MANSOURA JOURNAL OF CHEMISTRY
Official Journal of Faculty of Science, Mansoura University, Egypt
E-mail: scimag@mans.edu.eg ISSN: 2974-4938

Garlic Mitigates Cadmium-Induced Renal Damage: A Histopathological Study in Sprague-Dawley Rats

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Received:2025/9/13
Accepted: 4/10/2025

Abstract: Background: Cadmium (Cd) is a toxic heavy metal that results from industrial pollution and accumulates mainly in the kidneys, causing cellular damage and impaired function, posing significant environmental and health risks worldwide.

Methods: Thirty-two male Sprague-Dawley rats were randomly divided into four equal groups: a control group, a Cd group (receiving $CdCl_2$ at 5 mg/kg body weight three times weekly), a garlic treatment group (receiving $CdCl_2$ at 5 mg/kg body weight three times weekly along with a diet containing 7% ground garlic), and a garlic protection group (fed a 7% garlic-supplemented diet for two weeks before and during six weeks of Cd, with $CdCl_2$ at 5 mg/kg body weight three times weekly). Tissue samples were collected to measure Cd accumulation in renal tissue, expression of the metallothionein (MT) gene, and for histopathological examination.

Results: Cd accumulates in renal tissues, increasing the expression of the MT gene, with a positive correlation observed between the two variables (Pearson's; $p<0.01$). Treatment with garlic reduces MT expression, especially in the protective group. Histopathological examination of renal tissues revealed structural damage in the Cd-exposed group, characterized by tubular degeneration, glomerular atrophy, interstitial nephritis, and vascular congestion. Conversely, both garlic groups demonstrated marked improvement in renal histology. The garlic protection group showed near-normal histological features with minimal pathological alterations, suggesting garlic's potential nephroprotective effect against Cd-induced toxicity.

Conclusion: Garlic exhibits both detoxifying and protective effects against Cd-induced nephrotoxicity. Notably, its prophylactic action, when administered before Cd exposure, proved more effective than co-administration as a therapeutic approach.

keywords: Cadmium, garlic, histopathology, nephrotoxicity

1.Introduction

Environmental contamination by heavy metals and trace elements (HMTE) has increased significantly due to their extensive industrial and agricultural use. Among these pollutants, cadmium (Cd) stands out as a toxic heavy metal with no known biological role. Its rapid dispersal in the environment poses serious health risks, primarily through accumulation in the food chain [1]. After entering the body, mainly via inhalation, Cd binds to red blood cells and gradually accumulates in vital organs, especially the kidneys and liver [2]. Because Cd is eliminated from the body very slowly through renal excretion, the kidneys become particularly vulnerable to its toxic effects over time [3].

One of the major mechanisms by which Cd exerts its nephrotoxic effects is through the induction of oxidative stress. Cd disrupts mitochondrial function by inhibiting the electron transport chain and displacing essential trace metals like copper and zinc in antioxidant enzymes. These actions lower the body's antioxidant defenses and lead to excess production of reactive oxygen species (ROS) [4], which cause significant damage to cellular

DNA, proteins, and lipids. [5].

Although the body has both enzymatic and non-enzymatic antioxidant systems, prolonged exposure to Cd can overwhelm these defenses, resulting in progressive renal injury, particularly within the tubular and glomerular regions [6]. In addition to oxidative damage, Cd-induced renal toxicity is also associated with alterations in the expression of genes involved in oxidative stress response, inflammation, and cellular repair, further contributing to the pathogenesis of kidney dysfunction [7].

Numerous natural compounds have been identified for their potential against heavy metal-induced toxicity, among which garlic (*Allium sativum*) shows significant promise due to its potent bioactive properties. Garlic is a well-known medicinal plant traditionally used for its protective effects on various organs, including the kidneys. It is rich in bioactive compounds such as allicin, S-allyl cysteine, and other sulfur-containing molecules [8], which are recognized for their antioxidant, anti-inflammatory, and metal-chelating properties [9]. These compounds contribute to enhancing the body's defense systems and support renal health by mitigating tissue damage [10].

The present study was designed to investigate the impact of cadmium exposure on metal accumulation, the expression levels of the MT gene, and renal histopathological alterations. Additionally, it aimed to explore the potential therapeutic and protective effects of garlic in mitigating cadmium-induced renal toxicity.

2. Materials and methods

Chemicals and Reagents

Cadmium chloride ($CdCl_2$) was purchased from Oxford Lab Chemicals, India (Catalog No. C-02022) and used without further purification. Fresh garlic cloves were obtained from a local market in Egypt, thoroughly washed, and minced just before use. Nitric acid (HNO_3, 69%) EMSURE® (CAS No. 7697-37-2) was obtained from Merck KGaA, Darmstadt, Germany. Hydrogen peroxide (H_2O_2, 30% w/w in H_2O, CAS No. 7722-84-1) was purchased from Sigma-Aldrich (Munich, Germany). For molecular biology procedures, RNA stabilization was performed using RNAlater® (Cat. No. AM7024, Invitrogen, USA). Total RNA was extracted using the RNeasy Mini Kit (Cat. No. 74104, Qiagen, Germany). cDNA synthesis was conducted using the RevertAid First Strand cDNA Synthesis Kit (Cat. No. K1622, Thermo Scientific, USA). Quantitative real-time PCR was carried out using the 2x QuantiTect SYBR Green PCR Kit (Cat. No. 208052, Qiagen, Germany). Primers were synthesized by Thermo Fisher Scientific (Waltham, MA, USA). All other chemicals and reagents were of analytical grade and obtained from standard commercial suppliers.

2.2. Experimental Animals and Housing Conditions

A total of 32 male Sprague-Dawley rats, aged 6–7 weeks and weighing between 200–250 g, were housed in polycarbonate cages, with four rats per cage. Animals were maintained under controlled environmental conditions: a 12-hour light/dark cycle, temperature of 24 ± 2 °C, and relative humidity of 50–70%. Rats had ad libitum access to standard laboratory chow containing 14–16% protein and no more than 8% fat, as well as free access to water throughout the experimental period.

All animal handling and experimental procedures were conducted in accordance with the guidelines of the Institutional Animal Ethics Committee of the National Research Center. Ethical approval for the study was obtained from the Animal Care and Use Committee at Mansoura University (Approval Code: MU-ACUC (SC, PhD.22.10.3)).

Experimental Design

The animals were randomly assigned to four equal groups (n = 8 per group). The **control group** received distilled water (5 mL/kg body weight) orally once daily, along with a standard diet, throughout the experimental period. The **Cd group** was administered cadmium chloride ($CdCl_2$) at a dose of 5 mg/kg body weight via oral gavage three times per week for six consecutive weeks [11]. The **garlic treatment group** received the same Cd dosing regimen as the Cd group, in addition to a daily diet supplemented with 7% freshly ground garlic for six weeks, reflecting a nutritionally relevant intake [12]. In the **garlic protection group**, rats were pre-fed the garlic-enriched diet for two

weeks prior to Cd exposure, which was then administered as described for the Cd group. Garlic supplementation continued throughout the remainder of the experiment. All animals were sacrificed at the end of the eight-week study period for subsequent analysis.

Sample Collection and Preparation

After completing the 8-week experimental period, the rats were humanely euthanized under general anesthesia using sodium thiopental. Their kidneys were carefully collected and either frozen at −80°C for Cd measurement and gene expression analysis or fixed in 10% formalin for later histopathological evaluation.

Methods

Assessment of Cadmium Content in Renal Tissues

Renal tissue samples were collected from all experimental animals for analysis. A measured portion of each tissue was digested by adding 3 mL of nitric acid (HNO_3) and 1 mL of hydrogen peroxide (H_2O_2) to the digestion vessels, which were then left at room temperature for 15 minutes to allow initial reaction. Following this, the samples underwent microwave-assisted digestion using a Speedwave Four system (Berghof Products, Germany) under a one-step program consisting of a power output of 1600 W, a temperature ramp to 200 °C, over 15 minutes, a 15-minute hold at this temperature, and a subsequent 15-minute cooling period [13]. Once cooled, the digested samples were diluted to a final volume of 10 mL with double-distilled water. The Cd concentration in the prepared solutions was then determined by inductively coupled plasma optical emission spectrometry (ICP-OES) using a Perkin Elmer Optima 7000 DV instrument (Germany).

RNA Extraction and Quantitative Real-Time PCR

Renal tissue samples were preserved at −80°C in RNAlater until processing. Total RNA was extracted using the RNeasy Mini Kit according to the manufacturer's instructions. RNA concentration and purity were assessed using a NanoDrop 2000C spectrophotometer, while RNA integrity was confirmed via agarose gel electrophoresis stained with ethidium bromide. First-strand complementary DNA (cDNA) was synthesized using a reverse transcription kit. Quantitative real-time PCR (qRT-PCR) was performed using SYBR Green-based detection. Specific primers targeting metallothionein (MT) and the housekeeping gene GAPDH were designed using the NCBI Primer-BLAST tool and synthesized commercially. The primer sequences used for MT1 were 5'-ACCTCCTGCAAGAAGAGCTG-3' (forward) and 5'- AAACTGGGTGGAGGTGTACG-3'(reverse), and for GAPDH were 5'-TGGGAAGCTGGTCATCAAC-3' (forward) and 5'- GCATCACCCCATTTGATGTT-3' (reverse). Relative gene expression levels were calculated using the 2^−ΔΔCT method, with GAPDH used as the internal control.

Histopathological Examinations

Renal tissue samples from all control and treated animals were first rinsed in 1X phosphate-buffered saline (PBS) and then fixed in 4% paraformaldehyde (PFA). Following fixation, the tissues were dehydrated through a graded ethanol series ranging from 70% to 100%, and subsequently embedded in paraffin wax. Sections of 5 μm thickness were cut using a Leica microtome (Leica Microsystems Ltd). These sections were mounted on slides, dewaxed in xylene, rehydrated through a descending ethanol series, and stained with hematoxylin and eosin (H&E) [14], Histological evaluation was performed under an Olympus CX51 light microscope (Olympus, Japan), and images were captured using an Olympus E-620 camera. Quantitative analysis was conducted with Cell* software (Olympus Soft Imaging Solution GmbH). Key pathological features such as tubular degeneration, necrosis, glomerular alterations, inflammation, and hemorrhage were assessed using a semi-quantitative scoring system as described by Shi et al. [15].

Statistical analysis

Statistical analyses were conducted using SPSS version 20 (MAS Medical and Scientific Equipment Co., IL, USA). Data normality was first assessed. Continuous variables are presented as mean ± standard deviation (SD), and comparisons between groups were performed using one-way ANOVA followed by

Tukey's post hoc test. A *p*-value of less than 0.05 was considered statistically significant. Pearson correlation coefficients (*r*) were calculated to examine the relationship between variables. Non-parametric data, such as the pathological scores from histological examinations, were analyzed using the Mann-Whitney U test and Kruskal-Wallis test as appropriate.

3. Results and Discussion

Cadmium (Cd) contamination in the environment poses significant health risks due to its toxic effects on various organs, particularly the kidneys. It is well-established that Cd exposure can impair kidney health [16]. In the present study, we focused on evaluating Cd-induced nephrotoxicity and examined the potential protective and therapeutic effects of garlic against this toxicity.

Concentration of Cd in Renal tissue

Figure 1 compares Cd levels in renal tissue. The concentration of Cd in the Cd group was 5.39 ± 0.73 mg/g, which was significantly higher compared to the control group (0.056 ± 0.01 mg/g), garlic treatment (3.23 ± 1.12 mg/g), and garlic protection groups (1.56 ± 0.42 mg/g) ($p < 0.01$). Both the garlic treatment and protection groups showed a significant elevation in renal Cd levels compared to the control group ($p < 0.01$) and a significant reduction compared to the Cd group ($p < 0.01$).

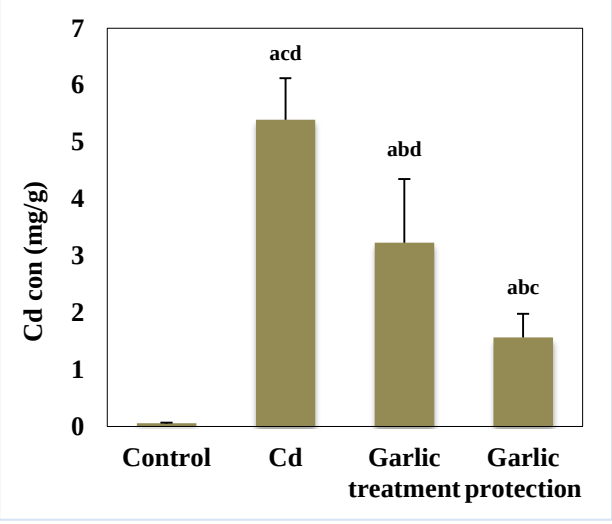

Figure 1: Comparison of the Cadmium concentration (mg/g) between different groups

Results are expressed as mean ± SD $p < 0.05$ was considered to be statistically significant. [a] Significantly different from control group; [b] Significantly different from Cd group; [c] Significantly different from garlic treatment group; [d] Significantly different from garlic protection group

Our findings revealed a marked accumulation of Cd in kidney tissues following Cd exposure, resulting in a significant elevation of Cd levels compared to the control group. This observation aligns with previous evidence indicating that Cd tends to accumulate in vital organs such as the liver and kidneys upon exposure [17].

In contrast, treatment with garlic showed a notable ability to counteract Cd accumulation, as evidenced by a significant reduction in Cd levels in the garlic-treated groups compared to the group exposed to Cd alone. This suggests that garlic plays a beneficial role in enhancing the detoxification process and limiting Cd retention in renal tissues. These findings are consistent with previously observed patterns, where Cd exposure led to elevated renal concentrations [18] , while garlic administration contributed to a measurable decrease in Cd accumulation within kidney tissue [19].

The ability of garlic to reduce heavy metal toxicity is largely attributed to its sulfur-containing compounds, particularly allicin, which exhibit strong metal-chelating properties. These bioactive compounds can bind to Cd ions, facilitating the formation of stable complexes and thereby limiting Cd accumulation in vital organs such as the kidneys and liver [20]. In addition to their metal-binding capacity, Organosulfur compounds in garlic, particularly allicin, possess a wide range of biological activities, including antitumor, antimutagenic, detoxifying, and antioxidant effects. Upon crushing or cutting garlic, allicin rapidly breaks down to form diallyl tetrasulfide, a lipid-soluble compound with enhanced antioxidant capacity and potential health benefits [21].

Expression of the MT gene

The nephrotoxic effects of Cd are believed to involve alterations in the expression of specific genes [7]. In the present study, we focused on evaluating the expression of the metallothionein (MT) gene, given its well-

established role in metal detoxification and cellular defense against Cd-induced toxicity.

MT is a low-molecular-weight protein rich in cysteine residues, playing a key role in the regulation and metabolism of various heavy metals. It contributes to essential physiological processes, such as the storage and transport of copper and zinc ions to metalloproteins and enzymes [22]. Additionally, MT helps maintain cellular redox balance by scavenging free radicals and facilitating the detoxification of toxic metal ions, including Cd, lead (Pb), and mercury (Hg). Due to these properties, MT is considered an important protective factor against Cd-induced toxicity [23].

The mRNA level of the MT gene (Figure 2) was significantly elevated in all rats exposed to Cd compared to the control group ($p < 0.01$). In the Cd group, the mRNA level of MT was higher compared to the garlic treatment and protection groups, with a significant increase observed in the garlic protection group ($p < 0.01$).

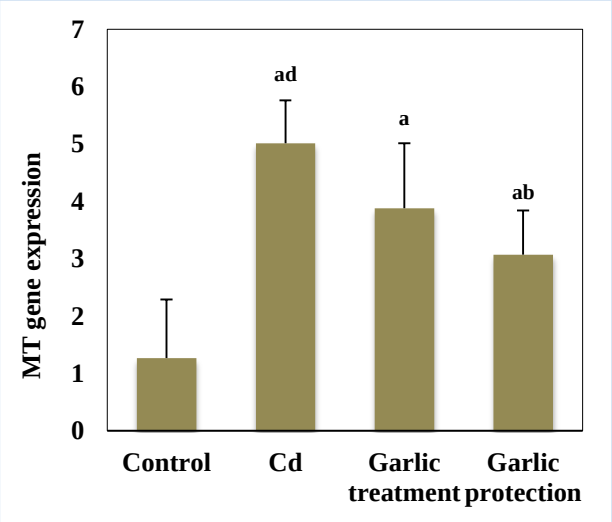

Figure 2: Comparison of MT gene expression between different groups

Results are expressed as mean ± SD $p < 0.05$ was considered to be statistically significant. [a] Significantly different from control group; [b] Significantly different from Cd group; [c] Significantly different from garlic treatment group; [d] Significantly different from garlic protection group

Figure 3 illustrates the relationship between Cd concentration (mg/g) and the expression level of the MT gene, which is known to play a crucial role in cellular responses to heavy metal exposure. The plot demonstrates a strong positive correlation between the two variables with a coefficient of determination (R^2) = 0.652, indicating that approximately 65.2% of the variation in MT gene expression can be explained by changes in Cd concentration. The Pearson correlation coefficient (r) ≈ is approximately 0.80, indicating a strong positive correlation. The P-value < 0.0001, suggesting that the relationship is highly statistically significant.

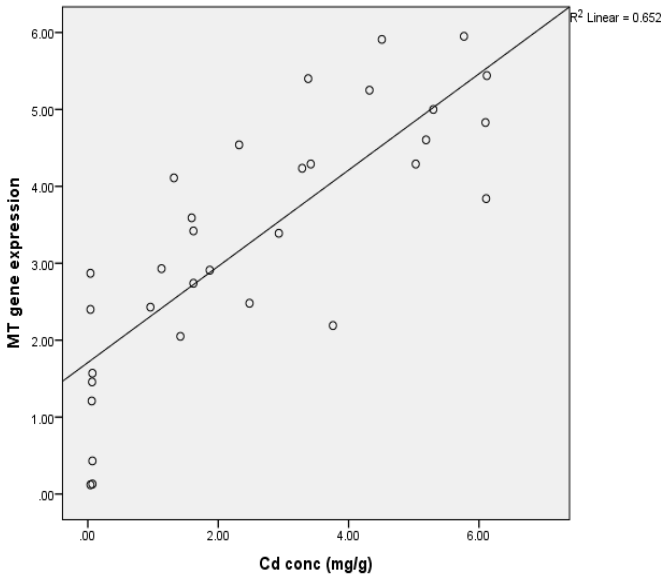

Figure 3: Scatter plot illustrating the statistical correlation between Cd concentration (mg/g) and MT gene expression

In the current study, exposure to Cd resulted in a significant upregulation of **MT** gene expression in rat kidney tissues compared to the control group. This elevated expression reflects the cellular response to Cd accumulation, as MT is induced to mitigate metal toxicity. The increased expression of MT may also be attributed to Cd-induced synthesis in the liver and its subsequent impact on renal tissues. These findings are consistent with previously observed patterns of MT induction in response to Cd exposure [24].

Once Cd enters the body, it exhibits a strong affinity for thiol groups and readily binds to MT, forming stable Cd-MT complexes. These complexes circulate systemically and are filtered by the glomeruli, after which they are reabsorbed by the proximal tubular epithelial cells in the kidneys. Prolonged exposure to Cd can lead to the depletion of MT, resulting in the release of free Cd^{2+} ions. These unbound ions

interact with thiol-containing cellular components, impairing the function of essential proteins and enzymes, and initiating a cascade of toxic effects that contribute to sustained renal injury [5]. Garlic has been shown to mitigate the genotoxic impact of heavy metals and alleviate associated oxidative stress and mitochondrial dysfunction, highlighting its potential as a protective agent [25].

Histopathological Evaluation of Renal Tissues

The histopathological findings in this study supported the molecular result. Figure 4 details the histopathological examination of renal tissues obtained from rats of different groups. The mean histopathological score of renal tissue significantly increased in the Cd group compared to the control group, indicating marked kidney injury. However, this score was notably reduced in both garlic treatment and protection groups, with the garlic protection group showing the most substantial improvement (Figure 4A).

Microscopically, the control group (Figure 4B) exhibited a normal renal architecture, including intact tubules and glomeruli. In contrast, the Cd group displayed prominent renal lesions, including multifocal cortical interstitial nephritis, degenerated tubules, hyaline casts, atrophied glomeruli, and perivascular inflammation and edema (Figure 4C). Further fibrotic changes, tubular damage, and vascular congestion were observed (Figure 4D).

The garlic treatment group demonstrated partial improvement, with reduced inflammatory infiltration and fewer degenerative changes (Figure 4E). Meanwhile, the garlic protection group appeared mostly normal, showing minimal vascular congestion and limited histological alterations (Figure 4F).

These outcomes are consistent with previous research that reported similar protective effects of garlic against Cd-induced renal injury [11]. Moreover, the reversal of histopathological damage was more pronounced when garlic was administered prior to Cd exposure, as seen in the protection group. Remarkably, in some animals from this group, kidney tissues closely resembled those of healthy controls, highlighting the strong nephroprotective potential of garlic.

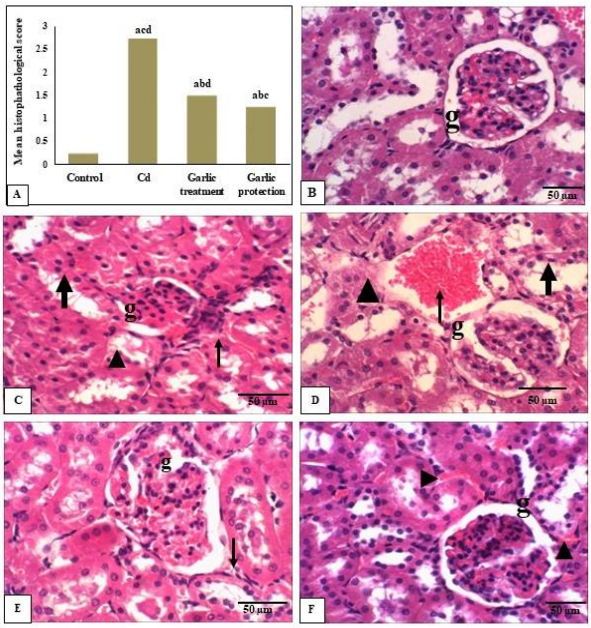

Figure 4: Histopathological examination of the renal tissues stained with hematoxylin and eosin (400X).

A) Mean histopathological score of renal tissue in different groups. Bars with different superscripts indicate statistically significant differences: $p < 0.05$ was considered to be statistically significant [a] Significantly different from control group; [b] Significantly different from Cd group; [c] Significantly different from garlic treatment group; [d] Significantly different from garlic protection group. B) control kidney mostly showing the normal histological appearance of tubules and glomeruli (g). C) Cd group showing multifocal mild cortical interstitial nephritis, separated degenerated tubules (black arrows) with few intraluminal hyaline casts (arrowhead) and atrophied glomeruli (g), mild perivascular aggregations of lymphocytes, plasma cells, and macrophages, admixed with perivascular edema (thin arrow). D) Cd group showing focal, coalescing mild fibrosis (arrowhead) with moderate tubular damage (thick arrow), intratubular aggregation of sloughed epithelium with congested glomeruli (g) and blood vessel (thin arrow). E) Garlic treatment group showing cortical improvement with focal low aggregation of inflammatory cells (thin arrow). F) Garlic protection group mostly appeared normal with a few, multifocal vascular congestion (arrow heads).

In conclusion, garlic demonstrates promising protective effects against Cd toxicity through its sulfur-containing compounds that chelate metals. However, while the results are encouraging, further studies are needed to clarify the precise molecular mechanisms involved and to evaluate optimal dosing and long-term safety. Future research should also explore garlic's effects in diverse models and clinical settings to confirm its potential as a natural therapeutic agent for heavy metal detoxification.

Acknowledgments

We would like to thank *Science and Technology Development Fund (STDF)* for the financial support of this study.

Funding

This work was funded by *Science and Technology Development Fund* (STDF, project ID: 41579).

Author's contribution

All authors contributed to the study's conception and design. Abdel-Aziz A. F. performed conceptualization. Heba H. Tarabay and Manar E. Elkady performed the methodology. Heba H. Tarabay wrote the first draft of the manuscript. Bedeir Ali-El-Dein reviewed and edited the manuscript. Bedeir Ali-El-Dein performed funding acquisition. Abdel-Aziz A. F. , Manar E. Elkady, and Bedeir Ali-El-Dein were supervisors. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Conflicts of Interest Statement

The authors have no competing interests to declare that are relevant to the content of this article.

Ethics approval

This study was performed in line with the ARRIVE guidelines (https://arriveguidelines.org), which are designed to enhance the reporting of research involving animals. Approval of this study was granted by the Local Institutional Review Board and the Animal Care and Use Committee, Mansoura University (MU-ACUC) (code no. MU-ACUC (SC, PhD.22.10.3)).

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