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## Safety Evaluation of Long Term Treatment of Methanol Sub-Fraction of Seeds of *Carica papaya* as a Male Contraceptive with Particular Emphasis on Carcinogenicity in Albino Rats

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**Abstract:** A preliminary study to evaluate if long term treatment of Methanol Sub-Fraction (MSF) of the seeds of *Carica papaya* as a male contraceptive would develop neoplastic lesions in vital organs was carried out in albino rats at 50, 250 and 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for a period of 24 months, with a minimum dose being one therapeutic dose. Pre-terminal deaths, 45% in males and 48% in females, well within the acceptance limit, were reported to be age related and not treatment related, resulted due to general/respiratory/gastrointestinal/urogenital disorders in both males and females of control and treated animals. Skin peeling, withering of fur leaving skin patches were observed in few of the animals after 18 months of treatment. Absence of spermatozoa in the cauda epididymis was evident in all the treated animals. No major structural changes compared to control were evident in the vital organs. Serum testosterone, serum electrolytes, tissue biochemical, hematology and clinical chemistry were comparable to those of control animals, suggesting no adverse effect of the test substance following long term treatment. The results provided evidence that the methanol sub-fraction of the seeds of *Carica papaya* does not lead any development of neoplastic lesion following life term treatment for 24 months in rats and is safe enough to be permitted for further trials as a male contraceptive.

**Key words:** Neoplastic lesion, sperm count, histology, hematology, clinical chemistry

### INTRODUCTION

It has been well established that the seeds of *Carica papaya* possess male contraceptive efficacy in rats, rabbits and langur monkeys (Lohiya and Goyal, 1992; Lohiya *et al.*, 1999, 2001, 2005a; Kamal *et al.*, 2003; Ansari *et al.*, 2008). The chloroform extract, benzene chromatographic fraction of the chloroform extract and its methanol sub-fraction and ethyl acetate sub-fraction and the isolated active compounds, viz., MCP-I and ECP-I yielded potential 100% reversible contraceptive efficacy in all the tested animal models (Pathak *et al.*, 2000; Lohiya *et al.*, 2001, 2005a, b, 2008). The methanol sub-fraction of the benzene chromatographic fraction, owing to its feasibility of mass production, has been identified as a candidate for male contraception (Lohiya *et al.*, 2001, 2005b). Preliminary chemical evaluation by IR, NMR<sup>-1</sup> and MS revealed that the product is the homogeneous mixture of free fatty acids (Unpublished observations). The dose at 50 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> has been standardized as one contraceptive dose.

After establishing efficacy in animal models, series of preliminary safety evaluations of MSF as a putative male contraceptive is being conducted. Initial acute and

repeated sub-chronic toxicity evaluation following 28 and 90 day repeated dose have already been reported (Lohiya *et al.*, 2006). In the present study, a preliminary attempt has been made to assess the development of neoplastic lesion in vital organs following long term treatment of MSF for a period of 24 months in Wistar albino rats in accordance with the guidelines developed by the Office of Prevention, Pesticides and Toxic Substances (OPPTS, 1996).

### MATERIALS AND METHODS

**Animals:** Adult Wistar albino rats of both sexes, eight week old, weighing 150-200 g were used in the present investigation. The animals were maintained in individual polypropylene cages in the Departmental Animal House Facility with 12:12 h light: dark schedule. Temperature was maintained at 25±3°C. Feeding schedule consisted of rat pellet diet, twice a day and water was provided *ad libitum*. Daily intake of food and water were quantitated precisely. Prior to initiation of experiments, the entire experimental protocol was submitted to the Institutional Animal Ethical Committee, reviewed and the approval obtained. The animals were maintained under veterinary supervision in accordance with Lohiya *et al.* (2006).



**Test material:** Fresh seeds of *Carica papaya* L. (Caricaceae) of honey dew variety were used in the present investigation. The methanol sub-fraction was obtained as indicated in the earlier publication (Lohiya *et al.*, 2006). In the present investigation, the MSF suspended in olive oil is being used as test material.

**Experimental design:** The animals were divided into the following 4 groups each consisted of 10 male and 10 female animals:

**Group 1:** Animals served as vehicle control treated orally with olive oil at 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> (w/v) for a period of 24 months

**Group 2:** Animals were treated orally with 50 mg of MSF kg<sup>-1</sup> b.wt. day<sup>-1</sup> for a period of 24 months

**Group 3:** Animals were treated orally with 250 mg of MSF kg<sup>-1</sup> b.wt. day<sup>-1</sup> for a period of 24 months

**Group 4:** Animals were treated orally with 500 mg of MSF kg<sup>-1</sup> b.wt. day<sup>-1</sup> for a period of 24 months

The duration of the carcinogenicity study is to cover the majority of the normal life span of the strain of the animal used.

**Parameters:** The following parameters were recorded:

**Food and water intake:** Individual consumption of food and water was recorded daily.

**Body and organs weight:** The body weight was recorded weekly and the weights of liver, kidney, spleen, adrenal, thyroid, lungs, heart, brain, gonads and accessory reproductive organs were recorded after completion of the experiments.

**Phenotypic symptoms:** Mortality and morbidity, changes in fur, skin, eyes and nasal mucous membrane, tremors, convulsions, salivation, diarrhea, lethargy, animal behavior, feeding pattern, changes in the level of motor activity, gait and posture, reactivity to handling or sensory stimuli, grip strength and bizarre behavior such as self mutilation, walking backward, etc., were recorded daily. Libido was recorded monthly.

**Routine toxicology investigations:** All the surviving animals were necropsied after completion of the experiment schedule. The blood samples were collected through cardiac puncture and used for hematological observations. The serum separated out by centrifugation at 3000 rpm for a period of 10 min at room temperature and subjected for the clinical chemistry, electrolytes and

hormone analyses. The testis, cauda epididymis, seminal vesicle and ventral prostate of the males and the ovary and uterus of the females were carefully excised free of surrounding tissues, weighed and used for the histology and reproductive tissue biochemistry. All the other vital organs were removed carefully, weighed and used for histopathology examination.

**Hematology:** Total RBC, WBC, hemoglobin and red cell indices were recorded according to Lynch *et al.* (1969).

**Clinical chemistry:** Serum protein, glucose, cholesterol, triglycerides (TGL), Serum Glutamate Pyruvate Transaminase (SGPT), Serum Glutamate Oxaloacetate Transaminase (SGOT), alkaline phosphatase (ALP), High Density Lipoprotein (HDL), creatinine, urea, bilirubin and creatine phosphokinase (CPK), were analysed, using commercial kits.

**Serum electrolytes:** Serum sodium, potassium, calcium, phosphorous, chloride, magnesium, iron and zinc were estimated colorimetrically using commercial kits.

**Hormone analysis:** Serum testosterone levels were estimated by ELISA, kits obtained from Biochem Immuno Systems, Italy, in all the surviving control and treated animals following completion of the experiment. The binding of antisera was 90%, serum pools gave intra assay coefficient of variation of 3.9% and inter assay coefficient of variation of 6.2% with 0.01 ng mL<sup>-1</sup> sensitivity.

**Sperm analysis:** The cauda epididymis was chipped in 1 mL of normal saline and the clear fluid was used for the analysis of sperm concentration, motility, viability and abnormality (Lohiya and Goyal, 1992).

The sperm concentration was calculated by hemocytometric method using Neubauer's haemocytometer at 1:19 dilution. The percent motility, viz., rapid linear progressive, slow linear progressive, vibratory and non motile, percent viability, assessed through eosin-nigrosin staining method and percent normal/abnormal spermatozoa through Papanicolaou staining procedure, were assessed under phase contrast microscope (Model: Optiphot, Nikon, Japan).

**Tissue biochemistry:** A portion of the reproductive organs, viz., testis, cauda epididymis, seminal vesicle and prostate of the male and ovary and uterus of the female were used for the evaluation of androgen sensitive biochemical markers, viz., cholesterol (King and Wooton, 1959), glycogen (Montgomery, 1957) and lactate



dehydrogenase (LDH) (Bergmeyer, 1965) of testis, sialic acid (Svennerholm, 1960), L-carnitine (Cooper *et al.*, 1988) and neutral  $\alpha$ -glucosidase (Cooper *et al.*, 1990) of epididymis, fructose of seminal vesicle and acid phosphatase (ACP) of ventral prostate (Karvonen and Malm, 1955), glycogen and sialic acid of ovary and uterus. Protein concentration for enzymatic analysis was estimated (Lowry *et al.*, 1951).

**Pathological examination:** Liver, kidney, spleen, adrenal, pancreas, thyroid, lungs, heart, brain, gonads and accessory reproductive organs were excised free of surrounding tissues, weighed and fixed immediately in Bouin's fixative. The fixed tissues were washed several times in distilled water, dehydrated in graded series of ethanol, cleared in benzene and embedded in paraffin wax. Five micrometer thick sections were stained with Harris' haematoxylin and eosin for routine histopathological examination.

The other organs, viz., salivary glands, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, brain, including cerebrum, cerebellum, medulla/pons pituitary, peripheral nerve (sciatic or tibial), spinal cord (cervical, mid thoracic and lumbar), eyes, trachea, pharynx, larynx, nose, aorta, heart, bone marrow, lymph nodes, spleen, urinary bladder, mammary glands of females and skin were fixed in Bouin's fluid, washed in distilled water and archived in 70% ethanol for future histopathological examination as and when necessary.

**Statistical analysis:** One way ANOVA was employed for statistical comparison. The values are expressed as Mean $\pm$ Standard Error (SEM) and  $p < 0.05$  level was considered significant.

## RESULTS

Pre-terminal deaths in eighteen males and nineteen females were recorded, out of forty males and forty females, thus the per cent survival rate was estimated to

be 45% in males and 48% in females. In most of the cases, the pre-terminal deaths were age related that resulted due to general/respiratory/gastrointestinal/urogenital disorders in both males and females of control and treated animals (Table 1). The external morphological characteristics, viz., skin, fur, eyes, mucous membrane and nose in most of the animals appeared normal. The animals, control as well as treated did not show tremors, convulsions, salivation, diarrhea, bizarre behaviour such as self mutilation, walking backward, etc. The gait and posture, reactivity to handling or sensory stimuli and grip strength were recorded normal. However, in few of the animals, irrespective of sex, including that of controls, skin peeling and withering of fur, leaving skin patches, were observed. Most of the control and treated animals were lethargic and showed the tendency of repetitive circling. However, in all these cases, the abnormalities were found after 18 months of treatment (Table 2). There were daily fluctuations among individual animals in food and water intake, which were comparable to those of control (data not shown). No significant changes observed in quarterly body weight of both males and females, compared to those of corresponding control. The weight of vital organs and reproductive organs did not show appreciable changes of both males and females compared to control animals. The sperm parameters revealed absence of spermatozoa in cauda epididymis, in all surviving animals of the treatment groups. The biochemical parameters of reproductive organs of the treated animals did not show significant changes and the values were comparable to those of control animals. Hematology did not show appreciable changes, although there were wide fluctuations, compared to those of control values. Levels of serum clinical biochemistry and serum electrolytes showed no appreciable changes, the values although showed mild fluctuations, were comparable to those of control animals. The serum testosterone level did not show appreciable changes, compared to controls. None of the above parameters showed gender differences (data not shown).

Table 1: Carcinogenicity test-summary of clinical signs and pre-terminal deaths

Parameters	Males (n = 10 each)				Females (n = 10 each)			
	Control (G-1)	50 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-2)	250 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-3)	500 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-4)	Control (G-1)	50 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-2)	250 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-3)	500 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-4)
General defects	4	5	4	5	5	4	5	5
Neurological defects	0	0	0	0	0	0	0	0
Respiratory defects	0	2	0	0	0	0	0	0
Eye defects	0	0	0	0	0	0	0	0
Gastro-intestinal defects	2	0	2	0	2	2	2	2
Skin defects	0	2	2	2	0	2	2	0
Urogenital defects	2	2	2	4	0	0	0	0
Pre-terminal deaths	5	4	4	5	5	4	5	5



Table 2: Carcinogenicity study: Summary of visible toxicological symptoms

Parameters	Males (n = 10 each)				Females (n = 10 each)			
	Control (G-1)	50 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-2)	250 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-3)	500 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-4)	Control (G-1)	50 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-2)	250 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-3)	500 mg kg <sup>-1</sup> b.wt. day <sup>-1</sup> (G-4)
Skin and fur	2	2	2	0	2	0	2	0
Eyes	0	0	0	0	0	0	0	0
Nasal mucous membrane	0	0	0	0	0	0	0	0
<b>Central nervous system</b>								
Tremors	0	0	0	0	0	0	0	0
Convulsions	0	0	0	0	0	0	0	0
<b>Autonomic activity</b>								
Salivation	0	0	0	0	0	0	0	0
Diarrhea	0	0	0	0	0	0	0	0
Lethargy	4	4	2	4	5	2	2	4
Animal behaviour	0	0	0	0	0	0	0	0
Feeding pattern	0	0	0	0	0	0	0	0
Changes in level of motor activity	0	0	0	0	0	0	0	0
Gait and posture	0	0	0	0	0	0	0	0
Reactivity to handling or sensory stimuli	0	0	0	0	0	0	0	0
Repetitive circling	4	2	2	2	2	0	2	2
Grip strength	0	0	0	0	0	0	0	0
<b>Bizarre behaviour</b>								
Self mutilation	0	0	0	0	0	0	0	0
Walking backward	0	0	0	0	0	0	0	0
Libido	0	0	0	0	0	0	0	0

In most of the cases, the symptoms appeared after 18 months of treatment

**Pathological examination**

**Thyroid:** The thyroid of the treated animals following 24 months of MSF treatment showed most of the follicles with eosinated colloid in the lumen, relatively low parafollicular cells, epithelium with low cuboidal cells and lesser secretory activity. There was no evidence of neoplastic lesion in the thyroid, due to treatment (Fig. 1, 2).

**Liver:** Treated animals showed structural similarities with that of control animals. No treatment related changes were observed. There was no evidence of neoplasm due to treatment (Fig. 3, 4).

**Kidney:** Following oral administration with MSF at 50, 250 and 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for 24 months, no appreciable changes observed in the structure of kidney tubules, compared to control. In majority of the treated animals, irrespective of doses, the cells of both proximal and distal convoluted tubules were less granular and less eosinophilic. However, endothelial cells between the tubules were numerous, highly eosinophilic and more prominent (Fig. 5, 6).

**Adrenal:** There were no appreciable changes in the structure of adrenal cortex and medulla, compared to control animals following daily oral administration with the MSF at 50, 250 and 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for 24 months. No neoplastic lesions were evident in the adrenal due to treatment (Fig. 7, 8).

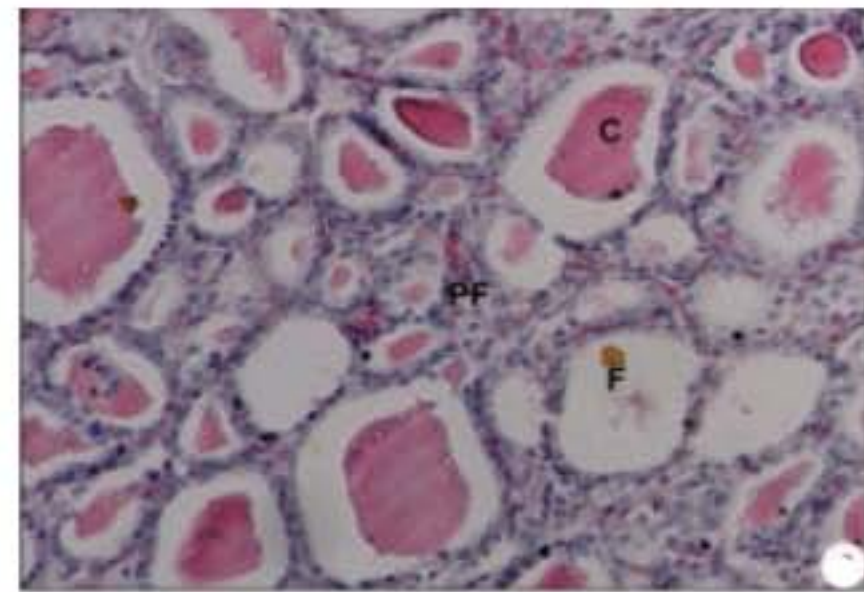


Fig. 1: Histology of the thyroid glands of the vehicle treated control animals following completion of 24 months study period. The thyroid follicles show evidence of normal secretory activity. The epithelial cells are low cuboidal and the cytoplasm of the cells appears granular. The lumen contains eosinated colloid. The lumen of few follicles appears empty. x100, F: Follicle, C: Colloid, PF: Parafollicular cells

**Testis:** Following 24 months of treatment, no evidences of major structural changes observed in the testis of all treated groups. Necrotic changes, however, in the Sertoli cells and germ cells were evident. Germ cells show atrophy and germ cells differentiation beyond spermatocytes was less discernible. Leydig cells showed atrophy (Fig. 9-11).



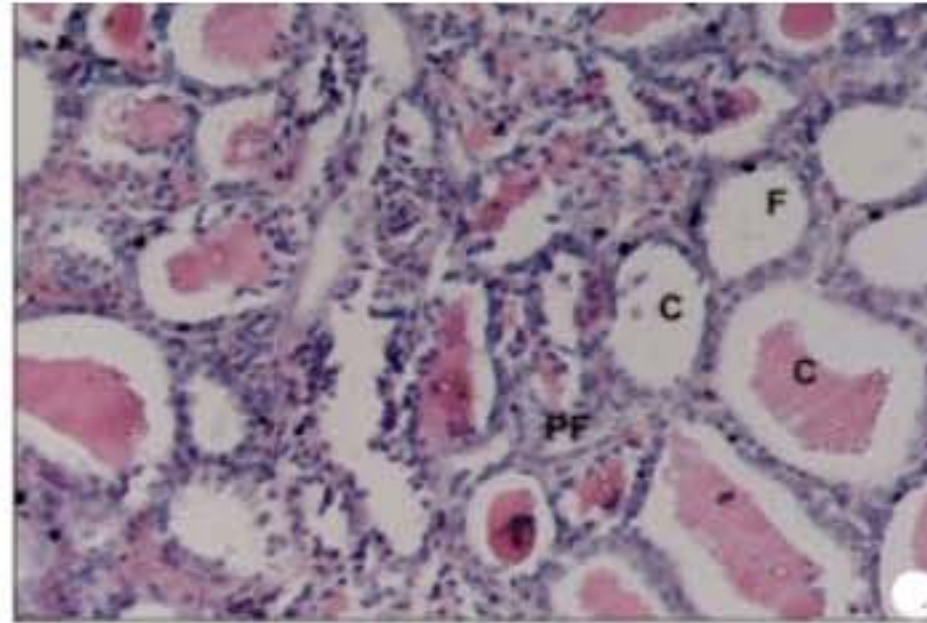


Fig. 2: Histology of the thyroid gland following daily oral administration with MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. No structural changes are evident compared to control. The thyroid follicles are indicative of normal secretory activity. x100, F: Follicle, C: Colloid, PF: Parafollicular cells

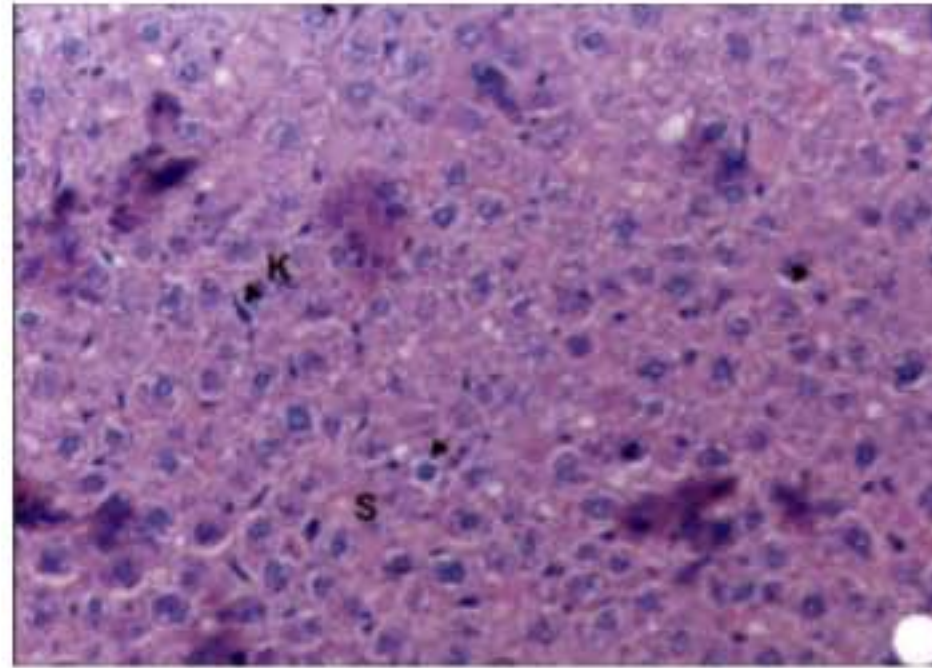


Fig. 3: Histology of liver of vehicle treated control animal following completion of 24 months study period. Vacuolization is evident in few of the cells (arrows). Kupffer cells are few, adjacent to sinusoids. x100, S: Sinusoids, K: Kupffer cells

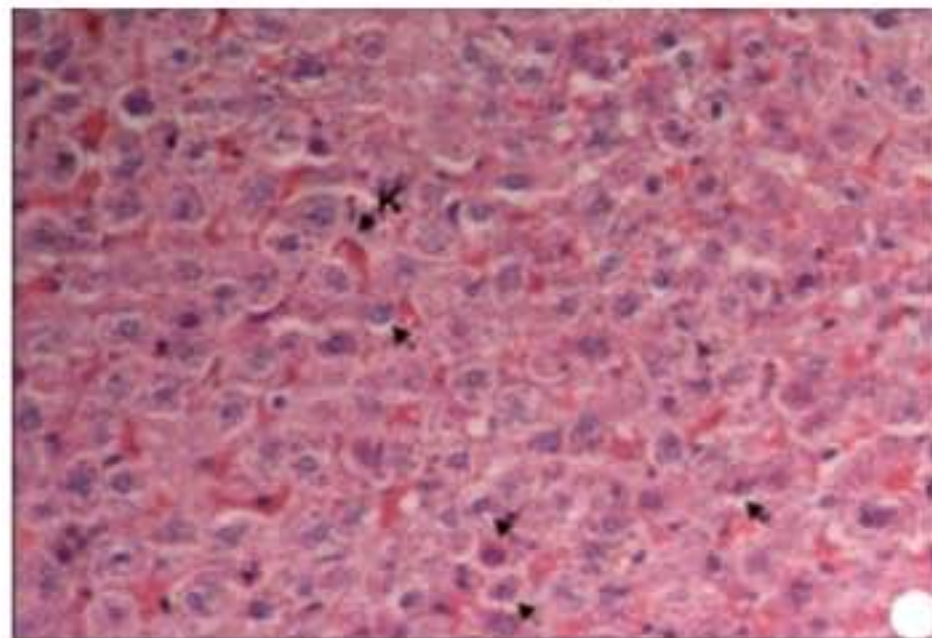


Fig. 4: Histology of liver following daily oral administration with MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. The Kupffer cells are relatively more. Many of the cells show vacuolization (arrows). No evidence of carcinogenic lesion. x100, K: Kupffer cells



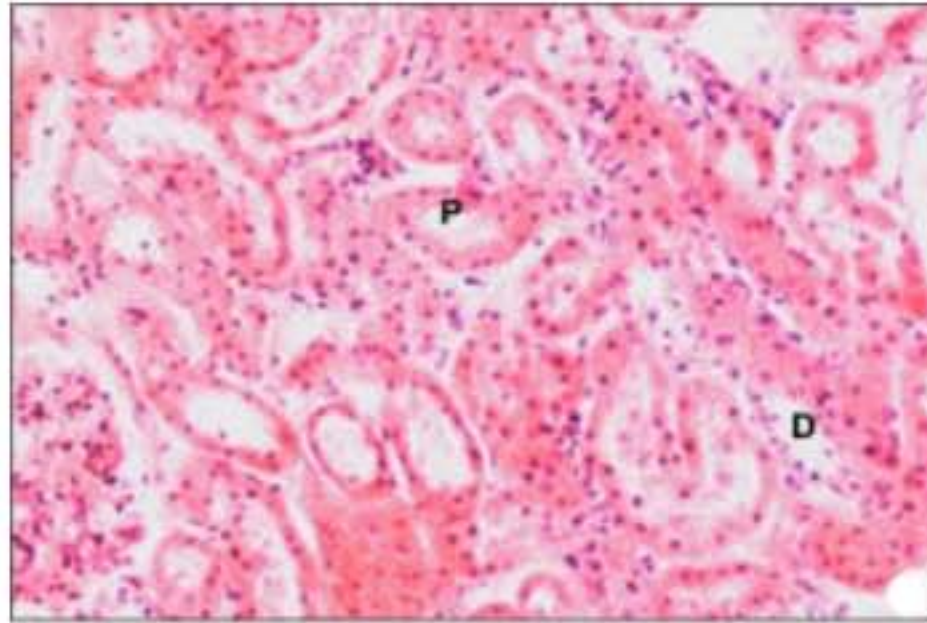


Fig. 5: Histology of the kidney of the vehicle treated control animals following completion of 24 months study period. The cells of proximal as well as distal convoluted tubules are less granular. Endothelial cells are more prominent. x100, P: Proximal convoluted tubule, D: Distal convoluted tubule

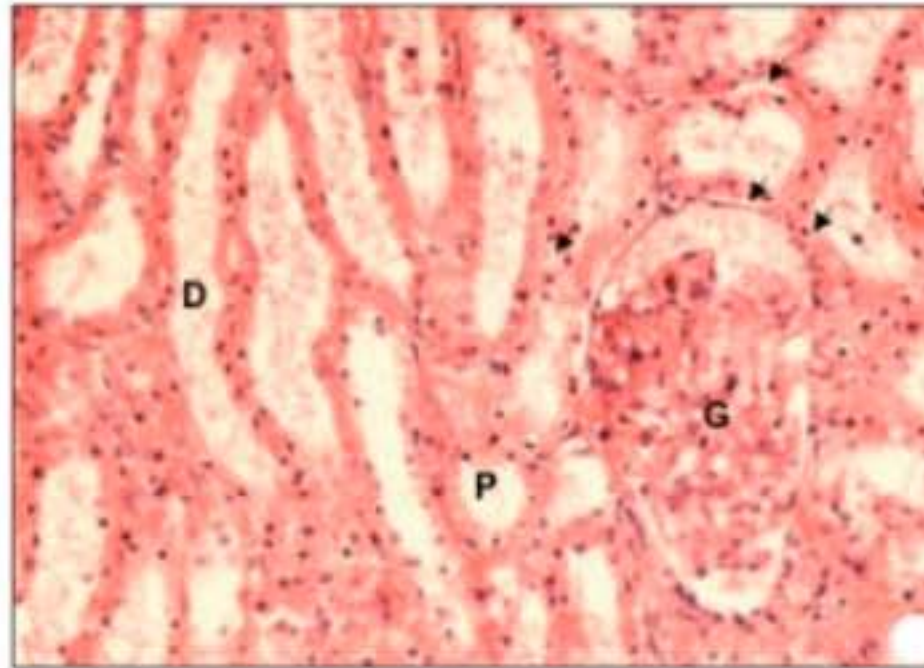


Fig. 6: Histology of the kidney following daily oral administration of MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. The cells are less eosinophilic and show vacuolization (arrows). Endothelial cells are more prominent. x100, P: Proximal convoluted tubule, D: Distal convoluted tubule, G: Glomerulus

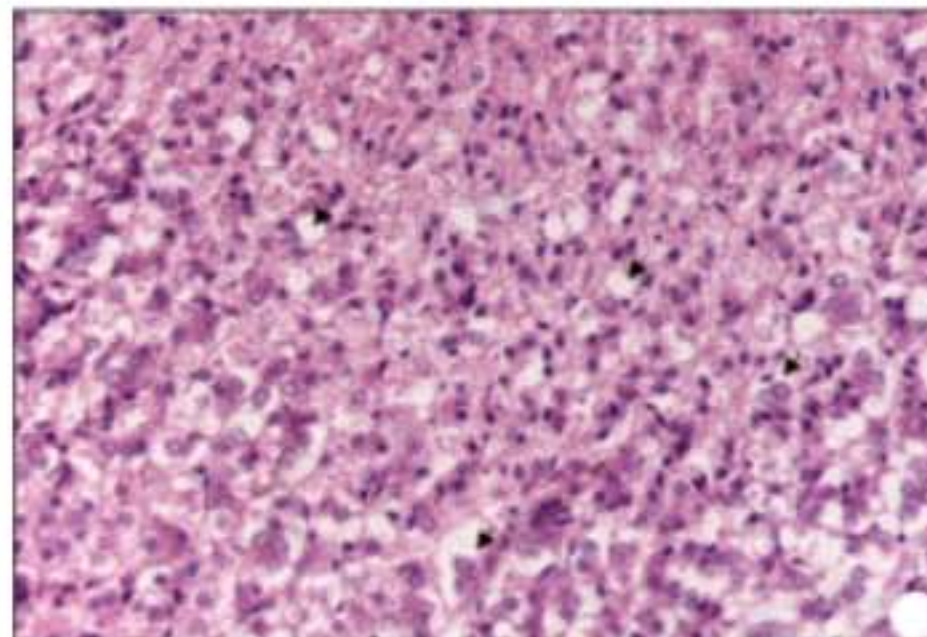


Fig. 7: Histology of the adrenal gland of the vehicle treated control animals following completion of 24 months study period. Intercellular spaces are relatively more. The cytoplasm of most of the cells show vacuolization (arrows). Some of the cells show nuclear pyknosis. x100



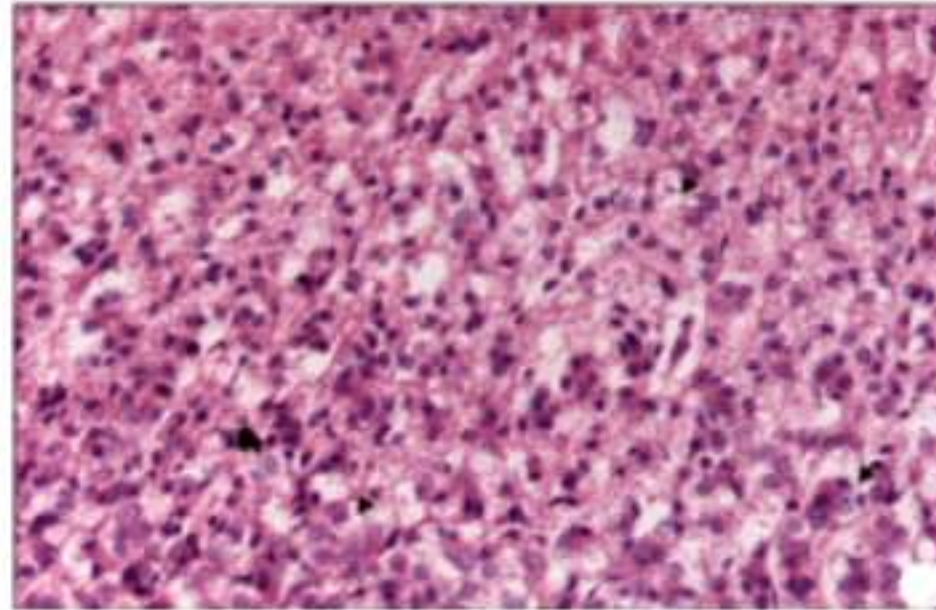


Fig. 8: Histology of the adrenal gland following daily oral administration of MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. The structural details are comparable to control animals. Most of the cells show vacuolization (arrows). x100

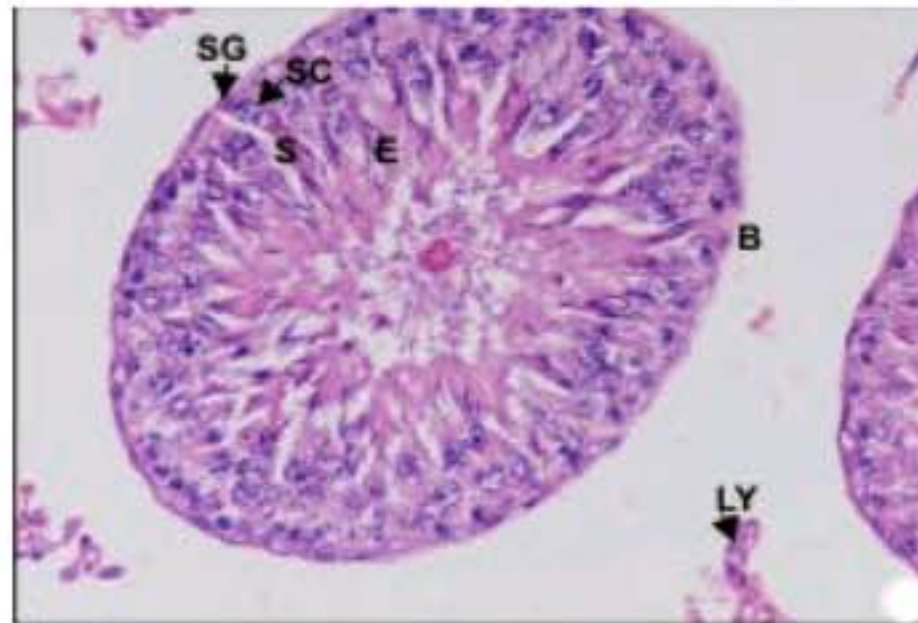


Fig. 9: Histology of the testis of the control animals following completion of 24 months study period. Vacuolization is evident in Sertoli cells and germ cells. The nuclei of spermatogonia and spermatocytes are pyknotic. The lumen contain cell debris and edematous material. x100, B: Basal lamina, SG: Spermatogonia, S: Sertoli cells, SC: Spermatocytes, E: Elongated spermatid, LY: Leydig cells

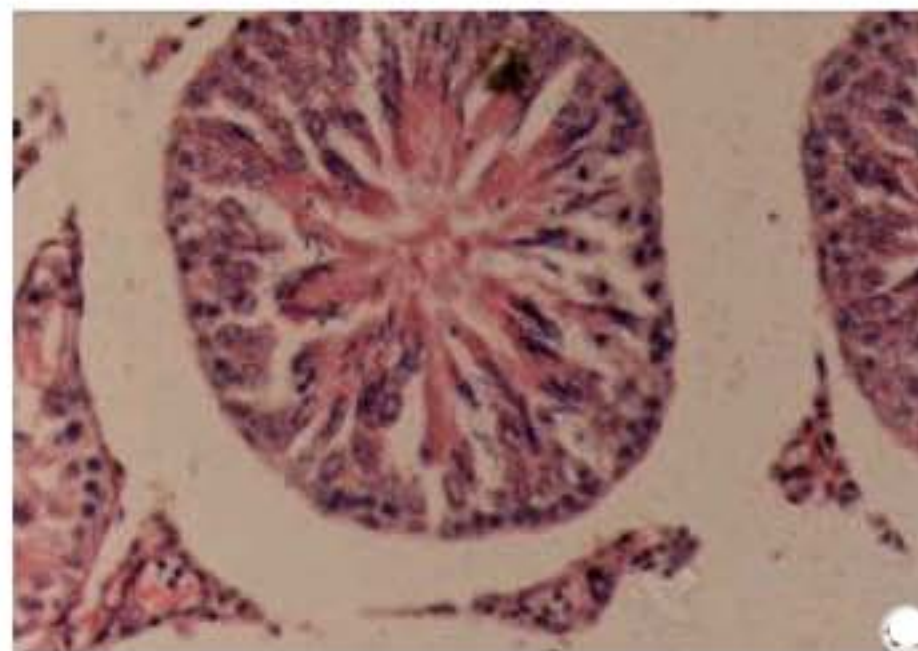


Fig. 10: Histology of the testis following daily oral administration with MSF at  $250 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. Germ cell differentiation is severely affected. The lumen contains edematous material. Leydig cells show atrophy. x100



**Epididymis:** The caput and cauda epididymis following completion of treatment at 50, 250 and 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for 24 months exhibited no structural alteration when compared with control. The epithelium showed evidence of normal secretory activity and the lumen was devoid of spermatozoa (Fig. 12, 13).

**Seminal vesicle:** The seminal vesicle of the treated rats following completion of 24 months study period

showed no evidence of structural changes or secretory pattern compared to those of control animals (Fig. 14, 15).

**Ventral prostate:** Treated animals did not show drastic changes in the structure or secretory pattern of ventral prostate following completion of treatment. No neoplasm was evident in the ventral prostate due to treatment (Fig. 16, 17).

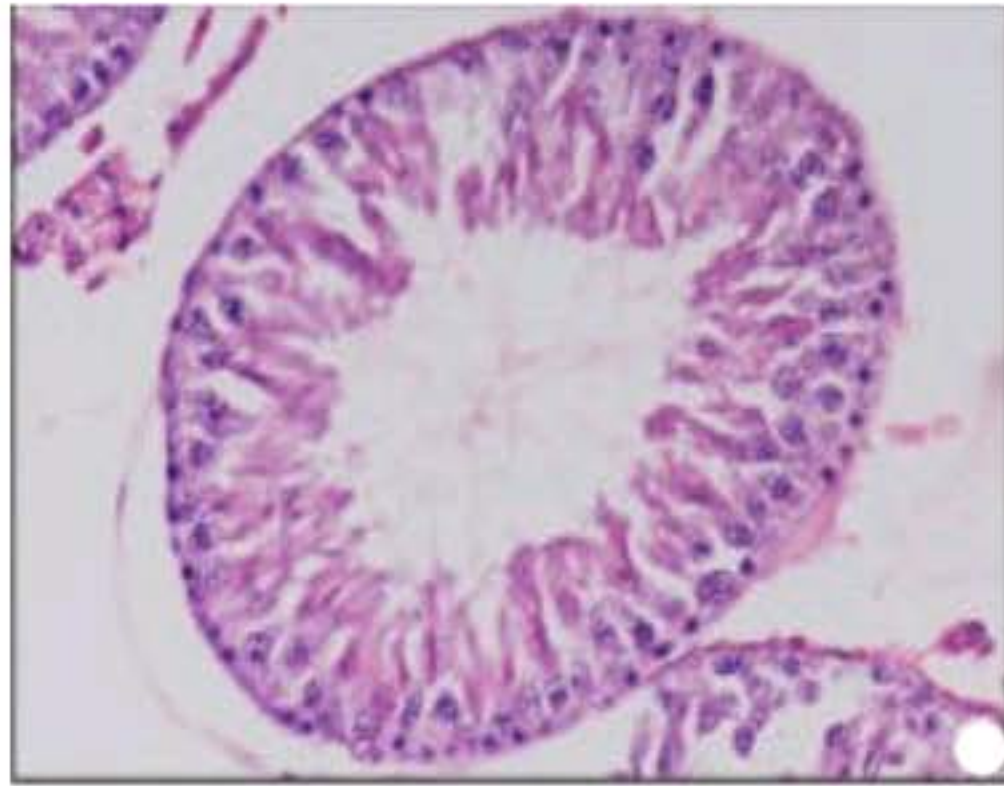


Fig. 11: Histology of the testis following daily oral administration with MSF at 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for 24 months study period. The germ cells show atrophy. Nuclear pyknosis is evident in spermatogonia and spermatocytes. Leydig cells show atrophy. x100

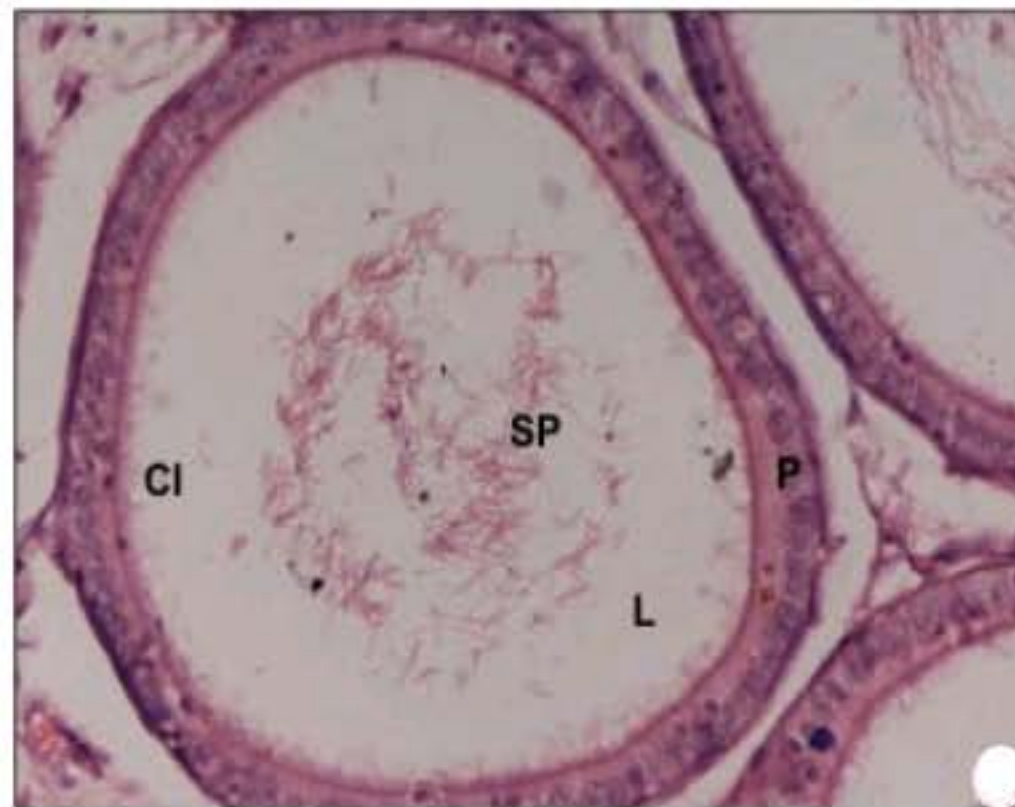


Fig. 12: Histology of the cauda epididymis of the vehicle treated control animal following completion of 24 months study period. The epithelium presents well-defined basal and principal cells. The principal cells are ciliated and contain granular cytoplasm. The lumen contains less spermatozoa. Inter tubular elements are comparatively less. x100, P: Principal cells, CI: Cilia, SP: Spermatozoa, L: Lumen, EP: Epithelium



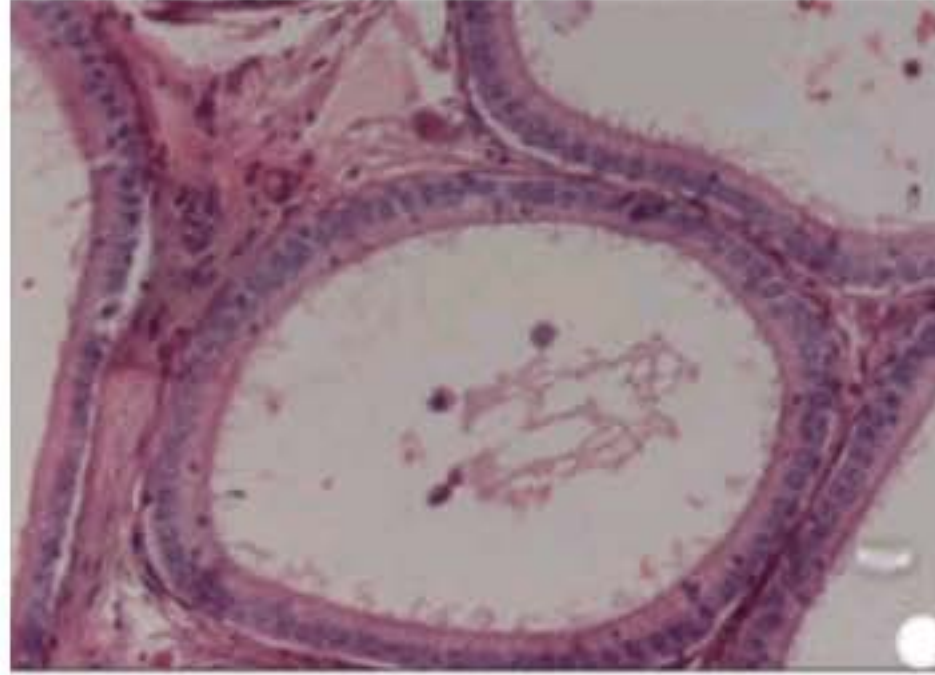


Fig. 13: Histology of the cauda epididymis following daily oral administration with MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. No structural changes are evident. The epithelium is indicative of normal secretory activity. The lumen contains phagocytes. x100

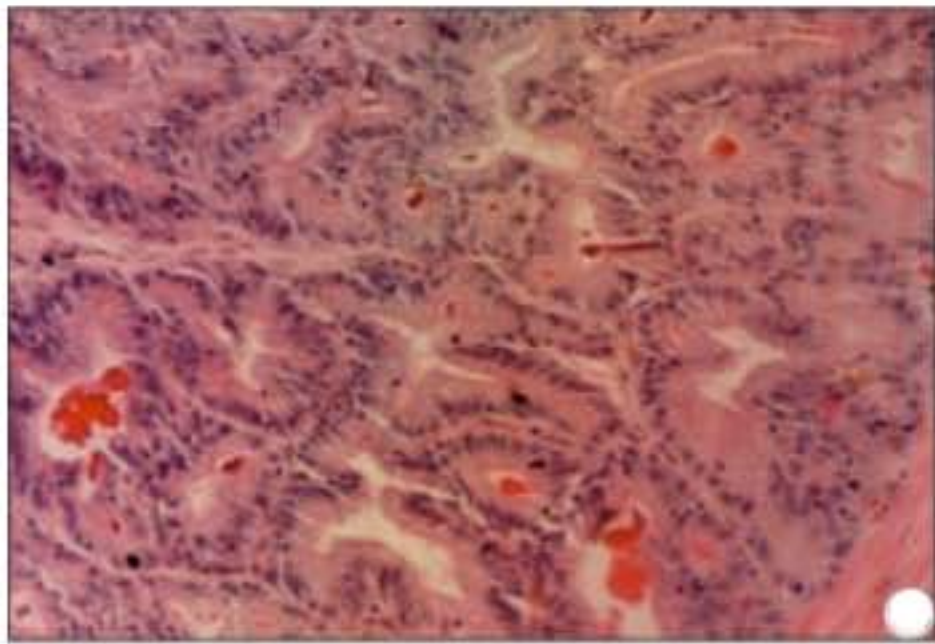


Fig. 14: Histology of the seminal vesicle of the vehicle treated control animal following completion of 24 months study period. The epithelium shows typical cryptic pattern and indicative of normal secretory activity. x100

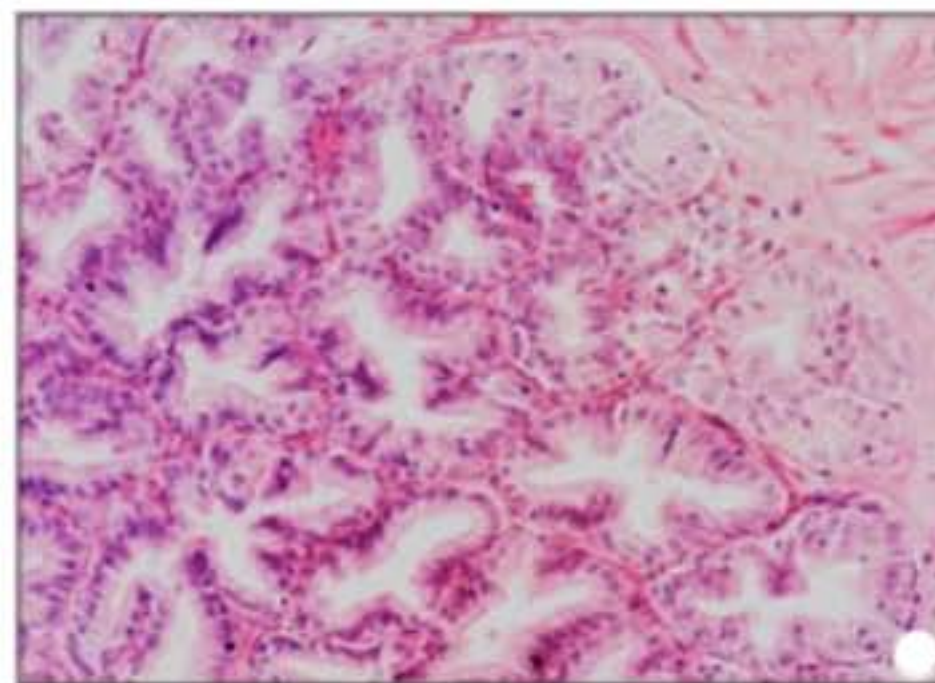


Fig. 15: Histology of the seminal vesicle following daily oral administration with MSF at  $500 \text{ mg kg}^{-1} \text{ b.wt. day}^{-1}$  for 24 months study period. No structural changes are evident. The epithelium is indicative of normal secretory activity. x100



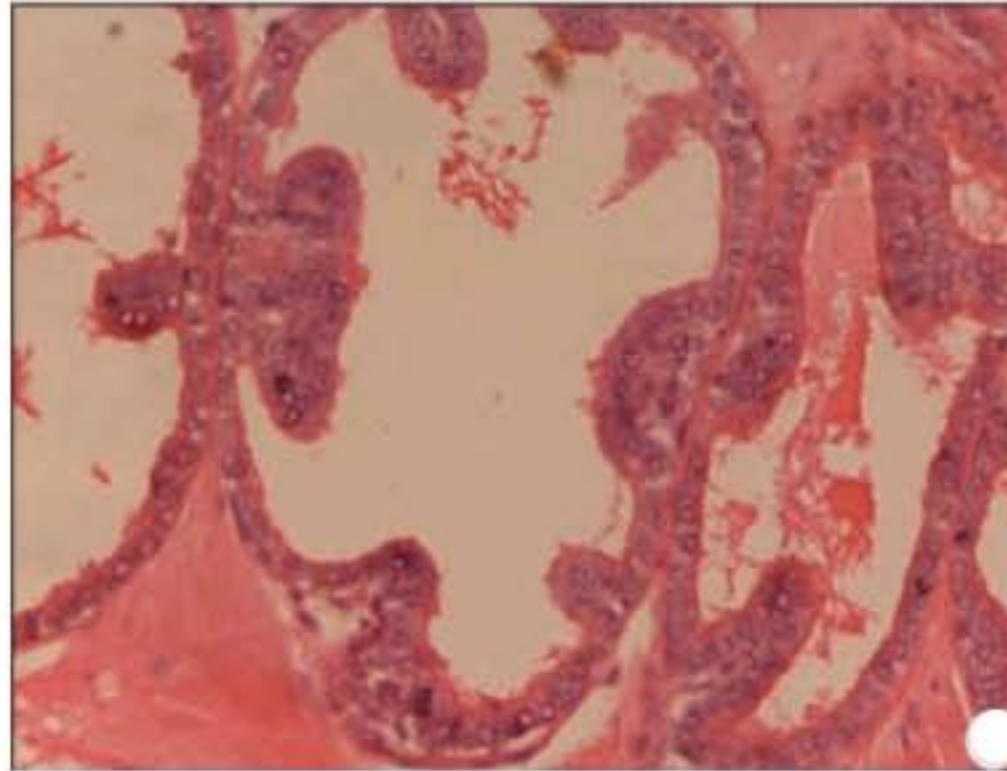


Fig. 16: Histology of the ventral prostate of the vehicle treated control animals following completion of 24 months study period. The epithelium of alveoli shows cryptic pattern. Few of the cells show vacuolization. x100

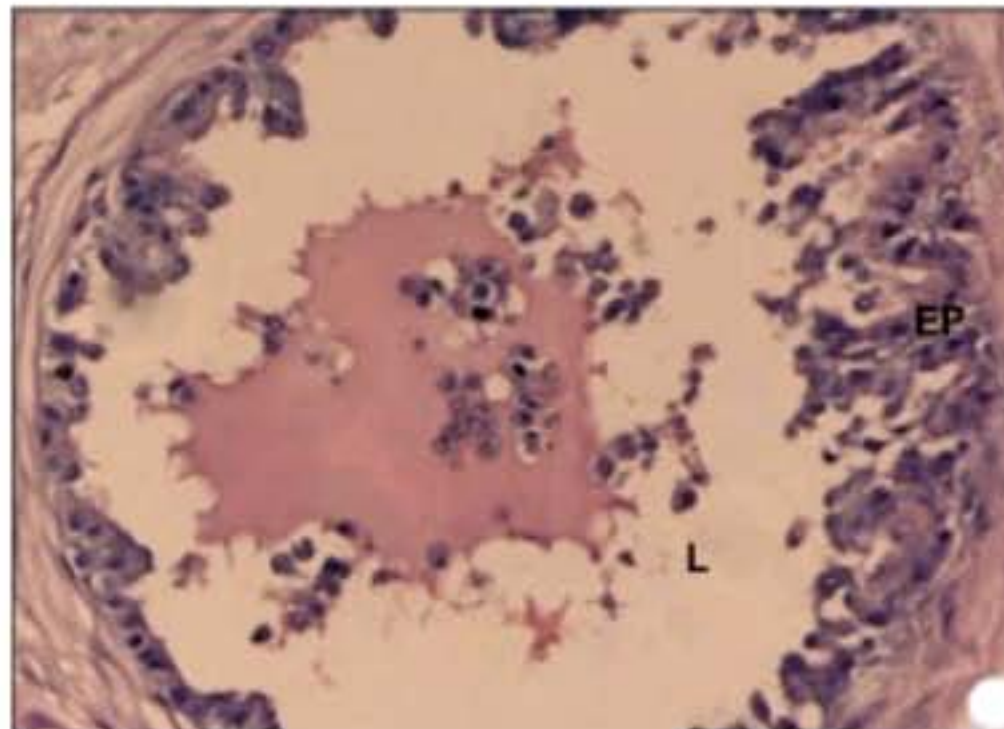


Fig. 17: Histology of the ventral prostate following daily oral administration with MSF at 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> for 24 months study period. Disorganization of epithelial cells is evident. The lumen contains secretory material and sloughed cells. No carcinogenic lesion is evident. x100, L: Lumen, EP: Epithelium

## DISCUSSION

Development of herbal drug products often entails unique issues that are different from other drug products. In the history of male contraceptive research, thus far three plant products viz., gossypol, *Tripterygium wilfordii* and *Ecballium ellaterium*, claimed to have better contraceptive efficacy in clinical trials, failed to reach the level of commercial launch, due to the side effects at unacceptable level (Morris, 1986; Waller *et al.*, 1986; Ansari *et al.*, 2008). Research on herbal male contraceptive warrants special attention in terms of

toxicity considering the lessons learnt from the previous herbal contraceptive products.

Earlier preliminary toxicity evaluation revealed that a single oral dose of MSF at 2000 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup>, over 14 day study period and daily oral doses of 50, 100, 250 and 500 mg kg<sup>-1</sup> b.wt day<sup>-1</sup> for 28 and 90 day study periods showed no overt general toxicity in exposed animals. Food and water intake showed daily fluctuations within control limits. Sperm density showed a significant decrease in all 28 and 90 day repeated dose treated animals, whereas total sperm motility inhibition was observed at 250 and 500 mg kg<sup>-1</sup> b.wt. day<sup>-1</sup> dose levels



at the 28 day time interval, but in all dose groups at the 90 day interval. However, hematological and serum clinical parameters including serum testosterone level were within control range following completion of observation period. Histopathology of vital organs, other than testis of 90 day treated animals which showed necrotic changes were recorded normal (Lohiya *et al.*, 2006).

The main objective of the present investigation was to observe, if long term treatment of the methanol sub-fraction of the seeds of *Carica papaya* for the major portion of the life span in animal model causes neoplastic lesions in vital organs. The study was carried out in accordance with the series of harmonized test guidelines developed by the Office of Prevention, Pesticides and Toxic substances (OPPTS, 1996). The test was performed on rats because of its relatively short life span, limited cost of maintenance, widespread use in pharmacological and biological, susceptibility to tumor induction and the availability of inbred or sufficiently characterized animals. MSF was administered at various dose levels, up to 10 times the contraceptive dose by daily oral route up to a period of 24 months. Survival record of treated animals, both males and females were similar to that of control groups. Death of animals during the study period was well within the acceptance criteria, that is less than 50% at 24 month study period.

MSF, used in the present investigation was from the same lot, with established stability of the test substance, evaluated periodically by potency test in rabbits. Prior to the initiation of the study, the test substance was subjected to characterization which revealed groups of free fatty acid esters. Highest dose was determined based on the findings from the 90 day repeat dose toxicity study (Lohiya *et al.*, 2006), to ensure that the dose used is adequate to assess the carcinogenic potential of the test substance.

The results revealed no treatment related toxicity. The mortality or morbidity observed during the treatment period were not related to treatment, as the effects were common in control as well as treated animals. Histopathological effects were minimal without showing significant changes due to treatment.

Gross pathological observations of vital organs revealed no neoplastic lesions. The lesser secretory activity with low cuboidal cells in thyroid, characteristic Kupffer cells, phagocytic in nature and prominent sinusoids between the hepatic cells of liver, less granular and less eosinophilic cuboidal cells of kidney, vacuolated cell cords of adrenal were reportedly age related, not treatment related as these changes were also observed in control animals and that these changes were common in senile animals (Bloom and Fawcett, 1975). However, highly vacuolated Sertoli cells, spermatogonia, spermatocytes, macrophages and sperm fragments in the

lumen, vacuolated Leydig cells in testis, absence of spermatozoa in the lumen of cauda epididymis and lesser secretory activity in alveoli of ventral prostate could be related to treatment as these are the major target organs for the test substance (Manivannan *et al.*, 2004).

In the present investigation, experimental redundancy to assess broad range of toxicity endpoints in multiple tissues and organs was followed. This was recommended not only to assure scientific rigor, but also to maximize the likelihood for detecting important target tissue effects. Clinical significance of serum constituent i.e., protein to determine various disease states like dehydration, multiple myeloma, chronic liver and renal diseases, glucose to determine diabetes mellitus, hypoglycemia, adrenal dysfunction and inborn errors of carbohydrate metabolism, cholesterol to determine the risk of coronary arterial occlusion, atherosclerosis, myocardial infarction, liver function, biliary function, intestinal absorption and thyroid function and adrenal diseases, creatinine to determine the renal function, the SGPT and SGOT to determine the liver function, creatine kinase to determine myocardial infarction, acute cerebrovascular diseases, muscular dystrophy and injury, bilirubin to determine liver function, urea to determine protein metabolism, kidney function, liver diseases, triglycerides to determine the coronary heart diseases, HDL cholesterol to determine the coronary heart diseases, alkaline phosphatase to determine hepatobiliary diseases, primary or secondary malignancies and post hepatic obstructive jaundice and all the electrolytes to determine the vital organ functions have been clinically correlated with hematology, histopathological findings and visible toxicological symptoms. In the present investigation, treatment related changes were minimal in total RBC, WBC and hematological indices, serum clinical chemistry, biochemical markers, electrolytes and in testosterone levels. All values were comparable to control values and within the historical control data of the test facility.

In conclusion, the results obtained in the present investigation, indicated that there are no gross pathological effects and no neoplastic lesion in vital organs following life term treatment of MSF, thus provided evidence that it is safe enough to be permitted for long term treatment as a male contraceptive in clinical trial.

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