Effect of Carthamus Tinctorius Safflower Aqueous Extract Against Nickel Chloride Induces Hematotoxicity and Immunotoxicity in Adult Male Rabbits

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Abstract
This study was designed to show the role of Carthamus tinctorius safflower aqueous extract against toxicity of nickel chloride (NiCl₂). Twenty male rabbits were used and divided into four groups (with 5 rabbits in each group); group (control group) received normal diet, group II received orally 100mg/kg NiCl₂ for six weeks, group III received 100mg/kg NiCl₂ and 100mg/kg extract six weeks, group IV received 100mg/kg NiCl₂ and 200mg/kg extract six weeks. Hematological parameters showed (RBC (Red blood cells), Hb (Hemoglobin), PCV (Packed cells volume) decreased and WBC (White blood cells) increased) significant changes (P < 0.05) compared with control group. Immunological parameters (IgG, IgA and IgM increased) and oxidative stress factors (MDA increased and GSH decreased) show significant changes (P < 0.05) compared with control group. While, safflower aqueous adverse the negative effects of NiCl₂ and causing ameliorative effects on all hematological parameters, hematological immunological parameters and oxidative stress factors showed no significant changes (P < 0.05) compared with control group. It was concluded that flower extract of Carthamus tinctorius has been antioxidant role against nickel chloride toxicity in rabbits.

Keywords: Carthamus tinctorius; Hematological parameters; Immunological parameters; Oxidative stress factors
Introduction

*Carthamus tinctorius* (Safflower) is a member of the family Compositae or Asteraceae, cultivated mainly for its seed, which is processed to edible oil (This oil is composed of typically linoleic acid, oleic acid and linolenic acid) and used as bird seed [1-2]. Safflower is a highly branched, herbaceous, winter annual, usually with many spines on the leaves. Plants are 30-150 cm length with globular flower heads and brilliant yellow, orange or red flowers [3]. *Carthamus tinctorius* has biological and pharmacological activities including cardioprotective [4], swelling associated with trauma, antidepressant [5], sedative, anti-inflammatory and anti-tumor activities [6-7], neuroprotective [8], inhibition of platelet aggregation, increase of peripheral blood flow, increase in the beating amplitude of cultured myocardial cell sheet and inhibition of tumor promotion in mice [9]. In Korea, Safflower was used for the promotion of bone formation and in the treatment of rheumatism and osteoporosis [10].

Nickel is a toxic metal that found into the environment by industrial activities, such as companies of batteries and paints. Thus nickel is an important metal in industries, and hence, the exposure to nickel compounds by contaminated water and foodstuffs lead to public health environmental problems [11]. After the body exposure to nickel, nickel penetrates all organs but mainly accumulates into liver, kidney, lungs and bone. Nickel can induce severe liver and kidney damage by changing several enzymes and ascorbate cholesterol metabolism along with histopathological changes [12-13].

Materials & methods

Animal model

Twenty adult males were collected locally (Kirkuk city markets). The weights and age ranged between (1.5-2 kg and 7-11 Mon respectively). Rabbits were kept under the same environmental conditions. All animals received free food and water. The animals were observed to avoid any possibility for infection.

Plant extraction

Flowers of *Carthamus tinctorius* were powdered, macerated in 200 ml distilled water for 1 h, the mixture was extracted by boiling for 60 min. After filtering, extract was autoclaved (at 121°C for 20 min) and then it was stored in a 4°C [4].

Experimental design

Nickel chloride (NiCl₂) was obtained from Kirkuk University / Science College. Rabbits administrated daily with Nickel chloride (NiCl₂) at a dose of 100mg/kg body weight orally [14]. Rabbits were used in this experiment and divided as follow (each group consists of five rabbits):

I. Negative control: rabbits received normal diet and used as control.

II. Positive control: rabbits received orally (100mg/kg) NiCl₂ for six week, and then killed.

III. Subjects received orally (100mg/kg) NiCl₂ and 100mg/kg extract in same time for six weeks, and then killed.

IV. Subjects received orally (100mg/kg) NiCl₂ and 200mg/kg extract in same time for six weeks, and then killed.

Blood samples collection

The blood samples were collected by cardiac puncture under anesthesia and put in test tubs, under anesthesia. After clotting, the blood sample tubes were centrifuged (5000 cycle/min for 10 min) to isolate blood serum. Serum was taken and stored by deep freezing to estimate the biochemical measurement.

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Measurements

1. **Hematological parameters**: red blood cell (RBC) count, hemoglobin (Hb) concentration, packed cell volume (PCV) and white blood cell (WBC) count was estimated utilizing fully automated hematological cell counter.

2. **Immunological parameters**: immunoglobulin type IgG, IgM and IgA was estimated by Single Redial Immuno Diffusion Assay (SRIDA) technique [15].

3. **Oxidative stress factors**: MDA (malonealdehyied), by thiobarbituric acid (TBA) according to method [16], and Glutathione (GSH) by using DTNB according to method [17].

Statistical analysis

The Data were analyzed using one-way ANOVA by statistical Minitab program. The data were presented as the mean ± SD. *P* < 0.05 was considered significant.

Results

Hematological parameters

The results of the present study showed significant changes (*P* < 0.05) in levels of RBC, Hb, PCV and WBC counts (4.69 ± 0.33; 9.4 ± 0.3; 28.66 ± 3.51 and 10.7 ± 0.69 respectively) in rabbits received nickel chloride compared with control rabbits (6.01 ± 0.17; 11.57 ± 0.38; 34.83 ± 2.75 and 5.8 ± 0.3 respectively). The same hematological parameters levels (5.41 ± 0.21; 10.3 ± 0.5; 30.67 ± 2.08 and 8.13 ± 0.32 respectively) in rabbits received nickel chloride and 100mg/kg flower extract show high significant changes (*P* < 0.05) compared with normal rabbits. While, the hematological parameters levels in received nickel chloride and 200mg/kg flower extract show no significant changes (*P* < 0.05) compared with normal rabbits as shown in table (1).

Immunological parameters

In the results of present study show significant changes (*P* < 0.05) in levels of IgG, IgA and IgM (3753.1 ± 252.9; 516.8 ± 24.11 and 274.5 ± 32.8 respectively) in rabbits received nickel chloride compared with normal rabbits (2978.2 ± 189.7; 280.8 ± 18.94 and 149.23 ± 24.15 respectively). The same immunological parameters levels (3221.3 ± 251.7; 375.5 ± 32.43 and 207.97 ± 13.53 respectively) in rabbits received nickel chloride and 100mg/kg flower extract show high significant increased (*P* < 0.05) compared with normal rabbits. While, the immunological parameters levels in rabbits received nickel chloride and 200mg/kg flower extract show no significant changes (*P* < 0.05) compared with normal rabbits as shown in table (2).

Oxidative stress factors

The results of present study show significant changes (*P* < 0.05) in levels of Malonealdehyied (*MDA*) and Glutathione hormone (*GSH*) levels (2.85 ± 0.41 and 0.265 ± 0.032) in rabbits received nickel chloride compared with normal rabbits (1.43 ± 0.15 and 0.626 ± 0.03). *MDA* and *GSH* levels (1.96 ± 0.14 and 00.435 ± 0.026) in rabbits received nickel chloride and 100mg flower extract show high significant changes (*P* < 0.05) compared with control rabbits. While, the levels of *MDA* and *GSH* (1.47 ± 0.31 and 0.641 ± 0.059) in rabbits received nickel chloride and 200mg/kg flower extract show no significant changes (*P* < 0.05) compared with control rabbits as shown in table (3).
Discussion

The exposure to highly nickel-polluted environments leads to different pathologic effects. Nickel was entering body by dermal absorption, ingestion and inhalation. Nickel was lead to fibrosis, cardiovascular and kidney diseases, DNA damage and cancer [18]. The results of hematological parameters (RBC, Hb, PCV decreased and WBC increased) is in agreement with Dahdouh et al. (2016) who referred that the NiCl2 has been hematotoxicity in mice. They found that mice treated with NiCl2 showed decreased in RBC (5.46±0.65X 10^6), Hb (10.1±0.08mg/dl) and PCV (32.7±0.13%) with WBC (8.05±0.40X10^3) increased compared to control mice (7.28±0.4 X10^6; 44.12±0.9; 13.37±0.6; 6.06± 0.22 respectively). They suggested that nickel leads to adverse effect on hematopoietic process and bone marrow activity [11]. Wu et al. (2014) referred that the NiCl2 has been immunotoxicity in broilers. They found that broilers treated with NiCl2 showed decreased in IgA+ B Cells and sIgA, IgA, IgG, IgM in the intestinal mucosal. They suggest that NiCl2 at high levels has intestinal mucosal humoral immunotoxicity in animals [19]. Also, in study of Bencko et al (1983) on 38 production workers exposed to nickel (compound not specified). They found significant increases in levels of immunoglobulin G (IgG), IgA, and IgM [20]. The results of oxidative stress factors (MDA increased and GSH decreased) in agreement with Sunderman et al. (1985) who referred that the NiCl2 lead to elevated the levels of sera MDA in rats [21]. Also, Chen et al. (1998) referred that the NiCl2 lead to increase the levels of sera MDA in rats [22].

C. tinctorius flowers were used as a medicinal herb. This herb has been considered to relieve the sting pain, clear the throat. Safflower oil is used as antiseptic, wound healer, reducing cholesterol level, relieving rheumatism and treatment of atherosclerosis [23]. The results of hematological parameters when used the flower extract against nickel chloride back to the normal ranges. Where, in study of Jawad et al (2013) referred that the extract of plant leads to an increase of hematological parameters (RBC and Hb). They suggested that the Carthamus tinctorius possess Eriodicytol that stimulating bone marrow to produce RBC [24].

Also, in study of Namjoo et al (2013) referred that the extract of plant leads to have no adverse effects on hematological parameters (RBC, Hb and WBC) [25]. About the role of Carthamus tinctorius in an immunity in this study, the results show that the immunoglobulin (G, A and M) back to normal ranges. In study of Wajadan (2015) to show the role of Carthamus tinctorius extract. Suggest that the Carthamus tinctorius extract in mice lead to stimulate the immune system to increase the WBC count and elevated the plasma proteins (albumin and globulin) [26]. The oxidative stress factors (MDA and GSH) back to normal ranges after using Carthamus tinctorius extract, where In study Hu & Wei-xing (2015) to showed the effect of Carthamus tinctorius extract against Diethylnitrosamine. They found that Carthamus tinctorius extract inhibited MDA formation in DEN-induced rat liver and they suggest that Carthamus tinctorius have antioxidant effects [27]. It was concluded that flower extract of Carthamus tinctorius has an antioxidant role against nickel chloride toxicity in rabbits.
References

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Table (1): The levels of RBC, Hb, PCV and WBC count

<table>
<thead>
<tr>
<th>Groups</th>
<th>RBC (IU/L)</th>
<th>Hb (IU/L)</th>
<th>PCV (IU/L)</th>
<th>WBC (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6.01 ± 0.17 a</td>
<td>11.57 ± 0.38 a</td>
<td>34.83 ± 2.75 a</td>
<td>5.8 ± 0.3 a</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg</td>
<td>4.69 ± 0.33 c</td>
<td>9.4 ± 0.3 c</td>
<td>28.66 ± 3.51 b</td>
<td>10.7 ± 0.69 c</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 100mg/kg Flo. extract</td>
<td>5.41 ± 0.21 b</td>
<td>10.3 ± 0.5 b</td>
<td>30.67 ± 2.08 ab</td>
<td>8.13 ± 0.32 b</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 200mg/kg Flo. extract</td>
<td>5.94 ± 0.24 a</td>
<td>11.47 ± 0.5 a</td>
<td>35 ± 3.61 a</td>
<td>6.1 ± 0.27 a</td>
</tr>
</tbody>
</table>

Note: different letters mean significant changes and same letters mean non-significant changes

Table (2): The levels of IgG, IgA and IgM in sera

<table>
<thead>
<tr>
<th>Groups</th>
<th>IgG (mg/dl)</th>
<th>IgA (mg/dl)</th>
<th>IgM (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2978.2 ± 189.7 a</td>
<td>280.8 ± 18.94 c</td>
<td>149.23 ± 24.15 c</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg</td>
<td>3753.1 ± 252.9 c</td>
<td>516.8 ± 24.11 a</td>
<td>274.5 ± 32.8 a</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 100mg/kg Flo. extract</td>
<td>3221.3 ± 251.7 b</td>
<td>375.5 ± 32.43 b</td>
<td>207.97 ± 13.53 b</td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 200mg/kg Flo. extract</td>
<td>2602.8 ± 148.2 a</td>
<td>262.33 ± 38.81 c</td>
<td>141.47 ± 11.51 c</td>
</tr>
<tr>
<td>Parameters</td>
<td>MDA (mmol/l)</td>
<td>GSH (mol/l)</td>
<td></td>
</tr>
<tr>
<td>----------------------------------------</td>
<td>--------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1.43 ± 0.15 c</td>
<td>0.626 ± 0.03 a</td>
<td></td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg</td>
<td>2.85 ± 0.41 a</td>
<td>0.265 ± 0.032 c</td>
<td></td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 100mg/kg Flo. extract</td>
<td>1.96 ± 0.14 b</td>
<td>0.435 ± 0.026 b</td>
<td></td>
</tr>
<tr>
<td>NiCl₂ 100mg/kg + 200mg/kg Flo. extract</td>
<td>1.47 ± 0.31 c</td>
<td>0.641 ± 0.059 a</td>
<td></td>
</tr>
</tbody>
</table>

**Table (3): The levels of MDA and GSH in sera**