* root powder, Tween 80

Collection and identification of plant

10 kg of *Carica papaya* (pawpaw) plant root was harvested from Gwagwalada Area Council, Abuja, Nigeria. The plant was authenticated by a Taxonomist at the Botany Department, Faculty of Sciences, Yakubu Gowon University (formerly University Of Abuja), and assigned scientific number UAPS307.

Ethical control

Ethical control for the use of laboratory animals was gotten from the University of Abuja Ethics Committee On Animal Use (UAECAU)

Extraction of plant material

Extraction was done at the Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, University of Abuja. The plant roots were thoroughly washed under running water, cut into smaller pieces to fasten the drying process and air-dried at room temperature for 3-5 days. The dried plant root material was pulverized into fine powder using a commercial blender.

A total of 1292 g of the powdered *Carica papaya* root was macerated in 3.875 L of 80 % methanol for 72 hours, with the mixture (extract and methanol) shaken vigorously every six hours to ensure thorough maceration. After maceration, the extract was first filtered with a sieve and then refiltered using clean Whatman filter paper (size 1). The filtrate was collected into a conical flask and concentrated using a water bath set at 40 °C for five days. The resulting extract was weighed to be 454 g, and the percentage yield was calculated.

Phytochemical Analysis

The phytochemical analysis was done according to methods described by (Alagbe *et al.*, 2022, Alagbe, 2026).

Experimental Animals

A total of 24 apparently fertile and healthy adult male Wistar rats weighing between 150 g - 220 g aged 12-14 weeks were used for the experiment. The rats were purchased from NVRI, Jos. They were acclimatized for two weeks before the commencement of the experiment, They were housed in stainless steel wire mesh cages (30cm by 30 cm), in a well-ventilated room with 12 hour light/dark cycle, with free access to clean drinking water and feed (Chikun feed).

Oral Acute Toxicity Test

Acute toxicity study was carried out using the procedure as described by Lorke (Lorke, 1983). Rats were divided into 6 groups (1–6) consisting of 3 rats per group. Group 1, 2, 3, 4, 5 and 6 were separately given 200, 400, 800, 1600, 3200 and 5000 mg/kg of the extract respectively. The extract was administered orally by oral lavage. The animals were monitored for 24 hours after treatment to check for signs of toxicity, and subsequently for 48 hours to observe any potential mortality.

Another group of three rats was assigned as the control group and animals in that group were given distilled water.

Experimental Design

A total of 24 male rats were randomly assigned into four treatment groups with six rats in each group, the rats were given the treatment as presented in Table 1

Table 1. Experimental treatment

Group	Number of Rats	Treatment
1 Control	6	Distilled water
2 (Low dose)	6	200 mg/kg of cPME
3 (High dose)	6	400 mg/kg of cPME
4 Positive (control)	6	(0.06mg) Dexamethasone

CPME - *Carica papaya* Methanol root extract

The control group was administered distilled water orally determined by their body weight, while graded doses (200 and 400 mg/kg) of *Carica papaya* root extract were given to treatment groups 1 and 2 respectively via oral lavage once daily every morning (7-8:45am) for thirty days. The plant extract was constituted using distilled water and Tween 80 solution to enhance its solubility.

At the end of the length of spermatogenesis (60 days), each experimental rat was euthanized humanely. Blood samples were obtained from each rat via the ocular retrobulbar plexus into EDTA vacutainer bottles and Gel sample bottles. A midline incision was performed in the scrotal region to expose the testes, and the left caudal epididymis was carefully isolated and excised. A sterile needle was then used to puncture the caudal epididymis, allowing spermatozoa to ooze out. The sperm sample was gently expressed onto a pre-warmed glass slide to maintain motility and viability. The slide was covered

with a cover slip and observed for mass motility under the microscope to verify the presence of actively motile spermatozoa. Sperm samples were collected from the caudal epididymis for analysis of pH, colour, and gonadal sperm reserve. The testes and epididymis were excised, cleared of fat, blotted dry, and weighed using a digital weighing balance following the technique as described by (Slott *et al.*, 1991, Ubah *et al.*, 2023)

Effect of *Carica papaya* on pH of Sperm

The pH of the sperm sample was assessed by briefly applying a strip of litmus paper to the specimen. After air drying, the observed color change was then compared with a standardized pH chart to determine the corresponding pH value. This is in accordance to technique reported by (Vinmec, 2024).

Effect of *Carica papaya* on Gonadal Sperm Reserve

The whole length of the left epididymis was measured using a meter rule. Subsequently, the caudal portion was carefully dissected, macerated, and homogenized in 2 mL of 1% formalin. The resulting mixture was transferred into a plain sample bottle for analysis of epididymal sperm concentration.

The left testis of each rat was weighed using a digital balance, and its length and width were measured with a meter rule. Testicular volume was determined via the water displacement method. The tunica albuginea was gently removed to expose the parenchymal tissue, which was homogenized in a sterile mortar and pestle to create a uniform suspension without compromising tissue integrity. To this homogenate, 10 mL of normal saline was added to facilitate dilution, and the mixture was filtered through a fine sieve to obtain a clear filtrate. The filtrate was transferred into a universal sample bottle, followed by the addition of 0.1 mL of Turk's solution for staining, along with 0.1 mL of penicillin-procaine and streptomycin to prevent microbial contamination using procedures adapted from (Ubah *et al.*, 2023).

Effect of *Carica papaya* on Hormones

Blood samples were obtained from each rat through the ocular retrobulbar plexus and collected into gel vacutainer tubes. The samples were centrifuged at 3000 rpm to separate the serum, which was subsequently transferred into plain sample bottles for analysis. Hormonal assays were conducted to determine serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH).

Statistical Analysis

All analyses was performed using statistical software, GraphPad Prism version 9.3.1.471 (GraphPad Software Inc., San Diego, CA, USA). The result were expressed in mean, standard error of mean and graphs. Analysis of Variance was carried out to compare the groups at $p < 0.05$.

Results

Percentage Yield of Plant Material

The *Carica papaya* plant Linn root extract gave a yield of 35.1 % w/w dry extract when macerated in 80 % methanol for 72 hours. The plant extract demonstrated a high concentration of Alkaloids (Carpaine), Flavonoids, Saponins, Sugars, Phenols, Tanins, Terpenoids, Proteins and Quinones. These compounds have high therapeutic value (Adewale *et al.*, 2021; Shittu *et al.*, 2021; Omokore and Alagbe, 2019).

Table 2. Phytochemical Analysis of *Carica papaya* root

Phytochemicals	Test	Result
Alkaloid	Dragendorff's	+++
Flavonoid	NaOH+HCl	++
Sugars	Benedict's	+
Tannis/Phenols	Ferric Chloride	-
Saponins	Frothing	++
Quinone	Borntragers	+
Proteins	Buuret	+
Terpenoids	Salkowski's	++
Carbonhydrates	Molisch	++

Key: +++ = Elevated concentration

++ = Moderate concentration

+ = Reduced concentration

- = Absent

Toxicity effect of *Carica papaya*

The toxicity study revealed that the methanol root extract of *C. papaya* was safe in rats up to 5000 mg/kg orally, with no deaths observed, thereby establishing the LD50 at 5000 mg/kg.

Effect of *Carica papaya* on pH of Sperm

A comparative assessment of sperm pH revealed that while the 200 mg/kg group and the control group showed slightly acidic values, a greater degree of acidity was observed in the 400 mg/kg group and was most pronounced in the dexamethasone-treated group.

Effect of *Carica papaya* and Dexamethasone Gonadal Sperm Reserve

The sperm count analysis (Figure 2) shows distinct group differences. Dexamethasone treatment significantly increased sperm count compared to the control (* $p < 0.05$), indicating a stimulatory effect on spermatogenesis. However, both 200 mg/kg and 400 mg/kg extract groups showed markedly lower sperm counts compared to dexamethasone (** $p < 0.01$), with values closer to the control, suggesting that the extract did not enhance sperm production. No significant difference was observed between the control and extract groups, indicating that the extract neither improved nor worsened sperm output at the tested doses. Overall, while dexamethasone boosted sperm count, the plant extract appeared neutral, failing to support spermatogenesis in contrast to the hormonal changes observed.

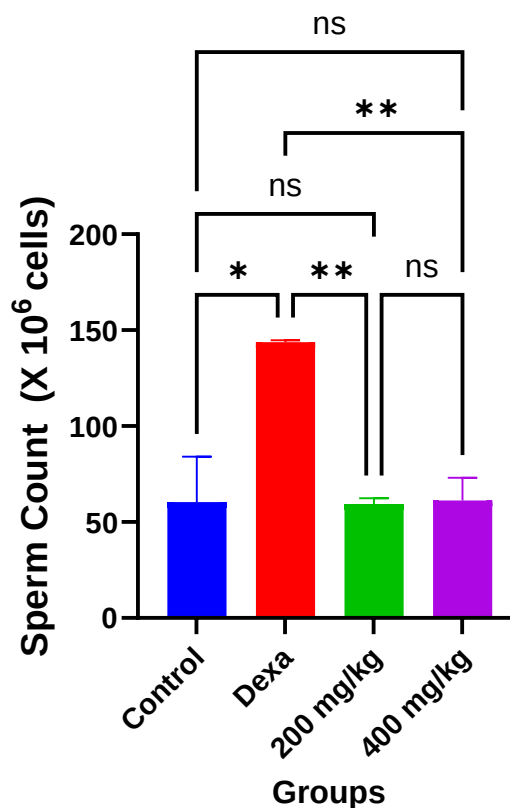


Figure 2: Sperm count of Control, Dexamethasone treated, 200 mg/kg and 400 mg/kg treated groups.

Effect of *Carica papaya* and Dexamethasone on Hormones

The hormonal assay results in Table 2 revealed significant alterations across groups. Dexamethasone administration markedly elevated testosterone levels (4.31) compared to the control (1.10), whereas both extract doses, particularly 200 mg/kg (0.38), significantly reduced testosterone, suggesting a possible suppressive effect of the extract on androgen production. For gonadotropins, FSH levels were very low in the control, dexamethasone, and 200 mg/kg groups (0.009, 0.004, and 0.001, respectively), but significantly increased in the 400 mg/kg group (0.04), indicating a stimulatory effect of the higher extract dose on pituitary function. Similarly, LH levels were elevated in the dexamethasone (0.01) and 400 mg/kg extract groups (0.019), compared to very low values in the control and 200 mg/kg groups, suggesting that dexamethasone and higher extract dose enhanced LH secretion. Overall, the extract appears to have a dose-dependent effect, with the lower dose suppressing testosterone and

gonadotropins, while the higher dose (400 mg/kg) enhanced FSH and LH, potentially modulating reproductive hormonal balance differently from dexamethasone.

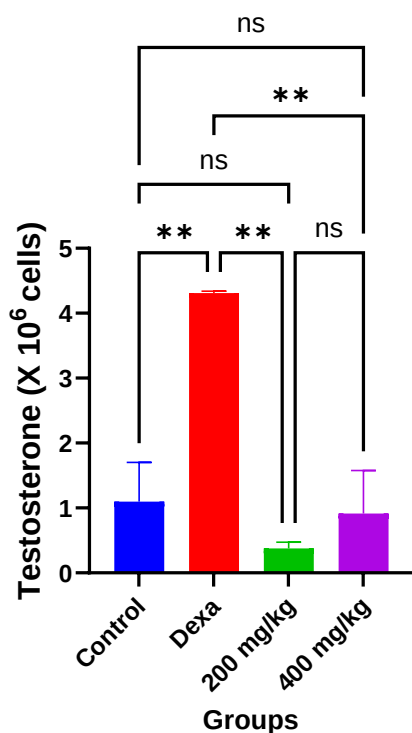


Figure 3

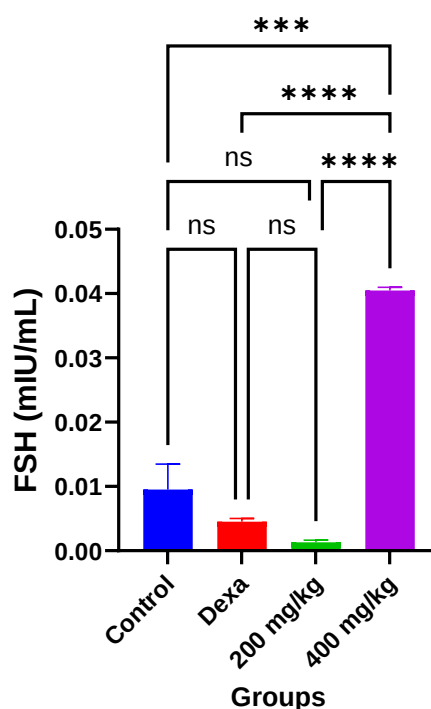


Figure 4

Discussion

The *Carica papaya* root extract exhibited a dose-dependent effect. At 200 mg/kg, it suppressed testosterone levels and did not improve sperm count, indicating a possible anti androgenic or spermatotoxic effect. At 400 mg/kg, the extract increased gonadotropins (FSH and LH) but still failed to enhance spermatogenesis. This aligns with prior studies showing that *C. papaya* extracts, particularly seeds and roots, possess antifertility properties by disrupting the hypothalamic pituitary gonadal axis and impairing sperm function (Nwaehujor *et al.*, 2014; Udoh & Kehinde, 1999).

Assessment of sperm pH revealed that both the control and 200 mg/kg *C. papaya* groups exhibited slightly acidic values within the normal physiological range (Adejuwon *et al.*, 2016), suggesting that low-dose extract does not disturb the seminal environment. In contrast, the 400 mg/kg extract and dexamethasone groups showed progressively higher acidity, with the greatest effect in the dexamethasone group. Lower sperm pH can impair motility and viability, which may contribute to the contraceptive effects observed in these groups (Agarwal *et al.*, 2016). These results suggest that alterations in seminal pH are a potential mechanism through which high-dose *C. papaya* extract and dexamethasone exert reproductive effects.

Conclusion

Conclusively, this study demonstrated that *Carica papaya* methanol root extract exhibit contraceptive potential probably because of the carpaine alkaloid present in the plant root. The extract produced dose-dependent hormonal changes, suppressed testosterone at low doses, and elevated gonadotropins at higher doses without enhancing spermatogenesis, which indicates that the plant material may possess spermatotoxic effects. The acidic pH level of methanol extract of *Carica papaya* on sperm cells suggests that the plant could be a potential contraceptive agent in males.

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