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ORIGINAL ARTICLE

# Melatonin mitigates oxidative stress in luniron-induced testicular injury in Wistar rats

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## Abstract

Numerous studies have demonstrated that natural antioxidants protect cells from contaminants' toxic effects. This study aims to investigate the potential protective effects of melatonin (MLT) against linuron-induced testicular toxicity and spermatogenesis damage. Rats were divided into four groups: the control group (no treatment), the MLT group that received MLT (10 mg/kg b.w), the LIN group that received LIN (120 mg/kg b.w), and (LIN/MLT) group treated with LIN and MLT. The investigated substances MLT and linuron (LIN) were given orally to the animals for 30 days. The results showed that linuron treatment-induced testicular dysfunctions demonstrated significant inhibition of pituitary–testicular axis hormone synthesis (diminution serum levels of testosterone, FSH, and LH) associated with spermatogenesis injury improved by low Johnsen scores and increased in testis CD117 expression. Furthermore, superoxide dismutase (SOD) and catalase (CAT) activities, as well as reduced glutathione (GSH) content, were significantly decreased. In contrast, there was a considerable rise in the activity of glutathione S-transferase (GST), glutathione peroxidase (GPx), malondialdehyde (MDA), and protein carbonyl (PCO). Our results established that oral MLT supplementation in LIN-treated rats restored plasma hormone levels and alleviated the adverse cytotoxic effects of LIN. According to the findings, MLT demonstrated potential as an endogenous antioxidant, effectively alleviating linuron-induced testicular oxidative injury.

**Keywords** Testis · CD117 expression · Luniron · Melatonin · Oxidative stress · Antioxidant

## Introduction

In the agricultural industry, hazardous chemicals and pesticides are frequently employed. As a result of rising worldwide demand, they are a practical and affordable solution to increase the quality and quantity of food items (Sharma et al. 2019). Due to their high consumption, these chemicals rank among the most pervasive environmental pollutants. The development of several diseases, such as metabolic disorders, neurotoxic and carcinogenic conditions, issues with reproduction, and other long-term health impacts, has been related to persistent exposure to these compounds (Kim et al. 2017; Bailey et al. 2018). Pesticides have negative impacts on target organisms in a variety of ways, and depending on a number of variables, including the amount, frequency of exposure, genetics, nutritional state, and the type of pesticide, they may have hazardous effects (Al-Attar et al. 2017; Hassaan and El Nemr 2020).

Linuron (LIN) (3-(3,4-chlorophenyl)-1-methoxy-1-methyl urea) is a phenyl-urea herbicide commonly used to control a variety of broadleaf and grass weeds in a wide range of crops, including soybean, cotton, corn, wheat, sugar cane, potato, and many other fruits and vegetables (Bai et al. 2017; Spirhanzlova et al. 2017). By blocking photosynthetic electron transport chain enzymes, their principal mode of action is the destruction of photosynthesis in the targeted weeds (Hayyat et al. 2016).

Linuron enters and lingers in surface waterways through agricultural runoffs (Patterson 2004). It is also widespread

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in the residues found on food and in drinking water (Pest Management Regulatory Agency 2014; Environmental Protection Agency, 2015). Recently, LIN was categorized as carcinogenic category 2 and harmful for reproduction category 1B. (EFSA, 2016). It has an anti-androgenic reputation (Lambright et al. 2000). Actually, a number of studies have shown that LIN is associated with a variety of adverse effects on the health of male reproductive organs in rats such as abnormal fetal development, the development of tumors in Leydig cells, and a reduction in the production of testosterone both in vitro and in vivo (Hotchkiss et al. 2004; Wilson et al. 2009; Ding et al. 2017). This herbicide causes deformities, infertility, or cell cancers by attacking the liver and red blood cells (MarxStoelting et al. 2014; Bai et al. 2017; Ding et al. 2017).

Previous research has shown that exposure to the herbicide damages cells and generates oxidative stress, which in turn causes an excess of reactive oxygen species (ROS) and lipid peroxidation (Leong et al. 2013; Tichati et al. 2021). These organisms continuously develop as by-products of typical metabolic processes. Exposure to substances like pesticides, which the cell is unable to counteract, speeds up their production. One or more biomolecules, such as proteins, nucleic acids, lipids, and carbohydrates, are damaged as a result (D'Souza 2017; Voronkovaet al. 2018). It is anticipated that reactive oxygen species (ROS) may cause a number of diseases, including liver problems and testicular atrophy (Bai et al. 2017).

Numerous researches showed that natural antioxidants shield cells from toxins brought on by pollutants. The pineal gland regularly produces melatonin (*N*-acetyl-5-methoxy tryptamine), a well-known animal hormone with a variety of biological actions (Reiter et al. 2010). Melatonin is present both in the plant world and in animals (Acosta et al. 2022). Its anti-inflammatory, antioxidant, and anti-cancer activities were identified in numerous researches (Eghbal et al. 2016; Favero et al. 2018). MLT protects against the toxicity of numerous environmental agents and chemical variables since it possesses antioxidant and preventive properties (Asghari et al. 2017; Król et al. 2018; Upadhyaya et al. 2018).

Melatonin increases antioxidant enzymes like catalase, glutathione peroxidase, and superoxide dismutase in rats (Magierowski et al. 2013; Manchester et al. 2015; Reiter et al. 2016). According to reports, melatonin may have the capacity to reverse the functional deficits linked to a variety of illnesses in the male reproductive system (Rocha et al. 2015). Additionally, because it controls the release of steroid hormones, MLT is essential for male reproduction. The lipophilic and hydrophilic characteristics of MLT, which are dispersed into subcellular organelles, enable it to cross the blood-testis barrier and the testicular cell membrane.

In order to assess several characteristics related to the gonadotropic axis response, antioxidant status, histological changes, and expression of the leucocyte marker CD 117 in the testis of linuron-exposed rats, the current investigation was carried out. Additionally, it looked into whether melatonin therapy could lessen the toxicity caused by linuron. Melatonin has a well-known part in mammals' seasonal and circadian rhythms. However, nothing is known about its general impact on the physiology of male reproduction.

## Materials and methods

### Chemicals

Linuron bought Linuchem, a commercial formulation of linuron with a CAS registration number of 1071–83-6 and a homologation number of 08 46 126, which contains linuron at a concentration of 50% (Fig. 1a) from Sigma Chemical Co., melatonin (St. Louis, MO, USA) (Fig. 1b).

### Animals and protocol design

The Pasteur Institute furnished 28 male albino Wistar rats, each weighing 250 ± 0.10 g and being 8–9 weeks old (Algiers, Algeria). The rats were kept in ventilated cabinets Bio-C36 (TecniPlast; Italy) for 30 days prior to the start of the experiment (temperature 22–25 °C; light 12-h light/dark cycle; relative humidity 50–5 °C). The "ONAB of Bejaia" in Algeria provided the regular feed for the rats, which were also given unlimited access to water. Twice every week, the bedding was changed. Before the studies, the animals were acclimated to these circumstances for a month.

The Ethical Committee of the Directorate General for Scientific Research and Technological Development at the Algerian Ministry of Higher Education and Scientific Research approved all the protocols used in this study in accordance with the International Guidelines for Laboratory Animal Care and Use (Council of European Communities) (JO86/609/CEE; permit number PNR/SF 08/2012). The rats were randomly separated into four groups of seven animals each to assess the toxicity of LIN.

Group 1 (control): For 30 days, rats were given 1 mL of distilled water by oral gavage.

Rats in group 2 (MLT-treated group) received an oral MLT treatment for 30 days at a dose level of 10 mg/kg/bw.

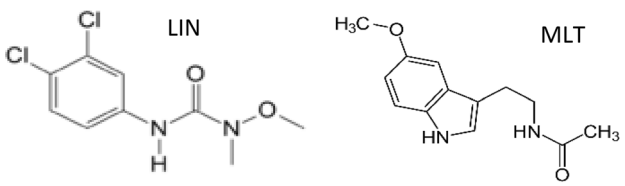



**Fig. 1** Chemical structure of linuron and melatonin

Rats in group 3 (LIN-treated) received an oral LIN treatment for 30 days at a dose of 1/10 of LD50 (120 mg/kg/bw/day).

Rats in group 4 (MLT + LIN) received MLT and LIN for 30 days in the same manner as groups (2) and (3).

The amount of LIN utilized in this experiment has been employed in other studies since it is toxic but not fatal to rats (Bai et al. 2017). According to Olukole et al. (2018), the MLT was dissolved in a DMSO saline solution. Regular checks were made on the animals' body weight, water and food intake, and body weight. The animals underwent an overnight fast at the conclusion of the trial, and their total body weight was noted. To reduce the stress on the animals, they were scarified by cervical decapitation.

## Tissue and blood samples

The blood was collected under ice in polyethylene heparin and centrifuged at 2500 × g for 15 min at 4 °C. The collected plasma was separated into Eppendorf tube and kept at − 20 °C until the hormonal parameter analysis.

## Testicular homogenate preparation

After the animals have been dissected, the testis was carefully taken out and weighed under ice. A 2 mL buffer solution of phosphate-buffered saline (PBS) (1:2 weight/volume, pH 7.4) was used to homogenize 1 g of testes. The cell suspension was centrifuged (9000 × g, − 4 °C, 15 min) after the tissues had been homogenized. The resulting supernatants were divided into Eppendorf tubes and kept at – 20 °C until the oxidative stress parameters were measured.

## ELISA pituitary–testicular hormone analysis

ELISA kits were used to measure the levels of testosterone, LH, and FSH in accordance with the manufacturer's instructions (SKFDIA Beckman Coulter, Inc., 33,560 testosterone, ref. 33,560; FSH, ref. 33,520; LH, ref. 33,510).

## Testicular redox status markers

### Level of malondialdehyde

Lipid peroxidation products in testis homogenates were determined as TBA-reactive metabolites expressed as the level of malondialdehyde (MDA) formation according to Buege and Aust (1978). Briefly, 100 µl of supernatant was sonicated with 50 µl of Tris-buffered saline (TBS) and 125 µl of trichloroacetic acid (TCA)-BHT to precipitate proteins, and then centrifuged at 1000 × g, 10 min, 4 °C. After that, 200 µl of the supernatant was mixed with 40 µl of HCl (0.6 M) and 160 µl of TBA dissolved in TBS. The mixture was heated in a water bath at 80 °C for 10 min. The pink-red complex is based on the peak absorbance at 532 nm which was proportional to the amount of TBARS formed. Values are expressed in nmol malondialdehyde (MDA) equivalents per mg of protein.

### Levels of protein carbonyl

Protein carbonyl concentrations were determined using the Levine et al. (1990) approach, which relies on derivatizing the carbonyl group with 2,4-dinitrophenylhydrazine (DNPH) to produce a stable 2,4-dinitrophenyl. At 370 nm, absorbance was measured. Millimole per milligram of protein was used to express protein carbonyl (PCO) concentration.

## Oxidative biomarkers

Glutathione (GSH) determination was carried out using Weekbeker and Cory's method (1988). The idea behind this assay is to measure the optical absorbance of 2-nitro-5-mercapturic acid, which is produced when glutathione's (-SH) groups reduce the compound 5,5-dithiol-bis-2-nitrobenzoic acid (DTNB). Deproteinization is carried out for this purpose in order to retain solely the (-SH) groups unique to glutathione. At 412 nm, the absorbance was measured. The amount of GSH was given as mmol GSH/mg protein. The glutathione-S-transferase (GST) activity was evaluated by Habig et al. (1974). The absorbance was evaluated at 340 nm at 30-s intervals for 3 min. The computed and expressed GST activity is given in units of nmol CDNB conjugate/min/mg protein.

The enzymatic activity of glutathione peroxidase (GPx) was determined by Flohe and Gunzler (1984). This approach is based on decreasing hydrogen peroxide ($H_2O_2$) in the presence of reduced glutathione (GSH). At 412 nm, the absorbance was measured. GPx activity was determined and represented as nmol/min/mg protein. The enzymatic activity of catalase was measured at 25 °C using Aebi's method (1984). Kinetic monitoring of the hydrogen peroxide ($H_2O_2$) elimination by the CAT enzyme was done at 240 nm. Calculated and represented as mmol of $H_2O_2$/min/mg of protein, CAT activity was determined. The activity of superoxide dismutase was measured. According to the method described by Beyer and Fridovich (1987) based on the ability of superoxide dismutase to prevent the reduction of nitro blue tetrazolium served as a measure of the enzyme's activity (NBT), the reaction medium contains a photosensitizer (riboflavin) and NBT, a molecule that can be reduced by O-generated by riboflavin. The SOD activity was estimated and represented as (international unit) UI/mg of protein after the absorbance was measured at 560 nm.

### Histopathological analysis

Fresh testicular tissue pieces were preserved in Bouin solutions for 24 h for histological analysis. They were dehydrated using a graduated ethanol solution before being imbedded in paraffin. Using a microtome (LEICA RM2235), the paraffin sections were cut into 5-µm-thick slices, which were subsequently deparaffinized and stained with hematoxylin and eosin (H&E) (Hould 1984). Using an LEICA DM 710 microscope, a single pathologist who was unaware of the experimental rat group independently assessed the histopathological analysis. Johnsen scoring was used to assess the spermatogenesis of the testes which ranges from 10 to 1, which was used to categorize the severity of germ cell injury (Johnsen 1970) (Table 1). The value of the Johnsen score in each testis was the mean point value from at least 20 seminiferous tubules, and it was used to compare long-term spermatogenesis between the control and treated groups.

### Immunohistochemistry detection of CD117 glycoprotein

Five-micrometer sections of a paraffin-embedded testis sample were cut, and they were afterwards deparaffinized and rehydrated using xylene and graded ethyl alcohol. Sections were treated with 3% hydrogen peroxide in deionized water for 30 min to suppress endogenous peroxidase activity. The sections were then stained for 4 min in deionized water with antigen retrieval solution after being washed with buffered tampons pH 7.6 and prepped for 45 min in 10 mM sodium citrate with a pH of 6.0 (Abcam Lab, UK). After that, sections underwent an overnight incubation at 4 °C with the primary antibody (anti-CD117 antibody ab10558). Slides were treated with biotinylated goat anti-rabbit antibody CD117 diluted 1:200 for 1 h at room temperature following numerous washes in 50 mM Tris/HCl, pH 7.6 (both from Novus Biological, Laboratories). Using a reliable source of 3,3′-diaminobenzidine (DAB) chromogen (DAB; Sigma, St. Louis, MO, USA), the peroxidase method was used for 1 h at room temperature. Sections were viewed and photographed using a Leica DM 710 optical microscope, Mayer's hematoxylin-counterstained sections, and a Leica microsystem camera (image processing software, LAZ EZ version 3). For image analysis, we used QuPath-0.3.2 software to determine the number of positive cells (Bankhead et al. 2017).

**Table 1** Johnsen scoring system for evaluating testicular damage

| Johnsen score | Description of histological criteria |
|---|---|
| 10 | Fully developed spermatogenesis |
| 9 | A few late spermatids, slightly delayed spermatogenesis, and an unorganized epithelium |
| 8 | Less than five spermatozoa per tubule, few late spermatids |
| 7 | No spermatozoa, no late spermatids, many early spermatids |
| 6 | No spermatozoa, no late spermatids, few early spermatids |
| 5 | No spermatozoa or spermatids, many spermatocytes |
| 4 | No spermatozoa or spermatids, few spermatocytes |
| 3 | Spermatogonia only |
| 2 | No germinal cells, Sertoli cells only |
| 1 | No seminiferous epithelium |

### Statistic data analysis

The means and standard error means were used to express all data (SEM). The treated groups were compared to the control groups using one-way analysis of variance (ANOVA), followed by Tukey's, where $p$ 0.05 was deemed significant. The GraphPad Prism application was used to run statistical tests (version 7.0; GraphPad Software, CA, USA).

## Results

### Treatment effects on rats' health and behaviors

Male rats exposed to LIN did not exhibit any unusual behaviors during the trial, such as head flapping, scratching, biting, circling, licking, or passive motions. There was no mortality in any group. In the control and MLT groups, the rats exhibited no symptoms of systemic poisoning. The rats in the LIN group, however, exhibited clinical symptoms including anorexia and weight loss. Additionally, the rats in the MLT + LIN group displayed some anorexia-related signs and weight loss.

### Treatment's effect on testicle and body weight

According to Table 2, the LIN group's body weight was significantly decreased in comparison to the control group (−9.13%, $p < 0.001$). The absolute and relative testis weights were affected by LIN exposure as well. A significant diminution in testicular weight was observed in LIN rats compared to the control and MLT groups (respectively, $p < 0.05$, $p < 0.001$).

### Pituitary testicular axis response to LIN treatment

Concentrations of plasmatic FSH, LH, and testosterone in all of experimental groups are represented in Fig. 2. The results show that LIN-treated rats had a negative impact on hormonal secretion revealed by significant diminution in plasma levels of FSH, LH, and testosterone compared

**Table 2** Effects of treatments on body weight, absolute and relative testes weights in control and experimental groups

| Parameters | Experimental groups | | | |
|---|---|---|---|---|
| | Control | MLT | LIN | MLT + LIN |
| Initial body weight (g) | 251.9 ± 3.30 | 252.71 ± 2.79 | 250.87 ± 3.04 | 250.02 ± 5.58 |
| Final body weight (g) | 275.28 ± 4.41 | 259 ± 4.42 | 237.3 ± 3.32*** | 235 ± 5.91*** |
| Absolute testes weight (g) | 3.25 ± 0.13 | 2.91 ± 0.15 | 2.64 ± 0.16* | 2.86 ± 0.15 |
| Relative testes weight (g) | 1.27 ± 0.06 | 1.20 ± 0.06 | 0.95 ± 0.04*** | 1.13 ± 0.08 |

Values are given as mean ± SEM. For groups of 7 animals each. Significant difference: MLT, LIN groups compared to the control (***$p < 0.001$, *$p < 0.05$)

to the control group ($p < 0.01$) (Fig. 2a–c). This decrease in levels was − 44%, − 65%, and − 62%, respectively. Nevertheless, MLT supplementation resulted in a considerable rise in these levels.

## Stress biomarker response

The effects of LIN exposure and MLT on the antioxidant defense system in the rat testis are summarized in Figs. 3 and 4. The results demonstrate a considerable increase in MDA and PCO levels in LIN-exposed rats, with values significantly higher than the control ($p < 0.01$; $p < 0.05$) (Fig. 3b, c). Rats exposed to LIN had a significant uptick on both the activity of antioxidant enzymes such GST and GST ($p < 0.05$) and GPx ($p < 0.001$) (Fig. 4a, b). Melatonin treatment has improved in these parameters. In addition, the data demonstrate a substantial reduction ($p < 0.05$) in GSH level, as well as CAT and SOD activities compared to the control group.

## Histopathological analysis

Slides of testis were observed to have morphological changes in seminiferous epithelium or in spermatogenesis stages that could be linked to LIN toxicity. Effectually, microscopic examination of the testis histology in control and melatonin-treated rats reveals normal testis tissue, including normal seminiferous tube structure (Ts), normal Leydig cell (Cl) in the interstitial tissue (TI), and no alterations to the stratified seminiferous epithelium (Es) (Fig. 5A, B). However, stratified seminiferous epithelium in LIN treatment groups showed substantial modifications by decreasing its density, uncommon germ cells with pycnotic nuclei (Np), absence of Leydig cells, a considerable drop in sperm counts, and vacuolization (V) (Fig. 5C, D). In comparison to rats just receiving LIN treatment, testis sections from rats getting the combined treatment (MLT + LIN) displayed fewer histological changes in the stratified seminiferous epithelium. As a result, the testis tissue injury intensity was significantly decreased (Fig. 5E, F).

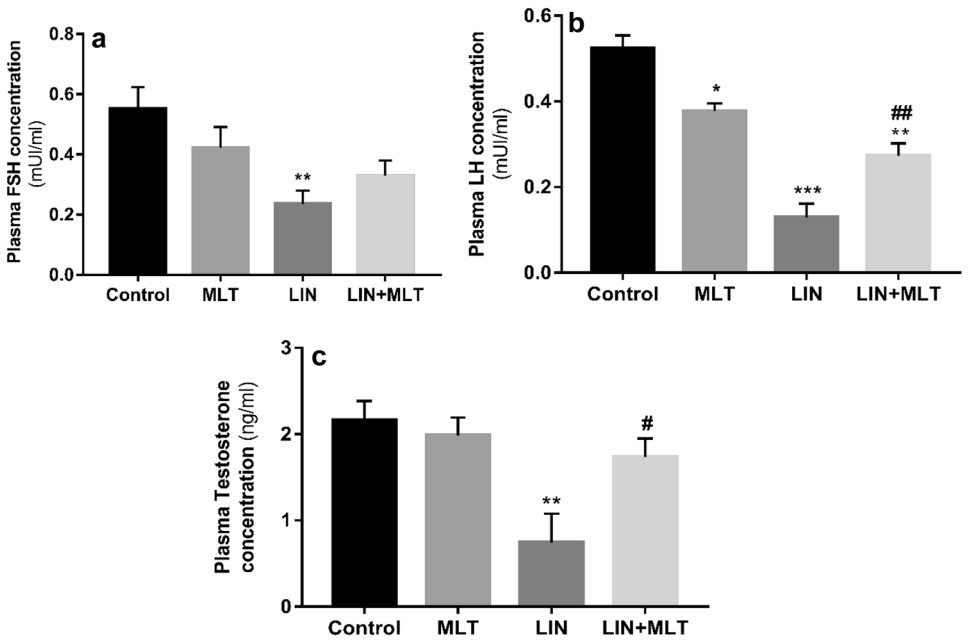



**Fig. 2** Effects of LIN (linuron) and MLT (melatonin) on the concentration of FSH (follicle-stimulating hormone) (**a**), LH luteinizing (**b**), and testosterone (**c**) in the plasma of animals after 30 days of treatment. Values are given as the mean ± SEM of 7 rats. Significant difference: MLT, LIN, and LIN + MLT versus control group (***$p < 0.001$, **$p < 0.01$, **$p < 0.05$). Significant difference: MLT + LIN versus LIN group ($^{\#}p < 0.05$, $^{\#\#}p < 0.01$)

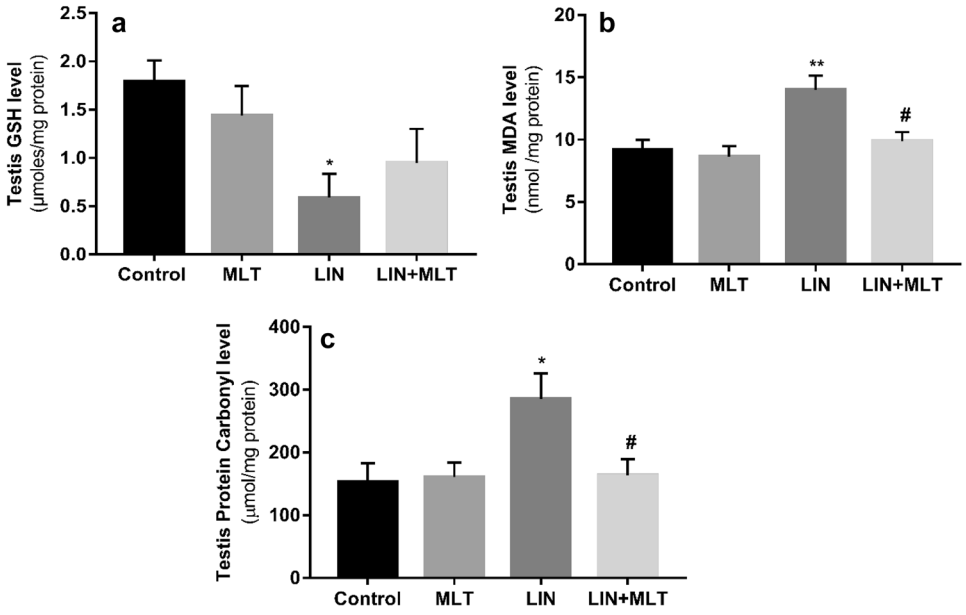



**Fig. 3** Effects of LIN (linuron) and MLT (melatonin) on the GSH (reduced glutathione) (**a**), MDA (malondialdehyde) (**b**), and PCO: protein carbonyl (**c**) levels in testis of animals after 30 days of treatment. Values are given as the mean ± SEM of 7 rats. Significant difference: MLT, LIN, and LIN + MLT versus control group (*$p < 0.05$, **$p < 0.01$). Significant difference: MLT + LIN versus LIN group (.$^{\#}p < 0.05$)

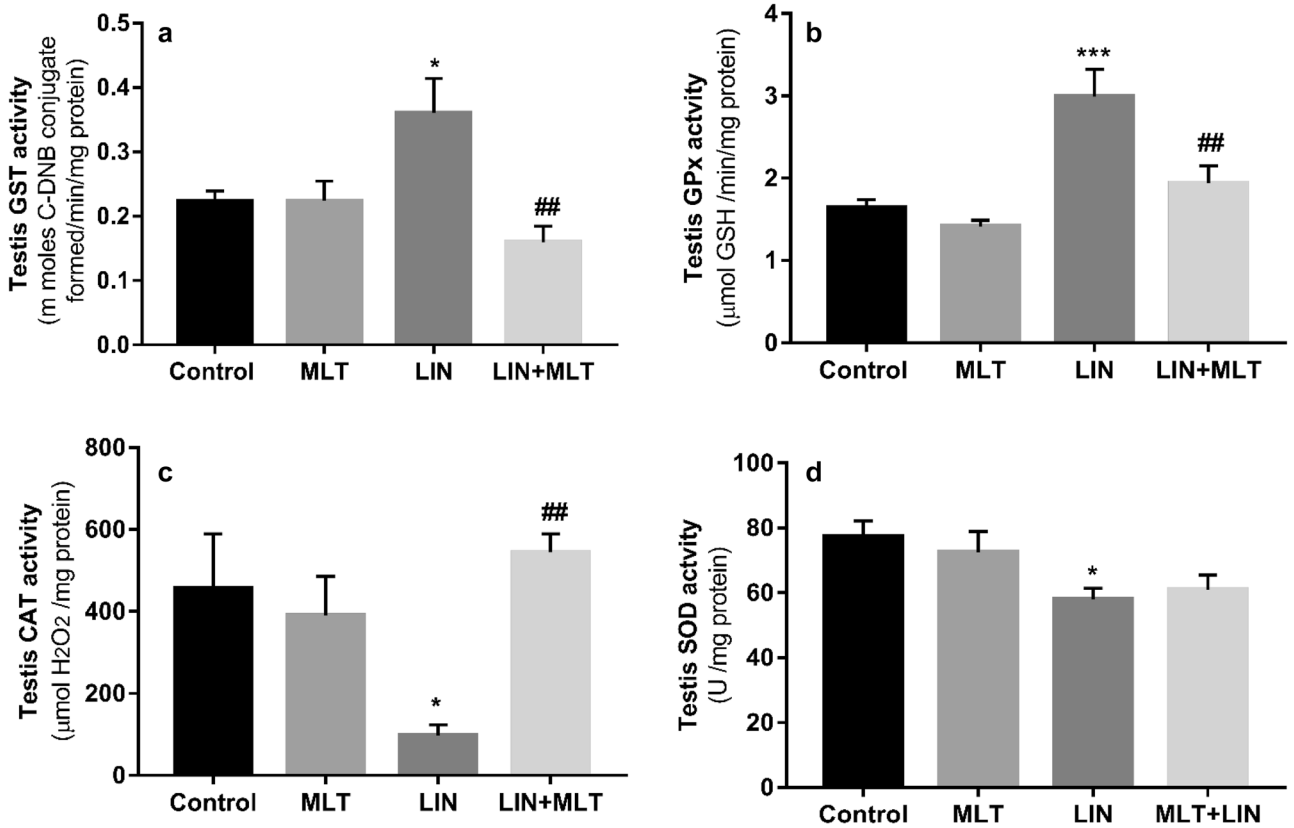



**Fig. 4** Effects of LIN (linuron) and MLT (melatonin) on the enzymatic activities of GST (glutathione-S-transferase) (**a**), GPx (glutathione peroxydase) (**b**), CAT catalase (**c**), and SOD (superoxyde dismutase) (**d**) in the testis of animals after 30 days of treatment. Values are given as the mean ± SEM of 7 rats. Significant difference: MLT, LIN, and LIN + MLT versus control group (***$p < 0.001$, *$p < 0.05$). Significant difference: MLT + LIN versus LIN group (.$^{\#\#}p < 0.01$)

### Treatment effect on inflammation response (testis CD 117 expression)

Testis from control and melatonin-treated rats show less CD 117 + cell expression than testis from LIN and LIN MLT-treated rats (Fig. 6A–D). Additionally, the number of CD117 + cells estimate using QuPath 3.2 software showed a significant increase in CD117 expression in the LIN-treated rats compared to the control and melatonin groups ($p$ 0.05) (Fig. 6E).

## Discussion

Toxic substances in the environment, such as pesticides, can contribute to the development of oxidative stress by free radical increasing free radical production, which can have negative effects like metabolic changes, neurological disorders, and fertility issues (D'Souza 2017; Kim et al. 2017; Kadar et al. 2017). Recent studies have shown the protective benefits of antioxidants in cells and tissues exposed to these substances in order to prevent oxidative stress (Król et al. 2018; Prathima al. 2020, Li et al. 2021). The ability of melatonin to mitigate luniron-damaging effects on the testis was examined in this study.

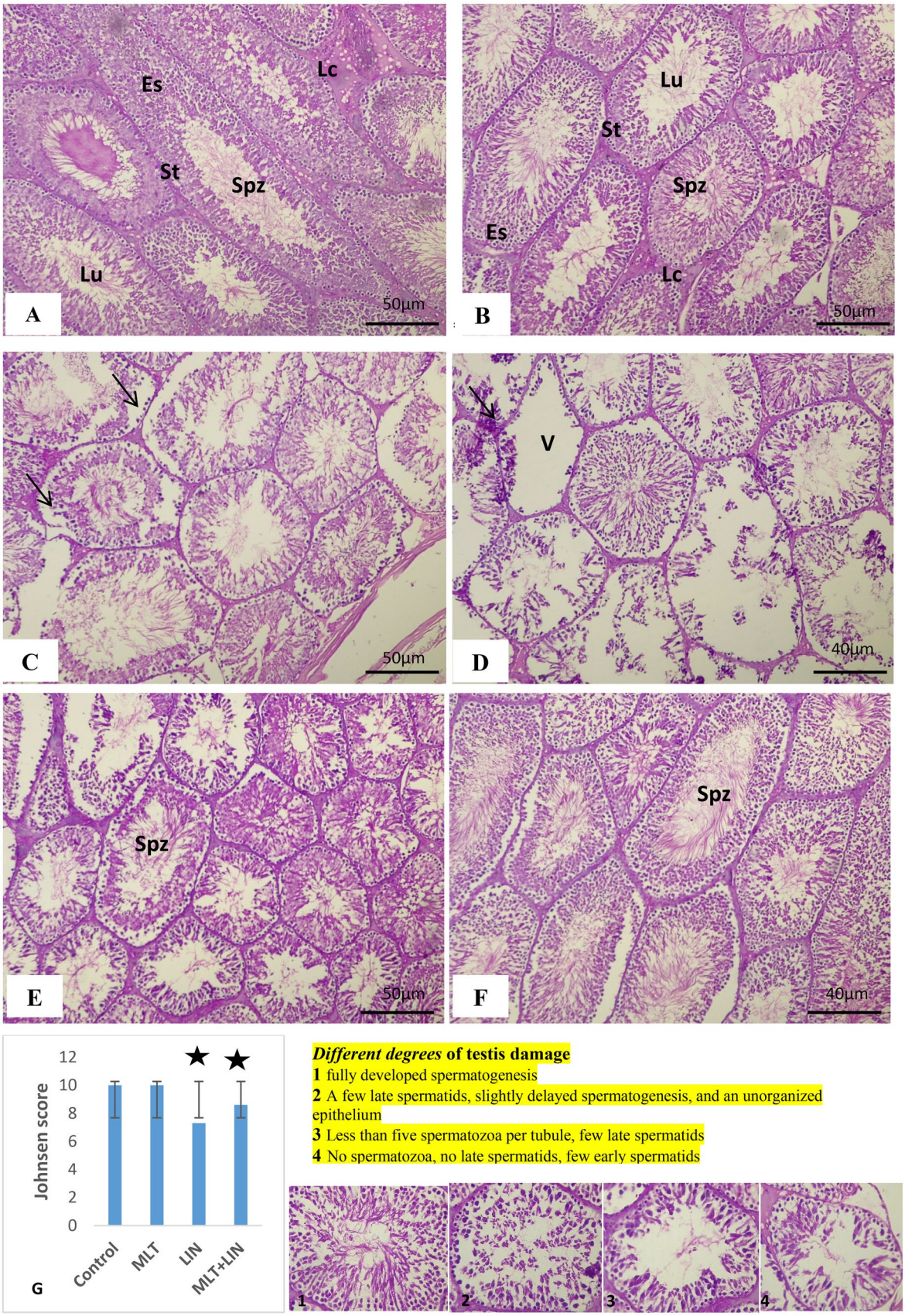
In the current study, LIN administration for 30 days at a dose of 120 mg/kg body weight resulted in a loss of body and testicular weight. The loss of body and target organ weight is regarded as a significant sign of the toxicity of LIN on the health of the rats. The decrease in body weight after pesticide administration was reported in several studies, and it was often associated with the decrease in food intake (Bhatti et al. 2011; Chiali et al. 2013). The results suggest that LIN can affect eating behavior of animals. This has been made clear by studies that suggest that ghrelin and serotonin, which regulate the hunger mechanism, might be altered by pesticides (Peris-Sampedro et al. 2015; Burke and Heisler 2015; Judge et al. 2016). Testicular weight is one of the important indicators for assessing male reproductive toxicity. According to our data, the absolute and relative testis weights were lower in the group exposed to the LIN than in the control group. This decline may be due to luniron's harmful side effects on testicular tissue (Bai et al. 2017; Prathima et al. 2020). However, MLT supplementation to LIN-treated rats restores absolute and relative testis weights. This may be due to increased food intake, which reduces the formation of free radicals, leading to tissue repair (Asghari et al. 2017; Li et al. 2021).

In our study, testicular hypoplasia observed after luniron exposure was accompanied with hormonal depletion. The androgen hormone class includes testosterone, a steroid hormone made from cholesterol; it is the primary hormone of the testicles produced by Leydig cells whose secretion was regulated by pituitary hormones including LH and FSH. It promotes male growth and development; it is also an important factor in the control of sperm formation (Leong et al. 2013). In our work, rats chronically exposed to LIN for 30 days induced a significant depletion in plasmatic testosterone, LH, and FSH concentration. Therefore, it would appear that the toxicity of the LIN was directly related to the process of biosynthesis gonadotropic hormones (Wilson et al. 2009). A few recent works on the use of pesticides, such as organophosphate and pyrethroid insecticides, have shown altered levels of pituitary gonadotropic hormones, as well as FSH, LH, and steroid hormones (Han et al. 2008; Anderson et al. 2008; Blonco-Munoz et al. 2010). Pesticides act through different mechanisms like inducing oxidative stress and lowering testosterone levels (Dehkhargani et al. 2011; Clair et al. 2012). Pesticides are responsible for decreasing testosterone concentration either by inhibiting the release of FSH and LH or also by inducing Leydig cell apoptosis (Slimani et al. 2011). Our histological findings according to Johnsen scoring system support these results and demonstrated that rats exposed to LIN developed some lesions of the seminiferous epithelium that were accompanied by a reduction in the density of germ cells and occasionally by the lack of Leydig cells inside the seminiferous tubules.

The administration of MLT resulted in an increase in FSH, LH, and testosterone levels with a notable recovery in testicular tissue. The protective effects of MLT against LIN-induced testis toxicity may be a result of its capacity to reduce oxidative stress and mitigate inflammation via a variety of pathways, including reducing lipid peroxidation and Leydig cell death (Li et al. 2020; Koohsari et al. 2020). MLT as an antioxidant agent can prevent alteration of enzymatic Leydig cell function induced by LIN treatment such as cytochrome P450 enzymes involved in synthesis of testosterone (Akingbemi et al. 2000).

Our data show an increase in CD117 glycoprotein expression in testis cells of rats treated with LIN compared to the control and MTL groups. CD117 is thought to play an important role in hematopoiesis, in spermatogenesis, and in carcinogenesis (Natali et al. 1992; Pietsch et al. 1998). Potti et al. (2005) demonstrated the presence of relation between the overexpression of CD117and pesticide exposure in human. In our study, we observed a significant diminution in the number of C117-positive cells in testis of rats treated with MLT. This result reveals the important role of MLT to regulate inflammatory pathway during luniron-induced testicular toxicity and the ability of this natural agent to protect and minimized spermatogenesis damage.

According to several investigations, the most frequent mechanism of pesticide toxicity is oxidative damage (Trea et al. 2020; Tichati et al. 2021; Li et al. 2021). Reactive oxygen species (ROS) overproduction leads to oxidative state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4 No spermatozoa, no late spermatids, few early spermatids
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◂**Fig. 5** Histological sections of the testes in rat's H&E staining. Control and melatonin-treated rats (**A, B**), luniron (**C**, **D**), and melatonin-luniron-treated (**E**, **F**) and Johnsen scores (**G**). **A** and **B** show a normal structure of seminiferous tubule (St), spermatozoa Spz concentrated in light (Lu), Leydig cell (LC) in interstitial tissue, and stratified seminiferous epithelium (Es). In luniron rats (**C**, **D**), the layer is less dense and has rare germ cells with pycnotic nuclei (arrow); Leydig cells are virtually absent and show vacuolization of seminiferous tubule (V). Restoration of Es in rats treated with melatonin (**E**, **F**). The sections were observed at 150- and 600-fold magnification (scale bars 40 to 50 µm)

within the testis and a concomitant decrease in antioxidant levels, which compromises the integrity of the cell membrane concluding in impaired spermatogenesis as observed in rats (Lee et al. 2007; Voronkova et al. 2018). The results of the current investigation, LIN rat's exposure resulted in a significantly lower testis GSH content with an increase in GST and GPx activity compared to the control group. Since proteins with aromatic amino acids are particularly vulnerable to oxidation and serve as a target for ROS, the conversion of GSH to GSSG triggers a cellular defense mechanism against oxidative damage (Aydin et al. 2010; Prathima et al. 2020). Additionally, our findings revealed a rise in PCO and MDA levels, which may be brought on by the creation of adducts between certain amino acid residues and lipid peroxidation products like MDA (Stadt-man and Levine 2006). Additionally, the rise in MDA levels sparked physicochemical changes that harmed the components of cell membranes and disrupted biomembranes (Leong et al. 2013; Tichati et al. 2019). Nevertheless, MLT supplementation considerably decreased levels of oxidative damage to proteins and lipids. MLT's ability to scavenge pro-oxidant

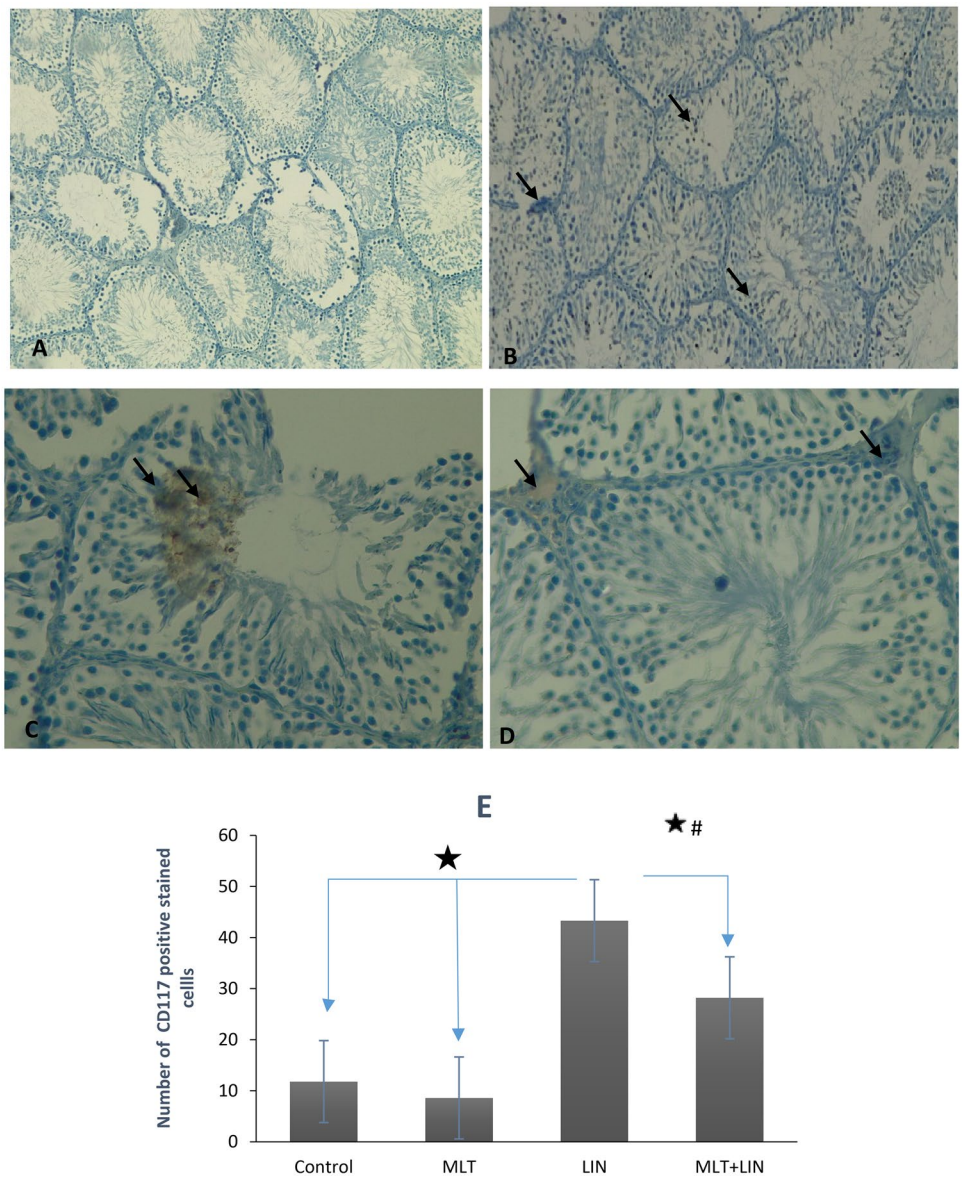


**Fig. 6** Immunohistochemical labeling for CD117 expression in liver of control (**A**), melatonin (**B**), luniron (**C**), and luniron/melatonin (**D**) treated rats at 300 × magnification. Control and melatonin rats' sections exhibit a lower number of CD 117 + cells compared with luniron-treated rats. The expression of CD117 was estimated by number of positively stained cells using QuPath (**E**). Data is represented as means ± SEM ($n = 5$). $^{*}p < 0.05$ compared with the control and MLT groups, $^{\#}p < 0.05$ compared with the corresponding LIN

chemicals or increase enzymatic antioxidant defense may be the reason for its protective benefits in our investigation against LIN-induced toxicity (Manchester et al. 2015; Reiter et al. 2016). The initial lines of defense against the harmful effects of free radicals are CAT and SOD. They convert superoxide radicals into the considerably more stable $H_2O_2$. The reduction of hydrogen peroxide by CAT must come after this function in order for it to be effective (Bhatti et al. 2014). This explains why, in our investigation, we noted that rats treated with LIN had lower activity levels of CAT and SOD. The considerable decrease may lead to an accumulation of free radicals in testis tissue. Rats that were subjected to LIN and other insecticides showed similar results (Leong et al. 2013; Prathima et al. 2020).

In contrast, administering MLT to rats receiving LIN treatment increased GSH levels and recovered the SOD and CAT enzymatic activity. The antioxidant characteristics of MLT, which easily pass the blood–brain barrier and infiltrate cells, may contribute to this impact (Murawska-Ciaowicz et al. 2011; Goc et al. 2017; Król et al. 2018). In this context, Nascimento et al. (2019) also revealed the protective benefits of MLT against cypermethrin-induced oxidative injury in the rat liver, serum, and brain.

We can allow from the present findings that melatonin may protect the testis from inflammation and damaging effects of reactive oxygen species produced by LIN exposure. The mechanism of melatonin's protecting effects involves possible stimulation of antioxidative enzyme activities and inhibition inflammatory factors. Melatonin is supposed as a safe substance with stumpy risk of side effects. It gives a perspective to promote studies of its free radical scavenger and anti-inflammatory properties in prevention of oxidative stress-dependent diseases, caused by pesticides such as linuron.

**Author contributions** Fouzia Trea: conceptualization, methodology, and presentation of the published work. Sakina Chaib: conducting an investigation and research process, particularly carrying out the tests. Lazhari Tichati: statistical application and review. Kheireddine Ouali: analysis and interpretation of histological and immunohistological samples.

**Data availability** Data and materials are available. The relevant author can provide the data reported in this work upon request.

## Compliance with ethical standards

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**Conflict of interest** The authors declare that they have no conflict of interest.

**Ethical approval and consent to participate** All the protocols used in this study were conducted according to the International Guidelines for Laboratory Animal Care and Use (Council of European Communities) (JO86/609/CEE) and approved by the Ethical Committee of BADJI Mokhtar University.

**Informed consent** For this type of study, informed consent is not required.

**Consent for publication** All the authors consent to the publication of the manuscript.

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