Hepatoprotective role of garlic (*Allium sativum*) on nickel-induced liver injury in albino Wistar rats

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ABSTRACT

This study was conducted to examine the protective effect of garlic on nickel induced impairment of liver function. Thirty-two female albino Wistar rats were divided into 4 groups. Control group fed a standard diet, the second group (Ga) received garlic 20 g/kg diet, while the third group (Ni) was given nickel 800 mg/L in their drinking water as $NiSO_4 6H_2O$. The fourth group (Ni+Ga) was treated daily with both nickel and garlic. The experiment was lasted for 28 days. The exposure to nickel led to significant decrease in body weight and food intake with an increase of liver weight. Nickel treatment also produced oxidative liver injury characterized by an increase of glucose, glutamate-pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT), alkaline phosphatase (ALP), bilirubin and malonaldehyde (MDA). Simultaneously, serum total proteins, liver reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GSH-Px), glutathione superoxide dismutase (SOD) were decreased. The supplementation of garlic restored a partial the previous parameters.

In conclusion, the present study demonstrated that garlic has potent antioxidants activity which revealed by the amelioration of nickel hepatotoxicity. In other words, garlic has a protective effect towards damages induced by nickel.

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1. INTRODUCTION

Pollution by heavy metals constitutes a serious interest in the world's population. These metals are used in many industries, and they have the ability to accumulate in living organisms posing a serious public health problem [1], Heavy metal hazards on human and animal health are increased and represent global environmental problems [2]. Nickel is an alloying element for steel and cast iron, yielding alloys and steel increases strength and resistance to corrosion and temperature. Additionally, Nickel compounds are used in preparation of Ni alloys, ground coated enamels, in cooling of ceramics and glass, electroplating, batteries, electronic components and to prepare nickel catalysts [3]. Nickel penetrates all organs and accumulates primarily in bone, liver, kidney and excreted through bile and urine [4]. Liver is the primary target for environ-mental and occupational toxicity and the major site for detoxification [5]. Nickel induced severe liver and kidney damage by altering several marker enzymes and ascor-bate-cholesterol metabolism. One of the harmful effects of nickel action in the body is to induce formation of reactive oxygen species (ROS) and increase lipid peroxidation in the cells [6]. Accumulation of nickel is responsible for reactive oxygen species (ROS) generation and the enhancement of LPO. Additionally, nickel has been associated with DNA oxidation and DNA strand breaks [7]. Herbs and spices, which are important part of the human diet, have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are known for their preservative, antioxidant, antimicrobial and various other medicinal values [8, 9]. Garlic (*Allium sativum*) is widely used as a seasoning in cuisines worldwide. Historically, garlic was used by the Egyptians in several therapeutic formulas [10, 11]. Garlic contains at least 100 bioactive volatile sulfur compounds and nonvolatile with medicinal values that contribute to its pharmacological uses, including S-allylcysteine, saponins, ajoene, and flavonoid phenolic compounds [12]. The biological responses of garlic have been largely attributed to reduction of risk factors for cardiovascular diseases and stimulation of immune function [13], antifungal [14], anticarcinogenic [15], anti-atherosclerotic [16], antimicrobial effects [17], anti-tumor promoting effects [18], and anti-thrombotic [19], anti-hypercholesterolemia, anti-coagulant and anti-hypertensive [20]. Thus, this work was carried out to investigate the possible protective properties of garlic extracts against nickel hepatotoxicity in albino Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Nickel sulphate ($NiSO_46H_2O$), 5, 5'dithiobis-(2-nitrobenzoic acid (DTNB) and reduced glutathione, epinephrine, thiobarbituric acid were purchased from sigma Chemical Co (St Louis, France) and all other chemicals used were of analytical grade.

2.2 Preparation of garlic

Garlic (*Allium sativum*) cloves were purchased from local market (EL-Harrouch, Skikida, East of Algeria), washed, peeled coarsely minced, and cut into small pieces' air dried, and pulverized with a blender to fine powder and preserved in airtight containers at room temperature until the formulation of experimental diet.

2.3 Animals

Thirty-two female albino (Wistar) rats with a body weight of 216-223g were obtained from Pasteur Institute, Algiers, Algeria. Animals were acclimated for two weeks for adaptation under the same laboratory conditions of photoperiod (12h light/12 h dark) with a relative humidity of 40% and room temperature of 22 ± 2°C. Food (Standard diet, supplemented by the ONAB, EL-Harouch, Algeria) and water were provided *ad-libitum*.

2.4 Experimental design

Animals were randomly divided into four groups (eight each). Control group received standard diet. The second group (Ga) received a standard diet supplemented with 20g/kg diet of garlic. The third group (Ni) was given nickel 800 mg/L in their drinking water as nickel sulphate ($NiSO_46H_2O$), the fourth group (Ni+Ga) was treated with both nickel and garlic. The doses of nickel and garlic used in the present experiment were selected on the basis of previous studies of Sidhu et al [3] and Kwon et al [21] respectively. 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. Treatments of rats continued for a period of three weeks. Body weight, food intake and water consumption were recorded regularly. At the end of the experiment, animals were sacrificed by decapitation without anesthesia to avoid animals stress. At the time of sacrifice, 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, total bilirubin, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and alkaline phosphatase (ALP) assays. Liver was removed immediately and one part was processed immediately for assaying lipid peroxidation (MDA), reduced glutathione (GSH) and antioxidant enzymes activities including glutathione peroxidase (GSH-Px), catalase (CAT) and superoxide dismutase (SOD). The other part was used for the histological study.

2.5 Analytical methods

2.5.1 Determination of biochemical parameters

Glucose, transaminases (GPT, GOT), Alkaline Phosphatase (ALP), total proteins, total bilirubin were assessed using Spinreact Laboratory Spain diagnostic kits and spectrophotometer (Jenway 6505, Jenway LTD, Essex, UK). The references of kits were as follow: glucose-41011, GOT-1001161, GPT-1001171, ALP-1001131, total proteins-1001291, total bilirubin-1001044.

2.5.2 Tissue preparation

About 1 g of liver was homogenized in 2 ml of buffer solution of phosphate buffer saline 1:2 (w/v; 1 g tissue with 2 ml TBS, pH 7.4). Then the homogenates were centrifuged at 10000 x g for 15 min at 4°C and the resultant supernatant was used for the determination of MDA, GSH, GSH-Px, CAT, SOD and liver proteins.

2.5.3 Estimation of lipid peroxidation (MDA)

The lipid peroxidation level in liver homogenate was measured as malondialdehyde (MDA), which is the end product of lipid peroxidation, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance (TBARS) to produce a red colored complex with a peak absorbance at 532 nm according to the method of Buege and Aust [22].

2.5.4 Estimation of reduced glutathione (GSH)

Liver GSH content was estimated using a colorimetric technique, as mentioned by Ellman [23] modified by Jollow et al [24], based on the development of yellow color when DTNB is added to compounds containing sulfhydryl groups. In brief, 0.8 ml of liver supernatant was added to 0.3 ml of 0.25% sulfosalicylic acid, and then tubes were centrifuged at 2500 x g for 15 min. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). The absorbance at 412 nm was recorded. Finally, total GSH content was expressed as n mol GSH/mg protein.

2.5.5 Determination of glutathione peroxidase (GSH-Px)

GSH-Px (E.C.1.1.1.1.9) activity was measured by the procedure of Floche and Gunzler [25]. Supernatant obtained after centrifuging 5% liver homogenate at 15000 x g for 10 min followed by 10.000 x g for 30 min at 4°C was used for GSH-Px assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M. pH 7.4). 0.2 ml of GSH (2 mM), 0.1 ml of sodium azide (10 mM), 0.1 H_2O_2 (1 mM) and 0.3 ml of liver supernatant. After incubation at 37°C for 15 min, the reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500 x g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1 M pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

2.5.6 Assay of catalase (CAT)

CAT activity was determined according to the method of Aebi [26]. The reaction mixture (1 ml) that contained 0.78 ml of phosphate buffer (0.1 M, pH 7.4), 0.2 ml of liver supernatant, and 0.02 ml of H_2O_2 (0.5 M) was prepared. The reaction was started by adding H_2O_2 and decomposition was monitored by following the decrease in absorbance at 240 nm for 1 min. The enzyme activity was calculated using an extinction coefficient of 0.043 mM−1/cm.

2.5.7 Estimation of superoxide dismutase (SOD)

Estimation of Superoxide dismutase SOD: The activity superoxide dismutase (SOD) was determined according to the method described by Misra and Fridonih [27] . Ten micro liters of tissue homogenate were added to 970µl of EDTA-Sodium carbonate buffer (0.05M) at pH 10.2. The reaction was started by adding 20µl of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min.

2.5.8 Protein determination

The protein content of tissues samples was measured by the method of Bradford [28] using bovine serum albumin as a standard.

2.5.9 Histological study

The histological examination of livers was carried out as follows: liver was dissected and immediately fixed in bouin solution for 24 h, processed by using a graded ethanol series, and then embedded in paraffin. The paraffin sections were cut into 5µm thick slices and stained with hematoxylin and eosin. The preparations were then observed under an optical microscope and photographed by the method of Hould [29].

2.5.10 Statistical analysis

Data are given 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 $p<0.05$.

3. RESULTS

3.1 Effect of treatment on body weight, liver weight and food intake

The findings illustrated in table 1 showed that body weight and food intake of animals exposed to nickel was significantly decreased by -36% and -35% respectively as compared to controls. Meanwhile, a significant increase of Ni-treated group in absolute and relative weights was noticed 57% and 35%. However, the previous parameters were obviously restored after garlic supplementation near controls values.

Table1: Initial body weight, final body weight, food intake, absolute and relative liver weights of control rats, treated with garlic (Ga), nickel (Ni), and nickel with garlic (Ni+Ga) after three weeks of treatment.

Parameter	Experimental groups			
	Control	Ga	Ni	Ni+Ga
Initial body weight (g)	217 ±16	219 ±15	216 ±23	222 ±15
Final body weight (g)	302±17	293±23	191±17***	263±11$^{*\#\#}$
Food intake(g/day/rat)	20±0.56	21±0.96	13±1.35***	16±0.77$^{***\#}$
Absolute liver weight (g)	6.11±0.23	6.19±0.38	9.64±0.73***	6.89±0.28$^{*\#\#}$
Relative liver weight (g)	2.70±0.05	2.77±0.06	3.66±0.11***	3.11±0.18$^{*\#\#}$

Values are given as mean± SEM, eight female rats each group.

*Statistically significant different from control group: *p<0.05, ***p<0.001; from nickel group: #p<0.05, ##p<0.01.*

3.2 Effect of treatment on serum markers of liver damage

Treatment with nickel caused significant increase (p<0.05) of serum glucose and bilirubin, highly significant (p<0.01) for GPT, ALP and very high significant (p<0.01) for GOT. Meanwhile the concentration of serum total protein was diminished (p<0.01). Nevertheless, the supplementation of garlic in combination with nickel produced a recovery in the above-mentioned biochemical parameters for total protein, (p<0.05) for bilirubin, GPT, GOT and p<0.001 for glucose, ALP (table 2).

Table 2: Concentrations of liver biochemical parameters of control rats, treated with garlic (Ga), nickel (Ni), and nickel with garlic (Ni+Ga) after three weeks of treatment.

Parameter	Experimental groups			
	Control	Ga	Ni	Ni+Ga
Glucose(g/l)	1.13±0.06	1.04±0.06	1.35±0.07*	1.05±0.03$^{\#\#\#}$
Direct bilirubin (mg/dl)	0.40±0.18	0.40±0.04	0.80±0.04*	0.55±0.14$^{\#}$
Total protein(mg/dl)	7.33±0.44	7.69±0.20	5.49±0.2**	6.06±0.26$^{**\#\#}$
GOT(U/L)	75.05±1.74	73.82±1.44	137.72±9.91***	112.72±5.17$^{\#}$
GPT(U/L)	44.33±5.67	46.08±3.68	65.32±6.88**	47.47±4.57$^{\#}$
PAL(U/L)	115±1.57	116.33±1.89	269.66±3.53**	224.83±2.36$^{***\#\#\#}$

Values are given as mean± SEM, eight female rats each group.
*Statistically significant different from control group: *p<0.05, **p<0.01, ***p<0.001; from nickel group: #p<0.05, ##p<0.01, ###p<0.001*

3.3 Effect of treatment on hepatic oxidative stress parameters

As seen from figures 1 and 2 the exposure to nickel produced a significant adverse effect on the liver redox status, which is indicated by an increase (p<0.05) of MDA level and a decrease of GSH (p<0.05) content, GSH-Px (p<0.01), catalase (p<0.001) and SOD (p<0.01) activities. However, treatments with garlic in association with nickel restored these stress oxidative biomarkers.

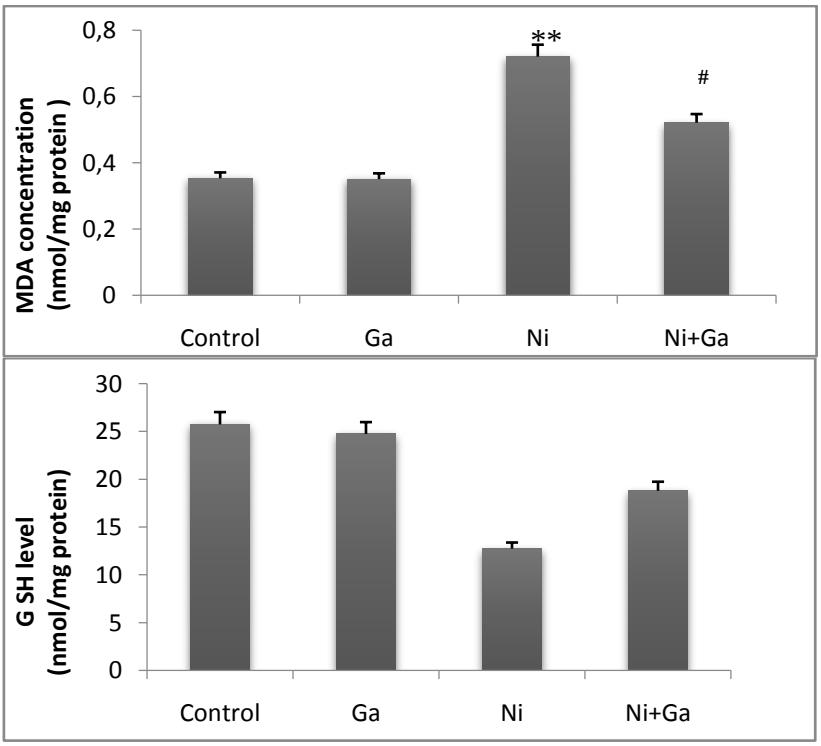

Figure 1. Liver MDA and GSH concentrations of control rats, treated with garlic (Ga), nickel (Ni), and nickel plus garlic (Ni+Ga) after three weeks of treatment. Values are given as mean± SEM, eight female rats each group. Statistically significant different from control group: *p<0.05, **p<0.01; from nickel group: #p<0.05.

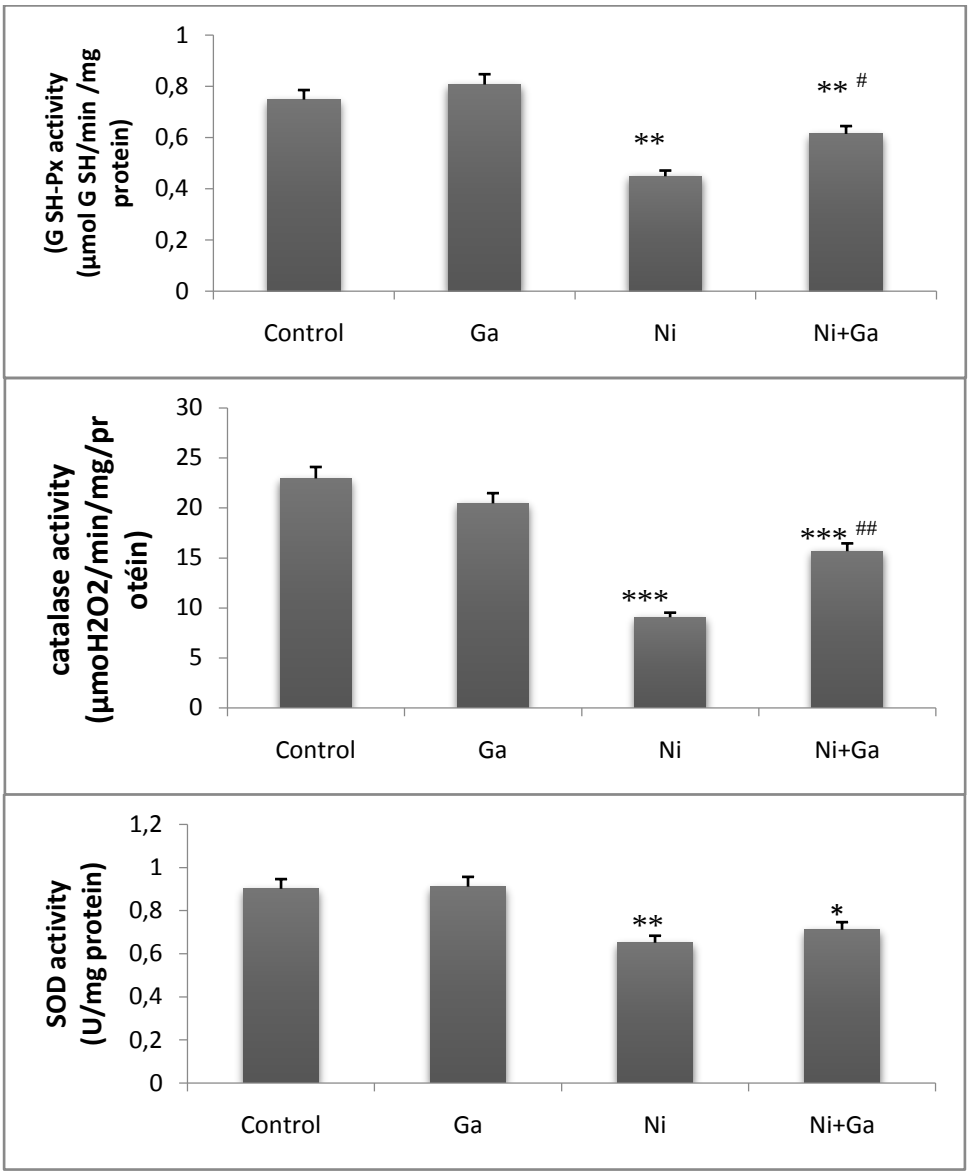

Figure 2. Liver GSH-Px, CAT, SOD activities of control rats, treated with garlic (Ga), nickel (Ni), and nickel with garlic (Ni+Ga) after three weeks of treatment. Values are given as mean± SEM, eight female rats each group. Statistically significant different from control group: *p<0.05, **p <0.01 ***p<0.001; from nickel group: $^{\#}$p<0.05.

3.4 Histological results

The mentioned biochemical alteration could be referred to the liver histological changes. Livers section of control group (fig. 3A) and garlic group (fig.3B) were showing a normal hepatic plate radiating from a thin walled central vein separated by blood sinusoids lined by endothelial cells. Histopathological studies indicated that the administration of nickel led a severe liver damage including extensive degeneration of hepatocytes with the presence of cellular debris within a central vein and necrosis, inflammation, cytological vacuolization (fig. 3C). The supplementation of garlic mentioned prominent recovery in the form of the hepatic histo-architecture such as the decreased cytoplasmic vacuolization and the normal sinusoidal spaces (fig. 3D).

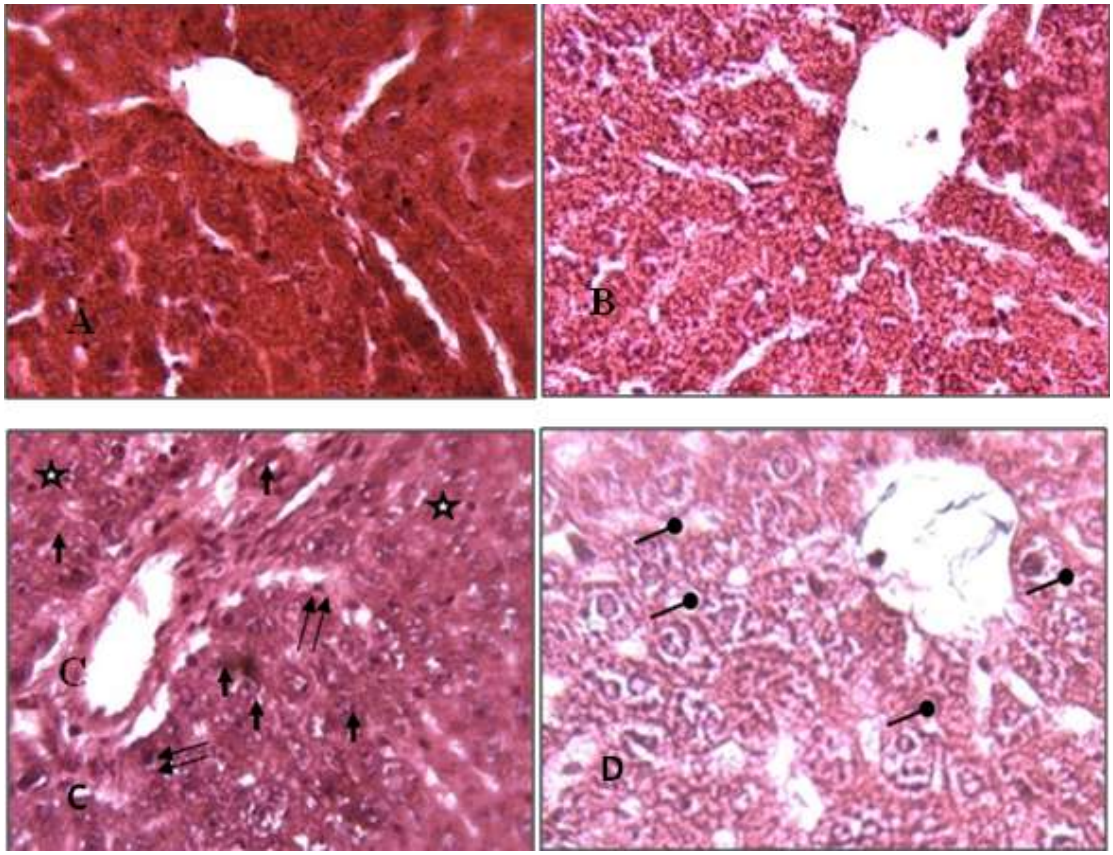

Figure 3. Light photomicrograph of liver sectional histology from control rats (A), garlic (B), nickel (C) and nickel+ garlic (D) (H&E. X 400).
: indicate a presence of cellular degeneration, Necrosesatocytaire. ★ *: Multifocal inflammatory of hepatocytes.* *: Hepatocyte vacuolization.* *: Regenerative cells.*

4. DISCUSSION

Heavy metals are toxic and do not have biodegradable properties, remaining in the ecosystem [30]. Nickel is omnipresent at low levels in the environment due to its abundance in the earth's crust. Nickel is used in industry for alloys, coins, batteries and electroplating but human industrial activities contaminate the environment resulting in unnatural high levels of nickel. Nickel compounds have been classified as class I carcinogens by the International Agency for Research on Cancer [31, 32]. It is a well-known cytotoxic metal that may affect various organs particularly liver cells [33, 34]. In garlic, there are at least 100 bioactive volatile sulfur compounds and nonvolatile with medicinal values that contribute to its pharmacological uses including S-allylcysteine, saponins, ajoene, and flavonoid phenolic compounds [12]. These bioactive constituents play an important therapeutic effect for many diseases such as risk of cardiovascular disease (anti-hypercholesterolemia, anti-coagulant and anti-hypertensive) [20]. Therefore, this study was devoted to determine the beneficial effects of garlic on nickel-induced hepatic injury in rats. The decreases in final body weight, food and water intake along with significant increase in liver weight were seen in nickel-exposed group. These findings are concomitant with previously published reports [35]. The diminution in weights might as a result of the overall augmentation degeneration of lipids and proteins [36] and a diminution of appetite. However, the increased of absolute and relative liver weights might be as a result of the selective accumulation of nickel in the liver [37] 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 variations in these physiological parameters were obviously reversed by garlic treatment. Many reports indicated that garlic compounds induce positive effects. In other words, garlic has an ameliorative effect against nickel-alteration in body weight. So, certainly that enhancement of body weight would result in a concomitant increase in the daily food consumption and promotion of protein synthesis [38, 39]. The augmentation in serum glucose is a common result of nickel toxicity and is usually linked with inhibition of insulin release from Langerhans 'islets [40] or, the high glycogen breakdown and new supply of glucose production from other non-carbohydrate sources such as proteins [41]. Meanwhile, there was an amelioration of blood glucose concentration in nickel animals treated with garlic. So, the decrease of glucose concentration in animals treated with *Allium sativum*, it can be explained that garlic has a hypoglycemic effect, which it helps to increase insulin levels and ensures better storage of hepatic glycogen due to one of its sulfur components like S-allyl cysteine [42, 43]. In the present investigation, higher activities of serum transaminases and ALP have been found in nickel-treated rats. That is indicative of cellular leakage and loss of functional integrity of the hepatic cell membranes implying

hepatocellular destruction, which gives a sign on the hepatotoxic effect of this metal [44]. Mreover, low total protein and high bilirubin concentrations in serum signify the state of the liver damage [45]. These findings confirm the work of Sidhu et al [3]. The diminution in serum total protein in nickel treated rats might be due to changes in protein synthesis and/or metabolism [46, 47] and the hyperbilirubinemia might be owing to excessive heme destruction and blockage of biliary tract. This obstruction may have resulted to mass inhibition of conjugation effect and release of unconjugated bilirubin from broken and dead hepatocytes [45]. Interestingly, the biochemical perturbations seem to be correlated with the liver histological alterations such as the presence of cellular debris within a central vein and a cytoplasmic vacuolization, plasma membrane destruction and cellular hypertrophy. Previous histological studies on liver have documented nickel-induced changes characterized by dilated sinusoids, vacuolization and the appearance of hepatic cells with distorted nuclei [48, 49]. Garlic is an antioxidant can stabilize the hepatic cell membrane and protect hepatocytes against the toxic effects of nickel which can decrease the leakage of enzymes to plasma [50]. Significant restoration of hepatic serum marker enzymes, total proteins, and bilirubin was noticed in the animals treated with garlic offering protection against Ni toxicity in rats. This means that garlic will reduce liver damage by maintaining the integrity of the cell membrane. Otherwise, the hepatoprotective activity of garlic unlikely due to its direct radical scavenging activity [51]. The findings obtained in this investigation confirm that chronic intoxication with nickel caused an increase of LPO level in livers of rats as indicated by the significant increase in MDA. This increase reflected an MDA attack of lipid membranes by free radicals induced by nickel. It has been reported that administration of nickel resulted in the accumulation of iron, which in turn generate ROS via Haber–Weiss and Fenton's reaction [52]. The significant decrease in GSH in nickel treated group was in accordance with previous reports [35].The decline of GSH level may be due whether to the higher affinity of sulfhydryl group of cysteine moiety of glutathione to of metals, forming thermo-dynamically stable mercaptide complexes with several metals [53].The results showed also that nickel administration induced a significant decrease GSH-Px, CAT and SOD activities, which confirm the work of Boulila et al [54]. This possibly is the consequence of the intracellular accumulation of ROS with subsequent development of livers injury, and might be due to their increased utilization in scavenging free radicals induced by the metal, consequently causing irreversible inhibition in their activities or due to direct binding of the metal to the active sites of these enzymes [55].In the present study, it was observed that garlic elicit antioxidant action by scavenging reactive oxygen species (ROS), enhancing the cellular antioxidant enzyme superoxide dismutase, glutathione peroxidase, catalase and increasing glutathione in the cells and significant decrease in MDA, which coincide with the work of Abdel-Daim [56,58] reported that garlic and its major organosulfur constituents had a scavenging effect on hydrogen peroxide, and inhibited the chain of oxidation induced by a hydrophilic radical initiation.

In conclusion, this investigation indicated that the exposure to nickel caused hepatotoxicity through affecting biochemical parameters, antioxidant parameters and histology of liver. However, the supplementation of garlic restored these disturbances provoked by nickel, which were undoubtedly through its antioxidant properties and inhibiting ROS generation.

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