International Journal of Veterinary Science

www.ijvets.com P-ISSN: 2304-3075 editor@ijvets.com

SHORT COMMUNICATION

Effects of Ripe *Carica Papaya* Seed Powder on Testicular Histology of Boars

Kipyegon AN*, HM Mutembei, VT Tsuma and JA Oduma[1]

Department of Clinical Studies, [1]Department of Veterinary Anatomy and Physiology, Faculty of Veterinary Medicine, University of Nairobi, P.O. Box 29053- 00625, Kangemi, Kenya

ARTICLE INFO

Received: July 24, 2012
Revised: July 28, 2012
Accepted: August 06, 2012

Key words:
Pawpaw seeds
Spermatogenesis
Testicular histology

***Corresponding Author**
Kipyegon AN
kipyegongeno@yahoo.com

ABSTRACT

The effect of oral administration of ripe *Carica Papaya* seed powder on testicular histology of *Sus scrofa domestica* boars has been addressed in the present article. Fifteen pubertal Large White boars were randomly selected and divided into two groups. Each boar in the experimental group received a daily dose of 300mg *C Papaya* mixed with 0.5 kg of conventional pig feed while the control group received a placebo. The experiment was carried out for 56 days in the University of Nairobi. After every two weeks, one boar from the control group and two from the experimental group were castrated and testicular tissue samples processed for histology. At the end of 56 days the remaining entire boars were maintained for 14 days and 60 days respectively without the papaya powder to assess reversibility. The test material had no effect on haematological parameters. However, histopathological changes of the seminiferous epithelium which appeared to be dependent upon duration of *C Papaya* consumption was noticed, these changes were reversible.

Although the mechanism(s) for the effect of papaya seed extract is not explained by this study, it is observed that papaya seed powder causes gradual disorganization, exfoliation and loss of spermatocytes and spermatids.

Cite This Article as: Kipyegon AN, HM Mutembei, VT Tsuma and JA Oduma, 2012. Effects of ripe *Carica Papaya* seed powder on testicular histology of boars. Inter J Vet Sci, 1(1): 1-4.

INTRODUCTION

In Kenya small scale pig farming is characterized by low inputs (Mutua *et al*., 2010) with farmers using locally available feedstuffs to feed the pigs (Phengsavanh *et al*., 2010). The most commonly used feedstuff includes; kitchen leftovers, sweet potato vines, fruits and vegetables. Among the fruits, most farmers prefer *Carica papaya* due its perceived anthelmintic effects. *C. Papaya* fruit is available all year round in tropical regions. The fruits and leaves have pharmaceutical and medicinal application which include: antimicrobial (Emeruwa, 1982), anthelmintic (Okeniyi *et al*., 2007) and antifertility (Lohiya *et al*., 2002) effects.

While the objective of the pig industry is to produce a large number of piglets efficiently and economically, reports have indicated a poor reproductive performance in small holder farms in Kenya (Wabacha *et al.*, 2004). This situation has been attributed to small litter sizes, high piglet mortality, poor feeding and breeding, diseases and reduction in the number of boars kept (Mutua *et al.*, 2011). This study was designed to investigate whether *C. Papaya* seeds given to pigs as feed may have any effect on the testicular histology of boars thus contributing to the poor reproductive performance alluded to above.

MATERIALS AND METHODS

Preparation of the test material

Ripe *C Papaya* seeds were obtained from groceries in Nairobi, Kenya. The seed were identified and authenticated by taxonomists of the Department of Land Resource Management and Agricultural Technology (LARMAT), University of Nairobi. The seeds were air dried for 3 weeks, ground to a fine powder using a domestic grinder, and kept at room temperature till usage.

Experimental animals

Fifteen healthy Large White boars from 2 litters and of the same age (120 days old) were purchased and housed in groups of five. The animals were allowed a period of one month to acclimatize before the experiment. The experiment was conducted in accordance with accepted humane practices as approved by the Faculty ethical committee.

Experimental design

The animals were divided into two groups: Group i, control animals (5 animals) - They were treated orally with 300 mg of maize germ in 0.5kg of growers mash daily for 56 days. Group ii, experimental animals (10 animals) – They were treated orally with 300 mg papaya seed powder mixed in 0.5 kg of growers mash daily for 56 days. Following completion of treatment phase, the uncastrated boars were maintained on commercial feed to assess testicular recovery period, one was castrated after two weeks and another after 60 days.

Castrations and Testicular Sample processing

Castrations were conducted under a plane of anaesthesia achieved by injection of a mixture of ketamine (2.2 mg/kg) and xylazine (4.4 mg/kg) intramuscularly. Excess connective tissues were trimmed off and the testis cut longitudinally into two halves. From each half five $1cm^3$ parenchyma samples were taken from between the tunica albugnea and the mediastinum testis and processed for H&E staining. Histological sections were examined for histopathology changes of the interstitial and semiferous epithelium (Germ cell and Sertoli cell). The evaluation of cell population was based on the counts of each cell type per spherically appearing seminiferous tubule sections.

Statistical analysis

Simple qualitative statistics was used to compare the treated and the controls at every stage of castration. At the end of the study the controls and treatment groups were compared using unpaired *t-test*. Values were expressed as mean± standard error and $P<0.05$ was considered statistically significant.

RESULTS

Haematology

The levels of the blood RBC, WBC, Hb, PCV, MCV, MCH and MCHC did not show significant changes throughout the experiment period, all were within the normal ranges (Table 1).

Non-treated group

The seminiferous epithelia in control animals contained Sertoli cells, spermatogonia, spermatocytes and round and elongating spermatids and spermatozoa (Fig. 1).

Treatment group

After 4 weeks of daily dosing, germ cells were desquamated and displaced to the seminiferous tubule lumen. There was a reduction in spermatocyte numbers ($P<0.05$), the spermatogonia and round spermatids appeared unaffected (Fig. 2 and Table 2).

After 8 weeks of daily dosing, the spermatogonia remained unaffected. Seminiferous epithelium degeneration was observed in the majority of the tubules. The lumen contained germ cell debris (Fig. 3 and Table 3).

The effects of papaya were not noticeable two months after stoppage of extract administration. Spermatogenesis had resumed fully (Fig. 4 and Table 4).

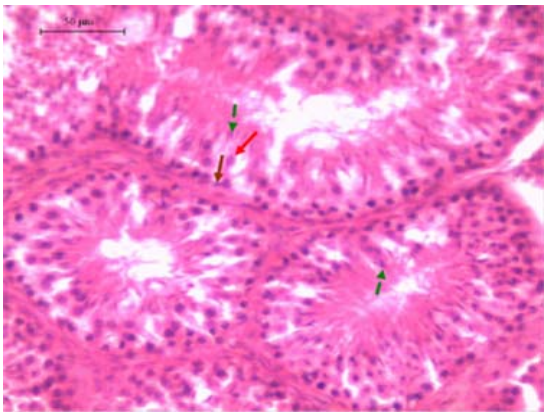

Fig. 1: Testicular histology photo of a 206 day old control animal showing different spermatogenic cells covering the entire process of spermatogenesis. Spermatogonia (brown arrow), spermatocytes (red arrow), and spermatids (green arrow); (transverse section x40 H&E stain).

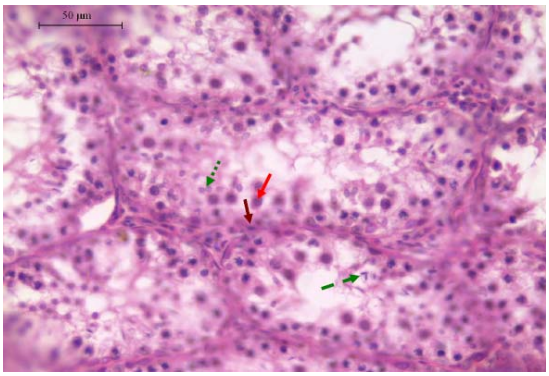

Fig. 2: Testicular histology photo in a 178 day old boar after 4 weeks of dosing showing a depleted seminiferous epithelium. The spermatogonia (brown arrow), spermatocytes (red arrow) and spermatids (green arrow) appear morphologically normal. (transverse section x40 H&E stain).

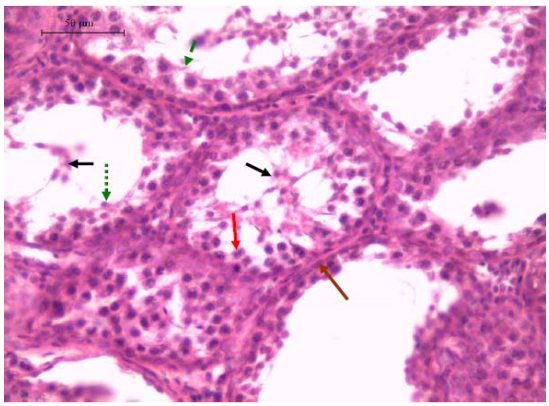

Fig. 3: Photo of testis of a 206 day old boar after 8 weeks of dosing showing a depleted seminiferous epithelium. Exfoliated germ cells (black arrow), spermatogonia (brown arrow), spermatocytes (red arrow) and spermatids (green arrow). (transverse section x40 H&E stain).

Table 1: The Summary of effect of C. papaya on haematological parameters of adult boar

Treatment	PCV (%)	Hb (g/dl)	WBC m/mm3	RBC m/mm3	MCH pg.	MCHC g/dl	MCV Fl
0	38.5	13.1	11.4	7	18.8	33.9	55.2
2 week	38.3	12.7	9.42	6.8	18.7	33.1	56.7
4week	40.4	13.5	15.9	7.1	18.6	32.75	57
6week	38.4	12.9	10.8	6.6	19.5	33.5	58.4
8week	38.1	12.1	12.3	6.9	17.4	31.7	55
Recovery	38.4	13.2	11.0	7	18.6	33.2	55.5

Table 2: Germ cell population in a 178 days old boar after 4 weeks of powder administration

	Testicular cell counts (number/10 cross section)		
	Spermatogonia	Spermatocyte	Round spermatids
Control	27.8±4.4	23.2±3.5	64.8±9.6
Treated	32.6±2.5	18.1±5.4	74.1±3.6

Table 3: Germ cell population in 206 days old boar after 8 weeks of powder consumption

	Testicular cell counts (number/10 cross section)		
	Spermatogonia	Spermatocyte	Round spermatids
Control	28.7±8	38.7±5.4	75.3±10.8
Treated	27.8±6.3	29.5±9.9	19.5±8

Table 4: Germ cell population after cessation of powder consumption

	Testicular cell counts (number/10 cross section)		
	Spermatogonia	Spermatocyte	Round spermatids
Control	27.4±4.4	25.5±3.5	19.4±12.5
Treated	28.3±5.4	29.5±9.4	24.1±11.1

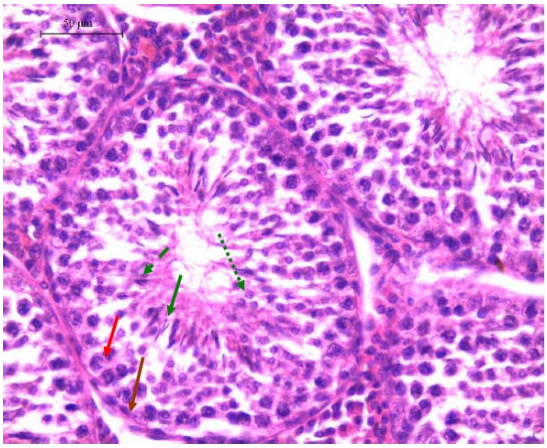

Fig. 4: Photo of a 280 day old boar testis 60 days after treatment withdrawal. The seminiferous epithelium is comparable with that of a control animal. It contains spermatogonia (brown arrow), spermatocytes (red arrow) and spermatids (green arrow). (transverse section x 40 H&E stain).

DISCUSSION

Small holder pig farming is an important source of income in rural population; pigs are sold to earn family income to meet basic needs. In this regard an efficient reproductive performance is essential since it determines the herd size and influence the number of pigs available for sale (Lanada *et al*., 2005). In an effort to increase the profit margins small scale pig farmers shun commercial pig rations for swill which is a low cost feed. This practice may be a silent cause of the poor reproductive performance reported in small scale pig farming (Mutua *et al*., 2011) and could be attributed to plants such as *C Papaya* which has previously been shown to possess antifertility properties in monkeys, rats, dogs and humans (Udoh and Kehinde, 1999; Lohiya *et al*., 2002). This study revealed that consumption of *C. Papaya* which seasonally makes up the bulk of pig feed in the tropics causes histopathological changes in the testis, with profound effects on the germinal epithelium affecting the spermatocytes and the spermatids numbers. The histological changes observed were those of germ cell desquamation and loss a finding similar to that of Udoh *et al.*, (2005) when he administered crude ripe *C Papaya* seeds to male Winstar rats. There seemed to be no effect on the Sertoli cells, Leydig cells and spermatogonial cells within the duration of administration examined.

The testes in the males perform two main functions; steroidogenesis and spermatogenesis which are both vital in fertility. Spermatogenesis occurs within the seminiferous tubules of the testis with support of Sertoli cells (Hess *et al*., 2008).Intact Sertoli cell architecture is important in anchoring germ cells as they develop, therefore their disruption will most likely lead to germ cell desquamation observed in this study due to the possible disruptive effect of *C. Papaya* seed powder on Sertoli cell architecture, cellular tight junctions and on germ cell cytoplasmic bridges.

It is noted that after one cycle of spermatogenesis (39 days), there is gradual reduction in spermatocyte numbers leading to loss of spermatids as the proceeding stages mature. Lohiya *et al.*, (2002) in his study, observed a gradual decline in sperm counts, motility and sperm viability in Langur monkey 30 days after administration of *C Papaya* seed extract. Such stage specific spermatocyte loss has also been seen with glycol ethers (Creasy *et al*., 1985) and may indicate that papaya seed prevents any further division of the spermatogonia to spermatocyes.

The testicular changes observed in this study were reversible upon withdrawal of papaya seed administration; this agrees with the findings of Lohiya *et al*., (2002) when he administered C Papaya seed to Langur monkeys. This may be attributed to the observation that papaya seed powder had little or no effect on the spermatogonial population even after prolonged treatment. The blood parameters measured in this study were within the normal ranges for the pig and did not differ between the control and treatment groups, these findings are similar to these of Lohiya *et al* (2002) after administration of papaya seed to Langur monkeys. This suggests that the effect of the

papaya seed powder on the testicular histology in the pig may be at the local testicular level, and not due to systemic effects. However, in the Nile Tilapia, Ayotunde and Ofem, (2008) observed an increase in white blood cell count and lymphocyte percentages after the fish were fed on papaya seed powder for 96 hours. Probably, various species respond differently to oral administration of papaya seed powder.

From the current study, the testicular effects of oral administration of *Carica papaya* seed powder seem to be influenced by the duration of papaya administration. The findings of this study may partly explain the observed reproductive failures in small scale pig farms who utilize papaya seeds and other remains as pig feed and is recommended that further surveys be carried out in these farms to confirm this finding.

REFERENCES

Ayotunde EO and BO Ofem, 2008. Acute and Chronic Toxicity of Pawpaw (*Carica papaya* seed) Powder to Nile Tilapia*Oreochromis niloticus* (Linne 1757), Fingerlings.Advancement in Environmental Biology2 (3): 101-107.

Creasy DM, JC Flynn, TJB Gray and WH Butler, 1985.A quantitative study of stage specific spermatocyte damage following administration of ethylene glycol monomethyl ether in the rat. Experimental molecular pathology, 43: 321-336.

Emeruwa AC, 1982. Antibacterial substance from *Carica papaya* fruit extract. Journal of Natural Products, 45: 123-7.

Hess RA and L Renato de Franca, 2008. Spermatogenesis and cycle of the seminiferous epithelium. *Adv Exp Med Biol*; 636: 1–15.

Lanada B, J Lee, SJ More, S Basito and AT Alberto, 2005. A longitudinalstudy of sows and boars raised by smallholder farmers in the Philippines. Preventive Veterinary Medicine, 70: 95–113.

Lohiya NK, B Minavanna, P.K Mishra, N Pathak, S Sriram, SS Bhande and S Panneerdoss, 2002. Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in langur monkey. Asian Journal of Andrology, 4(1) 17-26.

Mutua FK, CE Dewey, SMArimi, E Schelling, WO Ogara and M Levy, 2011. Reproductive performance of sows in rural communities of Busia and Kakamega Districts, Western Kenya.African Journal of Agricultural Research, 6(31): 6485-6491.

Mutua FK, SM Arimi, CE Dewey, WO Ogara and E Schelling, 2010. Farmer perceptions on rural pig rearing in Kakamega District, Western Kenya. *Nordic J Afr Stud*, 19 (1): 43-57

Okeniyi AO, TA Ogunlesi, AO Oyelami, AL Adeyemi, 2007. Effectiveness of *Carica papaya* seed against human intestinal parasitosis: A pilot study. Journal of Medicinal Food,10(1) 194-196.

Phengsavanh P, B Ogle, W Stur, BE Frankow-Lindberg and JE Lindberg, 2010. Feeding and performance of pigs in small holder production systems in northern Lao PDR. Tropical animal health and production, 42 (8): 1627-1633

Udoh FV, PB Udoh and EE Umoh, 2005. Activity of alkaloid extract of *Carica papaya* seeds on reproductive functions in male wistar rats. Pharmaceutical Biology, 43(6): 563-367.

Udoh P and A Kehinde, 1999. Studies on antifertility effects of pawpaw seeds (Carica papaya) on the gonads of male albino rats. Phytotheraphy Research, 13 (3): 226-228.

Wabacha JK, JM Maribei, CM Mulei, MN Kyule, KH Zessin and W Oluoch-Kosura, 2004. Characterization of smallholder pig production in Kikuyu Division, Central Kenya. Preventive Veterinary. Medicine, 63: 183-195.