

·Complementary Medicine·

## Sperm motility inhibitory effect of the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in langur monkey, *Presbytis entellus entellus*

Nirmal K. Lohiya, Boomi Manivannan, Shipra Goyal, Abdul S. Ansari

Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur 302004, India

### Abstract

**Aim:** To assess the contraceptive efficacy of the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in langur monkeys. **Methods:** The test substance was given p.o. to five monkeys at 50 mg/kg body weight/day for 360 days. Control animals ( $n = 3$ ) received olive oil as vehicle. Sperm parameters as per World Health Organization standards, sperm functional tests, morphology of testis and epididymis, haematology, clinical biochemistry, serum testosterone and libido were evaluated. Following completion of 360 days treatment the animals were withdrawn from the treatment and the recovery pattern was assessed by semen analysis and sperm functional tests. **Results:** Total inhibition of sperm motility was observed following 60 days of treatment that continued until 360 days study period. Sperm count, percent viability and percent normal spermatozoa showed a drastic decline following 30 days of treatment. Sperm morphology showed predominant mid piece abnormalities. Sperm functional tests scored in sterile range. Histology and ultrastructure of testis revealed vacuolization in the Sertoli cells and germ cells. Loss of cytoplasmic organelles was evident in spermatocytes and round spermatids. Histology and ultrastructure of epididymis of treated animals were comparable to those of control animals. Hematological and serum clinical parameters and testosterone levels fluctuated within the control range throughout the study period. Recovery was evident following 60–120 days of treatment withdrawal. **Conclusion:** The results suggest that the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* shows contraceptive efficacy without adverse toxicity, mediated through inhibition of sperm motility. (*Asian J Androl* 2008 Mar; 10: 298–306)

**Keywords:** male contraception; *Carica papaya* seeds; sperm motility inhibition; testis; epididymis; langur monkeys

Correspondence to: Prof. Nirmal K. Lohiya, Centre for Advanced Studies, Department of Zoology, University of Rajasthan, Jaipur, 302004, India.  
Tel: +91-141-270-1809, +91-141-270-3552  
Fax : +91-141-270-1809  
E-mail: lohiyank@hotmail.com, lohiyank@rediffmail.com  
Received 2007-01-29 Accepted 2007-07-23

### 1 Introduction

The contraceptive efficacy of the seeds of *Carica papaya* at various level of extraction/purification has been well established in rats, rabbits and langur monkeys [1]. In our earlier preliminary investigations, aqueous, methanol, ethanol, ethyl acetate and chloroform extracts of the seeds of *Carica papaya* have been shown to produce 100% sterility in rats. In rabbits, aqueous extract

was ineffective and chloroform extract produced azoospermia after 120 days of treatment [1]. In langur monkeys, treatment with chloroform extract resulted in azoospermia after 90 days of treatment [2]. Among the various crude extracts tested, the chloroform extract was found to be more effective in eliciting contraceptive efficacy in rats, rabbits and langur monkeys. We have attempted to purify the chloroform extract in column chromatography eluted with benzene. The resultant benzene chromatographic fraction was further purified by solvent subfractionation using methanol and ethyl acetate. At each stage of purification, the purified fractions were tested for its contraceptive efficacy in animal models (i.e. rats, rabbits and langur monkeys) to establish, if there are losses of biological activity of the fractions in purification process. Initial attempts to identify the chemical composition of the purified fractions through high performance liquid chromatography (HPLC), nuclear magnetic resonance<sup>-1</sup> (NMR<sup>-1</sup>), and mass spectroscopy (MS) revealed that the product is a homogenous mixture of fatty esters (unpublished observations). However, in the partial purified form, these fractions showed no loss of biological activity, yielding total sperm motility inhibitory effect in rats [1, 3] and azoospermia in rabbits [4]. It is also pertinent to mention that column chromatography of chloroform extract eluted with methanol or ethyl acetate in place of benzene, did not produce significant contraceptive efficacy in either rats [1] or rabbits [4]. All these effects were free of toxicity, evidenced from routine hematology and clinical chemistry and reversible following withdrawal of treatment [1].

Although there are established species specificity, i.e. sperm motility inhibition in rats and azoospermia in rabbits, owing to the proven contraceptive efficacy of the benzene chromatographic fractions resulting in 100% sterility in these animal models, a further insight of the study in a non-human primate model is warranted to extrapolate the findings to human. Therefore, in the present investigation, an attempt was made to assess the contraceptive efficacy, toxicological profiles and mode of action at tissue level of benzene chromatographic fraction of the chloroform extract in langur monkeys, an animal model close to human in reproductive anatomy and physiology [5].

## 2 Materials and methods

### 2.1 Test material

Fresh seeds of *Carica papaya* of honey dew variety

were used in the present investigation after authentication in the Department of Botany, University of Rajasthan, Jaipur, India (voucher No. RUBL 16590). The seed extracts were prepared as per the standardized protocol [3] for oral administration to langur monkeys.

### 2.2 Animals

Ten adult male langur monkeys *Presbytis entellus entellus* Dufresne, weighing 15 kg–18 kg [5] were procured from places in and around Jaipur, India and were kept for quarantine in individual metallic cages (105 cm × 75 cm × 75 cm) in the Departmental Primate House Facility for a period of 2 months. The animals were fed with roasted wheat cakes, seasonal vegetables and fruits, and water was provided *ad libitum*. Routine pathological tests and semen analysis were carried out to assess the health and reproductive status and only healthy animals ( $n = 8$ ) were selected for the investigation. The experiments were conducted in accordance with accepted humane practices as approved by the Departmental Research/Ethical Committee and the Guidelines for Care and Use of Animals for Scientific Research [6] were strictly followed. Complete veterinary care and supervision were provided to the animals throughout the course of the investigation.

### 2.3 Experimental design

#### 2.3.1 Pretreatment phase

Prior to the onset of the experiments, at least three pretreatment semen and blood samples at 10-day intervals were collected and subjected to routine analysis of semen, hormone, hematology and serum clinical biochemistry, and the values were used as individual reference control for comparison.

#### 2.3.2 Treatment phase

Five animals were treated with the benzene chromatographic fraction of the seeds of *Carica papaya* using a sterile feeding syringe at the standardized dose regimen, 50 mg/kg body weight/day p.o. for 360 days [2]. Three animals served as vehicle treated control. The animals were habitual for the dosage and did not show dislike/discomfort during treatment.

#### 2.3.3 Recovery phase

Following completion of 360 days treatment, all the animals were withdrawn from the drug treatment. The pattern of recovery was assessed through semen analy-

sis and sperm functional tests.

#### 2.4 Parameters

The following parameters were analyzed:

##### 2.4.1 Body weight

Body weight of the animals before, during the treatment and recovery periods was recorded monthly.

##### 2.4.2 Semen analysis

Semen samples were collected every 10 days before treatment and every 30 days during treatment and recovery periods by penile electro stimulation [5] for the following analyses:

###### 2.4.2.1 Physical characteristics

Semen volume, ejaculation time, pH value, colour, consistency, sperm concentration, motility, viability and morphology were analyzed according to the World Health Organization Method Manual [7].

###### 2.4.2.2 Scanning electron microscopy

Washed spermatozoa were immediately fixed in 2.5% glutaraldehyde in phosphate buffer for 30 min and washed thrice in phosphate buffer (0.1 mol/L; pH 7.2). A thin film of spermatozoa was smeared on a scanning electron microscopy stub with silver paint, air dried, sputter coated with gold and observed under a scanning electron microscope (Leo 435 VP, Eindhoven, the Netherlands).

###### 2.4.2.3 Sperm functional tests

The acrosome intactness test for the status of acrosome [8], the sperm mitochondrial activity index test, indicating motility disorders, flagellar and mitochondrial defects [9] and hypo-osmotic swelling test, indicating the membrane integrity [10], were carried out using washed spermatozoa. Scores below 50% in the acrosome intactness and mitochondrial activity index tests and below 60% in the hypo-osmotic swelling test were considered infertile.

##### 2.4.3 Histology and ultrastructure

###### 2.4.3.1 Light microscopy

Following completion of the treatment schedule and recovery period, the animals were anaesthetized with sodium thiopentone (20 mg/kg body weight, i.v.). Biopsies were obtained from testis and epididymis and a por-

tion of the tissues were fixed immediately in Bouin's fluid for 24 h, dehydrated in ethanol, cleared in xylene and embedded in paraffin wax. Five micron thick sections were double stained with Harris' hematoxylin and eosin for observation.

###### 2.4.3.2 Electron microscopy

For ultrastructural studies, the remaining biopsies of testis and cauda epididymis were cut into small pieces (1 mm) and fixed immediately in 2.5% glutaraldehyde in phosphate buffer (0.1 mol/L; pH 7.2) for 24 h. After primary fixation, the tissues were washed thoroughly in phosphate buffer, post fixed in 1% OsO<sub>4</sub> for 6 h, washed in phosphate buffer, followed by distilled water, dehydrated in acetone, infiltrated and embedded in low viscosity spurr media and polymerized at 60°C for 48 h. The semithin sections of 1 mm were stained with toluidine blue and ultrathin sections were stained with uranyl acetate and lead citrate and observed under transmission electron microscope (Philips CM-10, Eindhoven, the Netherlands).

##### 2.4.4 Toxicological investigations

###### 2.4.4.1 Hematology

Total red blood corpuscles (RBC), white blood corpuscles (WBC), hemoglobin and standard hematological indices (i.e. packed cell volume [PCV], mean corpuscular volume [MCV], mean corpuscular hemoglobin [MCH], mean corpuscular hemoglobin concentration [MCHC]) were recorded monthly [11].

###### 2.4.4.2 Clinical biochemistry

Serum total protein, glucose, cholesterol, creatinine, creatine kinase (CK), serum aspartate aminotransferase (S AST), serum alanine aminotransferase (S ALT) were estimated through Autoanalyzer (Erba Smartlab, Mumbai, India) using reagent kits (Transasia Biomedicals, Mumbai, India).

##### 2.4.5 Hormone analysis

Serum testosterone was assayed quarterly by enzyme immunoassay using reagent kits (Biochem Immuno Systems, Italia, Italy) and the values were recorded using a microplate reader (Tecan, Grödig, Austria).

##### 2.4.6 Libido

Libido was assessed monthly through response to electro-stimulation and mounting behavior [5].

### 2.5 Statistical analysis

Whenever appropriate, paired *t*-test was used for statistical comparison. The values are expressed as mean  $\pm$  SD and  $P < 0.05$  was considered significant. Data were analyzed using SPSS version 10.0 software (SPSS, Chicago, IL, USA).

## 3 Results

### 3.1 Body weight

The body weight of the treated animals did not show significant changes compared to that of control animals throughout the study period (data not shown).

### 3.2 Semen analysis

#### 3.2.1 Physical characteristics

The ejaculation time, volume, pH, color, consistency, liquefaction time and liquefaction status did not show significant changes compared to pretreatment values in both control and treated animals throughout the study period.

#### 3.2.2 Sperm concentration

A gradual decline in sperm count compared to pretreatment/control values was recorded from 30 days of treatment onwards and at 360 days study period, sperm count ranged between  $70 \times 10^6/\text{mL}$  and  $80 \times 10^6/\text{mL}$ . The treatment withdrawal resulted in a gradual increase in sperm count from 30 days. Sperm count comparable to that of the pretreatment values was achieved following 120–150 days of treatment withdrawal. Control animals showed normal sperm concentration throughout the study period (Figure 1A).

#### 3.2.3 Sperm motility

A drastic reduction, compared to pretreatment/control values in percent sperm motility ranged between 19% and 26% was observed within 30 days of treatment. A total and uniform inhibition of sperm motility was observed after 60 days of treatment that continued until 360 days study period. Following treatment withdrawal, sperm motility resumed after 30 days; however, with teratozoospermia, motility increased gradually and attained pretreatment levels after 120 days of treatment withdrawal. Control animals exhibited normal sperm motility throughout the study period (Figure 1B).

#### 3.2.4 Sperm viability

The percent viable spermatozoa showed a drastic

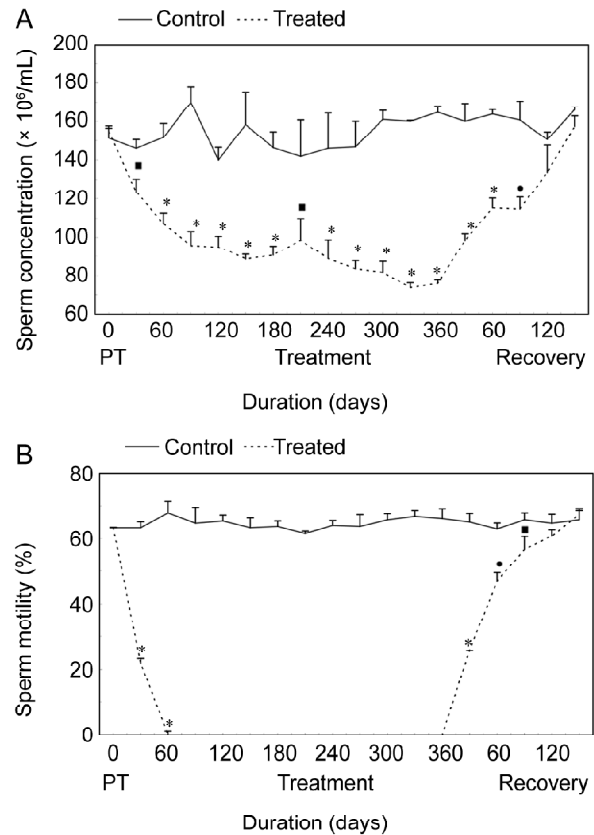


Figure 1. Sperm concentration (A) and sperm motility (B) in langur monkeys, during different phases of treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days. PT, pretreatment. ■  $P < 0.05$ , ●  $P < 0.01$ , \* $P < 0.001$ , compared with the control.

decline (range 10%–30%) from 30 days of treatment compared to control/pretreatment values, which continued throughout the 360 days study period, indicating negative fertility. Treatment withdrawal resulted in a gradual improvement in percent sperm viability. The pretreatment levels were achieved after 90 days of treatment withdrawal. Control animals exhibited normal sperm viability throughout the study period (Figure 2A).

#### 3.2.5 Sperm abnormality

The percent abnormal spermatozoa showed a steep increase compared to control/pretreatment values from 30 days treatment period onwards (range 68%–72%), which continued throughout the 360 day treatment period. Following treatment withdrawal, the percent abnormal spermatozoa resumed to pretreatment level within 30–60 days. Control animals did not show drastic

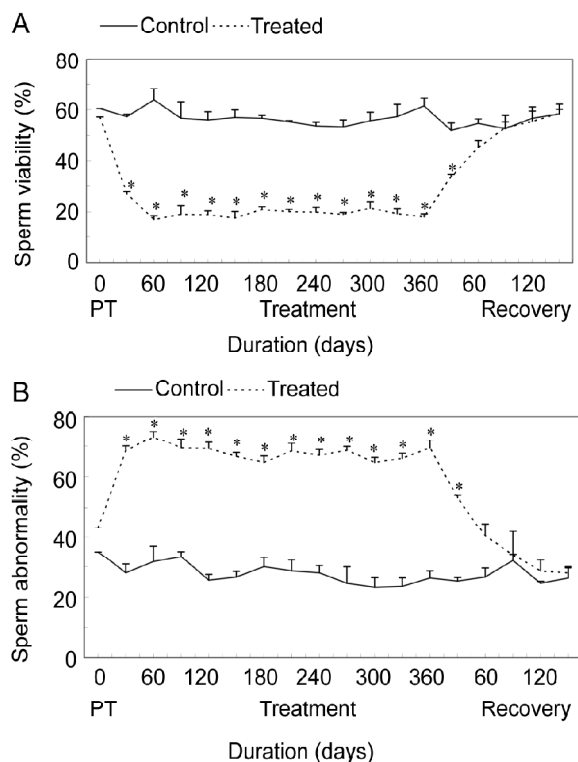


Figure 2. Sperm viability (A) and sperm abnormality (B) in langur monkeys, during different phases of treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days. PT, pretreatment. \* $P < 0.01$ , compared with the control.



Figure 3. Scanning electron microscopy of spermatozoa of langur monkey following 30 days of treatment with the benzene chromatographic fraction of chloroform extract of *Carica papaya*. Note all the spermatozoa are abnormal. Bent mid-piece and coiled tail are the typical abnormalities.  $\times 2\,000$ .

changes from their pretreatment values throughout the study period (Figure 2B).

### 3.3 Scanning electron microscopy of spermatozoa

Scanning electron microscopy of the spermatozoa showed abnormalities mainly at mid-piece and neck regions from 30 days of treatment. Coiled tail was also frequently observed (Figure 3). Treatment withdrawal resulted in normal morphology after 90 days.

### 3.4 Sperm functional tests

All sperm functional tests scored in sterile range from 30 days of treatment, which fluctuated within the sterile range throughout the 360-day study period. Treatment withdrawal resulted in a gradual increase and the normal parameters showing the fertile score, comparable to the pretreatment level were achieved after 90 to 120 days of treatment withdrawal. All sperm functional tests in control animals scored in the fertile range throughout the study period (Figure 4).

### 3.5 Histology and ultrastructure

#### 3.5.1 Morphology of testis

##### 3.5.1.1 Light microscopy

Following completion of the 360-day treatment period, testis histology revealed affected tubules. Although in most of the seminiferous tubules all stages of spermatogenesis were present, at certain places the spermiogenesis was arrested at stage I of the spermatogenic cycle. Vacuolization in Sertoli cells and germ cells were evident in the majority of the seminiferous tubules. Sertoli cells and round spermatids were vulnerable to vacuolization. Few of the spermatocytes showed evidence of nuclear pyknosis. Leydig cells, however, appeared normal (Figure 5).

##### 3.5.1.2 Electron microscopy

Ultrastructure of the testis of the treated animals, following completion of the 360-day treatment period, showed increased vacuolization in the Sertoli cells and germ cells compared to the control. The nucleus of the Sertoli cell appeared normal with deep indentation and dense chromatin material and the entire cytoplasm showed vacuolization and loss of cytoplasmic organelles. Mitochondria were few and vacuolated. Nuclear degeneration and loss of cytoplasmic organelles were evident in spermatocytes and round spermatids and the nuclear membrane of these cells were ill-defined.

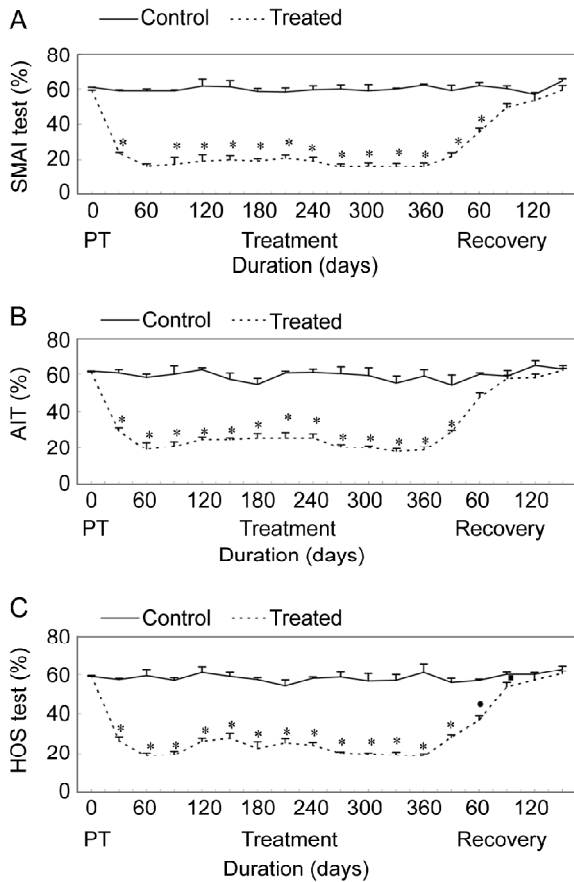


Figure 4. Sperm mitochondrial activity index (SMAI) test (A), acrosome intactness (AIT) test (B) and hypo-osmotic swelling (HOS) test (C) in langur monkeys, during different phases of treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days. PT, pretreatment. ■  $P < 0.05$ , ●  $P < 0.01$ , \* $P < 0.001$ , compared with the control.



Figure 5. Histology of the testis of the langur monkey following oral treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days. Note: vacuolization in the Sertoli and germ cells, particularly the round spermatids.  $\times 100$ .

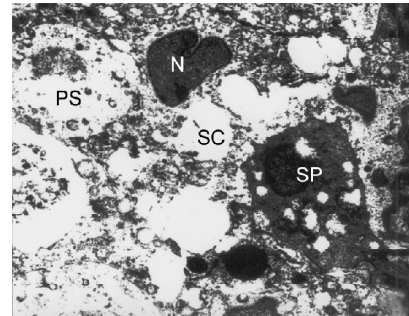


Figure 6. Ultrastructure of testis of the langur monkey following treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days showing the Sertoli cells (SC). The nucleus (N) shows indentation and dense chromatin material. Cytoplasm shows vacuolization and loss of cytoplasmic organelles. The nuclei of the primary spermatocyte (PS) and of round spermatid (SP) showed degenerative features.  $\times 2\ 800$ .

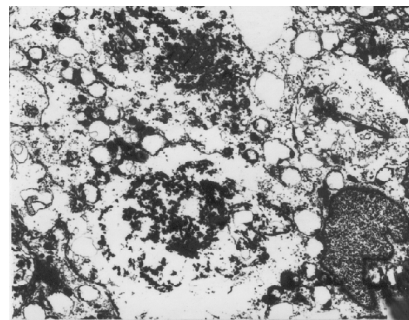


Figure 7. Ultrastructure of testis of the langur monkey following treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days showing spermatocytes. The nuclear pyknosis is evident.  $\times 3\ 600$ .

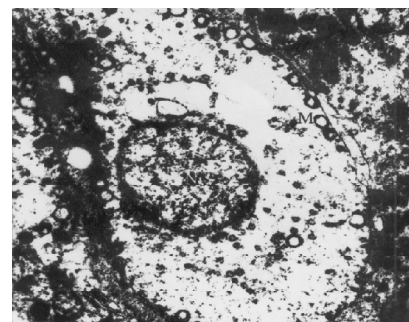


Figure 8. Ultrastructure of testis of the langur monkey following treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days showing the round spermatid. Note vacuolization in the cap region. Cytoplasmic organelles are relatively sparse. The peripheral mitochondria (M) shows vacuolization.  $\times 3\ 200$ .

Vacuolization in the acrosomal cap and peripheral mitochondria of round spermatids was also evident (Figures 6–8).

### 3.5.2 Morphology of epididymis

#### 3.5.2.1 Light microscopy

Histology of the cauda epididymis following 360 days treatment with benzene chromatographic fraction presented no appreciable changes to that of the control animals. The basal and principal cells appeared normal and comparable to that of control animals. Lumen contained dense spermatozoa. However, few phagocytes were evident in the pool of spermatozoa. Inter-tubular elements appeared normal (figure not shown).

#### 3.5.2.2 Electron microscopy

Ultrastructure of the principal cells of cauda epididymis following 360 days of treatment was comparable to that of the control. The nucleus was round with prominent double layered nuclear membrane and the nucleoplasm contained dense chromatin material. Occasionally the nucleus showed an irregular pattern. Mitochondria in the cytoplasm were round or elongated with prominent cristae. Supranuclear cytoplasm was characterized by the presence of numerous well-defined Golgi bodies, tubules of granular endoplasmic reticulum and rosettes of glycogen granules, showing evidence of active protein secretion (figure not shown).

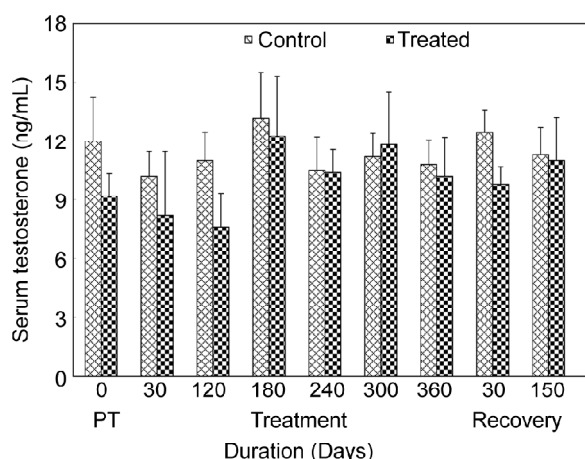


Figure 9. Serum testosterone levels in langur monkeys, during different phases of treatment with the benzene chromatographic fraction of chloroform extract of the seeds of *Carica papaya* at 50 mg/kg body weight/day for 360 days. PT, pretreatment.

### 3.6 Toxicological investigations

There were no appreciable changes, although wide fluctuations were observed in the levels of total RBC, WBC, hemoglobin (Hb), PCV, MCV, MCH and MCHC and the serum protein, glucose, cholesterol, creatinine, CK, serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxalate transaminase (SGOT). The values fluctuated within the pretreatment range in both control and treated animals throughout the study period (data not shown).

### 3.7 Hormone assay

Serum testosterone levels did not show appreciable changes from that of the pretreatment values in both control and treated animals throughout the study period (Figure 9).

### 3.8 Libido

The libido of the treated animals remained unaffected. All the animals, control and treated, showed normal mounting and ejaculatory behavior throughout the study period.

## 4 Discussion

The seed extracts of *Carica papaya* produce variable responses towards contraceptive efficacy in rats, rabbits and langur monkeys. The effects are post-testicular, affecting the cauda epididymal sperm motility in rats, and testicular effects lead to azoospermia in rabbits and langur monkeys [1–4]. In the present investigation, treatment with benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* resulted in decline in sperm concentration after 30 days of treatment and total sperm motility inhibition after 60 days of treatment with reduced viability and increased sperm abnormality. Sperm functional tests including ultrastructure of spermatozoa, indicated that these spermatozoa are functionally infertile, suggesting significant contraceptive efficacy of the test material.

The mechanism by which the extract of the seeds of *Carica papaya* induces the sperm motility inhibition in all treated animals in the present investigation is poorly understood. It has been reported that there are three major sites of action of an epididymal antifertility agent; namely, (i) the peritubular muscle, hastening sperm transport leading to ejaculation; (ii) on the epithelium by altering the composition of epididymal fluid and (iii) on the

spermatozoa by affecting their enzymes [12]. As there are no appreciable changes in the histology and ultrastructure of epididymis in the treated animals, it is likely that the effect lies directly on the epididymal spermatozoa [13].

The excessive generation of reactive oxygen species might result into dramatic loss of sperm function and motility [14]. Decrease in activity of antioxidant enzymes in the epididymis increase reactive oxygen could damage the epididymal environment and sperm function [15]. Al-Majed *et al.* [16] demonstrated that treatment with Yohimbe induced chromosomal aberrations, spermatozoal abnormalities and reduction in sperm count, motility and fertility in Swiss albino mice as a result of depletion of antioxidants. Likewise, in the present study, the testis histology and ultrastructure depicted inhibition of spermatogenesis and an increased percentage of abnormal spermatozoa.

In the triptolide treated rats, Huynh *et al.* [17] described two phenotypic effects on mature and maturing germ cells. The first action appears early on the epididymal sperm and its subsequent action is directly on the germ cells of testis. Interestingly, in the triptolide-treated rats, no ultrastructural differences in the epididymal epithelium were observed between control and treated rats, but the treated rats exhibited total motility inhibition with severe sperm abnormalities and varied sperm concentration in the cauda epididymis [17]. In the present investigation, the treatment with the seed products of *Carica papaya* altered testis histology but not that of epididymis. Even the ultrastructure of epididymis showed normal configuration with active protein synthetic machinery, which provides a clue that the test substance directly acted upon the germ cells of the testis, particularly at the level of spermatid maturation and produced malformed spermatozoa, resulting in increased abnormalities with motility inhibition of spermatozoa in the ejaculation.

It has been reported that the Sertoli cells of a regressed testis do not contain conspicuous stacks of granular endoplasmic reticulum, which is consistent with a reduced protein secretory function of the Sertoli cells [18]. Huynh *et al.* [17] reported ultrastructural defects in the Sertoli cells and spermatids of the triptolide treated rats. In the present investigation, treatment with a benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* showed vacuolization in the Sertoli cell cytoplasm and loss of cytoplasmic

organelles, particularly at the basal region, suggesting loss of metabolic activity of the cell. Degeneration and maturational arrest of germ cells (i.e. spermatocytes and spermatids), therefore, could be due to the Sertoli cell factors responsible for germ cell maturation. It has also been described that gossypol and gossypol acetic acid produce vacuolization and degeneration of mitochondria in the mid-pieces of late spermatids in rats and hamsters that are pathognomonic to gossypol or its derivatives [19]. A similar unique effect on the mitochondria of late spermatids and on the voided spermatozoa has been observed in monkeys treated with chloroform extract of the seeds of *Carica papaya* [2]. In the present study, the sperm functional tests (i.e. acrosome intactness test for the acrosome status and the ability of the spermatozoa to penetrate the oocyte, sperm mitochondrial activity index test for motility disorders and flagellar and mitochondrial defects in the mid-piece and the hypo-osmotic swelling test for membrane integrity [8–10]), particularly the tests for mitochondria and membrane integrity, indicated defective sperm production from the testis [16]. Incidentally, in the present study, ultrastructure of the spermatocytes and spermatids showed nuclear degeneration and vacuolized mitochondria.

It is concluded here that the sperm motility inhibitory effect could be a result of a selective action of the test substance on developing germ cells, possibly mediated through Sertoli cells, leading to inhibition of mitochondrial activity of these cells, which might alter the respiratory chain, generating a cytotoxic effect on the germ cell proliferation, as in the case of gossypol treated animals [19, 20]. It is further evidenced by increased mid-piece defects and vacuolization in the mitochondria in the voided spermatozoa of the seed extract-treated animals in the present study, as reflected from sperm ultrastructure. However, further evidence needs to be generated at the level of glycolytic pathway of the testis, more particularly on the developing germ cells and spermatozoa of the treated animals to substantiate this view. The blood parameters (i.e. RBC, WBC, Hb, standard hematological indices, the serum clinical parameters and the serum testosterone level, which presented normal values throughout the study period) suggest systemic safety of the test material.

Available evidence indicates that the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* possesses contraceptive efficacy, effected through defective germ cell proliferations, as



evidenced from testis ultrastructure. The sperm motility inhibitory action in the present study seems to be more advantageous in that it would avoid delayed reversibility as in the case of gossypol treated animals [20]. The results achieved so far meet the essential criteria for the male contraceptive (i.e. being orally effective, non-steroidal, free of toxicity, reversible and possibly cost effective). However, further purification of the chromatographic fraction, identification of bioactive compounds, their characterization and pharmacokinetic properties of the purified compounds are required for product development and mass application. Further relevant studies are currently in progress.

### Acknowledgment

The investigations were supported by the Ministry of Health and Family Welfare/Indian Council of Medical Research, Government of India, New Delhi and the Special Assistance Programme (Phase III), University Grants Commission, New Delhi. Ultrastructural studies were carried out at EM-SAIF-DST Facility, Department of Anatomy, All India Institute of Medical Sciences, New Delhi, India.

### References

- 1 Lohiya NK, Manivannan B, Mishra PK, Pathak N. Prospects of developing a plant based male contraceptive pill. In: Chowdhury SR, Gupta CM, Kamboj VP, editors. Current Status in Fertility Regulation: Indigenous and Modern Approaches. Lucknow: Central Drug Research Institute; 2001, p99–119.
- 2 Lohiya NK, Manivannan B, Mishra PK, Pathak N, Sriram S, Bhande SS, *et al.* Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in langur monkey. *Asian J Androl* 2002; 4: 17–26.
- 3 Pathak N, Mishra PK, Manivannan B, Lohiya NK. Sterility due to inhibition of sperm motility by oral administration of benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in rats. *Phytomedicine* 2000; 7: 325–33.
- 4 Lohiya NK, Mishra PK, Pathak N, Manivannan B, Jain SC. Reversible azoospermia by oral administration of the benzene chromatographic fraction of the chloroform extract of the seeds of *Carica papaya* in rabbits. *Adv Contracept* 1999; 15: 141–61.
- 5 Lohiya NK, Manivannan B, Sharma RS, Panneerdoss S, Garg S. Langur monkey, *Presbytis entellus entellus*: an animal model for the study of human reproduction. In: Puri CP, Ganguly NK, editors. National Centre for Primate Breeding and Research: Vision, Challenges and Opportunities. Mumbai: National Institute for Research in Reproductive Health; 2005, p279–90.
- 6 Indian National Science Academy. Guidelines for Care and Use of Animals for Scientific Research. New Delhi: Indian National Science Academy; 2000.
- 7 World Health Organization. WHO Laboratory Manual for Examination of Human Semen and Sperm-Cervical Mucus Interaction. New York: Cambridge University Press; 1999.
- 8 Gopalkrishnan K. Standardized procedures in human semen analysis. *Curr Sci* 1995; 68: 353–62.
- 9 Gopalkrishnan K, Hinduja IN, Anand Kumar TC. Assessment of mitochondrial activity of human spermatozoa: Motility/viability in fertile/infertile men. *Mol Androl* 1991; 3: 243–50.
- 10 Jeyendran RS, Van der Ven HH, Perez-Pelaez M, Carbo BG, Zaneveld LJ. Development of an assay to assess the functional integrity of the human membrane and its relationship to other semen characteristics. *J Reprod Fertil* 1984; 70: 219–28.
- 11 Lynch MJ, Raphael SS, Melor LD, Spare PD, Inwood MJH. Medical Laboratory Technology and Clinical Pathology. Philadelphia: W. B. Saunders Company; 1969.
- 12 Cooper TG, Yeung CH. Approaches to post-testicular contraception. *Asian J Androl* 1999; 1: 29–36.
- 13 Raji Y, Akinsomisoye OS, Salman TM. Antispermatic activity of *Morinda lucida* extract in male rats. *Asian J Androl* 2005; 7: 405–10.
- 14 Aitken RJ, Clarkson JS, Hargreave TB, Irvine DS, Wu FC. Analysis of the relationship between defective sperm function and the generation of reactive oxygen species in case of oligozoospermia. *J Androl* 1989; 10: 214–20.
- 15 D'Cruz SC, Mathur PP. Effect of piperine on the epididymis of adult male rats. *Asian J Androl* 2005; 7: 363–8.
- 16 Al-Majed AA, Al-Yahya, Al-Bekairi AM, Al-Shabanah OA, Qureshi S. Reproductive, cytological and biochemical toxicity of Yohimbe in male Swiss albino mice. *Asian J Androl* 2006; 8: 469–76.
- 17 Huynh PN, Hikim AP, Wang C, Stefanovic K, Lue YH, Leung A, *et al.* Long-term effects of triptolide on spermatogenesis, epididymal sperm function, and fertility in male rats. *J Androl* 2000; 21: 689–99.
- 18 Kasturi M, Ahmed RN, Pathan KM, Manivannan B, Aladakatti RH. Ultrastructural changes induced by leaves of *Azadirachta indica* (Neem) in the testis of albino rats. *J Basic Clin Physiol Pharmacol* 2002; 13: 311–28.
- 19 Kumar M, Sharma S, Lohiya NK. Is it still possible to develop gossypol as an ideal male contraceptive pill? A review. In: Joshi SC, Ansari AS, editors. Advances in Reproductive Toxicology. Jaipur: Pointer Publishers; 2006, p244–61.
- 20 Sharma S, Kumar M, Goyal RB, Manivannan B, Lohiya NK. Reversible antispermatic effect of gossypol in langur monkeys (*Presbytis entellus entellus*). *Adv Contracept* 1999; 15: 15–27.

Edited by Dr Ralf Henkel