Original Research Article

IJCRR
Section: Healthcare
Sci. Journal
Impact Factor
4.016

# EFFECT OF ISOPROTURON TOXICITY ON TESTIS OF ALBINO RATS

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## ABSTRACT

**Objectives:** As pesticides hold a unique position among environmental contaminants; the objective of the present study was to evaluate the toxic effects of Isoproturon, a substituted phenylurea herbicide on testis of albino rats.

**Methods:** The compound, suspended in groundnut oil, was administered orally to rats for 60 days at the doses of 400 mg/kg and 800 mg/kg body weight for two groups respectively. Body weight, testis weight and seminiferous tubular diameter were measured. Histological study was done on haematoxylin- eosin stained 5µm thick paraffin sections.

**Results:** Decrease in weight gain and seminiferous tubular diameter was observed in experimental rats which was highly significant. The difference in testis weight was not significant statistically in experimental group as compared to control group. Light microscopic study showed degenerative changes in the testis.

**Conclusions:** Degenerative changes in the gametogenic cells indicate adverse effects on the functional ability of these cells and are highly conclusive of reproductive toxicity caused by isoproturon.

**Key Words:** Isoproturon, Testis, Seminiferous tubules, Leydig cells

## INTRODUCTION

Exposure to pesticides can range from mild skin irritation to birth defects, tumors, genetic changes, blood and nerve disorders, endocrine disruption, and even coma or death.[1] Developmental effects have been associated with pesticides. Recent increases in childhood cancers in throughout North America, such as leukemia, may be a result of genotoxic and non-genotoxic pesticides due to somatic cell mutations.[2] Insecticides targeted to disrupt insects can have harmful effects on the nervous systems of mammals, due to basic similarities in system structure. Both chronic and acute alterations have been observed in those who are exposed. Pesticides can act in the promotion and proliferation of cancer while causing hormone imbalance. DDT and its breakdown product DDE, with levels still present in the environment, despite its ban, are known to disturb estrogenic activity and possibly lead to breast cancer. Exposure to pesticides, for example DDT, in fetal stages has been proven to alter male penis size in animals to that much smaller than average as well as develop undescended testicles. Exposure to pesticides may occur in postnatal early stages of development, in utero, and even if either parent was exposed before conception took place. Reproductive disruption has the potential to occur by chemical reactivity and through structural changes to a system.[3]

Of the potential health risks associated with exposure to chemical or physical agents, a prominent concern is that these agents may interfere with the ability of individuals to produce normal and healthy children. Concern about the susceptibility of the male reproductive system to drugs or environmental agents has assumed an increasing extent. A large number of toxic chemicals in the environment are known to interfere with the endocrine system. Sexual development during the prenatal and neonatal period is under hormonal control and is therefore sensitive to exogenous substances with an endocrine effect. The present work primarily focuses on toxic effects that involve testicular and spermatogenic processes that are essential for reproductive success. Occupational or experimental exposure to herbicides alters the testicular steroidogenic function resulting in spermatogenic failure and decreased male fertility. Isoproturon is a substituted phenylurea [3-(4-isopropylphenyl)-1,1-dimethylurea], a herbicide possessing broad spectrum activity was taken to

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**Received:** 29.04.2016 **Revised:** 13.05.2016 **Accepted:** 30.05.2016

see its toxic effects on testis of albino rats. Literature suggests that very few studies have been done on testicular toxicity of isoproturon while recently it is widely used because of its broad spectrum activity. Therefore, the present study is a step to fill this lacuna.

## MATERIAL AND METHODS

The present study was conducted in the Department of Anatomy, King George's Medical University, Lucknow, Uttar Pradesh, India. Twenty four male albino rats weighing 50-80 g were used for the present experiment. Animals were obtained from animal house of Indian Institute of Toxicology & Research, Lucknow. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in stainless steel cages at temperature 22±3°C and relative humidity 30–70%.[4] Light dark cycle (photoperiod) was controlled between 8.00 a.m. to 5.00 p.m. They were fed on the standard pellet diet and tap water ad libitum. Animal care was as per Indian National Science Academy (INSA) guidelines for Care and Use of Animals in Scientific Research.[5] After acclimatization for 2 weeks in laboratory conditions, animals were divided into 3 groups of 8 rats each. Group-1 served as control. Group 2 & 3 received 400 mg and 800 mg isoproturon/ Kg body weight respectively in 0.2 ml of groundnut oil orally, 6 days/week for 60 days. Body weight of all the rats i.e. control and treated were taken on day zero, at every 10 days interval and at the end of experiment (day 60) with the help of weighing machine. All treated rats along with their controls were anaesthetized by intraperitoneal administration of Nembutol (30 mg/Kg body weight). Fixation of testis was achieved by in- vivo perfusion with Bouin's fixative. After completion of the perfusion, the scrotum was skinned and an incision was given with the scalpel and forceps. The testis was isolated from the scrotum and weighed. Testis was cut into small pieces and kept in Bouin's fluid for 24-48 hours. Processing of tissue was done for making paraffin blocks. 5µm thick sections were cut with a sharpen knife on a rotary microtome. The sections were adhered on clean glass slides smeared with a thin layer of Mayer's egg albumin. The slides were then dried in an incubator and staining was done with haematoxylin and eosin (H&E).

Quantitative histopathology was done with the help of micrometer. It was done to know the alterations in the diameter of seminiferous tubules. Total number of tubular cross-sections was counted in 3-5 fields/testis at a magnification of 10x40 and testicular damage was assessed by determining the percentage of damaged tubules against the total tubules. Approximately, 20 circular cross-sectioned seminiferous tubules were randomly selected and two transverse diameters of each tubule was measured in order to calculate the mean tubular diameter.

Statistical analysis was done by Student's t test. The difference was considered statistically significant, if the P value was $< 0.05$.

## OBSERVATIONS AND RESULTS

### Appearance

Rats treated with isoproturon appeared to be dull, drowsy, emaciated and weak.

### Body weight

The mean body weight (g) of 8 rats in control group was found to be increased from 59.38±6.41 at zero day to 73.50±7.25 after 60 days. Rats treated with low dose of isoproturon exhibited less weight gain as compared to control and the value was highly significant. It was 70.50±7.09 at day zero while 78.28±6.93 after 60 days. The mean body weight of rats in high dose group increased from 71.13±5.14 to 72.26±5.34 at day zero and after 60 days respectively. Weight gain was very less and was highly significant as compared to control and low dose group (Table 1, Figure 1).

**Table 1: Body weight (g) in control and isoproturon treated rats during the course of 60 days**

| Group | Days | Mean±SD | Change in Mean±SD | % Weight gain | t value | p value |
|---|---|---|---|---|---|---|
| Control | 0 | 59.38±6.41 | - | - | - | - |
| | 60 | 73.50±7.25 | 14.13±2.23 | 23.79 | 17.90 | p<0.001* |
| Isoproturon (low dose) | 0 | 70.50±7.09 | - | - | - | - |
| | 60 | 78.28±6.93 | 7.76±2.10 | 11.10 | 10.46 | p<0.001* |
| Isoproturon (high dose) | 0 | 71.13±5.14 | - | - | - | - |
| | 60 | 72.26±5.34 | 1.14±0.78 | 1.60 | 4.12 | p<0.001* |

p value<0.001* is highly significant

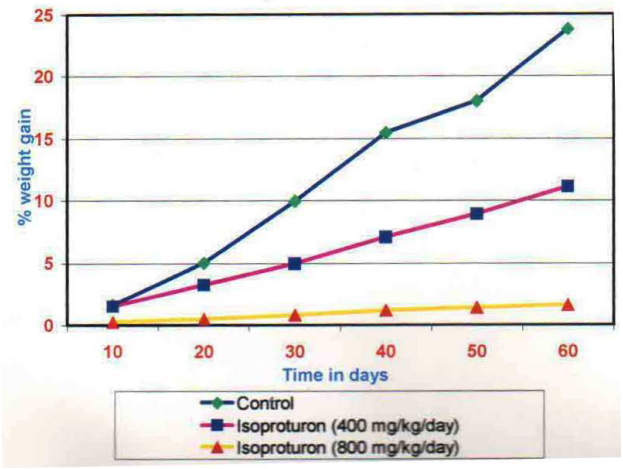


**Figure 1:** Graph showing % weight gain in control and isoproturon treated rats

## Testis weight

One testis of each rat was weighed and the mean testis weight (g) of 8 rats after 60 days in control group was 1.15±0.06, in isoproturon low dose group 1.10±0.04 and in high dose group 1.11±0.05. The difference in testis weight was not significant statistically in experimental group as compared to control group (Table 2).

**Table 2: Weight of testis of rats following oral administration of isoproturon for 60 days**

| Group | Weight of testis (g) Mean±SD | t value | p value |
|---|---|---|---|
| Control | 1.15±0.06 | - | - |
| Isoproturon (low dose) | 1.10±0.04 | 1.68 | p>0.05 |
| Isoproturon (high dose) | 1.11±0.05 | 1.34 | p>0.05 |

## Seminiferous tubular diameter

The mean seminiferous tubular diameter (µm) of 8 rats in control group was 247.35±10.79, in low dose group 219.31±7.55 and in high dose group 176.65±7.08 after 60 days. The value decreased in experimental groups as compared to control and was highly significant statistically (Table 3).

**Table 3: Effect of isoproturon treatment on seminiferous tubular diameter (µm) after 60 days**

| Group | Seminiferous tubular diameter (µm) Mean±SD | t value | p value |
|---|---|---|---|
| Control | 247.35±10.79 | - | - |
| Isoproturon (low dose) | 219.31±7.55 | 4.26 | p<0.001* |
| Isoproturon (high dose) | 176.65±7.08 | 10.95 | p<0.001* |

p value<0.001* is highly significant

## Histopathology

Light microscopic study of testis of control rats exhibited seminiferous tubules cut in various planes of sections. The seminiferous tubules were lined by stratified cuboidal epithelium which consists of cells in various stages of spermatogenesis collectively referred to as cells of spermatogenic series. Next to basement membrane lie the spermatogonia, having spherical nuclei. Non-spermatogenic cells, called Sertoli cells having characteristically ovoid shaped nucleus was seen towards the basement membrane. In the interstitial spaces, Leydig cells having endocrine function and connective tissue cells were present (Figure 2).

Testis of isoproturon treated animals revealed distorted seminiferous tubules, decreased diameter of seminiferous tubules, depressed spermatogenesis, loss of sperms, degeneration and desquamation of gametogenic cells from the basement membrane, accumulation of cellular mass in the lumen of tubules, oedematous exudation in the interstitial spaces which was more marked in the central region, vacuolation in the interstitium was also evident, some of the Leydig cells were showing signs of degeneration (Figures 3-5). All these findings were much significant with high dose of isoproturon.

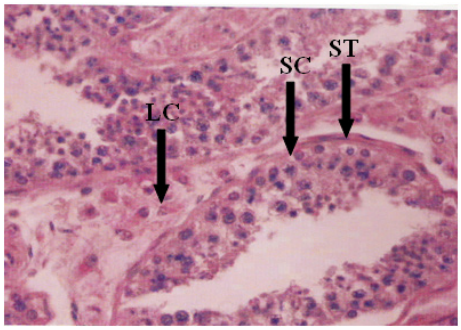


**Figure 2:** Photomicrograph of testis of control rat (H&E, x400) showing seminiferous tubule (ST), Sertoli cell (SC) and Leydig cells (LC).

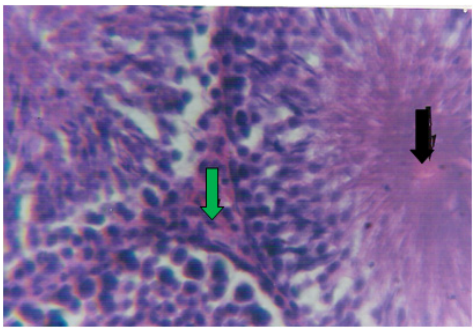

**Figure 3:** Photomicrograph of Isoproturon treated (H&E, x400) showing central cellular mass (black arrow) and degenerated Leydig cells (green arrow).

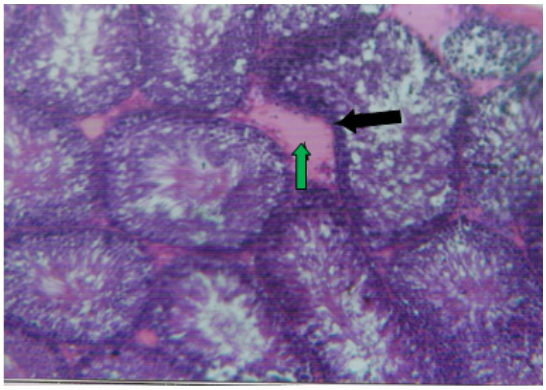

**Figure 4:** Photomicrograph of testis of Isoproturon treated rat (H&E, x400) showing distorted seminiferous tubule (black arrow) and interstitial oedema (green arrow).

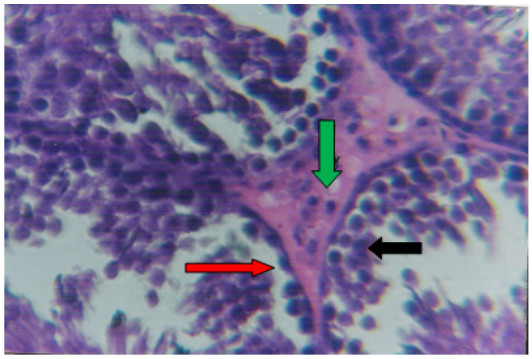

**Figure 5:** Photomicrograph of Isoproturon treated group (H&E, x400) showing vacuolation in oedematous interstitial space (green arrow), depressed spermatogenesis (black arrow) and desquamation of gametogenic cells (red arrow).

## DISCUSSION

Rats treated with Isoproturon appeared to be dull and drowsy. Dikshith et al (1990) noted mild to moderate toxic effects which was more pronounced in male rats.[6] Sarkar and Gupta (1993) studied the effect of isoproturon on pregnant rats and their offspring. Dose-related depression and drowsiness of pregnant rats along with decreased maternal body weight, litter size, foetal weight, crown-rump and trans-umbilical lengths were observed.[7] In the present study also, the rats appeared to be emaciated which runs parallel with the finding of Sarkar et al (1995). Emaciation of rats may be due to direct cumulative toxicity of the pesticide.[8] None of the rat was found to be dead in the present experiment, thereby showing that these doses were not lethal.

Isoproturon significantly decreased the body weight of rats at both low and high dose in the present study. Sarkar et al (1995) also noticed significant decrease in weekly body weight of rats at high dose (800 mg/kg).[8] The cause for this loss in weight gain may be due to their organ directed toxicities. Loss of appetite during experimental period also explains the loss in body weight.

Isoproturon did not alter the testicular weight even with the highest dose as revealed by the study of Sarkar et al (1995).[8] The difference in testis weight was not significant statistically in experimental group as compared to control group in the present study also. No alteration in weight of testis can be related to the damage to the testis may be detected only at doses higher than those required to produce significant effects in other measures of gonadal status. In his study, reduction in weight of epididymis was noticed which was explained that it was due to decrease in sperm reserve.

Seminiferous tubular diameter was decreased in experimental groups as compared to control and was highly significant statistically in the present study. Sarkar et al (1997) also observed that the mean diameter of seminiferous tubules was diminished by 17 and 37% with medium (400 mg/kg) and the highest doses (800 mg/kg), respectively, over the mean values of control rats, but this diminution was significant only at the highest dose level. Consequently, the number of tubular cross-sections per microscopic field was increased. Concomitant and significant increase in the percentage of damaged tubules was also detected. There was evidence of significantly decreased number of tubules with proper spermatogenesis.[9] The possible cause for decrease diameter of tubules may be attributed to oedema in the interstitial spaces which compresses the nearby tubules and atrophy of the tubules.

Marked histopathological changes in the present study was noticed. Distorted seminiferous tubules, depressed spermatogenesis, loss of sperms, degeneration and desquamation of gametogenic cells from the basement membrane, accumulation of cellular mass in the lumen of tubules, oedematous exudation in the interstitial spaces which was more marked in the central region and vacuolation in the interstitium was evident. Some of the Leydig cells were showing signs of degeneration. All these findings were much significant with high dose of isoproturon. These findings run parallel with those noted by Sarkar et al (1995).[8] The exact mechanism through which isoproturon induced these alterations in spermatogenesis is not clear. There is a possibility that all these effects of isoproturon on both testicular and epididymal components are the result of appearance of the factor (s) from the peripheral circulation due to hepatic and/or renal toxicity of the agent. Hepatic malfunction and renal failure are known to influence testicular functions.[10] Lox (1984)[11] and Kiran et al (1985)[12] stated that malfunctioning of liver and kidney causes general systemic toxicity which alter testicular functions. According to Sarkar et al (1997), high dose of isoproturon cause impairment of androgen biosynthetic process which affects spermatogenesis and induces maturational anomalies

of sperm cells. It decreased epididymal sperm count and percent of motile sperms. The seminiferous tubular diameter was also reduced. Leydig cells maintain high concentration of testosterone in the testicular fluid. Therefore, degenerative changes in them indicate some adverse effects on the functional ability of these cells to synthesize testosterone.

## CONCLUSION

The present study indicates the potential of isoproturon to affect the function, fertilizing capability and survival of spermatozoa. Therefore, correlative ultrastructural, histochemical and quantitative biochemical studies are likely to enable further insight into this interesting area of research.

## ACKNOWLEDGEMENTS

I express my gratitude to the staff of the Department of Anatomy for assistance in providing infrastructure facilities and necessary help. A special appreciation to my head of the Department for his suggestions and constant encouragement in fulfilling this task.

Authors acknowledge the immense help received from the scholars whose articles are cited and included in references of this manuscript. The authors are also grateful to authors / editors / publishers of all those articles, journals and books from where the literature for this article has been reviewed and discussed.

**Source of Funding:** Nil

**Conflict of interest:** All authors have none to declare.

**ABBREVIATIONS USED:** DDT- Dichloro-diphenyl-trichloroethane, DDE- Dichloro-diphenyldichloro-ethylene, INSA- Indian National Science Academy, IAEC- Institutional Animal Ethical Committee, H&E- Haematoxylin and eosin, ST- Seminiferous tubule, SC- Sertoli cell, LC- Leydig cells

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