Olea europaea leaf extract can modulate the reproductive state of cadmium-poisoned rats

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ABSTRACT

Cadmium is a widely used heavy metal and is considered a powerful endocrine disruptor, with adverse effects on fertility and the body's physiological activities. The aim of the study is to investigate the benefits of the aqueous extract of fresh olive leaves (*Olea europaea* L.) in reducing cadmium toxicity. In this context, 80 male *Wistar* rats were divided into eight groups and treated daily for one month by gavage. The negative control received a standard diet; three positive control groups (LEO1, LEO2, and LEO3) respectively received different extracts (0.25, 0.5, and 1 g/kg rat), one group received fresh cadmium chloride solution (40 mg/kg rat), and the last three groups received a combination of LE + $CdCl_2$ with the same dosages. The results demonstrated cadmium led to a decrease in fertility parameters (concentration, mobility, LH, and testosterone), with a significant decrease in GSH and an increase in MDA levels. Supplementation of olive leaf extract significantly improved all parameters of the three combined treatment groups with different degrees. In conclusion, olive leaf extract was able to mitigate cadmium-intoxicated rats and preserved reproductive parameters.

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1. INTRODUCTION

Increased environmental pollution due to industrialization has become a major health concern worldwide [1; 2]. Heavy metals used in many applications continue to have very harmful effects on the environment and human health [3]. Cadmium is present in water, soil, and air, and it is toxic even at low concentrations. It also tends to accumulate in living organisms [4]. Due to its high toxicity and prolonged persistence in the environment, Cd, which is not essential, requires special attention [5]. Cadmium is a widespread environmental contaminant from industrial and agricultural activities, causing unavoidable exposure [6]. It is easy for it to enter the food chain and endangering human health [7]. Long-term exposure to cadmium in the environment and occupational activities has been associated with several pathophysiological disorders affecting vital organs [8; 9].

Mechanisms that induce testicular toxicity due to this metal include lipid peroxidation (LPO) and over production of reactive oxygen species (ROS). Cadmium has negative effects on various organs of the body, but its reproductive toxicity in men is of particular concern due to its potential for infertility [10]. The presence of cadmium impairs spermatogenesis, reduces sperm quantity and quality, and causes structural and functional abnormalities of the testis [11].

Metal toxicity neutralizers are commonly found in medicinal plants. They are considered a valuable source of antioxidant compounds due to their free radical scavenging ability and potential health benefits associated with their consumption [12]. The olive tree, known as *Olea europaea* L., is an iconic tree of very ancient origin. The main use of

the olive tree has traditionally been based on the oil extracted from the olive fruit due to its economic and nutritional values. However, in recent decades, interest has gradually shifted to the olive leaf due to its important pharmaceutical values [13; 14; 15]. The leaves have been used in human nutrition in the form of extracts, herbal teas, and powders [16].

In this context, this study focuses on assessing the toxicity of cadmium by evaluating reproductive parameters (spermogram, sex hormones) and oxidative stress (testes) in Wistar rats and on the mitigating effect of olive leaf *Olea europaea* at different concentrations on this toxicity.

2. EQUIPEMENT AND METHODS

2.1 Biological material

This work was carried out on 80 young male Wistar rats from the Pasteur Institute of Algiers, weighing on average 250 grams. The animals were raised in plastic cages (42.5 x 26.5 x 15 cm. Animals were exposed to laboratory conditions.

2.2 Preparation of olive leaf extract

Fresh leaves of the olive tree (*Olea europaea L.*) were sampled and the plant part was ground by adding 10 ml of distilled water using a blender. After filtering the mixture, the obtained aqueous crude extract was administered to rats daily by gavage.

2.3 Preparation of cadmium chloride

Cadmium chloride $CdCl_2$ was dissolved in a volume of distilled water. The obtained solution was administered by gavage.

2.4 Animal treatments

Male Wistar rats were divided into 8 groups: The control group received standard diet (in the form of pellets); the LEO1, LEO2 and LEO3 groups were treated with olive leaf extract at doses of 0.25, 0.5, and 1 g/kg body weight of rats, respectively. The cadmium group was treated with cadmium chloride solution (40 mg/kg body weight). The other 3 groups were treated with a combination of cadmium and leaf extract (Cd+LEO1, Cd+LEO2, Cd+LEO3) at the same dosage. The treatment was administered by gavage for one month.

2.5 Blood sampling

After 30 days of treatment, rats were fasted overnight and then sacrificed in the morning by decapitation. Once the dissection was completed, the testes were removed and weighed. In order to assess oxidative stress parameters, part of this organ was stored in a freezer at -20 °C.

2.6 Spermogramme study

Sperm was collected from a small opening made at the epididymis. The sperm was then diluted in physiological serum (NaCl 0.9%) to study the concentration and mobility of spermatozoa carried out by the machine (Sperm Class Analyzer).

2.7 Estimation of LH and testosterone

The enzyme immunoassay method was used for the determination of luteinizing hormone and testosterone according to the technical sheet of the Diametra ELISA kit.

2.8 Estimation of stress oxidative merkers

Glutathion (GSH)

The glutathione assay was performed according to the method of [17]. The principle of this assay is based on the measurement of the optical absorbance of 2-nitro-5-mercapturic acid.

The latter results from the reduction of 5,5'-dithiobis-2-nitrobenzoic acid (Ellman's reagent, DTNB) by the (-SH) groups of glutathione. For this, a deproteinization of the homogenate is essential in order to keep only the thiol groups specific to glutathione.

To obtain the homogenate, 200 mg of the testicle was placed in 8 mL of 0.02 M ethylene diamine tetra acetic acid (EDTA) solution, cold ground using an ultrasonic grinder at a temperature of 4 ° C.

Malondialdehyde (MDA)

MDA is one of the end products formed during the decomposition of polyunsaturated fatty acids by free radicals. The testicular MDA level was evaluated according to the method of [18].

500 mg of the testis was placed in 5 mL of phosphate buffer solution (0.1 M, pH 7.4), then cold ground (4 °C) using an ultrasonic grinder for 30 seconds to obtain a homogenate.

The protein concentration is determined according to the method of [19] which is based on the use of Coomassie blue (G 250) as a reagent. The latter reacts with the amine groups (–NH2) of the proteins to form a blue complex.

2.9 Statistical analysis

Statistical results are expressed as mean ± standard error. Data analysis was performed by ANOVA test comparing the means of each parameter analyzed two by two, using Graph Pad Prism software. Results are considered significant when P≤0.05 is represented by (*).

3. RESULTS

3.1 Testicular absolute weight

According to the results obtained in Figure 1, a significant decrease was recorded in the group treated with Cd alone compared to the different treatment groups. A significant increase in the combined groups (Cd+LEO) compared to the group treated with Cd alone was recorded.

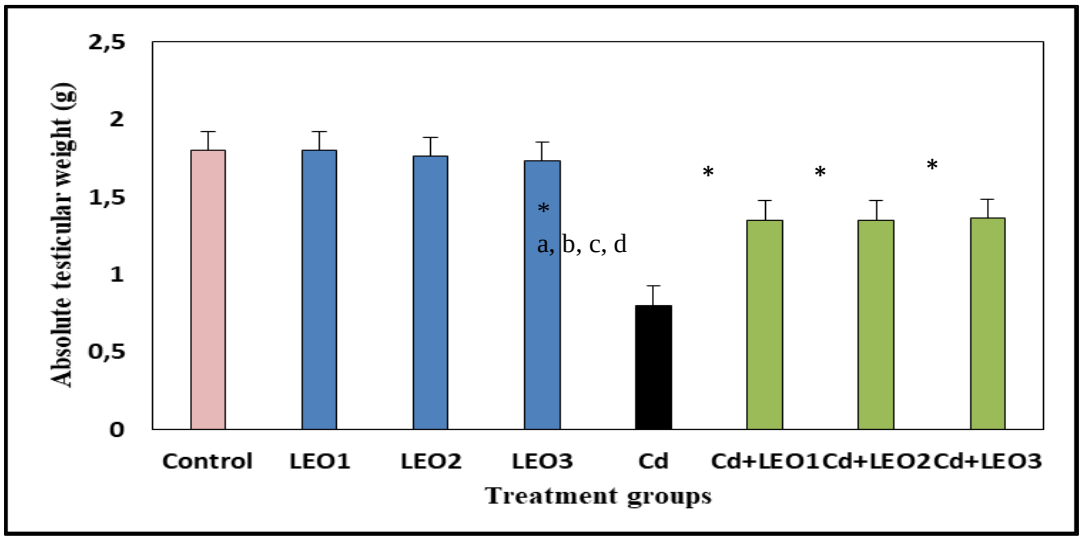

Figure 1: Change in absolute testicular weight (g) in different groups after 30 days of treatment.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

3.2 Spermatozoa concentration

According to the result presented in Figure 2, a significant decrease in sperm concentration in the Cd alone group compared to: (control, positive controls and the three groups combined).

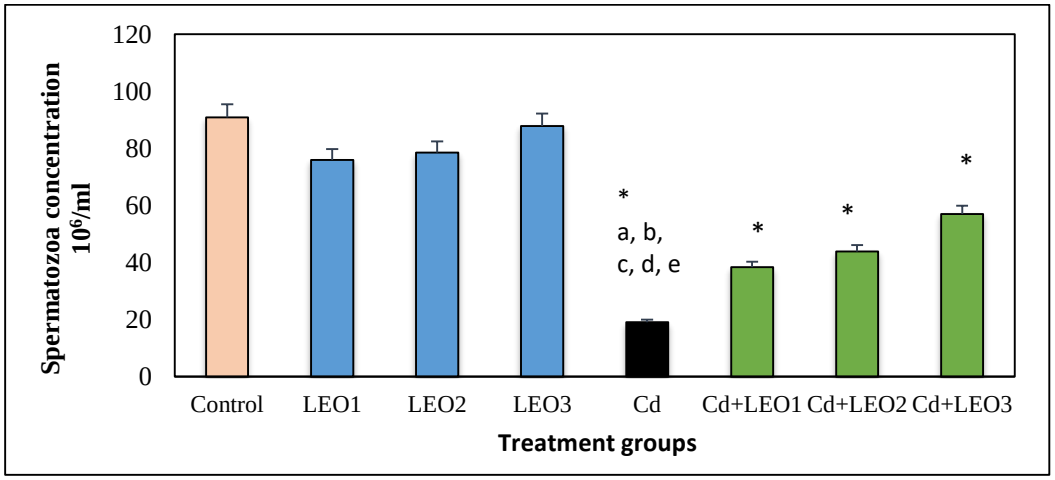

Figure 2: Sperm concentration (10^6/ml) in different treatment groups.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.3 Spermatozoa motility

A significant decrease in sperm motility was recorded in the group treated with cadmium alone compared to the control and positive control groups. On the other hand, a significant increase was observed in the combined groups (Cd+LEO1, Cd+LEO2 and Cd+LEO3) compared to the group treated with Cd alone.

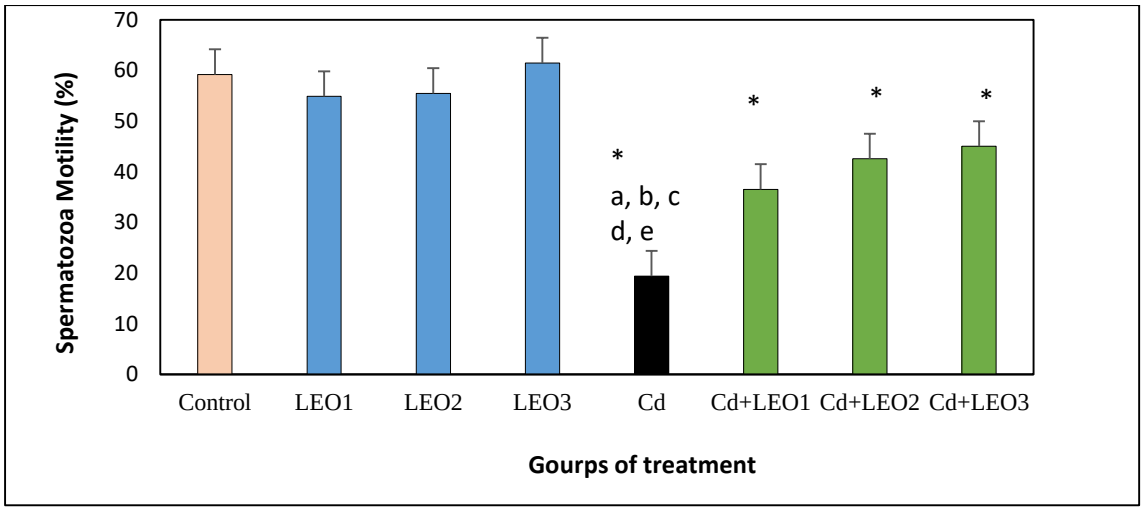

Figure 3: Sperm motility (%) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

3.4 Testosterone

Figure 4 showed a significant decrease in testosterone levels in the cadmium-exposed group compared to the control, positive controls, and groups treated with the combination of cadmium and olive leaves at different doses.

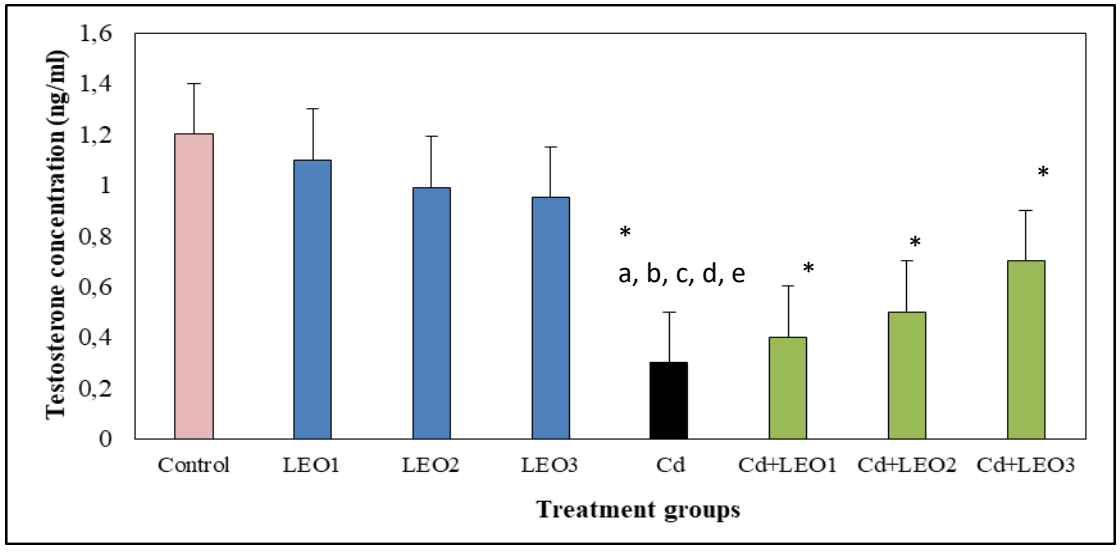

Figure 4: Variation in testosterone concentration (ng/ml) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.5 Luteinizing hormone (LH)

A significant decrease was recorded in rats exposed to cadmium alone compared to control, positive control and combined groups.

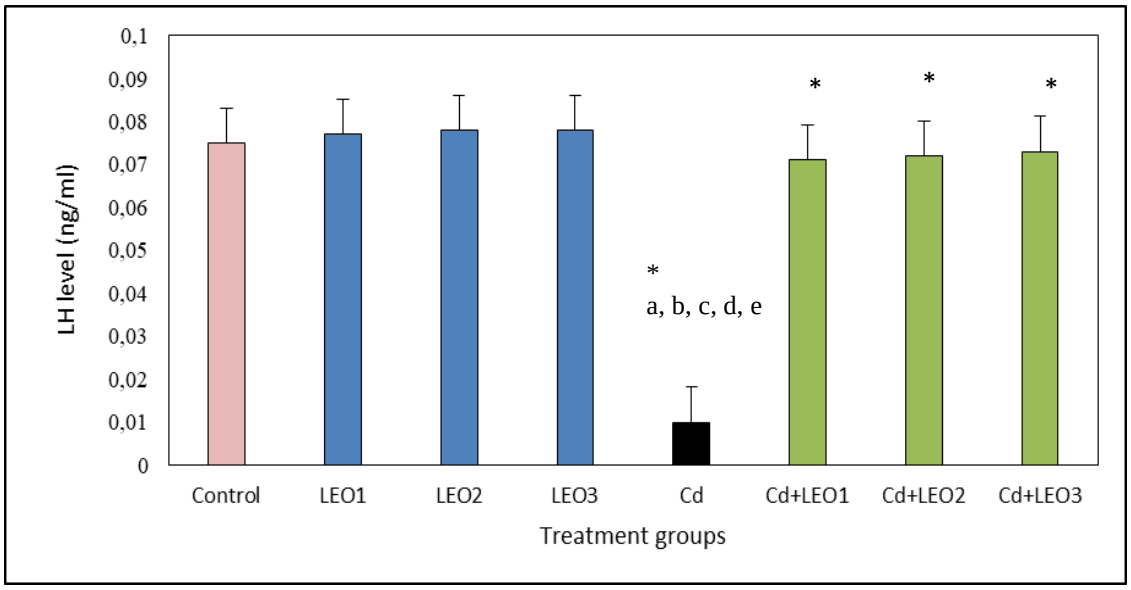

Figure 5: Variation in LH level (ng/ml) in different treatment groups.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.6 Testicular glutathione

According to the results obtained in Figure 6, a significant decrease in glutathione level was recorded in the group treated with Cd alone compared to the control groups, LEO1, LEO2, LEO3 and Cd+LE groups.

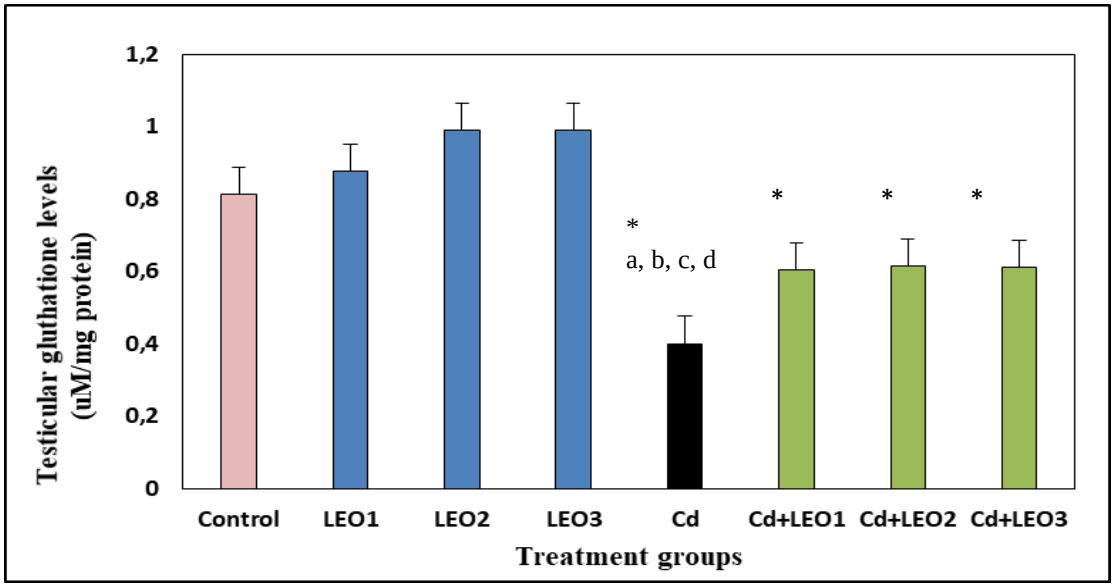

Figure 6: Variation in testicular glutathione levels (µM/mg protein) in rats exposed to cadmium and olive leaf extract after 30 days of treatment.

a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE.

3.7 Testicular malondialdehyde

The results mentioned in Figure 7, indicated a significant increase in the MDA rate concerning the group exposed to cadmium alone compared to the control, positive control and Cd+LEO1, Cd+LEO2 and Cd+LEO3 groups, respectively.

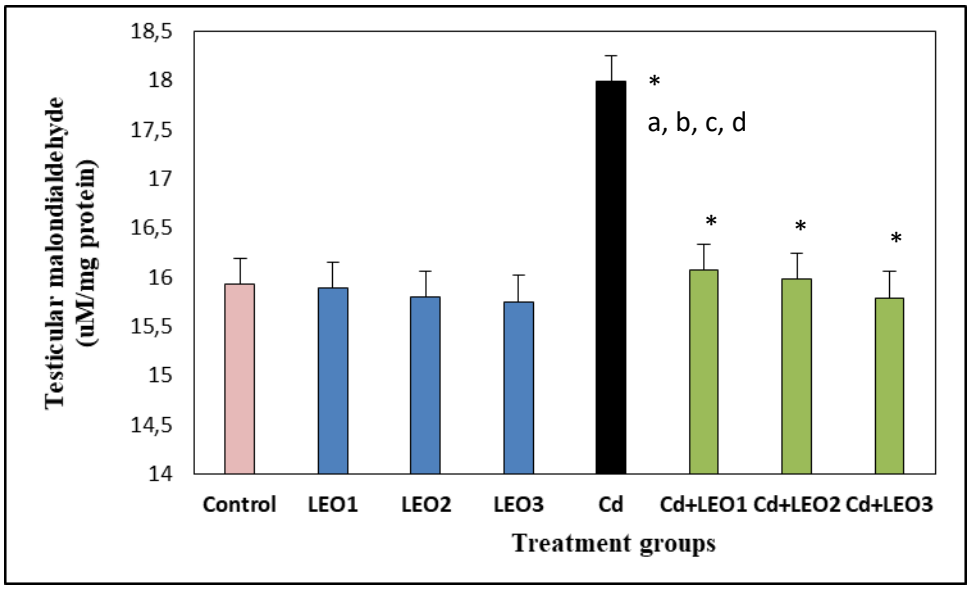

Figure 7: Variation in testicular MDA levels (µM/mg protein) of rats exposed to cadmium and olive leaf extract after 30 days of treatment.
a: Cd vs control; b: Cd vs LEO1; c: Cd vs LEO2; d: Cd vs LEO3; e: Cd vs LE

4 DISCUSSION

In recent years, the persistence of cadmium contamination has become a major concern due to its adverse effects on the reproductive organs. These properties make them vulnerable to free radical attack, which can lead to alterations in various membrane enzymes, as well as affect the structural integrity and fluidity of the membrane [20].

In our study, sperm motility and concentration were significantly reduced in rats exposed to cadmium alone compared to the control, positive control, and combined groups. The results obtained are similar to those of other studies [21]. However, studies conducted on the effects of cadmium on human sperm motility, concentration, and viability have shown that this metal not only significantly reduces these parameters; changes in ATP (adenosine triphosphate) production, which provides the energy required for sperm movement, are also observed [22].

It has been suggested that cadmium-derived ROS can propagate through lipid membranes and cause disturbances in various reproductive mechanisms. Chronic exposure to cadmium has been shown to impair mammalian reproductive functions by causing spermatogenesis impairment, sperm motility impairment, sperm morphology alteration, and decreased acrosome reaction rate [11]. Germ cells containing high concentrations of polyunsaturated fatty acids exhibit low antioxidant capacity.

Cadmium has detrimental effects on the action of the hormones LH and testosterone, which play an important role in spermatogenesis, while not ignoring its effects on Leydig cells responsible for testosterone secretion. In addition, its effects on the composition and effect of the hormone testosterone acting on the hypothalamic-pituitary axis explain the reason for the decreased fertility in poisoned rats. Recent studies [23] have shown that cadmium chloride causes the degradation of stem cells (spermatogonia), alteration of Sertoli cells, and spermatogenesis disorders.

With regard to oxidative stress markers, the results showed a significant decrease in testicular glutathione levels in the metal-exposed group. Cadmium appears to bind to the SH group of GSH, affecting its antioxidant activity, and it also has the ability to alter the activity of antioxidant enzymes. Co-administration of leaf extract to rats resulted in an increase in testicular GSH concentration and a decrease in MDA concentration. Studies conducted by several authors [24; 25] have shown that chronic exposure to cadmium chloride induces metabolic changes in rats, including a significant decrease in glutathione (GSH) antioxidant parameters along with an increase in morphological and malonic acid peroxidation indices, including methanogen dialdehyde (MDA). Known functions of glutathione include its role in biosynthetic pathways, detoxification, and redox homeostasis. Glutathione can interact with proteins in a variety of ways through thiol-disulfide exchange and other processes to protect body tissues and organs.

5 CONCLUSION

This study revealed that the toxicity caused by cadmium chloride after 30 days of exposure resulted in various alterations in reproductive function (testosterone and LH), sperm quality (concentration and motility) by disruption of the hypothalamic-pituitary-gonadal axis in male Wistar rats. In addition, cadmium increased the testicular MDA level, with a decrease in the GSH concentration. However, co-administration of the aqueous extract of fresh olive leaves with cadmium preserved these parameters in the normal physiological state, which determines the benefits of the plant constituents.

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