PROTECTIVE EFFECT OF *ROSMARINUS OFFICINALIS* EXTRACT ON THE NEPHROTOXICITY CAUSED BY NICKEL CHLORIDE IN WISTAR RATS

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**ABSTRACT**

Nickel is a common environmental pollutant inducing nephrotoxicity. Oxidative stress has been proposed as a possible mechanism involved in this toxicity. The present study aimed to elucidate the potential protective effect of methanolic extract of *Rosmarinus officinalis* (RO) against nephrotoxicity induced by Nickel Chloride (NiCl2). The antioxidant activity and the metal chelating power of the plant extract were evaluated. An in vivo study on 28 rats divided into equal four groups including the first group as the control; the second received 100 mg/kg bw of RO extract; the third was exposed to 10 mg/kg bw of NiCl2, and the last was treated with the combination of the extract and NiCl2 by gavage for 28 days. Oxidative stress parameters, biochemical biomarkers, and histopathological examination of the kidney were determined. Analysis of the results showed that the plant has significant antioxidant and metal-chelating power. In addition, exposure of rats to NiCl2 caused a disturbance of renal function (urea, creatinine, and uric acid). This exposure also induced a renal oxidative stress, which results in increased MDA level and GST activity a decrease in antioxidant status (CAT, GPx, and SOD activity) and alteration in tissue architecture of kidney was observed. Co-administration of RO (extract and NiCl2) restored most of the parameters cited above to values close to normal. Therefore, the present study revealed the ability of RO to bind to NiCl2 and protect kidney tissue from NiCl2-induced oxidative damage.

Keywords: Oxidative stress, Nickel, *Rosmarinus officinalis*, Nephrotoxicity, Rat

INTRODUCTION

Nickel (Ni) is an environmental pollutant, a silver-white metal present under several oxidative states, and causes cytotoxicity (*Amudha et Pari*, 2011). Several exhibition routes to nickel: Digestive (food and water), respiratory, and skin (mining, fusion, refining, welding, galvanoplasty, food processing, and the elimination of Ni waste) (*Dahmane et al.*, 2016; *Begum et al.*, 2022).

Nickel toxicity has been attributed to the disruption of the cellular redox system (*Salah et al.*, 2021). Cellular oxidative homeostasis is maintained by a non-enzymatic antioxidant defense system such as reduced glutathione, metallothionein, and an enzymatic defense system such as glutathione peroxidase (GPx), superoxide dismutase (SOD), glutathione-s-transferase (GST) and catalase (*Lushchak*, 2011). While nickel exposure causes a decrease in non-enzymatic antioxidants leading to a decrease in antioxidant enzyme activity and therefore an increase in reactive oxygen species (ROS) and oxidative damage (*Pari et Prasath*, 2008; *Ijomone et al.*, 2018). This oxidative damage is representative of nephrotoxicity, hepatotoxicity, hematotoxicity, reproductive toxicity, and an increased risk of cancer (*Xu et al.*, 2015; *Goodman et al.*, 2009; *Adjroud et Moufok*, 2009).

Nephrotoxicity is a major risk in preclinical toxicology studies due to direct cell and tissue damage, obstruction of renal excretion, hemodynamic changes, and inflammation. Kidney plays a major role in plasma infiltration and maintenance of metabolic homeostasis. Nephrotoxicity in response to pollutants can impair renal excretory activities and cause alteration in kidney physiology and structure (*Zhang et Sun*, 2015; *Shang et Falah*, 2019).

Since antiquity, plants have been used for the healing of various diseases, pain, and certain organ dysfunctions (*El-Demerdahe et al.*, 2021; *Njoua*, 2021). *Rosmarinus officinalis* (*Rosemary*) is an herb whose leaves are widely used in cooking as a condiment and flavoring material for food (*Fu et al.*, 2007). Rosemary leaves and flowers contain different phenolic compounds with significant antioxidant activity due to their richness in phenolic compounds (*Benkhedir et al.*, 2022; *Ngo*, 2011). Several studies have shown that rosemary is antibacterial (*Koy et al.*, 2020), anti-inflammatory (*Benincà et al.*, 2011), cytoprotective (*Rajgopal et al.*, 2019), antioxidant and antidiabetic properties (*Benkhedir et al.*, 2022; *Naimi et al.*, 2017).

Therefore, the present study aims to evaluate the possible protective role of the methanolic extract of *Rosmarinus officinalis* leaves against nickel (NiCl2) nephrotoxicity in male rats.

MATERIAL AND METHODS

Chemicals

Most of the chemicals used in this study (Nickel chloride [NiCl2, CAS N°: 7718-54-9], nitro blue tetrazolium, N-[1-naphthyl]-ethylene diamine, and Tris-HCl, thiobarbituric acid and trichloroacetic acid) carry the registered trademark Sigma (St. Louis, MO). Louis, MO). Except for “nickel chloride”, which was used to cause toxicity to rats, all other chemicals and reagents were used for analytical purposes.

*Rosmarinus officinalis* extract preparation

The fresh *Rosemary* leaves used in this study are from the Hammamet, Tebessa region Northeast Algeria. A quantity of these leaves (100 g) was dried by exposure to air, then methanol was added to it at normal temperature three times within 24 hours, after which the extract was evaporated, and a dry powder was extracted and stored at -4°C.

In vitro study on *Rosmarinus officinalis* extract

**Assay chelating metal**

The Chelate Metal activity is determined by the ferrozine formation complex (Fe3+), according to the procedure described by Decker and Welch (1990). 40 µl extract with different concentrations (50-800 µg/mL) mixed with 40 µl methanol and 40 µl iron chloride (0.2mM) in a 96-microplate well. 80 µl of 0.5 ferrozine was added to the mixture to trigger a reaction; the reaction mixture was incubated for 10 min and measured at 562 nm with EDTA as a standard the percentage inhibition of the ferrozine complex (Fe3+) was calculated according to the...
In vivo study of Rosmarinus officinalis extract

Treatment of animals

Twenty-eight (28) Wistar Albino rats (140 ± 25g), provided by the Institut Pasteur (Algiers, Algeria) were used in this study. All rats were placed under the same favorable experimental conditions of temperature (T° 25 ± 2°C) and humidity (45%). The animals were divided into 4 groups of 7 rats each and treated orally for 28 days. as follows:

- Control group (C): received drinking water.
- Extract of RO group (RO): (treated with 100mg/kg of Rosemary) (Kayashima et al.,2020).
- Nickel chloride group (Ni): (treated with 10 mg/kg of NiCl₂) (Iqbal. et al.,2020).
- Mixture group (Ni-Ro): (treated with the association of NiCl₂: 10 mg/kg and 100 mg/kg of extract) for 28 days.

Preparation of Samples

After the end of the treatment period, rats were sacrificed, blood was collected, and kidneys were extracted:

- Blood plasma was prepared by centrifugation (1500 g/15 min/4 °C) and stored at -20 °C for biochemical tests.
- The kidneys were weighed, used part of it for histological study, and the rest for use in the analyses of antioxidants.

Biochemical analysis markers in plasma

Plasma biochemical markers: creatinine, urea, and uric acid were measured using commercial kits from Spreacpt Spain.

Analysis of Reduced GSH and MDA

Assay of MDA is performed using the method of Esterbuer et al., (1992). However, GSH levels were estimated in the rats’ kidneys using a method of Ellman (1959) modified by Jollow et al (1974), were expressed as μg GSH/mg protein.

Analysis of Antioxidant enzymes activity


Histological study

After placing rat kidney samples for more than two days in a 10% formalin solution, they were dried in successive concentrations of alcohol baths, cleaned with xylene, and then dipped in paraffin. Finally, they were cut to a thickness of 5 mm, stained with hematoxylin and observation under the microscope.

Statistical Study

The data is expressed as an average standard deviation (SD). The variations between various groups were measured by ANOVA one-way analysis followed by the Dunnett multiple comparison test, non-matches performed the comparisons between treated groups.

RESULTS AND DISCUSSION

Antioxidant activity and metal chelating of Rosmarinus officinalis extract

The possibility of Rosmarinus officinalis extract (RO) to prevent the formation of a ferrozine complex is presented in Table 1. This activity was compared with EDTA as a standard (p< 0.05). The CUPRAC results showed significance (p<0.05) to the standards that have BHT, BHA, and α-tocopherol.

Table 1 Antioxidant activity and metal chelating of Rosmarinus officinalis extract (comparison with BHT, BHA, EDTA, and α-tocopherol).

<table>
<thead>
<tr>
<th></th>
<th>CUPRAC (IC₅₀ μg/ml)</th>
<th>Metal chelating (IC₅₀ μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NT)</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>BHT</td>
<td>9.62±0.09</td>
<td>NT</td>
</tr>
<tr>
<td>BHA</td>
<td>3.64±0.11</td>
<td>NT</td>
</tr>
<tr>
<td>EDTA</td>
<td>NT</td>
<td>96.68±1.04</td>
</tr>
<tr>
<td>α-Tocopherol</td>
<td>20.26±0.06</td>
<td>NT</td>
</tr>
</tbody>
</table>

Oxidative stress parameters

Based on the results shown in Figure1, a single dose of NCl₂ resulted in a very highly significant decrease (p< 0.01) in kidneys reduced glutathione (GSH) content and the activity of glutathione peroxidase (GPx), was recorded in the Nickel (10 mg/kg bw of NiCl₂) treated groups compared to the control group. In contrast, there was no significant change in the RO-treated group and a significant decrease (p<0.05) in the combination (RO-Ni) treated group compared to the control. Our results show a significant increase (p<0.01) in the enzyme activity of renal glutathione-s-transferase (GST) (figure 2), in the Ni group compared to the control group. There is also a significant (p< 0.05) increase in GST enzymatic activity in the RO-Ni group compared to the control. A highly significant 60% decrease in renal catalase activity (figure 1) was observed in the Ni group of animals compared to the control. In contrast, renal catalase activity was also reduced to 24% in the co-exposure group (Ni-RO). Similarly, for the enzymatic activity of superoxide dismutase (SOD), we recorded a highly significant decrease (p< 0.01), in the group treated with nickel (Ni), and significant (p<0.05) in the combination group (RO-Ni) compared to controls and treated with RO alone.

Figure 1 Kidneys GSH content, GPx, and CAT activities of control rats treated with Rosmarinus officinalis (RO), nickel (Ni), and Rosemary plus nickel (RO-Ni) after 28 days of treatment. Values are given as means ± SD, seven rats in each group. A statistically significant difference (p<0.05): compared to the (a) control group, (b) Ni group, (c) (RO-Ni) group.
**Figure 2** Kidneys SOD, GST activities, and MDA content of control rats treated with Rosmarinus officinalis (RO), nickel (Ni), and Rosemary plus nickel (RO-Ni) after 28 days of treatment. Values are given as mean±SEM, seven rats in each group. A statistically significant difference (p<0.05): compared to the (a) control group, (b) Ni, (c) (RO-Ni) group.

### Blood biochemical assay

The results show a significant increase in serum levels of urea, creatinine and uric acid (p<0.05) in rats treated with nickel (10 mg/kg bw of NiCl2), compared to controls and to the RO group. Treatment of the NiCl2 group with RO significantly reduced (p<0.05) the concentrations of these metabolites compared to the group treated with nickel alone.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Uric Acid (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7.10±0.21</td>
<td>49.81±5.07</td>
<td>6.55±2.33</td>
</tr>
<tr>
<td>RO</td>
<td>7.59±0.411</td>
<td>46.93±4.907</td>
<td>5.23±3.18</td>
</tr>
<tr>
<td>Ni</td>
<td>9.08±0.3232</td>
<td>66.55±2.333</td>
<td>69.50±4.576</td>
</tr>
<tr>
<td>Ni-RO</td>
<td>7.40±0.3444</td>
<td>42.64±4.964</td>
<td>56.87±4.642</td>
</tr>
</tbody>
</table>

Table 2 Concentrations of kidneys urea, creatinine, and uric acid of control rats, treated with rosemary (RO), nickel (Ni), and rosemary with nickel (RO-Ni) after 28 days of treatment.

**Histopathology study**

Renal tissue for rats treated with 10 mg/kg bw of NiCl2, shows severe acute tubular necrosis and enlarged glomeruli cells showing a reduction in Bowman space. The convoluted distal and proximal tubules are dilated with a flattened epithelial lining (Figure 3C). The kidneys of rats treated with a combination of Rosmarinus officinalis extract and NiCl2 (RO-Ni) (figure 3D) show a similar appearance to the control (Figure 3A).

**DISCUSSION**

The kidney is recognized as the most targeted organ by xenobiotics (Mohammadi and Ahmedizedah 2018). Many drugs, chemicals, and heavy metals have been shown to change their structure and function (Kim et al., 2019). Animal testing and human studies are convincing in terms of adverse metabolic effects and nephrotoxicity of nickel compounds (Salimi et al., 2020). Rosmarinus officinalis is an aromatic plant used heavily in Mediterranean places and contains a strong antioxidant power against free radicals induced by oxidizing stress that causes tissue damage. The production of free radicals in the body make different disorders, such as the inactivation of the enzymatic defense system, and the degradation of proteins (Demirci-Čekić, 2022; Gonzalez et al., 2020).

Urea, creatinine, and uric acid are markers of renal function, produced by cell metabolism, and excreted by the kidneys. Kidney failure due to an alteration of the kidney’s functions makes the kidneys incapable of excreting these products by causing their increase in the blood (Iqbal et al., 2020 Akinwumi et al., 2020). So the increase in these markers in the blood in the group of rats treated by nickel results from a nephrototoxic effect of this metal. Previous research on the nephrotoxicity of Ni in mice and rats has shown similar increase in urea and creatinine (Akinwumi et al., 2020, Kadi et Dah Douh, 2016). This nephrotoxicity was studied by Shang et al., 2019, who worked on nanoparticles of nickel (NiNPs), and found a high serum creatinine. This hypercreatininemia is due to an accumulation of NiNPs in the kidneys leading to a decrease in glomerular filtration and therefore renal failure (Tiwari et al., 2017; Gasmi, 2018; Shang et al., 2019; Khan et al., 2022; Singh et al., 2022).

The administration of the rosemary extract in the group treated by nickel (RO-Ni), has protected the renal tissue from the damage which can be caused by Ni, this effect is proven by normal renal function and the study of Histological kidneys. These results confirm those obtained by many researchers (El-Demerdech et al., 2021; Gasmi, 2020; Vos et al., 2013). These preventive effects of rosemary extracts (Rosmarinus officinalis) could be due to their antioxidant power (Benkhedir et al., 2022). Nickel has caused a reduction in antioxidant markers (GSH, GPX, CAT, SOD), associated with an induction of the GST enzyme and lipid peroxidation (MDA).

Reduced glutathione (GSH) plays an essential role in maintaining the integrity of cells thanks to their reductive properties and their participation in cell metabolism. While the antioxidant enzymes GPX, CAT, GST, and SOD, are essential enzymes for the preservation of the functions of normal cells. The sod catalyzes the conversion of the anion superoxide into O2 and H2O, the latter breaks down into H2O by the Catalase (Salah et al., 2022; Dahmen et al., 2019; Guo et al., 2021; Dahmane et al., 2016). While GPX uses GSH to reduce hydroperoxides and protect cell membranes (El-Demerdech et al., 2021, Kalaiselvi et al., 2013). While GST is a detoxification enzyme (Ghosh et al., 2012). The decrease in enzyme activity; GPX, CAT, and SOD could be used as an indicator of toxicity by heavy metals (Salah et al., 2022; Nehru et Anand, 2005). The increase in GST activity is an adaptive response against oxidizing stress induced by nickel (Kazeem et al., 2020). The Rosmarinus officinalis (RO) plays a fundamental role in trapping free radicals and protecting cells from oxidizing stress (Hegazy et al., 2016, Benkedir et al., 2022, Eldemerdesh 2021).

Benkhedir et al., (2022), Eldemerdesh (2021), and Abdel-Gawad et al., (2020), reported that the components of RO extracts are capable of inhibiting lipid peroxidation, the generation of free radicals, and preserving renal function. In addition, that RO increases the activity of GPX, CAT, and SOD enzymes with a reduction in MDA in diabetic subjects. According to McMahon and Waikar (2013), and Wright et al., (1998), the increase in GST activity is associated with damage to the distal and proximal tubules in animal models treated with heavy metals.
The histological study of the kidneys shows tubular and glomerular necrosis in the group treated with 10 mg/kg bw of nickel. These histological modifications reflect the alterations of renal function. These histological disturbances are confirmed by the increase in serum urea and creatinine which are indicators of renal functioning and their increase means damage to the function of nephrons (Mahlmoudi et al., 2015).

The RO administration in rats treated with nickel (RO-Ni), has protected renal cells from damage that can be caused by nickel, which results in a normal histological section. Our results are in accordance with those found by Akinwumi et al., (2020) on the effect of Nickel on the toxicity of NCI1, and with those of El-Demerdech et al. (2021), on the effect of RO on the Chrome toxicity (CrCl).

CONCLUSION

According to this in vivo study, we can conclude on the one hand the injection of rats by nickel has induced nephrotoxicity which results in lipid peroxidation and a depletion of the enzymatic and not enzymatic antioxidant defence, degeneration of tubular cells and glomerular. On the other hand, the administration of the extract of Rosmarinus officinalis protected the renal cells from radical damage to nickel, because of their richness in phenolic compounds.

Conflict of interest: All authors declare no conflict of interest.

REFERENCES


Khan, I., Bilal, A., Shakeel, K., & Malik, F. T. (2022). Effects of Nickel Toxicity on the toxicity of NiCl2 on the effect of Dunaliella sp., lipid extract rich in chlorophyll a and b and their increase means damage to the function of nephrons

Lafort, J., Khalil, N., & El-Feki, A. (2016). Protective effect of Dunaliella sp., lipid extract rich in chlorophyll a and b and their increase means damage to the function of nephrons


