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RESEARCH ARTICLE

Protective Effect of *Angelica sinensis* Extract and *Origanum majorana* Oil on Hepatic and Renal Toxicities Induced by Nickel Chloride in Male Albino Rats

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Abstract

The present study was accomplished to evaluate the efficacy of *Angelica sinensis* root extract and *Origanum majorana* oil on subchronic toxicity of nickel chloride ($NiCl_2$) in albino rats by studying the changes in the biochemical parameters, lipid peroxidation and antioxidant status related to the liver and kidneys. This study was carried out on sixty male albino rats with average body weight (120 ± 10 g), which were divided into 6 equal groups. Gp.(1): were kept as control on distilled water, rats in Gp. (2) received $NiCl_2$ (0.75 mg/kg BW), Gp. (3) received *A. sinensis* root extract (300 mg/kg BW) and Gp. (4) received *O. majorana* oil (0.5 ml/kg BW). $NiCl_2$ intoxicated rats in Gp. (5) and Gp. (6) were administered *A. sinensis* root extract and *O. majorana* oil at the same former doses, respectively. The treatments were daily and orally for 4 weeks. Blood and tissue samples were collected for performing the different experimental analysis after 4 weeks from starting the treatments. Comparing with the control group, results showed that $NiCl_2$ significantly increased serum alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase activities and serum bilirubin, triglycerides, total cholesterol, urea and creatinine concentrations, besides hepatic and renal malondialdehyde concentration, while serum total proteins, albumin and hepatic and renal glutathione, glutathione peroxidase, superoxide dismutase and catalase levels were significantly decreased. *A. sinensis* root extract and *O. majorana* oil has no adverse effects and worked on amelioration the various alterations in estimated analytes in combined treated groups. In conclusion, $NiCl_2$ caused hepatic and renal injuries besides the induction of lipid peroxidation and reducing the antioxidant enzymes activities in these organs. Moreover, both of *A. sinensis* root extract and *O. majorana* oil provided significant protection against harmful effects of $NiCl_2$ with superiorty of *A. sinensis* root extract .

Keywords: *Angelica sinensis extract*, Antioxidants, Nickel chloride toxicity, *Origanum majorana oil*..

Introduction

Nickel (Ni) is a nutritionally essential trace element as well as one of heavy metals. The exposure to excess or few amount of it may produce toxicity or deficiency symptoms [1,2]. Human beings and animals expose to nickel mainly through food, drinking water and inhalation. The food is the most important pathway among these pathways. Nickel compounds can enter the food chain and may have a toxic effect on living organisms [3]. Nickel chloride ($NiCl_2$) is a water-soluble Ni salt, which being more readily absorbed according the way in which it is consumed [4]. A number of studies showed various hepatic and renal toxicity pictures besides hematological adverse effects following the exposure to $NiCl_2$ [5,6].

Nowadays, there is an increasing interest in the using of medicinal plants globally due to growing recognition of natural products and the public direction towards the "back to nature" slogan, which might be safer without adverse effects [7]. *Angelica sinensis* is one of the important medicinal plants. Their root has been used for long years in traditional Far East medicine and is highly recommended for

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several disease conditions. Recently, the biomacromolecules of the *A. sinensis* root attracted the attention for its various bioactivities, such as hepatoprotective, hematopoietic, immunomodulatory and antioxidant activities [8, 9, 10, 11].

Origanum majorana is publicly known as sweet marjoram. It is an aromatic medicinal plant. Wide extent of pharmacological activities including hepatoprotective, cardioprotective, antioxidant, anti-inflammatory and antimicrobial activities has been recorded for this plant and its oil recently in the medical field [12]. The purpose of the current study was to estimate the efficacy of *A. sinensis* root extract and *O. majorana* oil on $NiCl_2$ toxicity in male albino rats. The evaluation was done by studying changes in the biochemical parameters, lipid peroxidation and antioxidant status related to the liver and kidneys.

Materials and Methods

Experimental animals

Sixty male albino rats at the age of two months old, weighted 120 ± 10 g and in a good health condition were purchased from the Laboratory Animal Unit, Faculty of Veterinary Medicine, Zagazig University, Egypt. The rats were housed in the prepared metal cages under perfect hygienic conditions, given balanced feed with water *ad-libitum* and observed for 7 days before starting the experimental procedures.

The protocol of this study was in accordance with the ethical regulations for the protection of animals' welfare, which followed by the Faculty of Veterinary Medicine, Zagazig University, Egypt.

Chemical

Nickel chloride (CAS Number: 7718-54-9) was obtained from El-Gomhoria Company, Zagazig, Sharkia Governorate, Egypt. It is yellow-orange powder and was dissolved in distilled water.

Medicinal plants

Angelica sinensis (root) was obtained from Saleh el Attar Sons Company, Al-Azhar, Cairo, Egypt. Plant root was scientifically identified by the Department of Pharmacognosy, Faculty of Pharmacy, Zagazig University Zagazig, Sharkia Governorate, Egypt. *Origanum majorana* oil was obtained from Sekem Company, Egypt. It is dark yellow liquid with sweet and calming odor.

Angelica sinensis processing

Crude parts of *A. sinensis* dry root (600 g) were crushed and extracted 3 times with 95% (v/v) ethanol for 8 h then the supernatant was collected and filtered by using of filter paper, after that was concentrated through rotary evaporation in vacuum at 40°C for ethanol removal. The residue was lyophilized to remove the water. The *A. sinensis* extract (51g) was obtained and stored in a closed glass bottle for daily administration at 4°C. Finally, the extract was prepared in 100 mg/ml with using distilled water according to proper dosage [13].

Experimental design

Sixty rats were divided randomly into six groups (n=10) as the following:

Gp. (1): control group was kept on distilled water , Rats in Gp. (2) received $NiCl_2$ daily at a dose of 0.75 mg/kg BW on the basis of LD_{50} which equal to 105 mg/kg (used dose equal to $LD_{50}/140$) as a 0.5 ml/ rat [14,15]. Gp. (3): received *A. sinensis* root extract daily (300 mg/kg BW) [13], Gp. (4) were given *O. majorana* oil daily (0.5 ml/kg BW) [16]. $NiCl_2$ intoxicated rats in Gp. (5) and Gp. (6) Gp. (5) were administered *A. sinensis* root extract and *O. majorana* oil at the same former doses, respectively. All treatments were given orally by esophageal tube for 4 weeks. All rats were weighed weekly to adjust dose volumes and observed for recording of any clinical signs daily.

Sampling

Blood samples (n=10/ group) were collected after 4 weeks from starting the treatments from the retro-orbital venous plexus of anesthetized animals. Each sample was

about 2 ml and was put in a test tube (sterile, labelled and without anticoagulant) for serum separation to be used for performing the different biochemical tests.

Tissue specimens: Pieces of hepatic and renal tissues (n=10/ group) were removed, then weighed (0.5 g), washed out with ice-cold saline to remove the blood and homogenized in cold 50 mM phosphate buffer. Tissue homogenate was centrifuged for approximately 15 min at 5000 rpm to remove the debris. Finally, the pellets were discarded and supernatants were collected for estimation of lipid peroxidation level and antioxidants status.

Biochemical and antioxidant studies

Serum was used to determine alanine aminotransferase (ALT) [17], aspartate aminotransferase (AST) and alkaline phosphatase (ALP) activities [18], total and direct bilirubin [19], total proteins [20], albumin [21], total cholesterol (TC) [22], triglycerides (TGs) [23], urea [20] and creatinine [24] concentrations. Furthermore, hepatic and renal malondialdehyde (MDA) [25] and glutathione (GSH) [26] concentrations and glutathione peroxidase (GPx) [27], superoxide dismutase (SOD) [28] and catalase (CAT) [29] activities were detected in the tissue samples. Indirect bilirubin concentration was estimated by subtracting value of direct bilirubin from total bilirubin as well as globulins concentration was estimated by subtracting value of albumin from total proteins [30].

Statistical analysis

The data of this study were analyzed by using SPSS software. One-way of variance (ANOVA) is significant at $\mathbf{P} \leq 0.05$ [31]. The differences among the groups were calculated by Duncan's multiple range test. Means in the same column followed by different letters were significantly different and the highest value was represented by the letter (a).

Results and Discussion

In the present study, the rats received $NiCl_2$ for 4 weeks (gp.2) showed some clinical signs such as dullness, weakness, lethargy, ataxia and salivation with no mortality. These clinical signs were possibly associated with overall subchronic toxicity of $NiCl_2$ which is a one of water-soluble nickel compounds that have been shown to be more toxic than the less soluble ones. On the other hand, $NiCl_2$ has no significant retention and cumulative effect in the body and this may be the reason for no mortality in the present study besides the low used dose rate of $NiCl_2$ [15,32].

Concerning the changes in liver enzymes' activities and bilirubin fractions concentration, the rats in gp. (2) showed a significant increase in the hepatic enzymes activities (ALT and AST) in comparison with the control group (Table 1). The hepatic injury which occurred in gp. (2) may be due to that the nickel is a transition metal which induce formation of reactive oxygen species (ROS) and increase the level of lipid peroxidation in the cells. Intermediate products of lipid peroxidation and free radicals are capable of inducing deleterious effects on the cell membrane and releasing of liver cystol enzymes (ALT &AST) into the bloodstream [33,34]. The significant increase in serum ALP activity following the administration of $NiCl_2$ in comparison with the control group may indicate the harm to one of the organs richen with ALP isoforms such as liver (bile canaliculi), kidney (proximal tubules), bone and intestine. Hepato-biliary disorders and cholestatic liver condition may be the main cause of the increasing activity of ALP enzyme in gp. (2) as it is associated with increase of serum ALT and AST [35,36]. The significant increase in the concentration of serum bilirubin (total, direct and indirect) following the administration of $NiCl_2$ (gp.2) in comparison with the control group indicated the decreasing of the hepatic functional capacity, which in turn leads to decrease the hepatic uptake, conjugation and excretion of bilirubin [35].

Table 1: Some serum liver enzymes and bilirubin concentrations of rats in the different groups at 4th week of the experiment

Parameters / Groups	ALT (U/L)	AST (U/L)	ALP (U/L)	Total bilirubin (mg/dl)	Direct bilirubin (mg/dl)	Indirect bilirubin (mg/dl)
Gp.(1) Control	13.80[d] ±1.06	22.60[d] ±1.50	57.20[d] ±1.28	2.47[d] ±0.02	0.41[d] ±0.02	2.05[d] ±0.02
Gp.(2) $NiCl_2$	68.60[a] ±2.22	59.60[a] ±1.32	97.40[a] ±1.86	4.53[a] ±0.15	0.89[a] ±0.01	3.63[a] ±0.14
Gp.(3) *A. sinensis* extract	13.20[d] ±0.86	21.60[d] ±1.96	55.80[d] ±1.49	2.31[d] ±0.08	0.44[d] ±0.02	1.87[d] ±0.08
Gp.(4) *O. majorana oil*	13.00[d] ±1.22	21.00[d] ±1.58	52.60[d] ±1.07	2.38[d] ±0.06	0.43[d] ±0.01	1.95[d] ±0.07
Gp.(5) $NiCl_2$ + *A. sinensis* extract	41.20[c] ±2.31	35.20[c] ±1.93	68.40[c] ±1.77	3.49[c] ±0.09	0.57[c] ±0.02	2.92[c] ±0.08
Gp.(6) $NiCl_2$+ *O. majorana oil*	55.60[b] ±1.63	46.60[b] ±1.80	87.40[b] ±2.18	4.02[b] ±0.50	0.74[b] ±0.02	3.27[b] ±0.04

Mean values ±SE with different superscripts within the same column are significantly different ($p < 0.05$) and the highest value was represented with the letter a. $NiCl_2$: nickel chloride, ALT:Alanine aminotransferase, AST:Aspartate aminotransferase, ALP:Alkaline phosphatase.

On the other hand, administration of *A. sinensis* root extract (gp.3) and *O. majorana* oil (gp.4) alone did not cause any alterations in these parameters when compared with the control group. This may indicate that these naturally occurring therapeutic agents haven't any hazardous effect on hepatic tissue and its function. Our results related to liver enzymes activities agreed with the same previously obtained by Lim and Kim [37] in rats administered *A. sinensis* root extract and in rats administered *O. majorana* oil [7,38].

However, the co-administration of *A. sinensis* root extract to $NiCl_2$ intoxicated rats (gp. 5) significantly decreased serum liver enzymes activities and bilirubin fractions concentration in comparison with the $NiCl_2$-exposed group. These results indicated the ability of *A. sinensis* root extract to protect against $NiCl_2$-induced hepatic tissue injury which related to the prevention of GSH partially depletion in the hepatic tissue as GSH is serving to determine the tissue susceptibility to oxidative damage. Also, the extract has the ability to attenuate the lipid peroxidation and scavenge free radicals induced by $NiCl_2$ [39].

Meanwhile, the coadministration of *O. majorana* oil to $NiCl_2$ intoxicated rats (gp. 6) has the same effect of *A. sinensis* root extract on serum liver enzymes activities and bilirubin (total, direct& indirect) concentration, but in a lower degree, which may be due to the possible hepatoprotection mechanisms of *O. majorana* oil, which related to its polyphenolic compounds and the mechanisms express in the forms of preventing lipid peroxidation, the free radical scavenging effect, and improvement of the antioxidant/detoxification system in liver [7].

Serum proteinogram (Table 2) in this study, revealed significant hypoproteinemia and hypoalbuminemia in the rats which received $NiCl_2$ (gp.2) in comparison with the control group and this perhaps attributed to the reduction in protein synthesis by injured liver, increased loss by damaged kidney and/or raise rate of protein catabolism by nickel element [40,41]. While, non-statistical significant change in the serum globulins concentration in this group may not mean the normal condition, but globulins level may increase under the impact of metal toxicity and in the same time the hepatic impairment may reduce production of some globulin fractions so the net result of this is non-significant change of serum globulins [42,43].

Table 2: Serum protein and lipid profiles of rats in the different groups at 4th week of the experiment

Parameters Groups	Total proteins (g/dl)	Albumin (g/dl)	Globulins (g/dl)	Total cholesterol (mg/dl)	Triglycerides (mg/dl)
Gp.(1) Control	7.70^{a}	5.22^{a}	2.48	170.80^{c}	118.40^{d}
	±0.19	±0.06	±0.02	±2.70	±1.96
Gp.(2) $NiCl_2$	5.09^{d}	3.07^{d}	2.02	221.20^{a}	185.00^{a}
	±0.03	±0.03	±0.43	±2.92	±1.41
Gp.(3) *A. sinensis* extract	7.65^{a}	5.22^{a}	2.43	152.00^{d}	119.40^{d}
	±0.15	±0.04	±0.16	±3.14	±2.99
Gp.(4) *O. majorana* oil	7.59^{a}	5.26^{a}	2.33	154.80^{d}	118.40^{d}
	±0.23	±0.10	±0.17	±1.15	±2.40
Gp.(5) $NiCl_2$ + *A. sinensis* extract	6.49^{b}	4.10^{b}	2.39	170.40^{c}	139.00^{c}
	±0.07	±0.05	±0.10	±2.42	±1.22
Gp.(6) $NiCl_2$+ *O. majorana* oil	5.97^{c}	3.67^{c}	2.30	200.20^{b}	170.80^{b}
	±0.04	±0.07	±0.07	±3.46	±1.77

Mean values ±SE with different superscripts within the same column are significantly different (**p** < 0.05) and the highest value was represented with the letter a. No letters indicate (**p** > 0.05). $NiCl_2$: nickel chloride.

Furthermore, the administration of *A. sinensis* root extract (gp.3) and *O. majorana* oil (gp.4) did not cause any significant changes in the serum total proteins, albumin and globulins concentrations in comparison with the control group which mean that there is no negative effect on the protein synthesis and catabolism after using these natural substances. Concerning to *A. sinensis* root extract, our results which related to total proteins were in agreement with the same previously obtained by Lim and Kim [37].

Moreover, the administration of *A. sinensis* root extract and *O. majorana* oil to $NiCl_2$ intoxicated rats (gps. 5 and 6), respectively, showed improvement in the values of protein profile in comparison with the group exposed to $NiCl_2$ alone and this improvement was more obvious in gp. (5). This may indicate the ameliorative role of active substances in these natural agents on hepatic and renal functions as well as overcoming the toxic effect of $NiCl_2$. Concerning to *O. majorana* oil with $NiCl_2$, our results were in agreement with [16,38].

By monitoring the results of serum lipid profile tests in this study, significant hypercholesterolemia and hypertriglyceridemia were observed in rats of gp.(2) in comparison with the control group (Table 2) such results may be due to the hepatic injury under the impact of $NiCl_2$ toxicity and consequently the defective excretion of cholesterol into bile besides the defects in the LDL-receptor which interfere with cholesterol uptake from the blood stream, as well as the decreasing of hepatic lipase activity, which breaks up the triglycerides and finally may lead to hypertriglyceridemia [44,34].

Moreover, rats administered *A. sinensis* root extract (gp.3) and *O. majorana* oil (gp.4) alone showed significant hypocholesterolemia in comparison with the control group. Such results may be due to the ability of *A. sinensis* root extract to promote the clearance of cholesterol, increase lipoprotein lipase (LPL) activity and decrease serum cholesterol concentration partially through the increase of uptake by LDL receptors [45]. While, the hypocholesterolemic effect of *O. majorana* oil could be attributed to the presence of isoflavones, which prevent the intestinal absorption of cholesterol by competition for its absorption sites [46].

On the other hand, the significant hypocholesterolemia and hypotriglyceridemia were observed after administration of *A. sinensis* root extract and *O. majorana* oil to rats with $NiCl_2$ toxicity in (gps. 5 and 6), respectively in comparison with the group exposed to $NiCl_2$ alone and the modulating effect for natural substances appeared more prominently in gp.(5). These results may be partially due to the different hypolipidemic mechanisms of these natural substances as mentioned above as well as the role of its different active biological substances aiding in reducing the liver injury induced by $NiCl_2$.

Regarding to *O. majorana* oil with $NiCl_2$, our results were in agreement with previous studies [16,38,47] that the co-administration of *O. majorana* oil with different toxic substances reduced the serum cholesterol and/or triglycerides levels in comparison with the intoxicated group.

Regarding to the changes in some kidney function tests, $NiCl_2$ intoxicated rats (gp. 2) showed a significant increase in the concentrations of urea and creatinine in the serum comparatively with the control group as illustrated in Table (3).These results may be due to the nephrotoxic effect of the nickel, as a result of oxidative stress and overproduction of free radicals that can cause renal cell death, and consequently disruption of glomerular filtration rate and a decrease in reabsorption at renal epithelium [48,49].

Table 3: Mean values of serum urea and creatinine of rats in the different groups at 4th week of the experiment

Parameters Groups	Urea (mg/dl)	Creatinine (mg/dl)
Gp.(1) Control	23.26^{d}±1.17	0.79^{d}±0.01
Gp.(2) $NiCl_2$	51.92^{a}±1.07	1.83^{a}±0.03
Gp.(3) *A. sinensis* extract	23.00^{d}±1.18	0.80^{d}±0.03
Gp.(4) *O. majorana oil*	23.54^{d}±1.49	0.81^{d}±0.01
Gp.(5) $NiCl_2$ + *A. sinensis* extract	36.52^{c}±1.25	1.03^{c}±0.02
Gp.(6) $NiCl_2$+ *O. majorana oil*	42.70^{b}±0.76	1.43^{b}±0.02

Means± SE with different superscripts within the same column are significantly different ($p < 0.05$) and the highest value was represented with the letter a. $NiCl_2$: nickel chloride.

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On the other hand, administration of *A. sinensis* root extract (gp.3) and *O. majorana* oil (gp.4) alone did not cause any alterations in the aforementioned renal function parameters. This may indicate that there is no risk on renal tissue and its function from these natural substances and its active principles and this was in line with the previous findings with regard to *A. sinensis* root extract [37].

The administration of *A. sinensis* root extract and *O. majorana* oil to $NiCl_2$ intoxicated rats (gps. 5 and 6), respectively, alleviated renal dysfunction by reducing the serum creatinine and urea levels in comparison with the gp.(2) and the administration of *A. sinensis* root extract to $NiCl_2$ intoxicated rats (gp.5) showed improving effect more prominently than the intoxicated group administered *O. majorana* (gp. 6). These may be related to ferulic acid, the active constituent of *A. sinensis*, which can play a renal protective role via its dual ability to deal with the formation of oxidative protein and retain the antioxidant enzymes expression [50]. However, the renal protective action of *O. majorana* oil could arise from the activities of its minor and major compounds which relief the impairment of the cellular functions via prevention lipid peroxidation and the improvement of the antioxidant status in the renal system [51].

Concerning the results of lipid peroxidation in hepatic and renal tissues as shown in Tables (4 and 5), the rats in gp. (2) which received $NiCl_2$ revealed a significant increase in the hepatic and renal MDA level in comparison with gp.(1) and this possibly due to the potential stimulation of a peroxidative chain reaction under the influence of the nickel (transitional metal). Furthermore, nickel tends to increase iron and copper concentrations in the liver and iron in the kidneys. Perhaps, nickel disturbs homeostatis of these two essential metals resulting in their interorgan relocation and subsequent elevation of its concentrations, which might be expected to further enhance LPO in the hepatic and renal tissues [52, 53].

In this study, the administration of *A. sinensis* root extract and *O. majorana* oil to $NiCl_2$ intoxicated rats (gps. 5 & 6), respectively, decreased significantly the concentration of MDA in hepatic and renal tissues comparing with the $NiCl_2$-exposed group and the ameliorative effect for *A. sinensis* (gp.5) appeared more prominently

than *O. majorana* (gp.6). This result may be related to the ability of active constituents of herbal plants to decline the amount of oxidation and to react with the oxygen metabolites and subsequently decreasing rate of lipid peroxidation [54, 55].

Table 4: Levels of hepatic malondialdehyde and some antioxidant markers of rats in the different groups at 4th week of the experiment

Parameters / Groups	MDA (nmol / g tissue)	GSH (mmol / g tissue)	GPx (U/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Gp.(1) Control	24.22^{d}	4.32^{a}	116.38^{a}	6.78^{a}	3.50^{a}
	±0.76	±0.21	±1.66	±0.49	±0.21
Gp.(2) $NiCl_2$	65.82^{a}	0.90^{c}	50.16^{d}	1.01^{d}	0.84^{d}
	±1.87	±0.01	±0.82	±0.02	±0.02
Gp.(3) *A. sinensis* extract	22.69^{d}	4.41^{a}	117.15^{a}	6.96^{a}	3.49^{a}
	±0.66	±0.22	±1.19	±0.64	±0.17
Gp.(4) *O. majorana oil*	23.04^{d}	4.28^{a}	115.32^{a}	6.65^{a}	3.51^{a}
	±0.89	±0.13	±4.86	±0.15	±0.22
Gp.(5) $NiCl_2$ + *A. sinensis* extract	37.72^{c}	2.53^{b}	87.85^{b}	4.44^{b}	1.91^{b}
	±1.27	±0.14	±3.24	±0.26	±0.03
Gp.(6) $NiCl_2$+ *O. majorana oil*	49.21^{b}	1.31^{c}	66.71^{c}	2.53^{c}	1.33^{c}
	±0.54	±0.01	±2.33	±0.18	±0.04

Mean values ± SE with different superscripts within the same column are significantly different ($p < 0.05$) and the highest value was represented with the letter a. MDA: malondialdehyde, GSH: Glutathione, GPx: Glutathione peroxidase, SOD:Superoxide dismutase, CAT:Catalase and $NiCl_2$: nickel chloride.

Table (5): Renal malondialdehyde level and some antioxidant markers of rats in the different groups at 4th week of the experiment

Parameters / Groups	MDA (nmol/ g tissue)	GSH (mmol/ g tissue)	GPx (U/g tissue)	SOD (U/g tissue)	CAT (U/g tissue)
Gp.(1) Control	12.48^{c}	2.92^{a}	98.52^{a}	4.76^{a}	1.63^{a}
	±0.24	±0.06	±1.15	±0.20	±0.05
Gp.(2) $NiCl_2$	47.61^{a}	0.87^{d}	51.48^{d}	1.18^{d}	0.57^{d}
	±3.51	±0.02	±2.97	±0.05	±0.01
Gp.(3) *A. sinensis* extract	12.98^{c}	2.86^{a}	97.05^{a}	4.73^{a}	1.70^{a}
	±0.57	±0.12	±3.06	±0.15	±0.01
Gp.(4) *O. majorana oil*	12.12^{c}	2.98^{a}	98.76^{a}	4.98^{a}	1.69^{a}
	±0.42	±0.01	±0.62	±0.03	±0.04
Gp.(5) $NiCl_2$ + *A. sinensis* extract	26.63^{b}	1.80^{b}	84.92^{b}	3.12^{b}	0.96^{b}
	±2.14	±0.05	±2.69	±0.09	±0.02
Gp.(6) $NiCl_2$+ *O. majorana oil*	30.71^{b}	1.07^{c}	72.08^{c}	2.11^{c}	0.82^{c}
	±1.65	±0.04	±1.29	±0.07	±0.02

Mean values ± SE with different superscripts within the same column are significantly different (**p** < 0.05) and the highest value was represented with the letter a. $NiCl_2$: nickel chloride, GSH: Glutathione, GPx:Glutathione peroxidase, SOD: Superoxide dismutase and CAT:Catalase .

Evaluation of antioxidant defense system in the hepatic and renal tissues in this study as shown in Tables (4 and 5), declared a significant decline in GSH concentration as well as GPx, SOD and CAT activities in rats with $NiCl_2$ toxicity (gp.2) comparatively with the control gp.(1) and this may be due to the inhibition or consumption of the enzymatic and non enzymatic antioxidants by excess ROS produced after exposure to Ni metal [56].While, the administration of *A. sinensis* root extract and *O. majorana* oil to $NiCl_2$ intoxicated rats (gps. 5 and 6), respectively

showed a significant increase in the previously mentioned antioxidants in comparison with the gp.(2). As well as, *A. sinensis* root extract modulates positively the values of the previously mentioned parameters than *O. majorana* (gp.6). This effect may be due to that *A. sinensis* root extract has the ability to upregulate the gene expression of antioxidative enzymes, and enhancing the activities of these enzymes [55]. Also, *O. majorana* oil contains several active principles which increase antioxidant activities that protect against $NiCl_2$ oxidative stress [57].

Conclusion

Overall from the results of this study, it can be concluded that the exposure to $NiCl_2$ caused hepatic and renal injuries besides the induction of lipid peroxidation and reducing the antioxidant enzymes activities. Administration of *A. sinensis* root extract and *O. majorana* oil provided a significant protection against harmful effects induced by $NiCl_2$, especially *A. sinensis* root extract which has superior ameliorating effect than *O. majorana* oil.

Conflict of interest

The authors have no conflict of interest to declare.

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الملخص العربي

التأثير الوقائي لمستخلص حشيشة الملاك وزيت البردقوش على السمية الكبدية والكلوية الناجمة عن كلوريد النيكل في ذكور الجرذان البيضاء

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تم اجراء هذه الدراسة لتقييم فعالية مستخلص جذر حشيشة الملاك وزيت البردقوش على السمية تحت المزمنة لكلوريد النيكل في الجرذان البيضاء من خلال دراسة التغيرات في قياسات الدم البيوكيميائية ، حالة بيروكسيد الدهون و مضادات الأكسدة المتعلقة بالكبد والكلى. أجريت هذه الدراسة على عدد ستين جرذا ذكور بمتوسط وزن جسم (١٢٠ ± ١٠ جم). تم تقسيمهم إلى ٦ مجموعات متساوية .المجموعة الأولى: مجموعة ضابطة أعطيت الماء المقطر، تلقت الجرذان فى المجموعة الثانية كلوريد النيكل (٠.٧٥ مجم / كجم من وزن الجسم)،المجموعة الثالثة مستخلص جذر حشيشة الملاك (٣٠٠ مجم / كجم من وزن الجسم) ،المجموعة الرابعة: تلقت زيت البردقوش(٠.٥ مل / كجم من وزن الجسم).الجرذان المسممة بكلوريد النيكل فى المجموعة الخامسة والسادسة تم اعطائها مستخلص جذر حشيشة الملاك زيت البردقوش بنفس الجرعات السابقة على التوالى. التجريع كان يوميا عن طريق الفم لمدة ٤ أسابيع. تم تجميع عينات دم وأنسجة لإجراء التحليلات التجريبية المختلفة بعد أربع أسابيع من بدء المعاملات .مقارنة مع المجموعة الضابطة،أظهرت النتائج أن كلوريد النيكل زاد معنويا من نشاط الألانين أمينوترانسفيريز،الأسبرتيت أمينوترانسفيريز و الفوسفاتيز القلوي ومستويات البيليروبين، الدهون الثلاثية ، الكوليسترول الكلي ، اليوريا ، الكرياتينين إلى جانب تركيز المالونديالدهيد الكبدي والكلوي، بينما انخفض معنويا تركيز البروتين الكلي والألبيومين في السيرم والجلوتاثيون، الجلوتاثيون بيروكسيداز ، السوبر اكسيد ديسميوتيزو الكتاليز الكبدي والكلوي. مستخلص جذر حشيشة الملاك وزيت البردقوش ليس لهما أي آثار سلبية وعملا على تحسين الأختلالات المختلفة في التحليلات المقاسة في مجموعات العلاج المشترك .فى الختام كلوريد النيكل تسبب في أضرار كبدية وكلوية بالإضافة إلى الحث على أكسدة الدهون والحد من أنشطة الأنزيمات المضادة للأكسدة في هذه الأعضاء. علاوة على ذلك خلاصة جذر حشيشة الملاك وزيت البردقوش قدما حماية كبيرة ضد التأثيرات الضارة لكلوريد النيكل مع تفوق خلاصة جذر حشيشة الملاك .