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Toxicological Impacts of Certain Herbicides on Reproductive System and Thyroid Gland in Male Rats

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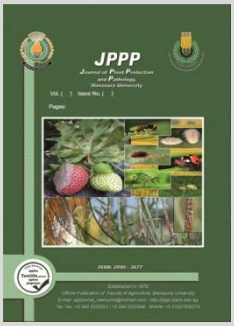

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ABSTRACT

This study examined the effects of four commonly used herbicides (Glyphosate, Dicamba, S-metolachlor, and Cyhalofop-butyl**)** on male rats. Animals were orally administered 1/10 LD_{50} of the tested herbicides for four weeks /three times a week, at the end of the experiment, biochemical, hormonal, sperm, and thyroid parameters were investigated. The results indicated that exposure to tested herbicides increased malondialdehyde (MDA) levels. cyhalofop-butyl showed the highest value (12.64 nmol/mL) compared to the control given oil only (4.12 nmol/mL). Antioxidant enzymes such as catalase and superoxide dismutase (SOD**)** were significantly reduced in all treated groups, especially in the cyhalofop-butyl group (8.76 ng/mL and 7.86 U/mL, respectively). Reproductive hormones were also markedly decreased. The cyhalofop-butyl group had the lowest testosterone (2.44 ng/mL), luteinizing hormone (1.76 mIU/mL), and follicle-stimulating hormone (1.44 mIU/mL) compared to untreated group (testosterone: 4.5 ng/mL in oil; LH: 3.04 mIU/mL; FSH: 2.44 mIU/mL). Sperm quality was dramatically affected, with significant decreases in progressive motility, increased dead and abnormal sperm, and lower the total number of sperms, particularly in dicamba and cyhalofop-butyl treated rats. Additionally, thyroid hormone profiles were disrupted, where glyphosate and Dicamba increased T3 and T4 levels (T3: 2.18 - 4.02 T4: 14.2–17.34 ng/mL respectively), reducing TSH (0.31and 0.33 mIU/mL respectively), suggesting possible hyperthyroid-like effects. The most important findings demonstrated that the tested herbicides, especially cyhalofop-butyl and dicamba, can cause serious oxidative, hormonal, and reproductive damage in male rats. These findings raise concerns about the endocrine-disrupting potential of these herbicides and the need for more regulatory evaluation and safer alternatives.

Keywords: reproductive system, endocrine disorder, herbicides

INTRODUCTION

Herbicides play an essential role in agriculture systems globally by effectively eliminating unwanted plant species and improving crop yields. Among the most widely used herbicides are glyphosate**,** dicamba**,** s-metolachlor**,** and cyhalofop-butyl**,** each one of them valued for its selective action and commercial availability. However, their widely spread usage has led to many significant human and environmental health issues, such as environmental persistence**,** bioaccumulation, and chronic exposure which induce adverse health effects in agricultural workers, consumers and ecosystems. (Gad, *et al.,* 2022; Mesnage *et al*., 2013)

Glyphosate [N(phosphonomethyl) glycine] is one of the most widely used herbicide (Dayan, *et al*., 2020). It is an organophosphorus compound; it is a non-selective systemic biocide with broad-spectrum activity and it was introduced in 1974 for the control of weeds (Duke, 2018). In agriculture, it is mainly used to protect soybean, corn, cotton, and pasture crops. It may also be used as a plant growth regulator that hastens certain grains and legumes harvest when applied as a drying agent. Non agricultural uses of glyphosate include forestry, house maintenance, and vegetation control on industrial lands and transportation routs (such as train tracks and highways) (Équiterre [Internet])

Dicamba, $C_8H_6C_{l2}O_3$, is another globally used herbicides, a post-emergence broad-leaf benzoic acid (3,6-dichloro-2 methoxybenzoicacid) (CASAFE 2019). It is chlorinated derivatives, this herbicide was developed to control glyphosate resistant strains weeds and it is widely used on lawns, grasslands, and several crops (maize, rice, cotton).

Cyhalofop-butyl, $C_{20}H_{20}FNO_4$, was first developed by Dow AgroSciences in 1987, which is an aryloxyphenoxypropionate (APP) herbicide (phenoxy herbicide) widely used in paddy fields all over the world (Bleau *et al*., 1996; Cao *et al.*, 2016).

S-metolachlor (SM) (chloroacetanilide) is the ISO common name for a reaction mixture of 80–100% 2-chloro-2′-ethyl-*N*-[(1 *S*)-2-methoxy-1-methylethyl]-6′-methylacetanilide and 20–0% 2-chloro-2′-ethyl-*N*-[(1*R*)-2-methoxy-1-methylethyl]-6′-methylacetanilide (IUPAC) (Authority, *et al.*, 2023). s-metolachlor stimulates oxidative stress, cytotoxicity, and developmental toxicity in non-target organisms. (Liu, and Xiong 2009) and (Greenlee, *et al.,* 2004).

In spite the fact that herbicides are widely used in modern agriculture to control weed, concerns have been growing over their potential to act as endocrine-disrupting chemicals (EDCs), the compounds that interfere with hormonal systems in the body. Furthermore, studies have shown that these compounds can disrupt the balance of reproductive hormones like testosterone**,** luteinizing hormone (LH)**,** and follicle-stimulating hormone (FSH), which are essential for male fertility and reproductive health (Gore *et al.,* 2015; Mnif *et al.,* 2011). These damages may reduce sperm production, damage testicular tissue, and impair fertility (Mesnage *et al.,* 2013). These outcomes are often linked to

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oxidative stress, which refers to an imbalance between the production of free radicals and the body's ability to neutralize them. This access of free radical might attack cells and tissues, including those in the testes (Aitken and Roman, 2008).

Although research on the thyroid-disrupting effects of herbicides is still limited, there is growing evidence that these chemicals may also disturb the hypothalamic–pituitary–thyroid (HPT) axis **(**the system that controls thyroid hormone production). Some investigations have report that exposed agricultural workers experienced an increase in T4 and decreased in T3 levels (Gasnier *et al.,* 2009; Romano *et al.,* 2010). These findings raise new concerns about how herbicides might affect both metabolism and reproductive function through their effects on thyroid hormones.

Therefore, the present study aims to evaluate and compare the toxicological effects of four different herbicides glyphosate, dicamba, S-metolachlor**,** and cyhalofop-butyl, which are commonly used locally, glyphosate, dicamba, s-metolachlor**,** and cyhalofop-butyl, on both male reproductive parameters and thyroid hormone profiles of male rats. Moreover, the current study tested the impact of tested herbicides on oxidative stress biomarkers malondialdehyde (MDA) and antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), hormonal profiles (testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH), sperm motility, viability, morphology, and count, alongside measurements of T3 (triiodothyronine), T4 (thyroxine), and TSH (thyroid-stimulating hormone)**.** The aim of this study is to better understand not only the direct reproductive effects of these herbicides, but also how thyroid disruption might be involved in reproductive dysfunction.

MATERIAL AND METHODS

Tested materials

Glyphosate 48%, its chemical name is N-(phosphonomethyl) glycine, trade name is Roundup, and the molecular formula is $C_3H_8NO_5P$. Dicamba 48%, its chemical name is 3,6-dichloro-2-methoxy benzoic acid, the trade name is Weedmaster . and the molucular formula $C_8H_6Cl_2O_3$. S-metolachlor 96%, its chemical name is 2-chloro-N-(2-ethyl-6-methylphenyl)-N-[(2S)-1-methoxypropan-2-yl] acetamide, the trade name is Dual Magnum, and the molecular formula is $C_{15}H_{22}C1NO_2$. Cyhalofop-buty 10%, its chemical name is butyl (2R)-2-[4-(4-cyano-2-fluorophenoxy) phenoxy] propanoate, the trade name is Clincher 10 EC, and the molecular formula is $C_{20}H_{20}FNO_4$. The herbicides formulations that were selected for the current study are widely used in Egypt and they obtained from the Central Laboratory for Pesticide, Egypt, they were supplied from Syngenta Company (USA).

Ethical Statement

This study's experimental protocol and animal care followed the ethical standards of the Faculty of Agriculture, Mansoura University, Egypt. The study's ethical approval code number is MU-ACUC(AGR.R24.09.10).

Animals and treatments

Male waster rats weighing 180 ± 20 g were inspected prior to the experimental period and judged to be healthy by a licensed veterinarian. They were maintained under temperature-controlled conditions (25 ◦C), and a normal photoperiod of 12h of darkness and 12h of light during the period of the experiment. Animals were randomly divided into six groups of five rats for each. The first and the second groups were maintained untreated throughout all the experimental period and received corn oil and saline, respectively (control groups), according to the solubility of the herbicides. The third, fourth, fifth and sixth groups received orally 1/10 of the LD_{50} (WSSA,1994 and U.S. National Library of Medicine1995) of formulated form of glyphosate (LD_{50} 5000 mg/kg dissolved in saline), dicamba (LD_{50} 2000 mg/kg dissolved in saline), s-metolachlor (LD_{50} 2000 mg/kg dissolved in corn oil) and Cyhalofop-butyl (LD_{50} 2000 mg/kg dissolved in corn oil) respectively, three times a week for four weeks. Throughout the investigation, clinical evaluation and body weight measurements were performed once a week. Accordingly, dosages of herbicides were daily prepared and adjusted weekly for body weight changes and given at approximately the same time each morning. Animal procedures and management protocols were carried out according to the Animal Welfare Policy of pesticides department, Faculty of agriculture, Mansoura University. At the end of the experimental period, rats were starved overnight and then scarified under slight ether anesthesia.

Sample collection and preparation of subcellular fractions.

Before sacrifice, a sample of blood was taken to determine biochemical parameters in serum. Then, the abdomen was opened, the epididymis was taken to assess sperm parameters and the testes were removed for histopathological analysis

Biochemical parameters

Lipid peroxidation marker malondialdehyde (MDA) and antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were measured in blood serum using the following kits, respectively: MD 2529, CA 2517, and SD 2521. All kits were purchased from Biodiagnostic, Giza, Egypt.

Hormonal parameters

Testosterone levels in serum, along with follicle-stimulating hormone (FSH) and luteinizing hormone (LH), were measured using ELISA kits. The testosterone ELISA kit was obtained from MyBioSource, Southern California, San Diego, USA (Catalog No: MBS766199), while the FSH (Catalog No: CSB-E06869r) and LH (Catalog No: CSB-E12654r) kits were purchased from Cusabio. All assays were performed according to the manufacturers' instructions.

Assessment of Sperm Parameters (Motility and Morphology)

In this study, semen samples were collected from the cauda epididymis for analysis. To assess progressive sperm motility (PM%), the samples were diluted with 0.9% NaCl. Three microscopic fields per sample were examined using a phase-contrast microscope (Leica DM500, Leica Mikrosysteme Vertrieb GmbH) at 37 °C. To evaluate sperm viability and morphology, the same semen samples (300 sperm cells per sample) were stained with a mixture of 10% nigrosine and 5% eosin. Live (unstained), dead (stained), and morphologically abnormal spermatozoa were then identified under contrast microscopy at 400× magnification according to (Bearden and Fuquay (1980)).

Thyroid Hormones Evaluation

Serum concentrations of T3 (triiodothyronine), T4 (thyroxine), and TSH (thyroid-stimulating hormone) were

estimated utilizing the Enzyme-Linked Immunosorbent Assay (ELISA). The commercial kits of ELISA from purchased from Monobind Inc. (USA), following the manufacturer's instructions. Absorbance was informed with a microplate reader, then the hormone levels were deliberated using standard curves.

Histopathological Examination

Some of the right-side testes from various groups were fixed in Bouin's solution for 24 hours described by Zhen *et al.,* . The samples were then processed through a series of increasing alcohol concentrations, followed by treatment with xylene and embedding in paraffin. Sections of 5 µm thickness were cut from the paraffin blocks, deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) for histological examination of the seminiferous tubules under a light microscope.

Statistical analysis

Five animals were included in each experimental group (control and treatment groups), and the results are presented as the mean ± standard deviation (SD). Statistical analysis was conducted using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test at a 5% significance level, utilizing Costat Statistical Software (1990).

RESULTS AND DISCUSSION

The effect on biochemical parameters

The results in table (1) reveale that all tested herbicides (glyphosate, dicamba, s-metolachlor, and cyhalofop-butyl) caused significant alterations in oxidative stress markers and reproductive hormone levels compared to the control groups. cyhalofop-butyl showed the most significant oxidative damage, with malondialdehyde (MDA) levels at 12.64 nmol/mL, catalase at 8.76 ng/mL, and superoxide dismutase (SOD) at 7.86 U/mL. These data suggested that cyhalofop-butyl may strongly promote lipid peroxidation as well as weaken enzymatic antioxidant defenses. glyphosate also induced oxidative stress, reflected in MDA levels of 11.20 nmol/mL, catalase at 10.06 ng/mL, and SOD at 8.92 U/mL. dicamba had similar oxidative outcomes, with malondialdehyde (MDA) at 10.70 nmol/mL, catalase at 9.42 ng/mL, and SOD at 8.84 U/mL. s-metolachlor, while still inducing oxidative imbalance, presented slightly lower MDA (9.50 nmol/mL) and somewhat higher catalase (10.24 ng/mL) and SOD (8.70 U/mL) levels.

The alterations in oxidative stress are most likely caused by chemical toxicity Huang, *et al*., (2018). While creatures digest toxic compounds, they will produce a huge number of reactive oxygen species (ROS), such as superoxide free radicals, hydrogen peroxide, etc. These reactive oxygen species will damage proteins, lipid membranes, DNA bases, and other substances in the creature, end with cell damage and apoptosis. The most important job of antioxidant enzyme is to balance the active oxygen in creature and bypass the oxidative damage Cerqueira and Fernandes (2002). It has been demonstrated that SOD and catalase are crucial enzymes in biological defense system, which can scavenge excessive reactive oxygen species (ROS). Antioxidant enzymes are sensitive to oxidative stress thus, they are often used as biological indicators to examine whether the body is under oxidative stress or not. In addition, MDA content is an important marker of oxidative stress (Monteir *et al*., (2006); Dogan, *et al.,* (2011)). Moreover, this study measured the activity of MDA, catalase, and SOD to examine the alterations of oxidative stress of male rats exposed to the selected herbicides. The data revealed that the content of MDA increased, and the activity of catalase and SOD decreased. Therefore, the level of oxidative stress elevated with the tested herbicides exposure, suggesting that tested herbicides could induce oxidative stress in male rats.

Moving to the effect of tested herbicides on reproductive hormones, notably, cyhalofop-butyl again caused the most severe hormonal suppression, with testosterone at 2.44 ng/mL, luteinizing hormone (LH) at 1.76 mIU/mL, and follicle-stimulating hormone (FSH)at 1.44 mIU/mL. Dicamba resulted in testosterone at 2.72 ng/mL, LH at 1.98 mIU/mL, and FSH at 1.52 mIU/mL. glyphosate-treated rats showed testosterone at 3.00 ng/mL, LH at 2.14 mIU/mL, and FSH at 1.75 mIU/mL. s-metolachlor had the least hormonal impact, with testosterone at 3.12 ng/mL, LH at 2.14 mIU/mL, and FSH at 1.72 mIU/mL.

Table 1. the effect of the tested herbicides on some biochemical parameters and reproductive hormones.

Treatments	Catalase (CAT) (ng/mL)	malondialdehyde (MDA) (nmol/mL)	Superoxide dismutase SOD(U/mL)	Testosterone (ng/mL)	luteinizing hormone (LH)(mIU/mL)	follicle-stimulating hormone FSH(mIU/mL)
Glyphosate	10.06^{c}	11.20^{b}	8.92^{c}	3.00^{d}	2.14^{c}	1.75^{c}
Dicamba	9.42^{d}	10.70^{b}	8.84^{cd}	2.72^{e}	1.98^{d}	1.52^{d}
S-metolachlor	10.24^{b}	9.50^{c}	8.7^{d}	3.12^{c}	2.14^{c}	1.72^{c}
Cyhalofop-butyl	8.76^{e}	12.64^{a}	7.86^{e}	2.44^{f}	1.76^{e}	1.44^{d}
Control Oil	15.08^{a}	4.12^{e}	19.00^{a}	4.5^{b}	3.04^{b}	2.44^{b}
Control Saline	15.16^{a}	5.24^{d}	18.16^{b}	4.74^{a}	3.3^{a}	2.78^{a}
LSD	0.0859	0.5520	0.1979	0.0923	0.0825	0.0900

Data are expressed as the mean ± standard deviation (SD) based on five animals in each group. Superscripts within the same column indicate significant **differences between groups ($p < 0.05$)**

Statistically, significant differences ($p < 0.05$) reflected by the LSD values confirm that herbicide exposure to tested herbicides can cause disruption in oxidative balance and suppresses reproductive hormones, with cyhalofop-butyl applying the most severe effect, followed by dicamba, glyphosate, and s-metolachlor in descending order regarding toxicity. These results agree with other publications which showed that herbicides can cause reactive oxygen species formation and decreasing antioxidants and finally led to oxidative damage in different tissues including testes Tang., *et al.,* (2017); Sule *et al.,* (2022). Thus, oxidative stress appears to be a vital key pathway where these herbicides exert reproductive toxicity. In a 12-week study administering Roundup® (a glyphosate-based herbicide), significant reductions in serum testosterone, LH, and FSH were reported, linked with decreased sperm quality and histopathological

changes (Owagboriaye, *et al.,* 2017). More notably, Cheng*, et al*., (2021) revealed that cyhalofop-butyl exposure might dramatically elevate oxidative stress in zebrafish larvae. These findings closely match our outcomes.

In contrast, Mathias, *et al.,* (2012) stated that metolachlor caused an increase in serum concentrations of testosterone, and FSH but did not alter the LH level. This data differs slightly from our data, where testosterone and gonadotropins were suppressed, this might suggest dosage, developmental stage, or formulation differences may account for these contradictions.

Effect of tested herbicides on sperm parameters.

The data in table (2) clearly shows that exposure to the tested herbicides (glyphosate**,** dicamba**,** s-metolachlor, and cyhalofop-butyl) had a significant impact on sperm quality in male rats when compared to the control groups. All herbicide-treated groups showed significant reduction in progressive motility **(**PM%**),** increased in dead and abnormal sperm, and decreased sperm count, hence, this indicated a malfunction in male rats' reproductive system.

In control group, over 60% of sperm showed progressive motility, specifically, 64.6% in the saline group and 60.6% in the oil group. But this dropped sharply in treated animals. The most dramatic reduction was observed in rats treated with cyhalofop-butyl, where motility fell to just 11.2%, followed by dicamba at 14.6%, and S-metolachlor at 18.4%. Even Glyphosate, often regarded as a milder herbicide in this case, significantly reduced motility to 25.0%. This severe decline reflects compromised sperm function, making fertilization process is less likely to happen.

It was also noticed that there was an increase in the number of dead and abnormal sperm in herbicide-treated rats. Again, cyhalofop-butyl and Dicamba caused the most damage, increasing both dead and abnormal sperm rates well far away from those in the control groups. These types of modifications are considerable because they suggest that the herbicides are not only affecting movement, but also the structural integrity and the time being alive of the sperm cells Duan, *et al.,* (2022).

Sperm production was also affected, the rats in the saline control group had an average count of 180.2 $\times 10^6$, and those in the oil group had 174.4 $\times 10^6$. By contrast, the sperm count in cyhalofop-butyl-treated rats dropped to 96.2 $\times 10^6$, with dicamba at 100.6 $\times 10^6$ and glyphosate at 122.4 $\times 10^6$. Even s-metolachlor, though slightly less harmful, caused 134.2 $\times 10^6$.

These consistent declines in motility PM %, viability, and sperm count suggest that the tested herbicides damage spermatogenesis, this might happen through oxidative stress mechanisms and endocrine interference. The most sever action belong to cyhalofop-butyl, followed by dicamba and glyphosate, also aligns with our outcomes related to oxidative and hormonal biomarkers.

These results matching with the outcomes from Owagboriaye, *et al.,* (2017), who notified a motility (PM) reduction following Roundup exposure. Moreover, ÖZGÜR, (2025) found matching results regarding sperm quality parameters when he tested the effect of Glyphosate on a type of fish called Capoeta trutta in the Upper Euphrates Basin. According to the results, the sperm quality parameters were decreased significantly. Notably, our findings also are in line with research by Akande, *et al.,* (2024) who documented that Pesticide exposure significantly impairs sperm parameters in Wistar rats, including motility, viability, and count, and increases abnormal morphology. Another study is done by Duan, *et al.,* (2022) who found that the fertility of the male of zebrafish (Danio rerio) decreased when exposed to (0.1, 1 and 10 ug/L of cyhalofop-butyl.

On the other hand, Fernandes*, et al.,* (2007) found that there was no any considerable change between the treated groups and the untreated group in plasma testosterone concentrations, sperm morphology, parameters of sperm production every day, and sperm reserves in the epididymis or any sexual parameters that measured in male rats when he treated the rats with 125 or 250 mg/kg of diuron per day for one month.

Table 2. Effects of Selected Herbicide Exposure on Sperm Quality Indicators in Male Rats.

Treatments	progressive motility (PM)% (Mean ± SD)	Dead Sperm% (Mean ± SD)	Abnormal Sperm% (Mean ± SD)	Sperm count (mean± SD ($\times 10^6$))
Glyphosate	$25.0^{(e)} \pm 1.22$	$25.0^{(e)} \pm 1.22$	$25.0^{(e)} \pm 1.22$	$122.4^{d} \pm 1.82$
Dicamba	$14.6^{(d)} \pm 0.55$	$14.6^{(d)} \pm 0.55$	$14.6^{(d)} \pm 0.55$	$100.6^{e} \pm 0.89$
S-metolachlor	$18.4^{(d)} \pm 1.52$	$18.4^{(d)} \pm 1.52$	$18.4^{(d)} \pm 1.52$	$134.2^{c} \pm 0.84$
Cyhalofop-butyl	$11.2^{(e)} \pm 1.25$	$11.2^{(e)} \pm 1.25$	$11.2^{(e)} \pm 1.25$	$96.2^{f} \pm 1.30$
Control Oil	$60.6^{(b)} \pm 0.89$	$60.6^{(b)} \pm 0.89$	$60.6^{(b)} \pm 0.89$	$174.4^{b} \pm 2.51$
Control Saline	$64.6^{(a)} \pm 0.55$	$64.6^{(a)} \pm 0.55$	$64.6^{(a)} \pm 0.55$	$180.2^{a} \pm 0.84$
LSD	1.50	1.50	1.50	2.074192

Data are expressed as the mean ± standard deviation (SD) based on five animals in each group. Superscripts within the same column indicate significant differences between groups ($p < 0.05$)

Effect on thyroid gland

The data in table (3) clearly shows that herbicide exposure significantly disrupt the delicate balance of thyroid function in male rats. Animals treated with tested herbicides showed elevated levels of T3 and T4, alongside an obvious drop in TSH levels, compared to the control groups.

One of the most damaging treatments was cyhalofop-butyl. It raised T4 to 18.34 ng/mL and **T3** to 4.34 ng/mL, while suppressing TSH to 0.25 mIU/L. These changes suggest a hyperthyroid-like state, where the thyroid is overstimulated, but the regulatory hormone (TSH) is downregulated most likely as a feedback response to excessive thyroid hormone levels. A similar hormonal pattern was observed with Dicamba, which also elevated T3 (4.02 ng/mL) and T4 (17.34 ng/mL) while lowering TSH (0.31 mIU/L).

Glyphosate exposure had a moderate, but it is still a significant effect, with T4 averaging 14.2 ng/mL, T3 at 2.18 ng/mL, and TSH suppressed to 0.33 mIU/L. Interestingly, S-metolachlor showed slightly lower T4 (12.8 ng/mL) and higher TSH (0.366 mIU/L) than other herbicide treatments, suggesting a somewhat milder thyroid disruption. Still, its T3 level (2.8 ng/mL) was elevated compared to the control (1.00 ng/mL**),** indicating that it may interfere with thyroid metabolism, possibly by impacting deiodinase activity.

Romano, *et al*., (2009) suggested that the commercial formulation of glyphosate is a potent endocrine disruptor in vivo, causing disorder in the reproductive development of rats when it is given during the puberty period. These changes are agreed with previous research showing that thyroid hormones can impair reproductive system by reduce testosterone synthesis, and damage spermatogenesis Chandrasekaran *et al.,* (2015) and Wagner *et al.,* (2008). To sum up, these results indicated that glyphosate and other herbicides pose a real threat to thyroid hormones**,** even at doses that considered safe by regulators, the implications of which exceed the reproductive function to broader endocrine health.

Table 3. the effect of selected herbicides on thyroid hormones

Treatments	Thyroxine (T4) (ng/mL)	Triiodothyronine (T3) (ng/mL)	Thyroid-stimulating hormone (TSH)(mIU/L)
Glyphosate	14.20 ± 0.38 ^{c}	2.18 ± 0.56 ^{c}	0.33 ± 0.03 ^{b}
Dicamba	17.34 ± 0.56 ^{b}	4.02 ± 0.54 ^{a}	0.31 ± 0.03 ^{b}
S-metolachlor	12.80 ± 0.28 ^{d}	2.80 ± 0.26 ^{b}	0.37 ± 0.04 ^{b}
Cyhalofop-butyl	18.34 ± 0.41 a	4.34 ± 0.21 ^{a}	0.25 ± 0.03 ^{b}
Control Oil	9.46 ± 0.45 ^{e}	1.00 ± 0.19 ^{d}	1.04 ± 0.25 ^{a}
Control Saline	8.14 ± 0.30 ^{f}	0.86 ± 0.11 ^{d}	1.02 ± 0.22 ^{a}
LSD	0.5334	0.4676	0.1806

Data are expressed as the mean ± standard deviation (SD) based on five animals in each group. Superscripts within the same column indicate significant **differences between groups (p < 0.05)**

Histopathological effects of the tested herbicides on testes.

Figure (1) Represents photomicrographs of testes from different treatment groups. Panel A treated with saline shows typical histological structure of the seminiferous tubules, spermatogenic cells (thick arrow) and leydig cell (thin arrow). Moving to panel B - Oil group- reveals also normal histological perspective. Panel C (glyphosate-treated testes) exhibit marked degeneration of the germinal epithelium, with vacuolated seminiferous tubules, disorganization of spermatogenic cells (black arrow), and loss of mature spermatozoa. These features are consistent with the increased MDA levels and decreased antioxidant enzyme activity (catalase and SOD), suggesting oxidative stress-induced cellular damage and impaired spermatogenesis. This is supported by significantly reduced testosterone levels observed in the biochemical analysis. Panel D, Dicamba exposure leads to severe disruption of the seminiferous tubules, pyknotic nuclei (blue arrow), and sparse spermatogenic cells (black arrow), indicating apoptotic changes. These alterations align with Dicamba's observed hormonal suppression (notably low testosterone, LH, and FSH) and elevated oxidative markers, suggesting both endocrine disruption and direct cytotoxicity to testicular cells. Panel E (s-metolachlor): moderate histological changes are seen, including partial degeneration and focal vacuolation. Spermatogenic cell (black arrows) alignment appears slightly disrupted, though less severe than in glyphosate and Dicamba groups. These findings correlate with intermediate oxidative damage and lesser hormonal suppression, reflecting a relatively milder toxic profile. Panel F (cyhalofop-butyl): testicular structure is severely compromised, showing tubular atrophy, marked spermatogenic vacuolation predominantly observed in spermatid cells (thin arrow), accompanied by pyknosis of spermatogonia (thick arrow) and a noticeable loss of germ cells. These changes are consistent with this herbicide's highest recorded MDA level, lowest antioxidant enzyme activities, and most suppressed hormonal profile (testosterone, LH, FSH), marking it as the most cytotoxic among tested compounds.

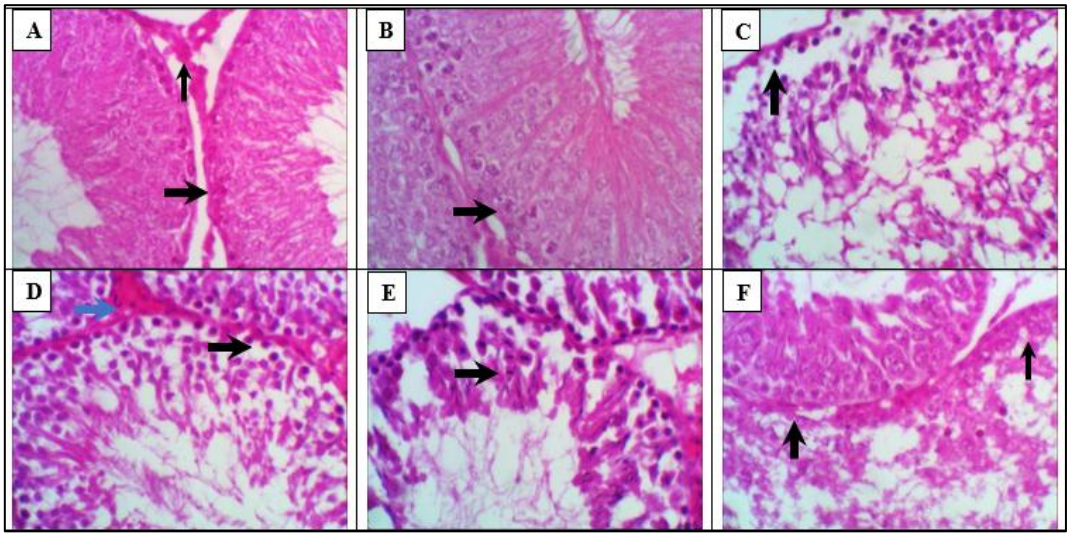

Fig 1. Representative photomicrographs of testes from different treatment groups A The control group displayed a normal histological structure of the seminiferous tubules, spermatogenic cells(thickarrow) and leydig cell (thin arrow). B) Oil group showing a typical histological structure of seminiferous tubules and spermatogenic cells (thick arrow). C) treated with 1/10 of LD50 of glyphosate showing marked decrease in spermatogenic cells with necrobiotic changes (black arrow). D) treated 1/10 of LD50 of Dicamba showing marked decrease in number of germ cell (mostaly spermatogonia) (black arrow), and necrobiotic alterations in leydig cells (blue arrow). E) treated 1/10 of LD50 of S-metolachlor caused disrupted testicular structure characterized by extensive necrosis in multiple layers of spermatogenic germ cells, primarily affecting spermatids and spermatozoa (black arrows). F) Testes treated with Cyhalofop-butyl exhibited degenerative changes, including pronounced spermatogenic vacuolation mainly in spermatid cells (thin arrow) and pyknosis of spermatogonia (thick arrow). (Hematoxylin and Eosin stain),400X.

The most severe histopathology happened in the group treated with cyhalofop-butyl then dicamba group, followed by glyphosate and S-metolachlor groups. These histological changes closely linked with the observed biochemical and sperm disorder, reinforcing the hypothesis that oxidative stress-induced damage and endocrine disruption gather to impair testicular structure and function.

Hariti, *et al.,* (2024) stated that when the Wister rats treated with glyphosate-based herbicides for a short period, this led to disruption of spermatogenesis. Additionally, Hashim *et al.,* (2020) documented exposure to glyphosate caused mitochondrial degeneration, nuclear condensation, vacuolation, widened intercellular spaces, and malformed sperm heads, reflecting severe damage at the cellular level (Hashim *et al.,* 2020). Therefore, the results of Zhu, *et al.,* (2015) declared that exposure to dicamba (0, 0.05, 0.5, 5, and 50 μg/L) for 40 days caused inhibition of spermatogenesis in male testes of rare minnow (Gobiocypris rarus), he also added that sex hormone homeostasis and normal reproduction of fish could be affected by dicamba.

This study highlights the potential reproductive and endocrine risks related to selected herbicides, glyphosate, dicamba, s-metolachlor, and cyhalofop-butyl. Our findings clearly showed that exposure to these compounds significantly affect the oxidative balance in male rats, with significant increases an increase in malondialdehyde (MDA) levels and a decrease in enzymes with antioxidant properties such including catalase and superoxide dismutase (SOD). cyhalofop-butyl was the most damaging, with MDA levels reaching 12.64 nmol/mL, catalase at 8.76 ng/mL, and SOD at 7.86 U/mL, causing severe oxidative stress.

These biochemical imbalances were closely accompanied by hormonal disruption, including a decrease in testosterone, Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), both of which are vital for male reproductive function. For example, testosterone levels fell as low as 2.44 ng/mL in cyhalofop-butyl-exposed rats, compared to 4.5 ng/mL in the oil control group. These hormonal changes were associated with observed declines in sperm quality, including reduced motility, increased abnormalities, and lower sperm counts.

From toxicological view, these results emphasize growing concerns about the unexpected consequences of herbicide exposure on non-target organisms. The observed effects, especially the link between oxidative stress, hormonal imbalance, and sperm dysfunction, highlighted the need for stricter regulatory examination, especially for herbicides that may act as endocrine disruptors. Future studies should aim to study the long-term exposure for these herbicides and utilize safer alternatives.

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التأثيرات السامة لبعض مبيدات الحشائش على الجهاز التناسلي و الغدة الدرقية في ذكور الفئران

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الملخص

أجريت التجربة علي ذكور الفئران لدراسة تأثير أربعة من مبيدات الحشائش شائعة الاستخدام في جمهورية مصر العربية و هي الجلوفوسات، الديكمبا، س-ميتولاكلور و سيهالوفوب بيوتيل. عوملت فئران كل مجموعة (خمس فئران) بعشر الجرعة النصفية المميتة للمبيد لمدة اربع أسابيع ثلاث مرات في الأسبوع. في نهاية التجربة، تم اخذ عينات السيرم لتقدير الهرمونات التناسيلية ومضادات الاكسدة والحيوانات المنوية. وجد ان كل المبيدات المختارة أدت الي زيادة malondialdehyde (MDA و الذي هو دلالة علي زيازة معنوية مؤشرات الاجهاد التاكسدي. يعتبر مبيد السيهالوفوب بيوتيل الاعلي تأثيرا 12.64 nmol/mL مقارنه مجموعة بالمقارنة التي أعطيت زيت فقط 4.12 nmol/mL. بالنسبة للانزيمات المضادة للاكسدة مثل انزيم catalase and superoxide dismutase (SOD) حدث لها انخفاض معنوي في كل المعاملات و خاصة في المجموعة المعاملة بمبيد السهالوفوب بيوتيل 8.76 ng/mL and 7.86 U/mL علي التوالي. كما حدث أيضا انخفاض في الهرمونات التناسلية كان مبيد السهالوفوب الأكثر تأثيرا علي الانزيمات المختبرة testosterone 2.44 ng/mL وLH 1.76 mIU/mL و FSH 1.44 mIU/mL مقارنه بالمجموعة المقارنة testosterone: 4.5 ng/mL in oil; LH: 3.04 mIU/mL; FSH: 2.44 mIU/mL علي التوالي. حدث أيضا انخفاض معنوي في قدرة الحيوانات المنوية علي الحركة الي الامام ، و حدث زيادة في عدد الحيوانات المنوية و كذلم الحيوانت المنوية المشوه، و انخفاض في عدد الحيوانات المنوية خاصة في المجموعات المعاملة بكل من مبيد السيهالوفوب بيوتيل و الديكمبا. كما حدث خلل في النظام الهرموني للفئران المعاملة. أدت المعاملة بمبيد الجلوفوسات و مبيد الديكمبا الي زيادة كل من T4 و T3 (T3: 2.18 - 4.02 T4: 14.2–17.34 ng/mL) و انخفاض TSH (0.31–0.33 mIU/mL) مما يتوقع حدوث زيادة نشاط في الغدة الدرقية. تدل هذه النتائج على ان مبيدات الحشائش المختبرة و خاصة مبيد السهالوفوب بيوتيل و مبيد الديكمبا الي تأثيرات معنوية جادة علي الهرمونات التناسيلية و مضادات الاكسدة و اخداث خلل في الجهاز التناسلي للفئران المعاملة. تدق هذه النتائج ناقوس الخطر خاصة وان لها تاثير سلبي على هرمونات الغدة الدرقية و بالتالي الحاجة الماسة الي التقييم المستمر لهذه المبيدات و البحث عن حلول بديلة اكثر امانا.