Original article

Cytomorphologic distinctions in accessory reproductive tissues and extra-gonadal testosterone milieu in *Carica papaya*-treated male rats

Kusemiju, TO.*, Yama, OE. and Okanlawon, AO.

Department of Anatomy, Faculty of Basic Medical Sciences, University of Lagos, Idi-Araba Lagos, Nigeria
*E-mail: ktaiwoolabisi@yahoo.com

Abstract

Introduction: In this study, we reviewed the effects of contraceptive doses of *Carica papaya* (CP) bark extract on the accessory reproductive organs and peripheral testosterone of rats. **Methodology:** Sixty mature 6 weeks old male Sprague-Dawley rats randomized into 3 main groups; each further subdivided into 2 groups A and B; 10 rats/subgroup. Group 1: The control fed distilled water (2-4 mL.day^{-1}). Group 2: administered CP (50 mg .mL^{-1}.kg^{-1}.day^{-1}), while Group 3: treated orally at a higher dose of 100 mg .mL^{-1}.kg^{-1}.day^{-1}. At the end of the experimental period, rats were sacrificed. Venous blood obtained, testes and accessory organs harvested for microscopic study. **Results:** The extracts produced a dose dependent alteration in testes and accessory organs in the rats. *Carica papaya* significantly decreases peripheral testosterone concentration in both groups. There was a significant reversal in the testosterone concentration of the rats in the group administered CP 50 mg.mL^{-1}.kg^{-1}.day^{-1} than in those treated with CP (100 mg.mL^{-1}.kg^{-1}.day^{-1}) this suggests injury inflicted in the low dose group were nominal. **Conclusion:** This study has demonstrated a time dependent reversible alterations in the morphologies of testes and accessory organs treated with contraceptive doses of CP. The reversal outcome suggests that it is safe to exploit its contraceptive use.

Keywords: *Carica papaya*, testosterone, sprague-dawley rats, accessory organs.

1 Introduction

In a synopsis of our dated trials at developing apposite non-hormonal oral contraceptive agent for men that would be marketable; attempts were made at properly investigating *Carica papaya* (CP) bark extract. First we established qualitatively the antifertility effect of CP extract on the testes in orally treated rats (KUSEMIJU, NORONHA and OKANLAWON, 2002; KUSEMIJU, OSINUBI, NORONHA et al., 2010); then extended the scope of our research to include analysis of blood data in rats which appraised organ-specific toxicity (KUSEMIJU, YAMA, NORONHA et al., 2011). Furthermore the effects of the extract on testicular proteins, oxidative stressors as well as testicular morphometry were described in separate studies (KUSEMIJU, YAMA and OKANLAWON, 2012). In this present study, a lateral extension of the impact of the extract on the morphologies of adjunct reproductive tissues in male rats will be considered.

The organs associated with sperm production include testes, epididymis, seminal vesicle and prostate gland; we shall briefly discuss their anatomic-physiology. The testis has both exocrine and endocrine function. The exocrine product is chiefly the sex cells, spermatozoa, and thus the testis may be referred to as a cytogenic gland. Testosterone the endocrine product is an internal (interstitial) secretion by certain specialized cells, Leydig cells (SVECHNIKOV, LANDREH, WEISSER et al., 2011). Testosterone plays a vital role in the control and maintenance of the process of spermatogenesis (GUIDO, ARIANE, EVI et al., 2010). Seminal fluid comprises secretions produced by the prostate gland 30%, seminal vesicles 60% and Cowper's gland the remaining fluid to the semen (CHARLOTTA, 2008).

The epididymis functions in the transport and storage of the sperm cells that are produced in the testes. It brings the sperm to maturity, since the sperm that emerge from the testes are immature and incapable of fertilization. During sexual arousal, contractions force the sperm into the vas deferens. The epididymis plays an essential role in male fertility, and disruption of epididymal function can lead to obstructive azoospermia (O'HARA, WELSH, SAUNDERS et al., 2011).

The seminal vesicle an accessory reproductive gland produce secretions important for semen coagulation and may promote sperm motility, increase stability of sperm chromatin, and suppress the immune activity in the female reproductive tract (ZHANG and JIN, 2007). Therefore the function of seminal vesicle is important for fertility. Parameters as sperm motility, sperm chromatin stability, and immuno-protection may be changed in case of its hypofunction (GONZALES, 2001).

The prostate is a compound tubuloalveolar gland, which contains between 30-50 glands that empty into the prostatic urethra via 15-30 ducts. A calcified concretion, which may exceed 1 mm in diameter, has been disputed (TISSELL and SALANDAR, 1984). The rat prostate is a complex structure with several distinct anatomical lobes. Physiologically the prostate fluid contains proteolytic enzymes and protein

decomposition enzymes that help sperm through the numerous barriers allowing smooth combination of the sperm and egg cells (MOORE, DALLEY and AGUR, 2006). Other functions include semen liquefaction, and improving the survival rate of sperm.

A host of herbal agents when introduced into the body can disrupt the process of spermatogenesis and can cause both morphological and functional changes within the cells. This can be used to assess the reproductive capacity in experimental animals. Some plants extract have been reported to affect spermatogenesis thereby serving as fertility regulating agents acting by interfering with either sperm production or sperm maturation, sperm storage or even transport in the genital tract (CUNNINGHAM and HUCKIN, 1979). Therefore the maleness in terms of the ability to procreate is hinged solely on the capacity to produce semen, reflective in properly functioning reproductive organs as described earlier. This research attempt at studying the histological perturbation/cytomorphologic differences in these major tissues connected with production of semen in rats treated with contraceptive doses of *Carica papaya* bark extract and how it relates to testosterone ratio.

2 Material and methods

2.1 Anthology and preparation of plant materials

The bark of the *Carica papaya* plant was obtained from a forest in Lagos and authenticated in the Department of Botany, University of Lagos by Professor J. Olowokudejo, a taxonomist and given an ascension number LUH 2151. The bark of the plant was dried in an electric oven at 40 °C for 4 days and crushed to obtain a coarse powder that was used for the extraction in the Pharmacognosy Department of the Faculty of Pharmacy, University of Lagos. The water-soluble extract were prepared and screened for their effects on the reproductive organs in the male Sprague-Dawley rats.

2.2 Animal reserve

Sixty mature 6 weeks old male Sprague-Dawley rats were used in this study (Table 1). They were procured from the Animal House of the College of Medicine, University of Lagos, Idi-Araba authenticated by a taxonomist in the department of Zoology of same institution (MALAKA, 2005, personal communication). The animals were domiciled in plastic cages of size 15" × 10" × 9" in the Rat Control Room of the Anatomy Department at room temperature (28 ± 2 °C) on a reversed day-night cycle (12:00 AM to 12:00 AM). Food pellets and water were provided *ad libitum*. The animals were kept for at least one week to acclimatize to laboratory conditions before experimentation.

2.3 Animal ethics and Modus operand of experimentation

The etiquette of conducting tests on the rats was approved by the Department of Anatomy Ethical Committee for use of laboratory Animals University of Lagos, Nigeria. At the meeting of the departmental committee held on 8th April, 2008, the work was reviewed and accepted to be in conformity with the international guiding principles for research involving animals as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals (AMERICAN..., 2002).

The rats were randomized into 3 groups (1-3); 20 rats/group (Table 1). Each group was further subdivided into 2 groups A and B; 10 rats/subgroup. Group 1: The control vehicle gavaged distilled water (2-4 mL.day^{-1}) was used to establish the baseline reference values. Group 2: administered with *Carica papaya* bark extracts orally at a low dose of 50 mg .mL^{-1}.kg^{-1}.day^{-1}, using canula and syringe while Group 3: were treated orally at a higher dose of 100 mg.mL^{-1}.kg^{-1}.day^{-1}. Doses were administered once a day to the rats for 4 and 8 weeks. All the 3 groups were subjected to the same feeding regime.

2.4 Euthanization and autopsy schedule

At the end of the 4 weeks experimental period, Groups 1A, 2A and 3A rats were sacrificed. The procedure was done under mild anaesthesia (intra-peritoneal injection of 7 mg.kg^{-1} body weight Ketamine HCl) (YAMA, NORONHA and OKANLAWON, 2011). The abdomen entered in layers via ventral incisions (laparotomy) and the testis, prostrate, epididymis and seminal vesicle from each rat in each group harvested and fixed in Bouin's fluid for histological analysis. The same procedure was repeated at the end of 8 weeks for the second half of the rats in groups 1B, 2B and 3B.

2.5 Blood collection and testosterone assay

Blood was obtained by left ventricular cardiac puncture and collected into a heparinised bottle (KUSEMIJU, YAMA, NORONHA et al., 2011). Each blood sample was spun at 2500 revolution per minute for 10 minutes in an angle-head desktop centrifuge at 25 °C. Serum samples were, assayed for testosterone in batches with control sera at both physiological and pathological levels by Standard Qualitative Enzyme linked immunosorbent assay (ELISA) technique with Microwel kit from Syntrobioreseach Inc. California, U.S.A. Assay procedure (TIETZ, 1995; YAMA, DURU, OREMOSU et al., 2011) was as follows: 10 µL of

Table 1. Experimental protocol of Sprague-Dawley rats.

Group	Treatment regimen	Duration	Dose
1A	CPBE	4	50 (low dose)
1B	CPBE	8	50 (low dose)
1C	$CPBE_{8WK}$-DW_{8WK}	16	50 (low dose)
2A	CPBE	4	100 (high dose)
2B	CPBE	8	100 (high dose)
2C	$CPBE_{8WK}$-DW_{8WK}	16	100 (high dose)
3A	Distilled water	4	4-5 mL
3B	Distilled water	8	4-5 mL
3C	Distilled water	16	4-5 mL

CPBE: *Carica papaya* bark extract; $CPBE_{8WK}$-DW_{8WK}: CPBE for 8 weeks followed by distilled water for another 8 weeks; (n = 10); Duration in weeks; Dose units [1 & 2 (mg.mL^{-1}.kg^{-1}.day^{-1})].

standard sample and control were dispensed into desired number of coated wells. 100 µL of testosterone-conjugated reagent was dispensed into each well followed by 50 µL of anti-testosterone reagent. The contents of the well were mixed vigorously for 30 seconds and incubated for 90 minutes at room temperature. Later, the wells were washed with distilled-ionized water. 100 µL of testosterone binding reagent was dispensed into each well and incubated for 20 minutes. Reaction at this level was terminated with 100 µL of 1N HCL acid and color intensity measured on a microwell automatic reader E1 times 180. Testosterone concentrations of the various samples were estimated from calibration curve plotted with 6 standards 0, 0.1, 0.5, 2.0, 6.0 and 18, all run in duplicate.

2.6 Histological study

Microscopic study was similar to methods described by Duru, Yama, Noronha et al. (2011). After 48 hours the organs were removed from Bouin's fluid and further fixed in fresh Bouin's fluid for another 72 hours. Each testis was sliced athwart into slabs of about 0.5 cm thick and dehydrated in varying degree of alcohol (70%, 90%). From 90% alcohol to 3 changes of absolute alcohol for 1 hour each, then into chloroform for about 10 hours and later transferred into fresh chloroform for about 30 minutes. The tissues were placed in 3 changes of molten paraffin wax for 30 minutes each in an oven at 57 °C. They were placed vertically in molten paraffin wax inside a plastic mould and left overnight to cool and solidify. They were later trimmed and mounted on wooden blocks. Serial sections were cut using a rotary microtome at 5-micron thickness. Sections were floated in a water bath and picked by albuminized slides and dried on the hot plate at 52 °C. To stain, the slides were de-waxed in staining racks and placed in staining wells containing xylene and rehydrated in varying degree of alcohol (absolute, 90%, and 70%) and then to water for 5 minutes after which they were stained with haematoxylene for 3 minutes. Excess haematoxylene was washed off with water and differentiated with 1% acid alcohol. Sections were rinsed under running tap water and then left for 5 minutes for bluing. Sections were counterstained with 1% eosin and washed off with water. They were dehydrated with 70%, 90% and absolute alcohol and cleared in xylene to remove all traces of water. A drop of mountant was placed on the surface of the slide and covered with a 22 by 22 cm cover slip.

2.7 Statistical technique

The data obtained were expressed in Mean ± Standard deviation (SD). Analysis of variance (ANOVA) with Scheffer's post-hoc test was used to analyze the significance of difference and a probability of $p < 0.05$ was considered significant.

3 Results and discussion

There was a significant decrease in the peripheral testosterone level in both low and high dose groups treated with *Carica papaya* bark extract for 4 and 8 weeks compared to the control group (Table 2). However, the reduction was more pronounced with the high than the low dose group in all cases. For the low dose group treated for 4 weeks, the serum testosterone level fell from 1.29 ± 0.14 to 0.90 ± 0.14 mg.mL^{-1} and for the high dose regime the serum testosterone level fell from 1.29 ± 0.14 to 0.75 ± 0.10 mg.mL^{-1}. In the 8 weeks treatment, a more pronounced effect was observed; from 1.30 ± 0.15 to 0.30 ± 0.08 at low dose; and 1.30 ± 0.15 to 0.25 ± 0.07 mg.mL^{-1} at high dose (Table 2). The serum testosterone concentration of the reversal group was more pronounced with the low dose extract reversal group than the high dose extract reversal group. At a low dose treatment with extract, the serum testosterone level reversed from 1.28 ± 0.15 to 0.92 ± 0.15 and at the higher dose the reversal was from 1.28 ± 0.15 to 0.28 ± 0.07 mg.mL^{-1}.

The administration of Sprague-Dawley rats with *Carica papaya* bark extract for 4 and 8 weeks showed significant alteration in the histology of the testis, prostate, epididymis, and seminal vesicle.

The testis (Figure 1a) shows control testis with seminiferous tubules containing germ cells up to the level of spermatozoa (i.e. spermatogenesis). In the samples with low dose treatment for 4 weeks, the seminiferous tubules showed focal areas with marked hypospermiation and coagulative necrosis of the seminiferous tubules (Figure 1b). Samples with higher dose treatment for 4 weeks showed an extensive necrosis of the seminiferous tubules and damage to the germ cells (viable germ cells were not seen). There was also destruction to the basement membrane, focal area of disorganization and sloughing. All these resulted in hypospermiogenesis and consequently hypospermatogenesis (Figure 1c). The low and high doses given for 8 weeks showed a more extensive damage with the nuclei of the cells not seen and very scanty Leydig cells (Figure 1d-f).

The epididymis of the control rats contained numerous tubules lined by epithelium, projecting in the lumen containing oesinophilic material (Figure 2a). In the samples with low dose treatment for 4 weeks, there was a thick basement membrane of the tubules with some coagulative necrosis. Few viable germ cells were seen within the lumen. Some tubules were lined with 1 to 2 layers of pseudostratified, tall columnar epithelial cells and contained fibronoid exudates with some spermatogenic cells (Figure 2b). At a higher dose treatment for 4 weeks several tubules lined with 1 to 2 layers of cells were seen. The lumen contained

Table 2. Effect of dose variation of *Carica papaya* bark extract on testosterone concentration in rats.

Group (n = 10)	Low dose extract (50 mg.mL^{-1}.kg^{-1}.day^{-1})			High dose extract (100 mg.mL^{-1}.kg^{-1}.day^{-1})			Controls/distilled Water		
	1A (4 wks)	1B (8 wks)	1C (Rev)	2A (4 wks)	2B (8 wks)	2C (Rev)	3A (4 wks)	3B (8 wks)	3C (16 wks)
Testosterone (mg.mL^{-1})	0.90 ± 0.14[a]	0.30 ± 0.08[a]	0.92 ± 0.15	0.75 ± 0.10[a]	0.25 ± 0.07[a]	0.28 ± 0.07[a]	1.29 ± 0.14	1.30 ± 0.15	1.28 ± 0.15

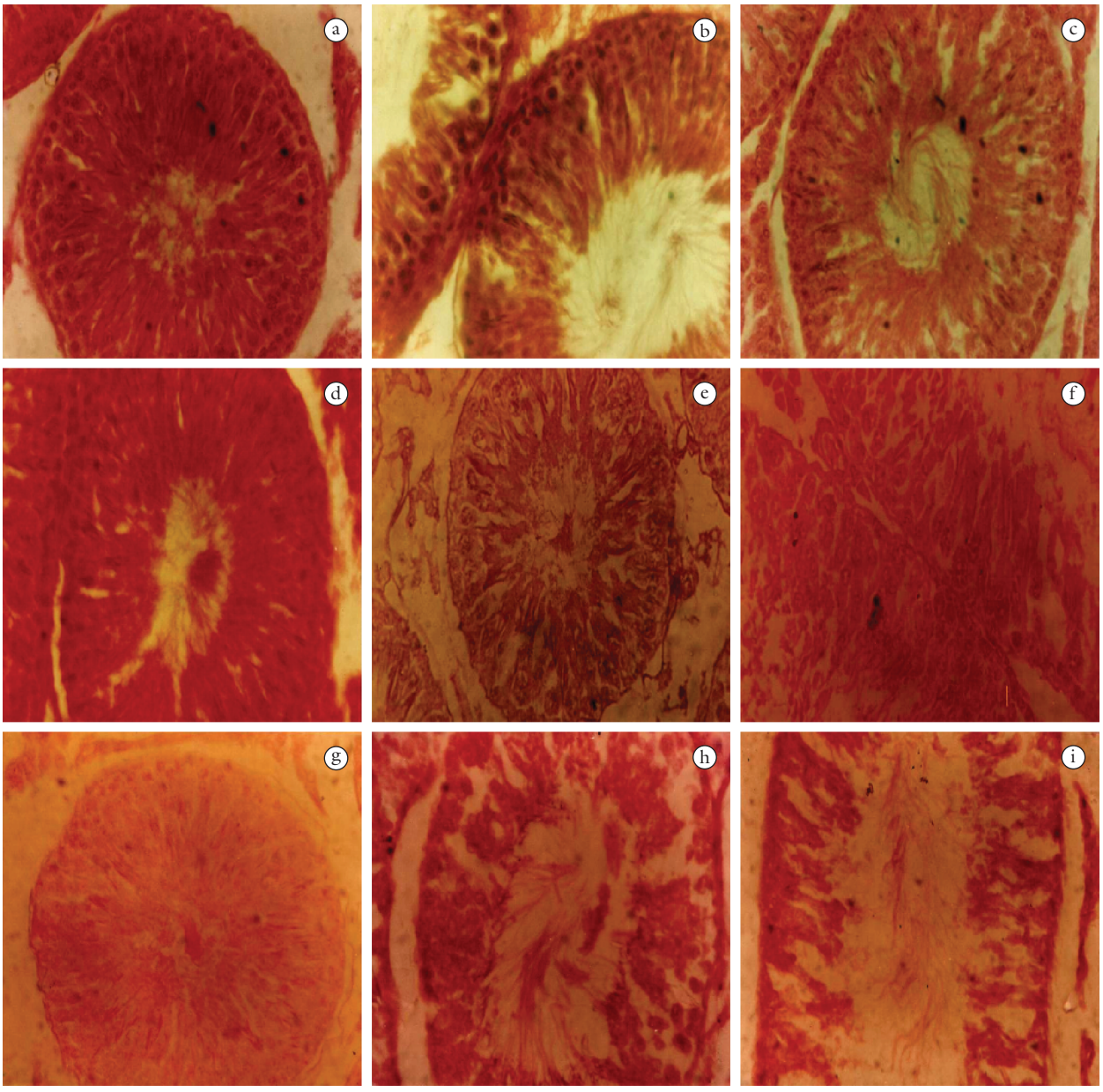

Figure 1. Photomicrograph of the testis of: a) control rat at 4 weeks; b) rat given low dose extract for 4 weeks; c) rat given high dose extract for 4 weeks; d) control rat at 8 weeks; e) rat given low dose extract for 8 weeks; f) rat given high dose extract for 8 weeks; g) control rat after 8 weeks reversal period; h) rat with low dose extract after 8 weeks reversal period; i) rat with high dose extract after 8 weeks reversal period. Magnification ×400; Stain: Hematoxylene & Eosin.

fibroblasts with necrotic materials. There was also extensive fibrosis of the tubules and disruption of the epithelial lining (Figure 2c). When the extract was given at the lower dose for 8 weeks, there was fibrosis of the interstitial spaces with cystic dilation of tubules, and severe thickening of the surrounding capsule. This was similar to the result obtained in high dose for 4 weeks (Figure 2d-f).

The seminal vesicle of the control rats consisted of numerous ducts, which were thrown into folds. The epithelial lining is made of pseudostratified low cuboidal cells and the lumen contained thick secretions (Figure 3a). At the low dose treatment for 4 weeks, the structures appeared almost the same as in control, with numerous tubules lined with folds of epithelia and eosinophillic cells in multilayers (Figure 3b). At the higher dose treatment for 4 weeks, there was disruption of epithelial lining with necrosis of the epithelial cells (Figure 3c). At the lower dose given for 8 weeks, the result was similar to the high dose at 4 weeks (Figure 3e). The result obtained from the higher dose for 8 weeks was necrosis of the tubules; thickening of the wall and appearance of deeply eosinophillic materials (Figure 3f).

The control prostate gland was lined with multilayered epithelium and contained oesinophilic secretion in their lumen (Figure 4a). At the low dose treatment for 4 weeks, there were numerous glandular structures lined by tall

to low columnar epithelium. The cells had uniformly oesinophilic cytoplasm with poor outlined and faint nuclei. This is consistent with coagulative necrosis of the epithelium (Figure 4b). At the higher dose treatment for 4 weeks, there was extensive coagulative necrosis of the epithelium, alteration of the epithelium and proliferation of epithelial lining in some areas (Figure 4c). This was the same with the low dose treatment for 8 weeks (Figure 4e), but at a higher dose treatment for 8 weeks, the effects were more severe. There was necrosis of the cell (cell death), fibromuscular hyperplasia, scanty prostatic acini, and multilayering of the epithelium in some glands (Figure 4f).

In the testes, epididymis, prostate and seminal vesicle of rats in which reversibility studies were observed; comparing the controls [Figures 1-4 (g)] to the extract treated low and high doses [Figures 1-4 (h, i)]. Only slides from rats treated low dose slightly mirrored the base line control which indicates that the reversal impact were only relative. This feature supports values from testosterone concentration.

Deductively a heavy consumption of *Carica papaya* bark could cause impaired seminal and prostate function as revealed by histological analysis. The extensive necrosis of the epithelial lining and the epithelia cells could result in impairment of the functioning of these organs although matching biochemical parameters were not estimated. The significant decrease in viable germ cells within the epididymis with fibrinoid

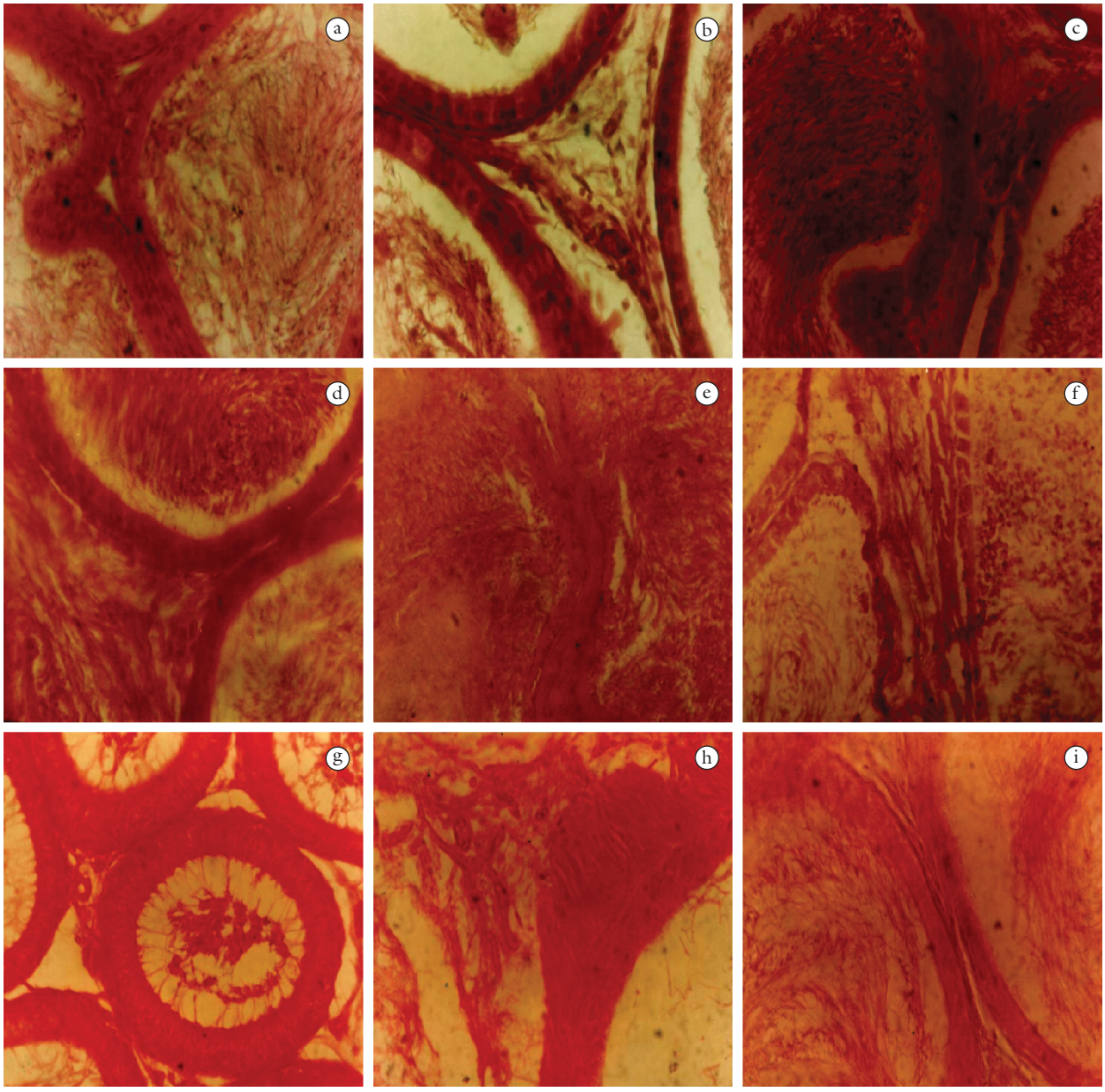

Figure 2. Photomicrograph of the epididymis of: a) control rat at 4 weeks; b) rat given low dose extract for 4 weeks; c) rat given high dose extract for 4 weeks; d) control rat at 8 weeks; e) rat given low dose extract for 8 weeks; f) rat given high dose extract for 8 weeks; g) control rat after 8 weeks reversal period; h) rat with low dose extract after 8 weeks reversal period; i) rat with high dose extract after 8 weeks reversal period. Magnification ×400; Stain: Hematoxylene & Eosin.

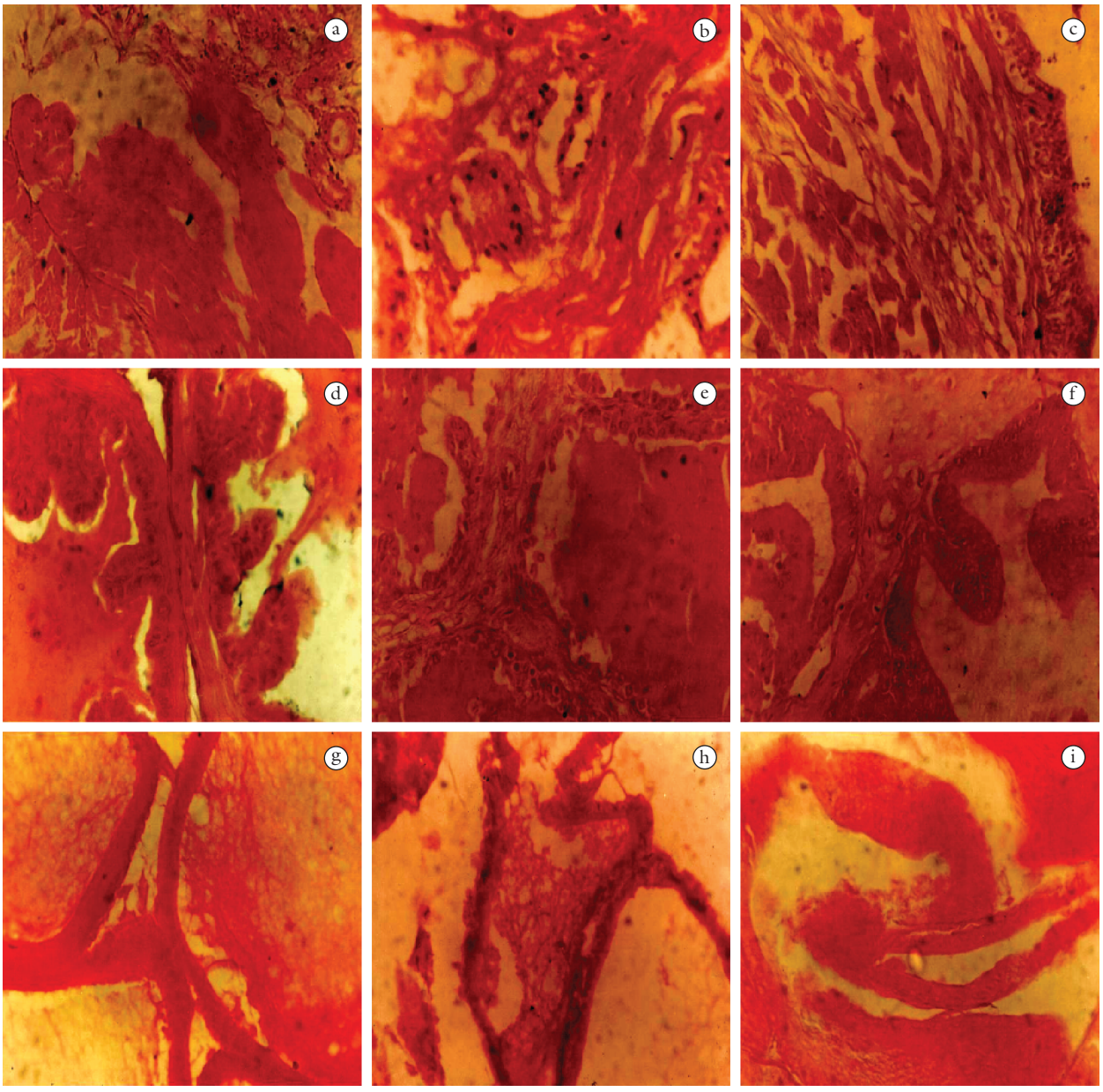

Figure 3. Photomicrograph of the prostate of: a) control rat at 4 weeks; b) rat given low dose extract for 4 weeks; c) rat given high dose extract for 4 weeks; d) control rat at 8 weeks; e) rat given low dose extract for 8 weeks; f) rat given high dose extract for 8 weeks; g) control rat after 8 weeks reversal period; h) rat with low dose extract after 8 weeks reversal period; i) rat with high dose extract after 8 weeks reversal period. Magnification ×400; Stain: Hematoxylene & Eosin.

exudates suggests a resultant decrease in fertility. This could provide evidence of a deleterious effect of the extract on the epididymis. The cytoarchitectural perturbation of the accessory sex organs caused by the extract administration is probably the result of the decreased secretory activity supported by histological analysis. This strong inhibitory effect results in reduced availability of androgens (JAROW, CHEN, ROSNER et al., 2001). Findings from this study have further reiterated our prior qualitative proofs of the bark of *Carica papaya* as a potential male contraceptive agent and that its effect is reversible when used at a lower dose (KUSEMIJU, NORONHA and OKANLAWON, 2002).

In conclusion, the extracts significantly affect the testes and accessory organs at high doses. This toxicity is however dose-dependent. *Carica papaya* significantly decreases peripheral testosterone level in both low and high dose groups. There was a significant reversal in the testosterone concentration of the rats in the low dose group than in the high dose group indicating that damage done to the testes and accessory glands in the low dose group was minimal. The reversal effect suggests that it is safe to use this contraceptive. Males may finally have access to contraceptive options beyond condoms sand vasectomies.

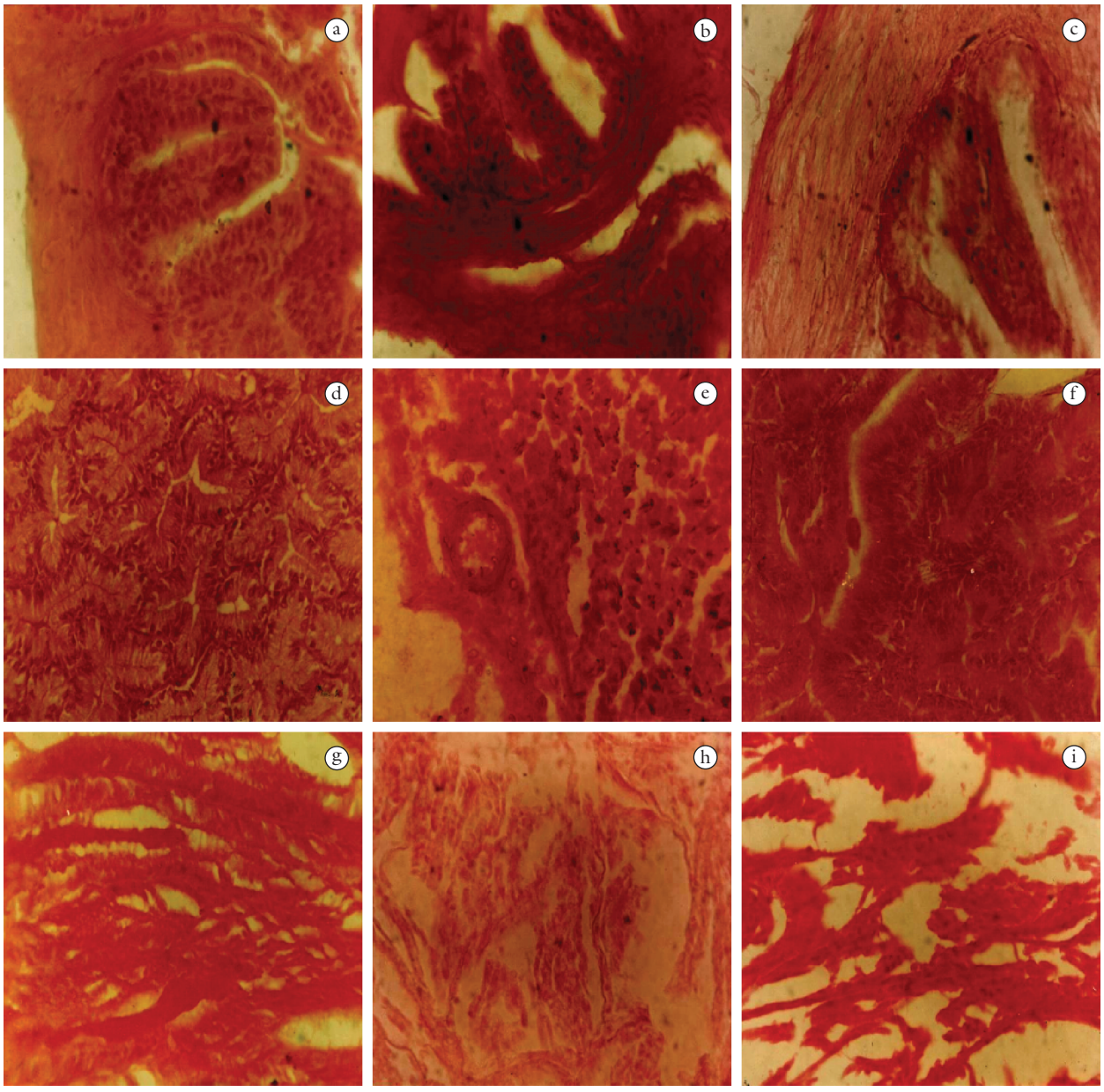

Figure 4. Photomicrograph of the seminal vesicle of: a) control rat at 4 weeks; b) rat given low dose extract for 4 weeks; c) rat given high dose extract for 4 weeks; d) control rat at 8 weeks; e) rat given low dose extract for 8 weeks; f) rat given high dose extract for 8 weeks; g) control rat after 8 weeks reversal period; h) rat with low dose extract after 8 weeks reversal period; i) rat with high dose extract after 8 weeks reversal period. Magnification ×400; Stain: Hematoxylene & Eosin.

References

American Physiological Society – APS. Guiding principles for research involving animals and human beings. *American Journal of Physiolog - Regulatory Integrative and Comparative Physiology*, 2002, vol. 283, p. 281-283.

CHARLOTTA, S. *The Kallikrein-Related Peptidases hK2 and PSA with Emphasis on Genetic Variation, Secretion, and Sperm Motility*. Sweden: Lund University, 2008. p. 12-13. [Ph.D. Thesis].

CUNNINGHAM, GR. and HUCKIN, C. Persistence of complete spermatogenesis in the presence of low intra-testicular concentration of testosterone. *Endocrinology*, 1979, vol. 105, p. 177-87. PMid:446406. http://dx.doi.org/10.1210/endo-105-1-177

DURU, FIO., YAMA, OE., NORONHA, CC. and OKANLAWON, AO. Alterations in morphometry and malondialdehyde levels in adult Sprague-Dawley rat testis in three obstructive vasectomy models: Effect of Melatonin. *Asian Journal of Pharmaceutical and Clinical Research*, 2011, vol. 4, n. 2, p. 27-30.

GONZALES, GF. Function of seminal vesicles and their role on male fertility. *Asian Journal of Andrology*, 2001, vol. 3, n. 4, p. 251-8. PMid:11753468.

GUIDO, V., ARIANE, W., EVI, D., JOHANNES, VS. and KAREL, D. Androgens and spermatogenesis: lessons from transgenic mouse models. *Philosophical Transactions of the Royal Society B*, 2010, vol. 365, p. 1537-1556. http://dx.doi.org/10.1098/rstb.2009.0117

JAROW, JP., CHEN, H., ROSNER, W., TRENTACOSTE, S. and ZIRKIN, BR. Assessment of the androgen environment within the human testis: Minimally invasive method to obtain intra testicular fluid. *Journal of Andrology*, 2001, vol. 4, p. 640-645.

KUSEMIJU, O., NORONHA, C. and OKANLAWON, A. The effect of crude extract of the bark of *Carica papaya* on the seminiferous tubules of male Sprague-Dawley ratsBottom of Form. *The Nigerian Postgraduate Medical Journal*, 2002, vol. 9, n. 4, p. 205-209. PMid:12690680.

KUSEMIJU, TO., OSINUBI, AA., NORONHA, CC. and OKANLAWON, AO. Effect of *Carica papaya* bark extract on the testicular histology of male SD rats. *Nigerian Quarterly Journal of Hospital Medicine*, 2010, vol. 20, n. 3, p. 133-137. PMid:21033322.

KUSEMIJU, TO., YAMA, OE., NORONHA, CC. and OKANLAWON, AO. Left ventricular cardiac blood analysis in male Wister rats treated with aqueous extract of *Carica papaya* bark. *Asian Journal of Pharmaceutical and Clinical Research*, 2011, vol. 4, n. 4, p. 41-43.

KUSEMIJU, TO., YAMA, OE. and OKANLAWON, AO. Effect of *Carica papaya* bark extract on oxidative stress parameters in testes of male albino rats. *International Journal of Applied Research in Natural Products*, 2012, vol. 4, n. 4, p. 1-6.

MOORE, KL., DALLEY, AF. and AGUR, AMR. Pelvis and Perineum. In MOORE, KL., DALLEY, AF. and AGUR, AMR. *Clinically Oriented Anatomy*. 5th ed. Lippincott Williams & Wilkins, 2006. chap. 3, p. 379-423.

O'HARA, L., WELSH, M., SAUNDERS, PT. and SMITH, LB. Androgen receptor expression in the caput epididymal epithelium is essential for development of the initial segment and epididymal spermatozoa transit. *Endocrinology*, 2011, vol. 152, n. 2, p. 718-29. PMid:21177831. http://dx.doi.org/10.1210/en.2010-0928

SVECHNIKOV, K., LANDREH, L., WEISSER, J., IZZO, G., COLÓN, E., SVECHNIKOVA, I. and SÖDER, O. Origin, development and regulation of human Leydig cells. *Hormone Research in Paediatrics*, 2011, vol. 73, n. 2, p. 93-101. PMid:20190545. http://dx.doi.org/10.1159/000277141

TIETZ, NW. *Clinical Guide to Laboratory Tests*. 3rd ed. Philadelphia: W.B. Saunders Company, 1995. p. 578-80.

TISSELL, LE. and SALANDAR, H. Anatomy of the human Prostate and its three paired lobes. *Progress in Clinical And Biological Research*, 1984, vol. 145, p. 55-65. PMid:6371831.

YAMA, OE., DURU, FIO., OREMOSU, AA. and NORONHA, CC. Testicular oxidative stress in Sprague-Dawley rats treated with *Momordica charantia*: The effect of antioxidant supplementation. *Bangladesh Journal of Medical Science*, 2011, vol. 10, n. 2, p. 104-111.

YAMA, OE., NORONHA, CC. and OKANLAWON, AO. Mosaic-cellular patterns of seminiferous tubules in rats gavaged *Momordica charantia* for four intervallic spermatogenic phases. *Journal of Morphological Science*, 2011, vol. 28, n. 4, p. 1-6.

ZHANG, XD. and JIN, BF. The role of seminal vesicles in male reproduction and sexual function *Zhonghua Nan Ke Xue* (*National journal of Andrology*), 2007, vol. 13, n. 12, p. 1113-6.

Received January 31, 2012
Accepted October 26, 2012