SPERMICIDAL AND HORMONAL RESPONSE OF WISTAR RATS TREATED WITH ETHANOL SEED, LEAF AND PULP EXTRACTS OF CARICA PAPAYA (LINN)

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ABSTRACT

Majority of the world’s population is dependent on traditional medicine, which is particularly true for poorer sections of the population in the developing countries. This reliance is not without its attendant problems and benefits. This research was therefore, undertaken to evaluate the spermicidal and hormonal effects of C. papaya seed, leaf and pulp extracts on Wistar rats. Seed, leaf and pulp extracts at 100, 200 and 300 mg/kg BW of rats each were orally administered for 30 days. The rats were arranged in a 3x4 factorial experimental layout using completely randomized design (CRD). Blood samples collected from the various treated male rats in different treatment groups were used for hormonal assay. The results showed that there were significant effect (P < 0.05) of C. papaya (seed, leaf and pulp) extracts on sperm count, sperm motility and hormonal profile of rats. The results showed that seed, leaf and pulp extracts of C. papaya have anti-fertility, and also enhanced the production of reproductive hormones especially, the pulp extract.

Key words: Carica papaya, sperm count, hormonal profile, rats.

INTRODUCTION

It has also been surmised that synthetic drug consumers in developed countries are becoming disillusioned and disenchanted with modern health-care and are therefore seeking alternatives. This “paradigm shift” and recent resurgence of plant remedies however, is brought about by several factors, which include; the effectivenes of plant remedies/ medicines, side effect of most synthetic drugs, low toxicity of some plant extracts and the development of science and technology to tackle health related problems (Kong et al., 2003). Interestingly, attention has been given to the medicinal value of herbal remedies for safety, efficacy and economy (Glombitza et al., 1993; Mahabir and Gulliford, 1997).

Carica papaya is a plant that needs to be elaborately researched upon, because of its anti-fertility and pharmaceutical potentials (Udoh and Kehinde, 1999; El-Moussaoui et al., 2001; Lohiya et al., 2002; Ikpeme et al., 2007). C. papaya is a soft-wooded perennial plant that has a life span of 5-10 years, although commercial plantations are usually replanted (Chay-Prove et al., 2000). It normally grows as a single-stemmed tree with a crown of large palmate leaves emerging from the apex of the trunk, but plant stands may become multi-stemmed when damaged (Villegas, 1997). Papaya flowers are borne on inflorescences which appear in the axis of the leaves. Female flowers are held close to the stem as single flowers or in clusters of 2-3 (Chay-Prove et al., 2000). Nutritionally, Carica papaya fruit is a good source of calcium and an excellent source of vitamins A and C (Nakasone and Paull, 1998). Oloyede (2005) analyzing the chemical and mineral composition of unripe pulp of Carica papaya reported that potassium, calcium, and magnesium were in high proportion. It may not be far-fetched to assert that distortion in fertility in male mammals such as rat is directly correlated with the distortion or disruption of spermatogenesis and the hormone regulatory system. The purpose of this study therefore, was to evaluate the efficacy of C. papaya seed, leaf and pulp extracts on the reproductive performance of male rats, where there are paucity of information especially, on the leaf and pulp extracts. This was to have a holistic overview of the plant.

MATERIALS AND METHODS

Plant collection and preparation of extraction

Seeds, leaves and unripe pulp of C. papaya (Solo variety) used for the study were collected from State Housing Lane, Ediba, Calabar and certified in the herbarium unit of the Department of Botany, University of Calabar, Calabar, Nigeria. The seeds and leaves were washed and dried at
room temperature (25°C) while the pulp was oven dried (Continent, MG800G) at 40°C for 15 minutes. The dried plant materials were then pulverized with an electric blender (4250 Braun, Germany) to a powdery form. Each plant material was subjected to ethanol extraction in a soxhlet extractor (M & B, UK). The pastry samples were further concentrated in a rotary evaporator (Sigma, USA) at a controlled temperature of about 70°C for 1 hour. The semi solid residue from the extracts was the stored at 5-6°C in the refrigerator. One gram of each extract was dissolved in 100 ml of normal saline to give 100 mg/l.

Experimental animal and administration of extract

Seventy-two healthy wistar rats of about two months old weighing between 150-180 g were obtained from the Animal Unit, Zoology and Environmental Biology Department, University of Calabar, Calabar. They were housed in cages under standard laboratory conditions of temperature range of 25-29°C and 12h light/dark cycle throughout the experimental periods. The rats were left to acclimatize for two weeks with free access to water and feed. Four experimental groups of six rats each per extract were used for the study in 3x4 factorial experimental layouts using completely randomized design. Male rats in group 1 served as control and received 1 ml of normal saline and normal chow. Rats in group 2 received 100 mg/kg BW while rats in groups 3 and 4 received 200 mg/kg BW and 300 mg/kg BW of each extract, respectively for 30 days through oral gavage. Blood samples were collected from the treated male rats through cardiac puncture under chloroform anesthesia. The male rats were then sacrificed.

Estimation of mean sperm count

Epididymes from the three rats in the different treatment groups were surgically removed and weighed. The epididymes were carefully macerated to release the semen containing sperm cells (Ekaluo et al., 2008). Each petri dish containing the epididymes from each treated group was diluted with 0.1 ml of normal saline. The diluted semen was transferred into a specimen bottle and incubated for 30 minutes. After this, the semen was introduced gently into the Improved Neubauer chamber and covered with a cover slip and the sperm cells counted in the chamber (WHO, 1992).

Evaluation of sperm motility

Immediately after dissection, the epididymal contents were dropped on a glass slide and viewed under the light microscope to determine the motile and non-motile sperm cells. The motile and non-motile sperm cells were distinguished by the movement of sperm cells (WHO, 1992).

Hormonal assay

The blood samples were spun at 2500 rpm for 10min using Wisperfuge model 1384 centrifuge (Tamson, Holland) at 10-25°C. Serum samples were assayed for levels of testosterone, follicle stimulating hormone (FSH), luteinizing hormone/interstitial cell stimulating hormone (LH/ICSH), estrogen (estradiol) and prolactin using the Microwell enzyme linked immunoassay (ELISA) technique; using analytical grade reagents (Syntron Bioresearch Inc., USA) (Ekaluo et al., 2010).

Data collection and analyses

Data from semen and hormonal analyses were subjected to the analyses of variance (ANOVA) while differences in means were separated using least significant difference (LSD) according to Obi (2002).

RESULTS

Effect of C. papaya seed, leaf and pulp extracts on sperm count.

Result indicates that there were significant differences (P < 0.05) in the sperm count of male rats, which were dose-dependent. At 100 mg/kg BW, the seed and pulp extracts treated rats had lower sperm count than did the rats treated with leaf extract. There were no significant differences (P > 0.05) amongst the sperm count of rats treated with 200 and 300 mg/kg BW of the three extracts. Increasing dosage of the three plant part extracts caused a significant reduction in the sperm count, which was lowest at 300 mg/kg BW (Table 1 & Fig. 1).

![Fig. 1: Effect of seed, leaf and pulp extracts of C. papaya on the sperm count of rats](image)

Effect of C. papaya seed, leaf and pulp extracts on sperm motility

Sperm motility result for male rats showed that there were significant differences (P < 0.05) in the different C. papaya extracts administered and this effect was dose-dependent. It also showed that rats treated with the seed extract had the highest mean non-motile sperm cells of
Table 1: Effects of seed, leaf and pulp extracts of C. papaya on sperm count and hormonal parameters of male wistar rats

<table>
<thead>
<tr>
<th>Extract</th>
<th>Dosage (mg/kg BW)</th>
<th>Sperm count (x10^3/µl)</th>
<th>LH (mIU/ml)</th>
<th>FSH (mIU/ml)</th>
<th>TST (ng/ml)</th>
<th>PLT (ng/ml)</th>
<th>EST (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>0</td>
<td>7.3±0.02</td>
<td>1.23±0.06</td>
<td>1.93±0.04</td>
<td>0.80±0.01</td>
<td>4.80±0.50</td>
<td>0.4±0.01</td>
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<tr>
<td></td>
<td>100</td>
<td>6.2±0.03</td>
<td>1.40±0.07</td>
<td>1.67±0.51</td>
<td>0.90±0.30</td>
<td>5.33±0.23</td>
<td>0.8±0.03</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>5.4±0.10</td>
<td>1.57±0.09</td>
<td>1.40±0.53</td>
<td>0.93±0.03</td>
<td>5.47±0.42</td>
<td>0.9±0.02</td>
</tr>
<tr>
<td></td>
<td>300</td>
<td>3.0±0.20</td>
<td>1.67±0.28</td>
<td>1.27±0.60</td>
<td>0.97±0.02</td>
<td>6.30±0.25</td>
<td>1.2±0.04</td>
</tr>
<tr>
<td>Leaf</td>
<td>0</td>
<td>7.3±0.02</td>
<td>1.23±0.04</td>
<td>1.93±0.04</td>
<td>0.80±0.01</td>
<td>4.80±0.50</td>
<td>0.4±0.01</td>
</tr>
<tr>
<td></td>
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<td>7.1±0.30</td>
<td>1.47±0.05</td>
<td>2.0±0.06</td>
<td>1.87±0.40</td>
<td>4.30±0.60</td>
<td>0.6±0.03</td>
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<tr>
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<td>200</td>
<td>4.6±0.12</td>
<td>1.67±0.04</td>
<td>2.23±0.07</td>
<td>2.0±0.80</td>
<td>3.7±0.70</td>
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<tr>
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<td>300</td>
<td>3.0±0.02</td>
<td>1.70±0.02</td>
<td>2.30±0.28</td>
<td>2.8±0.51</td>
<td>3.57±0.40</td>
<td>1.1±0.06</td>
</tr>
<tr>
<td>Pulp</td>
<td>0</td>
<td>7.3±0.02</td>
<td>1.23±0.06</td>
<td>1.93±0.04</td>
<td>0.80±0.01</td>
<td>4.80±0.50</td>
<td>0.4±0.01</td>
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<tr>
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<td>1.40±0.06</td>
<td>2.20±0.04</td>
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<td>2.60±0.09</td>
<td>3.57±0.45</td>
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<tr>
<td></td>
<td>300</td>
<td>3.5±0.23</td>
<td>1.77±0.04</td>
<td>3.13±0.46</td>
<td>3.83±0.29</td>
<td>6.17±0.80</td>
<td>3.5±0.07</td>
</tr>
</tbody>
</table>

*Means followed with the same letter along vertical array indicate no significant difference (P > 0.05).

Table 2. Effects of seed, leaf and pulp extracts of C. papaya on the percentage sperm motility

<table>
<thead>
<tr>
<th>Treatment groups (mg/kg BW)</th>
<th>Number of spermatocoea observed</th>
<th>% Mean motile sperm cells.</th>
<th>% Non-motile sperm cells.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Seed</td>
<td>Leaf</td>
</tr>
<tr>
<td>Control</td>
<td>200</td>
<td>85.5±2.12</td>
<td>87.3±2.43</td>
</tr>
<tr>
<td>100</td>
<td>200</td>
<td>52.83±3.21</td>
<td>51.46±4.21</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>46.83±0.94</td>
<td>50.63±2.75</td>
</tr>
<tr>
<td>300</td>
<td>200</td>
<td>34.17±1.33</td>
<td>35.18±1.98</td>
</tr>
</tbody>
</table>

*Means followed with the same letter indicate no significant difference (P > 0.05).

65.83%, followed by those with the leaf and pulp extract treatments had 64.82% and 59.44%, respectively (Table 2).

Effect of C. papaya seed, leaf and pulp extracts on hormonal profile

Seed, leaf and pulp extracts of C. papaya significantly (P < 0.05) affected the reproductive hormones assayed at varying treatment concentrations. The seed extract reduced the level of FSH as the dosage increased while the leaf and pulp extracts increased its level with increasing concentration of treatment (Table 1). The result also indicated that at 300 mg/kg BW, the leaf and pulp extracts had the same effect on LH while the seed extract had a lowering effect.

The pulp extract significantly (P < 0.05) increased testosterone secretion in the rats more than extracts of leaf and seed, especially at 300 mg/kg BW. The seed extract and pulp extracts enhanced the production of prolactin while the leaf extract reduced it with increasing extract dosages. It was observed from the result that there was no significant difference (P > 0.05) on the level of prolactin in the rats administered with 200 mg/kg BW for seed extract and 100 and 200 mg/kg BW of the pulp extract. Though there were significant differences observed on the level of estradiol in the rat, the seed and leaf extracts did not significantly (P > 0.05) differ in their effect on the hormone with increasing dosage. Generally, the pulp extract had a more boosting effect on the reproductive hormone than other extracts.

DISCUSSION

Mohammed et al. (2004) reported that the cellular alteration of the testicular cells maligned spermatogenesis. The administration of the C. papaya extracts significantly (P < 0.05) reduced the sperm count from 7.3 x 10³/ml to 3.0 x 10³/ml in the rats treated with 300 mg/kg BW of the extract (Table 1). It does show that there could have been alterations of the cellular machinery underlying the production of sperm cells. Though the exact mechanism of these effects is not clear, it might be due to substances which are present in the extract as reported by Chino et al. (1995); Joshi and Chino (1996) and Pathak et al. (2000). It is possible that the increase in the extracts concentration may have resulted in the alteration of the testicular DNA. The dose-dependent effects of C. Papaya extracts on sperm count are similar to those reported by Lohiya et al. (2000). Sharpe (1992) pointed out that the chemical action during the spermatogonial phase will probably have great effect on sperm output than chemical action during the spermatid phase. Probably, the bioactive compounds in the C. papaya extracts might have exerted their effect at the spermatogenic stage of spermatogenesis.

Result showed that the seed, leaf, and pulp extracts of C. papaya significantly affected the motility of sperm cells (Table 2). Verma and Chino (2001) reported a decrease in sperm motility in male albino rats treated with C. papaya. The result on the sperm motility indicated that though there was significant effect of the extracts, the leaf and pulp extracts affected the sperm motility more than did the seed extract. It might be that the chemicals...
contained in the extracts came in contact with seminal fluid resulting in functional or structural impairment of sperm cells (Letz, 1990). It is also possible that the bioactive compounds might have caused immobilizing or weakening effects on the sperm cells and those bioactive compounds responsible for the reduction in sperm count may not be involved in the immobilization of sperm motility. This could explain the discrepancy noticed between the sperm count and motility. The understanding therefore is that some bioactive substances in *C. papaya* extracts may have permeated the blood-testis barrier, leading to sperm motility reduction.

It is obvious that any alteration either in the cells that produce reproductive hormones or in the pathway of synthesis will inevitably affect spermatogenesis, and consequently sperm count, therefore the extracts may have acted indirectly via the hypothalamo-hypophyseal-gonadal axes. Result of the hormonal analysis showed that the extracts significantly (P < 0.05) enhanced or inhibited the hormonal profiles of the rats. Specifically, the result suggests that the pulp extract increased the secretions of FSH, testosterone and estradiol when compared to those of LH/ICSH and prolactin. It is probable that the bioactive compounds in the pulp extract had enhanced LH/ICSH secretion which in turn stimulated the interstitial cells of the testes to increase the production of testosterone (Table 1) (Gelain et al., 2005). It has also been reported that testosterone synergizes with FSH in spermatogenesis and spermiogenesis (Greenspan and Stawler, 1997; Gelain et al., 2005). The testosterone enhancing ability of the pulp extract did not influence significantly the sperm count. It appeared that the amount of testosterone that was increased as a result of the pulp extract was not enough to cause an increase in the sperm count. The level of prolactin was affected more by the seed, leaf and pulp extracts compared to the pulp extract. This agreed with the report of Pastuszewska et al. (2006). One can thus infer that these bioactive components might have caused the positive increase in the hormonal levels. Since tannins are not present in the pulp extract, this could be the reason the pulp extract had no significant effect on the level of prolactin.

It is an implicit indication that the level of testosterone, estradiol and FSH might be enhanced by the administration of the pulp extract. According to Mooradian et al. (1987), testosterone is required for the growth, development and maintenance of male reproductive organs. It then implies that using pulp extract of *C. papaya* to enhance testosterone production could be a worthwhile venture to ensure a good fertility. This goes to suggest that normal hormonal secretion in any animal system might not be all it takes for effective and efficient functioning of the reproductive system, especially during spermatogenesis. Our results showed that seed, leaf and pulp extracts of *C. papaya* have anti-fertility properties, but might also enhance the production of the sex hormones especially, the pulp extract.

**REFERENCES**


