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Subchronic Methomyl Exposure Alters Oxidative Balance, Damages Reproductive and Adrenal Tissues in Male Rats

La Exposición Subcrónica al Metomilo Altera el Equilibrio Oxidativo y Daña los Tejidos Reproductivos y Suprarrenales en Ratas Macho

Faiza Zaida[1]; Mohamed El Fadel Ousmaal[1,2]; Kahina Chabane[1,3]; M'hammed Amine Khene[1,4]; Lynda Ainouz[1,5]; Saâdia Mameri[6]; Jean Giaimis[7] & Ahsene Baz[1]

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SUMMARY: Methomyl (MET) is a carbamate pesticide whose prolonged exposure has been associated with systemic toxicity in mammals. The reproductive system and adrenal glands are among the most sensitive targets of chemical-induced oxidative imbalance. The aim of this study was to evaluate the effects of subchronic exposure to MET on oxidative stress and histological damage in the reproductive system and adrenal glands of adult male rats. Animals were randomly divided into two groups: the control group and the MET-treated group, which received a daily oral dose of MET (1mg/kg body weight) for 90 consecutive days. Histopathological analysis revealed multiple types of tissue damage characterized by degenerative and necrotic lesions in the epididymis, accessory sex glands and adrenal glands in the MET-treated group. These alterations were associated with oxidative stress, as evidenced by a significant increase in malondialdehyde, a marker of lipid peroxidation, along with a decrease in glutathione levels and reduced activity of antioxidant enzymes such as glutathione S-transferase and glutathione peroxidase in the affected organs. This study provides compelling evidence that 90-day exposure to MET induces histological damage in the reproductive system and adrenal glands, potentially mediated by disrupted redox homeostasis in these tissues.

KEY WORDS: Methomyl; Subchronic toxicity; Oxidative stress; Reproductive organs; Adrenal glands.

INTRODUCTION

In response to global food demand, pesticide use has significantly increased over recent decades, aimed at facilitating large-scale farming by protecting crops throughout their growth cycle (Meng *et al.*, 2014). Pesticides are widely employed in agriculture and gardening to enhance crop yields and ensure plant protection during development (Van Scoy *et al.*, 2013). However, these compounds may pose serious risks to human health and the environment due to their accumulation in ecosystems. Numerous studies have reported the presence and persistence of pesticides in water, soil, air, and food products (Strathmann & Stone, 2001). Several investigations have revealed high levels of these chemicals or their derivatives in the blood, breast milk, and urine of individuals who either regularly use them or live in highly exposed areas (Atallah *et al*., 2023). The risks associated with pesticide use are particularly concerning, with their effects on vital organs being a major consequence.

Methomyl (MET) is a carbamate-based insecticide widely used in agriculture for pest and insect control (Meng *et al*., 2014). This broad-spectrum insecticide is classified by the U.S. Environmental Protection Agency (EPA) as highly hazardous (Class 1B) due to its toxicity to many non-target species, including animals and humans, and its anticholinesterase activity, which poses multiple health

[1] Laboratory of Biology and Animal Physiology, Ecole Normale Superieure de Kouba, Algiers, Algeria.
[2] Laboratory of Valorization and Bioengineering of Natural Resources (LVBRN), Faculty of Sciences, University of Algiers, Benyoucef Benkhedda, Mourad, Algeria.
[3] Laboratory of Biology and Organism Physiology, University of Sciences and Technology, Houari Boumediene (USTHB), Algiers, Algeria.
[4] Department of Biology, Faculty of Natural Sciences, Life and Earth Sciences, University of Ghardaia
[5] Research Laboratory of Food Hygiene and Quality Insurance System (HASAQ), Higher National Veterinary School ENSV, Oued Smar-Algiers, Algeria.
[6] Laboratory of Anatomopathology, Mustapha Bacha Hospital, Algiers, Algeria.
[7] UMR Qualisud-Faculty of Pharmacy, University of Montpellier I.

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hazards (Van Scoy *et al*., 2013). Numerous studies have reported harmful effects of carbamates, particularly MET, including reproductive toxicity (Aboushouk *et al*., 2021), hepatic dysfunction (Ayse & Yusuf, 2025), immune system disturbances (Radad *et al*., 2009), renal damage (Sakr *et al*., 2018), digestive toxicity (Chabane *et al*., 2022), and neurological effects (Amine *et al*., 2020). Additionally, MET has been identified as a potent genotoxic agent capable of inducing in vivo DNA damage, conferring mutagenic and carcinogenic potential, and contributing to increased mortality rates (Lu & Liu, 2022).

Several studies have suggested that the toxic effects of pesticides on peripheral organs are linked to their ability to interfere with the endocrine system, particularly adrenal function (Atallah *et al*., 2023; Li & Robaire, 2025). Exposure to pesticides in rats has been shown to induce structural alterations in the adrenal glands (Kaptaner *et al*., 2024), along with increased secretion of catecholamines and glucocorticoids in the medulla and adrenal cortex, respectively (Akhtar *et al*., 2009). Such adrenal disruptions may have serious consequences for the organism, including impaired physiological stress response (Atallah *et al*., 2023). MET is considered an endocrine disruptor that affects embryonic development and reproductive cycles, even in non-target organisms (Toledo *et al*., 2019). Multiple studies in various species have demonstrated its reproductive toxicity in males, manifested as decreased fertility index, reduced weights of reproductive organs, lower serum testosterone levels, altered sperm characteristics, and tissue lesions (Aboushouk *et al*., 2021). Lipid peroxidation and reduced levels of antioxidant molecules have also been observed in male gonads following MET exposure (Mahmoud *et al*., 2024), indicating the presence of oxidative stress, a key mechanism underlying carbamate-induced toxicity. Moreover, MET exposure has been shown to impair early embryonic development in mice and fish by inducing mitochondrial dysfunction, apoptosis, and autophagy in blastocysts, thereby affecting embryo viability (He *et al*., 2022).

Pesticide exposure is widely recognized as a major factor in oxidative stress generation, primarily through the overproduction of reactive oxygen species (ROS) (Aboushouk *et al*., 2021). MET, in particular, is known for its capacity to generate excessive ROS, such as superoxide anions (O2-) and hydroxyl radicals (•OH), leading to oxidative stress and lipid peroxidation (Mahmoud *et al*., 2024). Oxidative stress induction is a primary pathway through which pesticides exert toxicity, damaging DNA, proteins, and cellular lipids (Lu & Liu, 2022). Furthermore, oxidative stress is increasingly recognized as a critical component in the pathogenesis of various disorders, including reproductive dysfunction (Mahmoud *et al*., 2024) and adrenal gland alterations (Kaptaner *et al*., 2024). Given that the male reproductive system (epididymis, seminal vesicles, and prostate) is particularly sensitive to environmental toxins (Aboushouk *et al*., 2021) we propose to investigate the effects of MET on the epididymis, accessory sex glands, and adrenal glands. This study focuses on the impact of subchronic MET exposure on oxidative stress and histopathological alterations in the reproductive tract, and adrenal glands of adult male rats.

MATERIAL AND METHOD

Chemicals

Methomyl (MET), or S-methyl N-(methylcarbamoyloxy) carbamate (CAS No. 16752-77-5), is a carbamate insecticide. A commercial formulation of MET (Lannate® 25 % WP), containing 25 % active ingredient, was purchased from a local agricultural supply store and used for the experiments. All other chemicals used in this study were obtained from Sigma Chemical Company (St. Louis, France).

Animals

The study was conducted on 20 adult male Wistar rats (8-12 weeks old; weighing 227-257 g), obtained from the Pasteur Institute (Algiers, Algeria). The animals were housed in polypropylene cages and maintained under standard laboratory conditions (temperature: 24±2 °C, 12 h light/12 h dark cycle, and 40-60 % relative humidity). They were fed standard rat pellets and had free access to food and water throughout the experiment. All procedures were carried out in accordance with the guidelines of the Algerian Association of Experimental Animal Sciences (AASEA) and were approved by the local ethics committee of Houari Boumediene University of Science and Technology (USTHB), Algeria (Approval No. 45/DGLPAG/DVA.SDA.14).

Experimental Design

After a 2-week acclimatization period, the rats were randomly assigned into two groups (n=10 per group). Control group (C): received 1 mL of distilled water orally, serving as the vehicle for MET, once daily for 90 days. Methomyl-treated group (MET): received MET orally at a dose of 1 mg/kg body weight (equivalent to 1/16 of the oral LD50) once daily for 90 consecutive days. The MET dose and the 90-day subchronic exposure duration were determined based on earlier toxicological studies (Chabane *et al*., 2022).

Organ collection

The reproductive organs and adrenal glands were carefully dissected and examined macroscopically for gross abnormalities. The organs were cleaned of fat and weighed to determine both absolute and relative weights (the latter calculated by dividing the organ weight by the body weight of the animal). A portion of each organ was fixed in 10 % neutral buffered formalin for histological evaluation. The remaining portions (epididymides, seminal vesicles, prostate, and adrenal glands) were stored at -80 °C for subsequent oxidative stress parameter assays.

Oxidative stress analysis

Preparation of tissue samples. Tissue samples from epididymides, seminal vesicles, prostate, and adrenal glands were homogenized (1:10 w/v) in cold physiological saline (0.9 % NaCl) using a Polytron homogenizer (T25 Ultra Turax; IKA-Werke, Staufen, Germany), then centrifuged at 10,000 rpm at 4°C for 20 min. Protein contents in supernatants were estimated by the procedure of Bradford (1976) using bovine serum albumin as the standard. Supernatants were aliquoted and stored at -20°C for biochemical analyses.

Determination of malondialdehyde levels. Malondialdehyde (MDA) concentration was quantified in tissue supernatant using the thiobarbituric acid (TBA) method as described by Ohkawa *et al*., (1979). A volume of 100 µL of each sample was mixed with a solution containing 375 µL of 0.8 % TBA, 375 µL of 20 % acetic acid (pH 3.5), and 50 µL of 8.1 % sodium dodecyl sulfate. After incubation in a boiling water bath for 1 h followed by rapid cooling in an ice bath, the samples were centrifuged at 3000 g for 10 min. The absorbance of the supernatant was measured at 532 nm and MDA levels were expressed as nmol/mg protein in tissues ($\varepsilon = 1.56 \times 10^5\ M^{-1} \cdot cm^{-1}$).

Estimation of reduced glutathione levels. Reduced glutathione (GSH) levels were determined according to the method of Ellman (1959), as modified by Jollow *et al*. (1974). The assay is based on the oxidation of GSH by 5,5'-dithiobis-2-nitrobenzoic acid (DTNB). For this purpose, protein precipitation was carried out by mixing 0.3 mL of tissue homogenate with of 0.25 % sulfosalicylic acid, followed by incubation at 4 °C for 1 h. After centrifugation at 3000 g for 10 min, 0.5 mL of the supernatant was collected and mixed with 0.025 mL of Ellman's reagent (DTNB, 0.01 M) and 1 mL of phosphate buffer (0.1 M, pH 7.4). The absorbance was measured at 412 nm, and GSH levels were expressed as nmol/mg protein in tissues.

Antioxidant enzyme activities

Glutathione peroxidase activity. Glutathione peroxidase (GPx) activity was determined according to the method of Flohé & Günzler (1984). For this assay, 0.3 mL of tissue homogenate was mixed with 0.2 mL of GSH (2 mM), 0.3 mL of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of sodium azide (10 mM), and 0.1 mL of hydrogen peroxide (1 mM). After incubation at 37°C for 15 min, the reaction was stopped by adding 0.5 mL of 5 % TCA. The samples were then centrifuged at 3000 g for 10 min, and 0.1 mL of the resulting supernatant was mixed with 0.2 mL of phosphate buffer and 0.7 mL of DTNB (0.4 mg/mL). Absorbance was measured at 420 nm, and GPx activity was expressed as nmol/mg protein in tissues.

Glutathione S-transferase activity. Glutathione S-transferase (GST) activity was measured following the method of Habig *et al*. (1974), which is based on the conjugation of GSH with 1-chloro-2,4-dinitrobenzene (CDNB). A mixture of 20 µL of tissue homogenate, 100 µL of GSH (0.1 M), 50 µL of CDNB (0.02 M), and 830 µL of phosphate buffer (0.1 M, pH 7.4) was used. Absorbance was measured at 340 nm initially and at 3-minute intervals.

Histological analysis

For histological examination, epididymides, seminal vesicles, prostate, and adrenal glands were fixed in 10 % formalin, dehydrated in graded ethanol, and embedded in paraffin wax using an automated tissue processor (LEICA EG 1160, Wetzlar, Germany). Sections were cut at 3 mm using a Leica rotary microtome and stained with hematoxylin and eosin (H&E) for general tissue structure assessment. Observations were made at 100× and 400× magnifications under a Zeiss Axioskop-40 light microscope, and images were captured using an Axiocam digital camera.

Statistical analysis

The normality of data distribution was assessed using the Kolmogorov-Smirnov test, and since all variables showed non-normal distribution, comparisons between groups were performed using the non-parametric Mann-Whitney U test. Results are presented as median with interquartile range (IQR), and statistical significance was set at $p < 0.05$. All statistical analyses were conducted using GraphPad InStat software (Prism 5.0, San Diego, CA).

RESULTS

Effects of subchronic MET exposure on body weight. At the end of the experiment, a non-significant decrease (P =

0.35) in body weight was observed in the MET-treated rats compared to the control group (Table I). Similarly, the percentage of body weight gain was lower in MET-exposed animals than in controls, though this difference was not statistically significant (P = 0.41).

Effects of subchronic MET exposure on organ weights. Post-treatment analysis showed no statistically significant differences in either the absolute or relative weights of the studied organs between the MET-treated and control groups (Table I).

Effects of subchronic MET exposure on oxidative stress

Effects of MET on MDA levels. As shown in Figure 1A, MET treatment significantly increased MDA levels in epididymis, and prostate by 110 % (MET: 172.4 [IQR: 129.1-194.1 nmol] vs control: 81.95 [IQR: 54.59-99.07 nmol], P = 0.002), and 152 % (MET: 150.1 [IQR: 91.97-220.9 nmol] vs control: 59.63 [IQR: 31.09-69.36 nmol], P = 0.004), respectively, compared to the control group. A highly significant elevation was also noted in the adrenal glands (MET: 103.8 [IQR: 94.24-116.3 nmol] vs control: 37.97 [IQR: 33.86-49.82 nmol], 173 %, P = 0.028). MDA levels in the seminal vesicles increased by approximately 56 %, though this change was not statistically significant (P = 0.30).

Effects of MET on GSH levels. GSH levels were moderately decreased in MET-treated rats, although the reductions were mostly non-significant in seminal vesicles (39 %, P = 0.132), and adrenal glands (113 %, P = 0.100) (Fig. 1B). Similarly, statistically significant reductions in GSH levels were observed in the epididymis and prostate by 123 % (MET: 57.54 [IQR: 46.87-66.34 nmol] vs control: 128.4 [IQR: 118.9-143.4 nmol], P = 0.0022) and 125 % (MET: 122 [IQR: 91.2-208.1 nmol] vs control: 274.7 [IQR: 214.2-344 nmol], P = 0.0087), respectively.

Effects of MET on GST activity. After 90 days of MET exposure, a significant reduction (157 %) was also recorded in the epididymis (MET: 346.3 [IQR: 230.6-368.5 nmol] vs control: 891.2 [IQR: 668.6-1291 nmol], P = 0.002). Although not statistically significant, GST activity declined in the prostate (60 %, P = 0.06), seminal vesicles (37 %, P = 0.13), and adrenal glands (89 %, P = 0.100) (Fig. 1C).

Effects of MET on GPx activity. As shown in Figure 1D, MET treatment resulted in decreased GPx activity across all examined tissues. Reductions were observed in adrenal glands (42 %, P= 0.200). Significant reductions were noted in the epididymis (101.21 %, MET: 124.2 [IQR: 102.9-148.4 nmol] vs control: 249.9 [IQR: 193.3-376 nmol], P = 0.0022) and prostate (105 %, MET: 129.8 [IQR: 106.1-176.2 nmol]

Table I. Effects of subchronic MET exposure on body weight, weights of accessory reproductive organs, and adrenal glands in rats.

	Control (C)	MET	P
Initial body weight (g)	177.2 (IQR: 150.3–197)	174.5 (IQR: 151.9–207)	0.98
Final body weight (g)	254,3 (IQR: 218–325.2)	253,1 (IQR: 205.8–286.6)	0.35
Body weight gain (%)	50.28 (IQR: 17.5–87.4)	42.77 (IQR: 17.3–61.7)	0.41
Absolute right epididymidis weight (g)	0,56 (IQR:0,30-1,48)	0,59 (IQR: 0,45-0,66)	0.19
Relative right epididymidis weight (g/100 g b.w.)	0,21 (IQR: 0,55– 0,12)	0,22 (IQR: 0,16–0,27)	0.24
Absolute seminal vesicles weight (g)	1,30 (IQR: 0,70–1,57)	1,05 (IQR: 0,75–1,60)	0.33
Relative seminal vesicles weight (g/100 g b.w.)	0,48 (IQR: 0,29–0,62)	0,42 (IQR: 0,26–0,66)	0.52
Absolute Prostate weight (g)	0,63 (IQR: 0,38–0,99)	0,53 (IQR: 0,36–0,74)	0.15
Relative Prostate weight (g/100 g b.w.)	0,24 (IQR: 0,15–0,37)	0,22 (IQR: 0,16–0,27)	0.18
Absolute right adrenal gland weight (g)	0,03 (IQR: 0,02–0,04)	0,02 (IQR: 0,03–0,01)	0.11
Relative right adrenal gland weight (g/100 g b.w.)	0,012 (IQR: 0,007–0,403)	0,009 (IQR: 0,017–0,006)	0.30

Values are presented as median with interquartile range (n = 10). Statistical significance between control and MET-treated rats was assessed using the Mann–Whitney test, and differences with $p < 0.05$ are indicated by asterisks.

vs control: 267.1 [IQR: 197.5-390.5 nmol], P = 0.0087). An 80 % decrease, which was statistically significant, was also observed in the seminal vesicles (MET: 211.3 [IQR: 127.3-329.9 nmol] vs control: 380.7 [IQR: 325.7-514.2 nmol], P = 0.041).

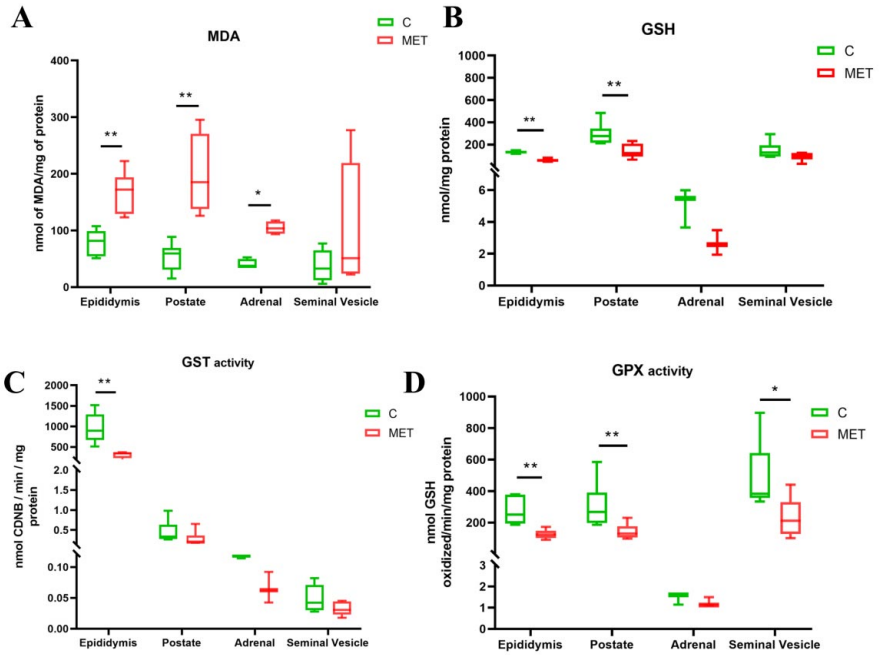

Fig. 1. Effect of MET on oxidant/antioxidant biomarkers in epididymis, accessory glands and adrenals glands of rat. A: MDA level (nmol of MDA/mg of protein). B: GSH level (nmol/mg protein). C: GST activity (nmol CDNB/min/mg of protein). D: GPx activity (nmol GSH/mg of protein). Values are given as median with interquartile range. Significant differences were analyzed using Mann-Whitney test and indicated by *P < 0.05, **P < 0.01 compared with C group (n=10).

Effects of subchronic MET exposure on the histological structure of organs

Epididymis. Control epididymis exhibited normal histological architecture and sperm density (Figs. 2A, 2C). In MET-treated rats, the caput epididymis showed hypertrophied clear cells with nuclear degeneration and increased vesicles (Fig. 2B). Metaplastic changes in epithelial lining, interstitial fibrosis, and degeneration of cauda epididymal tubules were evident. Cytoplasmic rupture, cellular debris, and abnormal round germ cells were noted in the lumen, often with reduced or absent sperm density. Severe interstitial edema and inflammatory infiltration were also detected (Fig. 2D).

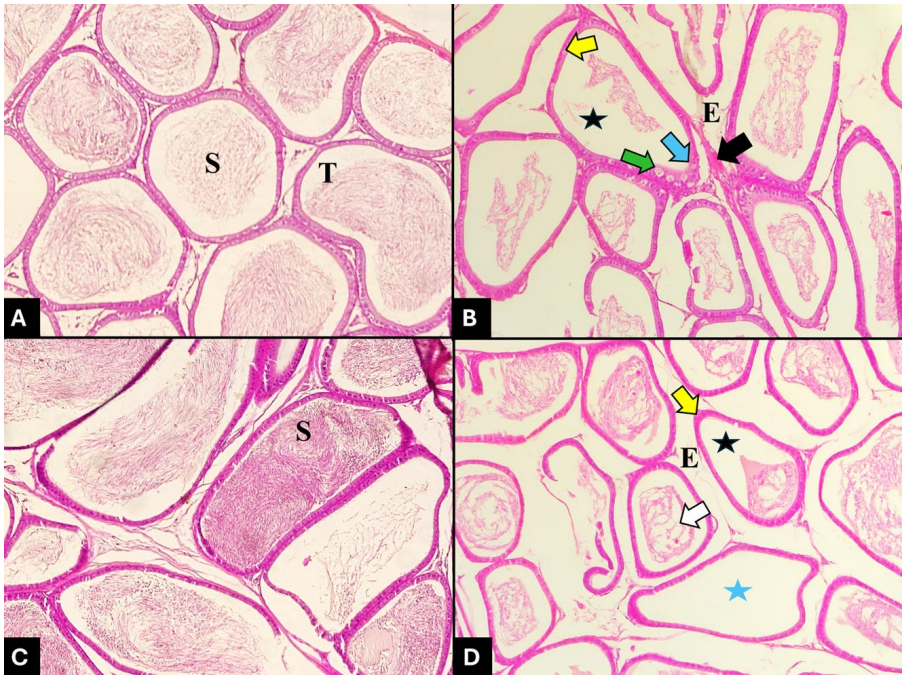

Fig. 2. Histopathological evaluation of epididymis in rats treated with MET during 90 days (X 100). A: Normal architecture of epididymal caput in C group. B: Epididymal caput of MET-treated rats. C: Normal architecture cauda epididymis in C group. D: Cauda epididymis of MET. The micrographs were stained with H&E. T: tubules; S: spermatozoa; E: oedema; black arrow: Smooth muscle layer; yellow arrow: Lining epithelium; green arrow: vacuolation of few germinal epithelial cells; blue arrow: mucosal epithelial hyperplasia; white arrow: cellular debris; black star: oligospermia; blue star: aspermia).

Prostate and Seminal vesicles. The control group's prostate showed acini lined with columnar epithelium, basal nuclei, pink secretions in the lumen, and a surrounding smooth muscle-rich stroma (Fig. 3A). In MET-treated rats, histology revealed epithelial hyperplasia, papillary projections, and reduced luminal secretion (Fig. 3B). The interstitial space appeared expanded with infiltration of mononuclear inflammatory cells.

In controls, seminal vesicles displayed normal folded columnar epithelium, a fibro-muscular layer, and secretory material in irregular lumina (Fig. 3C). MET exposure led to epithelial atrophy, detachment, structural disorganization, and fold destruction. The fibro-muscular layer showed edema and vacuolization (Fig. 3D).

Adrenal glands. Control adrenal glands exhibited typical histological features: an outer dense connective capsule, cortex (zona glomerulosa, fasciculata, reticularis), and inner medulla (Fig. 4A). In MET-exposed rats, cellular disorganization was observed in all three cortical zones. The capsule appeared swollen and partially detached due to subcapsular edema (Figs. 4B). Zona glomerulosa lost its ovoid cluster arrangement, while zona fasciculata cells were disordered and enlarged with lipid droplet accumulation. Dilated blood vessels and fibrous connective tissue surrounding endocrine clusters were evident (Fig. 4C). The zona reticularis contained vacuolated, balloon-like cells. The adrenal medulla exhibited hemorrhages and severe necrosis, with infiltration by inflammatory cells, blood clots, and cell debris (Fig. 4E).

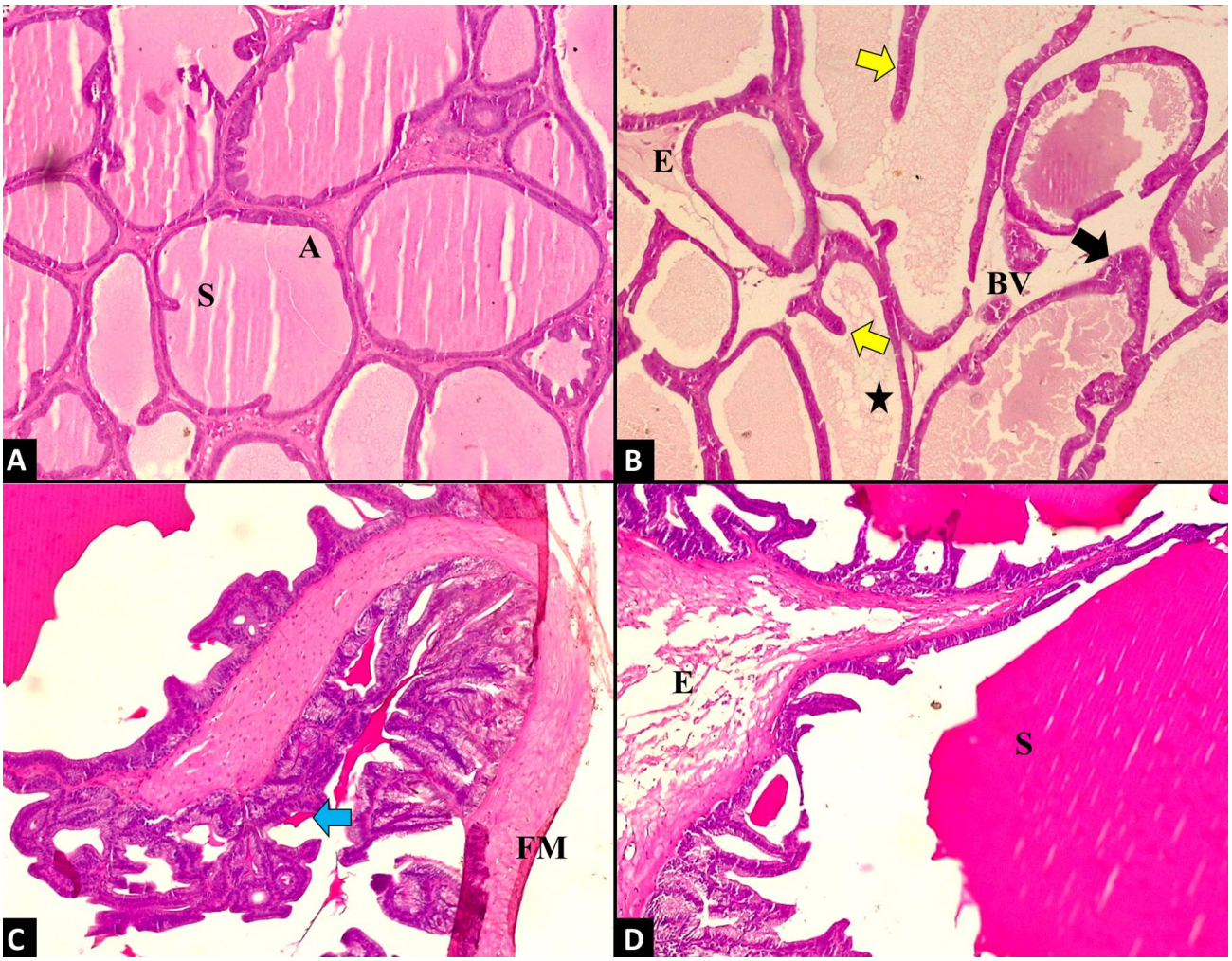

Fig. 3. Histopathological evaluation of accessory glands (prostate, vesicular seminalis) in rats treated with MET during 90 days (X 100). A: Prostate gland of the C group. B: Prostate gland of MET-treated rat. C: The seminal vesicles of the C group. D: The seminal vesicle of rat exposed to MET. The micrographs were stained with H&E. A: acicni; E: oedema; S: secretions; FM: fibro-muscular layer; black arrow: inflammatory cells; yellow arrow: papillary folds; blue arrow: mucosal folds; black star: few secretions.

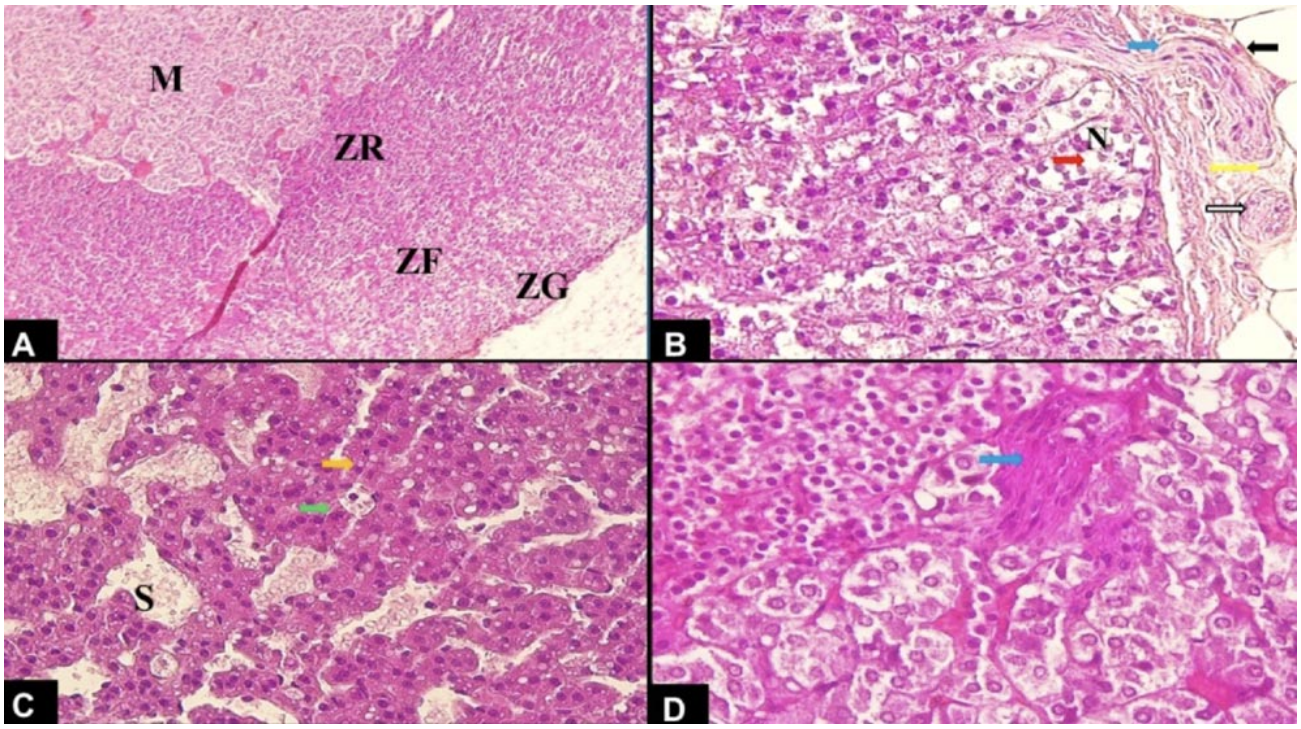

Fig. 4. Histopathologic changes of adrenal gland in MET-treated rats for 90 days. A (X100): adrenal glands of the C group. B (X400) the zona glomerulosa of MET-treated rats. C (X400): the zona fasciculata of MET. D (X400) the adrenal medulla of the MET. The micrographs were stained with H&E. ZG: zona glomerulosa; ZF: zona fasciculata; ZR: zona reticularis; M: medulla; yellow arrow: Lamellar separation of the capsule and subcapsular spindle cell hyperplasia; Red arrow: disturbed arrangement of ZG cells with ballooned vacuoles; blue arrow: mononuclear cell infiltration; white arrow: nodular hyperplasia; orange arrow: lipid droplets; green arrows: Ballooned vacuole; black arrow: haemorrhagic necrosis sites; N: multiple pleomorphic nuclei, S: sinusoidal capillaries.

DISCUSSION

This study investigated the impact of subchronic methomyl (MET) exposure (1 mg/kg b.w.) on oxidative stress parameters and histopathological alterations in epididymis, accessory sex glands and adrenal glands in adult male rats.

In toxicological research, changes in body and organ weights are widely used as indicators of general health deterioration and compound toxicity. Our findings suggest that MET adversely affects rat growth, as evidenced by a reduced body weight gain in treated rats compared to the control group. These results are consistent with previous studies reporting weight reduction after exposure to various carbamate compounds (MET, carbosulfan, carbofuran, thiodicarb) in rats (Al-Shinnawy, 2008; Chabane *et al*., 2022). The limited weight gain observed may be attributed to appetite suppression, reduced food intake, or metabolic and hormonal disturbances (Al-Shinnawy, 2008). In fact, Li *et al*. (2024), reported that subchronic MET exposure in zebrafish led to impaired energy metabolism, notably via downregulation of isocitrate dehydrogenase activity and acetyl-CoA accumulation.

Our results also showed decreased absolute and relative weights of the epididymis and accessory sex glands (seminal vesicles, prostate) and adrenal glands. These findings align with studies by Aboushouk *et al*., (2021) and Atallah *et al*. (2023), who reported decreased reproductive organ weights following pesticide treatment. Similar alterations in adrenal gland weights have also been documented (Akhtar *et al*., 2009; Li & Robaire, 2025).

Previous studies have shown that pesticide toxicity is often linked to ROS overproduction, leading to lipid peroxidation and cellular damage in tissues including the epididymis, brain, and adrenal glands (Atallah *et al*., 2023; Li & Robaire, 2025). Indeed, carbamate pesticides and their metabolites promote lipid peroxidation, leading to membrane damage and enzyme inactivation (Mahmoud *et al*., 2024). The rise in MDA levels following MET exposure may result from the insecticide or its metabolites interacting with polyunsaturated fatty acids (PUFAs) (Mahmoud *et al*., 2024), leading to ROS overproduction that disrupts cellular functions including steroidogenesis and gametogenesis

(Yang *et al.*, 2025). These ROS, including superoxide anions, hydroxyl radicals, and hydrogen peroxide, enhance reproductive vulnerability, disrupt the tricarboxylic acid cycle, and damage essential biomacromolecules such as mitochondrial DNA, proteins, and lipids, ultimately causing apoptosis and structural disorganization in cells (Aboushouk *et al*., 2021). Furthermore, several studies have shown that MET induces oxidative damage via downregulation of Nrf2 and its downstream antioxidant genes (HO-1, NQO1), while upregulating apoptotic markers such as Bax and caspase-3 (Hassanen *et al*., 2025). These transcriptional disturbances impair cellular redox balance and promote mitochondrial dysfunction, which may explain the histopathological lesions observed in the present study.

Histopathological changes combined with oxidative stress indices are increasingly recognized as biomarkers of pesticide toxicity. Many studies have demonstrated that chronic MET exposure causes degeneration of male reproductive organs (Hassanen *et al*., 2025). Our data show that MET induces significant elevation in MDA and reduction of GSH and enzymatic antioxidants (GST, GPx) in reproductive tissues. These results echo those of Aboushouk *et al*. (2021), who found a marked increase in the epididymis MDA in MET-treated rats. Similar oxidative stress imbalances have also been reported in the prostate (Soerensen *et al*., 2025), and seminal vesicles (Mesallam *et al*., 2025) in pesticide-exposed rats.

Histological analysis revealed major structural damage in the reproductive organs of MET-exposed rats, including hypertrophy of epithelial cells in the epididymal head, tissue disorganization in the cauda, atrophy in seminal vesicle glands, and epithelial hyperplasia in the prostate. Aboushouk *et al*. (2021), confirmed chronic pesticide exposure caused epididymal degeneration in male rats. Our findings are also consistent with Hassanen *et al*. (2025), who observed several histopathological lesions in rats exposed to chlorpyrifos. However, Adamkovicova *et al*. (2014), found no such damage after 90 days of diazinon exposure. The differential response may stem from varying pesticide properties, treatment duration, dosage, administration route, or animal sensitivity.

Another important aspect is that the prostate is particularly vulnerable to pesticide-induced toxicity due to its hormonal dependence, lipid-rich composition, and active xenobiotic metabolism. Epidemiological data link occupational pesticide exposure, especially in agricultural workers, to a higher risk of prostate cancer (Soerensen *et al*., 2025). Several pesticides, including carbamates, disrupt androgen signaling and hormonal homeostasis, while the gland's high PUFAs content increases susceptibility to lipid peroxidation and oxidative stress. Experimental evidence further indicate that pesticide-induced prostatic toxicity involves both ROS-mediated and androgen-dependent mechanisms (Soerensen *et al*., 2025). Altogether, these findings highlight oxidative stress and endocrine disruption as key mechanisms underlying pesticide-related prostate injury.

Alongside epididymal histology, adrenal gland examination revealed several alterations in MET-treated rats, including disorganization and destruction of both medullary and cortical cells. These findings may be related to oxidative imbalance within the adrenal glands. MDA levels were significantly elevated in adrenal tissues, while GPx, GST, and GSH levels were slightly but non-significantly decreased compared to controls. Previous studies have shown that chronic chlorpyrifos or subchronic atrazine exposure induces oxidative stress in adrenal glands (Kaptaner *et al*., 2024) supporting the notion that oxidative damage underlies the adrenal histopathology observed. Similar histological changes in adrenal glands, such as cell disorganization, vacuolization, and inflammation, have been reported by Atallah *et al*., (2023) and Li & Robaire (2025). However, Akhtar *et al*. (2009), reported no adrenal damage with certain pesticides, suggesting variability based on treatment conditions. Moreover, MET-treated rats showed inflammatory infiltration in the adrenal medulla and cortical disorganization, particularly in the zona glomerulosa and zona fasciculata. These observations are consistent with the findings of Li & Robaire (2025). Neutrophil infiltration, fibrosis, and adrenal hyperplasia observed in MET-exposed rats, possibly reflecting immune system activation and systemic stress (Radad *et al*., 2009).

CONCLUSION

In conclusion, this study highlights the detrimental effects of MET on male reproductive physiology and adrenal gland integrity. It emphasizes the need for preventive and therapeutic measures against MET-induced toxicity, particularly in the context of public health. Given the risks posed by pesticides, cautious and controlled use is imperative. A major challenge in modern agriculture is to maintain food production while minimizing environmental and health hazards. To achieve sustainable agroecosystems, pesticide use must be reduced through crop system diversification. If current agricultural practices persist, irreversible environmental damage may occur. In this context, biocontrol offers a promising alternative to promote ecological intensification and reduce health risks.

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RESUMEN: El metomilo (MET) es un plaguicida carbamato cuya exposición prolongada se ha asociado con toxicidad sistémica en mamíferos. El sistema reproductivo y las glándulas suprarrenales se encuentran entre los órganos más sensibles al desequilibrio oxidativo inducido por sustancias químicas. El objetivo de este estudio fue evaluar los efectos de la exposición subcrónica al MET sobre el estrés oxidativo y el daño histológico en el sistema reproductivo y las glándulas suprarrenales de ratas macho adultas. Los animales se dividieron aleatoriamente en dos grupos: el grupo control y el grupo tratado con MET, que recibió una dosis oral diaria de MET (1 mg/kg de peso corporal) durante 90 días consecutivos. El análisis histopatológico reveló múltiples tipos de daño tisular caracterizados por lesiones degenerativas y necróticas en el epidídimo, las glándulas sexuales accesorias y las glándulas suprarrenales en el grupo tratado con MET. Estas alteraciones se asociaron con estrés oxidativo, evidenciado por un aumento significativo de malondialdehído, un marcador de peroxidación lipídica, junto con una disminución de los niveles de glutatión y una actividad reducida de enzimas antioxidantes como la glutatión S-transferasa y la glutatión peroxidasa en los órganos afectados. Este estudio proporciona evidencia contundente de que la exposición a MET durante 90 días induce daño histológico en el sistema reproductivo y las glándulas suprarrenales, potencialmente mediado por una alteración de la homeostasis redox en estos tejidos.

PALABRAS CLAVE: Metomilo; Toxicidad subcrónica; Estrés oxidativo; Órganos reproductivos; Glándulas suprarrenales.

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Corresponding author:
Faiza Zaida
Laboratory of Biology and Animal Physiology
Department of Natural and Life Sciences
Ecole Normale Superieure de Kouba
Algiers
ALGERIA

E-mail: faiza.zaida@g.ens-kouba.dz