

Effect of ethanol extract of *Carica papaya* seeds on the histology of the epididymis of adult male albino mice

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Abstract - Epididymis play a very important role in the male reproduction. It is also the job of the epididymis to bring the sperms to maturity since the sperms that emerge from the testes are immature and incapable of fertilization. Contraceptive efficacy of *C. papaya* seed extracts have also been well documented in various animal models. This study examines the effect of oral administration of ethanol extract of *C. papaya* seeds on the histology of epididymis of the adult male albino mice. 60 healthy adult male albino mice were divided into 4 Groups (Group I to IV) of 15 mice each. 50 and 70 mg/kg/day of the extract were orally administered to Groups II and III respectively while Group I served as placebo (control). The daily administration was carried out for a period of 45 days after which the animals of Group I, II and III were sacrificed. The epididymis were obtained and processed for light microscopy. Reversibility studies were carried out in Group IV animals in which the higher dose treatment was withdrawn for another 45 days to elucidate that any induced effects were transient. Microscopic examination revealed that there were milder changes in the histology of epididymal tubules of group II (low dose) as compared to the control whereas the animals of Group III fed on higher dose show drastic changes such as vacuolation in the tubules, loose contacts of the principal cells in the epithelium of epididymis. The lumen of many tubules showed degenerated late spermatids and spermatozoa. After 45 days of withdrawal, a nearly normal pattern of tubules was regained. The study concludes that the *C. papaya* seeds ethanol extract altered the epididymal histology to influence male reproductive functions, which confirmed its antifertility property and its reversibility.

Index Terms- antifertility, *C. papaya* seed, epididymis, histology, vacuoles

I. INTRODUCTION

The epididymis is an important part of male reproductive system. It is a highly convoluted tubule that links the rete testes and the ductus deferens. The primary functions of transport, maturation and storage of spermatozoa released from the germinal epithelium of the seminiferous tubules are served by it (Flickinger et al 1978; Adebayo and Olurode, 2010). The acquisition of fertilizing capacity by the spermatozoa in the epididymis is an active process in a way that they have to be subjected to the micro environment of epididymis which is essentially controlled by the epididymal epithelium as it is responsible for the synthesis of proteins and sialic acid which are directly poured into the lumen (Chinoy et al.,1995; Johnson et al ., 2000). Contraceptive efficacy of a number of plants have been studied in various animals (Lohiya et al.,2002;Verma et al., 2006;Jahan et al.,2009; Mishra et al.,2009;Abu et al.,2012)

The quest for the development of a male contraceptive particularly from the natural sources has led to the discovery of the contraceptive efficacy of the seeds of *C. papaya*. Antifertility of various extracts of seeds of *C. papaya* have shown great promise in various experimental animals (Lohiya et al., 1999; Udoh and Kehinde., 1999; Pathak et al.,2000; Lohiya et al., 2006).

However, the information on the effect of ethanol extracts on the epididymal histology which play important role of conferring maturity to spermatozoa is scanty and this forms the stimulus for the present study.

II. MATERIALS AND METHODS

- A. **Preparation of Extract:** Seeds of ripe papaya, *Carica papaya* of Honey Dew variety were collected and shade dried. Dried seeds were then powdered (Lohiya et al., 2006). A suspension of 30g of papaya seeds powder in 250ml of ethanol was prepared and soxhalated for 3 hours. The content was filtered

successively through ordinary filter paper and WhatmanNo. 1 filter paper. The filtrate and residue were collected separately. The residue was then resuspended in the same amount of ethanol and soxhlatedfor 3-4 times for complete extraction of ethanol soluble components. The filtrate was collected, pooled and evaporated on a water bath to dryness. The residue was stable and dissolved in propylene glycol (Dose I-12mg/ml and DoseII-14mg/ml)

- B. Experimental Design: 60 healthy,sexually mature male albino mice weighing between 30g and 35g were used in the present study. The animals were obtained and housed in polypropylene cages in the Small Animal Colony, Department of Zoology, PAU, Ludhiana. They were kept under controlled & hygienic condition and provided with food and water ad libitum during acclimatization period and course of experiment.

Experimental design consisted of two phases :

Treatment:

Group I (Vehicle treated Control) - the animals were given 0.1 ml propylene glycol orally.

Group II- The animals were orally administered 50mg/kg /day of ethanol extract of *C. papaya* seeds.

GroupIII- Ethanol extract were orally administered at a dose of 70 mg/kg/day.

2.Recovery Phase:Group IV consist of the mice which were orally administered ethanol extract of *C. papaya* seeds at higher dose and then the treatment was withdrawn for another 45 days.

Daily oral administration was sustained for a period of 45 days after which the animals of GpI,II and III were humanely sacrificed under chloroform anesthesia. The animals of group IV were sacrificed after 90 days total (45days treatment and 45 days of withdrawal). Epididymis were dissected out and freed from surrounding tissue. For histomorphological studies, the epididymis of mice of different groups were fixed in alcoholic Bouin's for 24 hours. Routine paraffin processed H&E stained tissues were then prepared for light microcopy. Different tubules of epididymis were examined and different morphological changes in the epididymal tubules were observed.

III. RESULTS

Results of light microscopy of the cross sections of epididymis obtained from the control group (Group I) as shows in Plate I depicts a regular and circular duct with a pseudostratified columnar epithelium containing a collection of late spermatids& spermatozoa.

Light microscopy examination of cross sections of Group II revealed milder changes in the epithelium. The principal cells started losing contacts with each other. No drastic changes were observed as shown in Plate II .Cross sections of Group III (higher dose) revealed severe changes in the epididymal epithelium. The shape of principal cells changed, loose contacts occurred and large vacuoles appeared in the supranuclear position as shown in Plate III

As shown in Plate IV, in Group IV animals (recovery phase) the cross sections showed that the recovery occurred in the epithelial cells of epididymis revealing the fact that the antifertility effect of *C. papaya* seeds is reversible and transient.

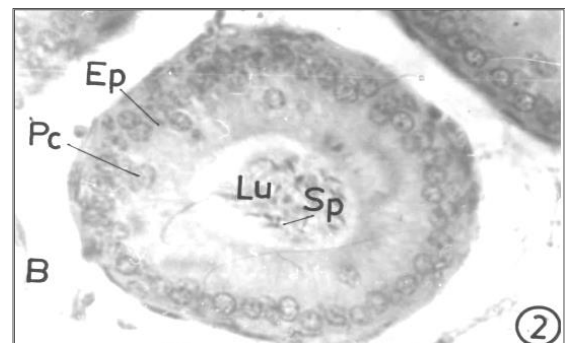


PLATE I. T.S of epididymis of control mice showing normal pattern of tubules

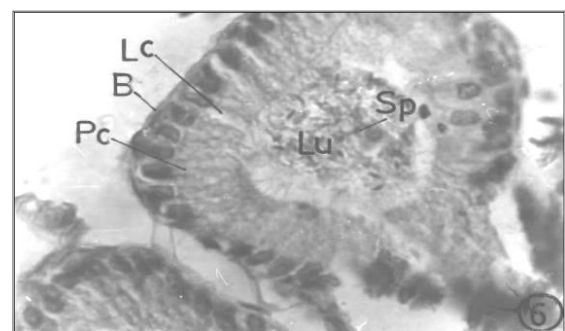


PLATE II. T.S of epididymis of mice treated with 50mg/kg /day showing loose contacts of principal cells

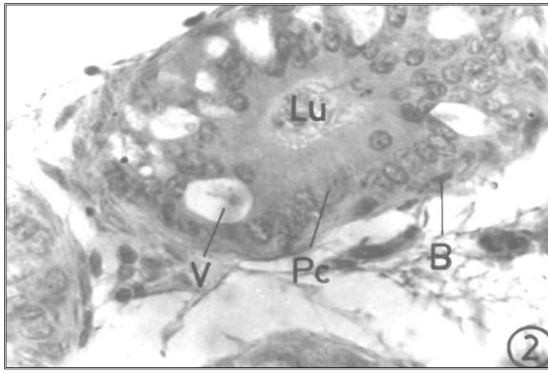


PLATE III. T.S of epididymis of mice treated with 70 mg/kg/day showing large vacuoles in principal cells

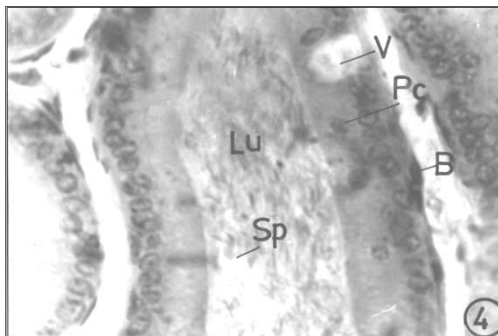


PLATE IV. T.S of epididymis of mice after withdrawal of treatment showing normal pattern of tubules

IV. DISCUSSION

The altered histological features observed in cross sections of the epididymis of animals fed on lower (Group II) dose and higher dose (Group III) indicates that the ethanol extract of *C. papaya* seeds 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 the Group III animals as compared to the animals of Group II revealed the fact that the changes in the epididymal epithelium are dose dependent.

The changes might be due to the reduced target organ response to androgen and/or their patent metabolites. These might be related to the greater sensitivity of these organs to androgens for maintenance of their structure and metabolism (Verma and Chinoy.,2002). The vacuolation was due to the mitochondrial swelling and the consequent hypoxia, which causes vacuoles in the principal cells (Udoh and Udoh.,2005). The recovery of the epididymal epithelium in Group IV animals suggested that the contraceptive efficacy of the *C. papaya* seeds are reversible and transient which is consistent with the findings of Udoh and

Kehinde.,1999; Lohiya et al .,2008 ;Manivannan et al .,2009; Oyekunle and Omope.,2010)

V. CONCLUSION

In conclusion, the results obtained from the present study of the effect of oral administration of ethanol extract of *C. papaya* seeds cause severe changes in epididymal epithelium which in turn affects the protein secretion and influences the sperm maturation hence confirmed its contraceptive efficacy .The antifertility effects are reversible and transient so it can be used for functional sterility. Therefore *C. papaya* seeds extract have shown great promise in the quest for the development of natural based male contraceptive.

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