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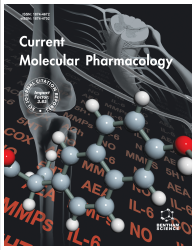


RESEARCH ARTICLE

# Effect of Chrysin and Chrysin Nanocrystals on Chlorpyrifos-Induced Dysfunction of the Hypothalamic-Pituitary-Testicular Axis in Rats

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**Abstract:**

***Aims and Background:***

The escalating global concerns regarding reproductive health underscore the urgency of investigating the impact of environmental pollutants on fertility. This study aims to focus on Chlorpyrifos (CPF), a widely-used organophosphate insecticide, and explores its adverse influence on the hypothalamic-pituitary-testicular axis in Wistar male rats. This study explores the potential protective effects of chrysin nanocrystal (CHN), a flavonoid with known antioxidant and anti-inflammatory properties, against CPF-induced impairments in male Wistar rats.

***Methods:***

Chrysin nanocrystals were prepared using a solvent precipitation method. Six sets of male Wistar rats were subjected to 30 days of treatment, comprising a control group, a group treated solely with CPF, groups treated with CHN at doses of 5 mg/kg and 10 mg/kg, and groups co-treated with CPF and CHN. Serum levels of reproductive hormones, enzyme biomarkers of testicular function, oxidative stress, and inflammatory biomarkers were assessed. Additionally, histological examinations were conducted on the hypothalamus, testes, and epididymis.

***Results:***

CHN exhibited antioxidant and anti-inflammatory properties, effectively counteracting CPF-induced reductions in Luteinizing Hormone (LH), serum testosterone, Follicle-Stimulating Hormone (FSH), and testicular enzyme biomarkers. Moreover, CHN enhanced antioxidant defenses, as evidenced by decreased malondialdehyde (MDA) and increased glutathione (GSH) levels in the hypothalamus, and testes, epididymis. Inflammatory markers, including nitric oxide (NO), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), were significantly reduced in CHN co-treated groups compared to the CPF-only group. Histopathological analyses confirmed the protective effects of CHN on tissue integrity.

***Conclusion:***

Chrysin nanocrystal demonstrated promising potential in mitigating CPF-induced reproductive deficits in male rats through its anti-inflammatory and antioxidant properties. This study provides valuable insights into therapeutic interventions against environmental toxin-induced reproductive toxicity, emphasizing the potential of chrysin nanocrystals as a protective agent in the context of CPF exposure.

**Keywords:** Chrysin, Chrysin nanocrystal, Chlorpyrifos, Hypothalamic-pituitary-testicular axis, Rat, Follicle-Stimulating Hormone (FSH).

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## 1. INTRODUCTION

The increasingly evident decline in reproductive health among both animal and human populations is a major global concern [1]. Recent research shows that nearly 186 million people worldwide struggle with infertility, with more than half of these cases attributed to male infertility [1]. The impact of environmental pollutants on reproductive disorders is widely acknowledged [2]. Both wildlife and humans often encounter toxic chemicals from various sources, including the food we consume and industries. CPF, a prevalent organophosphate insecticide, is commonly used for households and agriculture applications [3]. In 2007, it was recorded as the pesticide with the highest utilization across the United States, with an

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estimated total usage of 7 billion pounds [3]. Certainly, CPF remains a leading organophosphate insecticide on a global scale.

The widespread application of CPF and its rapid distribution in the environment pose potential threats to both intended and unintended organisms within the ecosystem [4]. Primary exposure routes to CPF include inhaling it, absorbing it through the skin, and ingesting it through contaminated food [4]. Overexposure to CPF can lead to harmful sub-lethal effects or even death [4]. Both experimental and epidemiological studies assessing the dose response to CPF have indicated that exposure can also cause reproductive and developmental toxicity [5 - 8]. Research on population health has revealed that exposure to CPF significantly decreases both head circumference and birth weight in infants [9 - 11]. Moreover, exposure to CPF has been linked to a decrease in human semen quality, marked by reduced sperm concentration and motility, as well as heightened damage to sperm DNA [10].

The mechanisms by which CPF induces reproductive toxicity are believed to include a reduction in testicular antioxidant defense systems, the induction of lipid peroxidation, inhibition of steroidogenic enzymes, and apoptosis in animal models [11]. Therefore, gaining an understanding of these mechanisms is crucial for enhancing our knowledge of effective therapeutic interventions for individuals exposed to CPF-induced reproductive damage [11]. Flavonoids have attracted widespread attention globally due to their powerful pharmacological effects [12]. Chrysin, a dietary phytochemical found abundantly in various plant extracts such as honey, propolis, and blue passion flower (*Passiflora caerulea*), holds significant economic and medicinal value [12]. Researchers have evidently demonstrated the fascinating anti-inflammatory and antioxidant activities of chrysin [13 - 15].

However, scientific data are scarce regarding the potential protective effects of chrysin against reproductive toxicity induced by CPF exposure. In addition, despite its beneficial functions, the limited water solubility and bioavailability of chrysin pose challenges in introducing it as a new drug. To address this challenge, nanotechnology has provided a water-soluble and biocompatible nanoparticle form of chrysin with physicochemical properties that make it a promising option for drug delivery [16]. Therefore, this study aims to assess the effect of Chrysin Nanocrystals (CHN) on Chlorpyrifos (CPF)-induced functional alterations in the hypothalamic-pituitary-testicular axis and the epididymis. These structures play pivotal roles in sperm maturation, protection, concentration, transport, and storage.

## 2. METHODS

### 2.1. Chemicals

CPF was obtained from Jiangsu Co., Ltd, China, while chrysin was sourced from Sigma Aldrich. Oxidative parameters were evaluated using NavandSalamat (NS) diagnostic kits, including the Nalondi™ Lipid Peroxidation Assay Kit-MDA, Nasdox™ Glutathione Assay Kit, and Nasdox™ Nitric Oxide Assay Kit-NO.TNF-α and IL-6 were purchased from eBioscience.

### 2.2. Preparation of Chrysin Nanocrystal

Chrysin nanocrystals were synthesized *via* a solvent precipitation method [17]. In this approach, commercial chrysin was dissolved in acetone at predetermined concentrations of 5 and 10 mg/ml. The solution was subsequently loaded into a syringe connected to a syringe pump. The drug solution was rapidly injected (8 ml/min) into the anti-solvent solution (deionized water) at a ratio of 1 part drug solution to 250 parts water while being stirred magnetically (at speeds ranging from 300 to 1000 rpm). The chrysin nanoparticles obtained were filtered, frozen at -30°C, and then subjected to vacuum drying. Morphological analysis of the chrysin nanocrystals was conducted using Scanning Electron Microscopy (SEM) (VP 1450 Co., VP 1450). The samples were dispersed on an SEM stub, coated with gold *via* sputtering, and observed under SEM.

### 2.3. Experimental Design

Forty male Wistar albino rats were obtained from the experimental animal Lab center at Birjand University of Medical Sciences. The rats were kept under standard conditions including a temperature range of 22-25ºC, a 12-hour light/dark cycle, and free access to food from Behparvar Karaj Co., Iran before and during experiments in the Lab center at Birjand University of Medical Sciences. Fourteen days before the experiment, the animals were transferred to another room with standard conditions for acclimation. The Animal Ethics Committee of Birjand University of Medical Sciences approved all procedures.

The experiment involved six groups of 6 male Wistar rats, each receiving specific treatments for 30 consecutive days as outlined below:

#### *2.3.1. Control Group*

Rats received oral administration of corn oil alone at 0.5 cc.

#### *2.3.2. CPF Group*

Rats were orally administered CPF alone at a dosage of 10 mg/kg.

#### *2.3.3. CHN 5 Group*

Rats received oral treatment with CHN at 5 mg/kg.

#### *2.3.4. CHN 10 Group*

Rats received oral treatment with CHN at 10 mg/kg.

#### *2.3.5. CPF + CHN 5*

Rats were orally co-administered CPF at a dosage of 10 mg/kg along with CHN at a dosage of 5 mg/kg.

#### *2.3.6. CPF + CHN 10*

Rats were orally co-administered CPF at a dosage of 10 mg/kg along with CHN at a dosage of 10 mg/kg.

Following the last treatment, using ketamine (50 mg/kg bw) and xylazine (10 mg/kg bw), blood was collected from the heart.

Serum samples were acquired by centrifuging clotted blood at 3000 g for 10 minutes, followed by preservation in a frozen state at -20°C until hormone concentrations were analyzed using an ELISA strip reader. The hypothalamus, testes, and epididymis were meticulously excised, weighed, and prepared for both biochemical and histological analyses.

### 2.4. Biochemical Assessment

Pituitary and testicular hormone assay: Serum levels of pituitary and testicular hormones, including Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH), were evaluated using commercial enzyme immunoassay kits designed for rats, following the manufacturer's instructions.

Lactate dehydrogenase-X (LDH-X) and testicular activities of alkaline phosphatase (ALP) were determined as part of the biochemical analyses.

Assessment of oxidative stress and inflammation biomarkers involved homogenizing samples from the hypothalamus, testes, and epididymis in 50 mM Tris-HCl buffer (pH 7.4) containing 1.15% potassium chloride. Following homogenization, the mixture was centrifuged at 12,000 g for 15 minutes at 4°C, and the resulting supernatant was utilized for biochemical assays, including GSH, MDA, NO, TNF-alpha, and IL-6.

### 2.5. Histological Assessment

After sample collection, the testes, hypothalamus, and epididymis tissues of rats were placed in 10% formalin. The samples underwent immersion in ethanol, followed by clarification with xylenol. Subsequently, they were embedded in liquid paraffin at 60°C and sectioned into slices measuring 4-5 μm. Histological analysis was conducted using Hematoxylin-eosin (H & E) stain. For each sample, three sections were prepared and blindly assessed by an experienced pathologist.

### 2.6. Analysis Methods

A one-way analysis of variance (ANOVA) was performed, and the Tukey post-hoc test was used to make multiple comparisons. Statistical analyses were conducted using the InStat 3.0 program. The results are expressed as mean ± SD, and differences with $p < 0.05$ are considered significant.

## 3. RESULTS

### 3.1. SEM Analysis

The detailed analysis and explanation of these parameters and figures were comprehensively covered in our previous study [16].

### 3.2. Chrysin Nanocrystal Alleviated CPF Effects on Reproductive Hormones and Enzyme Biomarkers of Testicular Function

Table **1** demonstrates the impact of CHN on serum concentrations of reproductive hormones (FSH, LH, and testosterone), as well as marker enzymes of testicular function, in CPF-exposed rats. In comparison to the control group, rats exposed solely to CPF exhibited a notable decrease in serum testosterone, FSH, and LH levels, accompanied by a simultaneous reduction in testicular LDH and ALP activities.

However, co-administration of CHN at doses of 5 and 10 mg/kg body weight significantly alleviated the CPF-induced decreases in enzyme biomarker activities associated with testicular function and restored serum hormone levels, in contrast to animals treated solely with CPF.

### 3.3. Chrysin Nanocrystal Enhanced Antioxidant Status in the Epididymis, Testes, and Hypothalamus of CPF-exposed Animals

Table **2** presents the effect of chrysin nanocrystals on oxidative stress indices in rats exposed to CPF. The administration of CPF alone led to a significant reduction in GSH levels, while MDA levels increased ($p < 0.05$) in the epididymis, testes, and hypothalamus compared to the control group. However, co-treatment with CHN significantly improved ($p < 0.05$) GSH content and reduced MDA levels in the hypothalamus. Epididymis, and testes compared to rats were treated with CPF alone.

**Table 1. Serum concentrations of reproductive hormones FSH, LH, and testosterone, and also the marker enzymes of testicular function in experimental animals.**

| Parameters | Groups | | | | | |
|---|---|---|---|---|---|---|
| | C | CPF | NCH5 | NCH10 | CPF+NCH5 | CPF+NCH10 |
| **FSH (ng/ml)** | 12.78 ± 0.59 | 6.47 ± 1.09 *** | 12.52 ± 0.54 +++ | 12.59 ± 0.73 +++ | 12.50 ± 0.65 +++ | 12.73 ± 0.55 +++ |
| **LH (ng/ml)** | 11.46 ± 1.20 | 7.57 ± 0.76 *** | 7.57 ± 0.44 ++ | 10.25 ± 0.84 +++ | 11.25 ± 1.30 +++ | 11.34 ± 1.08 +++ |
| **Testosterone (ng/ml)** | 3.94 ± 0.62 | 2.20 ± 0.66 | 3.75 ± 0.87 | 3.61 ± 0.55 | 3.63 ± 0.57 | 3.73 ± 0.49 |
| **LDH (U/mg protein)** | 369.70± 34.21 | 152.07 ± 17.24 *** | 364.96 ± 42.04 +++ | 324.45 ± 43.78 +++ | 330.62 ± 32.10 +++ | 312.03 ± 2187 +++ |
| **ALP (U/mg protein)** | 271.30 ± 26.10 | 175.40 ± 10.15 *** | 283.33 ± 14.65 +++ | 294.67 ± 9.50 +++ | 288.56 ± 25.37 +++ | 292.87 ± 33.21 +++ |

**Note:** Data are shown as means ± SD for each group (n=6).

C: Control, CPF: Chlorpyrifos, N=CHN5: Chrysin Nanocrstal (5 mg/kg), CHN10: Chrysin Nanocrystal (10 mg/kg), CPF+ CHN5: Chlorpyrifos + Chrysin Nanocrystal (5 mg/kg),

CPF+ NCH10: Chlorpyrifos + Chrysin Nanochrystal (10 mg/kg).

Significant difference between the data of the C group *vs*. other groups: ***; $p < 0.001$.

Significant difference between the data of the CPF group *vs*. other groups: ++; $p < 0.01$, +++; $p < 0.001$.

**Table 2. Oxidative stress and inflammatory indices in the hypothalamus, testes, and epididymis of the experimental animals.**

| Tests | Groups | | | | | | |
|---|---|---|---|---|---|---|---|
| | Tissue | C | CPF | CHN5 | CHN10 | CPF+CHN5 | CPF+CHN10 |
| GSH (μM/mg tissue) | Hypothalamus | 6.57 ± 0.67 | 3.80 ± 0.37 *** | 6.26 ± 0.48 +++ | 6.18 ± 0.35 +++ | 5.98 ± 0.81 +++ | 6.12 ± 0.21 +++ |
| | Testes | 9.35 ± 1.20 | 5.48 ± 1.25 *** | 9.01± 0.82 +++ | 9.32 ± 0.92 +++ | 10.00 ± 1.81 +++ | 10.18 ± 1.10 +++ |
| | Epididymis | 8.13 ± 0.49 | 6.52 ± 0.79 ** | 7.77 ± 0.82 + | 8.08 ± 0.26 ++ | 7.87 ± 0.80 + | 8.19 ± 0.59 ++ |
| MDA (nM/mg tissue) | Hypothalamus | 0.67 ± 0.06 | 1.16 ± 0.07 *** | 0.70 ± 0.09 ++ | 0.74 ± 0.29 ++ | 0.80 ± 0.15 + | 0.79 ± 0.27 + |
| | Testes | 3.10 ± 0.85 | 4.89 ± 1.10 ** | 3.48 ± 0.59 + | 3.50 ± 0.56 + | 2.93 ± 0.76 ++ | 3.11 ± 0.60 ++ |
| | Epididymis | 1.00 ± 0.15 | 2.18 ± 0.37 *** | 1.21 ± 0.60 +++ | 1.03 ± 0.17 +++ | 1.05 ± 0.16 +++ | 1.06 ± 0.14 +++ |
| NO (μM/mg tissue) | Hypothalamus | 8.08 ± 0.89 | 10.77±0.90 *** | 8.16 ± 0.53 +++ | 8.72 ± 0.94 ++ | 8.60 ± 1.41 ++ | 8.80 ± 0.56 ++ |
| | Testes | 12.39±1.27 | 15.31±1.30 * | 12.62 ±1.30 + | 12.47±2.39 + | 12.11 ± 0.62 ++ | 11.93 ± 1.24 ++ |
| | Epididymis | 9.75 ± 0.92 | 11.64±0.60 ** | 10.26± 0.70 + | 9.80± 0.50 ++ | 10.00± 0.82 ++ | 10.21±0.82 + |
| TNF-α (pg/mg tissue) | Hypothalamus | 0.27 ± 0.14 | 0.48 ± 0.07 * | 0.30 ± 0.05 + | 0.28 ± 0.08 + | 0.26 ± 0.09 ++ | 0.24 ± 0.12 ++ |
| | Testes | 0.70 ± 0.08 | 1.14 ± 037 ** | 0.75 ± 0.10 ++ | 0.80 ± 0.10 + | 0.73 ± 0.09 ++ | 0.77 ± 0.11 ++ |
| | Epididymis | 0.51 ± 0.06 | 0.81 ± 0.07 ** | 0.52 ± 0.09 ++ | 0.54 ± 0.21 ++ | 0.60 ± 0.08 + | 0.55 ± 0.10 ++ |
| IL-6 (pg/ mg tissue) | Hypothalamus | 0.15 ± 0.02 | 0.25 ± 0.03 * | 0.16 ± 0.02 + | 0.17 ± 0.04 + | 0.17 ± 0.06 + | 0.16 ± 0.06 + |
| | Testes | 0.37 ± 0.05 | 0.77 ± 0.20 *** | 0.36 ± 0.07 +++ | 0.40 ± 0.08 +++ | 0.38 ± 0.06 +++ | 0.40 ± 0.07 +++ |
| | Epididymis | 0.28 0.04 | 0.50 0.1 *** | 0.25 0.10 +++ | 0.34 0.05 + | 0.32 0.07 ++ | 0.34 0.05 + |

**Note:** Data are shown as means ± SD for each group (n=6).

C: control, CPF: Chlorpyrifos, CHN5: Chrysin Nanocrstal (5 mg/kg), CHN10: Chrysin Nanocrystal (10 mg/kg), CPF+ CHN5: Chlorpyrifos + Chrysin Nanocrystal (5 mg/kg),

CPF+ NCH10: Chlorpyrifos + Chrysin Nanochrystal (10 mg/kg).

Significant difference between the data of the C group *vs*. other groups: *; $p < 0.05$, **; $p < 0.01$, ***; $p < 0.001$.

Significant difference between the data of the CPF group *vs*. other groups: +; $p < 0.05$, ++; $p < 0.01$, +++; $p < 0.001$.

### 3.4. Chrysin Nanocrystal Suppressed Inflammatory Biomarkers in CPF-treated Animals

Table **2** displays the effect of CHN on the biomarkers of inflammation in rats exposed to CPF. Treatment with CPF alone significantly increased IL-6, TNF-a, and NO, levels in the hypothalamu, testes, and epididymis, compared to the control group. Co-administration of CHN at 5 and 10 mg/kg significantly reduced ($p < 0.05$) IL-6, TNF-a, and NO levels in the treated animals compared to those treated with CPF alone.

### 3.5. Histopathological Findings

In the histological images of the hypothalamus from the control group, normal follicles were observed, outlined with follicular epithelium, enclosing an internal eosinophilic lumen containing colloid (Fig. **1A**). In Fig. (**1B**), histopathological sections of the thalamus in the CPF group showed neuropil vacuolation and thalamic edema, characterized by the formation of empty spaces around blood vessels or cells in the hypothalamus histological section. Photomicrographs of the hypothalamus histological section in CHN5, CHN10, CPF + CHN5, and CPF + CHN10 groups exhibited a normal hypothalamus without leukocytes or edema in the parenchyma (Figs. **1C**, **1D**, **1E**, and **1F**). Histological images of the testes in the control group showed normal seminiferous tubules with a well-organized germinal epithelium (Fig. **2A**). In Fig. (**2B**), the histological section of the testes in the CPF group showed degeneration of the germinal epithelium. Photomicrographs of the histological section of the testes in CHN5, CHN10, CPF + CHN5, and CPF + CHN10 groups exhibited a normal testicular structure (Figs. **2C**, **2D**, **2E**, and **2F**).

## 4. DISCUSSION

The widespread trade of agricultural produce often leads to the pervasive impact of harmful Organophosphate (OP) contaminants [18]. The global health impact of exposure to CPF is substantial because of its severe toxicity in both animals and humans [18]. The toxicity of the reproduction system stands out as a significant adverse effect of OPs [19]. Male infertility has been identified as contributing to 50% of infertility cases worldwide, with previous research implicating CPF as a key risk factor for male infertility [19]. The current study demonstrates the effective alleviation of reproductive dysfunction associated with CPF exposure in male rats through the use of CHN.

The regulation of reproductive hormonal levels and spermatogenesis in mammals is primarily controlled by the hypothalamic-pituitary-testicular axis [20]. In this study, treatment with CPF resulted in notable reductions in serum levels of pituitary hormones, particularly LH and FSH, along with decreases in the gonadal hormone testosterone.This suggests the adverse impact of pesticides on the male reproductive axis.

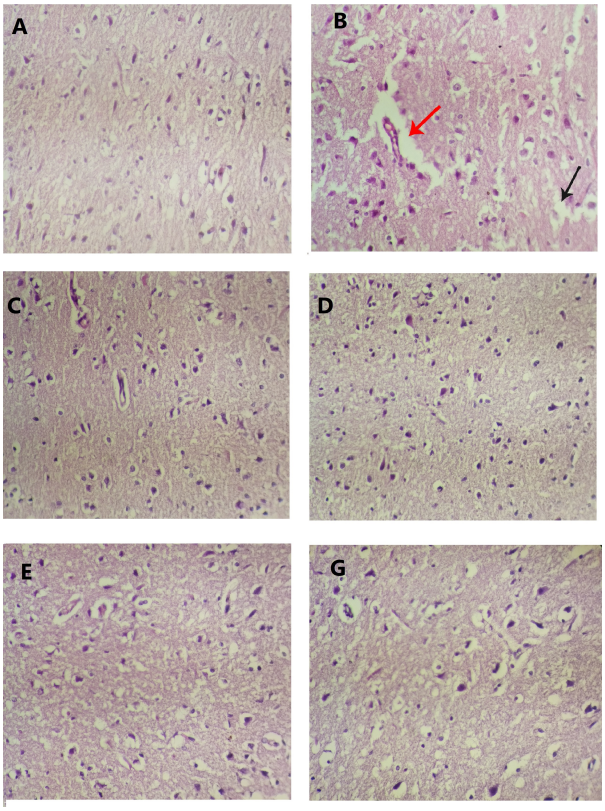


**Fig. (1).** Photomicrographs of hematoxylin and eosin (H&E X 400)-stained sections of the hypothalamus of control rats (**1A**), CPF (**1B**), CHN5 (**1C**), CHN10 (**1D**), CPF + CHN5 (**1E**), CPF + CHN10 (**1F**) groups.

The decline in serum testosterone levels indicates the suppressing effect of CPF on testicular steroidogenesis, previously associated with CPF's toxic effect on Leydig cells [21]. However, the replenishment of serum LH, FSH, and testosterone levels in animals co-administered CPF and CHN (5 and 10 mg/kg) clearly demonstrates the protective effect of CHN against CPF-induced endocrine deficits in the experimental rats. Furthermore, the detrimental consequence of CPF exposure is evident from the activities of enzymes crucial for spermatogenesis in the testis [22]. The decrease in testicular LDH in rats exposed to CPF alone indicates a dysfunction in the metabolic pathway of lactate, which is necessary for spermatogenic cells in rats [7]. Moreover, the reduced ALP activities noted in rats treated with CPF alone suggest an inhibitory impact of CPF on the phosphorylative function of ALP in the utilization of glucose by germ cells during spermatogenesis [23]. The recovery of testicular LDH and ALP activities in rats administered both CPF and CHN (5 and 10 mg/kg) suggests the protective influence of CHN against CPF-induced toxicity in testicular germ cells.

To elucidate the potential mechanisms underlying the protective effects of CHN against CPF-induced male reproductive toxicity, we investigated biochemical indices, specifically oxidative stress and inflammation in the testes, epididymis, and hypothalamus of experimental animals.

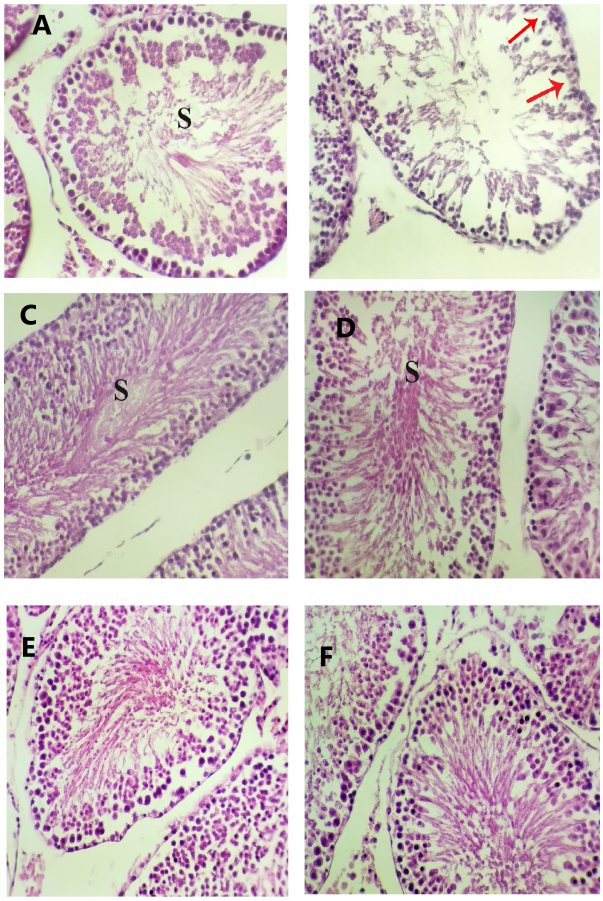


**Fig. (2).** Photomicrographs of hematoxylin and eosin (H&E X 400)-stained sections of the testes of control rats (**2A**), CPF (**2B**), CHN5 (**2C**), CHN10 (**2D**), CPF + CHN5 (**2E**), CPF + CHN10 (**2F**) groups.

The enzymatic and non-enzymatic antioxidant defense systems play a crucial role in maintaining cellular redox balance and combating oxidative stress [24]. Hence, the significant increase in testicular, epididymal, and hypothalamic MDA levels in animals treated with CPF alone indicates that CPF led to heightened Reactive Oxygen and Nitrogen Species (RONS) generation [25]. This, in turn, overwhelmed the antioxidant capacity, resulting in oxidative-RONS-mediated injury in the rats [26]. The decrease in GSH levels illustrated in animals treated with CPF alone indicates a suppression of their antioxidant function related to the accumulation of cellular Reactive Oxygen and Nitrogen Species (RONS) and subsequent oxidative damage in the epididymis, hypothalamus, and testes [26]. This finding aligns with previous studies reporting CPF-induced oxidative stress in rat testes. However, the concurrent decrease in MDA levels and increase in antioxidant content in rats co-treated with CPF and CHN suggest the antioxidant effect of CHN, as previously

documented [27].

TNF-α plays a crucial role in regulating cytokine production during inflammatory responses, triggering the generation of NO through the nitric oxide synthase pathway in the cell [28]. An increase in cellular NO levels is linked to nitrosative stress, which is reported to cause damage to cellular proteins, nucleic acids, and lipids following the reduction of antioxidant defense systems [29]. In the current investigation, exposure to CPF alone markedly increased levels of action in IL-6, and NO, in the epididymis, hypothalamus, and testes of the treated rats. This finding indicates the induction of inflammation. Uncontrolled production of pro-inflammatory cytokines, such as IL-6 and TNF-α, in the testes is detrimental to spermatogenesis and is widely recognized to contribute to male infertility [30]. Therefore, the reduction in IL-6, NO, and TNF-α levels in the epididymis, hypothalamus, and testes following co-exposure to CHN (5 and 10 mg/kg) suggests that CHN induced an anti-inflammatory mechanism to mitigate CPF-induced testicular toxicity.

The protective effect of CHN (at doses of 5 and 10 mg/kg) on the histological integrity of the examined tissues is apparent from the significant reduction in lesions observed in rats co-administered CPF and CHN. The preservation of these histological structures is consistent with the biochemical findings, corroborating the beneficial effects of CHN on CPF-induced male reproductive dysfunction in rats. In addition, it was observed that the administration of CHN (5 and 10 mg/kg) for 15 days did not induce significant changes in the levels of GSH, MDA, IL-6, NO, TNF-α, and histopathological alterations in the epididymis, testes, and hypothalamus of rats compared to the control group.

## CONCLUSION

The improvement observed in the hypothalamic-pituitary-testicular axis in experimental rats, ameliorating CPF-induced deficits, can be associated with the anti-inflammatory and antioxidant properties inherent in CHN. CHN demonstrates a comprehensive protective role, safeguarding against CPF toxicity and preventing damage to cellular macromolecules. Thus, it is possible that CHN (5 and 10 mg/kg) may mediate the detoxification of CPF-induced reproductive toxicity in male rats by inhibiting oxidative stress and inflammation. It is recommended to design an experimental study to evaluate the CHN effect on the reproductive system after 30-day of exposure to CPF and its possible mechanism action.

## AUTHORS' CONTRIBUTION

It is hereby acknowledged that all authors have accepted responsibility for the manuscript's content and consented to its submission. They have meticulously reviewed all results and unanimously approved the final version of the manuscript.

## LIST OF ABBREVIATIONS

| | | |
|---|---|---|
| **CPF** | = | Chlorpyrifos |
| **CHN** | = | Chrysin Nanocrystal |
| **GSH** | = | Glutathione |
| **MDA** | = | Malondialdehyde |
| **FSH** | = | Follicle-Stimulating Hormone |
| **CHN** | = | Chrysin Nanocrystals |
| **SEM** | = | Scanning Electron Microscopy |
| **LDH-X** | = | Lactate dehydrogenase-X |
| **ANOVA** | = | Analysis Of Variance |
| **RONS** | = | Reactive Oxygen and Nitrogen Species |

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the ethics committee of the National Institutes for Medical Research Development (NIMAD), Iran, Approval number: IR.NIMAD.REC.1400.004.

## HUMAN AND ANIMAL RIGHTS

All procedures conducted in this animal research adhered to the standards outlined in the Guide for the US National Research Councils and the Guide for the Care and Use of Laboratory Animals.

This study adheres to internationally accepted standards for animal research, following the 3Rs principle. The ARRIVE guidelines were employed for reporting experiments involving live animals, promoting ethical research practices.

## CONSENT FOR PUBLICATION

Not applicable.

## AVAILABILITY OF DATA AND MATERIALS

The data supporting the findings of this study can be obtained from the corresponding author [S.S.] upon making a reasonable request.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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Declared none.

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