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Research Article

EFFECT OF SEEDED AND SEEDLESS *CARICA PAPAYA* FRUIT EXTRACTS ON THE HISTOARCHITECTURE OF REPRODUCTIVE STRUCTURES IN MALE ALBINO RATS

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ABSTRACT

The present study was carried out to assess the effect of semi-ripe fruit pulp extract of *Carica papaya* (seeded and seedless fruits) on male albino Wistar rats. The aim of the present investigation is to analyse the mechanism of action of aqueous extract of seeded papaya fruit pulp and seed extract administered as a combined dose and seedless variety papaya fruit pulp extract on testicular function in male albino rats.The experimental animals were divided into three groups,
Group - I served as control, experimental
Group - II received combined papaya pulp and seed extract from seeded fruits and experimental
Group - III received papaya pulp extract from seedless fruits.
Experimental groups were orally administered with the extract (1gm / kg / body weight) for 60 days. These results indicate that *Carica papaya* semi-ripe seeded and seedless fruit pulp extract possess antifertility effects.

INTRODUCTION

Medicinal plants and herbal preparations have recently received considerable attention and have been found to be promising choice over modern synthetic medicines (Wills *et al* 2000; Sharma *et al.,* 2001). All over the world, 80% of population use traditional medicine for primary medical problems. In spite of considerable development in contraceptive technology, search for male and female antifertility agent from plants continues to be a potential area of investigation (Shukla and Dixit, 2011; Goverdhan Naik *et al*., 2014). *Carica papaya* has been exploited and documented for its antifertility properties in male animal models (Lohiya *et al*., 2005; Kusemiju, *et al*., 2012). Crude extracts from seeds of *Carica papaya* has been shown to induce variable responses depending on the dose, duration and route of administration in laboratory animals (Udoh and Kehinde, 1999; Kamal *et al.,* 2003; Verma and Chinoy, 2002). *Carica papaya* seeds have been emerging as potential antifertility drug and used as fertility control agent in animal models and even on human beings (Udoh and Kehinde, 1999; Manivannan *et al.,* 2009; Udoh *et al.,* 2009).

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They contain active compounds such as caricin, an enzyme carpasemine, a plant growth inhibitor and oleanolic glycosides (Emeruwa,1982) the last of which caused sterility in male rats (Das, 1980). The fruit and dry powder of papaya seed have contraceptive effects with no adverse toxicity on male albino rats (Unny *et al.,* 2003). The seeds of papaya has also been reported to have contraceptive effects in adult Langur monkeys (Lohiya *et al.,* 2002). A compound isolated from the seeds of *Carica papaya*, an alkaloid 1,2,3,4 tetrahydropyridin-3-yl-octanoate proved to have anti-fertility effects. The compound was evaluated against rat spermatozoa *in vitro* at a concentration of 12.5 ng/µL. The compound decreased the motility, viability and morphology of the spermatozoa (Euis Julaeha *et al.,* 2011). Most of the studies have so far focused on the effect of unripe fruit and seeds, leaves of *Carica papaya* in male fertility regulation. A novel approach has been made in this study to decipher the effects of semi- ripe stage of papaya fruit pulp and seeds on the reproductive structures in male albino rats.

MATERIALS

Papaya fruits: Semi- ripe seeded and seedless varieties of *Carica papaya* fruits were commercially obtained from wholesale fruit market in Chennai. The fruit specimen was

identified and authenticated by Dr P.Jayaraman, Plant Anatomy Research Centre (PARC), Chennai, Tamil Nadu, India. A voucher specimen with number (PARC / 2013 / 2319) has been deposited in the herbarium of the same department.

Preparation of aqueous extracts from fruit pulp and seeds of papaya fruits

The fruits were washed with double distilled water, and the outer skin and the inner seeds were removed. The fruit pulp was sliced. The seeds and sliced fruit pulp were separately air dried in shade. The dried pieces of fruit pulp and seeds of seeded variety and the fruit pulp of seedless variety were pulverized separately into a coarse texture form using an electrical blender. The powdered fruit pulp was macerated with cold water and passed through a fine muslin cloth. The filtrate was collected and dried. The dried material was stocked in an airtight plastic container. The dried fractions of the fruit pulp and seeds were preserved at $4^{o}C$ in a refrigerator for further use. Two different types of aqueous extracts were prepared. The fruit pulp and seed of seeded variety were combined and the fruit pulp of seedless variety were prepared with required amount of distilled water. A fresh sample of required amounts was prepared from the stock prior to administration of extracts to the animals.

Experimental animals

Healthy male albino Wistar strain rats (*Rattus norvegicus*) were purchased from The King Institute of Preventive Medicine and Research, Guindy, Chennai. Rats weighing 155 ± 25 g were used in the present study. The animals were maintained in polypropylene cages with metal grill top under standard environmental conditions of temperature 25 ± $2^{o}C$ and proper ventilation. They were exposed to a 12h light: 12h dark cycle and provided with water *ad libitum*. The animals were fed with standard balanced pelleted diet (Sai Durga Foods, Bengaluru). Animals were treated humanely. Care and supervision was provided throughout the period of study. The study protocols were duly approved by the Animal Ethical Committee Regn.No. CPCSEA/CA/ORG/2006/65/4 Studies were performed in accordance with the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

METHODS

Acute oral toxicity study

In order to assess the toxic effects and tolerance limit and to determine a safe dose, acute oral toxicity study was carried out as per the CPCSEA guidelines. The rats were fasted for 3-4 hours before administration of extracts. The extracts were administered in a single dose by using gastric intubation. Three groups of six rats each, were used in each group.

Testing of tolerance limit for seeded variety (fruit pulp and seed powder combined)

The fruit pulp powder and powered seed of seeded variety were taken in equal proportion, combined and mixed thoroughly in distilled water for oral administration. The combination of the extract was treated with a dose of 1000 mg , 2000 mg, 3000 mg, 4000 mg and 5000 mg / kg bw and mortality was observed for 96 hr and the LD_{50} was determined (Miller and Tainter, 1944; Weils,1952). The test dose was given at 9.00 AM. Animals were observed initially after dosing at least once during the first 30 minutes, periodically during the first 24h. 100%, 67%, 50% and 33% mortality was observed in 5000 mg, 4000 mg, 3000 mg and 2000 mg doses respectively. No mortality was observed in 1000mg dosage. 50% mortality was observed in 3000 mg / kg bw. From this one- third of the dose which would be safe for the animals were determined and the combined preparation was administered orally for a period of 60 days to the experimental group- I of rats

Testing of tolerance limit for seedless variety fruit pulp

Toxicity and tolerance limit were also carried out for rats administered with seedless variety papaya fruit pulp. The pulp extract was treated with at a dose of 1000 mg , 2000mg, 3000 mg, 4000 mg and 5000 mg / kg bw. 83%, 67%, 50% and 17% mortality was observed in 5000 mg, 4000 mg, 3000 mg and 2000 mg doses respectively. No mortality was observed in 1000 mg dosage. 50% mortality was observed in 3000 mg / kg bw. From this one- third of the dose which would be safe for the animals were fixed and the aqueous preparation was administered orally for a period of 60 days for experimental group- II rats. Conditions of tremors and convulsions were also observed. The animals were further observed for 48h post treatment for signs of toxicity and death before fixing the final dosage.

Dose Determination of various extract

Final doses were determined for the experiment after testing the tolerance limit for both the types of extracts.500 mg of the dried fruit pulp powder and 500 mg of powdered seed from seeded semi-ripe papaya fruit (1000 mg in combination) was dissolved in water (1.0 gm dissolved in 1.0 ml distilled water) for experimental group – I animals. 1000 mg fruit pulp powder from seedless papaya was dissolved in water (1.0 gm dissolved in 1.0 ml distilled water) for experimental group - II animals. Animals in each group received the same dose throughout the treatment period.

Route of administration of extracts

The extracts prepared were orally administered via gastric intubation using an orogastric tube comprising a 16-G polyethylene catheter fitted with a hypodermic syringe (volume of 10 ml). Administration of extracts were carried out every morning after a 24 hour interval for 60 consecutive days.

Experimental design

The animals were weighed and divided into three groups of equal weight. Each group consisted of six animals, maintained in separate cages.

Group I: Control: Male animals which received normal feed and water.

Group II: **Experimental Group- I** : Experimental male animals which received normal feed and water, oral administration of seeded semi- ripe papaya fruit pulp and seed

extract combined (1000 mg/kg body weight/day) for a period of 60 days.

Group III: **Experimental Group- II** : Experimental male animals which received normal feed and water, oral administration of seedless semi- ripe papaya fruit pulp extract alone (1000 mg/kg body weight/day) for a period of 60 days.

The animals were acclimatized to laboratory conditions for 15 days with normal feed and water before the start of the experiment. Initial and final body weights were recorded prior to and after treatment. The seeded semi- ripe papaya fruit pulp and seed extract and the seedless variety semi-ripe papaya fruit pulp extract was given orally through a gastric intubation daily at 9.00 A.M. The animals were sacrificied by cervical dislocation on the 61st day, 24 hour after administration of last dose.

Histological Studies

For histological studies of testis and epididymis the tissues were fixed in 10% formalin. They were processed routinely for paraffin embedding and sectioned to 5µ thickness for staining by haematoxylin and eosin (H&E) method (Luna, 1968) for histopathological examination

RESULTS

Histology of testis

In the control group rats, the seminiferous tubules were surrounded by intertubular connective tissues containing the germinal epithelium. Basement membrane on which rests actively dividing spermatogonia and sparsely distributed sertoli cells whose apices reach the lumen of the tubules. Spermatocytes, early and late spermatid were seen arranged in a concentric manner from the basal layer of the spermatogonia towards the lumen of the seminiferous tubules (Fig. 1a, b). In animals treated with seeded *Carica papaya* fruit pulp and seed extracts combination, histological studies revealed that the extract would have shown the effect on disruption in the arrangement of seminiferous tubules, depleted germinal epithelium.

Transverse section of testis of control rats

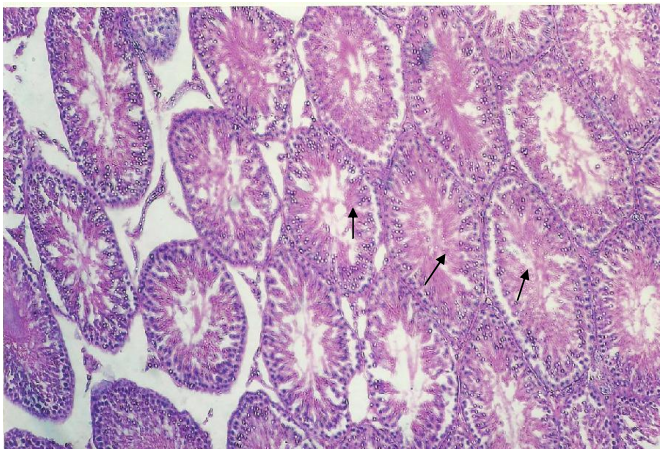

Fig. 1a. Normal germinal epithelium showing compact arrangement of seminiferous tubule containing spermatogonia and supporting cell (arrow) and lumen containing spermatids

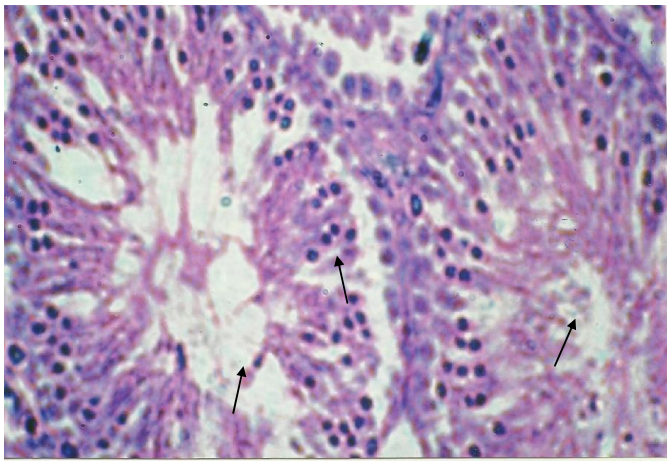

Fig. 1b. Concentric arrangement of different stages of spermatocytes. Primary and secondary from basal layer to lumen of seminiferous tubules (arrow) (x 100)

Transverse section of testis of experimental male rats treated with semi-ripe seeded papaya fruit pulp and seed extract combination for 60 days

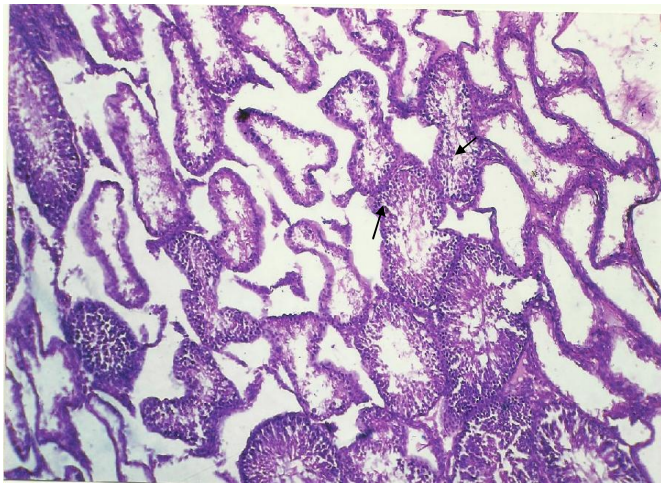

Fig. 2a. Acellular, fused, disorganised and distorted (arrow) seminiferous tubules. Severe disintegration of spermatocytes and supporting cells (x100)

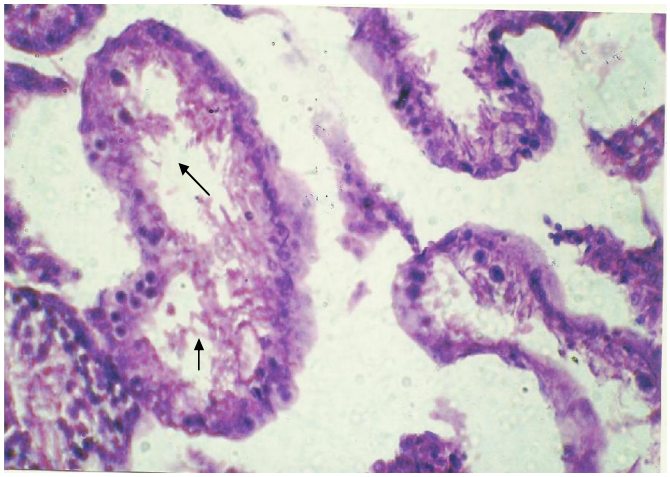

Fig. 2b. Lumen of seminiferous tubules containing empty spaces (arrow), scattered Sertoli cells. Degeneration of primary and secondary spermatocytes spermatids and spermatogenic arrest (x 400)

No late spermatids in some sections. The seminiferous tubules were lined by only few necrotic germ cells with scattered sertoli cells which suggest that the treatment caused the reduction in the number of spermatozoa which leads to alterations in kinetics of spermatogenesis. The seminiferous tubules presented significant degenerative changes. The changes involved by the treatment consisted of the damage of the germinal epithelium and degeneration of spermtocytes and spermatids. The interstitium was highly reduced (Fig. 2a, b). Not much changes was noticed in the testis of rats treated with seedless *Carica papaya* fruit pulp extracts except for the formation of vacuoles in the testis when compared to control rats (Fig. 3a,b).

Tranverse section of testis of experimental rats treated with semi-ripe seedless papaya fruit pulp extract for 60 days

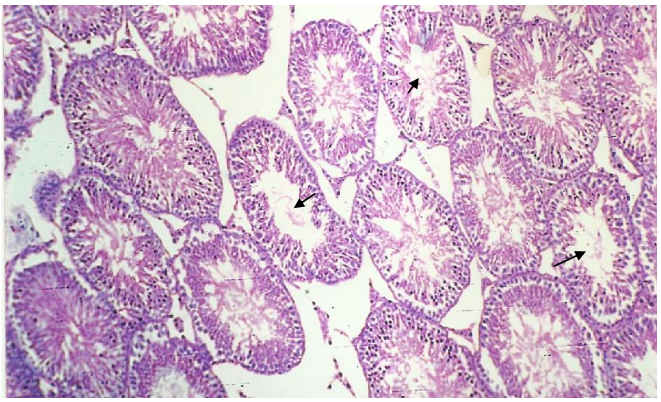

Fig. 3a. Compact arrangement disturbed by formation vacoules (arrow) in the tubules (x100)

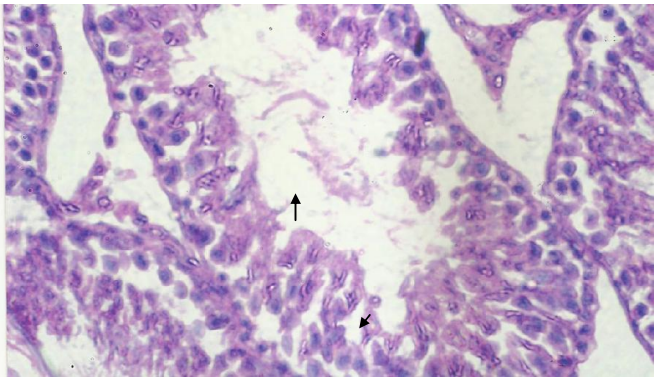

Fig. 3b. Widening of spaces between neighbouring tubules (arrow) interstitium was not affected Lumen with cellular debris, hypercellularity of Leydig cells (x400)

Histology of caput and cauda epididymis

The epididymis showed a normal structure. The epithelial cells of the caput were tall, columnar with nuclei arranged in a row near the thin basement membrane. The epithelium of the cauda consisted of low cubodial cells. The lumen of the ductules was larger in the cauda and smaller in the caput segments of sterocilia were more profuse in the caput region than in the cauda. Both the portions of the epidymis were full of spermatozoa. Intertubular connective tissue and vascularity was observed to be normal in both caput and cauda epididymis. Normal structure of caput epididymia (Fig. 4) and cauda epidydimis (Fig. 7). The histological alterations were more visible in caput in animals treated with seeded *Carica papaya* fruit and seed extracts combination. The inter tubular spaces were wider in caput. The stereocilia were lacking in caput. The lumen of ducts contained cellular debris. The interstitial spaces were filled with loose connective tissue. In caput the lumen of tubules contained little or no spermatozoa, fibroblasts with necrotic materials were present (Fig. 5). When compared to control the changes were not much pronounced in the caput epididymis (Fig. 6) in the rats treated with seedless *Carica papaya* fruit pulp extracts.

Transverse section of caput epididymis in control rats

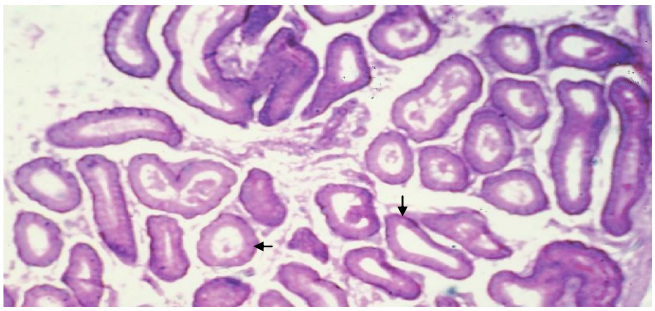

Fig. 4. Epithelial cells tall, columnar nuclei arranged in (arrow), thin basement membrane. with intra- tubular connective tissue (x100)

Transverse section of caput epididymis of experimental rats treated with seeded semi-ripe papaya fruit pulp and seed extract combination for 60 days

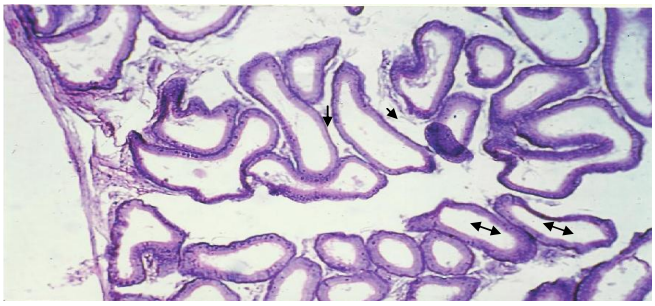

Fig. 5. Inter tubular spaces wider (arrow) lumen empty with very less or no spermatozoa. Large vacoules in the supranuclear position (double headed arrow) (x100)

Transverse section of caput epididymis of experimental rats treated with seedless semi-ripe papaya fruit pulp extract for 60 days

Fig. 6. Empty lumen with absence of spermatozoa (arrow). Intertubular connective tissue observed. Vacoules like spaces appear in few lumen (x100)

Transverse section of cauda epididymis in control rats

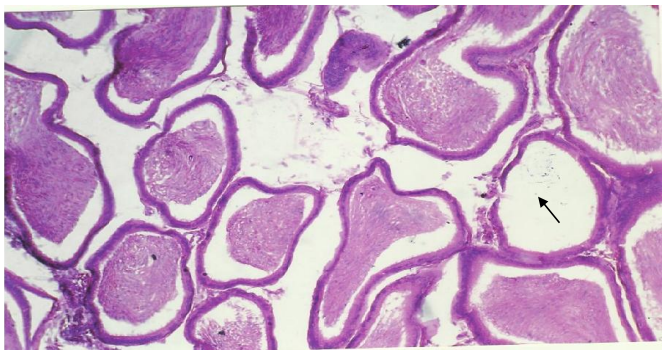

Fig. 7. Low cuboidal cells epithelium. Lumen is large in size. Intertubular connective tissue observed (arrow) (x100)

Transverse section of cauda epididymis in experimental rats treated with seeded semi-ripe papaya fruit pulp and seed extract combination for 60 days

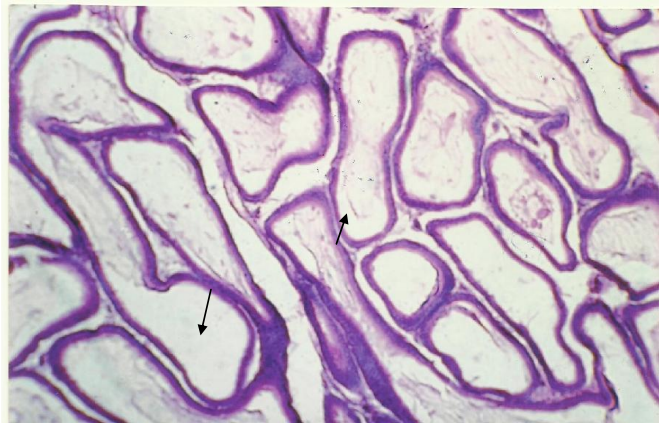

Fig. 8. Intra tubular spaces widened with fibrous material. Basement membrane, irregular shape and increased size of tubules (arrows). Epithelial cells distorted (x100)

Transverse section of cauda epididymis in experimental rats treated with seedless semi-ripe papaya fruit pulp extract for 60 days

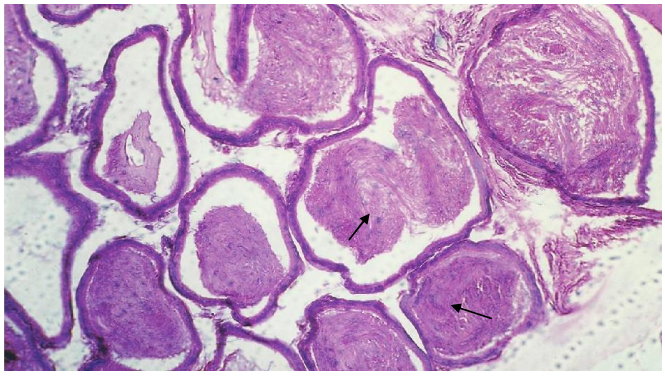

Fig. 9. Interstitial space filled with loose connective tissue. Cellular elements in the luminal regions (arrows) (x 100)

Similarly, cauda epididymis in treated animals showed irregular shape and increase in size of the tubules. The epithelial nuclei were observed to have migrated into the lumen of the tubules, distorted and nuclear pyknosis appeared among the epithelial cells. In cauda the intertubular spaces were observed to be filled with fibrous material. In cauda there were very little spermatozoa which had clumped (Fig. 8). When compared to control the changes were not much pronounced in the cauda epididymis (Fig. 9) in the rats treated with seedless *Carica papaya* fruit pulp extracts.

DISCUSSION

Treatment of rats with seeded fruit pulp extract caused various degrees of damage in all reproductive tissues, when compared to treatment with seedless fruit pulp of semi-ripe payapa. The seminiferous tubules were disfigured spermatogenesis was arrested at spermatogonial stage. The leydig cell was mostly atrophied. The interstitial spaces were filled with oedematous fluid. Similar results were reperted in other studies (Verma *et al*., 2002; Oyekunle and Omope, 2010). The degeneration of leydig cell reflects the depletion of androgen levels and absence of germinal cells (ie) spermatocytes, spermatids as these stages are androgen dependent (Beardsley and O'Donnell, 2003). Studies on leaf powder extracts of neem on testis of rat include seminiferous tubular atrophy along with abnormal histological appearance of seminifereous epithelium and Leydig cells (Dafalla *et al.,* 2012). Similar observations related to the unique nature of changes in the seminiferous tubule treated with different parts of plant extracts in male albino rats have been identified with perspective to male infertility from Carica *papaya* seeds (Udoh *et al.,* 2005a).

It is not known if vacuole formation occurs as a direct consequence of germ cell necrosis or is a non specific response to Sertoli cells to androgen deprivation (Kerr *et al.,* 1993). The concurrent appearance of numerous smaller vacuoles represent a morphological indicator of Sertoli cell damage (Ghosh *et al.,* 1992). Further studies are required to identify the exact sites and reasons for vacuole formation in spermatogenesis. The result of the present study revealed arrest of spermatogenesis at the spermatogonial stage. It may be because of the effect of the treatment on circulating levels of LH and FSH (Gupta *et al.,* 2004). Further, the atrophy of Leydig cells, under the influence of the present treatment may have resulted in the lowering of testosterone level indicating antiandrogenic activity. As reported earlier it is likely that the extracts exert their effects on spermatogenesis through the alteration of the hypothalamus-pituitary- gonadal axis function and regulation (Punitha and Shettu, 2014). Structural alterations induced by the treatment result due to antiandrogenic properties present in the fruit extracts (Udoh *et al.,* 2013).

The altered histological features observed in cross section of the epididymis of animals administered on seeded semi-ripe papaya fruit pulp and seedless semi-ripe papaya fruit pulp indicates that the aqueous extracts are harmful to the epididymis which plays an important role in the synthesis of proteins and sialic acid of epididymal fluid (Turner *et al.,* 1995). Drastic changes in experimental group-I animals as compared to the animals of experimental Group -II revealed the fact that the changes in the epididymal epithelium depends upon the constituents of the fruit pulp administered. The changes might be due to the reduced target organ response to androgen or their metabolites (Kasturi *et al.,* 1995; Verma and Chinoy, 2002). The vacuolation was due to the mitochondrial swelling and consequent hypoxia, which caused vacuoles in the

principle cells (Udoh and Udoh, 2005b). Phytochemical characterization of this extract with investigation into the biological activity of the resultant products is expected to unravel the compounds in the extract which alter reproductive functions. Functional sterility was induced in male rats by the treatment, which shows potential as a male contraceptive. Plant products as contraceptives will be more acceptable for economic reasons in terms of self reliance and the possible practicability for a male pill approach in countries where population pressure is high.

REFERENCES

Beardsley, A. and O'Donnell, L. 2003. Characterization of normal spermiation and spermiation failure induced by hormone suppression in adult rats. *Biol. Reprod*. 68, 1299-1307.

Dafalla, M.H., Konozy, E.M.H. and Haseeba Ahmed Saad, 2012. Biochemical and histological studies on the effects of *Azadrichta indica* seeds kernel extract on albino rats. *Int J Med Plant Res,* 1(6): 82-92

Das, R P. 1980. Effect of papaya seeds on the genital organs and fertility of male rats. *Ind J Expt Biol*, 18: 408-409.

Emeruwa, A.C. 1982. Antibacterial substances from ripe and unripe fruits of *Carica papaya*. *J Nat Prod* 45: 123-127

Euis Julaeha, Yunita Permatasari and Tri Mayanti, 2011. Antifertility compounds isolated from seeds of *Carica papaya*. *Proc 2nd International Seminar on Chemistry*, 417-420

Ghosh, S.P., Bartke, A., Grasso, P. Reichert, L.E. Jr. and Russsell, L.D. 1992. Structural manifestations of the rat Sertoli cell to hypophysectomy: a correlative morphometric and endocrine study. *Endocrinology*. 131(1): 485-497.

Govardhan Naik, A., Lakshman, J. and Changamma, C. 2014. A study on the effect of betel leaf stalk extract in male albino rats. *Int J Biol Pharma Res* 5(3): 285-289

Gupta, R.S., Kachhawa, J.B and Chaudhary, R. 2004. Antifertility effects of methanolic pod extract of *Albizzia lebbeck*. *Asian J Androl*, 6, 155–159.

Kamal, R., Gupta, R.S. and Lohiya, N.K. 2003. Plants for Male fertility regulation. *Phytother. Res*. 17: 579-590

Kasturi, M., Manivannan, B., Nazeer Ahmed, R., Shaikh, P.D. and Pathan, K.M. 1995. Changes in epididymal structure and function of albino rats treated with *Azadirachta indica* leaves. *Ind J Exp. Biol*., 33(10): 725-729.

Kerr, J.B., Millar, M., Maddocks, S. and Sharpe, R.M. 1993. Stage dependent changes in spermatogenesis and Sertoli cells in relation to the onset of spermatogenic failure following withdrawal of testosterone. *Anat. Rec*. 235, 547-559.

Kusemiju, O., Yama, O.E. and Okanlawon, A. 2012. Effect of *Carica papaya* bark extract on oxidative parameters in testes of male albino rats. *Int J Appl Res Nat Prod*, 4(4), 1-6.

Lohiya, N.K., Mishra, P.K., Pathak, N., Manivannan, B., Bhande, S.S., Panneerdoss, S. and Sriram, S. 2005. Efficacy trial on the purified compounds of the seeds of *Carica papaya* for contraception in albino rat. *Reprod Toxicol.,* 20: 135-148.

Lohiya, N.K., Mishra, P.K., Pathak, N., Maniyannan, B., Sriram, S., Bhande, S.S. and Panneerdoss, S. 2002. Chloroform extract of *Carica papaya* seed induced long term reversible azoospermia in langur monkey. *Asian J Androl*, 4(1), 17-26.

Luna, L.G. 1968. Manual of histological staining. *3rd ed. McGraw Hill Book Company, New York.*

Manivannan, B., Mittal, R., Goyal, S., Ansari, A.S. and Lohiya, N.K. 2009. Sperm characteristics and ultra structure of testes of rats after long-term treatment with the methanol subfraction of *Carica papaya* seeds. *Asian J Androl*. 11: 583-599.

Miller, L.C. and Tainter, M.C. 1944. Estimation of the LD_{50} and its errors by means of the logarithimic-probit graph paper. *Proc.Soc.Exp.Biol.Med* 57: 261-264.

Oyekunle, O.A. and Omope, M.M. 2010. Evaluation of andrological indices and testicular histology following chronic administration of aqueous extract of *Carica papaya* leaf in Wistar rat. *Afr J Pharma Pharmacol,* 4(5): 252-255.

Punitha, N. and Shettu, N. 2014. Effect of *Carica papaya* fruit extract on hormonal profile in male albino rats. *Int I Curr Res*., 6(5), 6564-6566.

Sharma, R.S., Rajalakshmi, M., Jeyaraj, D.A. 2001. Current status of fertility control methods in India. *J Bio Sci,* 26, 391-395.

Shukla, S. and Dixit, S. 2011. *In silico* identification of drug targets for antifertility from natural products by differential reaction content analysis of metabolic pathways. *Malay J Med Sci*, 18(3): 13-17

Turner, T.T., Miller, D.W. and Avery, E.A. 1995. Protein synthesis and secretion by the rat caput epididymis *in vivo* influence of the luminal microenvironment. *Biol. Reprod*., 53: 1012-1019.

Udoh, P.B. and Kehinde, A. 1999. Studies on antifertlity effect of paw paw seeds *(Carica papaya*) on the gonads of male albino rats. *Phytother Res*, 13(3), 226-228.

Udoh, P.B., Udoh, F.V., Umoren, E.B., James, U.W., Okeke, C.P. and Agwu, B. 2009. Effects of Caricapyrl-99 seed alkaloid extract on the serum levels of sex hormones and pituitary gonadotropins in male albino rats. *Nig J Physiol Sci* 24(1):13-15.

Udoh E. Nkeiruka and Nwaehujor O. Chinaka, 2013. Antifertility effects of *Carica papaya* methanol leaf extracts in male wistar rats. *J.Pharmacol Toxicol* 1-7

Udoh, F.V., Udoh, P.B. and Umon, E.E. 2005a. Activity of alkaloid extract of *Carica papaya* seeds on reproductive functions in male Wistar rats. *Pharma Biol,* 43, 563-567.

Udoh, P.B., Essien, I. and Udoh, F. 2005b. Effects of *Carica papaya* (paw paw) seeds extract on the morpohology of pituitary-gonadal axis of male Wistar rats. *Phytother Res,* 19(12), 1065-1068.

Unny, R., Chauhan, A.K., Joshi, Y.C., Dobhai, M.P. and Gupta, R.S. 2003. A review on potentiality of medicinal plants as the sources of new contraceptive principles. Phytomedicines. *Int J Phytother Phytopharmacol*, 10: 233-260

Verma, R.J. and Chinoy, N.J. 2002. Effect of papaya seed extract on contractile response of cauda epididymal tubules. *Asian J Androl*, 4(1): 77-78.

Verma, P.K., Sharma, A., Mathur, A., Sharma, P., Gupta, R.S., Joshi, S.C. and Dixit, V.P. 2002. Effect of *Sarcostemma acidum* stem extract on spermatogenesis in male albino rats. *Asian J Androl*, 4(1): 43-47.

Weils, C.S. 1952. Tables for convienient calculation of median effective dose (LD_{50}) and instructions in their use. *Biometrics* , 249-263

Wills, R.B.H., Bone, K. and Morgan, M. 2000. Herbal products: Active constituents, mode of action and quality control. *Nutr. Res. Rev*, 13: 47-77.

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