Effect of Aqueous extract on carica papaya seed and back on the Testes and sperm morphology of male wister rats.

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Abstract- *Carica papaya* is a major type of fruit that is been consumed for relaxation or medicinal purpose, and this plant is made of different parts which seen to have different health benefit. The study is aimed to evaluate the effect of aqueous extract of *Carica papaya* seed and peels (back) on the testes and sperm morphology of male albino wistar rats. 35 adult male wistar rats weighing 180g divided into seven (7) groups A-G were used for the study and the research lasted for 4weeks. Group A served as the control, while B, C, D for the seed and E, F, G for the peels at 100mg/kg, 200mg/kg and 400mg/kg for both plant part. At the 29 days the rats were weighed and sacrificed by chloroform sedation, testis were harvested and fix in bosin fluid for histological analysis, sperm morphology test was done. The result of this study showed that LD50 of both seed and peels of carica papaya are both toxic, as peels was found to be more toxic at 1000mg instant death. The result reveals significant decrease in weight across all experimental group especially in peels group, in the examined histo-pathological analysis showed no alteration or any pathological concern the slides in the seed and a slight changes in the peels group F and G. Conclusively the oral administration of aqueous extract of *carica papaya seed* and peels extract showed sign of toxicity in the experimental rats and there for can't be used in treatment of fertility problems.

Index Terms- carica papaya, seed, peels, testis.

I. INTRODUCTION

It is a fact that as many as 80% of the world's population depend on traditional medicines for their primary health care (WHO, 2000). This is sequel to the fact that medicines derived from plants have made large contributions to human health and well being (Iwu *et al.*, 1999). According to Yinger and Yewhalaw (2007), the reason for this prevalence is due to the facts that traditional medicine has remained the most affordable and easily accessible source of treatment in the primary health care system of resource poor communities.

At least 35,000 plant species have been recognized (Kong *et al.*, 2003) and large proportion have been used for the treatment of several human ailments for thousands of years (Yakubu *et al.*, 2007)

The testes (testicles) are the male gonads-paired ovoid reproductive glands that produce sperms (spermatozoa) and male hormones, primarily testosterone.

The testes are suspended in the scrotum by the spermatic cords, with the left testes usually suspended more inferiorly than the right testes (Moore *et al.*, 2006). Sperm is produced in the seminiferous tubules at a rate of about 2×108 per day in the adult male. Each testicle has about 250-1000 seminiferous tubules in its lobules, with each tubule measuring about 150-250µm in diameter and about30-70 cm in length. The combined length of the tubules of one testis is about 250 m. Each tubule is a convoluted loop linked via a short, narrower segment, the straight tubule, to the rete testis, a labyrinth of epithelium-lined channels embedded in the mediastinum testis. Ten to twenty efferent ductules connect the rete testis to the head of the epididymis.

Each seminiferous tubule is lined with a complex specialized stratified epithelium called germinal or seminiferous epithelium. The basement membrane of this epithelium is covered by fibrous connective tissue, with an innermost layer containing flattened, smooth muscle-like myoid cells, which allow weak contractions of the tubule. Interstitial cells occur in the connective tissue between the seminiferous tubules.

The seminiferous epithelium consists of two types of cells: Non-dividing supporting or sustentacular cells (Sertoli cells) and proliferative cells of the spermatogenic lineage. The cells of the spermatogenic lineage comprise four to eight concentric cell layers and their function is to produce the cells that become sperm. The part of sperm production that includes cell division through mitosis and meiosis is called spermatogenesis. The final differentiation of the haploid male germ cell is calledspermiogenesis(Mescher, 2010).Spermatogenesis is controlled by follicle stimulating hormone (FSH) of the anterior pituitary gland (Chaurasia, 2010).

Pawpaw (Carica papaya) is a common fruit available throughout the year in the tropics. The fruits, leaves, seeds and latex are used as a cure for many tropical diseases hence the common name "medicine tree" or "melon of health". The major active ingredients (carpine, chymopapain, papain, bactericidal aglycone, benzyl isothiocyanate, aglycoside, sinigrin, the enzyme myrosinand carpasemine) are present in the seeds. The fleshy part of the fruits (mesocarp) is a delicacy and nutrient-rich drinks of

high demand are produced from them. However, some of the active substances (e.g. carpine and papain) from pawpaw are toxic. Carpine are present in traces in the black seeds of C. papaya. In large quantities, it is used to lower the pulse rate and depress the nervous system. (Walter Last, 2008) The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as papain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer. The juice has been in use on meat to make it tender, (Wilson, 1994). The seed is used for intestinal worms when chewed. The root is chewed and the juice swallowed for cough, bronchitis, and other respiratory diseases. The unripe fruit is used as a remedy for ulcer and impotence, (Elizabeth, 1994). Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier. (Atta,1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, (Elizabeth, 1994).

II. Material and Method

Thirty-five (35) male Wister rats weighing between 150-180g were housed in wooden cages and allowed to acclimatize for two weeks before administration. The rats were feed with rat chow and water throughout the duration of the experiment. Rats were handled according to global best practices. Carica papaya was gotten from a garden in Anambra state and the peels were gotten and slice into equal part and seed were removed, both dried and aqueous extract was prepared.

Table: 1.0: Animal grouping and dose distribution

GROUP	ANIMAL	EXTRACT	DOSAGE	DURATION
1	5	Nil	Nil	28
2	5	Aqueous extract of C.P seed	100mg/kg	28
3	5	Aqueous extract of C.P seed	200mg/kg	28
4	5	Aqueous extract of C.P seed	400mg/kg	28
5	5	Aqueous extract of C.P peel	100mg/kg	28
6	5	Aqueous extract of C.P peel	200mg/kg	28
7	5	Aqueous extract of C.P peel	400mg/kg	28

The animals were kept under adequate sanitary condition with natural sun-light and adequate ventilation.

On the 29 day the animals were weighed as it was done before, after which were sacrificed by chloroform sedation and the testes was harvested and fixed in Bouins Fluid for histopathological studies.The data obtained were analyzed using statistical package for social sciences (SPSS) version 20.0 and the result expressed as mean ± standard Error of mean (SEM) significant differences of the result were established by one-way Analysis of Variance (ANOVA) and the acceptances level of significance was $p< 0.05$ for all the results.

Daily oral administration of carica papaya seed extract for 30days did not induce any obvious symptom of toxicity in rats, weight, deaths was not recorded and no obvious clinical signs were found in any groups throughout the experimental period.

Physical observation of the treated rats throughout the study indicated that none of them showed signs of toxicity in their skin, fur, eyes, mucus membrane, or behavioural changes, diarrhoea, tremors, salivation, sleep, and coma. Although rats in group 4(high dose) showed relative weakness immediately after administration.

Gross observation of the organs showed the no change in color, hypertrophy and abnormal fat deposition.

III. Results

PHYSICAL AND BEHAVIOURAL CHANGES

Table: 2.1: Comparison of rats weight before and after experiment.

	Period	N	Mean ± Std
Group A	Initial	5	93.75±4.79
	Final	5	182.50±6.46
Group B	Initial	5	101.25±2.50
	Final	5	153.00±10.13
Group C	Initial	5	120.00±8.17
	Final	5	155.00±5.78
Group D	Initial	5	110.00±11.55
	Final	5	182.00±2.83
Group f	Initial	5	120.00±8.17
	Final	5	180.00±2.83

Group g	Initial	5	110.00±11.55
	Final	5	146.00±10.16
Group h	Initial	5	120.00±8.17
	Final	5	153.00±10.13

All data were analysed using student dependent T-test and were considered significant at P<0.05, P≤0.05 means significant, and P>0.05 means not significant. Result from table 4.1 showed an significant (P<0.05) increase in the body weight when the test groups were compared to the normal control group (group A).

Table 2.2: Comparing the organ weight of the experimental groups to that of the control.

R. O. W (G)	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Testes	1.61±0.16	1.21±0.02*****	1.51±0.02*	2.55±0.02*	1.20±0.02*	2.42±0.02*	2.56±0.02*

Data was analysed using one-way Anova, followed by multiple comparison using LSD and data was considered significant at P<0.05. ROW= Relative organ weight.

Table2.3: COMPARING TESTESTERONE LEVELS OF THE EXPERIMENTAL GROUP TO THAT OF THE CONTROL.

Testosterone	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7
Levels (ng/ml)	2.75±0.13	7.13±0.25*****	4.88±0.26*	3.13±0.17*	3.25±0.19*	3.42±0.24*	6.56±0.32*

Data was analysed using one-way Anova, followed by multiple comparism using LSD and data was considered significant at P≤0.05 and insignificant at P> 0.05. Table4.3 shows Significant increase in the *mean* testosterone level of test groups compared to normal control group.

HISTOLOGICAL RESULTS:

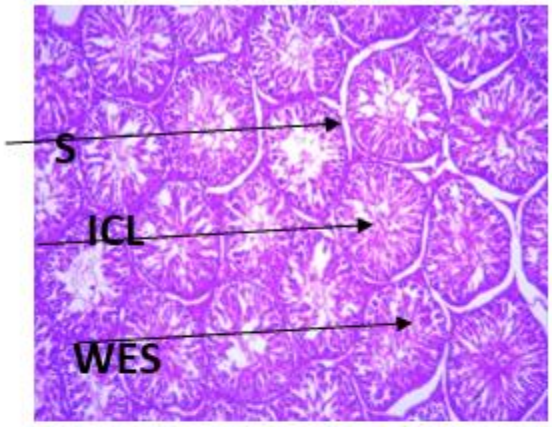

Plate 1: Grp 1. Normal seminiferous tubule (s) with interstitial cells of liydig (ICL) and well enhance spermatogenesis (WES). Features appears normal.

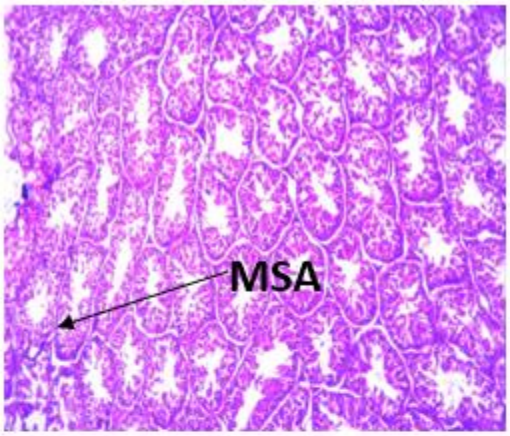

Plate 2: Grp 2.section administered with 100mg/kg of seed showed moderate degeneration with moderate spermatogenic arrest (MSA)

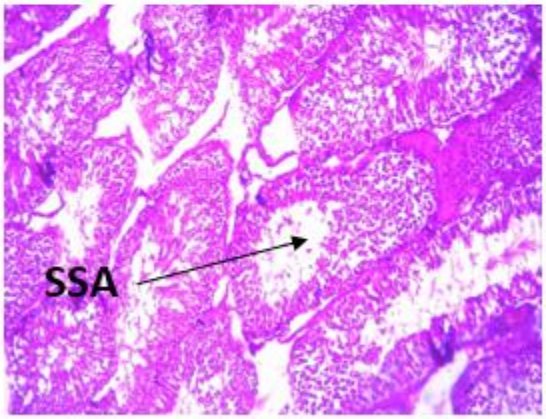

Plate 3: Grp 3. Section of 200mg/kg of seed shows severe degeneration with sever spermatogenic arrest (SSA)

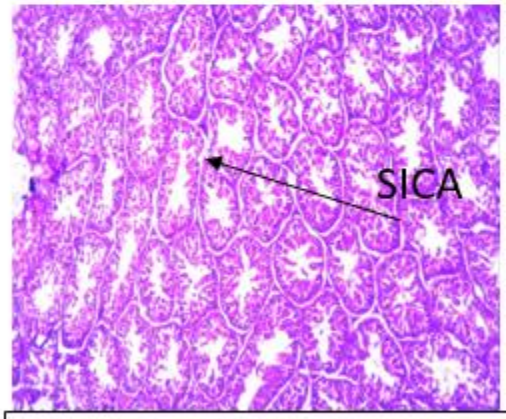

Plate 4: Grp 4. Section of 400mg/kg of seed shows degeneration with wall of interstitial cells scattered and arrest (SICA)

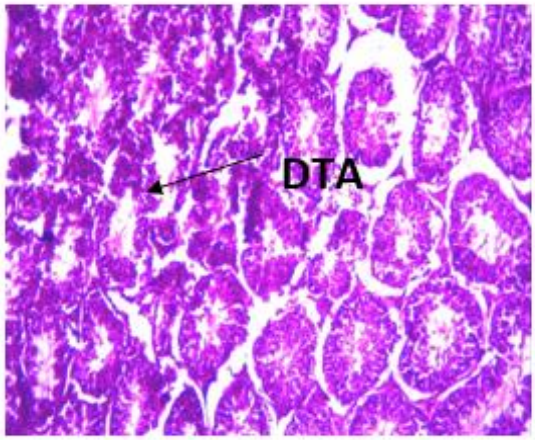

Plate 5: Grp 5. Section administered with 100mg/kg of peel shows disorganization of testicular architecture (DTA)

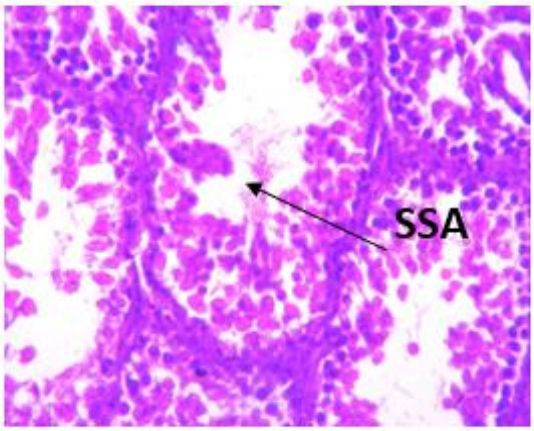

Plate 6: Grp 6. Section administered with 200mg/kg of peel shows severe degeneration with sever spermatogenic arrest

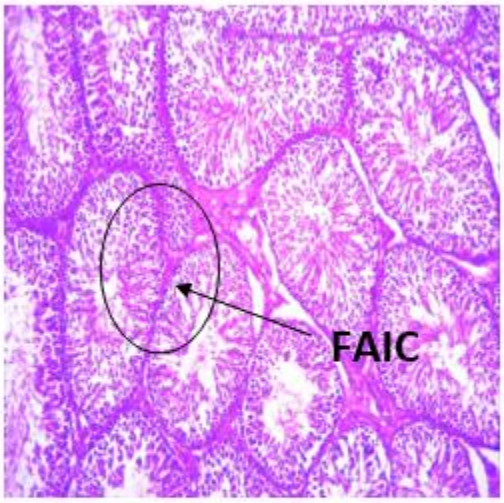

Plate 7: GRP 7. Section administered with 400mg/kg of peel showed focal aggregate of imflammatory cells (FAIC) around the seminiferous tubules.

IV. Discussion

Medicinal plant over decades have been in use for treatment of many illness and countries like Nigeria herbal remedies are mostly used as conventional drugs other than the orthodox drugs. Carica papaya seed and peel extract exhibited a lot of medicinal relevance (Walter Last, 2008). The papaya fruit, as well as all other parts of the plant, contain a milky juice in which an active principle known as parpain is present. Aside from its value as a remedy in dyspepsia and kindred ailments, it has been utilized for the clarification of beer; the juice has been in use on meat to make it tender, (Wilson, 1994). The unripe fruit is used as a remedy for ulcer and impotence, (Elizabeth, 1994). Fresh, green pawpaw leaf is an antiseptic, whilst the brown, dried pawpaw leaf is the best as a tonic and blood purifier. (Atta,1999). Chewing the seeds of ripe pawpaw fruit also helps to clear nasal congestion, (Elizabeth,1994). The green unripe pawpaw has a therapeutic

value due to its antiseptic quality; this makes all part of the fruit useful and creates interest to evaluate on the testes.

Result seen in table 2.1 showed changes in the body weight in both the peel and seed of the papaya, this shows that the seed and peels did not reduce appétit to stop food intake, and further result seen in table 2.2 which was organ weight increase in all the group, which is a sensitive indicator of toxicity (Thanabhorn *et al*., 2006; Norazmir and Ayub, 2010). The result of body weight and organ weight showed significant weight increase across the test group, which signifies that the extract didn't impair appetite. Also, the body weight of the rat increased in size as well as the testicular weight significantly increase in size which in support with Sumaia *et al*.,(2015)which stated that the increase in the testicular weight of the rats is due to the presence of Alkaliods, Flavonoids and Tanin in *carica papaya* seeds.

The significant increase in the levels of serum testosterone across the experimental group when compared with control group and this could be due to the activities in the functions of the anterior pituitary and Leydig cells. The increase in the activity of the 17- ketosteroid reductase enzyme that converts androstenedione to testosterone might be another possible reason for increased testosterone production (Panneerdoss S. 2002).

The histology of the tastes treated with 100ml, 200ml and 400ml of *c*arica *papaya* seed extracted showed severe degeneration with spermatogenic arrest, while peel showed disorganization of testicular architecture and inflammatory cells around the seminiferous tubules. These findings agree with the result obtained by Lohiya *et al*., (1999) that the histology of the testes revealed arrest of spermatogenesis beyond the level of spermatocytes. Nwachi *et al*., (2001) also reported that there was suppression of spermatogenesis in rats treated with extracts from different parts of the papaya but Udoh *et al*., (2005) who studied the effects of oral administration of *caricapapaya* seeds extract of 50m/kg and 200mg/kg on the morphology of the tests revealed gradual degeneration of germ cells, sertoli cells and leydig cells as well as germinal epithelium.

V. Conclusion

Based on the available scientific evidence *carica papaya seed and peel* extract has the capacity to regulate water balance, increase testicular weight and suppress spermatogenesis, fertility in rats despite in the treatment of warts, cancers, tumors, syphilis, alkalizine, urine, hemorrhoids and other ailments.

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