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Biochemical and Reproductive Impacts of Profenofos in Male Rats: Evidence of Natural Recovery Without Intervention

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Abstract: *Profenofos, an organophosphate pesticide, is known for its deleterious effects on the male reproductive system, primarily through oxidative stress and endocrine disruption. This study aimed to evaluate both the biochemical and reproductive toxicity induced by profenofos and the potential for spontaneous recovery in male rats without therapeutic intervention. Adult male Wistar rats were administered profenofos (25 & 50 mg/kg body weight) orally for 45 days, followed by a 45-day recovery period without treatment. Profenofos exposure significantly reduced serum testosterone levels, total protein, and antioxidant enzymes (SOD and GSH), while increasing lipid peroxidation (LPO). Sperm count, motility, and viability were also markedly impaired. After 45 days of withdrawal, partial but significant recovery was observed in most biochemical parameters, including a non-significant recovery of antioxidant enzyme levels. Testosterone levels and sperm quality showed non-significantly improvement, indicating the capacity of the reproductive system for natural recovery over time. However, full restoration to baseline values was not achieved. These findings suggest that while profenofos induces significant reproductive toxicity, endogenous repair mechanisms can mediate partial recovery in the absence of treatment, highlighting the resilience and limitations of natural physiological repair processes.*

Introduction

Indian agriculture, shaped by climate and topography, features unique cropping patterns across Kharif and Rabi seasons. While chemical pesticides and fertilizers boosted yields post-independence, overuse has harmed ecosystems and health. Biofertilizers and IPM offer sustainable alternatives. Despite low per-hectare usage, India leads in pesticide production. Recent trends focus on organic farming and reducing pesticide reliance to ensure food security and environmental safety. Pesticides are chemical agents used to control pests like insects, weeds, fungi, and rodents, protecting crops and improving yields. They include insecticides, herbicides, fungicides, and more. Though effective, improper use can harm human health and the environment, causing acute poisoning, chronic diseases, and ecological damage. Responsible application and sustainable alternatives are vital for long-term agricultural safety and productivity.

Improper use of organophosphate (OP) pesticides adversely affects male reproductive health, causing reduced sperm count, motility, abnormal morphology, and hormonal imbalances such as decreased testosterone and increased FSH and LH. [1] Studies confirm these findings across various OPs like chlorpyrifos, malathion, and methyl parathion. Factors like education, PPE usage, and pesticide practices influence outcomes. [2] Histological damage and organ weight loss were observed in animal models. Despite similarities with prior research, variations in study design, pesticide use patterns, and environmental conditions affect results. Lifestyle and occupational factors further complicate assessing OP impact on reproductive hormones and fertility across different populations and time periods.

Profenofos, a toxic organophosphate pesticide, is widely used in agriculture but poses serious health and environmental risks. It inhibits acetylcholinesterase, affecting neural function in pests and non-target species, including humans.[3] Linked to reproductive toxicity, profenofos can impair fertility, alter hormones, and damage organs. Due to its hazards, many countries are phasing it out.[4] Reproductive toxicity is one of the harmful health effects of exposure to organophosphate pesticides (OPs). The disruption of the normal functioning of the male and female reproductive systems due to OPs can lead to infertility, reduced fertility and abnormal hair growth.[5] Several studies have proven the toxicity of OPs to reproduction. For example, a study reported that male agricultural workers exposed to OPs had decreased sperm motility and count.[6] Abnormal menstrual cycles and reduced fertility have also been reported in women exposed to OPs.[7] In addition to affecting fertility, exposure to OPs during pregnancy can have harmful effects on the developing fetus. One study found that OP treatment during pregnancy resulted in reduced fetal weight and skeletal malformations in children.[8] These effects are believed to be due to OPs' ability to disrupt normal hormonal functions, particularly the function of the endocrine system.[5] All things considered; it is clear that exposure to OPs can have harmful effects on reproductive health. To minimize the risk of reproductive toxicity and other health problems associated with exposure to OPs, it is imperative to follow proper safety procedures when handling these compounds, including wearing protective gear and clothing and properly disposing of waste.[5] DDT can be replaced with toxic pesticides such as OPP

Organophosphorus Pesticides

More than 200 organophosphates (OPs) are extensively used in domestic pest management, agriculture and public health.[10] However, they present serious health hazards, especially in terms of reproductive damage. Mammals exposed to OPs may experience infertility, abortion, delayed puberty, impaired gamete production, altered sexual behavior and premature reproductive aging.[11,12] Research on Wistar rats shows that the weight of the prostate gland, epididymis and seminal vesicles decreased after long-term exposure to methyl parathion.[13,14] Other OPs such as profenofos, chlorpyrifos and malathion have also been shown to have comparable effects.[15-19] According to population research, OP treatment is associated with a higher incidence of dementia, non-Hodgkin's lymphoma and male reproductive dysfunction.[20,21] [22–24] Prenatal exposure has been linked to shorter life expectancy and neurological problems in children. [25–26]

In agriculture, a pesticide called profenofos is widely used to control pests on crops such as vegetables, cotton, maize, soybeans and corn. In agriculture, a pesticide called profenofos is widely used to control pests on crops such as vegetables, cotton, maize, soybeans and corn. Profenofos is an organophosphate pesticide that is hazardous to humans if eaten, inhaled or absorbed through the skin. Profenofos enters the body and is quickly processed and excreted, primarily through the liver and kidneys. Metabolites of profenofos, particularly 3,5,6-trichloro-2-pyridinol (TCP), are eliminated in the urine. The World Health Organization (WHO) classifies profenofos as a highly hazardous (toxicity class II) pesticide with moderate acute toxicity following oral and skin application. (WHO 2004) The acute hazardous action of profenofos is inhibition of acetylcholinesterase activity, which causes toxicity in humans.[**27**] Chronic exposure to profenofos can cause TCP and other metabolites to accumulate in the body, which can lead to long-term health consequences such as nerve damage, liver and kidney damage, and cancer.[28] Profenofos exposure can affect the anatomy of the testes, reducing sperm motility and count in male animals.[29] Exposure to profenofos can cause the spermatic canal to shrink, reducing sperm quantity and quality. Profenofos is known to be toxic to the spermatic canal, which are small, coil-like tubes that produce sperm cells in the testes.[30] Sertoli cells are specialized cells found in the testes that support the nutrition and structural development of sperm cells.[31] Decreased testosterone levels may prevent male animals exposed to profenofos from reproducing. Studies show that exposure to profenofos may reduce the activity of enzymes needed to produce testosterone, thereby

reducing the amount of testosterone produced.[32] Low testosterone levels can lead to a number of health problems specific to men, including erectile dysfunction, decreased libido, and decreased fertility.[33] In addition to reproductive health, decreased testosterone levels can also affect bone health, muscle mass, and general energy levels.

Materials and Methods

The excessive use of pesticides in contemporary farming has boosted crop production but also produced serious environmental and health consequences. Millions of cases of pesticide poisoning occur each year, with long-term effects such as cancer, neurological disorders, diabetes, respiratory diseases, and reproductive disorders. Farmers and workers in developing countries are frequently exposed to pesticides, causing acute effects such as headaches, vomiting, and seizures, as well as chronic diseases such as asthma, endocrine disruption, and birth defects. Pesticide residues in food pose an even greater threat to consumers' health. Given the growing global public health concerns, it is important to address pesticide-related diseases. [31, 32]

Test Compound Used

- **Pesticides:** An organophosphate insecticide called profenofos is used to manage pests in both public health and agriculture. It inhibits acetylcholinesterase, disrupting nerve function in insects but also harming birds, mammals and humans. First approved in the US in 1982, it has been widely used on crops such as maize, soybeans and cotton. However, due to its high toxicity and environmental persistence, it is being phased out in many countries. Profenofos negatively affects male fertility by altering essential trace elements and enzymes involved in spermatogenesis. Its structure allows for modifications, creating various organophosphate derivatives with different toxic properties. [33]
- **IUPAC Name of Profenofos:** (O-4-bromo-2-chlorophenyl O-ethyl S-propyl phosphorothioate)
- **Molecular formula:** $C_{11}H_{15}BrClO_3PS$

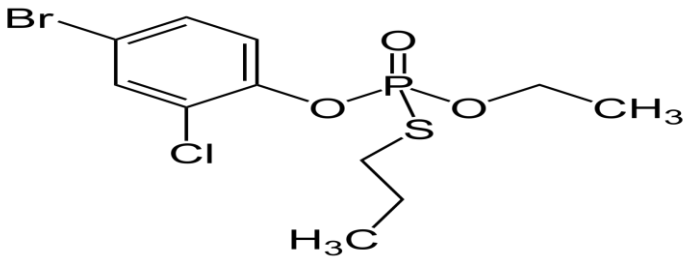

Figure: Profenofos Structure

We are investigating how male reproductive toxicity of albino mice is affected by profenofos. Although profenofos affects the body and reproductive system in many ways, we are focusing on how it specifically affects male reproductive health. In addition, we are examining the rate of natural recovery 45 days after exposure to both low and high pesticide doses.

Animal Model

For the experiment, healthy adult male albino rats (Rattus norvegicus) weighing 150–200 g will be used. Clean polyporphylene cages with chrome-plated grills will house the animals. The animals will mostly be fed a normal pellet diet that is purchased from Ashirwad Industries in Chandigarh. As an alternate feed, they will also occasionally be fed gram and wheat seeds that have germinated or sprouted. Throughout the experiment, they will have unlimited access to clean water. The rats will receive antibiotics when they become infected.

- **Doses:** The mice were orally treated with a low dose (25 mg/kg body weight/day) and a high dose (50 mg/kg body weight/day) of Profenofos.
- **Observation:** During a period of 45 days, the present study analyzed the reproductive toxicity of profenofos at doses of 25 and 50 mg/kg b.w.t. Vital rat organs, including the male reproductive system, were negatively affected in a dose-dependent manner. The toxicological effects of profenofos were minor at low doses but severe at high doses.

Result

Biochemical Findings

- **Serum Analysis**

Alanine Aminotransferase (ALT) and Aspartate Aminotransferase (AST) (Table: 1)

Rats in groups II and III showed a significant (P≤0.05) (P≤0.01) increase in both alanine and aspartate aminotransferase activity following a 45-day treatment with profenofos. Rats at both dose levels showed a non-significant decrease in alanine and aspartate aminotransferase activity when allowed to recover naturally (Groups IV & V).

Table 1

Treatment	Group	Alanine amino Transferase (ALT)	Aspartate amino Transferase (AST)	Acid phosphatase	Alkaline phosphatase	Bilirubin
		Units/ml		KA Units		mg%
Control (received vehicle olive oil only)	**GI**	127.20 ±3.79	70.55 ±2.00	4.61 ±0.47	63.32 ±2.21	0.27 ±0.09
25mg/kg b.wt./day of Profenofos	**GII**	153.63* ±5.15	88.65* ±4.34	6.38* ±0.57	52.51* ±2.30	2.56* ±0.51
50mg/kg b.wt./day of Profenofos	**GIII**	166.25** ±7.14	94.21** ±2.78	7.18* ±0.26	45.28* ±2.84	2.85* ±0.34
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	145.28 ns ±4.76	82.93 ns ±4.81	5.82 ns ±0.71	53.14 ns ±3.42	2.51 ns ±0.34
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	157.46 ns ±4.68	89.85 ns ±5.72	6.38 ns ±0.24	47.53 ns ±2.69	2.79 ns ±0.59

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Acid phosphatase and Alkaline phosphatase (Table: 1)

In groups II and III of rats treated to profenofos, there was a significant (P≤0.05) increase in acid phosphatase activity and a significant (P≤0.05) decrease in alkaline phosphatase activity. Groups IV and V, which were allowed to recover naturally at both dose levels, showed a non-significant drop in acid phosphatase levels and an increase in alkaline phosphatase levels.

Bilirubin (Table: 1)

When compared to controls, the bilirubin levels rose significantly (P≤0.05) in both profenofos-administered groups. Rats at both dosing levels showed a non-significant drop in blood bilirubin levels when allowed to recover naturally (Groups IV & V).

Total Protein (Table: 2)

Compared to control rats, there was a significant (P≤0.05) (P≤0.01) increase in protein content in both groups that received profenofos. Rats at both dosing levels showed a non-significant drop in blood total protein levels when allowed to recover naturally (Groups IV & V).

Table 2

Treatment	Group	Total Protein	Phospholipid	Triglyceride	Total Chol.
		mg/ dl			
Control (received vehicle olive oil only)	**GI**	18009.20 ±564.34	143.70 ±3.81	91.66 ±2.49	96.33 ±2.58
25mg/kg b.wt./day of Profenofos	**GII**	23983.62* ±1122.93	223.40** ±6.45	130.67** ±4.73	135.58** ±5.46
50mg/kg b.wt./day of Profenofos	**GIII**	30212.22** ±764.41	244.89** ±7.36	152.67** ±5.56	160.38** ±7.31

25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	21887.80 ns ±856.43	184.68* ±5.95	111.55* ±3.68	117.25 ns ±3.14
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	25780.80 ns ±856.43	204.68* ±5.95	137.55* ±3.68	131.25 ns ±3.14

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Phospholipid (Table: 2)

A significant (P≤0.05) (P≤0.01) increase in serum phospholipid levels was seen following 45 days of administering 20 and 30 mg/kg b.wt./day doses of profenofos. Serum phospholipid levels significantly (P≤0.05) decreased in rats (Groups IV & V) when they were allowed to recover naturally at both dosing levels.

Triglyceride (Table: 2)

Rats treated to profenofos exhibited a marked increase in blood triglyceride levels at both dosage levels when compared to control animals. Triglyceride levels in serum significantly (P≤0.05) decreased in rats allowed to recover naturally at both dose levels (Groups IV & V).

Total cholesterol (Table: 2)

Rats treated to profenofos had significantly higher serum cholesterol levels (P≤0.05) (P≤0.01) than the control groups. Rats at both dosing levels showed a non-significant drop in serum total cholesterol levels when allowed to recover naturally (Groups IV & V).

Tissue Biochemistry

- **Glycogen**

Testes (Table: 3)

Rats treated to profenofos showed a substantial decrease in testicular glycogen (P≤0.01) at both dosage levels. At both doses, the decrease was 73.18% and 68.96%, respectively, in comparison to control rats. The increase (P≤0.05) in testicular glycogen levels was 62.96% and 71.42% only (Groups IV & V) when rats were allowed to recover naturally at both treatment levels.

Heart (Table: 3)

Rats given profenofos exhibited a significant (P≤0.01) decrease in cardiac glycogen levels in both groups as compared to control animals. Glycogen levels in the heart of rats (Groups IV & V) increased little (P≤0.05) when they were allowed to recover naturally at both treatment levels.

Liver (Table: 3)

Rats given profenofos demonstrated a significant (P≤0.01) decrease in the liver's glycogen level in both toxicated groups. The amount of glycogen in the heart of rats (Groups IV & V) increased somewhat (P≤0.05) when they were allowed to recover naturally at both treatment levels.

Table 3

Treatment	Group	Glycogen (mg/g)		
		Testes	**Heart**	**Liver**
Control (received vehicle olive oil only)	**GI**	2.61 ±0.09	2.26 ±0.09	3.68 ±0.11
25mg/kg b.wt./day of Profenofos	**GII**	0.81** ±0.13	1.03** 0.17	0.79** ±0.22
50mg/kg b.wt./day of Profenofos	**GIII**	0.70** ±0.62	0.75** ±0.13	0.58** ±0.25
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	1.32* ±0.52	1.41* ±0.49	1.05* ±0.55
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	1.20* ±0.39	1.56* ±0.41	1.02* ±0.32

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

- **Cholesterol**

Testes (Table: 4)

Following exposure to profenofos at both dose levels, the cholesterol content of the testes rose considerably (P≤0.01) by 40.79% and 52.18% as compared to controls. Rats at both dosing levels showed a non-significant drop in testicular cholesterol levels when allowed to recover naturally (Groups IV & V).

Heart (Table: 4)

The hearts of rats exposed to varying levels of profenofos for 45 days showed a significantly elevated (P≤0.05) (P≤0.01) cholesterol content. Rats at both dosing levels showed a non-significant drop in cardiac cholesterol levels when allowed to recover naturally (Groups IV & V).

Table 4

Treatment	Group	Cholesterol (mg/g)				Fructose (mg/g)
		Testes	Heart	Liver	Adrenal Gland	(Seminal Vesicle)
Control (received vehicle olive oil only)	**GI**	5.27 ±0.23	10.64 ±0.08	7.41 ±0.27	10.60 ±0.65	4.74 ±0.19
25mg/kg b.wt./day of Profenofos	**GII**	7.42** ±0.05	13.25* ±0.06	11.09** ±0.36	20.15** ±0.45	2.80* ±0.24
50mg/kg b.wt./day of Profenofos	**GIII**	8.02** ±0.26	14.03** ±0.31	12.40** ±0.64	24.38** ±0.74	1.88** ±0.82
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	6.48 ns ±0.36	12.87 ns ±0.27	10.42 ns ±0.22	17.02* ±0.65	1.80 ns ±0.39
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	7.41 ns ±0.29	13.33 ns ±0.31	11.28 ns ±0.29	20.36* ±0.37	1.20 ns ±0.31

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Liver (Table: 4)

In both groups treated with profenofos, the liver's cholesterol content increased to significant (P≤0.01) levels. Rats at both profenofos-treated dose levels showed a non-significant reduction in liver cholesterol levels when allowed to recover naturally (Groups IV & V).

Adrenal Gland (Table: 4)

Both profenofos-treated groups showed a significant (P≤0.01) increase in the adrenal gland's cholesterol content following oral administration of the drug. There was a non-significant drop in the adrenal gland's cholesterol level when rats were allowed to recover naturally at both dosage levels (Groups IV & V).

Fructose (Table: 4)

Seminal Vesicles

Rats given profenofos showed a substantial (P≤0.05) (P≤0.01) decrease in seminal vesicular fructose at dose levels treated with profenofos. Fructose levels in seminal vesicles (Groups IV & V) increased non-significantly when rats were allowed to recover naturally at both dosage levels.

Protein

Testes (Table: 5)

Following treatment to profenofos, testicular protein was significantly (P≤0.05) elevated in both groups when compared to control rats. At both dose levels, the elevation was 12.60% and 14.83%. When given time to recover spontaneously, rats at both treatment levels (Groups IV & V) displayed a non-significant decrease in testicular protein levels.

Cauda Epididymis (Table: 5)

After 45 days of both dose levels of profenofos treatment, the cauda epididymis's protein content increased significantly (P≤0.05) (P≤0.01). When given the opportunity to recover naturally, rats at both dosage levels displayed a non-significant decrease in protein levels (Groups IV & V).

Seminal Vesicle (Table: 5)

Rats treated to profenofos in groups II and III had significantly higher (P≤0.01) protein concentrations in their seminal vesicles than did controls. At both treatment levels, allowing mice to recover naturally resulted in a non-significant drop in the protein level in the seminal vesicle (Groups IV & V).

Ventral Prostate (Table: 5)

The protein levels of the ventral prostate increased significantly (P≤0.05) (P≤0.01) in groups II and III after receiving profenofos treatment. At both treatment levels, allowing rats (Group X & XI) to recover naturally resulted in a non-significant drop in the protein level in their ventral prostates.

Vas deferens (Table: 5)

The vas deferens protein levels in rats administered both profenofos increased significantly (P≤0.01). Allowing rats to recover naturally at both dose levels resulted in a non-significant decrease in the protein level in the vas deferens (Groups IV & V).

Table 5

Treatment	Group	Protein (mg/g)				
		Testes	Cauda Epididymis	Seminal Vesicle	Ventral Prostate	Vas Deferens
Control (received vehicle olive oil only)	GI	252.16 ±4.61	209.64 ±5.91	234.50 ±5.87	227.39 ±7.41	255.47 ±8.62
25mg/kg b.wt./day of Profenofos	GII	283.95* ±7.64	234.79* ±7.50	251.49** ±7.53	231.40 * ±5.58	272.85** ±6.65
50mg/kg b.wt./day of Profenofos	GIII	289.56* ±6.62	240.31** ±3.54	258.58** ±74.28	239.82** ±5.36	279.14** ±7.23
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	GIV	277.58 ns ±5.07	227.34 ns ±5.12	247.36 ns ±4.75	229.25 ns ±3.98	266.95 ns ±5.28
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	GV	283.16 ns ±4.89	233.68 ns ±4.69	253.47 ns ±5.87	234.79 ns ±5.04	273.46 ns ±4.26

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Sialic Acid

Testes (Table: 06)

Oral profenofos administration led to a significant (P≤0.05) (P≤0.01) reduction in testicular sialic acid at both dose levels during a 45-day period, by 14.71% and 26.83%, respectively, in comparison to controls. The sialic acid content in the testes increased non-significantly (P≤0.05) in rats who were allowed to recover naturally at both treatment levels (Groups IV & V).

Cauda epididymis (Table: 06)

In groups II and III, rats exposed to profenofos at both dose levels showed a substantial decrease in the amount of sialic acid in their cauda epididymis (P≤0.05) (P≤0.01). The amount of sialic acid in the cauda epididymis (Group X & XI) rose non-significantly when rats were given both dose levels and allowed to recover normally.

Seminal Vesicle (Table: 06)

When compared to control animals, rats given profenofos exhibited a statistically significant (P≤0.01) decrease in the amount of sialic acid in their seminal vesicles. Sialic acid levels in seminal vesicles (Groups IV & V) increased non-significantly in rats that were allowed to recover naturally at both treatment levels.

Table 6

Treatment	Group	Sialic acid (mg/g)					Ascorbic acid (mg/g)
		Testes	Cauda Epididymis	Seminal Vesicle	Ventral Prostate	Vas Deferens	Adrenal Gland
Control (received vehicle olive oil only)	**GI**	5.03 ±0.15	4.51 ±0.12	5.38 ±0.11	5.36 ±0.11	5.31 ±0.15	4.58 ±0.64
25mg/kg b.wt./day of Profenofos	**GII**	4.29* ±0.28	3.98* ±0.09	4.37** ±0.12	4.44* ±0.08	4.66* ±0.26	2.06** ±0.51
50mg/kg b.wt./day of Profenofos	**GIII**	3.68** ±0.34	3.65** ±0.19	4.21** ±0.32	4.12* ±0.35	4.52* ±0.12	1.08** ±0.43
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	4.58 ns ±0.21	4.18 ns ±0.16	4.55 ns ±0.18	4.63 ns ±0.31	4.79 ns ±0.19	2.85 ns ±0.52
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	4.02 ns ±0.14	3.88 ns ±0.25	4.41 ns ±0.22	4.47 ns ±0.29	4.67 ns ±0.15	1.73 ns ±0.38

(Mean ± SEM of 5 animals (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

The levels of sialic acid in the ventral prostate of mice exposed to profenofos were significantly (P≤0.05) lower than those of control animals. The sialic acid level in the ventral prostate (Group X & XI) rose non-significantly but significantly (P≤0.05) (P≤0.01) at 200 and 300 mg when rats were given both dose levels and allowed to recover normally.

Vas Deferens (Table: 06)

In comparison to control animals, rats that were given oral profenofos showed a significant (P≤0.05) decrease in the amount of sialic acid in their vas deferens. The amount of sialic acid in the vas deferens (Group X & XI) rose non-significantly at 100 mg when rats were given both dose levels and allowed to recover normally.

Ascorbic acid (Table: 06)

Adrenal gland

The ascorbic acid level in the adrenal glands decreased significantly (P≤0.05) (P≤0.01) in both profenofos-treated groups after oral administration. In contrast, rats given both dose levels and left to recover spontaneously displayed a nonsignificant rise in ascorbic acid levels in the adrenal gland (Groups IV & V).

Oxidative Stress and Antioxidant Parameters (Table: 07)

At low dose levels, rats treated with profenofos exhibited a highly significant (P≤0.01) decrease in GSH, and testicular levels of catalase and SOD were significantly (P≤0.05) reduced. At higher dose levels, rats treated with profenofos exhibited a highly significant (P≤0.01) fall in comparison to control animals. Testicular LPO levels in rats administered profenofos increased statistically significantly (P≤0.01) as compared to the control group. The recovery group only marginally recovered in comparison to the groups treated with profenofos at both dose levels.

Table 7

Treatment	Group	Catalase (n mole of H_2O_2 consumed/min/mg protein)	SOD (Units/mg protein)	GSH (n mole/gm)	LPO (n mole MDA/mg protein)
Control (received vehicle olive oil only)	**GI**	77.51 ±2.26	22.21 ±1.28	32.57 ±2.40	0.79 ±0.05
25mg/kg b.wt./day of Profenofos	**GII**	60.10* ±1.98	11.71* ±1.16	13.51* ±1.10	1.25** ±0.12
50mg/kg b.wt./day of Profenofos	**GIII**	51.19** ±1.35	9.66** ±1.45	10.38** ±1.41	2.52** ±0.19
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	63.09 ns ±2.23	13.88 ns ±2.15	17.84 ns ±1.73	1.02ns ±0.11
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	54.87 ns ±1.74	12.36 ns ±2.31	14.86 ns ±1.27	2.21 ns ±0.09

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Radioimmunoassay (RIA) (Table: 08)

Serum levels of testosterone, luteinizing hormone, and follicle-stimulating hormone significantly decreased (P≤0.05) (P≤0.01) in rats administered profenofos at both dose levels for 45 days.

Table 8

Treatment	Group	Testosterone	Follicle Stimulating Hormone (FSH)	Luteinizing Hormone (LH)
		mg/ml	MLU/ml	
Control (received vehicle olive oil only)	**GI**	5.01 ±0.43	0.78 ±0.09	4.83 ±0.48
25mg/kg b.wt./day of Profenofos	**GII**	1.23** ±0.20	0.29** ±0.04	2.36** ±0.37
50mg/kg b.wt./day of Profenofos	**GIII**	0.78** ±0.46	0.14** ±0.09	1.69** ±0.63
25mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days	**GIV**	2.13 ns ±0.27	0.37 ns ±0.03	2.89 ±0.24
50mg/kg b.wt./day of Profenofos for 45 days and then kept without any treatment for next 45 days.	**GV**	1.59 ns ±0.31	0.30 ns ±0.07	2.24 ns ±0.32

(Mean ± SEM of 5 animals) (Group II and III compared with Group I)
ns = non-significant (Group IV compared with Group II)
* = Significant (P≤0.05) (Group V compared with Group III)
** = Highly significant (P≤0.01)

Conclusion

Exposure to profenofos resulted in marked biochemical and reproductive toxicity in male Wistar rats. Rats treated with 25 and 50 mg/kg body weight of profenofos for 45 days showed a significant reduction in serum testosterone levels, total protein, and antioxidant enzyme activities (SOD and GSH) compared to control animals (P≤0.05). Concurrently, a significant increase in lipid peroxidation (LPO) was observed, indicating elevated oxidative stress. Sperm parameters were adversely affected, with

significant decreases in sperm count, motility, and viability noted in both treatment groups. Following a 45-day recovery period without any therapeutic intervention, partial improvement was observed in several parameters. Total protein and serum testosterone levels demonstrated a non-significant upward trend toward recovery, while LPO levels decreased, indicating a partial attenuation of oxidative stress. However, the activities of antioxidant enzymes SOD and GSH remained significantly lower than control values ($p < 0.05$), suggesting incomplete recovery of antioxidant defense mechanisms. Sperm count, motility, and viability exhibited modest, non-significant improvements but did not return to baseline levels. Overall, while the recovery phase indicated some degree of spontaneous physiological repair, the restoration of both biochemical and reproductive functions remained incomplete, particularly at the higher dose of profenofos. These findings underline the persistent impact of profenofos toxicity and the limited capacity of natural recovery mechanisms to fully reverse its effects without therapeutic support.

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