Effect of thymoquinone on cadmium-induced toxicity of Leydig cells in adult male albino rats: a histological, immunohistochemical, and biochemical study
Ayman M. Mousa, Maysara M. Salem and Abeer M. El-Mahalaway

Introduction
Leydig cells are the testosterone-producing cells of the testis. The adult Leydig cell (ALC) population ultimately develops from undifferentiated mesenchymal-like stem cells present in the interstitial compartment of the neonatal testis. Four distinct stages of ALC development have been identified and characterized. These include stem Leydig cells, progenitor Leydig cells, immature Leydig cells, and ALCs [1].

Background
Cadmium (Cd) is an important cause of infertility among occupational workers. Thymoquinone (TQ) is a medical extract from Nigella sativa (NS) and is used as a natural remedy for many diseases because of its antioxidant and anti-inflammatory properties.

Aim of the work
The aim of the study was to study the histological, immunohistochemical, and biochemical effects of TQ on Cd-induced toxicity of Leydig cells in adult male albino rats.

Materials and methods
Forty adult male rats were included in the study and were divided equally into four groups: group I, group II, group III, and group IV. Group I was the control group. Group II received a daily oral dose of Cd at 0.5 mg/kg by means of a gastric tube for 6 weeks. Group III received a daily oral dose of TQ at 100 mg/kg through a gastric tube for 6 weeks. Group IV received TQ, followed by Cd, at the same dose for 6 weeks. Specimens of the right testis were taken and processed for light microscopy (stained with H&E) and electron microscopy (stained with toluidine blue for semithin sections) to evaluate the structure of Leydig cells. Morphometric study of Leydig cells stained immunohistochemically by testosterone Ab-1 was carried out, and biochemical measurements of serum total testosterone and luteinizing hormone were taken, followed by statistical analysis.

Results
Microscopic study of group II showed apoptotic shrunken Leydig cells with deeply acidophilic cytoplasm and deeply stained nuclei, whereas electron microscopic results showed few mitochondria, few Smooth endoplasmic reticulum (sER), large cytoplasmic vacuoles, and heterochromatin margination. This was confirmed immunohistochemically by significant decrease in the area% of testosterone Ab-1 and biochemically by significant decrease in serum total testosterone and significant increase in serum luteinizing hormone. Group III showed similar results to group I, whereas group IV showed improvement in the histological, morphometric, and biochemical changes described for group II.

Conclusion
TQ has a protective effect on Cd toxicity-induced Leydig cell damage as it has antioxidant and anti-inflammatory activities. It is suggested that workers dealing with Cd consume a diet rich in TQ, such as Nigella sativa.

Keywords:
cadmium, Leydig cells, thymoquinone

Effect of thymoquinone on cadmium-induced toxicity of Leydig cells in adult male albino rats: a histological, immunohistochemical, and biochemical study
Ayman M. Mousa, Maysara M. Salem and Abeer M. El-Mahalaway

Introduction
Leydig cells are the testosterone-producing cells of the testis. The adult Leydig cell (ALC) population ultimately develops from undifferentiated mesenchymal-like stem cells present in the interstitial compartment of the neonatal testis. Four distinct stages of ALC development have been identified and characterized. These include stem Leydig cells, progenitor Leydig cells, immature Leydig cells, and ALCs [1].
and intratesticular concentrations of testosterone in rats on day 24 [3].

Cadmium (Cd) is a widely used heavy metal that is implicated in many industrial applications like electric batteries, electronic components, pigments, and fertilizer. However, Cd is an environmental pollutant that is highly toxic to all living organisms. Heavy metals like Cd become toxic when they are not metabolized and can accumulate in most human organs and may produce severe toxic effects in many organs including the kidney, liver, testis, lungs, pancreas, placenta, and bone [4].

Levels of Cd in the body increase over time and, because of its slow elimination, once absorbed by an organism it remains resident for many years [5]. Food, water, and polluted air are sources of human exposure to Cd. Another source is cigarette smoking, as tobacco plants accumulate Cd from the soil. The type of Cd intake plays a significant role in its absorption level because the inhaled Cd is much higher than the absorption level from the gastrointestinal system. After absorption, Cd is transferred by blood to all organs of the body, where a small protein called metallothionein with a high binding affinity to Cd stores it. Cd has a half-time of 20–30 years in the human body. The highest concentration of Cd in the body is found in the liver and kidneys because of the high Mt concentration. Cd has high toxicity, because it induces cancer through multiple mechanisms. Some of them are induction of oxidative stress, aberrant gene expression, inhibition of DNA repair, and inhibition of apoptosis [6].

Over many centuries humans have been turning to nature to discover substances for the treatment of human diseases. *Nigella sativa* (NS) is an amazing natural herb with a rich historical and religious background for remedial action against several diseases. It belongs to the Ranunculaceae family and has been shown to contain NS seeds [7]. In folklore medicine, the seed is associated with diverse therapeutic benefits for treatment of bronchial asthma, eczema, dysentery, hypertension, headache, and obesity [8,9]. However, the effect of TQ on the histological structure of interstitial Leydig cells has not been comprehensively investigated and is an active area of research [10].

The aim of this study was to evaluate the histological, immunohistochemical, and biochemical effects of TQ on Cd-induced toxicity of Leydig cells in adult male albino rats.

### Materials and methods

**Used drugs**

Cd chloride powder was purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Each 5 mg of Cd chloride was dissolved in 50 ml of distilled water (0.1 mg/ml). TQ powder was purchased from Sigma-Aldrich. Each 1000 mg of TQ was dissolved in 100 ml of distilled water (10 mg/ml).

**Experimental animals**

The experiment was performed in the animal house of Zagazig Faculty of Veterinary Medicine, Zagazig University, and was approved by the animal and research committee, Benha Faculty of Medicine. Forty adult male albino rats aged 7 weeks and weighing 150–200 g were used in the experiments, as normal spermatogenesis occurred in rats older than 45 days [11]. All animals were housed in metal animal cages at a temperature ranging from 22 to 25°C, under a 12-h light–dark cycle. They were fed a routine diet (rat chow and tap water) ad libitum and divided into four groups of 10 rats each.

- **Group I** (the control group): rats in this group were given 1 ml of distilled water orally once daily through an orogastric tube for 6 weeks.
- **Group II** (the Cd group): rats in this group were given 0.5 mg/kg of Cd orally once daily through an orogastric tube for 6 weeks [12].
- **Group III** (TQ group): rats in this group were given 100 mg/kg of TQ orally once daily through an orogastric tube for 6 weeks [13,14].
- **Group IV** (Cd and TQ group): rats in this group were given 0.5 mg/kg of Cd and then 100 mg/kg of TQ orally once daily through an orogastric tube for 6 weeks.

**Histological study**

At the end of the experiment, the rats were anesthetized by ether inhalation and killed. The right testes were excised and the testicular specimens were divided into two parts: One part was processed to obtain paraffin sections that were stained with H&E for light microscopic examination [15], whereas the second part was processed to obtain semithin sections that were stained with toluidine blue for light microscopic examination [15], whereas the second part was processed to obtain semithin sections that were stained with toluidine blue for light microscopic examination [15] and ultrathin sections for electron microscopic examination with a JEOL-TEM 1010 microscope (Tokyo Japan) [16] in the EM Unit, Faculty of Medicine, Tanta University, to verify the histological details of interstitial Leydig cells.

**Immunohistochemical study**

Paraffin sections of 5 µm thickness were used for immunostaining with avidin–biotin–peroxidase complex for testosterone Ab-1 (for Leydig cell activity). The sections were placed on pretreated organosilane slides, deparaffinized, and rehydrated. Thereafter, endogenous peroxidase activity was blocked by using 10% H₂O₂, followed by three washes in phosphate buffer; antigen retrieval was performed by heating in citrate buffer (pH6) in a microwave (4 min at high pressure followed by 4 min at low pressure). Nonspecific reactions were blocked with 1/100 normal horse serum. After careful blotting, a group of slides was incubated with the primary antibody anti-rat testosterone Ab-1 (Dako, Glostrup, Denmark).
for 15 min [17]. The slides were rinsed in PBS, and buffer bath and biotinylated secondary antibody (horse antimouse) were applied to it for 30 min. The slides were then rinsed in avidin–biotin–peroxidase complex (Elite ABC reagent, Ontario, Canada) for 30 min. Freshly prepared diaminobenzidine in distilled water was added for 15 min. Finally, all sections were counterstained with Mayer’s hematoxylin, dehydrated, and mounted. For negative control, the primary antibody was replaced by PBS. The reaction of testosterone Ab-1 was observed as dark brown granules in the cytoplasm of active Leydig cells, compared with the positive control (prostate) [17].

**Morphometric study**

The mean area% of testosterone Ab-1 immunoreaction in Leydig cells was quantified in 10 images of high-power magnification ×1000 for each group using Image-Pro Plus program version 6.0 (Media Cybernetics Inc., Bethesda, Maryland, USA) in the Pathology Department, Faculty of Medicine, Benha University.

**Biochemical study**

The rats were subjected to thoracoabdominal incision. Blood samples were collected from the descending aorta into clean test tubes, and quantitative measurement of total testosterone (TT) and LH levels in the serum of all male rats was carried out using ELISA kits, (Alexandria, Egypt) [18].

**Statistical analyses of the data**

The TT and LH blood levels and testosterone Ab-1 immunoreaction in group II, group III, and group IV were compared with those of group I using the t-test, with P less than 0.05 as the level of statistical significance. Statistical analyses were carried out using IBM SPSS Statistics software for Windows, version 20 (IBM Corp., Armonk, New York, USA) [19].

**Results**

Group I (the control group): histological examination of testicular sections in group I stained with H&E and toluidine blue showed interstitial tissue in between the normal STs surrounded by the basement membrane. The interstitial tissue contained rounded or oval Leydig cells, a few macrophages, and blood vessels. Each Leydig cell exhibited ill-defined outlines with lightly stained acidophilic cytoplasm and vesicular nucleus with granular chromatin and prominent nucleolus (Fig. 1). Some Leydig cells contained lipid droplets (Fig. 2). Electron microscopic examination of group I showed Leydig cells with large rounded indented euchromatic nuclei and prominent nucleoli. The cytoplasm contained many mitochondria, cisternae of sER, some lipid droplets, and a few lysosomes (Fig. 3). Immunohistochemically stained sections of group I showed positive expression of testosterone Ab-1 reaction in the form of brown granules in the cytoplasm of Leydig cells (Fig. 4).

Group II (the Cd group): histological examination of testicular sections in group II stained with H&E and toluidine blue showed apoptotic changes in most Leydig cells. Many Leydig cells were shrunken with deeply acidophilic cytoplasm, some cytoplasmic vacuoles, and deeply stained nuclei, whereas a few Leydig cells appeared normal. Some macrophages with kidney-shaped nuclei appeared beside shrunken Leydig cells (Figs 5 and 6).

Electron microscopic examination of group II showed that most of the Leydig cells had multiple cytoplasmic vacuoles, a few cisternae of sER, and a few mitochondria in their cytoplasm. The nuclei had an irregular nuclear envelope and heterochromatin margination as a sign of apoptosis compared with group I (Fig. 7). Other fields revealed some Leydig cells with normal nuclei, and their cytoplasm showed multiple lipid droplets, a few cisternae of sER, and a few mitochondria. Some macrophages showed aggregation of multiple electron-dense residual bodies (Fig. 8). Immunohistochemically stained sections of group II showed minimal expression of testosterone Ab-1 reaction in the cytoplasm of most Leydig cells compared with group I (Fig. 9).

Group III (the TQ group): histological and immunohistochemical examination of testicular sections in group III showed that most of the Leydig cells were similar to those of group I.

Group IV (the Cd and TQ group): histological examination of the testicular sections of group IV stained with H&E and toluidine blue showed obvious improvement in Leydig cells when compared with those of group II; most of the Leydig cells were similar to those of group I, with acidophilic cytoplasm and large rounded vesicular nuclei with granular chromatin and prominent nucleolus. However, a few Leydig cells had deeply stained nuclei (Figs 10 and 11).

Electron microscopic examination of group IV showed that most of the Leydig cells were more or less similar to those of group I. Leydig cells had a nuclei with irregular nuclear envelope, peripheral heterochromatin, and a prominent nucleolus. The cytoplasm had numerous normal mitochondria, a few degenerated mitochondria, some lipid droplets, and a few cytoplasmic vacuoles (Fig. 12). Immunohistochemically stained sections of group IV showed that most of the Leydig cells had a positive expression of testosterone Ab-1 reaction (Fig. 13).

**Morphometric results**

The mean area% of testosterone Ab-1 immunoreaction for Leydig cells in all groups in the present study is presented in Table 1 and Histogram 1. There was a significant decrease in group II compared with group I. A nonsignificant increase was seen in group III compared with group I and a nonsignificant decrease in group IV compared with group I for testosterone Ab-1 immunoreaction.

**Biochemical results**

The mean blood level of TT and LH in all experimental groups is shown in Table 2 and Histogram 2. There was a significant decrease in the mean TT blood level and a significant increase in the mean LH blood level in group
II compared with group I. In addition, a nonsignificant increase in the mean TT blood level and a nonsignificant decrease in the mean LH blood level was seen in group III compared with group I and a nonsignificant decrease in the mean TT blood level and a nonsignificant increase in the mean LH blood level of group IV compared with group I.

**Figure 1.** A photomicrograph of a section of the testis in group I showing rounded or oval Leydig cells (LC) with acidophilic cytoplasm and oval or rounded nuclei around a blood vessel (BV). Macrophages appear with a kidney-shaped nucleus (MP). Notice a part of seminiferous tubule (ST).

H&E, ×1000.

**Figure 2.** A photomicrograph of a semithin section of the testis in group I showing rounded or oval Leydig cells (LC) around a blood vessel (BV) with rounded or oval nuclei and some lipid droplets (L) in their cytoplasm. Macrophages appear with a kidney-shaped dark nucleus (MP), seminiferous tubule is the land mark of ledig cells site (ST).

Toluidine blue, ×1000.

**Figure 3.** An electron photomicrograph of a section of the testis in group I showing a Leydig cell with a large indented euchromatic nucleus (N) with little heterochromatin and prominent nucleolus. The cytoplasm contains many mitochondria (M), smooth endoplasmic cisternae (sER), a few lysosomes (Ly), and some lipid droplets (L).

Uranyl acetate and lead citrate, ×20 000.

**Figure 4.** A photomicrograph of a section of the testis in group I showing positive expression of testosterone Ab-1 reaction (+VT) by Leydig cells (LC).

Testosterone Ab-1 immunostaining, ×1000.

**Figure 5.** A photomicrograph of a section of the testis in group II showing Leydig cells (LC) with deeply acidophilic cytoplasm, cytoplasmic vacuoles (V), and The arrow point to the deeply stained nuclei (D). Macrophages (MP) appear with a kidney-shaped nucleus.

H&E, ×1000.

**Figure 6.** A photomicrograph of a semithin section of the testis of group II showing Leydig cells (LC) with cytoplasmic vacuoles (V) and deeply stained nuclei (D). Macrophages (MP) appear with a kidney-shaped nucleus.

Toluidine blue, ×1000.
Figure 7. An electron photomicrograph of a section of the testis in group II showing a Leydig cell with indented electron-dense nucleus (N) and clumps of heterochromatin (HC). The cytoplasm has few mitochondria (M), a few sER, and many cytoplasmic vacuoles (V).
Uranyl acetate and lead citrate, × 20 000.

Figure 8. An electron photomicrograph of a section of the testis in group II showing one Leydig cell that has an oval nucleus (N), a few mitochondria (M), and a large cytoplasmic vacuole (V), and is studded with lipid droplets (L). A part of a macrophage (MP) appears with multiple electron-dense residual bodies (RB) and many cytoplasmic vacuoles (V).
Uranyl acetate and lead citrate, × 11 700.

Figure 9. A photomicrograph of a section of the testis in group II showing minimal expression of testosterone Ab-1 (-VT) reaction by Leydig cells (LC).
Testosterone Ab-1 immunostaining, × 1000.

Figure 10. A photomicrograph of a section of the testis in group IV showing Leydig cells (LC) with acidophilic cytoplasm and large oval nuclei around a blood vessel (BV). A few cells have deeply stained nuclei (D).
H&E, × 1000.

Figure 11. A photomicrograph of a semithin section of a testis in group IV showing oval Leydig cells (LC) around a blood vessel (BV). Most Leydig cells have oval nuclei. A few Leydig cells have deeply stained nuclei (D). A macrophage (MP) appears with a kidney-shaped nucleus.
Toluidine blue, × 1000.

Figure 12. An electron photomicrograph of a section of the testis in group IV showing a Leydig cell with an oval nucleus (N) that has peripheral heterochromatin (He) and prominent nucleolus (nu). The cytoplasm has multiple normal mitochondria (M), a few degenerated mitochondria (m), a few lipid droplets (L), and a few cytoplasmic vacuoles (V).
Uranyl acetate and lead citrate, × 20 000.
Discussion

Leydig cells are the principal source of androgen in the testis; they release their hormones into the abundant extracellular fluid of the interstitium and then diffuse into the ST to exert their local effect on spermatogenesis; their morphological changes are usually reflected on the state of steroidogenesis [20].

Cd is one of the main environmental and occupational pollutants in industrialized countries [21].

TQ is a major active compound derived from NS (one of the natural antioxidants), which is considered a promising dietary chemopreventive agent [22].

Histological examination of the testicular sections of group II showed degenerative changes in most of the Leydig cells, which manifested in the form of deeply acidophilic cytoplasm, many cytoplasmic vacuoles, and deeply stained nuclei. Electron microscopic examination of this group showed that most of the Leydig cells had apoptotic changes in the nuclei with margination of heterochromatin. Their cytoplasm had abnormal multiple cytoplasmic vacuoles, a few cisternae of SER, and a few mitochondria. Vacuolation of the cytoplasm of Leydig cells exposed to Cd was attributed to a reduction in the activity of ATPase, which results in failure of the sodium pump mechanism responsible for water and electrolyte control. Water accumulated within Leydig cells, leading to vacuolar degeneration [23]. This was confirmed by the immunohistochemical examination of Leydig cells in group II that showed a significant decrease in testosterone Ab-1 immunoreaction in the

<table>
<thead>
<tr>
<th>The mean area% of testosterone Ab-1 immunoreaction in Leydig cells in all experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Mean area %</td>
</tr>
<tr>
<td>±SD</td>
</tr>
<tr>
<td>P value</td>
</tr>
</tbody>
</table>

*Non-significant at P<0.05.
**Significant at P<0.05.

<table>
<thead>
<tr>
<th>The mean and SD blood level of total testosterone and luteinizing hormone (ng/ml) in all experimental groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
</tr>
<tr>
<td>TT</td>
</tr>
<tr>
<td>Mean</td>
</tr>
<tr>
<td>SD</td>
</tr>
<tr>
<td>P value</td>
</tr>
</tbody>
</table>

LH, luteinizing hormone; TT, total testosterone.
*Non-significant at P<0.05.
**Significant at P<0.05.
cytoplasm of most Leydig cells. Finally, the biochemical study revealed a significant decrease in TT and increase in LH.

These results are in accordance with those of a study that reported that Cd induces cell apoptosis in different organs such as the liver, kidney, and testis [24]. Another study reported inhibited reproduction in many species after Cd environmental exposure, as Cd toxicity reduces testicular blood flow and induces death among Leydig cells by apoptosis rather than by necrosis [25].

The environmental sources of Cd for humans and animals are food, drinking water, polluted air, and cigarette smoking. Exposure to a high level of Cd is associated with serious health hazards such as azoospermia in smokers, as a single inhaled cigarette contains 1.5 µg of Cd [26].

Other investigators reported that Cd can reduce testosterone synthesis at various levels and deteriorate spermatogenesis after a daily oral dose of 0.5 mg/kg Cd for more than 6 weeks [27]. Some researchers [28,29] proved that the mechanism of Cd toxicity on Leydig cells involves the damage of the vascular endothelium.

Other investigators [30,31] have demonstrated that Cd induces mitochondrial damage and oxidative stress, which forms excess ROS. Normally, ROS are balanced by natural antioxidant enzymes, but when an imbalance occurs between these ROS and natural antioxidants an oxidative stress condition is created, and the mitochondrial injury causes release of cytochrome-\(\text{c}\) and activation of caspase pathways that lead to apoptosis.

The mechanisms of action of Cd-induced cell injury were mediated through the production of free radicals and the oxidized products of oxidative stress such as superoxide anion, hydrogen peroxide, and hydroxyl radicals. These ROS of oxidative stress are capable of alternating and damaging cell compounds as well as membranes and leading to increase lipid peroxidation, DNA damage, and apoptotic cell death [30].

Also Cd had a toxic effect on many enzymes dependent on iron, such as cytochrome P450, where its disruption will interfere with testicular steroidogenesis. Leydig cells contain 10-fold more P450 compared with Sertoli cells, and thus Leydig cells are more sensitive to increased Cd level [32].

The biochemical results coincide with those of other authors [33], who have reported that Cd can modify hormone levels by affecting the hypothalamic–pituitary–testicular axis in different ways, not only through its effects on Leydig cells. However, the levels of testosterone could be either decreased or increased, depending on the nature of Leydig cell changes within the gonad.

Leydig cells are not capable of maintaining the normal testosterone production if destroyed [34]. The results of the present study were in accordance with those of other investigators [35], who reported that components of cigarette smoke (e.g. nicotine and Cd) cause oxidative stress and defects in steroidogenesis and play a role in Leydig cell dysfunction, as well as act directly on germ cell apoptosis.

Testicular macrophages are normal residents of the interstitial compartment and play an important role in regulating steroidogenesis of Leydig cells and maintain homeostasis within the testis [36]. They engulfed the apoptotic fragments of Leydig cells. This was confirmed by a number of studies that indicated that macrophages produce interleukins and phagocytose pathogenic organisms in the interstitial compartment of the testis [37].

The histological, immunohistochemical, and biochemical findings of group III resembled those of group I, as TQ does not exert any deleterious effect on Leydig cells at the dose level used in this study [13]. This result was in accordance with a study that reported that TQ has many medicinal properties, such as antioxidant, anti-inflammatory, antitumor, analgesic, and antimicrobial effects, which protect many organs against oxidative stress or against drug-induced damage [38].

The histological, immunohistochemical, and biochemical findings of group IV revealed marked improvement in various changes produced by Cd in group II. These results were in agreement with the findings of some investigators who reported the protective effect of TQ against many toxicants in human and experimental animals, as TQ had antioxidant activities and was a free radical scavenger and a lipid peroxidation inhibitor as it increased antioxidant enzyme activity and improved mitochondrial function [39].

Other authors [40] suggested that Cd exposure greatly diminished several antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, which counteract oxidative stress; therefore, antioxidant agents such as TQ may prevent testicular Cd toxicity. Also, TQ had a potent antiapoptotic and anti-inflammatory effect through inhibition of proinflammatory cytokine production in activated mast cells [41].

**Conclusion**

Cd results in pronounced oxidative stress and Leydig cell damage. TQ is a natural material that can ameliorate the damaging effect of Cd as it has antioxidant and anti-inflammatory enzyme activities. We advise workers who are exposed to Cd to consume a diet rich in TQ such as NS to protect themselves from the toxic effects of Cd.

**Acknowledgements**

**Conflicts of interest**

There are no conflicts of interest.

**References**


تأثير الثيموكينون على سميه الكادميوم المستحدثة لخلايا ليدج في ذكور الجرذان البيضاء البالغة (دراسة هستولوجية، هستوكيميائية مناعية وبيوكيميائية)

أيمن محمد موسي، ميسرة محمود سالم، عبير مصطفي المحلاوي
قسم الأنسجة و بيولوجيا الخلية - كلية الطب - جامعة بنها

المقدمة: يعتبر الكادميوم من أهم أسباب العقم عند الحرفيين. أما الثيموكينون فهو مثلا فيت فضائي للعديد من الأمراض من خلال انشطتها المضادة للأكسدة والالتهابات.

الهدف: استهدف البحث دراسة التأثيرات الهستولوجية والهستوكيميائية المناعية والبيوكيميائية للثيموكينون ضد سميه الكادميوم المستحدثة لخلايا ليدج في ذكور الجرذان البيضاء البالغة.

طرق البحث ومواده: تضمنت الدراسة عدد أربعين ذكرًا من الجرذان البيضاء البالغة وقد قسمت إلى أربعة مجموعات بكل مجموعة عشرة جرذان كانتا: المجموعة الأولي استخدمت كمجموعة سليمة (ضابطة)، والمجموعة الثانية كانت مجموعة (الكادميوم) التي أعطيت جرعات يومية من الكادميوم (5 مجم لكل كيلو جرام من وزن الجسم) باستخدام أنبوب المعدة لمدة 5 أسابيع، أما المجموعة الثالثة فكانت مجموعة (الكادميوم و الثيموكينون) والتي أعطيت جرعات يومية من الثيموكينون ثم الكادميوم بنفس الطريقة والجرعات السابقة. وفي نهاية التجربة تم أخذ عينات من الخصية اليمنى لكل جرذ وتجهيز العينات للدراسة الهستولوجية للقطاعات المصبوغة بالهيماتوكسلين والأيوسين والقطاعات المصبوغة بلون الازرق الميكروسكوبية (الجارنات الدهنية والخلايا الدهنية). كما تم التحليل المصور للدراسة الهستوكيميائية المناعية (ال أجسام المضادة وهرمون اللوتنة LH (LH) لخلايا ليدج.)

نتائج البحث: أظهرت الدراسة الهستولوجية لقطاعات الخصية في المجموعة الأولى (الكادميوم) نتائج مماثلة للمجموعة الضابطة. أما المجموعة الثانية (الكادميوم و الثيموكينون) فظهر تحسين واضح في جميع التغيرات التي ظهرت سابقا في المجموعة الثانية (الكادميوم).

الاستنتاج: للثيموكينون دور وقائي ضد التأثير السام للكادميوم على خلايا ليدج حيث أقل تلف الخلايا لأنه مضاد للأكسدة ومضاد للالتهابات. لذا ينصح بتناول المواد الغنية بالثيموكينون مثل حبة البركة للحرفيين المعرضين للتعامل مع الكادميوم.