

EFFECT OF PARA-CHLOROPHENYLALANINE ON MALE RATS : HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES IN THE TESTES

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Summary : Spermatogenically active testes of rat challenged by 100 mg/kg body weight of p- Chlorophenylalanine for 45 days displayed marked and drastic changes in the seminiferous epithelium. Degenerative changes followed by immense necrosis of germ cells lead to complete breakdown of seminiferous tubules. Leydig cells, however, remained unaffected histologically in the treated animals. Among the accessory sex organs, epididymis alone showed a marked decrease in its weight. A biochemical study in the drug treated rats revealed a significant accumulation of glycogen in the testes accompanied by increase in the activities of enzymes like the succinic dehydrogenase, glucose-6-phosphatase, ATP-ase and acid phosphatases. However, a marked decrease was noticed in the activities of enzymes like alkaline phosphatase, phosphohexoseisomerase and lactate dehydrogenase. No significant change was found in the protein, DNA and RNA concentrations in the drug treated testes. The histological and biochemical changes induced in the testes by p-CPA suggest the deleterious effect of the drug on the seminiferous tubules of the testes.

Key words : p-Chlorophenylalanine
histopathology

testes
biochemistry

accessory sex organs
enzymatic changes

INTRODUCTION

p-Chlorophenylalanine (p-CPA) has been the pharmacological drug of choice in studies on sexual behaviour and associated aggressive behaviour in animals for the past two decades. p-CPA has been shown to induce hypersexuality in male rats and cats (10) both in the presence or absence of testosterone (8,18) and this sexual behaviour was reversible by low doses of 5-hydroxytryptophan administration. This drug acts by selectively inhibiting serotonin synthesis in the brain, pineal and other tissues by inhibi-

ting tryptophan conversion to 5-hydroxytryptophan, the precursor of serotonin (12). Consequent to this decrease in serotonin, the prolactin secretion is also inhibited in intact male rats but not in orchidectomised or ovariectomised rats (7). As prolactin is known to regulate steroidogenesis as well as plasma FSH secretion and also to maintain accessory sex organs, any decrease in this hormone synthesis will affect the male sexual function. Since, p-CPA affected both sexuality as well as prolactin levels in rats, it would be interesting to observe if the drug had any effect on the male reproductive organs as well, if given for a long term. Hence, in the present investigation, the possible effect of p-CPA on the organ weights and on testicular histology and biochemistry has been studied.

MATERIAL AND METHODS

Pubertal male albino rats of Wistar strain (140-160 gm body wt.) were used in the present investigation. They were housed in a well ventilated, temperature controlled ($28\pm 1^\circ\text{C}$) room with a 14 hrs light and 10 hrs darkness schedule. They were fed with standard rat balance pellet diet (Gold Mohur-Hindustan Lever Ltd., India) and drinking water was made available *ad libitum*.

The animals were divided into two sub-groups, Group-A served as control and received the vehicle only (peanut oil), and Group-B were given p-CPA (Sigma, USA), 100 gm/kg body wt/rat/day for 45 days intraperitoneally. All the animals were sacrificed 24 hrs after the last dose. The animals were weighed prior to sacrifice. The testes, epididymis, seminal vesicles and prostate were weighed immediately. For gross histological studies the testes were fixed in Bouin's fixative and 7 micron thick paraffin sections were stained with heamatoxylin and eosin.

The following biochemical estimations were carried out in the tissues of both controls and p-CPA administered rats.

Enzyme estimations :

Glucose-6-phosphate dehydrogenase (G6PDH- E.C. 1.1.1.49) : G6PDH was estimated by the method of King (11) using NADP as co-factor and Glucose-6-phosphate as substrate.

Succinic dehydrogenase (Succinate-TTC oxidoreductase, E. C. 1.1.3.99.1) : SDH was measured in the whole homogenate by the method of Nachlas *et al.* (15).

Lactate dehydrogenase (L-lactate : NAD oxidoreductase, E. C. 1.1.1.27) : LDH was assayed with lactate substrate by method of King (11).

Phosphohexose isomerase (D-Glucose-6-phosphate-ketol-isomerase, E.C. 5,3.1.9) : PHI was measured by the method of King (11).

Acid and alkaline phosphatase (Orthophosphoric monoester : phosphohydrolase, E.C. 3.1.3.1 and E.C. 3.1.3.2, respectively) : A modified method of Bessey *et al.* (3) and Andersch and Szczypinski (1) was used for the estimation of acid and alkaline phosphatase (ACP and ALP, respectively).

Adenosine triphosphatase (Adenosine-triphosphate phosphohydrolase, E.C.3.6.1.3) : The assay of this enzyme was carried out according to the method of Shiosaka *et al.* (19).

Glycogen : The method of Hassid and Abraham (9) was used for glycogen estimation.

Nucleic acids : Ribonucleic acid (RNA) and desoxyribonucleic acid (DNA) were extracted and estimated by the method of Schneider (16).

Protein : The total protein was determined by the Lowry method (13).

The data were statistically analysed using Student's 't' test.

RESULTS

Table I summarises the weight changes in the testes, epididymis, seminal vesicles and prostate after p-CPA treatment. There was no marked change in the body weight.

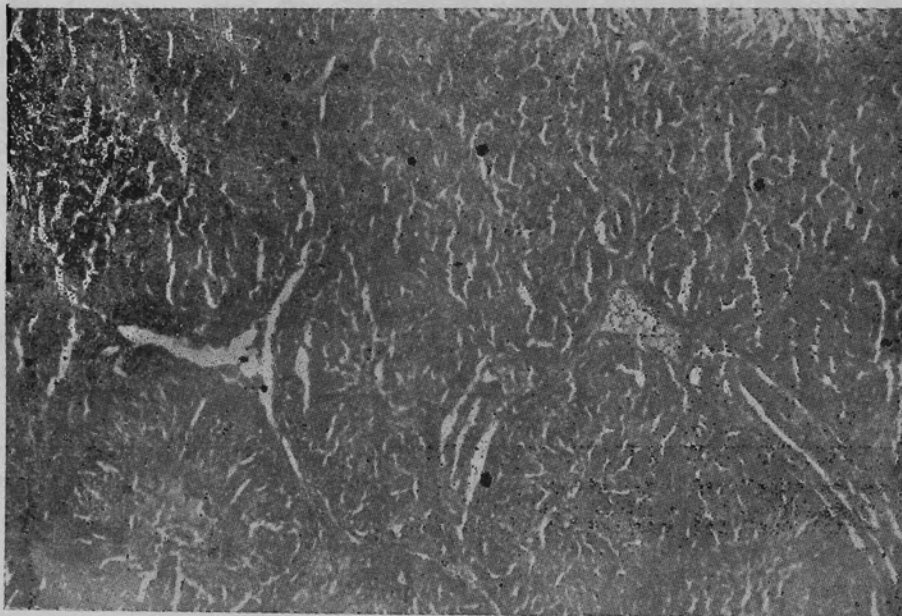


Fig. 1 : After p-Chlorophenylalanine treatment (p-CPA - 100 mg/kg body wt/day/rat for 45 days)

Note: Cytoplasmic degeneration, severe necrosis and obliteration of cellular contour. 350×H.E.

nor the weights of the testes, seminal vesicle and prostate. However, both caput and caudal segments of the epididymis showed marked reduction in their weights ($P < 0.05$) after p-CPA treatment.

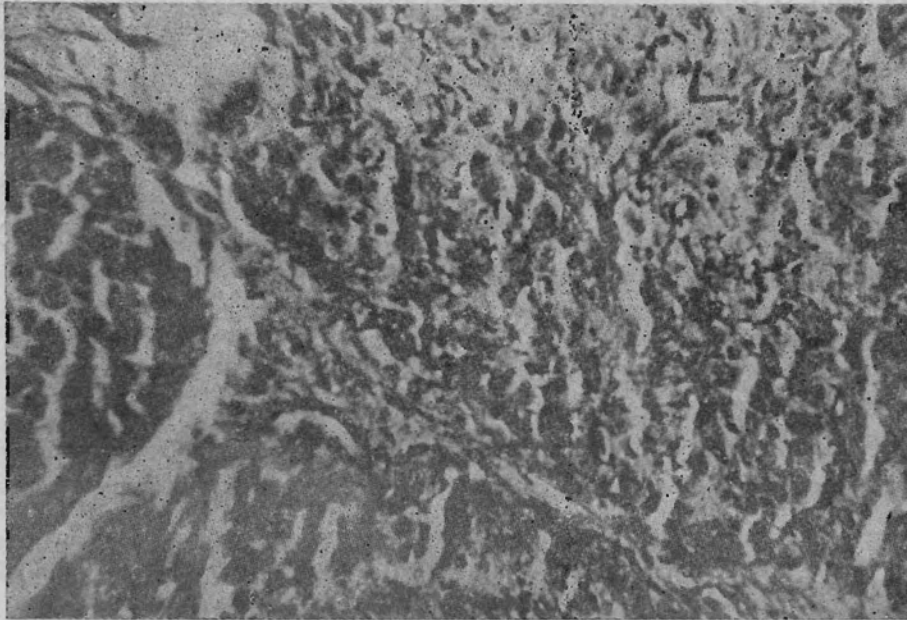


Fig. 2 : After p-Chlorophenylalanine treatment (p-CPA - 100 mg/kg body wt/day/rat for 45 days)

Note : cytoplasmic degeneration severe necrosis and obliteration of cellular contour.
1400 X. HE.

TABLE I : Changes in body weight, testicular, epididymal, seminal vesicular and prostatic weights after para-Chlorophenylalanine treatment@.

Groups	Body wt. (gm)	Testes (gm)	Epididymus (mg)		Seminal Vesicle (mg)	Prostate (mg)
			Caput	Cauda		
Control (5) (Vehicle treated)	195±4	1.493±0.076	231.80±12.80	173.16±10.72	278.00±25.24	197.20±14.01
p-CPA treated+ (8)	184±7	1.508±0.056	189.85±12.17*	139.40±19.52*	337.42±42.17	183.31±17.34

@ Each value is Mean ± S.E.M. Number of animals in each group is given in parenthesis. * $P < 0.05$.
+100 mg/kg body wt/day/rat for 45 days of p-Chlorophenylalanine treatment.

The histology of the testes revealed steady arrest in spermatogenesis in the drug treated animals (Fig. 1 and 2). There was desquamation of germinal epithelia and asynchronization of the different cell layers. The seminiferous tubules (ST) were reduced in size and diameter. The lumen of ST was congested with cell debris. There was a marked thickening of tunica albuginea and loosening of the layer at several places. The Leydig cells were not much affected. The interstitium was grossly oedematous. No cell in the ST is free from the necrotic effects of p-CPA. In controls, the spermatogenesis is normal with fully developed interstitial cells (Fig. 3 and 4).



Fig. 3 : Testes of control rat showing all stages of spermatogenesis. ST= seminiferous tubules; L = lumen 350X. HE.

Table II depicts the alterations in the biochemical parameters of the testes after p-CPA treatment. The activities of the dehydrogenase enzymes like SDH ($P < 0.001$) and G6PDH ($P < 0.01$) were markedly elevated compared to the controls. While the activity of acid phosphatase was increased markedly ($P < 0.001$) that of ALP was decreased ($P < 0.01$) after the drug treatment. An increase in glycogen was seen in the testes of these animals. Similarly, the ATP-ase enzyme activity was stimulated ($P < 0.001$). While LDH and PHI enzymes were reduced ($P < 0.01$), the nucleic acids were unaltered compared to controls.

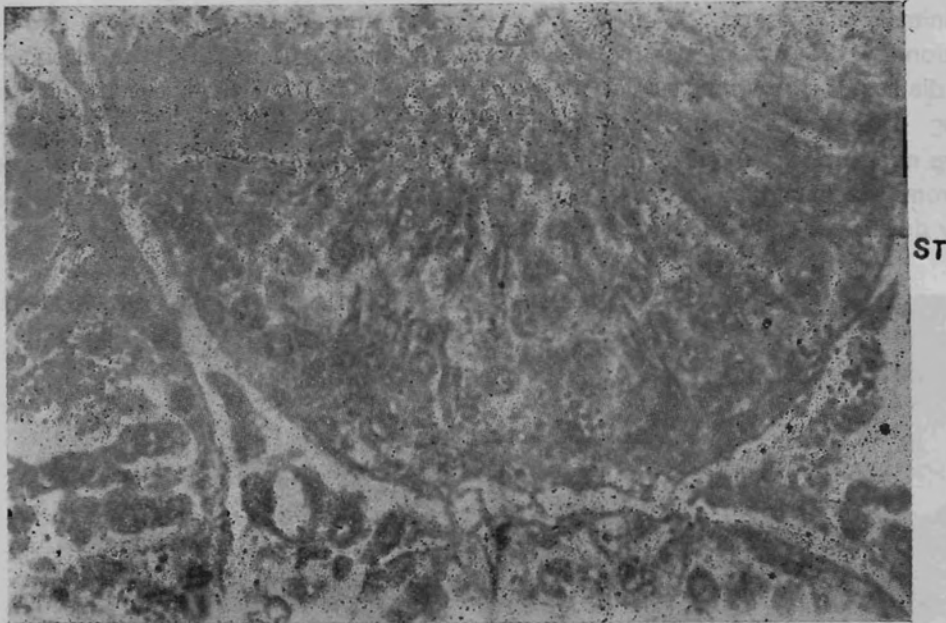


Fig. 4 : Testes of control rat showing all stages of spermatogenesis. ST= seminiferous tubules;
L = lumen 1400 X. HE

TABLE II : Levels of Nucleic acids, protein, glycogen and enzymes in the testes of control and p-CPA treated rats @.

Biochemical parameters	Control (vehicle)	p-CPA treated	P. value less than
SDH (μ moles of formazan/g/hr)	27.50 \pm 2.81	67.50 \pm 4.24	0.001
GG6PDH (m.I.U./mg protein)	56.48 \pm 7.65	84.28 \pm 4.42	0.05
LDH (m.I.U./mg protein)	364.17 \pm 39.72	103.50 \pm 11.46	0.001
PHI (m.I.U./mg protein)	99.19 \pm 8.54	43.82 \pm 4.07	! 0.01
Acid Phosphatase (μ moles/hr/gm tissue)	3.15 \pm 0.47	16.00 \pm 1.88	0.001
Alkaline Phosphatase (μ moles/hr/gm tissue)	39.62 \pm 6.08	3.52 \pm 0.43	0.001
ATP-ase (μ g Pi liberated/15 min at 37°C/ 100 mg tissue)	88.00 \pm 4.75	136.10 \pm 9.02	0.001
Glycogen (μ g/100 gm tissue)	46.65 \pm 4.77	76.44 \pm 5.81	0.001
Protein (μ g/100 mg tissue)	4733.59 \pm 713.28	4256.78 \pm 514.89	NS
Desoxyribonucleic acid (μ g/100 mg tissue)	66.50 \pm 6.48	60.24 \pm 1.52	NS
Ribonucleic acid (μ g/100 mg tissue)	1799.83 \pm 57.72	1619.40 \pm 148.60	NS

@ Each value is Mean \pm S.E.M. Controls=5 animals; p-CPA treated=8 rats. p-chlorophenylalanine was administered at 100 mg/kg body wt/day/rat/45 days.

DISCUSSION

p-Chlorophenylalanine brought about significant decrease in the weights of caput; and cauda epididymis alone with no change in the weights of the testes or other accessory sex organs. This decrease in epididymal weights could be due to the fact that no sperms or fluid is being pumped into the epididymis consequent to arrest of spermatogenesis by the drug.

Changes in testicular histology after p-CPA administration bear testimony to the deleterious potentialities of p-CPA. Probably p-CPA brings about this effect by a direct action on the testes interfering with the production of spermatozoa by acting directly on the germ cells. As the accessory sex organs weight point to normal androgen secretion, the Leydig cell function appears not to be adversely affected.

The biochemical observations, also in confirmation with the histological picture, indicates a direct interference of the drug with the testicular metabolism. SDH is one of key mitochondrial enzymes, whose level reflects the metabolic activity of any tissue. In the present study, p-CPA enhanced SDH levels significantly thereby indicating the increase in oxidative metabolism of the tissue. Similar increase in SDH has been reported after x-irradiation in the testes of Guinea pigs (17). Perhaps it may be the result of a protective mechanism of the animal in response to p-CPA treatment.

In rat testis, acid phosphatase has been shown to be present in lysosomes of Sertoli cells, spermatocytes and spermatozoa (14). Increase in acid phosphatase activity in the testes of p-CPA treated animals coincides with decrease in sperm concentration and appears to be due to cytolytic activities in these cells leading to probable disruption of lysosomal membranes and liberation of ACP for phagocytosis (14). ALP is known to be linked with cleave of phosphate esters of monosaccharides and also with exchange of substances between the cells. In this study, p-CPA by inducing a drastic decrease in ALP activity might have slowed down the transport mechanisms and decreased the energy metabolism in the testes. It may be possible that a mild suppression of testicular androgens, which are actively associated with the activities of these enzymes in the testes and other accessory sex glands (5), might have also caused a decrease in the activities of these enzymes. In fact, Aoki and Fawcett (2), have shown a hyporesponsiveness in Leydig cells caused by factor(s) released from the damaged seminiferous tubules.

The p-CPA induced accumulation of glycogen is probably the result of inhibition of glycolysis with normal glycogenesis. A decrease in glycolysis is possible for, both PHI

and LDH, enzymes of glycolytic pathway, are markedly inhibited by the drug treatment. Furthermore, the decreased LDH might result in accumulation of lactate, thus suggesting a decrease in cellular respiration (6). However, the p-CPA treatment enhanced G6PDH activity indicating the activation of the pentose phosphate pathway probably for nucleic acid synthesis or generation of NADPH₂ for lipid synthesis. As the levels of both RNA and DNA are unaltered it may suggest that both synthesis and degradation of these parameters might be occurring in the same ratio.

Thus, p-CPA appears to adversely affect the histology as well as the biochemistry of the testes of rat.

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