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Chloroform extract of *Carica papaya* seeds induces long-term reversible azoospermia in langur monkey

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Abstract **Aim:** To evaluate the antifertility activity of the chloroform extract of *Carica papaya* seeds by oral administration in langur monkey, *Presbytis entellus entellus*. **Methods:** The chloroform extract of *Carica papaya* seeds, 50 mg/kg/day, was administered orally for 360 days to adult male langur monkeys. The sperm characteristics by light and electron microscopy, the sperm functional tests, the semen biochemistry, the serum testosterone level, the Leydig cell function, and the histology and ultrastructure of testis were determined to evaluate the antifertility activity and the blood biochemistry and hematology, to evaluate the toxicology. **Results:** The extract gradually decreased the sperm concentration since days 30-60 of treatment with a total inhibition of sperm motility, a decrease in sperm viability and increase in sperm abnormality. Azoospermia was observed after day 90 of treatment and continued during the whole treatment period. Treatment withdrawal resulted in a gradual recovery in these parameters and 150 days later they reverted to nearly the pretreatment values. Morphological observation of the ejaculated sperm by light and scanning electron microscopy showed deleterious changes, particularly on the mid-piece. Sperm functional tests, viz., sperm mitochondrial activity index, acrosome intactness test and hypo-osmotic swelling test scored in the infertile range during treatment and returned to the fertile values 150 days after drug withdrawal. Histology of the testis revealed shrunken tubules, germ cell atrophy and normal Leydig cells. Ultrastructure of the testis showed vacuolization in the cytoplasm of Sertoli cells and germ cells. Loss of cytoplasmic organelles were evident in the spermatocytes and spermatids. Round spermatids showed loss of Golgi bodies, peripheral mitochondria and vacuolated cytoplasm, indicating maturational arrest. Leydig cell functional test indicated a mild inhibition of steroidogenic function. Haematology and serum biochemistry study disclosed no significant toxicological effect and the serum testosterone level was not affected. **Conclusion:** *Carica papaya* seed extract may selectively act on the developing germ cells, possibly mediated via Sertoli cells, leading to azoospermia. *(Asian J Androl 2002 Mar; 4: 17-26)*

1 Introduction

The contraceptive efficacy, reversibility and toxicity of the *Carica papaya* seed products have been investigated in rats and rabbits [1]. Oral administration of the aqueous, methanol, ethanol, ethyl acetate and chloroform extracts in rats revealed reversible contraceptive efficacy, but only the aqueous and chloroform extracts were without significant toxicity and impairment in libido [2]. Partial purification of the chloroform extract through silica gel column chromatography, eluted with benzene, chloroform and ethyl acetate in the order of polarity, showed total motility inhibition of cauda epididymal spermatozoa in rats with benzene and chloroform fractions [3,4]. Methanol and ethyl acetate sub-fractions of the benzene

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chromatographic fraction yielded similar results [1].

In rabbits, treatment with chloroform extract, benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate sub-fractions leads to azoospermia, while aqueous extract and the chloroform and ethyl acetate chromatographic fractions of the chloroform extract were ineffective, thus showing differential effect among species [1].

Owing to the proven contraceptive efficacy of chloroform extract of the *Carica papaya* seeds and its further purified products in rats and rabbits, a pre-clinical investigation has been initiated in langur monkey, a non-human primate close to the human anatomically [5] and in its reproductive exocrine and endocrine profiles [6,7], with a view to extrapolate the findings to the human. The present investigation reports the contraceptive efficacy, reversibility and toxicity, if any, of the chloroform extract of the seeds of *Carica papaya*.

2. Materials and methods

2.1 Animals

Eight adult male langur monkeys (*Presbytis entellus entellus* Dufresne), 6-7 years old, as identified by the musculature, sex skin in the rump and dentition [5] were procured from places around Jaipur and kept in individual metallic cages in the Department Primate House Facility. The animals were fed with roasted wheat cakes, seasonal vegetables and fruits and tap water was provided *ad libitum*. Routine pathological tests and semen analysis were carried out to assess the health and reproductive status of the animals and only healthy animals were selected for the investigation. The experiments were conducted in accordance with accepted humane practices as approved by the Departmental Research/Ethical Committee. Complete veterinary care and supervision were provided to the animals throughout the course of the investigation. The "guidelines for care and use of animals for scientific research" [8] were strictly followed.

2.2 Test materials

The seeds of *Carica papaya* Linn. (Caricaceae; Voucher No. RUBL 16590) of honey dew variety, were obtained commercially, shade dried and coarsely powdered. The powdered material was soxhleted with chloroform at 58°C for 12×3 h. The soxhleted material was concentrated under reduced pressure and the residue was used in the investigation.

2.3 Experimental design

2.3.1 Pretreatment phase (30 days)

Three pretreatment semen samples were collected at 10-day intervals for routine analysis and semen biochemistry. Blood samples were collected from the great saphenous vein for haematology and serum biochemistry and testosterone level determination.

2.3.2 Treatment phase (360 days)

Five animals were orally fed with the chloroform extract of the *Carica papaya* seeds, 50 mg/kg/day for 360 days, along with vegetables. Care was taken to ensure that the animal consumed the entire dose. Three animals served as control.

2.3.3 Recovery phase (150 days)

Following completion of the treatment, semen analysis was carried out at regular intervals to assess the recovery pattern.

2.4 Semen analysis

Semen samples were collected every 10 days before treatment and every 15 days during and after treatment by penile electrostimulation for the following analyses:

2.4.1 Routine examination

Semen volume, ejaculation time, pH, colour, consistency, and sperm concentration, motility, viability and morphology were assessed according to the WHO manual [9].

2.4.2 Scanning electron microscopy (SEM)

Spermatozoa were washed with phosphate buffer (0.01 mol/L, pH 7.2) and pelleted by centrifugation. The sperm pellets were fixed in 2.5% glutaraldehyde for 30 min and washed thrice in phosphate buffer followed by distilled water. A thin film of spermatozoa was smeared on a clean glass slide, air dried and mounted on SEM stub with silver paint, sputter coated with gold and observed under SEM (Leo 435 VP).

2.4.3 Sperm function tests

Washed spermatozoa were used for assessment of the acrosome intactness test [10], mitochondrial activity index test [11] and hypoosmotic swelling test [12]. Scores below 50% in the acrosome intactness and mitochondrial activity index tests and below 60% in the hypoosmotic swelling test were considered subfertile or infertile [13].

2.4.4 Seminal plasma biochemistry

Sperm free seminal plasma was used for the quantitative determination of fructose and acid phosphatase (ACP) [14], glycerophosphocholine (GPC) [15], lactate dehydrogenase (LDH) [16], protein, calcium and alka-

line phosphatase (ALP) (Reagent kits; Ranbaxy Laboratories Ltd, Mumbai).

2.5 Histology and ultrastructure of testis

Testicular biopsy was performed after completion of 360 days study period. For histology, the tissues were fixed in Bouin's solution, dehydrated in ethanol and embedded in paraffin wax. The sections cut at 5 μm were stained with haematoxylin and eosin.

The remaining portion of the testis was fixed in 2.5% glutaraldehyde, post-fixed in 1% OsO_4, dehydrated in acetone and embedded in araldite for ultrastructural studies. The ultrathin sections were stained with uranyl acetate and lead citrate and viewed under Philips transmission electron microscope (CM-10).

2.6 Leydig cell function test

The response of Leydig cells to 3β-hydroxysteroid dehydrogenase (3β-HSD) and the hCG-stimulated testosterone biosynthesis *in vitro* have been used as functional indicators for Leydig cells. Crude Leydig cell population was obtained by non-enzymatic dispersion and percoll density gradient centrifugation [17]. Purification of the Leydig cells was confirmed by 3β-HSD staining method. To 100 μL suspension of Leydig cell fraction, equivalent to 50,000 viable Leydig cells as identified by trypan blue staining, hCG 50 μL (mg/mL) was added and cultured for 6 h at 37 ¡æin a humidified atmosphere. Following completion of the culture, the spent media obtained by centrifugation was used for testosterone assay by RIA using NIDDK kits (Bethesda, USA), along with a parallel control without hCG. The sensitivity of the assay was 0.01 ng/mL.

2.7 Toxicology

2.7.1 Haematology

Blood samples were collected monthly and used for the analyses of red blood corpuscles (RBC), white blood corpuscles (WBC), haemoglobin (Hb), haematocrit (packed cell volume, PCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) [18-20].

2.7.2 Biochemistry

Serum glucose, protein, cholesterol, glutamate pyruvate transaminase (SGPT), glutamate oxalate transaminase (SGOT), lactate dehydrogenase (LDH), creatinine and creatine kinase (CK) were estimated colorimetrically using reagent kits (Ranbaxy Laboratories Ltd., Mumbai).

2.7.3 Hormone assay

Serum testosterone levels were assayed from frozen samples by RIA using NIDDK kits (Bethesda, USA). The sensitivity of the assay was 0.01 ng/mL.

2.8 Statistical analysis

Data were expressed in mean±SEM. Student's '*t*' test was employed for statistical comparison. $P<0.05$ was considered significant

3 Results

3.1 Semen analysis

3.1.1 Routine observation

Carica papaya did not significantly change the ejaculation time, semen volume, colour, consistency and pH of semen. Sperm concentration showed a gradual decline and azoospermia resulted in all treated monkeys after 90 days treatment that continued over the one year study period. On day 60 there was a total inhibition of sperm motility and a concomitant decrease in sperm viability; the per cent abnormal spermatozoa showed a gradual increase.

Following withdrawal of treatment, azoospermia continued for 30 days, severe oligospermia with sperm count less than 20 million/mL was observed up to day 60; a little later the sperm motility and viability increased gradually with a simultaneous increase in motility and viability and a decrease in per cent abnormal spermatozoa. At day 150 all these parameters, although at slightly lower levels, were comparable to pretreatment values (Tables 1 a,b,c,d).

Table 1a. Sperm concentration (10^6/mL) of langur monkeys after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^cP < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	161±11.38	150±1.74
Treatment		
30 days	156± 9.53	141±2.08^c
60 days	136± 8.77	118±2.60^c
90 days	134± 8.42	Nil
120 days	120± 9.41	Nil
150 days	146±19.44	Nil
180 days	161±14.54	Nil
210 days	165± 4.33	Nil
240 days	141±14.17	Nil
270 days	143± 5.24	Nil
300 days	141±13.00	Nil
330 days	138± 7.54	Nil
360 days	130±17.47	Nil
Post-treatment		
30 days	130±9.08	Nil
60 days	146±2.96	10±2.34^c
90 days	131±9.88	48±4.90^c
120 days	133±6.56	95±4.11^c
150 days	146±5.24	146±3.33

Table 1b. Sperm motility (%) of langur monkeys after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	68±4.41	66±1.30
Treatment		
30 days	61±1.85	Nil
60 days	58±5.21	Nil
90 days	61±1.52	-
120 days	61±3.05	-
150 days	63±1.66	-
180 days	61±2.18	-
210 days	61±1.45	-
240 days	61±0.66	-
270 days	65±1.45	-
300 days	66±3.53	-
330 days	63±6.25	-
360 days	60±1.15	-
Post-treatment		
30 days	67±1.73	-
60 days	64±2.64	Nil
90 days	62±4.33	12±2.68^{c}
120 days	58±4.16	40±1.86^{c}
150 days	60±0.88	61±2.64

Table 1c. Sperm viability of langur monkeys after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	57±3.48	58±1.85
Treatment		
30 days	52±1.45	52±1.36^{c}
60 days	54±2.90	42±1.85^{c}
90 days	55±1.20	-
120 days	57±1.45	-
150 days	57±2.18	-
180 days	58±2.31	-
210 days	59±4.16	-
240 days	56±2.08	-
270 days	54±1.00	-
300 days	55±2.18	-
330 days	55±1.52	-
360 days	58±2.51	-
Post-treatment		
30 days	57±1.52	-
60 days	53±0.57	Nil
90 days	53±2.40	19±1.65^{c}
120 days	52±1.85	42±1.22^{c}
150 days	53±1.20	54±1.97

After 30-60 days of treatment the spermatozoa (Papanicolaou stain) showed deleterious changes, particularly in the mid-piece. Typical changes included bent mid-piece and coiled tail. At day 120 post-treatment, the sperm morphology reverted to the pretreatment level (Figure 1).

3.1.2 SEM of spermatozoa

The pretreatment spermatozoa showed an oval head with distinct acrosomal and post-acrosomal region. The mid-piece was thick, encircled by spiral mitochondrial sheath. The annulus appeared as a constriction at the tail, which separated the mid-piece from the tail (Figure 2A). The spermatozoa of treated animals were well correlated with light microscopic studies, with bent mid-piece and coiled tail. In a few spermatozoa, head abnormalities were also evident, showing amorphous head and acrosome deformities (Figure 2B).

Table 1d. Abnormal spermatozoa (%) of langur monkeys after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	31±1.66	39±1.29
Treatment		
30 days	28±2.33	55±2.27^{c}
60 days	29±0.57	62±1.78^{c}
90 days	25±3.18	-
120 days	26±4.10	-
150 days	28±1.56	-
180 days	26±7.03	-
210 days	27±2.85	-
240 days	26±3.33	-
270 days	29±2.40	-
300 days	25±2.85	-
330 days	26±2.33	-
360 days	27±2.00	-
Post-treatment		-
30 days	27±1.45	80±1.63^{c}
60 days	27±4.36	61±2.48^{c}
90 days	25±5.00	39±2.27
120 days	26±2.40	37±2.46
150 days	22±5.05	

3.1.3 Sperm function tests

In all the sperm function tests, the first few ejaculates after treatment scored in the infertile range, which persisted in the whole treatment phase and early post-treatment phase. At 150 days post-treatment, they reached fertile scores comparable to the pretreatment values (Tables 2 a,b,c).

3.1.4 Seminal plasma biochemistry

The levels of fructose, ACP, GPC, LDH, protein, calcium and ALP in the seminal plasma were not significantly changed in the treatment animals, however, there was a tendency of reduction during the treatment phase (data not shown).

3.2 Histology of testis

3.2.1 Light microscope observation

The testis of control animals showed evidence of normal spermatogenesis with prominent Sertoli cells containing granular cytoplasm, spermatogonia, spermatocytes and spermatids and their differentiation into spermatozoa (Figure 3A).

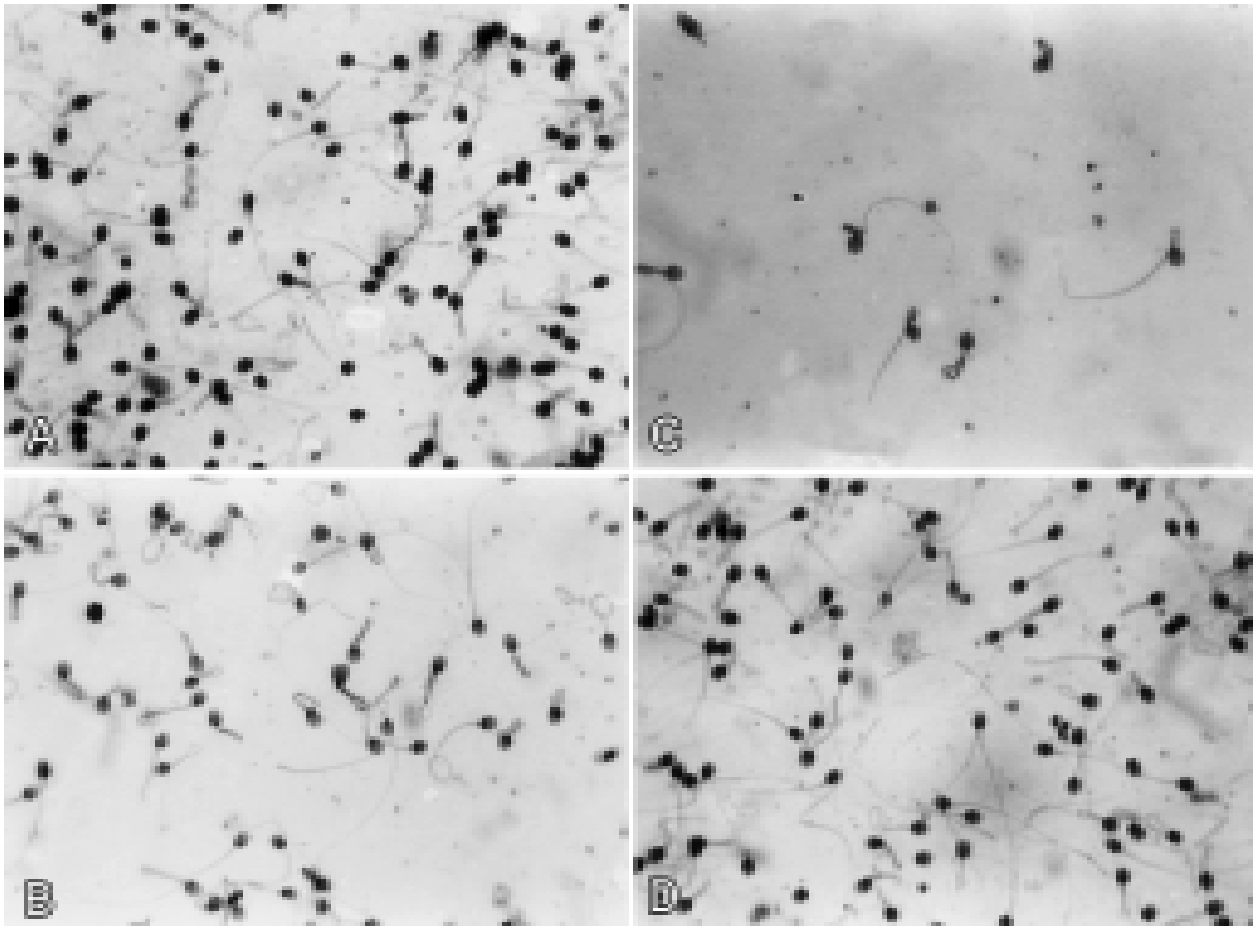

Figure 1. Sperm morphology of langur monkeys: <A> before treatment: normal spermatozoa; <B> after 30 days of treatment with chloroform extract of *Carica papaya* seeds: most spermatozoa showing bent mid piece and coiled tail; <C> after 60 days of treatment: a drastic reduction in number of spermatozoa, all spermatozoa showing bent mid piece; <D> after 120 days of treatment withdrawal: number and morphology of spermatozoa comparable to pretreatment level. Papanicolaou staining ×400.

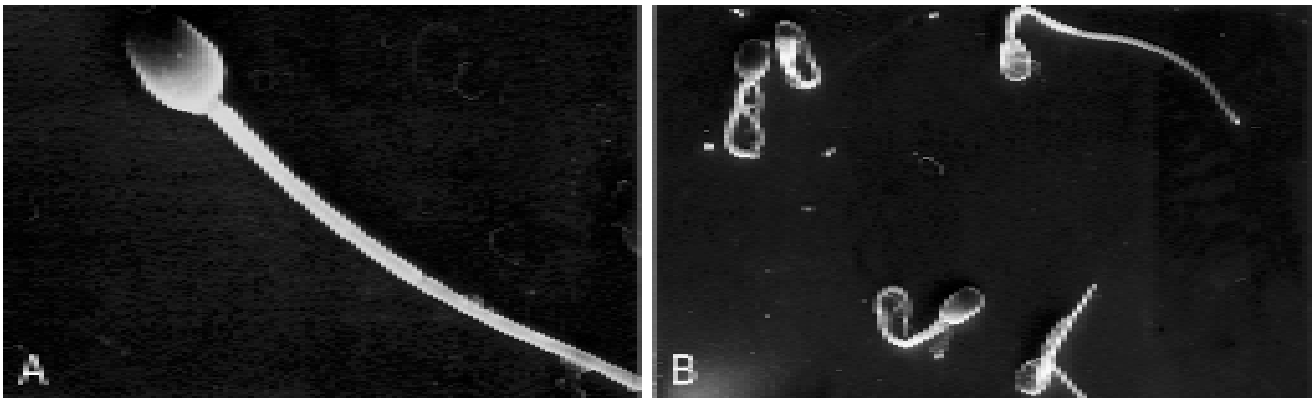

Figure 2. SEM study on sperm morphology of langur monkeys: <A> before treatment: normal morphology. × 4000; <B> after 60 days of treatment with chloroform extract of *Carica papaya* seeds. × 2000.

After completion of treatment, the testis revealed shrunken tubules. Spermatogonia appeared to be normal. Although spermatocyte differentiation was observed, the cells were atrophic with nuclear and cytoplasmic vacuolization. There were only a few round spermatids which were atrophic. Leydig cells appeared normal (Figure 3B).

3.2.2 Ultrastructure

In control animals, the ultrastructure of the Sertoli

Table 2a. Sperm mitochondrial activity index test (%) in langur monkey after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	62.6±2.33	59.2±2.64
Treatment		
30 days	58.3±1.66	25.0±3.27^{c}
60 days	59.6±0.88	14.0±2.70^{c}
90 days	62.0±2.08	-
120 days	59.0±3.21	-
150 days	60.3±1.45	-
180 days	63.0±3.05	-
210 days	58.0±1.15	-
240 days	58.3±2.02	-
270 days	58.3±1.66	-
300 days	59.3±2.33	-
330 days	60.0±1.00	-
360 days	59.6±3.38	-
Post-treatment		
30 days	60.0±0.02	-
60 days	61.6±1.76	10.4±1.63^{c}
90 days	63.0±0.05	28.8±0.86^{c}
120 days	59.0±0.57	41.0±2.43^{c}
150 days	59.3±2.96	58.4±1.89

Table 2b. Acrosome intactness test (%) in langur monkey after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	60.0±2.89	57.2±2.64
Treatment		
30 days	56.0±3.05	23.2±2.08^{c}
60 days	60.3±3.93	11.6±2.66^{c}
90 days	61.0±2.51	-
120 days	59.3±2.73	-
150 days	60.0±5.20	-
180 days	62.6±1.33	-
210 days	57.3±1.66	-
240 days	54.6±2.02	-
270 days	61.0±2.08	-
300 days	63.3±0.88	-
330 days	61.3±1.33	-
360 days	62.3±2.33	-
Post-treatment		
30 days	61.0±3.79	-
60 days	60.0±1.15	6.6±1.07^{c}
90 days	61.3±3.18	21.2±2.18^{c}
120 days	62.3±2.85	34.8±1.71^{c}
150 days	57.6±2.33	57.4±2.06

cells and germ cells showed characteristic nuclear and cytoplasmic features (Figure 4).

After completion of treatment, vacuolization was significant in the cytoplasm of Sertoli cells and germ cells. The Sertoli cells were ill defined with nucleus containing pale chromatin network and loss of cytoplasmic organelles. There were plenty of lipid droplets and few secretory granules. Loss of cytoplasmic organelles were also evident in the spermatocytes and spermatids; particu-

Table 2c. Hypo-osmotic swelling test (%) in langur monkey after treatment with chloroform extract of *Carica papaya* seeds. Mean± SEM, $^{c}P < 0.01$, compared with controls.

Phase	Control (*n*=3)	Treated (*n*=5)
Pretreatment	62.6±3.28	56.6±2.73
Treatment		
30 days	59.6±2.90	25.0±3.35^{c}
60 days	60.3±6.07	12.4±1.81^{c}
90 days	63.0±1.15	-
120 days	61.6±0.88	-
150 days	61.0±1.00	-
180 days	60.5±2.72	-
210 days	56.0±3.05	-
240 days	57.3±2.33	-
270 days	58.3±3.18	-
300 days	59.6±0.88	-
330 days	59.3±4.70	-
360 days	58.3±1.66	-
Post-treatment		
30 days	60.3±4.10	-
60 days	60.0±0.57	7.4±1.25^{c}
90 days	64.0±1.15	21.6±2.25^{c}
120 days	63.3±0.33	37.8±1.07^{c}
150 days	63.6±1.85	56.2±2.25

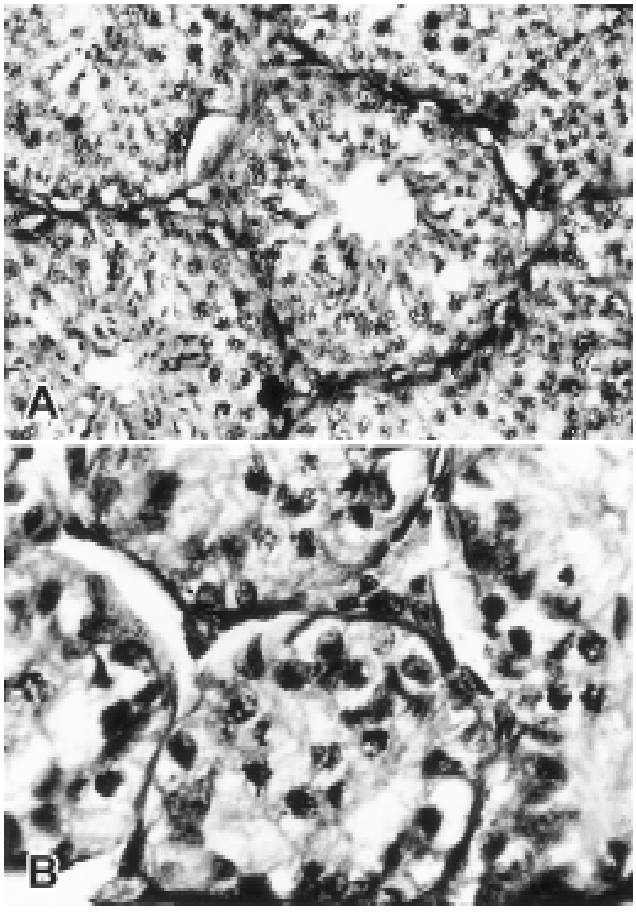

Figure 3. Testicular histology of langur monkey: <A> control: normal spermatogenesis; <B> treated with chloroform extract of *Carica papaya* seeds for 360 days: Tubules shrunken, showing disorganised germinal cells. Germ cell differentiation diminished at the level of spermatocytes. Leydig cells normal. × 100.

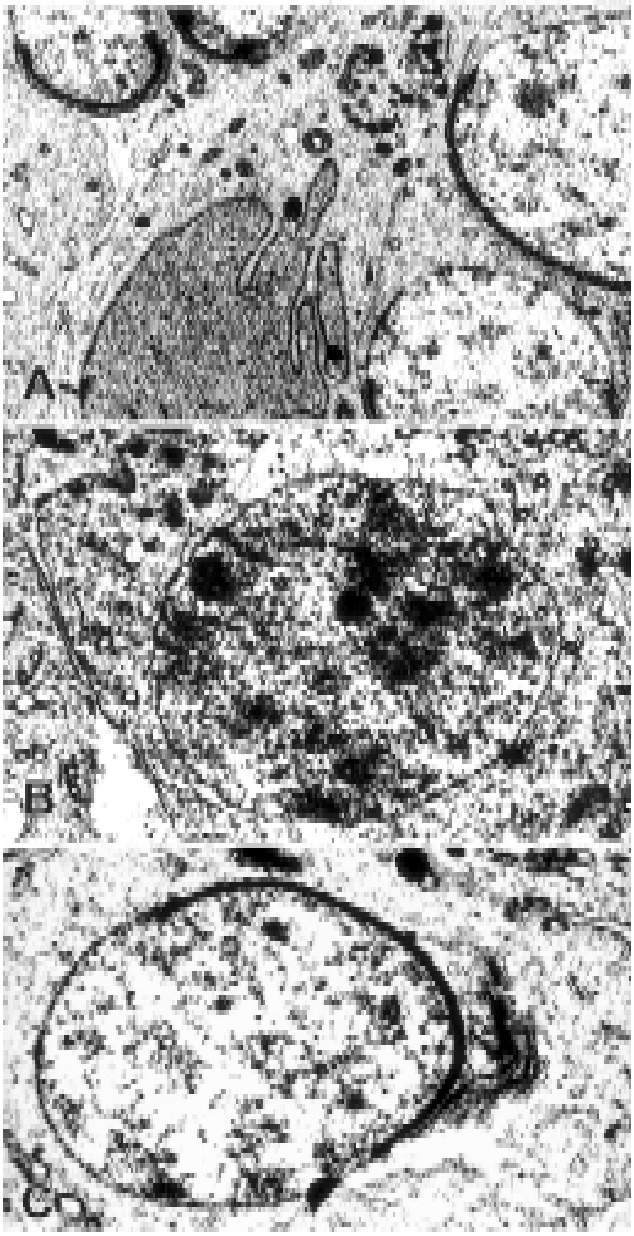

Figure 4. Testicular ultrastructure of control langur monkey: <A> showing normal Sertoli cell (Ser). Nucleus with deep indentation, Golgi bodies (G), mitochondria (M), smooth and rough endoplasmic reticulum (Rer), secretory granules (S) and coated vesicles (C) are well defined. Spermatids (Sp) with condensed nucleus, granular cytoplasm and peripheral mitochondria seen closely associated with Sertoli cells. ×5,600; <B> showing primary spermatocyte. Nucleus containing condensed chromatin material. Cytoplasm granular with well defined mitochondria. ×5,000. <C> showing spermatid at Golgi phase. Nucleus round with head cap. Golgi vesicles (Gv) concentrated around head cap. Mitochondria at the peripheral region. ×4,600.

larly in round spermatids the Golgi bodies and the peripheral mitochondria were virtually absent and the entire cytoplasm was vacuolated. Membrane damage was also evident (Figure 5).

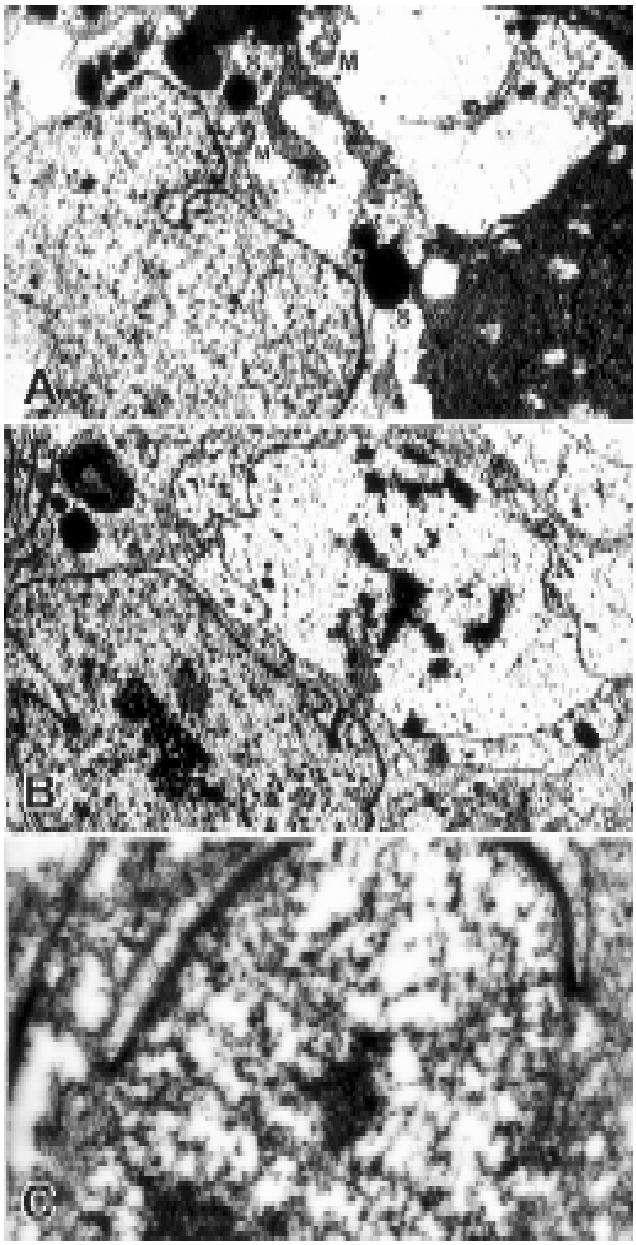

Figure 5. Testicular ultrastructure of langur monkey after treatment with chloroform extract of *Carica papaya* seeds for 360 days: <A> showing Sertoli cell. Nucleus containing relatively pale chromatin network. Cytoplasm showing vacuolization. Secretory granules (S) few and mitochondria (M) vacuolated. ×5.600. <B> showing primary spermatocyte. Nucleus pyknotic, cytoplasmic granules sparse. ×5,000. <C> showing round spermatid. Note membrane damage in nucleus. Other cytoplasmic organelles sparse. ×5, 600.

3.3 Leydig cell function test

The results of the *in vitro* hCG-stimulated testosterone biosynthesis of the Leydig cells revealed that these cells were capable of synthesizing testosterone (Table 3). However, the steroidogenic capacity of the Leydig cells of the treated animals was slightly inhibited as inferred from the 3β-HSD histochemistry and the test

Table 3. Effect of chloroform extract of *Carica papaya* seeds on hCG-stimulated testosterone biosynthesis (ng/mL) of Leydig cells *in vitro.* Mean± SEM, $^{c}P < 0.01$, compared with controls.

Group	Without hCG	With hCG
Control (*n*=3)	5.20 ± 0.01	6.92 ± 0.02
Treated (*n*=5)	4.00 ± 0.01^{c}	4.65 ± 0.05^{c}

osterone levels in the spent media of the treated animals which showed significant reduction, compared to the control levels.

3.4 Toxicology

The levels of the blood RBC, WBC, Hb, PCV, MCV, MCH and MCHC and the serum protein, glucose, cholesterol, SGPT, SGOT, LDH, creatinine and CK showed wide fluctuations in the study period, but they were within the pretreatment range throughout the course of the investigation (data not shown).

3.5 Hormone assay

Serum testosterone levels of the control and treated animals fluctuated within the pretreatment range throughout the study period (data not shown).

4 Discussion

The seeds of *Carica papaya* have been proven to possess male antifertility property. We have conducted a series of experiments with various extracts of the seeds of *Carica papaya* at different dose and duration regimens. Among the various extracts tested, the chloroform extract, the benzene chromatographic fraction of the chloroform extract and its methanol and ethyl acetate sub-fractions have shown reversible antifertility activity in rats and rabbits without adverse toxicity [1]. It is pertinent to note that *Carica papaya* possesses species variation, but the seed extract tested all show sperm motility inhibitory action in rats [4,21] and azoospermia in rabbits [22,23], while the aqueous extract and the chloroform chromatographic fraction of the chloroform extract showing contraceptive efficacy in rats [3,24] but failed to elicit significant response in rabbits[23,25].

In the present investigation, the chloroform extract of the seeds of *Carica papaya* in langur monkeys leads to azoospermia without adverse toxicity after 90 days of treatment, in a manner similar to that in rats and rabbits [1]. The sperm functional tests indicated that the voided spermatozoa after 30 and 60 days of treatment were in the infertile range. The effect is reversible as all the semenology parameters returned to pretreatment levels 150 days after treatment withdrawal.

The mechanism by which the chloroform extract of the seeds of *Carica papaya* brings about sperm motility inhibition/spermatogenic arrest in animals is not clear. In our earlier studies [22], it has been suggested that the spermatogenic arrest could be attributable to the estrogenic [26], androgenic [27,28] or antiandrogenic [29-31] property of the seed extract. Although the chloroform extract and the benzene chromatographic fraction of the chloroform extract has been reported to possess a mild estrogenic property [4,32], it is not likely that this property causes azoospermia in monkeys without severe side effects and change in hormonal pattern and libido. Androgenic and antiandrogenic properties seem to be less probable, as the seminal plasma biochemistry and serum hormonal pattern were all within the normal range. The increase in abnormal sperm count and total inhibition of sperm motility during the initial period of treatment suggest that the action of the drug could target the internal milieu of the epididymis.

Huynh et al.[33] in the triptolide treated rats described two phenotypic effects on mature and maturing germ cells. The first action appears early on the epididymal sperm and the subsequent action is directly on the germ cells of testis. However, in the present investigation, the chloroform extract of *Carica papaya* seeds alter the testicular but not the epididymal histology. Even the ultrastructure of epididymis shows normal configuration with active protein synthetic machinery (unpublished observations), suggesting that the drug acts upon the testicular germ cells. Interestingly, in the triptolide treated rats, no ultrastructural differences in the epididymal epithelium 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 [33-35].

Sertoli cells play an important role in germ cell maturation, but are highly susceptible to extraneous damage [36-39]. The chloroform extract of *Carica papaya* seeds induced cytoplasmic vacuolization and loss of cytoplasmic organelles in the Sertoli cells. Thus, degeneration and maturational arrest of germ cells, i.e., spermatocytes and spermatids, could be due to the Sertoli cell damage [37]. In the present study, a similar effect on the mitochondria of late spermatids and spermatozoa was also observed. It is therefore concluded that azoospermia could be due to a selective action of the drug on developing germ cells, possibly mediated via Sertoli cells, leading to inhibition of mitochondrial activity, which might alter the respiratory chain, generating a cytotoxic effect on the germ cell proliferation [40-43]. It is further evidenced by an increased mid-piece defects and vacuoliza-

tion in the mitochondria of the spermatozoa. However, further evidences are needed to substantiate this view.

Available evidences indicate that the chloroform extract of *Carica papaya* seeds is an orally effective, safe and readily reversible antifertility agent that meets the essential criteria for a male contraceptive.

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