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Original Article

Comparative study on the contraceptive effects of butanol and dichloromethane fractions of *Carica Papaya* male tree bark in male Sprague Dawley rats

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Abstract

BACKGROUND: The use of *Carica papaya* extract in traditional medicine suggests that it might have contraceptive effects on reproductive indices. Traditional healers in Nigeria have used male tree bark of *Carica Papaya* as a source of antifertility agent. . However, there are few information in scientific literature concerning the male tree bark extract of *Carica papaya* and media of preparation.
AIM: The aim of this study was to compare the contraceptive effects of butanol and dichloromethane fractions of male *Carica papaya* tree bark.
METHODOLOGY: The male tree bark of *Carica papaya* was dried and blended into powdery form, then dissolved in water. The solution was fractionated by column chromatography with petroleum ether and further fractionated using butanol and dichloromethane to isolate butanol (CPFB) and dichloromethane (CPFD) fractions respectively. A total of fifteen animals were divided into three experimental study groups A, B and C. Groups A and B received moderate doses of 75 mg/kg body weight of CPFB and CPFD for 28 days respectively while group C received distilled water. Animals were euthanized after 4weeks of administration, the testes were removed, and used for sperm count and motility evaluations. Blood sample were collected via left ventricular cardiac puncture and used to assay testosterone, luteinizing hormone and follicle stimulating hormone levels.
RESULTS: The fractions from both media have contraceptive effect, however the semen analysis of CPFB group showed significantly decrease ($p<0.01$) in reproductive hormones and corresponding decrease in sperm count and motility when compared with the CPFD group. Hence the CPFB group showed more contraceptive potential than the CPFD group when compared with the normal state of the control group.
CONCLUSION: The findings from this present study showed that the butanol fractionation of male sex *Carica papaya* tree bark demonstrated more contraceptive effects than the dichloro methane fractionate, making Butanol a better choice for fractionation in the contraceptive usage of male sex *Carica papaya* tree bark.

Keywords:
Antifertility, *Carica papaya*, Dichloromethane, Butanol, Sperm parameters

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INTRODUCTION

Modern family planning methods that aim to reduce high-risk pregnancies, decrease unsafe abortions, and birth spacing are becoming expensive (Wulifan *et al.,* 2015). Despite advances in contraceptive technology, 214 million women had unmet need for modern family planning in 2017 (Staveteig, 2017; Rademacher *et al*., 2018). Family planning has been promoted through several methods of contraception, although these contraceptives have several side effects as a result of their steroid contents (Ebrahim *et al*., 2018). The side effects have been identified as common reasons why women choose not to use contraceptives (Ebrahim *et al*., 2018). These side effects include; menstrual changes (heavy bleeding, amenorrhea or oligomenorrhea), changes in body weight, headaches, dizziness, nausea, and cardiovascular impacts. In addition, women may harbor fears of long-term effects of contraception, such as; infertility and childbirth complications (Rademacher *et al*., 2018; Beson *et al.,* 2018). A 2014 systematic review found a significant proportion of women that have attributed their unmet need for family planning to fear of side effects: 28% in Africa, 23% in Asia, and 35% in Latin America and the Caribbean (Chola *et al.,* 2015).

Male contraceptive offer potential for a significant shift in the reproductive responsibility between partners, enabling more equitable sharing of contraceptive duties regardless of gender (WHO, 2018). Survey suggest that two-third of women desire more unbiased sharing of contraceptive obligation, indicating substantial support for the development and adoption of male contraceptives (Reed *et al.*., 2023; Kretschmer, 2023). In more than four decades since women got the pill as birth

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control method, men contraceptives have gotten undesirable attention and results. Even though men have engaged in withdrawal method and vasectomy as ways of contraception but have proven to be inefficient and unaffordable respectively (Kretschmer, 2023). Therefore the drive for the search for safe, effective and viable contraceptive agent for men (Udoh *et al.*, 2015). *Carica papaya* (*CP*) plant belongs to the *Caricaceae* family and is commonly called papaya. It is cultivated in most countries with tropical climate (Gideon *et al.,* 2018. The male papaya tree is characterized by absence of fruits with the presence of many small, tubular blossoms, each with about 10 pollen-producing anthers, held in groups. Unlike the female papaya trees with blossoms that are waxy and yellowish-white, held on short-stalked branches (TEO, 2022). The quantitative phytochemistry of male and female tree leaves showed significant difference in their phytochemicals. Total phenolic contents were found to be 38.66 % and 54 % in female and male aqueous leaf extracts respectively while total phenolic contents in ethanol extracts of female and male were found to be 17.33 % and 28.66 % respectively. Phenolic contents in both aqueous and ethanol extracts of male tree leaves were found to be significantly different as compared to female (Syed *et al.,* 2019). More so, carpaine with other phytochemicals such as; papain and carmopapaine have been reported to contribute to the antifertility effects observed in male papaya bark tree and leaves (Julianti *et al.,* 2014; Syed *et al.,* 2019). Traditional healers in Nigeria have used the solutions of male tree bark of *CP* as a source of contraceptive agent, by dissolving the tree bark in water and following an administration procedure that is devoid of precision and accuracy (Aravind *et al.*, 2013). Therefore the need for scientific investigations on different media of solution and precision on the most effective medium that will exert contraceptive effect in the male *CP* tree bark using sperm count, sperm motility and hormonal assay as the study objectives.

MATERIALS AND METHODS

Collection and Preparation of *Carica papaya* (*CP*) Extracts

The bark of the ***C****arica papaya* plant was obtained from a pawpaw tree forest in Lagos and authenticated in the Department of Botany, University of Lagos by Mr. Nodza Gorge, a taxonomist in the Department of Botany, University of Lagos, Nigeria with herbarium number 100312. The bark of *CP* tree chop down using cutlass, dried in an electric over at 40°c for 4 days and crushed to obtain a course provider that was used for the extraction in the pharmacognosy Department of the Faculty of Pharmacy, University of Lagos.

250 g of powder was dissolved into 1000 ml of water. The solution was fractionated using petroleum ether chloroform: ethylacetate (8:2) and ethylacetate: methanol (3:7) as eluents.

Following partial purification, the petroleum ether fraction was divided into two fractions A and B. A was further subjected to butanol and B to dichloromethane fractionation respectively using preparative thin layer chromatography (TLC). Two components were obtained from the sub-fraction viz. CPFB and CPFD. This fractions were named base on the solvent of fractionation i.e the fraction from butanol was called *Carica papaya* fraction and the fraction from dichloromethane was named *Carica papaya* fraction dichloromethane (CPFD).The fractions were dissolved in 0.5% tween 20 before being used (Babalola, 2019).

Experimental Protocol

Fifteen male Sprague-Dawley rats weighing (100 g - 130 g) were used in this experiment. The animals were procured from the animal house of the College of Medicine, University of Lagos, Idi-Araba and kept in well-ventilated cages under room temperature of 12 hour light-dark cycle. Rat pellets and clean water were provided *ad libitum*. The animals were subjected to two weeks acclimatization to laboratory condition before experimentation.

The animals were divided into three study groups A, B and C of five animals each. CPFB and CPFD fractions were administered to groups A and B respectively at a dose of 0.75 ml of 75 mg/kg of each extract/day (Milind, 2011) while group C received the same volume of distilled water. All administrations were done orally daily for 4 weeks. The three groups were subjected to the same feeding regimen and at the end of the 4 weeks experimental period, animals were sacrificed, testes of the control and experimental rats were removed and the epididymis was incised to collect sperm for semen analysis (Osinubi *et al*., 2007). Blood samples were also collected by left ventricular cardiac puncture for hormone profile analysis.

Sperm count evaluation

The testis was harvested and the caudal part known as tail of the epididymis was incised from where spermatozoa is collected for semen analysis. Epididymal spermatozoa (5µl) was diluted in 95µl phosphate buffer saline (1:20 dilution). The well-mixed sperm sample was loaded into the counting chambers of hemocytometer. Hemocytometer was placed on the stage of the microscope with objective lens adjusted to 400× magnification. Hemocytometer was viewed and counting was done. The values were recorded and counting was repeated in each chamber and average count was documented. The number of sperm cells counted was used to calculate total sperm concentration (million/ml) using the formula:

Total sperm concentration= (number of sperm counted × dilution factor × chamber depth) / volume of chamber (Osinubi *et al*., 2007).

The average number of cells, cell density, dilution factor and cell concentration were then calculated by applying the formula below as described for a similar reproductive toxicity study:

- Average number of cell = (Total number of cell counted × Dilution factor)/Number of chambers or field counted
- Cell density = Average number of cells/Volume of chamber)

- Dilution factor = (Initial volume × Dilution 1 × Dilution 2 × Dilution n) / Final volume (Osinubi *et al*., 2007).

Sperm motility evaluation

The caudal epididymis was incised to expose the epididymal fluid. 5 µl of the fluid was collected with a micropipette, and delivered onto a glass slide and covered with a 22 mmX 22 mm cover slip. Each slide was viewed under a light microscope at 400X magnification. The sperm motility estimation was carried out at room temperature between 20°C and 25 °C. At the dimension of 200×10 Microscopic field was scanned systematically (using motility category and motility grade), each spermatozoon encountered was recorded. The motility was calculated in percentage and are classified as progressively motile against non-motile, procedure was repeated and the average was taken (Azu *et al.,* 2011).

Hormonal Assays for Testosterone (TT), Luteinizing hormone (LH) and Follicle stimulating hormone (FSH)

Blood was obtained by left ventricular cardiac puncture and collected into a heparinised bottle. Each blood sample was allowed to clot before Serum samples were collected and assayed for testosterone in batches with control sera at both physiological and pathological levels by Standard Qualitative Enzyme linked immunosorbent assay (EL-ISA) technique with Microwel kit from Syntrobioreseach Inc. California, U.S.A. 10µl of standard sample and control were dispensed into desired number of coated wells. 100 µl of testosterone- conjugated reagent was dispensed into each well followed by 50µl of antitestosterone reagent. The contents of the well were mixed vigorously for 30 seconds and incubated for 90 min at room temperature. Later, the wells were washed with distilled- dionised water. 100 µl of testosterone binding reagent was dispensed into each well and incubated for 20 mins. Reaction at this level was terminated with 100µl of IN HCL acid and color intensity measured on a micro well automatic reader El Measurement of organ weight times 180 (Osinubi *et al*., 2007).

Statistical Analysis

ANOVA with Scheffe's post-hoc test was used. All values were expressed as mean ± standard deviation. The Data obtained from sperm count and motility of the CPFB, CPFD and the control animals were pooled and expressed as mean ± SD (standard deviation). The differences between the control group and those of CPFB and CPFD was analyzed statistically by Scheffe's *post-hoc* test and the values of $p < 0.01$ was taken to imply statistical significance.

RESULTS

Sperm Count and Motility

The CPFB group shows low sperm count that is statistically significant ($p<0.01$) when compared with the control group. The CPFD group showed no significant different in sperm count when compared with control group as shown in Table 1. The CPFB group showed reduction in motile sperm which is highly significant ($p<0.01$) after four weeks of administration. The group CPFD of the extract showed no significance ($p<0.001$) in sperm motility as compared with the control group as shown in Table 1.

Table 1: Comparing the effects of CPFB and CPFD Extracts on Sperm Motility and Sperm Count in Male Sprague Dawley Rats.

GROUP	COUNT (mill/ml)	MOTILITY (%)
CONTROL	105.6±1.94	87.75±3.33
BUTANOL	71.56±2.07***	39.50±2.02***
DCM	98.13±2.63	67.50±6.85

Mean ± SD (n= 5) using ANOVA with Scheffe's *post-hoc* test; Values are represented in mean± SD (standard deviation) of 5 observations; using ANOVA with Scheffe's post-hoc test; *** indicates significance from control @$p<0.01$.

Hormonal Assays

The serum level of testosterone (TT) in CPFB group no significant difference while CPFD caused significant increase in serum testosterone level when compared to the control group as shown in the table 2 below. The CPFB and CPFD both showed statistically significant decrease in LH after administration for 4 weeks although the decrease is more significant in CPFB than in CPFD as seen in the table 2 below. The serum FSH level was significantly increased in the CPFD group and significantly decreased in the CPFB group when compared with the control group.

Table 2: Effects of CPFB and CPFD Extracts on TT, LH and FSH levels in Male Sprague Dawley Rats

GROUP	TT(ng/ml)	LH(ng/ml)	FSH(ng/ml)
CONTROL	0.58±0.12	4.6±0.06	3.73±0.20
CPFB	0.59±0.30	2.1±0.06***	2.80±0.46
CPFD	0.75±0.58**	3.5±0.17*	4.10±0.58

Values are represented in mean±SD (standard deviation) of 5 observations; ***indicates significance from control group @$p<0.01$ and insignificance @$p<0.001$ respectively.

DISCUSSION

This study was designed to compare the contraceptive effects of two fractions vis: the butanol fractionated solution and the dichloro methane fractionated solution of male *Carica Papaya* tree bark on the sperm parameters and reproductive hormones. The different parts of *Carica papaya* (seed, leaf and bark) have been documented to have contraceptive properties because of their phytochemicals such as; papain, carpaine and carmopapaine (Syed *et al., 2019*). The two fractions of the *Carica papaya* male tree bark extracts (CPFB and CPFD) showed contraceptive properties, although CPFB showed more contraceptive effect than the CPFD fraction. The effects of the fractions differ regarding the gonadotropins (LH, FSH and TT) levels as Butanol *Carica papaya*

fractionated extracts showed decrease in LH and FSH but an increase in serum TT level against the dichloro methane fractionated *Carica papaya* male tree bark extract which showed decrease only in serum LH but an increase in both FSH and TT levels. This proves that CPFB has more contraceptive effects due to its effect on the gonadotropins (LH and FSH) causing corresponding impact in semen analysis. The findings from this study support previously suggested findings that *Carica papaya* bark extract (ethyl acetate, butanol, dichloro methane and N-hexane fractioned) significantly affect the levels of gonadotropins (LH and FSH) leading to a decrease in sperm count and motility of testicular cells (Kushemiju *et al.,* 2019).

It is well known that the dysregulation of the gonadotropins have negative relative effect on spermatogenesis (Azu *et al.,* 2011; Oduwole *et al.*, 2018 ;Goldberg *et al.*, 2022). The findings from this study showed that the dysregulations of serum gonadotropins caused by the administration of butanol fractionated extract of *Carica papaya* male tree bark, negatively affect the process of spermatogenesis exhibited in reduction of sperm count and motility exerting contraceptive potency of the fractionate. The study also showed that dichloro methane fractionated *Carica papaya* bark extract has very low contraceptive effect as compare to the butanol fractionated one due to its insignificant effects on the gonadotropin hormones hence relative insignificant effect on spermatogenesis.

Conclusion: The butanol fraction of male *Carica Papaya* bark extract has more contraceptive potential than the dichloromethane fraction on testicular semen analysis and some reproductive hormones following 4weeks of administration.

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