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Research Article

Protective Effect of *Artimisia herba alba* Aqueous Extract upon Nickel Induced Hepatotoxicity in Albino Wistar Rats

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Abstract

Heavy metals cytotoxicity results in high oxygen species production, which leads to continuous injury and dysfunction of different organs including liver. Thus, the present investigation was carried out to evaluate the effect of aqueous extract of *Artemisia herba alba* leaf powder on nickel induced liver oxidative damage in rats. Male's albino Wistar rats were divided into four groups, eight each. The first group served as a control, the second group given orally aqueous extract of Artemisia (300mg/kg b.w), the third group was treated intraperitoneally with nickel (20mg/kg b.w) and the fourth group was given both nickel and artemisia. The treatment of all groups was lasted for consecutive three weeks. The biochemical liver parameters were determinated. Liver glutathione, malondialdehyde, catalase, glutathione peroxidase and glutathione-S-transferase were also estimated. Nickel exposure resulted in a significant increase of AST, ALT, ALP, liver MDA and GST and a decline of GSH, catalase and GSH-Px. However, the administration of Artemisia restored the previous biochemical parameters. From the results, it can be concluded that *Artemisia herba alba* has the ability to attenuate Ni-induced liver oxidative damage due to its antioxidants power.

Keywords: *Artemisia herba alba*; Cytotoxicity; Liver; Nickel; Rat; Oxidative Stress

Introduction

Nickel (Ni) is a metallic compound found abundantly in the Earth's crust. It exists in various forms, including elemental nickel (Ni), Nickel Oxide (NiO), Nickel Chloride ($NiCl_2$), nickel sulfate ($NiSO_4$), nickel carbonate ($NiCO_3$), nickel monosulfide (NiS) and nickel subsulfide (Ni_3S_2). Human is constantly exposed to nickel through air, drinking water, food consumption or tobacco smoking [1]. After entering into the body, nickel penetrates all organs, accumulating primarily in bone, liver and kidney and excreted in bile and urine [2]. Ni induces the production of free radicals. The later have been shown to promote cell damage [3]. In addition, depletion in reduced glutathione (GSH), Glutathione Peroxidase (GSH-Px) and Glutathione-S-Transferase (GST) activities were also observed after exposure to Ni [4]. The use of medicinal plants as interesting source of natural chemical substances is today the form of the most widespread medicine worldwide, because these compounds have a potential source of natural antioxidants and biologically active [5,6]. Artemisia is a genus of small herbs and shrubs found mainly northern temperate regions. It belongs to the important family Asteraceae, one of the largest families, which comprise about 1000 genera and more than 20000 species [7]. Within this family, Artemisia encompasses more than 500 species. A wide range of morphological and photochemical variability characterizes it, which is associated with different geographical origins of the samples [8]. Artemisia herba-alba ("desert wormwood" in English; "armoise blanche" in French and "shaih" in Arabic), is a medicinal and deserts of the Middle East (Egypt), North Africa (Tunisia, Algeria and Morocco) and Southern Europe (Spain and Italy), extending into the North western Himalaya [9]. Artemisia species isolated compounds are known mainly for their antidepressant, hepatoprotective, antimicrobial, antifungal, neuroprotective, antitumoral and insecticidal proprieties [10]. However, to the best of our knowledge, there is no study regarding the examination of Artemisia against nickel hepatic intoxication. Therefore, the aim of the present study is to assess the protective effects of Artemisia on Ni-induced hepatotoxicity in male albino (Wistar) rats.

Materials and Methods

Chemicals and reagents

Nickel sulfate, 2-thiobarbituric acid (TBA), Butylaled Hydroxytoluene (BHT), 5.5'-dithiobis-2-nitrobenzoic acid (DTNB), trichloroacetic acid (TCA), were obtained from Sigma Chemical Co. (St. Louis, France) and all other chemicals were of analytical grade.

Plant material and preparation of the extract

The plant, *Artemisia herba alba*, was collected in January 2016 from the region of Bordj Bou Arreridj (Northeastern Algeria; longitude: 4° 81' East, latitude 36° 08' Nord, altitude varies between 302m and 1,885m). The aerial parts of the plant were washed, dried at room temperature in the dark and finally ground into powder. The aqueous extract was prepared as follow: 6g of the grounded A. herba. Alba was dissolved in 25ml of boiling distilled water; the hot infusion was then left for 15 minutes at room temperature and filtered. The

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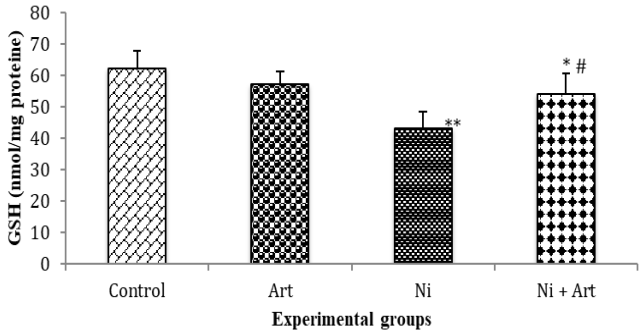

Figure 1: Reduced glutathione levels in liver of control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment. Significantly different from control: **p<0.001, *p<0.05; from Ni: #p<0.001. Values are given as mean ± SEM, number of samples = 8.

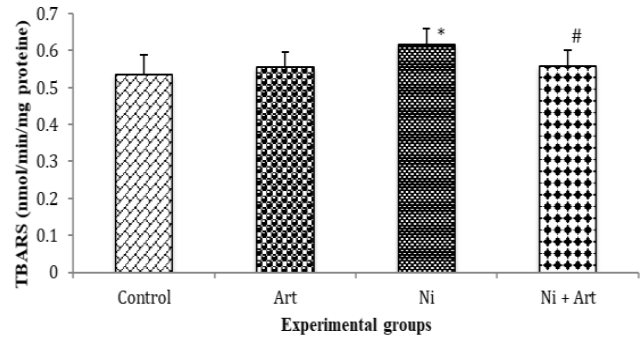

Figure 2: TBARS levels in liver of control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment. Significantly different from control: *p<0.01; from Ni: #p<0.05. Values are given as mean ± SEM, number of samples = 8.

obtained filtrate was used for the biological tests on animals.

Qualitative screening

Phenolics: To 2ml of aqueous extract, 1ml of 1% ferric chloride solution was added. Blue or green color indicates phenols [11].

Flavonoids: In a test tube containing 0.5ml of aqueous extract of the samples, 5 to 10 drops of diluted HCl and small amount of zinc were added and the solution was boiled for few minutes. Appearance of reddish pink or dirty brown color indicated the presence of flavonoids [12].

Tannins: To 2ml of aqueous extract 2ml of 5% FeCl3 was added. Formation of yellow brown precipitate indicates the presence of tannins [13].

Terpenoids: In a test tube 5ml of extract, 2ml of chloroform and 3ml of concentrated H_2S0_4 was mixed. A reddish brown coloration of the inter face was formed to show positive results for the presence of terpenoids [14].

Treatment of animals

Thirty two male albino (Wistar) rats (weighting 230 to 250g) were obtained from Pasteur Institute. Animals were acclimated for two weeks under laboratory conditions of photoperiod (12-h light/dark), relative humidity (40%) and temperature (24±2°C). A standard diet and water were provided ad-libitum. Rats were randomly divided

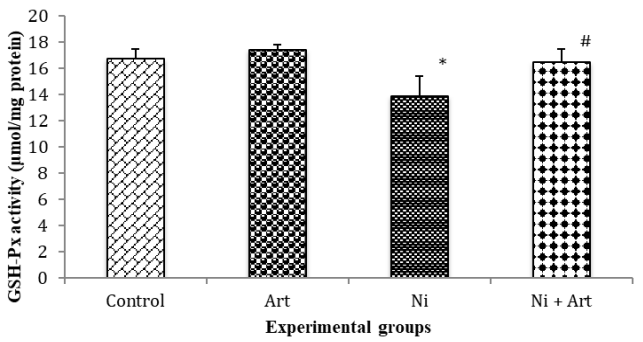

Figure 3: GSH-Px activity in liver of control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment. Significantly different from control: *p<0.01; from Ni: #p<0.01. Values are given as mean ± SEM, number of samples = 8.

into four groups of eight each as follows: The first group served as a control. The second group (Art) was treated orally with Artemisia herba. Alba aqueous infusion (300mg/kg b.w) [15]. The third group (Ni) was intraperitoneally injected with nickel as nickel sulphate ($NiSO_4$, $6H_2O$) at a dose of 20mg/kg b.w [16]. Rats in the fourth group (Ni+Art) were treated with both nickel and Artemisia. The experimental procedures were carried out according to the National Institute of Health Guide-lines for Animal Care and approved by the Ethics Committee of our Institution. The treatments of rats continued for a period of three weeks. At the end of the experiment, total body weight was recorded and animals were sacrificed by decapitation without anesthesia to avoid animals stress. Blood was transferred into ice cold centrifuged tubes. Tubes were then centrifuged for 10 minutes at 3000 rpm and serum was used for glucose, total protein, urea, AST, ALT and ALP assessments. Liver was immediately removed, weighed and one part was processed for assaying glutathione, MDA and antioxidant enzymes activities. The remaining part served for the histological study.

Estimation of serum biochemical parameters

Different biochemical parameters were assayed spectrophotometrically using commercially available kits from Spinreact (Spain, Refs: glucose-41011, total protein-1001291, urea-1001329, AST-1001165, ALT-1001175 and ALP-1001131).

Tissue preparation

About 1g of liver was homogenized in 2 ml of buffer solution of phosphate buffered saline solution 1:2 (w/v; 1g tissue in 2ml TBS, pH 7.4). The homogenates were centrifuged at 10,000g for 15 min at 4°C, the supernatants and the resultant supernatant was used for the determination of MDA, reduced glutathione, protein and the antioxidant enzymes including GSH-Px, GST and CAT.

Estimation of lipid peroxidation level

The lipid peroxidation level in liver homogenate was measured as malondialdehyde (MDA), which is the end of lipid peroxidation. It reacts with thiobarbituric acid (TBA)) to produce a red colored complex with a peak absorbance at 530nm [17].

Estimation of reduced glutathione level

The liver GSH content was estimated using a colorimetric technique, as mentioned by Ellman [18], and modified by Jollow et

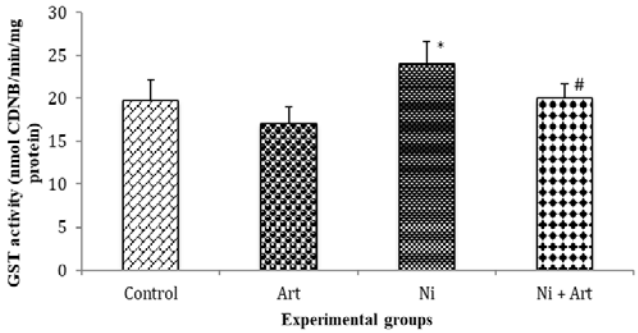

Figure 4: GST activity in liver of control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment. Significantly different from control: *p<0.05; from Ni: #p<0.01. Values are given as mean ± SEM, number of samples = 8.

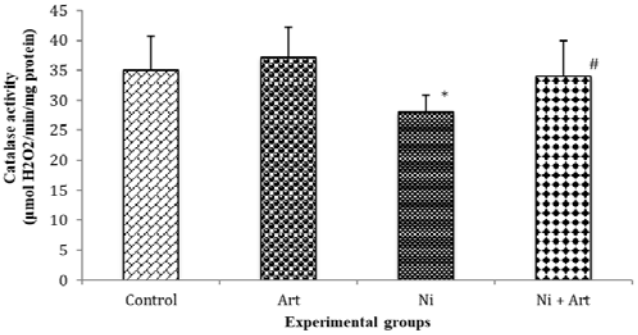

Figure 5: CAT activity in liver of control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment. Significantly different from control: *p<0.01; from Ni: #p<0.01. Values are given as mean ± SEM, number of samples = 8.

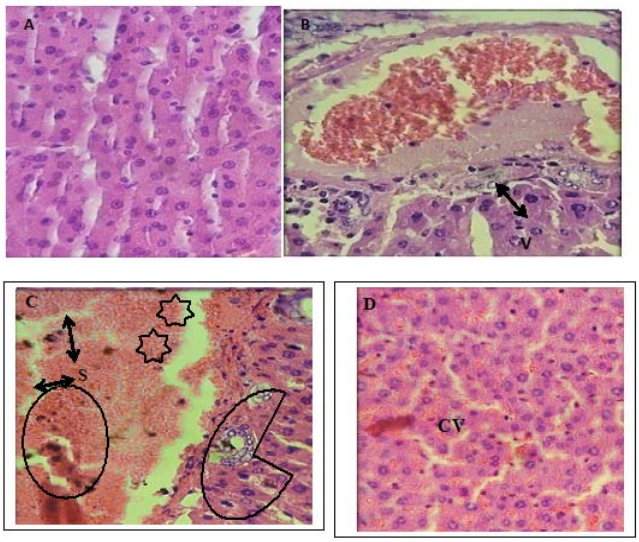

Figure 6: Effect of nickel sulphate (Ni) and *Artemisia herba alba* aqueous extract (Art) co-administered with Ni on histopathological damage in the liver. Control (A), treated with Art (B), treated with Ni(C) and Art co-administrated with Ni (D), H&E X400.
Arrows: CV; central vein, S; sinusoidal space, V; vacuolisation, (ᔓ); dilatation and vascular congestion in sinusoids, (◯); infiltration of mononuclear cells and ballooning degeneration, (✿) cells necrosis.

al [19], based on the development of a yellow color when DTNB [(5,5 dithiobis-(2-nitrobenzoic acid)] is added to compounds containing sulfhydryl groups. The GSH concentration (nmol GSH/mg protein) was obtained from the absorbance at 412nm.

Measurement of antioxidant enzymes activities

The activity of GSH-Px (EC 1.11.1.9) was measured by the procedure of Flohe and Gunzler [20], reading the absorbance at 420nm. Glutathione-S-transferase (EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig et al [21]. P nitrobenzyl chloride was used as substrate. The absorbance was measured spectrophotometrically at 340 nm at 30 s intervals for 3 min. The catalase (EC 1.11.1.6) activity was assayed by the method of Aebi [22]. Enzymatic reaction is initiated by adding an aliquot of 20ml of the homogenized tissue and the substrate (H_2O_2) to a concentration of 0.5M in a medium containing 100mM phosphate buffer (pH 7.4). Changes in absorbance were recorded at 240nm. CAT activity was calculated in terms of micromoles of H_2O_2 consumed per minute per milligram of protein.

Measurement of protein content

The protein content of tissues was determined by the method of Bradford [23] using bovine serum albumin as a standard.

Histopathological analysis

For histopathological analysis, the liver tissues were dissected and the tissue samples were immediately fixed in formalin solution, embedded in paraffin. The paraffin sections were cut into 5µm thick slices and stained with hematoxylin and eosin (H&E) for light microscopic examination. Then the sections were viewed under light microscope for histological examination [24].

Statistical analysis

Data are shown as means ± SEM. Statistical significance of the results obtained for various comparisons was estimated by applying one way analysis of variance (ANOVA) followed by Student's t-test and the level of significance was set at $p < 0.05$.

Results

Screening of the plant material revealed the presence of phenols, tannins, flavonoids and terpenoids. Qualitative estimations of bioactive constituents are summarized in Table 1.

Effects of treatments on body and liver weights

The variations in the body, absolute and relative liver weights of male rats in control and treatment groups are given in Table 2. During the course of present investigation, it was noticed that body weights of controls and Artemisia treated animals increased progressively throughout the study. However, body weight gain and food intake were decreased in rats treated with nickel with an increase of both absolute and relative liver weights. Meanwhile, the co-administration of Artemisia to nickel animals, led to a restoration of body weight, liver weight and food intake.

Effects of treatments on biochemical parameters

As seen in Table 3, treatment with Ni caused a significant increase in serum glucose, serum urea concentrations, AST, ALT and ALP activities with a decline in serum total proteins. Whereas, the previous

Table 1: Phytochemical screening of *Artemisia herba alba* L aqueous extract.

Bioactive Agents	Presence (+)/Absence (-)
Phenols	+++
Flavonoids	+
Tannins	+
Terpenes	+++

+, +++: indicating the level of the photochemicals in *Artemisia herba alba* plant.

Table 2: Changes in body, absolute and relative liver weights, and food intake in control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment.

		Experimental groups		
Parameters	Control	Art	Ni	Ni+Art
	(n=8)	(n=8)	(n=8)	(n=8)
Initial body wt(g)	242.83±7.25	238.5±2.81	245±13.06	247.16±9.97
Body wt gain/loss(g)	25.83±13.67	24±4.97	-25±21.73***	-17.66±8.23***
Food intake (g/day/rat)	16.50±1.10	17.67±1.55	12.61**±1.20	15.21#±0.40
Absolute Liver wt(g)	7.04±0.52	6.96±0.42	7.99*±0.84	7.40±0.70
Relative liver weight(g)	3.81±0.28	3.92±0.29	4.73**±0.69	4.58**±0.42

Significantly different from control: *p <0.05, **p<0.01, *** p<0.001; from Ni: #p<0.01.Values are given as mean ±SEM, n=number of animals.

biochemical parameters were ameliorated in Ni+ Art.

Effects of treatments on hepatic oxidative stress parameters

The GSH, TBARS (MDA), GSH-Px, GST and catalase values are shown in Figures 1-5, respectively. It can be seen that animals treated with nickel presented higher TBARS concentration and GST activity. Simultaneously the levels of GSH, GSH-Px and catalase activities were decreased. However, the supplementation of Artemisia resulted in an improvement and recovery of the above hepatic oxidative stress parameters.

Effects of treatments on liver histoarchitecture

Liver of the control group presented regular histological structure with a characteristic pattern of hexagonal lobules (Figure 6A). Accordingly, no histological alterations were observed in the liver of Artemisia-treated group (Figure 6B). In contrast, Ni-treated group showed slight histological alterations such as dilatation and vascular congestion in sinusoids, infiltration of mononuclear cells and ballooning degeneration (Figure 6C). Interestingly, the Ni–Artemisia group showed prominent recovery in the form of hepatic histoarchitecture (Figure 6D).

Discussion

It is known that plants rich in secondary metabolites such as phenolic compounds have antioxidant proprieties. According to the findings obtained, the phytochemical analysis showed that *Artemisia herba alba* extract contained high levels of total phenolic compounds, tannins, flavonoids and terpenoids. This is in agreement with the findings of Salhi et al [25]. A number of plant extracts have been investigated for their hepatoprotective and antioxidant effects against hepatotoxin-induced liver damage [26]. In the current study, rats treated with nickel presented lower body weight gain and food intake compared to the controls, which is consistent with several

Table 3: Changes of biochemical parameters in control and rats treated with Artemisia (Art), nickel (Ni) or their combination (Ni + Art) after 3 weeks of treatment.

Parameters	Control	Art	Ni	Ni+Art
	(n=8)	(n=8)	(n=8)	(n=8)
Glucose (g/l)	0.98±0.12	0.86*±0.03	1.73**±0.29	1.21**##±0.05
Total protein (g/dl)	6.13±0.31	7.10*±0.52	4.85**±0.11	5.69*##±0.11
Urea (mg/dl)	52.34±1.20	44.02**±2.21	58.17*±1.51	47.41###±1.78
AST (UI/L)	111.66 ± 21.31	106.61 ± 11.15	179.94**±16.23	123.63##±26.44
ALT (UI/L)	97.28±5.66	88.38*±7.31	149.07**± 5.76	113.11**##±6.24
ALP (UI/L)	519.72±13.87	451.19**±15.55	543.57**±12.89	524.14**#±13.94

Significantly different from control: *p<0.05, **p<0.01; from Ni: #p<0.05, ##p < 0.01, ###p<0.001.
Values are given as mean ±SEM, n= number of animals.

previously published studies [27,28]. Reduction in weights might be due to low food consumption, hormonal imbalance and a decrease in protein synthesis [29]. Meanwhile, administration of nickel induced a significant increase both absolute and relative liver weights. This might be explained by the accumulation of nickel in the liver [30] or nickel can lead to cell death by apoptosis of certain cell lines, due to the accumulation of toxic lipid derivatives such as ceramides. The later induce cellular hypertrophy of the target organ [31]. All these physiological changes were upturned in rats treated with nickel in association with *Artemisia herba alba* extract. The greater gain in body weight of these animals certainly resulted from an increase in daily food consumption (increase appetite) and promotion of protein synthesis. Moreover, Artemisia herba-alba extract might have the ability to reverse gluconeogenesis and control protein loss [32]. The increase in blood glucose of Ni-treated rats was supported by the findings of Dahdouh et al [27], who reported that the rise in serum glucose may indicate a disrupted carbohydrate metabolism resulting from enhanced break down of liver glycogen, possibly mediated by an increase of pancreatic release of glucagon and subsequently can lead to hypoinsulinemia, through inhibition of insulin release from rat islets. Thus, these alterations were found to cause a drastic variation in the insulin/glucagon plasma ration [33]. On the other hand, the elevation of blood glucose could be explained by the involvement of nitric oxide mediated pathways [34]. Nevertheless, the supplementation of Artemisia reduced glucose level. Tastekin et al [35] concluded that *Artemisia herba alba* could reduce the absorption of glucose from the intestine and inhibit the reabsorption of glucose by kidney tubules. Another possibility would be Artemisia increased sensitivity to insulin receptors [36]. A decline in serum proteins and an increase of urea levels were recorded in nickel group. These variations probably due to changes in protein synthesis and/or metabolism [37]. The findings indicated also an increase of serum transaminases and alkaline phosphatase activities. This it could be attributed to the hepatic damage resulting a release and leakage out of these enzymes from the liver cytosol into the blood stream, which gives an indication on the hepatotoxic effect of this metal [38-40]. Interestingly, the biochemical perturbations seem to be correlated with the liver histological alterations such as the presence of cellular debris within a central vein, dilatation, vascular congestion in sinusoids and a cytoplasmic vacuolization, plasma membrane destruction, infiltration of mononuclear cells, cells necrosis and cellular hypertrophy due possibly to the formation of highly reactive

radicals because of oxidative threat induced by nickel. Conversely, the co-treatments with Artemisia increased protein concentration and reduced urea levels, GOT, GPT, and ALP activities, suggesting that this plant offered a considerable level of hepatoprotection through a contributory hepatoprotective mechanism, which accelerates the regeneration process and the production of liver cells [41]. MDA is the most abundant individual aldehyde resulting from lipid peroxidation. So this aldehyde can be considered as an important indicator of lipid peroxidation [42]. The increase of MDA concentration in the present study is an indication of lipid peroxidation provoked by nickel intoxication. In other words, the increase of hepatic MDA in Ni-treated rats is possibly related to the decline in the GSH-Px activity, which scavenges hydroperoxides and lipid peroxides [43]. The diminution of glutathione level in nickel rats may be as result of oxidative stress, which has been occurred, in nickel toxicity. In other words, the reduced of antioxidant production was due to the increase of oxygen metabolites and to the elevated of free radicals, which cause a decrease in the activity of the anti-oxidant defense system [44,45] and several pathways have been proposed to show the depletion of GSH level in heavy metal toxicities. Firstly. The sulfhydryl group of cysteine moiety of glutathione has a high affinity of metals, forming thermo-dynamically stable mercaptide complexes with several metals [46]. These complexes are inert which can be excreted via the bile [47]. Secondly, GSH may be oxidized due to the interaction with the free radicals induced by nickel. Therefore, GSH level could be consumed during nickel detoxification [48,49]. Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage, which prevents biological macromolecules from oxidative injury and removes peroxides, free radicals, and superoxide anion generated within the cell. In the current study, the significant decrease in the antioxidant enzymes activities CAT and GSH-Px of liver proved their consumption during the breakdown of free radicals and H2O2 or the inhibition of the enzymes by free radicals [50]. In addition, the decrease in the activity of GSH-Px might result directly from the decreased level of GSH following Ni-exposure since GSH-Px activity depends on GSH level. Glutathione-S-Transferase (GST) enzyme has an important role in detoxification of xenobiotic, drugs and carcinogens and thus protects the cells against redox cycling and oxidative stress [51]. Increased GST activity following nickel exposure suggests a counteracting mechanism adopted by the system to eliminate nickel [52]. The co-administration of *Artemisia herba alba* aqueous extract showed a strong antioxidant activity via enhancement of GSH concentration, antioxidants enzymes activities and inhibition of lipid peroxidation production in hepatic tissue. It has been documented that Artemisia Herba plant contains wide variety of antioxidant molecules, such as phenolic acids and other natural antioxidants [53]. Several studies reported excellent linear correlations between phenolic contents and antioxidant capacity for plant samples indicating that extracts with highest polyphenol contents showed higher antioxidant activity [54-57].

Conclusion

Treatment with *Artemisia herba alba* restored the morphological and the biochemical changes and began to recover the normal tissue histology. So *Artemisia herba alba* aqueous extract has considerable hepatoprotective outcome against nickel-induced hepatic dysfunction and oxidative damage. Such protection is undoubtedly attributed to its antioxidant properties by chelating metal ions and scavenging the generated free radicals.

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References

1. Annangi B, Bonassi S, Marcosa R, Hernández A. Biomonitoring of humans exposed to arsenic, chromium, nickel, vanadium, and complex mixtures of metals by using the micronucleus test in lymphocytes. Mutation Research. 2016; 770: 140-161.
2. Ecological Soil. Screening Level for Nickel (ECOSSLs for Nickel) U. S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, NW, Washington DC (OSWER Directive). 2007; 9285: 7-76.
3. Sidhu, P, Garg, ML, Dhawan DK. Protective role of zinc in nickel induced hepatotoxicity in rats. Chemico-Biological Interactions. 2004; 150: 199-209.
4. Hfaiedh N, Allagui MS, Hfaiedh M, El Feki A, Zourgui Z, Croute et al. Protective effect of cactus (Opuntia ficus indica) cladode extract upon nickel-induced toxicity in rats. Food Chemical Toxicology. 2008; 46: 3759-3763.
5. Celiktas OY, Hames Kocabas EE, Bedir E, Verdar Sucan O, Baser KHC et al. Antimicrobial activities of methanolic extract and essential oils of Rosmarinus officinalis, depending on location and seasonal variations. Food Chemistry. 2007; 100: 553-559.
6. Tikare SN, Yendigeri S, Gupta AD, Dhundasi SA, Das KK et al. Effect of garlic (Allium sativum) on hematology and erythrocyte antioxidant defense system of albino rats exposed to heavy metals (nickel II & chromium VI). Indian Journal Physiology Pharmacology. 2012; 56: 137-146.
7. Abad MJ, Bedoya LM, Apaza L, Bermejo P. The Artemisia L, et al. genus: a review of bioactive essential oils. Molecules. 2012; 17: 2542-2566.
8. Bora KS, Sharma A. The genus Artemisia: a comprehensive review. Pharmaceutical Biology. 2011; 49: 101-109.
9. Mighri H, Akrouta A, El-jeni HH, Zaidia S, Tomi F, et al. Composition and intraspecific chemical variability of the essential oil from Artemisia herba-alba growing wild in a Tunisian arid zone. Chemistry Bio-diversity. 2010; 7: 2709-2717.
10. Bachrouch O, Ferjani N, Haouel S, Ben Jemâa JM. Major compounds and insecticidal activities of two Tunisian Artemisia essential oils toward two major coleopteran pests. Industrial Crops and Products. 2015; 65: 127-133.
11. Martinez A, Valencia G. Manual de practicas de Farmacognosia y Fitoquimia: 1999.1. Medellin: Universidad de Antiquia. Marcha fotiquimica. 2003; 59-65.
12. Sermakkani M, Thangapandian V. Phytochemical screening for active compounds in Pedalium murex L. Recent Research in Science and Technology. 2010; 2: 110-114
13. Jigna P, Sumitra VC. *In vitro* antimicrobial activity and phytochemical analysis of some Indian medicinal plant. Turkish Journal Biology. 2007; 31: 53-58.
14. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4: 685-688.
15. Boudjelal A, Siracusa L, Henchiri C, Sarri M, Abderrahim B, et al. Antidiabetic effects of aqueous infusions of Artemisia herba-alba and Ajuga iva in alloxan-induced diabetic rats. Planta Medica. 2015; 81: 696-704.
16. Pari L, Prasath A. Efficacy of caffeic acid in preventing nickel induced oxidative damage in liver of rats. Chemico-Biological Interactions. 2008; 173: 77-83.
17. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods enzymology. 1984; 105: 302-310.
18. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys. 1959; 82: 70-77.
19. Jollow DJ, Michell JR, Zampaglionic N, Gillete JR. Bromoibenzene-

induced Liver necrosis: Protective role of glutathione and evidence for 3, 4-Bromobenzene oxide as hepatotoxic metabolite. Pharmacology. 1974; 11: 151-169.

20. Flohe L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymology. 1984; 105: 114-121.

21. Habig WH, Pabst MJ, Jakoby WB. Glutathione-S-transferase the first step in enzymatic mercapturic acid formation. Journal Biology Chemistry. 1974; 249: 7130-7139.

22. Aebi H. Catalase *in vitro*. Methods Enzymology. 1984; 105: 121-126.

23. Bradford MM. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein binding. Anal Biochemistry. 1976; 72: 248-254.

24. Hould R. Techniques d'histopathologie et de cytopathologie. Ed Maloine. 1984; 19-21: 225-227.

25. Salhi N, Mohammed-Saghir SA, Terzi V, Brahmi I, Ghedairi N et al. Antifungal Activity of Aqueous Extracts of Some Dominant Algerian Medicinal Plants. Bio Med Research International. 2017; 1-6.

26. Tiwari P, Ahirwae D, Chandy A, Ahirwar B. Evaluation of hepatoprotective activity of alcoholic and aqueous extracts of Selaginella lepidophylla. Asian Pacific J Tropical Disease. 2014; 4: 81-86.

27. Dahdouh F, Kechrid Z, Djebar MR. Beneficial Effects of Vitamins (C+E) Supplementation against Nickel-induced Hepatotoxicity in Mice. Advances in Bioresearch. 2013; 4: 67-76.

28. Tikare SN, Yendigeri S, Gupta AD, Dhundasi SA, Das KK et al. Protective effect of α-tocopherol against hematotoxicity, hepatotoxicity and nephrotoxicity induced by nickel sulfate in male albino rats. Indian Journal Physiology Pharmacology. 2013; 57: 280-292.

29. Rao MV, Chawla SL, Sharma SR. Protective role of vitamin E on nickel and/or chromium induced oxidative stress in the mouse ovary. Food and Chemical Toxicology. 2009; 47: 1368-1371.

30. Cempel M, Janicka K. Distribution of nickel, zinc and copper in rat organs after oral administration of nickel (II) chloride. Biological Trace Element Research. 2002; 90: 215-226.

31. Lee YI, Lim SS, Baek,BJ, An JM, Nam HS, et al. Nickel (II)-induced nasal epithelial toxicity and oxidative mitochondrial damage. Environment Toxicology Pharmacology. 2016; 42: 76-84.

32. Rajagopal K, Sasikala K. Antihyperglycaemic and antihyperlipidaemic effects of Nymphaea stellata in alloxan-induced diabetic rats. Singapore Medical Journal. 2008; 49: 137-142.

33. Tikare SN, Das Gupta A, Dhundasi SA, Das KK. Effect of antioxidants L-ascorbic acid and alpha-tocopherol supplementation in nickel exposed hyperglycemic rats. Journal Basic Clinical Physiology Pharmacology. 2008; 19: 89-101.

34. Gupta S, Ahmad N, Husain MM, Srivastava RC. Involvement of nitric oxide in nickel-induced hyperglycemia in rats. Nitric Oxide. 2000; 4: 129-138.

35. Tastekin D, Atasever M, Adiguzel G, Keles M, Tastekin A, et al. Hypoglycaemic Effect of *Artemisia herba alba* in Experimental Hyperglycaemic Rats. Bulltin Veterinary Inst Pulawy. 2006; 50: 235-238.

36. Abou EL-Hamd HM, Magdi AES, Mohamed EH, Soleiman EH, Abeer ME, et al. Chemical Constituents and Biological Activities of *Artemisia herba alba*. Record Natural Products. 2010; 4: 1-25.

37. Das KK, Dasgupta S. Effect of nickel on testicular nucleic acid concentrations of rats on protein restriction. Biological Trace Element Research. 2000; 73: 175-80.

38. Kechrid Z, Dahdouh F, Djabar RM, Bouzerna N. Combined effect of water contamination with cobalt and nickel on metabolism of albino (Wistar) rats. Environ Health Sciences Engineering. 2006; 3: 65-69.

39. Das KK, Das SN, Dhundasi SA. Nickel: Molecular Diversity, Application, Essentiality and toxicity in human health. In: Blanc G and Moreau D, editors. Biometals, Molecular Structures, Binding Properties and Applications. New York, USA, Nova Science Publishers Inc. 2010; 33-58.

40. Adjroud O. The toxic effects of nickel chloride on liver, erythropoiesis, and development in Wistar albino pre-implanted rats can be reversed with selenium pretreatment. Environment Toxicology. 2013; 28: 290-298.

41. Abdallah HMI, Abdel-Rahman RF, Abdel-Jaleel GA, Abd El-Kader HAM, El-Marasy SA, et al. Pharmacological effects of ethanol extract of *Artemisia herba alba* in streptozotocin-induced type 1 diabetes mellitus in rats. Biochemistry Pharmacology. 2015; 4: 1-13.

42. Cemek M, Buyukben A, Buyukokuroglu ME, Aymelek F, Tur L, et al. Protective roles of vitamin E (α-tocopherol), selenium and vitamin E plus selenium in organophosphate toxicity *in vivo*: A comparative study. Pesticide Biochemistry and Physiology. 2010; 96: 113-118.

43. Klibet F, Boumendjel A, Khiari M, El Feki A, Abdennour C, et al. Oxidative stress related liver dysfunction by sodium arsenite: Alleviation by Pistacia lentiscus oil. Pharmaceutical Biology. 2016; 54: 354-363.

44. Gstraunthaler G, Pfaller W, Kotanko P. Glutathione depletion and *in vitro* lipid peroxidation in mercury or malate induced acute renal failure. Biochemistry Pharmacology. 1983; 32: 2969-2972.

45. Iscan M, Ada A, Coban T, Kapucuoglu N, Aydin A, et al. Combined effects of cadmium and nickel on testicular xenobiotic metabolizing enzymes in rats. Biological Trace Element Research. 2002; 89: 177-190.

46. Pari L, Amudha K. Hepatoprotective role of naringin on nickel-induced toxicity in male Wistar rats. European Journal of Pharmacology. 2010; 650: 364-370.

47. Aposhian HV. Biochemical toxicology of arsenic. Rev Biochemistry Toxicology. 1989; 10: 265-299.

48. Das KK, Buchner V. Effect on nickel exposure on peripheral tissues: role of oxidative stress in toxicity and possible protection by ascorbic acid. Review Environment Health. 2007; 22: 157-173.

49. Manna P, Sinha M, Sill PC. Arsenic-induced oxidative myocardial injury: protective role of arjunolic acid. Arch Toxicology. 2008; 82: 137-149.

50. Ihechiluru NB, Henry AN, Taiwo IE. Heavy metal bioaccumulation and oxidative stress in Austroaeschna inermis (Dragon fly) of the Lagos Urban ecosystem. Journal Environmental Chemistry Ecotoxicology. 2015; 7: 11-19.

51. Ognjanovic BI, Markovic SD, Dordevic NZ, Trbojevic IS, Stajn AS, et al. Cadmium-induced lipid peroxidation and changes in antioxidant defense system in the rat testes: Protective role of coenzyme Q10 and Vitamin E. Reproductive Toxicology. 2010; 29: 191-197.

52. Fatmi W, Kechrid Z, Nazıroğlu M, Flores-Arce M. Selenium supplementation modulates blood and tissue zinc, blood biochemical parameters and antioxidant system in diabetic rats fed zinc-deficient diet. Biological Trace Element Research. 2013; 152: 243-250.

53. Moufid A, Eddouks M. *Artemisia herba alba*: a popular plant with potential medicinal Properties. Pakistan Journal Biology Sciences. 2012; 15: 1152-1159.

54. Sengul M, Yildiz H, Gungor N, Cetin B, Eser Z, et al. Total phenolic content, antioxidant and antimicrobial activities of some medicinal plants. Pakistan Journal Pharmaceutical Sciences. 2009; 22: 102-106.

55. Riahi L, Chograni H, Elferchichi M, Zaouali Y, Zoghlami N, et al. Variations in Tunisian wormwood essential oil profiles and phenolic contents between leaves and flowers and their effects on antioxidant activities. Industrial Crops and Products. 2013; 46: 290-296.

56. Boudjelal A, Henchiri C, Siracusa L, Sari M, Ruberto G. Compositional analysis and *in vivo* anti-diabetic activity of wild Algerian Marrubium vulgare L. infusion. Fitoterapia. 2012; 83: 286-292.

57. Sekiou O, Boumendjel M, Taibi F, Boumendjel A, Messarah M. Mitigating effects of antioxidant properties of *Artemisia herba alba* aqueous extract on hyperlipidemia and oxidative damage in alloxan-induced diabetic rats. Arch Physiology Biochemistry. 2018; 26: 1-11.