

EFFECT OF CRUDE EXTRACT OF *Carica papaya* SEEDS ON THE REPRODUCTIVE EFFICIENCY OF MALE ALBINO RATS

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ABSTRACT

In this investigation, the effect of crude extract of *Carica papaya* (Linn Caricaceae) seeds was examined on the reproductive efficiency of male albino wistar rats. The ethanolic extract of *C. papaya* seeds was Soxhlet extracted. Three doses were estimated in acute toxicity test and administered orally, daily for three (3) days to four (4) groups of adult male rats weighing between 180-200g. Group one received normal saline (1ml/rat/d) as control, group two received 50mg/kg/d, while groups three and four received 100mg/kg/d and 150mg/kg/d, respectively. After treatment for three days the male rats of each group were mated with the normal female rats in the ratio of 1:1. Mating was confirmed by a vaginal plug. After mating, three male rats from each group were sacrificed and semen samples were quickly collected from the epididymes of each of the rats. Sperm count, motility and morphology, litter weights and sizes were measured. Results showed significant decrease in sperm cell count, motility and sperm head abnormality as compared to control. The result further revealed that mean litter sizes, litter weights at birth and weaning were significantly different ($P < 0.05$) among treatment groups. These results therefore allowed the suggestion that the ethanolic extract of the seed of *C. papaya* could probably affect the reproductive efficiency of male rats.

KEYWORDS: *Carica papaya* seed, albino rat, Reproductive efficiency, Sperm profile, epididymis.

INTRODUCTION

Reproductive efficiency of animals lies on their potency in discharging their reproductive functions, which however borders on the ability to procreate. For instance, in male animals, if spermatogenesis pathway is truncated by any genetic, physiological or hormonal factor, efficiency in reproduction is drastically hampered. Several plants have been identified to possess anti-fertility or contraceptive property (Udoh and Kehinde, 1999).

Carica papaya from the family of caricaceae is a perennial, seed propagated plant, which originated from Central America but now found everywhere. It is a plant that has been economically exploited. The fruits, leaves, latex, roots and seeds contains novel biologically active compounds such as saponin, glycoside, polyphenol, carpaine, tannin, papain, flavanols, alkaloids, quinions, etc (Franco et al., 1993; Udoh and Udoh, 2005). These bioactive compounds have been exploited extensively in the treatment of several ailments, which include arthritis, rheumatism, tuberculosis, malaria, cancer, among others (France et al., 1993; Adebisi et al., 2002).

Pathak et al. (2000) administered, orally, chloroform extracts of ripe seeds of *C. papaya* to rats for 30 days. It was found to cause infertility and irregular estrous cycle in females, decrease in sperm mortality, testis mass and sperm count (Pathak et al., 2000). Verma and Chinoy (2001) reported that aqueous extract of *C. papaya* seeds caused significant reduction in total protein and sialic acid content in both epididymal fluid and sperm pellets as compared with the control. Verma and Chinoy (2002) in a follow up experiment assessed the effect of *C. papaya* seeds extract on germinal epithelium and germ cells, revealed that oral administration of 100mg/kg BW of crude ripe seeds in male rats for 8 weeks caused degeneration of germinal epithelium and germ cells, reduction in the number of Leydig cells and presence of vacuoles in the tubules. Pathak et al. (2000) reported that total suppression of cauda epididymal sperm count, viability and an increase in percentage abnormal spermatozoa were observed

in male rats treated with 5 and 10mg/kg of benzene chromatographic fraction of chloroform extract for 60-150 days.

Based on the above facts about the activity of *C. papaya* seeds extract, it becomes necessary to carry out this investigation to ascertain the effect of the extract, *C. papaya* on the reproductive efficiency of male rats.

Preparation of plant extract

Mature fruits of *C. papaya* (paw-paw) were collected from Akansoko in Akpabuyo local government area, Cross River State, Nigeria between the months of January and March. The ripe fruits were cut open and the seeds carefully removed, washed to remove debris and sun dried for about 8 hours. The dried seeds were further subjected to oven drying at 40°C for 24 hours. The seeds were then pulverized into powder using electric blender (Model 4250 Braun, Germany). The powdered sample was soxhlet extracted in petroleum ether (M&B, UK) for 8 hours to remove the fats, and residue was re-extracted in ethanol for 72 hours. The ethanolic extract was suspended in chloroform and partitioned in equal volume of water overnight. The aqueous extract fraction was evaporated *in vacuo* at 45°C using Rotary evaporator (Sigma, USA).

About 1g weight of the dry ethanolic extract was dissolved in 10ml of normal saline to give 100mg/ml, which serves as the stock concentration.

Animals

Adult male rats (20) and adult female rats (20) weighing between 140-180g were obtained from the Biological Science Animal House of the Faculty of Science, University of Calabar, Nigeria. The rats were housed in a ventilated room at 30° under a 12h light-dark cycle. The rats were allowed to acclimatize for 7 days before the commencement of the experiment, and they had free access to laboratory feed (Agro feeds, Calabar, Nigeria) and water.

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Treatment

Twenty male rats were divided into four groups of 5 male rats per group. Group I served as control and received orally 0.2ml of corn oil while groups II, III and IV received orally 50mg/kg/d, 100mg/kg/d and 150mg/kg/d of the extract of *Carica papaya* for 3 days respectively. At the end of the treatment, the male rats in all the groups were mated with the normal adult fertile (untreated) female rats in the ratio of 1:1. After 72hrs contact time, the treated male rats were separated from the normal non-treated female rats. The mated female rats were under observation for 3 gestation periods.

3 male rats from the control group I and groups II to IV treated with the extract of *C. papaya* were sacrificed by cervical dislocation and epididymes dissected out for sperm analysis.

Similarly, 2 mated female rats from each of the experimental groups were sacrificed and dissected for dominant lethal mutation index (DLMI) estimated per uterine horn. The remaining female rats were allowed to go through full gestation period.

Analysis of semen

Epididymes from each rat was removed, weighed and minced with fine scissors in physiological or normal saline. After vigorous pipetting, the suspension was separated from tissue fragments by filtering through a 80µm stainless mesh. Then the sperm yield per milligram of epididymis) was determined using Improved Neubauer haemocytometer (Ekaluo et al., 2005).

The sperm suspension was diluted and dropped on a glass slide and viewed under light microscope to determine motile and non motile sperm cells. Motile and non-motile cells observed were expressed as percentages. (Ekaluo et al., 2005).

A fraction of each suspension was mixed with 1% eosin Y solution (10:1) and allowed to react for 30 minutes. The slides were examined under light microscope (Ekaluo et al., 2005).

Dominant lethal mutation index (DLMI)

Total implants which comprised both live implants and early foetal deaths (death implants) were scored. The induced dominant lethal mutation index was calculated for each of them, using the formula:

$$1- \frac{\text{Mean live implant in treated group}}{\text{Mean live implant in control group}}$$

(Odeigah, 1997)

Statistical analysis

Data collected were analyzed using analysis of variance (ANOVA) and the mean separated using least significant difference (LSD). Sperm motility was analyzed using contingency chi-square (χ^2) test. (Obi,2002).

RESULTS

The mean sperm cell counts ranged between 45190±19 and 38930±16 across the treatment groups with the mean of means 45213±14 (control), 4380±12 (group II), 41600±12 (group III) and 38897±22 (group IV), respectively. There was significant difference ($P < 0.05$) in the mean sperm cell counts among the treated groups, which was dose-dependent.

The mean percentage motile sperm cells were 85.50±1.5% for control while 52.83±1.0%, 46.83±1.8% and 34.17±0.9 were recorded for group I, II, III and IV, respectively. The result showed that there was a decrease in percentage sperm motility, which was dose-dependent.

Our results also showed that short hook, dunce cap, nubbed hook and long hook were the abnormalities observed, with short hook and dunce cap in highest frequency.

The total percentage frequency of sperm head abnormality increased from control group to group IV (2%, 4%, 7% and 10.5%)

Mean litter sizes at birth and weaning across the treatments were significantly different ($P < 0.05$). This suggested that the higher the concentration of the extract, the lower the fertility potential of the male.

Table 1: Effect of the extract of *C. papaya* treatment on sperm cells count in rats.

| Rep | Group I | Group II | Group III Control | Group IV |
|-----|------------|------------|----------------------|------------|
| 1. | 45,190d±19 | 43,820c±15 | 41,570b±21 | 38,860a±27 |
| 2. | 45,210d±26 | 43,830c±14 | 41,600b±34 | 28,900a±16 |
| 3. | 45,240d±20 | 43,870c±23 | 41,610b±12 | 38,930a±16 |

* Means followed with the same case letter in a given horizontal array indicate no significance ($P > 0.05$).

Table 2: Effect of *C. papaya* extract treatment on percentage sperm cell motility

| Treatment groups | No of spermatozoa observed | %mean motile | %mean non-motile |
|------------------|----------------------------|--------------|------------------|
| I (Control) | 200 | 85.5± 1.5 | 14.5±1.0 |
| II | 200 | 52.83± 1.0 | 47.17±1.5 |
| III | 200 | 46.83± 1.8 | 53.17±1.2 |
| IV | 200 | 34.17± 0.9 | 65.83±2.0 |

Table 3: Effect of *C. papaya* extract treatment on percentage sperm head abnormality

| Sperm head abnormalities | Group I | Group II | Group III | Group IV |
|--------------------------|-----------|-----------|-----------|-----------|
| Short hook | 1.5a±0.05 | 2.0a±0.3 | 3.0a±0.07 | 4.5b±0.6 |
| Dunce cap | 0.5a±0.4 | 1.0a±0.06 | 2.0a±0.03 | 2.5b±0.1 |
| Notched hook | 0.0a±0.0 | 0.5a±0.5 | 1.5±0.05 | 2.0b±0.6 |
| Long hook | 0.0a±0.0 | 0.5a±0.3 | 0.5a±0.04 | 1.5a±0.05 |

* Means followed with the same case letter in a given horizontal array indicate no significance (P > 0.05).

Table 4: Effect of *C. papaya* extract treatment on litter size and weights at birth and weaning (gram)

| Parity | Size | Group I | | Size | Group II | | Size | Group III | | Size | Group IV | |
|--------|----------|----------|------------|----------|-------------|------------|---------|-----------|------------|----------|----------|-----------|
| | | Weight I | Weight II | | Weight I | Weight II | | Weight I | Weight II | | Weight I | Weight II |
| 1 | 9.33±1.2 | 51.3±4 | 226.26±9 | 8.33±9 | 58.4±7 | 186.32±11 | 4.0±8 | 31.5±9 | 115.1±8 | 1.0±0.1 | 12.0±3 | 63.72±6 |
| 2 | 9.69±9 | 70.2±9 | 251.910 | 6.67±1.3 | 41.4±8 | 135.18±9 | 2.33±3 | 25.0±5 | 58.72±2 | 1.33±9 | 13.5±6 | 08.04±7 |
| 3 | 6.7±.9 | 74.0±.1 | 220.14±.8 | 6.0±6 | 31.8±6 | 134.5±.9 | 2.67±.8 | 24.8±.7 | 108.35±.7 | 1.33±.2 | 18.0±.4 | 66.69±.8 |
| | 8.57d±.9 | 65.17c±1 | 232.77c±11 | 7.0c±.7 | 43.87abd±.9 | 152.03bd±9 | 3.0b±.5 | 27.1a±.7 | 103.6abd±5 | 1.22a±.1 | 4.5a±.6 | 70.15a±5 |

• P > 0.05.

W1 = Weight at birth
WII=Weight at weaning

DISCUSSION

The administration of *C. papaya* seed extract to male albino rats for 3 days was observed to cause a significant reduction in the mean sperm count. This confirms the reports of Chinoy et al. (1995), Joshi and Chinoy (1996), Pathak et al. (2000), Udoh and Kehinde (1999). The dose-dependent effect of *C. papaya* seed extract on sperm count also agrees with the report of Lohiya et al. (2000). It could be that the exposure of the animals to the extract might have produced pituitary-hypothalamic or sex hormonal effect, which might have affected the process of spermatogenesis (Letz, 1990, Hole, 1993). It is also possible that the reduction in sperm count might have been caused by the bioactive chemicals in *C. papaya* seed extract during the spermatogonial stage (Sharpe, 1992).

Our results on sperm motility agree with the reports of Setty et al. (1977); Verma and Chinoy (2001); and Lohiya et al. (2000).

The results of the sperm morphology are similar to the report of Pathak et al. (2000), which stated that the abnormalities observed in the sperm cell morphology might have been as a result of alteration or mutation in testicular DNA that in turn disrupted the process of spermatzoa differentiation (Lister and McLean, 1997). Inferior sperm production could cause reproductive dysfunction (Randall et al., 2000). On the other hand, sperm count is one of the most sensitive tests for spermatogenesis and is highly correlated with fertility and conception rate of the females (Meistrich et al., 1982). It is therefore possible that inferior sperm cells (sperm head abnormality) and decrease in sperm count might have caused the difference in the litter sizes of the female rats mated with the treated male rats.

The results allow the conclusion that the extract of *C. papaya* possesses male contraceptive property.

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