Protective Role of Curcumin Against Combined Toxicity of Nickel-Chromium on The Enzymatic Antioxidants, Blood and Testicular Histopathology of Albino Rats

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Abstract

Recently, in particular, environmental contaminants have continued to be the most potential cause of many diseases, those of toxic metals which are capable of accumulating within vital organs of both humans and animals, inducing reactive oxygen species (ROS) production and causing severe health hazards within biological systems. Nickel (Ni) and chromium (Cr) are toxic elements producing highly reactive radicals which are able to disable enzymatic antioxidants through binding to their active sites. The present study has been designed to evaluate the protective role of curcumin against testicular dysfunction induced by nickel-chromium combination. The concentrations of Ni and Cr as well as the activities of antioxidants endogenous enzymes including; glutathione peroxidase (Gpx), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) were all determined in the testicular tissue while hematological biomarkers were being measured in the blood of experimental rats. Mixture metals resulted in significant variances within testicular tissues. However, oral administration of curcumin provided a significantly protective role against combined toxicity of nickel-chromium and reduced histopathological alterations due to antioxidant property represented in scavenging and chelating properties as concluded from the present investigations.

Key words: heavy metals, curcumin, testicular histopathology, antioxidants, oxidative stress, ROS.

1. Introduction

Heavy metals are able to interact with the different tissues of both human and animal since they are hardly broken down or discharged out the body instead, they gradually affect the vital processes allover internal organs till partial or complete malfunction. Such effects begin as soon as communicating with hormone, proteins and enzymes in order to form stable covalent complexes with these macromolecules [1, 2]. Nickel is used mainly in the manufacturing of stainless steel and nickel alloys, however, food is the main source of nickel exposure in the non-smokers in addition to normal people who don’t working in nickel industries. Moreover, water is a minor participator to the daily oral intake of nickel through naturally polluted groundwater or kettles made from non-resistance materials or even direct contacting to nickel-chromium plated taps [3]. Exposure to nickel has negative impacts on human health with high possible toxicity on liver, kidney, brain, lung and testis [4]. Chromium on the other hand, contributes in metal plating, production of pigments and dyes, corrosion inhibitors materials, leather tanning and other chemical synthesis applications [5, 6]. Testicular tissue is a major target for chromium-induced oxidative damage due to its high polyunsaturated membrane lipids thereby, chromium increases the risks reduced semen quality, sperm abnormalities leading to infertility for workers of welding industry; moreover, severe injuries have been appeared in seminiferous epithelium of the mice testis by the action of chromate concentration [7]. Oxidative stress is one of the critical mechanisms implicated in the nickel-chromium inducing toxicity.
as they are dangerous metals producing free radicals in biological systems through redox reactions carried out by such metals, these reactions create oxidative damage to proteins and DNA, in addition to disrupting the normal defense systems performed by enzymatic antioxidant [8, 9]. Since oxidative damages in various body organs are mostly related to toxic metals exposure, high solicitude has been given for the application of natural products to strengthen the cell antioxidant and to protect it from the toxic metals-induced damage [10]. Curcumin is the product obtained by solvent extraction of turmeric or the ground rhizomes of Curcuma longa L [11]. It is considered a natural class of anti-inflammatory and antioxidant which is concluded to be a potent inhibitor of reactive oxygen species (ROS) formation [12]. Curcumin is a lipid soluble antioxidant which reveals a typical radical trapping ability as a chain breaking; it is believed to be settle down within the membranous sub-cellular fraction of living cells to protect them from peroxidative damage [13]. Curcumin is able to nullifying heavy metal toxicity as it has a protective efficacy against metals induced toxicity while its recent applications such as nanocurcumin has a magnificent preventive efficacy against oxidative stress induced by toxic metals which lowering the reactive oxygen species along with the restoration of the blood glutathione level [14]. Many experiments have proved that curcumin has a protective role for the enzymes through its electron donor ability to the gastric peroxidase [15]. Therefore, there is little information about the testicular protection of curcumin against heavy metals toxicity. Hence, the purpose from this experimental study is to clarify the protective effects of curcumin against combined toxicity of nickel-chromium.

Experimental details

Material and Methods

Chemicals

All reagents and chemicals were of analytical grade quality with high purity. Nickel and chromium (SCP SCIENCE, Canada) were used for mixture preparation of oral administration solutions. Standard solutions (Merck, Germany) were used to create calibration curves for nickel and chromium analysis, while concentrated HNO₃ (65%, Merck, Germany) and H₂O₂ (30%, Sigma-Aldrich, Germany) were used for tissue digestion. All chemical and reagents for the examination of antioxidant status were purchased from Bio-diagnostic (Egypt).

Curcumin:

Curcumin extract was commercially purchased from Sigma Chemical Co, Germany and dissolved in commercial corn oil (2.5 ml/Kg B.W/ day). It was given to rats by oral gavage tube at a dose of 100 mg/Kg body weight / day [16].

Animals:

Thirty male Wistar rats weighing approximately 200g ± 10g were purchased from The Egyptian Holding Company for Biological Products & Vaccines, Cairo, Egypt and used as experimental animals. Rats were housed and maintained under standard controlled conditions of good ventilation, normal temperatures, and humidity range (temperature 25 ±4 °C, relative humidity of 35% to 60%, 12-h light-dark cycle). They were allowed free access to drinking water (metal-free water) unlike, feeding which was restricted to be introduced by limited amount (50 g) once daily for each group. During the experiment period (75 days), the feeding time was one hour before Ni-Cr dosing, while curcumin dosing was after two hours of metals administration. The experiment was carried out according to the Guide for the care and use of Laboratory Animals published by the National Institutes of Health (No. 85:23, 1996) and in compliance with the principles and guidelines of the Scientific Research Ethics Committee, Faculty of Science Al-Azhar University under Certificate Reference Number AZHAR11/2017. All rats underwent to good care and minimum pain suffering during the experiment, besides, anaesthetising before blood collection and dissection processes.

Study design and experimental procedure:

After ten days of acclimatization, rats were marked then placed into suitable cages by ratio of ten rats per cage and randomly divided into three groups: one negative control group and two experimental groups (Ni-Cr group and curcumin group). A long 75 days both experimental groups received a single dose (1 ml /200 g rat /day) of freshly prepared aqueous solution containing Ni-Cr mixture with concentrations exceeds the maximum limits of both WHO and Egyptian
standard regulation by 10 fold which are; 200, and 500 ppb for nickel, and chromium respectively. The curcumin group was post-treated by curcumin extract in a dose 100 mg/kg/day body weight (b.w.) for 75 days while the control group was neither treated nor contaminated. The treatment of all animals was performed by an oral gavage tube directly into stomach.

**Tissue Preparations:**

Blood samples were collected where retro-orbital venous plexus exist, after anesthetic administration. Suitable amounts of blood were collected in test tubes with anticoagulant (EDTA) for the measurement of hematological parameters. Testis organs were removed and separated into three parts. One tissue sample (0.5 g) was frozen and stored for the investigation of antioxidant status; a second was frozen and stored for toxic metals analysis and a third tissue sample was preserved in formalin for histopathological examination [35].

**Nickel-chromium Analysis:**

After the animals had been sacrificed, wet tissue samples of testis weighing about 0.5 g were placed in Teflon containers with 9 ml conc. HNO₃ and 1 ml H₂O₂ and digested using high performance microwave sample digestion (model Milestone ETHOS UP). Digestion was carried out according to the Milestone’s recommendations and USEPA [17, 18]. After the digestion program, the samples were transferred to 50 ml volumetric flasks and the volumes were completed to 50 ml using free-metal water (grade A). The amounts of Ni and Cr in the testicular tissues were determined by Inductively Coupled Plasma Optical Emission spectroscopy ICP-OES (ICap Thermo 7400, Thermo Fisher Scientific, Waltham, Ma, USA) according to APHA [19].

**Hematology Analysis**

Hematological parameters were measured by the CELL-DYN Ruby analyzer (Abbott, Abbott Park, IL, USA). Multi-angle polarized scatters separation was used for white blood cell and dual angle optical analysis for platelets count. The following hematological parameters were examined: white blood cell count (WBC) with WBC differential count (granulocytes, lymphocytes, and monocytes), red blood cell count (RBC), hemoglobin concentration (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT).

**Antioxidants Analysis:**

Testis were rapidly excised, washed in ice-cold 0.9% NaCl, then an exact weight of each organ (0.5 g) was grinded through homogenizer in 4 ml saline solution (NaCl 0.9%). Each sample was centrifuged at 4000 RPM for 15 minutes, the supernatant obtained were transferred into eppendorf tubes, and frozen to be analyzed for antioxidants biomarker. Assessment of the following parameters was performed: glutathione peroxidase (Gpx), glutathione reductase (GR), catalase (CAT), and superoxide dismutase (SOD) according to, [20, 21, 22, 23] respectively.

**Histopathological Analysis**

Testicular tissues were subjected to histopathological examination. Microscopic examinations on paraffin embedded 5 μm tissue sections with hematoxylin-eosin were performed. Each section was examined under an optical microscope [35].

**Statistical Analysis**

Statistical evaluation was performed using Graphpad Prism 6.0 Statistics software (Graphpad Inc. San Diego, CA, USA). One-way and two-way analysis of variance (ANOVA) tests were performed followed by Tukey's multiple comparisons test. p-Values less than 0.05were considered significant.

**Results**

**Concentrations of nickel and chromium in the testis:**

The experimental groups (Ni-Cr group and Ni-Cr + curcumin group) receiving a single dose (1 ml / 200g rat/day) of Ni-Cr mixture, provided significant elevations of Ni and Cr in the testis compared to the control group. The concentration of Ni in the testis of rats within Ni-Cr group exhibited a statistically high significant difference when compared to the control (p < 0.0001);
whereas, Cr showed elevation with statistically significant \((p < 0.001)\). Moreover, treatment with curcumin produced remarkable reduction in the concentrations of Ni and Cr in, testicular tissues when compared with Ni-Cr group, (Table, 1).

**Hematology:**

Acute exposure of rats to nickel and chromium in a mixture form via oral administration resulted in slight alteration in some haematological parameters. Comparing to control group, RBCs, WBCs and platelets showed slight elevation without any statistical differences in Ni-Cr group.

Furthermore, curcumin treating provided trivial reduction in the values of RBCs and PLTs with no statistical variances; in addition to barely increasing in WBCs value after curcumin. Similarly, trifle variances in HGB, HCT, MCV, MCH and MCHC were observed in both experimental groups compared to values obtained from control group. The change in WBCs differential (Lymphocytes, Monocytes and granulocytes) had a similar trend without any statistical differences comparing to control, (Table, 2).

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**Table (1): Concentrations of nickel and chromium (µg/g wet wt. ± SE) as well investigated antioxidants enzymes (U/g ± SE) in the testis tissues of rats from experimental groups.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Ni-Cr</th>
<th>Ni-Cr &amp; curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nickel</td>
<td></td>
<td>177.69 ± 1.51</td>
<td>1183.76 ± 102.25</td>
<td>788.61 ± 37.39</td>
</tr>
<tr>
<td>Chromium</td>
<td></td>
<td>412.77 ± 2.55</td>
<td>2634.89 ± 206.36</td>
<td>1633.53 ± 24.31</td>
</tr>
<tr>
<td>Glutathione peroxidise</td>
<td></td>
<td>13.53 ± 0.34</td>
<td>6.37 ± 0.51</td>
<td>10.65 ± 0.37</td>
</tr>
<tr>
<td>Glutathione reductase</td>
<td></td>
<td>7.83 ± 0.27</td>
<td>3.30 ± 0.28</td>
<td>4.79 ± 0.22</td>
</tr>
<tr>
<td>Superoxide dismutase</td>
<td></td>
<td>11.04 ± 0.24</td>
<td>5.92 ± 0.24</td>
<td>8.77 ± 0.33</td>
</tr>
<tr>
<td>Catalase</td>
<td></td>
<td>241.71 ± 4.35</td>
<td>114.87 ± 4.77</td>
<td>166.92 ± 6.24</td>
</tr>
</tbody>
</table>

Observed metals and antioxidant enzymes are expressed on wet tissues by means ± SE. Statistically significant differences \((p < 0.05)\) compared to control group are indicated by *, while those compared to heavy metals group are indicated by †. Statistical evaluation was performed using one-way ANOVA followed by Tukey's multiple comparisons test. * †††† \(p < 0.0001\); **** \(p < 0.0001\); *** ††† \(p < 0.001\); ** †† \(p < 0.01\); * † †\(p < 0.05\).

**Table (2): Values of haematological parameters ± SE in the different experimental groups.**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>Control</th>
<th>Ni-Cr</th>
<th>Ni-Cr &amp; curcumin</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBCs (10^6/mm^3)</td>
<td></td>
<td>7.65 ± 0.24</td>
<td>7.69 ± 0.26</td>
<td>7.47 ± 0.27</td>
</tr>
<tr>
<td>WBCs (10^3/mm^3)</td>
<td></td>
<td>10.18 ± 1.17</td>
<td>11.22 ± 3.00</td>
<td>11.75 ± 0.81</td>
</tr>
<tr>
<td>PLTs (10^3/mm^3)</td>
<td></td>
<td>677.75 ± 34.70</td>
<td>753.00 ± 18.19</td>
<td>662.25 ± 24.75</td>
</tr>
<tr>
<td>HGB g/dL</td>
<td></td>
<td>14.23 ± 0.23</td>
<td>14.30 ± 0.60</td>
<td>14.35 ± 0.39</td>
</tr>
<tr>
<td>HCT %</td>
<td></td>
<td>41.95 ± 1.19</td>
<td>41.40 ± 1.64</td>
<td>40.98 ± 1.67</td>
</tr>
<tr>
<td>MCV (µm^3)</td>
<td></td>
<td>54.75 ± 0.19</td>
<td>53.80 ± 0.49</td>
<td>54.75 ± 0.49</td>
</tr>
<tr>
<td>MCH pg</td>
<td></td>
<td>18.63 ± 0.32</td>
<td>18.64 ± 0.41</td>
<td>19.25 ± 0.29</td>
</tr>
<tr>
<td>MCHC g/dL</td>
<td></td>
<td>33.98 ± 0.45</td>
<td>34.56 ± 0.62</td>
<td>35.10 ± 0.56</td>
</tr>
<tr>
<td>Lymphocytes %</td>
<td></td>
<td>76.63 ± 1.83</td>
<td>77.82 ± 3.89</td>
<td>81.68 ± 0.79</td>
</tr>
<tr>
<td>Monocytes %</td>
<td></td>
<td>13.05 ± 1.08</td>
<td>12.60 ± 1.57</td>
<td>10.08 ± 0.37</td>
</tr>
<tr>
<td>Granulocytes %</td>
<td></td>
<td>10.33 ± 1.53</td>
<td>9.58 ± 2.47</td>
<td>8.25 ± 0.42</td>
</tr>
</tbody>
</table>

One-way ANOVA and Tukey's multiple comparisons tests, didn’t show any statistical significant differences \((p < 0.05)\)
Antioxidants Status:

After exposure to the heavy metals mixture, the activities of all endogenous antioxidant enzymes (GPx, GR, SOD, CAT) exhibited a downward trend in testis of rats at both, Ni-Cr and Ni-Cr-curcumin groups with statistically significant effects when compared to the control group. Administration of curcumin treatment demonstrated significant restoring in the activities of GPx, GR, SOD and CAT within testicular tissues with statistically significant elevations ($p < 0.01$, $p < 0.05$, $p < 0.0001$, $p < 0.01$, respectively) when compared to Ni-Cr group. Observed redox parameters in the testis of rats are presented in (Table,1).

Histopathological analysis:

Photomicrograph examination in the testis of control rats (section A) showed intact seminiferous tubules (white arrow) separated by interstitial tissue (black arrow). The Ni-Cr group (section B) showed degeneration and necrosis of spermatogonia (white arrow); necrosis of interstitial tissue (black arrow) and necrosis with sloughing of spermatocytes and spermatozoal zone (black arrow head). On the other hand, testicular tissue of rats at curcumin group (section C) showed moderate degeneration and separation of spermatogonia (white arrow) and focal area of necrosis (white arrow head), Figure, 1, A; B; C.

Figure (1): Histopathological investigations in the testis sections
A) control group; B) Ni-Cr group; C) Ni-Cr & curcumin group

DISCUSSION:

Nickel-chromium and curcumin:

Generally, accumulation of toxic metals in the testicular tissues is conditioned by the nature, dose, amount, exposure period and route type of the metals, since the toxicity can be carried through blood to many vital organs where deposition begins to occur and increase gradually until the symptoms of health hazards appear. The present study has proved that exposure to Ni-Cr mixture in high levels (10x) for 75 days via oral administration caused combined toxicity in male rats such toxicity biomarkers attained significant decreasing after curcumin treatment. Rats exposed to Ni-Cr mixture only, exhibited high significant increasing in the concentrations of Ni \((p<0.001)\) and Cr \((p<0.001)\) inside testicular tissues in comparison to unexposed rats. Clear signs for oxidative stress like; singlet oxygen, hydroperoxides \((\text{HO}_2^-)\), and hydrogen peroxide \((\text{H}_2\text{O}_2)\) appeared after Ni-Cr exposure. These signs were shown to enhance the formation of free radicals and overproduction of ROS leading to deactivation of antioxidant defense system, including; GPx, CAT, SOD and GR, as a clear mark for cellular damage \([24, 25, 26]\). Nickel element is able to raise structural changes and functional disruptions in various tissues and organs of the body \([27, 28, 29]\). Similarly, it was observed that, chromium intake promotes an early oxidative stress through undergoing an intracellular reduction, resulting in the generation of toxic intermediates as strong oxidants. These toxins generate excess of ROS and cause injury to cellular proteins, lipids and DNA as well as enhanced excretion of urinary lipid metabolites including malondialdehyde, formaldehyde, acetaldehyde, acetone and propionaldehyde \([30, 31, 32, 33, 34]\). Moreover, combined toxicity might be due to the differences in the metals bioavailability and their competitive affinity to protein transporters after oral exposure with possible synergistic effect \([35]\). Despite the presence of many complications because of metals effects, data detected that curcumin treatment provided statistically significant reduction \((p<0.01)\) in the concentrations of investigated metals (Ni, Cr) among testicular tissues. This reduction is probably due to the capacity of curcumin to prevent the damage of membranes or proteins and regulating their activity by specifically scavenging reactive oxygen species or by regulating specific enzymes and influencing cellular structures. Also, curcumin is able to reactivate the defense characteristics of enzymatic antioxidants, to protect testicular tissues as the alternative method for chelation therapy. Curcumin was shown to inhibit the formation of free radicals through scavenge action, and it has high efficacy against oxidative tissue damage arose by nickel-chromium \([7, 36, 37]\).

Antioxidant enzymes and Curcumin:

Oral administration of Ni-Cr mixture for 75 days produced lower level of glutathione peroxidase (GPx) in the testicular tissues \((p<0.001)\) at Ni-Cr group in comparison with control group, this is probably attributed to the testicular defensive mechanism under the effect of Ni-Cr within the testis as a result of free radicals production and ROS generation \([38, 39, 40]\). At the same time, rats exposed to Ni-Cr mixture and treated with curcumin showed statistically significant improvement \((p<0.01)\) in the activities of GPx among testicular tissues compared to Ni-Cr group which reinforces the protective role of curcumin in recovering the affected testis as normal rats through reducing the combined toxicity of Ni-Cr \([41, 42, 43]\). Moreover, the levels of glutathione reductase (GR) in the testis of rats exhibited statistically significant decreasing \((p<0.001)\) at Ni-Cr group and \((p<0.01)\) at treated group when compared to control. This probably occurred when Ni-Cr elements interfered with the disulfide bond of glutathione enzyme and inhibited its defense mechanism, as well prevented the optimal balance leaving testicular cells under oxidative damage \([38, 44]\). Furthermore, superoxide dismutase (SOD) enzyme attained statistically significance decreasing \((p<0.0001)\) within testicular tissues of rats from both experimental groups when compared to the control. This inhibition could be as a result of copper depletion which leads to decreased capability of cells to produce SOD and disrupting its pivotal role represented in producing \(\text{H}_2\text{O}_2\) in cells by a dismutation of superoxide radicals which are generated by the oxidative process \([45, 46]\). However, the antioxidant feature of curcumin enhanced the reversing effect of toxic mixture with statistical elevation \((p<0.0001)\) in SOD activity among Ni-Cr & curcumin group in comparison with Ni-Cr group. Comparing to control group, measured
levels of catalase enzyme in the testicular tissues showed remarkable statistically decreasing at Ni-Cr group (p<0.001) as well as curcumin treated group (p<0.01) which showed bio-signs for restoring catalase activities in the testicular tissues. Lower catalase activity was attributed to its ability to disable consumption of O₂ inside cells as a result of combined toxicity of Ni-Cr thereby, capturing H₂O₂ before it can escape the cell then converting it to water and molecular oxygen for maintaining the amount of O₂ in order to be repeated in chemical reduction or to interact with other toxins [47, 48].

Effect on Hematopoietic System:
The hematopoietic system is one of the most sensitive organs to assess the toxicity. The present study declared that exposure to Ni-Cr for 75 days was more than enough to cause haematological alterations by which early effects associated with increased toxic metals concentration in the testis and other soft tissues [49]. After oral exposure, red blood cells transported the Ni and Cr biomolecules within the circulatory system which enabled these metals to be absorbed at varied organs thereby accumulation occurred. The present data showed that both Ni-Cr administrations led to mild lymphocytosis as well as relative neutropenia in both experimental groups in comparison with control group. These effects could be varied between synergistic and antagonistic, thereby causing alteration in differential count of peripheral blood leukocytes in experimental rats groups as a result of activation of the immune system and oxidative stress induced by Ni-Cr mixture [50, 51]. The Authors who described similar effects were in conflict with some studies pointing to unchanged WBCs status. The current work investigated lymphocytosis and neutropenia [52]. However, others postulated lymphopenia and neutrophilic leukocytosis [53, 54]. Acute exposure to Ni-Cr produced mild alteration in RBCs, HGB, HCT, MCV, MCH and MCHC, in addition to a transient reactive thrombocytosis in Ni-Cr group, which returns to normal after curcumin treatment. Such mild changes might attribute to gastrointestinal absorption or fast clearance of Ni-Cr from the blood to the tissues including testis. The present observations are nearly in line with other authors who used different animal models, route of exposure, and dose regime [35, 55, 56, 57, 58, 59, 60].

Histopathological effects:
Histological examinations revealed that nickel-chromium mixture caused relative alterations in testis of rats from Ni-Cr group while rats from the control group almost showed no histopathological changes. The alterations in the testicular tissue could be attributed to the ability of Ni-Cr mixture to penetrate the testis barrier and impair testicular functions through disrupting serum testosterone level and activities of testicular steroidogenic enzymes in addition to consequently reduction in sperm count accompanied by abnormal morphology [61, 62]. Conversely, rats treated with curcumin exhibited depletion in histopathological changes induced by Ni-Cr mixture, since the number and morphological integrity of seminiferous tubule were being restored. Also most of the seminiferous tubules showed semi normal histo-architecture including; mild to moderate degeneration, less separation of spermatogonia, focal area of necrosis, mild degeneration of spermatogonia, congestion of blood vessels and focal area of necrosis. It was revealed that natural antioxidants like curcumin is able to modulate the toxic effects of heavy metals in histological examinations and attenuates the interstitial and seminiferous tubule alterations of testicular tissue [63, 63]. Although several studies regarding curcumin efficacy versus metals induced ROS toxicity have been conducted, there is an obvious lack of data on mechanisms underlying the curcumin antioxidant property against toxicity of other metals.

Conclusion:
Our results declared a combined toxicity of Ni-Cr through oral administration by which toxic effects induced in the testicular tissues of adult Wistar rats. The main toxicity mechanism of combined metals is oxidative stress which relatively proved by a disturbed structure in testicular tissues of Ni-Cr treated rats in addition to mild hematological alterations and significant decline in the levels of antioxidants enzymes under investigation. The present study also reinforced protective role of curcumin which reduced Ni-Cr deposition inside testis and improved the activities of investigated antioxidants through many suggested mechanisms including; lipid peroxidation inhibition, peroxidative prevention and neutralizing reactive species.
Acknowledgments:

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Authors’ contributions:

All authors contributed significantly to this work; Ayman El-Amawy and Dr. Samir Zaahkouk are the two first authors responsible for the Study conception, design, performing the experimental analysis and overall data presented here, Dr. Hesham Abdel-Rashid helped with the organization, references, tables, and data presentation; Dr. Bassem Elaraby was responsible for technical and scientific support during the experiment in addition to statistical analysis.

Data availability:

The datasets from which the current study was created are available from the corresponding author on reasonable request.

Compliance with ethical standards:

Conflict of interest:

The authors declare that they have no conflict of interest.

Ethical approval:

The experimental protocol was approved according to certificate reference number, AZHAR11/2017 of Institutional Animal Care and Use Committee, Faculty of Science, Al-Azhar University, Egypt.

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Consent for publication: Not applicable.

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