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

Hichem Saker[1], *Samira Boussekine*[1], *Salim Gasmi*[2*], *Abdkarim Benkhedir*[1], *Yasmine Benali*[3], *Chawki Bensouici*[4]

Address(es): Dr Salim Gasmi, PhD Cell Toxicology.
[1] Laboratory of Bioactive Molecules and Applications, Department of Applied Biology, Faculty of Exact Sciences and Natural and Life Sciences, Echahid Cheikh Larbi Tebessi University, Tebessa, 12400, Algeria.
[2] Department of Applied Biology, Echahid Cheikh Larbi Tebessi University, Tebessa, 12400, Algeria.
[3] Laboratory of Veterinary Pathological Anatomy and Cytology Laboratory, Veterinary Pathology Microbiology Department, Pasteur Institute Algiers, Little Staouéli road, Dely-Brahim, Algiers, 16000, Algeria.
[4] Biotechnology Research Center, Ali Mendjli, New City UV 03, BP E73 Constantine, 25000, Algeria.

*Corresponding author: Salim.gasmi@univ-tebessa.dz

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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 ($NiCl_2$). 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 $NiCl_2$, and the last was treated with the combination of the extract and $NiCl_2$ 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 $NiCl_2$ 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 $NiCl_2$) restored most of the parameters cited above to values close to normal. Therefore, the present study revealed the ability of RO to bind to $NiCl_2$ and protect kidney tissue from $NiCl_2$-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 oxidation 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 represented by 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-Demerdache et *al*., 2021; Njoya, 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, et Williams, 2011**). Several studies have shown that rosemary is antibacterial **(Kloy et *al*., 2020)**, anti-inflammatory (**Beninca 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 ($NiCl_2$) nephrotoxicity in male rats.

MATERIAL AND METHODS

Chemicals

ost of the chemicals used in this study (Nickel chloride [$NiCl_2$; 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 -4C°.

In vitro study on *Rosmarinus officinalis* extract

Assay chelating metal

The Chelate Metal activity is determined by the ferrozine formation complex (Fe^{2+}), 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 (Fe^{2+}) was calculated according to the

formula: **Fe^{2+} [%]** *chelating effect* **= [(As-Ac)/Ac] ×100** *(As Absorbance of extract solutions and Ac: Absorbance of the White control).*

Cupric-Reducing Antioxidant Capacities (CRAC) were determined by the method of **Apak et al., 2004**. Briefly, 50 µl of neocupronin in methanol, 60 µl of ammonium acetate solution (CH_3COONH_4), and 50 µl of ($CuCl_2$, $2H_2O$) were mixed with 40 µl of RO extract. Absorbance was measured at 450 nm against a reactive control after 1 hour of incubation time. Results were presented in mM Trolox/kg dry weight of the plant through the Trolox calibration curve.

In vivo study of *Rosmarinus officinalis* extract

Treatment of animals

Twenty-eight (28) Wistar Albinos 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 $NiCl_2$) (Iqbal. et al.,2020).*
- *Mixture group (Ni-Ro): (treated with the association of $NiCl_2$: 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, use 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 Spireact 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

The activity of GPx is measured by the method of **Flohe and Gunzler (1984).** The SOD assay is evaluated using the **Beauchamp and Fridovich (1971)** methods. The GST activity determined by the method of **Habig et *al*. (1974)**. The activity of CAT is performed using the **Cakmak and Horst (1991)** method.

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 ($p \leq 0.05$). 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 *Rosmarinu sofficinalis* extract (comparison with BHT, BHA, EDTA, and α tocophérol).

	CUPRAC (IC_{50} µg /ml)	Metal chelating (IC_{50} µg /ml)
RO	15.08±0.85[a,b,c]	63.56±0.97[d]
BHT	9.62±0.09	NT
BHA	3.64±0.11	NT
EDTA	NT	96.68±1.04
α -Tocophérol	20.26±0.06	NT

IC50: expressed as means ± SD (n=3), minimal significant level; P<0,05, significantly difference; [a] in respect to RO vs BHA (butylated hydroxyl-anisole); [b] in respect to RO vs BHT (butylated hydroxy toluene); [C] in respect to RO vs α –Tocopherol; [d] in respect to RO vs EDTA (etheylenediaminetetraacetic acid); NT: no treated

Oxidative stress parameters

Based on the results shown in Figure1, a single dose of $NiCl_2$ 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 $NiCl_2$) 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.

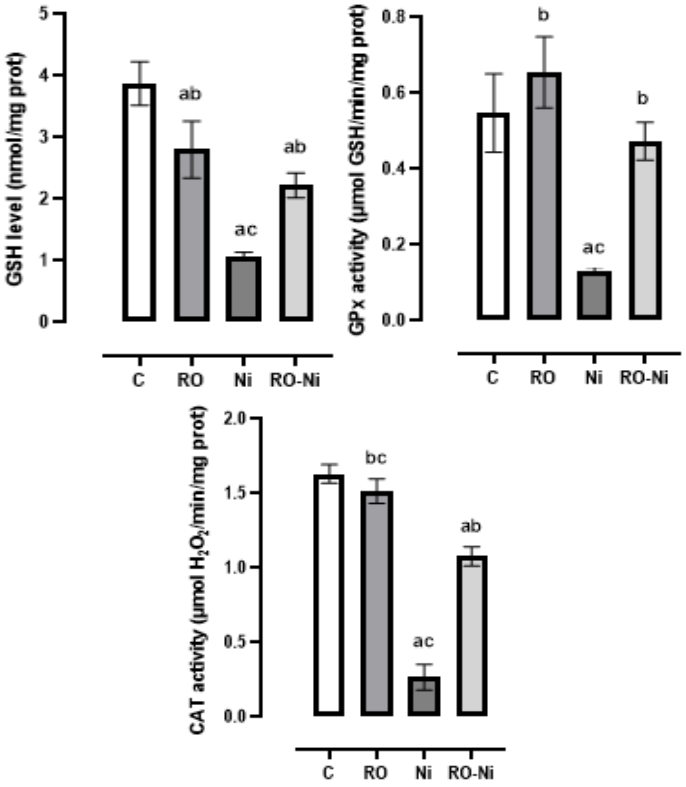


Figure 1 Kidneys GSH content, GPx, and CAT activities of control rats treated with Rosmarinus officinalis (RO), nickel (Ni), and Rosemary plus nickel (RO-Ni) after 28 days of treatment. Values are given as mean± SEM, seven rats in each group. A statistically significant difference ($p<0.05$): compared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

Catalase activity in the kidneys of rats treated with RO alone was not significantly different from those of the control. Following the treatment of rats with Nickel, we obtained a very highly significant increase ($p< 0.001$) in the level of MDA (figure 2), in the kidneys compared to the control group and a significant increase ($p<0.05$) recorded in RO-Ni group and a non-significant decrease recorded in the RO group.

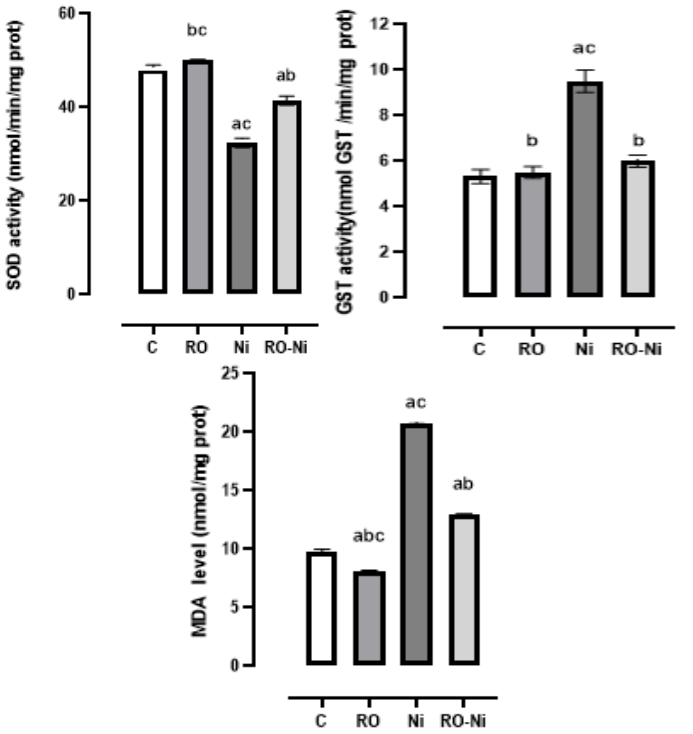


Figure 2 Kidneys SOD, GST activities, and MDA content of control rats treated with Rosmarinus officinalis (RO), nickel (Ni), and Rosemary plus nickel (RO-Ni) after 28 days of treatment. Values are given as mean± SEM, seven rats in each group. A statistically significant difference ($p<0.05$): compared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

Blood biochemical assaya

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 $NiCl_2$ group with RO significantly reduced ($p<0.05$) the concentrations of these metabolites compared to the group treated with nickel alone.

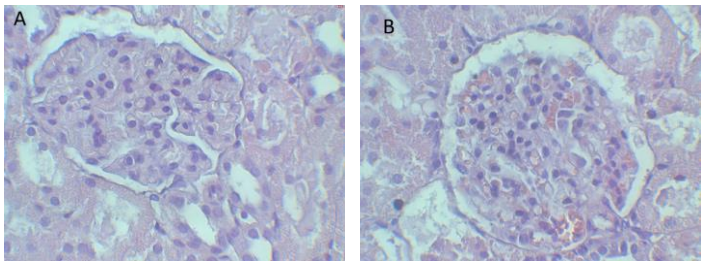
Table 2 Concentrations of kidneys urea, creatinine, and uric acid of control rats, treated with rosemary (RO), nickel (Ni), and rosemary with nickel (RO-Ni) after 28 days of treatment.

Parameters	Urea (mg/dL)	Creatinine (mg/dL)	Uric Acid (mg/dL)
Control	7.10±0.21	49.81±5.07	50.12±4.96
RO	$7.59±0.41^{C}$	$46.93±4.90^{C}$	$52.38±3.18^{C}$
Ni	$9.08±0.32^{a,b,d}$	$66.55±2.33^{a,b,d}$	$69.50±4.57^{a,b,d}$
Ni-RO	$7.40±0.34^{C}$	$42.64±4.96^{C}$	$56.87±4.64^{C}$

a: Comparison with the control group, b: Comparison with the RO group, c: Comparison with the Ni group, d: Comparison with the RO-NI combination group. Significant $p\leq0.05$.

Histopathology study

Renal tissue for rats treated with 10 mg/kg bw of $NiCl_2$, 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 $NiCl_2$ (RO-Ni) (figure 3D) show a similar appearance to the control (Figure 3A).



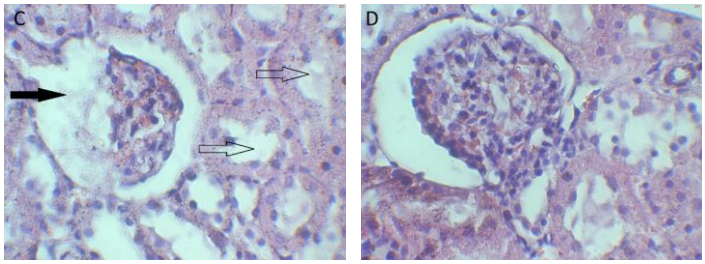


Figure 3 Sections of the kidney of rats exposed to RO and Ni (H&E, x400). **(A)** Control group with normal histoarchitecture of glomeruli. **(B)** RO-administered group showing normal renal histological picture similar to the controls; **(C)** Ni alone treated group showing severe tubular and glomerular necrosis; **(D)** rats treated with a mixture of RO and $NiCl_2$ (RO-Ni) showing restoration in the histological appearance with a marked reduction in Ni alone induced renal damage. Arrows indicate: ⟹ necrosis in epithelial cells of the proximal tubules and ➡ glomerular necrosis.

The results clearly indicate that kidney tissues that were damaged by the toxicity of Nickel, *Rosmarinus officinalis* treatment had significant protection against Nickel poisoning (Figure 3B).

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-Çekiç, 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 nephrotoxic effect of this metal. Previous research on the nephrotoxicity of Ni in mice and rats has shown a 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 hyper creatininemia 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; Yosr 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 O_2 and H_2O_2, the latter breaks down into H_2O 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, Benkhedir et *al*., 2022, Eldemerdech 2021)**.

Benkhedir et al., (2022), **Eldemerdech (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 **(Mahmoudi 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 *Nigella sativa* on the toxicity of $NiCl_2$, and with those of **El-Demerdech et *al* (2021)**, on the effect of RO on the Chrome toxicity ($CrCl_2$).

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

Abdel-Gawad. H, Taha. H, Aly Sayed. M.A. (2020) Ameliorative Effect of Rosemary Extract (Rosmarinus Officinalis L.) Against Ethion Bound Residues Induced Alterations in Experimental Animals, Adv Agri Tech Plant Sciences. 3 (1) 180045. Https://doi.org/10.21608/EJCHEM.2021.59950.3282

Adjroud O, Moufok S (2009) Effects of nickel chloride on hematological and developmental parameters in Wistar albino pregnant rats. Ass Univ Bull Environ Res 12(1):1–9. Https://doi.org/10.21608/AUBER.2009.149529

Akinwumi, K. A., Jubril, A. J., Olaniyan, O. O., & Umar, Y. Y. (2020). Ethanol extract of *Nigella sativa* has antioxidant and ameliorative effects against nickel chloride-induced hepato-renal injury in rats. Clinical Phytoscience, 6(1), 1-12. Https://doi.org/10.1186/s40816-020-00205-9

Amudha K, Pari L. (2011). The beneficial role of naringin, a flavonoid on nickel-induced nephrotoxicity in rats. Chem Biol Interact 193:57–64. https://doi.org/10.1016/j.cbi.2011.05.003

Begum, W., Rai, S., Banerjee, S., Bhattacharjee, S., Mondal, M. H., Bhattarai, A., & Saha, B. (2022). A comprehensive review of the sources, essentiality, and toxicological profile of nickel. RSC advances, 12(15), 9139-9153. Https://doi.org/10.1039/D2RA00378C

Beninca. J.P, Dalmarco. J.B, Pizzolatti. M.G, Frode. T.S, (2011) Analysis of the anti-inflammatory properties of Rosmarinus officinalis L in mice, Food Chem. 124 468–475. https://doi.org/10.1016/j.foodchem.2010.06.056.

Benkhedir, B., Boussekine,S., Saker, H., Gasmi, S., Benali, Y. (2022).Beneficial Effects Of Rosmarinus Officinalis And Thymus Numidicus On Key Enzymes Of Carbohydrate Metabolism In Alloxan-Induced Diabetic Rats, Journal of mbfs: Articles in press.

Beauchamp, C., & Fridovich, I. (1971). Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Analytical biochemistry*, *44*(1), 276-287. DOI: 10.1016/0003-2697(71)90370-8.

Dahmen-Ben Moussa, I., Bellassoued, K., Athmouni, K., Naifar, M., Chtourou, H., Ayadi, H., Dhouib, A. (2016). Protective effect of Dunaliella sp., lipid extract rich in polyunsaturated fatty acids, on the hepatic and renal toxicity induced by nickel in rats. Toxicology Mechanisms and Methods, 26(3), 221-230. Https://doi.org/10.3109/15376516.2016.1158340 .

Dahmen-Ben Moussa, I., Bellassoued, K., El Feki, A., Ayadi, H., &Dhouib, A. (2019). Effects of Dunaliella sp.-extract in reducing the toxicity of nickel in meat, liver, and kidneys in rabbits. Toxicology and Environmental Health Sciences, 11(3), 226-236. https://doi.org/10.1007/s13530-019-0408-2

Decker, E. A., & Welch, B. (1990). Role of ferritin as a lipid oxidation catalyst in muscle food. *Journal of Agricultural and food Chemistry*, *38*(3), 674-677 Https://doi.org/10.1021/jf00093a019

Demirci-Çekiç, S., Özkan, G., Avan, A. N., Uzunboy, S., Çapanoğlu, E., &Apak, R. (2022). Biomarkers of oxidative stress and antioxidant defense. Journal of pharmaceutical and biomedical analysis, 209, 114477. https://doi.org/10.1016/j.jpba.2021.114477

El-Demerdash Fatma M, Raghda A. El-Sayed, Mohamed M. Abdel-Daim (2021). Rosmarinus officinalis essential oil modulates renal toxicity and oxidative stress induced by potassium dichromate in rats. Journal of Trace Elements in Medicine and Biology. Https://doi.org/10.1016/j.jtemb.2021.126791 .

Ellman GL (1959) Tissue sulfhydryl groups. Arch Biochem Biophys 82(1):70–77 33. Jollow DJ,

Esterbauer, H., Gebicki, J., Puhl, H., & Jürgens, G. (1992). The role of lipid peroxidation and antioxidants in oxidative modification of LDL. *Free Radical Biology and Medicine*, *13*(4), 341-390.

Flohé, L., & Günzler, W. A. (1984). [12] Assays of glutathione peroxidase. In *Methods in enzymology* (Vol. 105, pp. 114-120). Academic Press.

Fu. Y, Zu. Y, Chen. L, Shi. X, Wang. Z, Sun. S, (2007) Antimicrobial activity of clove and rosemary essential oils alone and in combination, Phytother Res. 21 989–994. Https://doi.org/10.1002/ptr.2179

Gasmi, S. 2020. Neurotransmission dysfunction by mixture of pesticides and preventive effects of quercetin on brain, hippocampus and striatum in rats. Toxicol. Environ. Health Sci. 12, 203–212. https://doi.org/10.1007/s13530-020-00012-2

Gasmi S. 2018. Classic Labyrinth Test for Neurobehavioral Evaluation in Wistar Rats. Bioprotocol. https://bio-protocol.org/e3007

Ghosh, T., Mustafa, M. D., Kumar, V., Kumar Datta, S., Bhatia, M. S., Sircar, S., & Banerjee, B. D. (2012). A preliminary study on the influence of glutathione S transferase T1 (GSTT1) as a risk factor for late-onset Alzheimer's disease in the North Indian population. Asian journal of psychiatry, 5(2), 160-163. https://doi.org/10.1016/j.ajp.2012.02.023.

González-Minero, F. J., Bravo-Díaz, L., & Ayala-Gómez, A. (2020). Rosmarinus officinalis L.(Rosemary): An ancient plant with uses in personal healthcare and cosmetics. *Cosmetics*, *7*(4), 77.

Goodman JE, Prueitt RL, Dodge DG, Thakali S (2009) Carcinogenicity assessment of water-soluble nickel compounds. Crit Rev Toxicol 39(5):365–417. Https://doi.org/10.1080/10408440902762777

Guo, H., Yin, H., Zuo, Z., Yang, Z., Yang, Y., Wei, L., ... & Fang, J. (2021). Oxidative stress-mediated apoptosis and autophagy were involved in induced nephrotoxicity in the mice. Ecotoxicology and Environmental Safety, 228, 112954. https://doi.org/10.1016/j.ecoenv.2021.112954

Habig, W. H., Pabst, M. J., Fleischner, G., Gatmaitan, Z., Arias, I. M., & Jakoby, W. B. (1974). The identity of glutathione S-transferase B with ligandin, a major binding protein of liver. *Proceedings of the National Academy of Sciences*, *71*(10), 3879-3882.

Hegazy R, Salama A, Mansour D, Hassan A (2016) Renoprotective Effect of Lactoferrin against Chromium-Induced Acute Kidney Injury in Rats: Involvement of IL-18 and IGF-1 Inhibition. Plos ONE 11(3) : e0151486. Https://doi.org/10.1371/journal.pone.0151486

Ijomone, O. M., Olatunji, S. Y., Owolabi, J. O., Naicker, T., & Aschner, M. (2018). Nickel-induced neurodegeneration in the hippocampus, striatum and cortex; an ultrastructural insight, and the role of caspase-3 and α-synuclein. *Journal of Trace Elements in Medicine and Biology*, *50*, 16-23.

Iqbal, S., Jabeen, F., Peng, C., Ijaz, M. U., & Chaudhry, A. S. (2020). Cinnamomum cassia ameliorates Ni-NPs-induced liver and kidney damage in male Sprague Dawley rats. *Human & Experimental Toxicology*, *39*(11), 1565-1581.

Jollow, D. J., Mitchell, J. R., Zampaglione, N. A., & Gillette, J. R. (1974). Bromobenzene-induced liver necrosis. Protective role of glutathione and evidence for 3, 4-bromobenzene oxide as the hepatotoxic metabolite. *Pharmacology*, *11*(3), 151-169.

Kadi and, I. E., & Dahdouh, F. (2016). Vitamin C pretreatment protects from nickel-induced acute nephrotoxicity in mice. Arhiv za higijenu rada i toksikologiju, 67(3), 210-215. Https://doi.org/10.1515/aiht-2016-67-2753

Kalaiselvi, M., Gomathi, D., Ravikumar, G., Devaki, K., & Uma, C. (2013). Ameliorative effect of Ananus comosus peel on 7, 12 dimethylbenz (α) anthracene induced mammary carcinogenesis with reference to oxidative stress. Journal of Acute Disease, 2(1), 22-28. https://doi.org/10.1016/S2221-6189(13)60089-X

Kayashima, T., Nagao, K., Umino, M., Kaikiri, H., Shibata, S., & Matsubara, K. (2020). Anti-stress effects of rosemary (Rosmarinus officinalis L.) leaf extract on intestinal goblet cells and immobility of forced-swimming test in BALB/c mice. *Bioscience, Biotechnology, and Biochemistry*, *84*(11), 2385-2389

Khan, I., Bilal, A., Shakeel, K., & Malik, F. T. (2022). Effects of Nickel Toxicity on Various Organs of The Swiss albino Mice. Uttar Pradesh Journal of Zoology, 43(14), 1-12 ISSN: 0256-971X (P).

Kim, J. J., Kim, Y. S., & Kumar, V. (2019). Heavy metal toxicity: An update of chelating therapeutic strategies. Journal of Trace Elements in Medicine and Biology, 54, 226-231. https://doi.org/10.1016/j.jtemb.2019.05.003

Kloy. A, Ahmad. J, Yusuf. U, Muhammad. M, Antibacterial Properties of Rosemary (Rosmarinus Officinalis), South Asian Res J Pharm Sci 2 (2020), https://doi.org/10.36346/sarjps.2020.v02i01.002

Lushchak VI (2011) Adaptive response to oxidative stress: bacteria, fungi, plants, and animals. Comp Biochem Physiol C Toxicol Pharmacol 153(2):175–190. Https://doi.org/10.1016/j.cbpc.2010.10.004

Mahmoudi A, Ghorbel H, bouallegui Z, (2015). Oleuropein and hydroxytyrosol protect from bisphenol A effects in the livers and kidneys of lactating mother rats and their pups'. Exp Toxicol Pathol 67:7–8.

Mcmahon GM, Waikar SS (2013). Biomarkers in nephrology. Am J Kidney Dis. 62(1):165–78. Https://doi.org/10.1053/j.ajkd.2012.12.022

Mohammadi, A., & Ahmadizadeh, M. (2018). Effects of antioxidants on xenobiotics-induced nephrotoxicity. Journal of Renal Injury Prevention, 7(2), 56-57. Https://doi.org/10.15171/jrip.2018.14

Naimi.M, Vlavcheski. F, Shamshoum. H, Tsiani. E, (2017) Rosemary Extract as a Potential Anti-Hyperglycemic Agent: Current Evidence and Future Perspectives, Nutrients. 9 968, https://doi.org/10.3390/nu9090968

Nehru, B., & Anand, P. (2005). Oxidative damage following chronic aluminum exposure in adult and pup rat brains. Journal of Trace Elements in Medicine and Biology, 19(2-3), 203-208. https://doi.org/10.1016/j.jtemb.2005.09.004

Ngo. S.N, Williams. D.B, Head. R.J, (2011) Rosemary and cancer prevention: preclinical perspectives, Crit. Rev. Food Sci. Nutr 51 946–954. Https://doi.org/10.1080/10408398.2010.490883

Njoya, E. M. (2021). Medicinal plants, antioxidant potential, and cancer. In Cancer (pp. 349-357). Academic Press.https://doi.org/10.1016/B978-0-12-819547-5.00031-6

Pari L, Prasath A (2008) Efcacy of caffein acid in preventing nickel induced oxidative damage in the liver of rats. Chem Biol Interact 173(2):77–83. Https://doi.org/10.1016/j.cbi.2008.02.010

Rajgopal. A, Roloff. S.J, Burns. C.R., Fast. D.J, (2019) The cytoprotective benefits of turmeric, quercetin, and rosemary blend through activation of the oxidative stress pathway, Phcog Mag 15 449–454. Https://doi.org/10.4103/pm.pm_556_18

Salah, I., Adjroud, O., &Elwej, A. (2021). Protective effects of selenium and zinc against nickel chloride–induced hormonal changes and oxidative damage in the thyroid of pregnant rats. Biological Trace Element Research, 200(5), 2183-2194. https://doi.org/10.1007/s12011-021-02815-x

Salimi, A., Jamali, Z., Atashbar, S., Khezri, S., Ghorbanpour, A. M., &Etefaghi, N. (2020). Pathogenic mechanisms and therapeutic implication in nickel-induced cell damage. Endocrine, Metabolic & Immune Disorders-Drug Targets (Formerly Current Drug Targets-Immune, Endocrine & Metabolic Disorders), 20(7), 968-984. Https://doi.org/10.2174/1871530320666200214123118

Shang Ziyad Abdulqadir and Falah Mohammad Aziz (2019). Nickel Nanoparticles Induced Nephrotoxicity in Rats: Influence of Particle Size. Pakistan Veterinary Journal. Https://doi.org/10.29261/pakvetj/2019.106 .

Singh, M., Verma, Y., & Rana, S. V. S. (2022). Nephrotoxicity of nickel nano and microparticles in the rat-a comparative, time-dependent study with special reference to the antioxidant defense system. Inorganic and Nano-Metal Chemistry, 1-10 https://doi.org/10.1080/24701556.2022.2048307

Tiwari R, Singh R.D, Khan H, (2017) Oral subchronic exposure to silver nanoparticles causes renal damage through apoptotic impairment and necrotic cell death. Nanotoxicol 11:671-86. Https://doi.org/10.1080/17435390.2017.1343874

Wright, L. S., Kornguth, S. E., Oberley, T. D., & Siegel, F. L. (1998). Effects of Lead on Glutathione S-Transferase Expression in Rat Kidney: A Dose—Response Study. Toxicological sciences, 46(2), 254-259. Https://doi.org/10.1006/toxs.1998.2543

Xu S, He M, Zhong M (2015) The neuroprotective effects of taurine against nickel by reducing oxidative stress and maintaining mitochondrial function in cortical neurons. Neurosci Lett 590:52–57. Https://doi.org/10.1016/j.neulet.2015.01.065

Yosr Z, Hnia C, Rim T, Mohamed B (2013). Changes in essential oil composition and phenolic fraction in Rosmarinus officinalisl. Var. Typicus Batt. Organs during growth and incidence on the antioxidant activity, Ind Cro Prod 43 412–419. https://doi.org/10.1016/j.indcrop.2012.07.044

Zhang H and Sun S, 2015. NF-kb in inflammation and renal diseases. Cell & Biosciences. 5:63-72. Https://doi.org/10.1186/s13578-015-0056-4