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Heamatologic Indices and Anti-Spermatogenic Effects of Dietary Supplemented *Carica papaya* seeds on Wister Rats

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ABSTRACT

The continuous usage of indegigenous plants as an intervention tool for diseases that affect both humans and animals is as old as orthodox medicine in its self in this study we evaluated the overall effect of carica papaya seeds extract on wister rats to determine its heamatologic stimulatory effect and the anti-spermatogenic effect. A total of eighty rats where orally administered the various doses of the extract over a period of time and the heamatologic and spermatologic parameters where analyzed. From the results obtained there was a slight stimulatory effects as the total white blood cell increased according to the varying doses of 100mg and 200mg respectively (6.56 ± 0.38 and 6.96 ± 0.10) with mean packed cell volume of 38.0 ± 0.84 and 37.4 ± 1.03 also noticeable is the slight rise in the Lymphocytes from the control group to the varying doses of exposure 70 ± 2.06 and 68.6 ± 1.12 , the total Sperm cell count across the varying doses where $11.6 \times 10^6 + 0.5 \text{ cells/mm}^3$ and $11.3 \times 10^6 + 0.5 \text{ cells/mm}^3$ respectively. The above showed a a little marked increase the heamatologic parameters there was a slight increase in the lymphocyte although there was a marked decrease of sperm cell count from the control down the doses of exposure.

Key Words: Heamatologic, Anti-spermatogenic, Lymphocyte, White blood cell

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INTRODUCTION

The history of medicinal plants is intimately connected with the history of civilization. Records of early civilization in all parts of the world reveal that a considerable number of drugs used in modern medicine were in use even in ancient times. According to the world health organization, about 80% of the population in many third world countries still use traditional medicine (medicinal plants) for their primary health care due to poverty and lack of access to modern medicine (Silva, 1997). WHO therefore approved the use of herbal products for national policies and drug regulatory measures in order to strengthen research and evaluation of the safety and efficacy of these products (Saxena, 2001). Farnsworth *et al.* (1985) his co-worker reported that of the 119 plant derived drugs listed by WHO study, 74% were discovered as a result of chemical studies to isolate the active compounds responsible for the use of original plant in traditional medicine. *Carica papaya* (Pawpaw) is a herbaceous plant in the family *caricaceae*. It is believed to have its origin from the low lands of Eastern Central America, from Mexico to Panama (Nakasone and Paul, 1998). *Papaya* is cultivated mainly for their ripe fruit used as fruit desert. *Carica papaya* is a medicinal plant in that it contains substances that can be used for therapeutic purposes.

Medicinal plants have successfully been used to induce sterility in laboratory animals [1-3]. Pawpaw seed (*Carica papaya*) [4,5] reported high success in using. Pawpaw (*Carica papaya*) seeds had been used as fertility control agents in some animal models and even on human beings [6,7] respectively. Chloroform extract of papaya seeds tested in langen monkeys for one year, caused a steady decrease in sperm production with no sign of toxicity [8,9]. Crude extract fed to male rats deteriorated quantity and quality of the sperm [10,11]. At higher dose, it provided 100% contraception, but resulted in weight loss, possibly due to toxicity [7,12,9]. Suppression of spermatogenesis was observed in rats followed the administration of papaya seed extract [13]. The Oral administrations of extract induced reversible male infertility [14,17]. The biochemical studies on carbohydrate metabolism reveals the decreased oxidative metabolism [18]. Male reproduction is a multifaceted process that involves the testes, epididymis, accessory sex glands and associated hormones. Testes perform two highly

organized and intricate events, called spermatogenesis and steroidogenesis, which are vital for the perpetuation of life. Spermatogenesis, a highly dynamic and synchronized process, takes place within the seminiferous tubules of the testis with the support of somatic Sertoli cells, leading to the formation of mature spermatozoa from undifferentiated stem cells [19]. Pawpaw (*Carica papaya*) seeds contain antifertility properties, particularly of the seeds [20]. A complete loss of fertility has been reported in male rabbits, rats and monkeys fed an extract of papaya seeds [20,8,21].

The powdered seeds of *Carica papaya* have numerous applications worldwide; some includes its use in Northern India as an antihelminthic and their extract also used as anti-inflammatory and analgesic agents *Carica papaya* seeds is also said to possess Antimicrobial properties [20].. Some other uses of *Carica papaya* includes, for example, the use of dead leaves of *Carica papaya* that fall off the tree as abortifacient [20]. *Carica papaya* seed extract is currently being marketed as a nutritional supplement with purported ability "to rejuvenate the body condition and to increase energy", the product is said to improve immunity against common infection and body functioning. This study was designed to evaluate the acute hepatotoxic and nephrotoxic effects of orally administered aqueous and ethanolic extracts of *Carica papaya* seed in adult Wistar rat in low and high doses

Hence in the present study it is important to know how the steroid enzymes are modulating during spermatogenesis and antispermatogenesis and the immunostimulatory effects of the seeds extract.

MATERIALS AND METHODS

MATERIALS AND METHODS

Experimental animals: A total of Eighty healthy, adult Wistar rats weighing 32-48 g were acclimatized to the laboratory conditions for one week prior to the experiment. Ten animals were used for LD50 while fifteen were used for acute studies. The animals were bred and housed in polypropylene cages. The animals were fed rat pellet diet and layers mesh, exposed to approximately 12 h light: 12 h dark cycle and water was provided *ad libitum*. Animals were treated humanely;

Table 1.0 Hemaological indices

Table 1: Mean haematological indices of adult wistar rats after 6weeks Days of C. Papaya seed extracts administration Groups parameters	I Control normal	100 mg/kg	200 mg/kg
WBC ($\times 10^3/\text{mm}^3$)	6.80 \pm 0.18	6.56 \pm 0.38	6.96 \pm 0.10
PCV (%)	38.8 \pm 0.74	38.0 \pm 0.84	37.4 \pm 1.03
HB (g/100 mL)	13.1 \pm 0.277	12.64 \pm 0.287	12.64 \pm 0.43
Neutrophil (%)	30.2 \pm 1.77	27.6 \pm 2.42	29.2 \pm 2.85
Lymphocyte (%)	65.6 \pm 1.37	70 \pm 2.06	68.6 \pm 1.12
Eosinophil (%)	2.6 \pm 0.51	2.6 \pm 0.51	3.4 \pm 0.40
Monocyte (%)	0.8 \pm 0.37	1.4 \pm 0.25	1.4 \pm 0.25

Key: PM:Progressive Motility, NPM: Non Progressive Motility, IM: Immotile

	Control mean	Group I 100mg Mean	Group II 200mg Mean
PH	7.0 \pm 0.5	7.0 \pm 0.5	7.0 \pm 0.5
VOLUME			
VISCOUSITY	N	N	N
PUS-CELLS	Numerous	Numerous	Numerous
PM	90 \pm 0.5	65 \pm 0.5	55 \pm 0.5
NPM	10 \pm 0.5	15 \pm 0.5	20 \pm 0.5
IM	10 \pm 0.5	25 \pm 0.5	15 \pm 0.5
Total.SPERM CELL COUNT	22.3 $\times 10^6 \pm 0.5$ cells/ mm^3	11.6 $\times 10^6 \pm 0.5$ cells/ mm^3	11.3 $\times 10^6 \pm 0.5$ cells/ mm^3

Table 3 Sperm Cell Morphology

	NORMAL mean	Abnormal mean
Control	90 \pm 0.5	10 \pm 0.5
Group I	60 \pm 0.5	40 \pm 0.5
Group II	55 \pm 0.5	45 \pm 0.5

Veterinary care and supervision were provided throughout the period of study.

Phytochemical screening tests: Desirable amount of *Carica papaya* extract was used for phytochemical tests. The extract solution was tested for alkaloids, glycosides, flavonoids, saponins, sugars and tannins according to the protocol described by Trease and Evans (1983).

Extract preparation: Ripe *C. papaya* fruits of Homestead variety were obtained from a local market in Benin City Edo State between the months of January and February and authenticated at the Animal and Environmental Biological Science AEB of The University of Benin, Benin City- Nigeria. The voucher number 0911 was obtained. The seeds were removed, air dried under shade and coarsely powdered. A measure of 200 grams each of the powdered material was used for each extraction. There were soxhleted with ethanol and distilled water respectively. The soxhleted material was concentrated under reduced pressure and the obtained residue was weighed to calculate the yield and the extracts were used for the study.

Statistical analysis: The data obtained from the studies are represented as Mean \pm SEM. The data were analyzed by one way analysis of variance (ANOVA), 'P' value less than 0.05 was considered as statistically significant.

Result

Acute oral toxicity studies (LD50):

No mortality or morbidity was recorded in any of the animals used throughout the 14-day observation period following the oral administration of the different doses of the extracts of *C. papaya* seeds. There were no signs associated with oral toxicity. Animals did not show any sign of aggression or unusual behaviour during handling. The LD50 of the aqueous and ethanolic extract of *C. Papaya* seed was found to be above 2000 mg/kg.

Acute oral administration: The duration of administration was five days at doses of 100 mg/kg and 1000 mg/kg for the two extracts. There were no significant changes were observed in the treatment group compared to the control group.

The animals were not aggressive and did not exhibit any unusual behaviour during handling.

Haematological indices: The haematological indices observed after 5 days extract administration is as presented in Table 1. It indicated no significant difference within and between the groups.

Sperm quality assessment: The Spermatozoa quality was assessed to determine if the seed extract has any anti-spermic activities this is shown in Table 2 with a marked increased anti-spermic activity over a prolonged period of oral doses.

Result

Hematological findings

Result on the Hematological data is presented in the various figures below. Hematological values measured showed a significant ($p < 0.05$) elevation of RBC, WBC, Platelets, PCV and lymphocyte level in the treatment group. This increase in the various hematological parameters was dose dependent.

Spermatozoal quality assesment findings

Result on the spermatozoal data is presented in the various figures below. Spermatozoal values measured showed a significant ($p < 0.05$) Reduction of Progressive Motility Non Progressive Motility, Imotile, Viability, with distinct abnormal Sperm Morphology and Viability in the treatment group. This increase in the various hematological parameters was dose dependent Table 2 and 3

Discussion

The dietary supplemented extract of *Carica papaya seed* extract caused a decrease in all the sperm parameters studied in a dose dependent manner (Table 2 and 3). There was Similar observations suggestive of impaired spermatogenesis were made following oral administration of benzene extract of *Carica papaya* seeds (Lohiya et al., 1994)

It is well known that the blood epididymis barrier selectively regulates the flow of substances into and out of the epididymal lumen (Hinton and Howards, 1981). Substances of larger molecular weight and many Energy for spermatozoal

motility is derived from the oxidation of glucose and fructose. It is possible that hydro-ethanolic extract of *C. papaya* seed extract inhibited the uncoupling reaction of oxidative phosphorylation (Kalla and Vasudeva, 1981) which rendered the spermatozoa immotile.

Results of this study also revealed significant reduction ($P < 0.05$) in sperm count in the treatment groups indicating impaired spermatogenesis. The decline in viability of spermatozoa might be due to the spermicidal action of the extract. The decrease in spermatogenesis may be attributed to indirect effect of the extract on the hypothalamo-pituitary-gonadal axis and hence on gonadal function. Similar results on antifertility effect and reduced epididymal sperm count were reported with ethanolic extract of *Ricinus communis* (Sandhyakumary et al., 2003) and *Lagenaria breviflora*

(Saba et al., 2009).

The increase in percentage of abnormal spermatozoa are a consistent finding in testicular damage. An animal is considered infertile if more than 10% abnormalities are observed in the semen sample (Zemjanis, 1977). The increase in sperm abnormalities (Table 3) suggests that the aqueous ethanolic extract of *Carica papaya* could also destroy the internal structure of the testis. Disorganization of the histoarchitecture of testis and degenerative

changes observed in the present study were also reported with other medicinal plants found to have anti-spermatogenic property (Leigh and Fayemi, 2008; Rajiet al., 2005). The correlation between the production of fertile spermatozoa and histological integrity of the testis is well documented (Hafez and Hafez, 2000). Hematological findings also revealed elevated Lymphocyte in both the study groups dietary supplemented with *Carica Papaya* seed extract which further explains the immune stimulatory properties of the extract on Male Wister rats (Table 1).

Following the withdrawal of treatment, all the parameters measured had returned to near normal levels indicating that the effect of the extract was reversible. Exploitation of this extract could be used as normal contraceptive and the extract could be exploited as an immuno-stimulant.

Conflict of Interest:

There was no conflict of interest

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