



Hepatic and testicular toxicity induced by subchronic profenofos exposure in male Wistar rats

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Abstract

Organophosphate pesticides remain a significant environmental and health concern due to their widespread use and potential for systemic toxicity. Profenofos, a commonly applied organophosphate insecticide, has been associated with adverse effects beyond cholinesterase inhibition, yet its subchronic impact on metabolic and endocrine organs has not been fully characterized. This study investigated the effects of sub chronic profenofos exposure on hepatic integrity and testicular endocrine function in male Wistar rats using an integrated toxicological approach. Twenty adult male rats were orally administered profenofos at doses of 0, 20, 40, or 80 mg/kg body weight daily for 60 days. Profenofos exposure induced dose-dependent hepatic injury, characterised by vacuolar degeneration, sinusoidal dilation, hepatocellular necrosis, and significant elevations in SGPT and SGOT levels. Concurrently, testicular tissues exhibited progressive degeneration of seminiferous tubules, reduced Leydig-cell density, and a marked decline in testosterone immunoreactivity, indicating impaired steroidogenesis. The parallel structural and functional alterations in both organs support a systemic toxicological response to profenofos. Subchronic oral exposure to profenofos results in integrated hepatic and endocrine toxicity mediated by oxidative stress, metabolic overload, and steroidogenic disruption. By simultaneously evaluating liver and testicular endpoints within a single experimental framework, this study provides novel insight into the systemic nature of profenofos toxicity and underscores the need for comprehensive risk assessment of organophosphate pesticide exposure.

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Introduction

The widespread application of organophosphate pesticides has raised substantial environmental and public health concerns due to their persistence in ecosystems and potential for chronic exposure (1). These compounds remain extensively used in agriculture to maintain crop productivity,

yet their residues are frequently detected in soil, water sources, and food commodities, increasing the likelihood of long-term exposure in humans and animals (2-4). Among commonly applied organophosphates, profenofos is widely utilised because of its high insecticidal efficacy (5), and relatively low cost. Despite these advantages, accumulating toxicological evidence indicates that profenofos exposure

may induce systemic toxicity that extends beyond acute neurotoxic effects (6). From a biological perspective, the liver and reproductive organs are particularly vulnerable to organophosphate-induced toxicity (6,7). The liver serves as the primary site for xenobiotic metabolism and detoxification (8), rendering it highly susceptible to injury from reactive metabolites generated during biotransformation processes (9). The primary toxic mechanism of profenofos involves inhibition of acetylcholinesterase, resulting in acetylcholine accumulation and overstimulation of cholinergic pathways (10), thereby disrupting neuroendocrine regulation and contributing to secondary metabolic and reproductive dysfunction through sustained hormonal imbalance and cellular oxidative stress (11,12). However, growing evidence suggests that sublethal and prolonged exposure may elicit broader systemic effects (13,14), including endocrine disruption and hepatic dysfunction. These subchronic effects are toxicologically significant because they more accurately reflect environmental and occupational exposure scenarios, where repeated low-dose exposure (15) is common rather than acute poisoning. Furthermore, bioaccumulation and biomagnification of organophosphate residues may amplify these effects across trophic levels, underscoring the importance of comprehensive toxicological evaluation.

Concurrently, the male reproductive system represents a sensitive endocrine target, as steroidogenesis and spermatogenesis depend on tightly regulated hormonal pathways (16). Organophosphates, including profenofos, have been increasingly recognized as endocrine-disrupting chemicals capable of interfering with hormone synthesis, secretion, and regulation (11,12). In male mammals, exposure to these compounds has been associated with impaired spermatogenesis, reduced testosterone production, and structural damage to testicular tissue (17,18). These effects are often linked to oxidative stress mechanisms, whereby excessive production of reactive oxygen species leads to lipid peroxidation, mitochondrial dysfunction, DNA damage, and ultimately cellular apoptosis (19).

Similarly, hepatotoxic effects of profenofos have been documented in both aquatic and terrestrial models, characterised by enzymatic dysfunction, hepatocellular degeneration, and necrosis following subchronic exposure (20,21). From a toxicodynamic standpoint, profenofos undergoes hepatic metabolism predominantly via cytochrome P450 enzymes, producing reactive intermediates that contribute to oxidative injury and enzymatic inhibition (20). These metabolites may circulate systemically and interfere with steroidogenic processes in the testis, leading to reduced testosterone synthesis and impaired spermatogenesis. Histopathological studies have reported degeneration of seminiferous tubules, reduced Leydig-cell density, and cellular vacuolation following organophosphate exposure, changes that are consistent with biochemical

evidence of hormonal suppression and oxidative stress (22,23). However, many existing studies examine hepatic or reproductive toxicity in isolation, limiting understanding of how metabolic and endocrine disturbances interact under sub chronic exposure conditions.

Despite extensive documentation of the neurotoxic properties of profenofos, important gaps remain regarding its sub chronic effects on hepatic and endocrine systems at environmentally relevant doses. Most available data are derived from acute toxicity studies or residue analyses, with relatively few investigations addressing prolonged oral exposure and its combined impact on organ structure and function (3,4). Moreover, the relationship between histopathological alterations and corresponding biochemical or hormonal changes has not been comprehensively characterized within a single experimental framework.

Therefore, the present study was designed to evaluate the effects of sub chronic oral exposure to profenofos on the hepatic and reproductive systems of male Wistar rats. By integrating histopathological assessment of liver and testicular tissues with biochemical analysis of serum transaminases and immunohistochemical evaluation of Leydig-cell testosterone expression, this study aims to elucidate the systemic nature of profenofos toxicity. The integrative assessment of metabolic and endocrine endpoints represents a novel approach that provides mechanistic insight into how hepatic dysfunction and endocrine disruption may be linked under organophosphate exposure. These findings are expected to contribute to a more comprehensive understanding of profenofos toxicodynamics and to inform risk assessment strategies and regulatory policies concerning organophosphate pesticide use.

Materials and methods

Study design and overview

This study employed a true experimental design using a post-test only control group model to evaluate the toxicological effects of subchronic profenofos exposure on the hepatic and reproductive systems. The experimental approach was designed to assess structural, biochemical, and endocrine alterations following repeated oral administration of profenofos under controlled laboratory conditions.

Experimental animals

Twenty adults male Wistar rats (*Rattus norvegicus*), aged 8–10 weeks and weighing 180–200 g, were used as experimental subjects. Animals were acclimatized for 7 days prior to treatment to minimize stress and ensure uniform baseline physiological conditions. Rats were housed in polypropylene cages with wood-shaving bedding under standard environmental conditions (temperature 22–25°C; 12 h light–dark cycle) (24), and were provided ad libitum access to standard pellet feed and drinking water. Animal

health status and body weight were monitored throughout the experimental period.

Experimental grouping and profenofos administration

Following acclimatization, rats were randomly assigned into four experimental groups, each consisting of five animals. The control group received distilled water only, while treatment groups received profenofos at doses of 20, 40, or 80 mg/kg body weight. Dose selection was based on previous subchronic toxicity studies and represented low-to-high exposure ranges without inducing acute lethality (17,25). Profenofos was diluted in distilled water to obtain the desired concentrations and administered orally once daily using a gavage needle for 60 consecutive days. Body weight was recorded weekly to adjust dosing accurately.

Animal monitoring and exposure procedure

Prior to administration, rats were gently restrained to minimize stress. Profenofos solutions were freshly prepared every three days to ensure chemical stability. Animals were observed daily for changes in behaviour, food intake, locomotor activity, and clinical signs consistent with organophosphate exposure, including tremors or lethargy (26). Cage bedding was replaced regularly to maintain hygienic conditions and reduce environmental stressors.

Necropsy and sample collection

At the end of the exposure period, rats were anaesthetised using ketamine (80 mg/kg body weight) and xylazine (10 mg/kg body weight) administered intramuscularly (27). Following confirmation of deep anaesthesia, blood samples were collected via intracardiac puncture for biochemical and hormonal analyses. Animals were subsequently euthanised by cervical dislocation. The liver and testes were immediately excised, rinsed in physiological saline to remove residual blood, and fixed in 10% neutral-buffered formalin for histological and immunohistochemical evaluation.

Biochemical analysis of liver function

Blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum. Hepatic function was assessed by measuring serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxaloacetic transaminase (SGOT) activities using standard spectrophotometric methods, following established protocols (2,28). Serum cholinesterase activity was also measured to confirm systemic exposure to organophosphate compounds.

Histological processing and hematoxylin–eosin (he) staining histological examination

Fixed liver and testicular tissues were processed routinely by dehydration through graded ethanol, clearing in xylene, and embedding in paraffin. Sections of 5 µm

thickness were cut using a rotary microtome and mounted on glass slides. Hematoxylin–eosin staining was performed following standard histological procedures (29). In the testis, assessment focused on seminiferous tubule architecture, spermatogenic cell arrangement, and Leydig-cell distribution. Hepatic evaluation included assessment of hepatocellular degeneration, necrosis, sinusoidal dilation, and structural disorganisation. Lesions were scored semi-quantitatively using a modified histopathological scoring system (30).

Immunohistochemical detection of testosterone expression

Immunohistochemical analysis was performed to assess testosterone expression in Leydig cells. Paraffin-embedded testicular sections were deparaffinized and rehydrated, followed by antigen retrieval using citrate buffer (pH 6.0) at 95°C for 15 minutes. Endogenous peroxidase activity was blocked using 3% hydrogen peroxide, and non-specific binding was minimized by incubation with normal goat serum for 30 minutes. Sections were incubated overnight at 4°C with a primary anti-testosterone antibody at a dilution of 1:100. After washing, sections were incubated with a horseradish peroxidase-conjugated secondary antibody for 60 minutes at room temperature. Immunoreactivity was visualized using diaminobenzidine as the chromogen, with hematoxylin used as a counterstain (31). Leydig-cell testosterone expression was evaluated semi-quantitatively using the Immunoreactive Score system, which combines staining intensity and the proportion of positive cells to generate a composite score ranging from 0 to 12. Mean scores were calculated for each experimental group (25).

Statistical analysis

All data are presented as mean ± standard deviation. Data normality was assessed using the Shapiro–Wilk test. Normally distributed biochemical parameters were analyzed using one-way analysis of variance followed by Tukey's post-hoc test. Non-normally distributed data, including histopathological scores and immunoreactivity scores, were analyzed using the Kruskal–Wallis test with Bonferroni-adjusted post-hoc comparisons. Statistical significance was defined as $P < 0.05$. All analyses were performed using SPSS software version 26.

Ethical considerations and animal welfare

All experimental procedures were conducted in accordance with internationally accepted ethical standards for animal research and were approved by the Research Ethics Committee of Universitas Brawijaya (Approval No. 101-KEP-UB-2024). The study was designed and reported in accordance with the Animal Research: Reporting of *In Vivo* Experiments (ARRIVE) guidelines to promote transparency, reproducibility, and methodological rigor in

animal experimentation. In addition, the principles of Replacement, Reduction, and Refinement were applied to minimize animal use and suffering while maintaining scientific validity. The number of animals was restricted to the minimum required to obtain reliable statistical outcomes, and efforts were made to ensure appropriate experimental design, including clear definition of outcome measures and standardized procedures. Humane endpoints were established in advance, and animals were monitored daily for clinical signs of pain, distress, or adverse effects. Anesthesia and euthanasia were performed according to established veterinary protocols and institutional guidelines for laboratory animal care. Throughout the experimental period, animal housing, environmental conditions, and handling procedures complied with Indonesian regulations and welfare standards for laboratory animals (32).

Results

Serum Biochemical Parameters (SGPT and SGOT Levels)

Serum biochemical analysis demonstrated dose-related alterations in hepatic enzyme activity following sub chronic profenofos exposure (Table 1). Control rats (P1) showed SGPT and SGOT levels within the expected physiological range, indicating normal hepatocellular integrity. In the low-dose group (P2, 20 mg/kg BW), SGPT and SGOT values exhibited a mild increase compared with controls; however, these changes were not statistically significant. Similarly, the intermediate-dose group (P3, 40 mg/kg BW) did not show a significant elevation in transaminase levels relative to the control group. In contrast, rats exposed to the highest dose of profenofos (P4, 80 mg/kg BW) exhibited a significant increase in both SGPT and SGOT levels compared with all other groups ($P < 0.05$). SGPT levels increased to 32.14 ± 7.28 U/L, while SGOT levels rose markedly to 120.16 ± 22.19 U/L, representing approximately a twofold increase relative to controls. These findings indicate pronounced hepatocellular injury at higher profenofos exposure levels.

Hepatic Histopathological Finding

Histopathological examination of liver tissue revealed a clear dose-related progression of hepatic lesions following sub chronic profenofos exposure (Figure 1). In the control group (P1), hepatic architecture was preserved, with hepatocytes arranged in normal hepatic cords surrounding the central vein. Cells exhibited eosinophilic cytoplasm and centrally located nuclei, and sinusoids appeared intact, indicating normal liver morphology. In rats exposed to the lowest profenofos dose (P2, 20 mg/kg BW), mild hydropic degeneration was observed in scattered hepatocytes, particularly in the periportal regions. These changes were characterized by cytoplasmic swelling without overt nuclear damage, suggesting early cellular stress rather than

irreversible injury. Moderate histopathological alterations were evident in the intermediate-dose group (P3, 40 mg/kg BW). Liver sections showed more pronounced vacuolar degeneration, sinusoidal dilation, and the presence of necrotic hepatocytes with nuclear condensation and fragmentation. The normal lobular organization was partially disrupted, indicating progression from reversible injury to structural damage. In the highest-dose group (P4, 80 mg/kg BW), severe hepatic injury was apparent. Extensive hepatocellular necrosis, karyolysis, and marked inflammatory cell infiltration were observed, accompanied by loss of normal lobular architecture. Portal areas showed congestion and cellular disorganization, reflecting advanced toxic injury.

Table 1: Serum SGPT and SGOT levels following subchronic profenofos exposure

Groups	SGPT Levels (U/L)	SGOT Levels (U/L)
P1 (0 mg/mL)	17.76 ± 7.12^a	65.85 ± 7.61^a
P2 (20 mg/mL)	26.59 ± 2.68^{ab}	72.35 ± 16.67^a
P3 (40 mg/mL)	20.54 ± 5.5^a	68.73 ± 3.09^a
P4 (80 mg/mL)	32.14 ± 7.28^b	120.16 ± 22.19^b

Note: Different superscript letters indicate significant differences among groups ($P < 0.05$).

Semi-quantitative histopathological scoring confirmed these observations (Figure 2). Both hydropic degeneration and necrosis scores increased progressively with increasing profenofos dose. Statistical analysis using the Kruskal–Wallis test demonstrated significant differences between the control and high-dose groups ($P < 0.05$), supporting a dose-dependent pattern of hepatic damage.

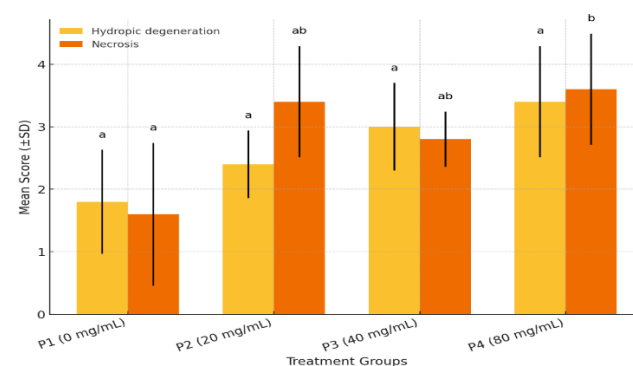


Figure 2: Semi-quantitative histopathological scores of hydropic degeneration and necrosis in the liver of male Wistar rats following subchronic profenofos exposure. Data are presented as mean $\pm$ standard deviation. Different superscript letters indicate statistically significant differences among groups ($P < 0.05$).

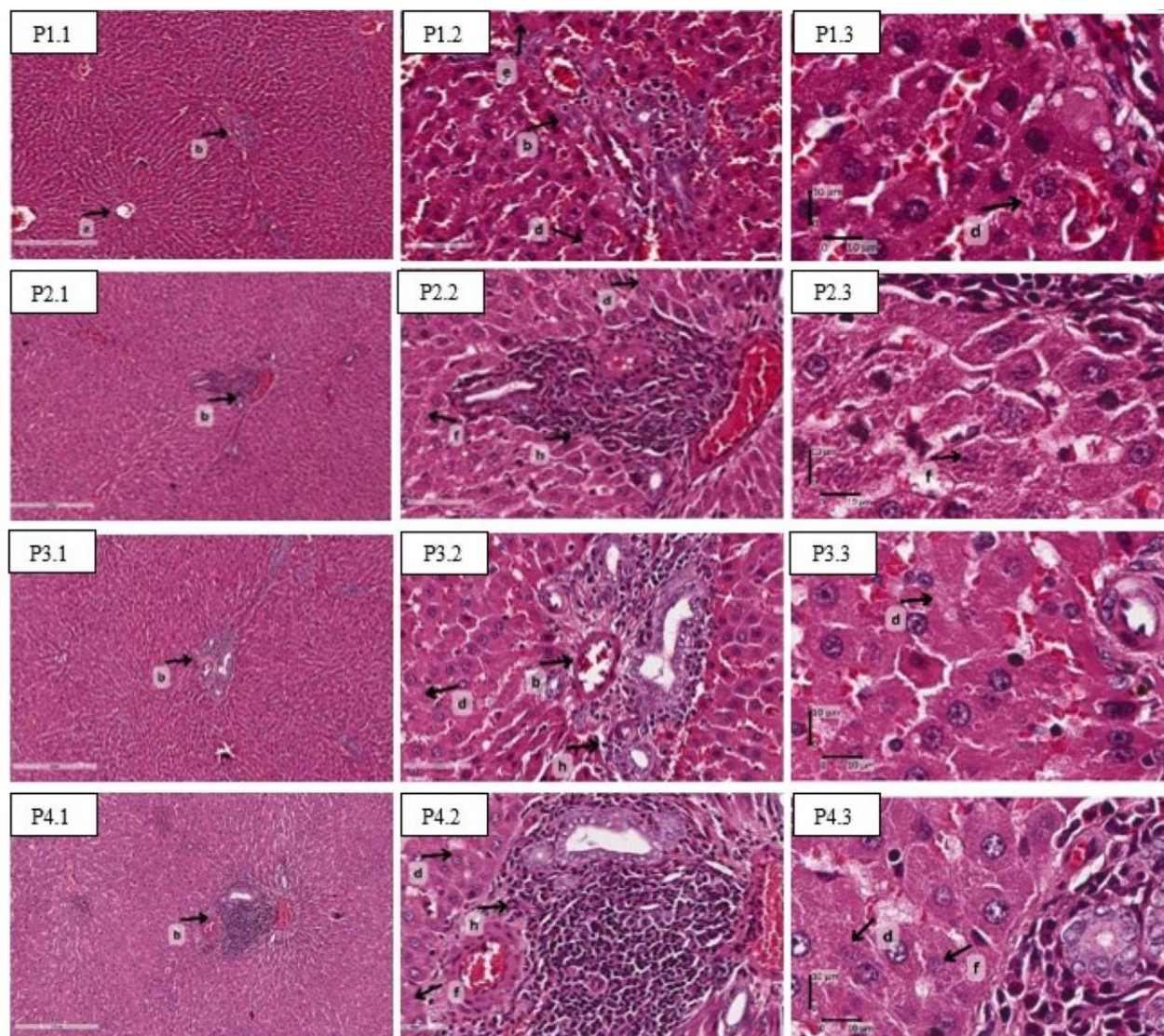


Figure 1: Histopathological alterations of the liver in male Wistar rats following subchronic profenofos exposure (hematoxylin-eosin staining). Representative micrographs from control (P1) and profenofos-treated groups (P2–P4) at magnifications of 100×, 400×, and 1000×. Control livers show normal hepatic architecture. Low-dose exposure (P2) reveals mild hydropic degeneration. Intermediate-dose exposure (P3) shows vacuolar degeneration, sinusoidal dilation, and scattered necrotic hepatocytes. High-dose exposure (P4) demonstrates extensive hepatocellular necrosis, nuclear fragmentation, inflammatory cell infiltration, and loss of lobular organisation. Annotations: (a) central vein, (b) portal triad, (c) sinusoids, (d) hydropic degeneration, (e) karyopyknosis, (f) karyorrhexis, (g) karyolysis, (h) inflammatory cells.

Histopathological Changes in Testicular Structure

Histological examination of testicular tissue demonstrated a clear dose-dependent pattern of structural deterioration following sub chronic profenofos exposure (Figure 3). In the control group (P1), seminiferous tubules exhibited normal architecture, with orderly layers of spermatogonia, spermatocytes, and spermatids arranged concentrically from the basement membrane toward the lumen. Sertoli cells appeared intact, and Leydig cells were

evenly distributed within the interstitial tissue, displaying normal morphology. In the low-dose group (P2, 20 mg/kg BW), mild histological alterations were observed. These changes included cytoplasmic vacuolation in spermatogenic cells and focal disorganisation of the germinal epithelium. Despite these alterations, the overall tubular structure remained largely preserved, and spermatogenic layers were still identifiable.

More pronounced degenerative changes were evident in the intermediate-dose group (P3, 40 mg/kg BW). Seminiferous tubules showed partial shrinkage and irregular luminal enlargement, accompanied by disrupted alignment

of spermatogenic cells. Exfoliation of germ cells into the tubular lumen was frequently observed, indicating impaired cell adhesion and early breakdown of spermatogenic integrity.

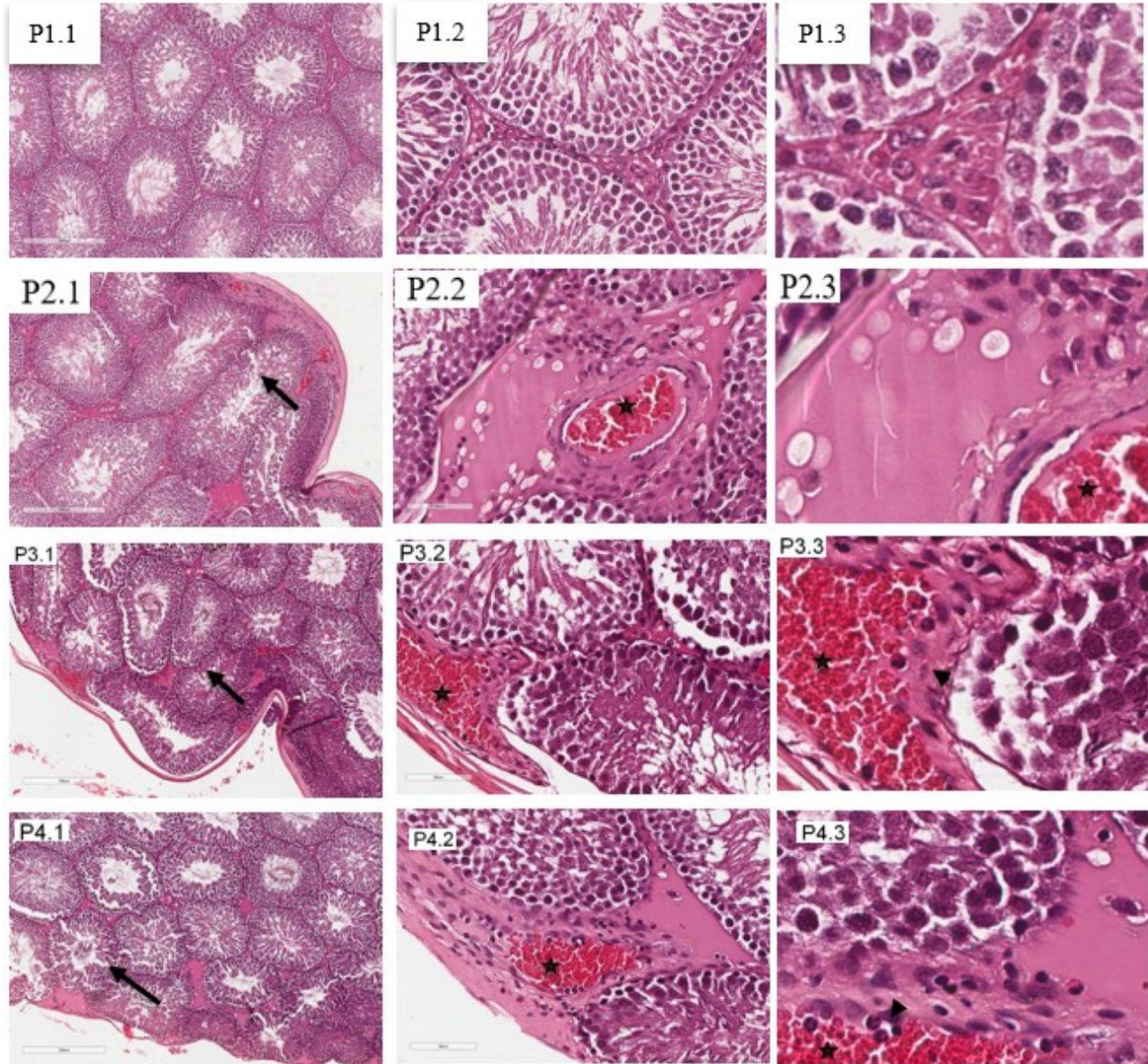


Figure 3: Histopathological alterations of the testis in male Wistar rats following subchronic profenofos exposure (hematoxylin-eosin staining). Representative micrographs of testicular tissue from control (P1) and profenofos-treated groups (P2–P4) at magnifications of 100×, 400×, and 1000×. Control testes show normal seminiferous tubule architecture and intact spermatogenic layers. Low-dose exposure (P2) reveals mild vacuolation of spermatogenic cells. Intermediate-dose exposure (P3) shows tubular disorganisation, germ-cell exfoliation, and interstitial congestion. High-dose exposure (P4) demonstrates severe degeneration, necrosis of spermatogonia, Sertoli-cell nuclear pyknosis, = interstitial edema, and reduced Leydig-cell density. Annotations: necrotic areas (black arrows), vascular congestion (asterisks), inflammatory cells (arrowheads).

In the high-dose group (P4, 80 mg/kg BW), severe testicular lesions were apparent. These included necrosis of spermatogonia, nuclear pyknosis in Sertoli cells, marked depletion of spermatids, and interstitial edema. Leydig cells appeared reduced in number and exhibited cytoplasmic condensation, suggesting impaired endocrine function. Semi-quantitative histopathological scoring confirmed a significant increase in lesion severity with increasing profenofos dose (Figure 4). Statistical analysis using the Kruskal–Wallis test revealed significant differences among groups ($H = 12.999$; $p = 0.005$), with post-hoc analysis indicating that P3 and P4 differed significantly from controls, whereas P2 did not.

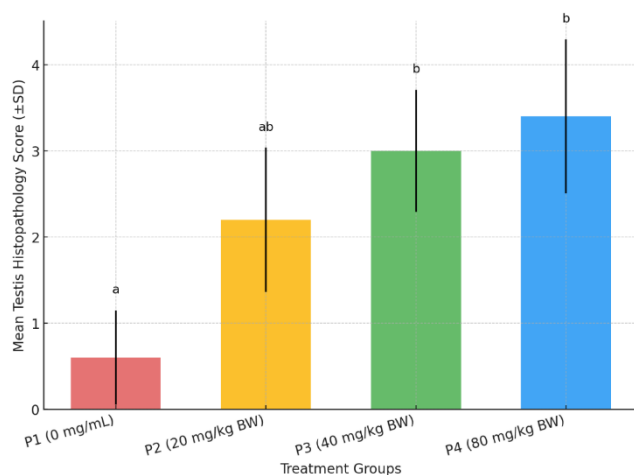


Figure 4: Semi-quantitative histopathological scores of testicular damage in male Wistar rats following subchronic profenofos exposure. Data are presented as mean $\pm$ standard deviation. Different superscript letters indicate statistically significant differences among treatment groups ($P < 0.05$).

Immunohistochemical Analysis of Testosterone Expression

Immunohistochemical staining revealed a clear dose-dependent reduction in testosterone expression within Leydig cells following subchronic profenofos exposure (Figure 6). In the control group (P1), Leydig cells exhibited strong cytoplasmic immunoreactivity, characterised by intense brown staining distributed uniformly across the interstitial tissue. The mean Immunoreactive Score (IRS) ranged from 8 to 9, reflecting strong staining intensity with more than 75% of Leydig cells positively stained.

In the low-dose group (P2, 20 mg/kg BW), testosterone immunoreactivity was moderately reduced. Leydig cells showed decreased staining intensity and a lower proportion of positive cells, resulting in a mean IRS of approximately 6. Despite this reduction, testosterone expression remained detectable in most interstitial regions. A marked decline in

testosterone expression was observed in the intermediate-dose group (P3, 40 mg/kg BW). Leydig cells exhibited weak immunostaining, with fewer than 25% of cells showing positive cytoplasmic reaction. The mean IRS decreased to approximately 3–4, indicating substantial impairment of steroidogenic activity. In the high-dose group (P4, 80 mg/kg BW), testosterone immunoreactivity was severely diminished. Most Leydig cells displayed faint or absent staining, with a mean IRS of approximately 2. Quantitative analysis confirmed a statistically significant, dose-dependent reduction in IRS values across treatment groups ($P < 0.05$) (Figure 5).

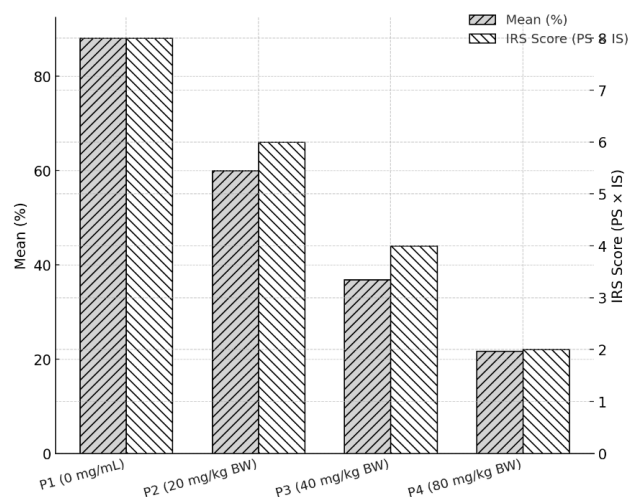


Figure 5: Immunohistochemistry (IHC) scoring rat testes.

Discussion

The present study demonstrates that sub chronic oral exposure to profenofos induces dose-dependent hepatic dysfunction, as reflected by significant elevations in serum SGPT and SGOT levels at higher exposure concentrations. Transaminase enzymes are sensitive indicators of hepatocellular injury, and their increased serum activity is commonly associated with membrane permeability disruption and hepatocyte necrosis (33,34). The marked elevation of SGPT observed in the high-dose group suggests leakage of cytosolic enzymes following damage to hepatocyte plasma membranes (35). In parallel, the increase in SGOT, which is partially localised within mitochondria, indicates more extensive cellular injury involving mitochondrial dysfunction (36). This pattern is consistent with toxicant-induced oxidative stress, where excessive reactive oxygen species compromise both cytosolic and mitochondrial integrity (37). These biochemical findings agree with previous studies reporting hepatotoxic effects of profenofos and other organophosphate pesticides. Dessouki,

et al. (38) demonstrated that profenofos exposure resulted in elevated serum transaminase levels accompanied by hepatocellular necrosis and sinusoidal dilation. Similarly, Jamalnia (30) reported significant increases in SGPT and SGOT following organophosphate exposure, correlating with histopathological evidence of liver degeneration.

The absence of significant SGPT and SGOT elevation in the P2 and P3 groups in the present study, where hepatic detoxification mechanisms and internal antioxidant defences may have partially compensated for toxic stress (39). Some investigations have shown minimal or non-significant changes in transaminase activity following low-dose or

short-term organophosphate exposure, suggesting the presence of adaptive hepatic responses (40) during early or mild toxic insult. From a mechanistic perspective, profenofos undergoes hepatic biotransformation via cytochrome P450 enzymes, generating reactive metabolites that promote lipid peroxidation and oxidative damage (41,42). When exposure exceeds the liver's detoxification capacity, cellular injury becomes evident, resulting in enzyme leakage into the circulation (43). The dose-dependent enzyme elevation observed in this study supports this toxicodynamic pathway and highlights the liver's central role in mediating systemic organophosphate toxicity.

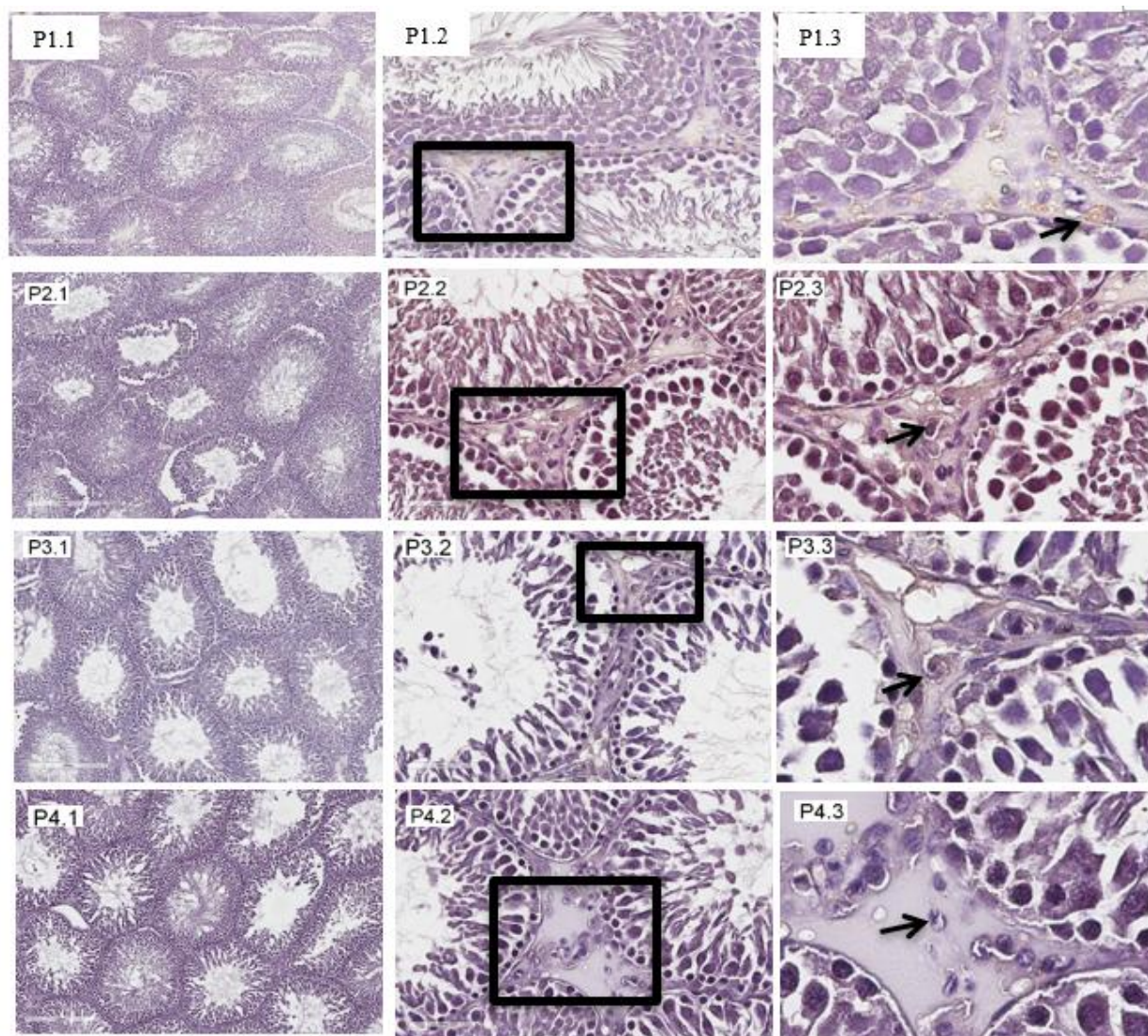


Figure 6: Testosterone expression in Leydig cells of treated rats (Immunohistochemical staining). Group (P1.1) 100×, Group (P1.2) 400×, Group (P1.3) 1000×, Group (P2.1) 100×, Group (P2.2) 400×, Group (P2.3) 1000×, Group (P3.1) 100×, Group (P3.2) 400×, Group (P3.3) 1000×, Group (P4.1) 100×, Group (P4.2) 400×, Group (P4.3) 1000×, Leydig cells (black arrow), interstitial area containing Leydig cells (black frame).

The histopathological findings provide strong morphological evidence that sub chronic profenofos exposure induces progressive hepatic injury in male Wistar rats (44). The transition from mild hydropic degeneration at low doses to extensive necrosis at higher doses reflects a shift from reversible cellular adaptation to irreversible hepatocellular damage. Hydropic degeneration observed in the P2 group likely represents an early response to toxic stress, characterized by impaired ionic regulation and water accumulation within hepatocytes. Such changes are commonly associated with mitochondrial dysfunction and early oxidative stress, where ATP depletion compromises membrane integrity (45). Similar early-stage hepatic alterations have been reported following organophosphate exposure in aquatic and terrestrial models (2,28). At higher exposure levels, the appearance of necrosis, nuclear fragmentation, and inflammatory infiltration suggests overwhelming of hepatic detoxification and antioxidant defence mechanisms. Once cellular repair capacity is exceeded, hepatocyte death ensues, leading to structural collapse of hepatic lobules and recruitment of inflammatory cells (46).

These histological changes are consistent with previous reports describing organophosphate-induced hepatotoxicity characterised by sinusoidal dilation, hepatocyte necrosis, and portal inflammation (30,38). However, this study has reported minimal histological changes at lower or shorter exposure regimens, suggesting that dose, duration, and metabolic capacity critically influence hepatic outcomes. The present findings extend this knowledge by demonstrating that prolonged oral exposure, even at sublethal doses, can result in substantial liver injury when exposure levels increase. The concordance between biochemical and morphological endpoints strengthens the validity of the integrated toxicological assessment employed in this study.

The analysis revealed a dose-dependent progression of hepatic injury following sub chronic profenofos exposure, characterized by distinct patterns of hydropic degeneration and necrosis. At lower doses, hepatocellular alterations were dominated by hydropic degeneration, reflecting an early and potentially reversible response to toxic stress associated with impaired ionic regulation and mitochondrial dysfunction. As the exposure dose increased, this pattern shifted toward a mixed lesion profile in which hydropic degeneration coexisted with necrotic changes, indicating a transition from adaptive cellular stress to irreversible injury. At the highest dose, necrosis became the predominant lesion, suggesting failure of compensatory mechanisms and widespread hepatocellular death. This non-linear relationship between hydropic degeneration and necrosis underscores the sequential nature of hepatocyte injury rather than a uniform escalation of all lesion types.

These findings are consistent with previous studies reporting organophosphate-induced liver damage characterized by vacuolar degeneration and necrosis, particularly at higher exposure levels (2,28). However, some studies have described minimal histopathological alterations at lower doses or shorter exposure periods, suggesting effective hepatic adaptation during early toxic insult. The present results extend these observations by demonstrating that prolonged sub chronic exposure shifts hepatocellular responses from reversible degeneration to irreversible necrosis as toxic burden increases. Once antioxidant and detoxification systems are overwhelmed, hepatocyte survival is compromised, leading to necrosis and inflammatory infiltration. The differentiation between early degenerative changes and advanced necrotic injury highlighted in this study provides important insight into the temporal and dose-related dynamics of profenofos hepatotoxicity and contributes novel evidence supporting its progressive, organ-specific toxic effects under sub chronic exposure conditions.

Importantly, the biochemical evidence of hepatic dysfunction observed here aligns with the histopathological alterations documented in liver tissue, strengthening the interpretation that profenofos induces genuine hepatocellular injury rather than transient enzymatic fluctuation. These results indicate that prolonged exposure to higher doses of profenofos can overwhelm hepatic adaptive mechanisms, leading to significant liver injury. Conversely, lower doses may elicit subclinical effects that are not immediately detectable through routine biochemical parameters but may still pose long-term health risks under continued exposure.

The present findings demonstrate that sub chronic profenofos exposure induces progressive, dose-dependent testicular damage, ranging from mild reversible alterations at low doses to severe degenerative and necrotic lesions at higher doses. The early appearance of cytoplasmic vacuolation and germinal epithelium disorganization in the P2 group likely reflects initial cellular stress associated with oxidative imbalance and disrupted membrane homeostasis. At this stage, spermatogenic function may still be partially preserved, consistent with the absence of statistically significant differences from controls in histopathological scores.

At higher exposure levels, the marked degeneration of seminiferous tubules and loss of spermatogenic cells observed in the P3 and P4 groups indicate failure of compensatory mechanisms. Spermatogenesis is a highly energy-dependent process regulated by Sertoli-cell support and androgen availability (47). Disruption of spermatogenesis through oxidative stress and endocrine interference can lead to germ-cell detachment, tubular atrophy, and impaired sperm production (48). Similar histopathological patterns have been reported in previous

studies of profenofos and other organophosphate pesticides, including seminiferous tubule degeneration and interstitial alterations (17,38).

However, this study has reported minimal testicular damage at lower or shorter exposure regimens, suggesting a threshold below which adaptive mechanisms may mitigate toxic effects. The present study extends these observations by demonstrating that prolonged sub chronic exposure results in a clear transition from adaptive cellular responses to irreversible tissue injury as dose increases. Mechanistically, organophosphorus compound, profenofos induced inhibition of cholinesterase activity, combined with oxidative stress and disruption of gonadotropin production and steroidogenic pathways (49), likely contributes to reduced testosterone availability and compromised Sertoli cell function (50), ultimately impairing spermatogenesis. By integrating morphological evaluation with dose stratification, this study provides novel evidence supporting the progressive nature of profenofos-induced reproductive toxicity under sub chronic exposure conditions.

The progressive reduction in Leydig-cell testosterone immunoreactivity observed in this study provides functional evidence of impaired steroidogenesis following sub chronic profenofos exposure. Testosterone synthesis in Leydig cells is highly dependent on mitochondrial integrity and enzymatic activity (51,52) within the steroidogenic pathway. Organophosphate induced oxidative stress is known to disrupt cholesterol transport into mitochondria and inhibit key enzymes involved in androgen biosynthesis, including 17 β -hydroxysteroid dehydrogenase and cytochrome P450-dependent enzymes (53). The dose-dependent decline in IRS values therefore reflects a direct toxic effect of profenofos on Leydig cell steroidogenic capacity.

These findings are consistent with previous reports demonstrating reduced androgen production following exposure to endocrine-disrupting chemicals, including organophosphate pesticides (18,19). However, this study has reported partial recovery or minimal changes in testosterone levels at lower exposure doses or shorter durations, suggesting the presence of adaptive endocrine responses under mild toxic stress. The present results extend this knowledge by showing that prolonged sub chronic exposure leads to progressive suppression of testosterone expression as exposure dose increases, indicating that compensatory mechanisms are eventually overwhelmed. The reduction in testosterone immunoreactivity closely parallels the histopathological alterations observed in the seminiferous tubules. Testosterone is essential for maintaining spermatogenesis and Sertoli cell function, and its depletion is expected to compromise germ cell survival and tubular integrity (54,55). The concomitant decline in testosterone expression and seminiferous tubule architecture observed in this study supports a functional link between endocrine disruption and structural degeneration of the testes. Similar

correlations between Leydig-cell dysfunction and testicular degeneration have been reported in organophosphate-induced reproductive toxicity models (56,57).

Beyond direct cytotoxicity, profenofos may also exert indirect endocrine effects through inhibition of acetylcholinesterase, leading to dysregulation of the hypothalamic–pituitary–gonadal (HPG) axis (57). It can alter hypothalamic signaling and suppress gonadotropin releasing hormone secretion, resulting in reduced luteinizing hormone stimulation of Leydig cells, which in turn diminishes testosterone production and compromises the maintenance of spermatogenesis, ultimately leading to a decline in spermatozoa quantity and quality. This combined mechanism of direct cellular injury and neuroendocrine disruption provides a plausible explanation for the pronounced reduction in testosterone expression observed at higher profenofos doses. Together, these findings highlight the multifactorial nature of profenofos induced endocrine toxicity and reinforce its classification as a potent endocrine disrupting compound under subchronic exposure conditions.

Exposure to profenofos in this study showed a pattern of simultaneous multi-organ damage to the liver and testes. These findings indicate that profenofos act not only as a direct cytotoxic toxin, but also as an endocrine disruptor. After entering the body, profenofos is metabolized primarily in the liver into oxon derivatives. These metabolites are known to be more reactive, acting as potent acetylcholinesterase (AChE) inhibitors and triggers for the formation of oxidative radicals. The reactive metabolites then circulate systemically and tend to accumulate in tissues with high steroidogenesis activity, such as the testes. Therefore, dual injury to the liver and testes reflects profenofos's ability to simultaneously disrupt metabolic detoxification processes in hepatocytes and inhibit hormone synthesis in the reproductive system.

Sequentially, the most likely mechanism explaining these effects begins with AChE inhibition, which triggers nerve overstimulation. This condition has the potential to disrupt neuroendocrine regulation, including GnRH release, thereby disrupting hormonal control of the reproductive axis. At the same time, oxon metabolites trigger oxidative stress characterized by lipid peroxidation, mitochondrial dysfunction, and activation of the apoptosis pathway, which ultimately exacerbates cell death in both the liver and testes. At the testicular level, oxidative stress and/or the direct effects of profenofos can inhibit steroidogenic enzymes, thereby decreasing testosterone biosynthesis in Leydig cells. Meanwhile, in the liver, “metabolic overload” occurs because this organ is the center of profenofos biotransformation; this process increases ROS production, reduces detoxification capacity, and leads to hepatocellular necrosis. This series of mechanisms is consistent with previous reports on organophosphates triggering oxidative stress in reproductive tissues and the liver (11,17,19).

The consistency of these research results is also evident when compared to previous studies. Dessouki *et al.* (38) reported degenerative and necrotic changes in liver and testicular tissue after profenofos administration, which parallels the pattern of tissue damage observed in this study. The decrease in testosterone expression found further strengthens the findings of Darbandi *et al.* (58) that oxidative stress in male reproductive organs can disrupt Leydig cell steroidogenesis. On the other hand, the biochemical response of the liver in this study is also in line with Jamalia (30), which shows that hepatocellular degeneration due to pesticide exposure is associated with increased transaminase activity.

Although consistent with previous report, this study provides additional contributions by integrating endocrine and hepatic indicators into a single integrated experimental framework. With this approach, the relationship between systemic toxicity becomes clearer, especially how metabolism in the liver can mediate the formation of reactive metabolites that then contribute to endocrine dysfunction in the testes. The pattern of damage increasing with dose, accompanied by hormonal suppression, also reinforces the hypothesis that chronic low-dose exposure—even below the acute toxicity threshold—can cause progressive accumulation of organ damage.

Conclusions

This study demonstrates that sub chronic oral exposure to profenofos induces coordinated hepatic and reproductive toxicity in male Wistar rats. Progressive hepatocellular degeneration accompanied by elevated serum transaminase levels confirms the liver as a primary site of toxic insult, while concurrent degeneration of seminiferous tubules and reduced Leydig-cell testosterone expression indicate impaired spermatogenesis and disrupted steroidogenesis.

The simultaneous involvement of hepatic dysfunction and endocrine impairment supports the view that profenofos toxicity is systemic in nature, arising from interconnected mechanisms that include oxidative stress, acetylcholinesterase inhibition, mitochondrial injury, and hormonal dysregulation. By integrating biochemical, histopathological, and immunohistochemical endpoints within a single experimental framework, this study clarifies the functional link between hepatic metabolism and reproductive endocrine disturbance under sub chronic organophosphate exposure.

Taken together, these findings highlight the potential health risks associated with repeated low dose profenofos exposure in environmental and occupational contexts and support the inclusion of both hepatic and endocrine biomarkers in toxicological risk assessment. This work contributes to a more comprehensive understanding of

profenofos toxicodynamics by demonstrating its multi-organ impact rather than isolated organ-specific effects.

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Conflict of interest

There is no conflict of interest

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سمية الكبد والخصية الناتجة عن التعرض للبروفينوفوس دون المزمّن في ذكور فئران ويستار

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الخلاصة

تُعدّ مبيدات الآفات الفوسفورية العضوية من القضايا البيئية والصحية المهمة نظرًا لاستخدامها الواسع وإمكاناتها السُمّية الجهازية. وقد ارتبط مبيد البروفينوفوس، وهو أحد المبيدات الحشرية الفوسفورية العضوية الشائعة الاستخدام، بآثار سلبية تتجاوز تثبيط إنزيم الكولينستيراز، إلا أن تأثيراته المزمّنة على الأعضاء الأيضية والغدد الصماء لم تُوصف بشكل كامل بعد. هدفت هذه الدراسة إلى استقصاء تأثير التعرض شبه المزمّن للبروفينوفوس على سلامة الكبد ووظيفة الغدد الصماء في الخصيتين لدى ذكور جرذان ويستار باستخدام منهج سُمّي متكامل. تم إعطاء عشرين جرّدًا بالغًا من الذكور جرعات فموية من البروفينوفوس بتركيزات ٠ و ٢٠ و ٤٠ و ٨٠ ملغ/كغ من وزن الجسم يوميًا لمدة ٦٠ يومًا. أدى التعرض للبروفينوفوس إلى إحداث أضرار كبدية تعتمد على الجرعة، تميّزت بالتشنك الفجوي، وتوسع الجيوب الدموية، ونخر الخلايا الكبدية، وارتفاع ملحوظ في مستويات إنزيمي SGPT وSGOT. وفي الوقت نفسه، أظهرت أنسجة الخصية تنكسًا تدريجيًا في الأنابيب المنوية، وانخفاضًا في كثافة خلايا لايدغ، وتراجعًا واضحًا في التفاعل المناعي لهرمون التستوستيرون، مما يشير إلى ضعف تكوين السيترويدات. وتدعم التغيرات المتوازنة البنوية والوظيفية في كلا العضوين وجود استجابة سُمّية جهازية للبروفينوفوس. ويؤدي التعرض الفموي شبه المزمّن لهذا المبيد إلى سمية كبدية وغدية صماء متكاملة، تتوسطها حالة الإجهاد التأكسدي، والعبء الأيضي، واضطراب تكوين السيترويدات. ومن خلال التقييم المتزامن لمؤشرات الكبد والخصية ضمن إطار تجريبي واحد، توفر هذه الدراسة رؤية جديدة حول الطبيعة الجهازية لسمية البروفينوفوس، وتؤكد الحاجة إلى تقييم شامل للمخاطر المرتبطة بالتعرض لمبيدات الآفات الفوسفورية العضوية.