THE BENEFIT EFFECT OF GINGER SUPPLEMENTATION AGAINST NICKEL-INDUCED HEPATOTOXICITY IN ALBINO WISTAR RATS

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ABSTRACT

Objective: This study was performed to study the potential capacity effect of ginger on the modulation effects of nickel-induced hepatotoxicity.

Methods: Thirty-two female albino Wistar rats were divided into four groups of eight each. One served as a control group, the second group (Gi) received ginger 20 g/kg diet, while the third group (Ni) was given nickel 800 mg/L in their drinking water as NiSO₄.₆H₂O and the fourth group (Ni+Gi) was treated daily with both nickel and ginger. The experiment was lasted for 21 days.

Results: The exposure to nickel led to a 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, cholesterol, triglyceride, total lipids, bilirubin, malondialdehyde (MDA) concentrations and glutamate-pyruvate transaminase, glutamic oxaloacetic transaminase, and alkaline phosphatase activities. Meanwhile, serum total proteins and liver reduced glutathione (GSH) levels, catalase, GSH peroxidase, and GSH superoxide dismutase activities were decreased. These results are substantiated with marked changes in the histopathology, whereas the supplementation of ginger resulted in a restoration of the previous parameters.

Conclusion: It seems that ginger supplementation is a potent factor for reducing the oxidative severity of nickel hepatotoxicity through its antioxidant action.

Keywords: Nickel, Ginger, Glutathione, Liver, Hepatotoxicity, Rats.

INTRODUCTION

Heavy metal hazards on human and animal health are increased and represent global environmental problems [1]. There are a large group of substances commonly found in the environment as natural elements or as a result of human anthropogenic activities. Nickel (Ni) is one of those elements that may exist in various forms [2]. The metal is widespread distribution in the environment due to its wide utilization in various industries. Ni is also a nutritionally essential trace element for several animal species, microorganisms, and plants [3]. Humans are generally exposed to Ni through food, water, and air produced from wide different sources including the production of stainless steel, alloys, catalysts, foundries, welding rods, jewelry, paints, and dental and medical implantation, coinage, electroplating, and batteries manufacture [4,5]. Moreover, Ni cannot be metabolized and accumulates in the body, where it can be found in high level in certain organs, especially liver, kidney, and excreted through bile and urine [6]. Liver is a vital organ that has many functions including detoxification of drugs, protein synthesis, secretion of bile, storage of glycogen, and vitamins [7]. Ni is a well-known cytotoxic metal that may affect various organs, particularly liver cells [8]. The metal generates reactive oxygen species (ROS), which lead to lipid peroxidation (LPO), oxidation of DNA, and proteins and results in cell apoptosis [9], immunotoxic [10], hematotoxicity [11], neurotoxic [12], reproductive toxic [13], pulmonary toxic [14], hepatotoxicity [15], and nephrotoxicity [16]. A number of medicinal plants have been described to acquire ROS scavenging and cytoprotective properties [17,18]. In recent years, the natural products and their active ingredients as sources for new drug discovery have attracted attention of many scientists. Ginger, the rhizome of Zingiber officinale, is one of the most widely used species of the ginger family (Zingiberaceae). In other words, it is the most commonly used spice over the entire world. It contains more than 60 different active ingredients including volatile (hydrocarbons and sesquiterpenes) and non-volatile (gingerols, shogaols, paradols, and zingerone) compounds, in addition to various minerals, vitamins, and enzymes [19-21]. Ginger is a strong antioxidant substance and may either mitigate or prevent generation of free radicals and protect the cells from lipid oxidation [22]. Hence, ginger is known to have many health benefits and applied to treat respiratory diseases [23], nausea [24], and a cholesterol-lowering herb [25]. Furthermore, it has reported that ginger possesses other several beneficial pharmacological effects including gastroprotective [26], antiviral [27], and antidiabetic [28]. Thus, the present study was designed to evaluate whether ginger supplementation could have a protective effect against Ni-induced hepatotoxicity in male albino Wistar rats.

MATERIALS AND METHODS

Chemicals
Ni sulfate (NiSO₄.₆H₂O), 5, 5’dithiobis-(2-nitrobenzoic acid (DTNB)) and reduced glutathione (GSH), epinephrine, and thiobarbituric acid chemicals were supplied by Sigma Chemical Co. (St. Louis, France) and all other chemicals used in the experiment were of analytical grade.

Preparation of ginger
Fresh Zingiber rhizomes was purchased from the local market, 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 preparation of experimental diet.

Animals
Thirty-two female albino (Wistar) rats with a body weight of 216–223 g were obtained from Pasteur Institute, Algiers, Algeria. Animals

Effect of nickel on liver function tests
The results of liver function tests are presented in Table 1. The level of liver enzymes such as aspartate transaminase (AST) and alanine transaminase (ALT) was significantly increased in Ni-treated groups in a dosedependent manner in comparison with the control group. Interestingly, these changes were significantly decreased with ginger supplementation. The liver total protein and albumin levels were significantly decreased in Ni-treated groups compared to those in the control animals. The results indicated that nickel administration significantly increased the level of total bilirubin in Ni-treated groups, whereas ginger supplementation significantly reduced these values. In addition, the level of serum glutamate-pyruvate transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and alkaline phosphatase (ALP) was significantly increased in Ni-treated groups as compared with those in the control group. Interestingly, these changes were significantly decreased with ginger supplementation. It is noteworthy that the level of serum total proteins and albumin in Ni+Gi group was significantly higher than Ni group, which might be due to the protective effect of ginger against Ni-induced liver injury. In the present study, Ni administration significantly decreased the level of liver reduced glutathione (GSH) content, catalase, GSH peroxidase, and GSH superoxide dismutase activities as compared with those in the control group. Interestingly, these changes were significantly increased with ginger supplementation. In addition, it is noteworthy that Ni+Gi group had the highest level of GSH content, catalase, GSH peroxidase, and GSH superoxide dismutase activities among all groups. Furthermore, the results of histopathology indicated that liver histology of Ni-treated groups was characterized by necrosis, vacuolar degeneration, and infiltration of inflammatory cells. Interestingly, these changes were significantly decreased with ginger supplementation. It is noteworthy that Ni+Gi group had the normal histology of liver among all groups.

Conclusion
The results of the present study indicated that ginger supplementation could have a protective effect against Ni-induced hepatotoxicity through its antioxidant action. The present study provides new insights into the potential role of ginger in the prevention and treatment of Ni-induced liver injury.
were acclimated for 2 weeks for adaptation under the same laboratory conditions of photoperiod (12 h light/12 h dark) with a relative humidity of 40% and room temperature of 22±2°C. Food and water were provided ad libitum.

Experimental design
Animals were randomly divided into four groups of eight each. One served as a control group. The second group (Gi) received a standard diet supplemented with 20 g/kg diet of ginger. The third group (Ni) was given Ni 800 mg/L in their drinking water as Ni sulfate (NiSO₄·6H₂O) and the fourth group (Ni+Gi) was treated with both ginger and Ni. The doses of Ni and ginger used in the present experiment were selected on the basis of previous studies of Siddhu et al. [29] and Kim et al. [30], respectively. The experimental procedures were carried out according to the National Institute of Health guidelines for animal care and approved by the ethics committee of our institution. Treatments of rats continued for a period of 3 weeks. Body weight and food intake 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 min at 3000 rpm and serum was used for glucose, total protein, direct bilirubin, glutamic-pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), and alkaline phosphate (ALP) assays. Liver was removed immediately and one part was processed immediately for assaying LPO (MDA), reduced GSH, and antioxidant enzyme activities including GSH peroxidase (Px), catalase (CAT), and superoxide dismutase (SOD). The other parts were used for the histological study.

Analytical methods
Determination of biochemical parameters
Glucose, GPT, GOT, ALP, total proteins, and direct bilirubin were assessed using Spinreact Laboratory Spain diagnostic kits and spectrophotometer (Jenway 6505, Jenway LTD, Essex, UK). The references of kits were as follows: Glucose-410111, GOT-1001161, GPT-1001171, ALP-1001131, total proteins-1001291, and direct bilirubin-1001041.

Measurement of hepatic oxidative stress parameters
Tissue preparation
About 1 g of liver was homogenized in 2 ml of buffer solution of phosphate-buffered saline 1:2 (w/v; 1 g tissue with 2 ml TBS, pH 7.4). Then, the homogenates were centrifuged at ×10,000 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.

Estimation of LPO (MDA)
The LPO level in liver homogenate was measured as malondialdehyde (MDA), which is the end product of LPO, and reacts with thiobarbituric acid (TBA) as a TBA reactive substance to produce a red-colored complex with a peak absorbance at 532 nm according to the method of Buege and Aust [31].

Estimation of reduced GSH
Liver GSH content was estimated using a colorimetric technique, as mentioned by Elman [32] modified by Jollow et al. [33], 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. Then, tubes were centrifuged at ×2500 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.

Determination of GSH-Px
GSH-Px activity was measured by the procedure of Flohé and Günzler [34]. Supernatant obtained after centrifuging 5% liver homogenate at ×1500 g for 10 min followed by ×10,000 g for 30 min at 4°C was used for GSH-Px assay. About 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 H₂O₂ (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 g for 5 min and the supernatant was collected. Subsequently, 0.2 ml of phosphate buffer (0.1 M, pH 7.4) and 0.07 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 412 nm.

Assay of CAT
CAT activity was determined according to the method of Aebi [35]. 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 H₂O₂ (0.5 M) was prepared. The reaction was started by adding H₂O₂ 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⁻¹cm⁻¹.

Estimation of SOD
SOD activity was determined according to the method described by Misra and Fridovich [36]. About 10 µl of tissue homogenate were added to 970 µl of EDTA-sodium carbonate buffer (0.05 M) at pH 10.2. The reaction was started by adding 20 µl of epinephrine (30 mM) and the activity was measured at 480 nm for 4 min.

Protein determination
The protein content of tissues samples was measured by the method of Bradford [37] using bovine serum albumin as a standard.

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 using a graded ethanol series and then embedded in paraffin. The paraffin sections were cut into 5 µm thick slices and stained with hematoxylin and eosin. The preparations were then observed under an optical microscope and photographed [38].

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

RESULTS
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 Ni were significantly decreased by ~36% and ~35%, respectively.

Table 1: Initial body weight, final body weight, food intake, and absolute and relative liver weights of control rats treated with ginger (Gi), nickel, and nickel plus ginger (Ni+Gi) after 3 weeks of treatment

| Parameter                | Experimental groups |
|--------------------------|---------------------|
|                         | Control | Gi | Ni | Ni+Gi   |
| Initial body weight (g)  | 217±16  | 223±15 | 216±23 | 216±13 |
| Final body weight (g)    | 302±17  | 291±10 | 191±17*** | 262±13*** |
| Weight (g)               | 20±0.49 | 13±1.35*** | 17±0.90*** |
| Food intake (g/day/rat)  | 6.11±0.23 | 6.16±0.32 | 9.64±0.73*** | 6.72±0.36*** |
| Absolute liver weight (g)| 2.70±0.05 | 2.82±0.10 | 3.66±0.11*** | 2.94±0.16*** |

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.
Effect of treatment on hepatic oxidative stress parameters

As shown in Figs. 1 and 2, the exposure to Ni 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), CAT (p<0.001), and SOD (p<0.01) activities. However, supplementation with ginger in association with Ni ameliorated most these stress oxidative biomarkers.

Histological results

Livers section of control group (Fig. 3a) and ginger group (Fig. 3b) was showing normal hepatic plates radiating from a thin-walled central vein separated by blood sinusoids lined by endothelial cells. In contrast, liver of nickel treated group had shown pathological alterations such as the presence of cellular debris within a central vein and cytoligical vacuolization, inflammation and necrosis (Fig. 3c). However, the supplementation of ginger mentioned prominent recovery in the form of the hepatic histarchitecture such as the decreased cytoplasmatic vacuolization and the normal sinusoidal spaces (Fig. 3d).

DISCUSSION

It is known that liver is the most target organ of toxicity [39,40]. A number of drugs, chemicals, and heavy metals have been shown to alter its structure and function. Animal experiments and human studies are conclusive about metabolic adverse effects and hepatotoxicity of Ni element [41]. Ginger is very strong antioxidant compound, which is well documented for the attenuation of oxidant mediated liver damage induced by various xenobiotics. Therefore, this study was devoted to determine the beneficial effects of this spice on Ni-induced oxidative liver injury in rats.

The findings of the present study showed a significant reduction in body weight of Ni exposed group. The decreased body weight is concomitant with previously reports [42,43]. This reduction in weights might as a result of the overall increased degeneration of lipids and proteins [9] and a decrease of appetite. The altered body weight was retrieved to near normal value due to the antioxidant effect of ginger. Many studies have reported that ginger showed a significant protective effect against damage induced by heavy metals such as arsenic and cadmium [44,45]. The results indicated also an augmentation of blood glucose in Ni animals. This may be linked with inhibition of insulin secretion from Langerhans’ islets and a block of glucose utilization by cells [46], or the high glycogen breakdown and new supply of glucose production from other non-carbohydrate sources such as proteins [47]. Meanwhile, there was an amelioration of blood glucose concentration in Ni animals treated with ginger. The effect of ginger on glucose reduction could be explained by the fact that gingerol; the most important bioactive compound of ginger has a variety of mechanisms to upregulate blood glucose [48,49]. In addition, it is reported that ginger inhibits α-amylase and α-glucosidase enzymes in carbohydrate digestion [50]. In the present investigation, higher activities of serum transaminases and ALP have been found in Ni-treated animals. That is an indicative of cellular leakage and higher activities of serum transaminases and ALP have been found in Ni-treated animals. That is an indicative of cellular leakage and high glycogen breakdown and new supply of glucose production from other non-carbohydrate sources such as proteins [47].

Table 2: Changes of biochemical parameters for control rats treated with ginger (Gi), nickel (Ni), and nickel plus ginger (Ni+Gi) after 3 weeks of treatment

| Parameter          | Control | Gi         | Ni         | Ni+Gi       |
|--------------------|---------|------------|------------|-------------|
| Glucose (g/l)      | 1.13±0.06 | 1.02±0.03  | 1.35±0.07  | 1.00±0.04***|
| Triglyceride (mg/dl)| 70.23±12.03 | 64.21±2.69 | 101.31±1.12 | 85.16±7.83* |
| Cholesterol (mg/dl)| 74.16±6.16 | 64.83±4.95 | 110±1.58*** | 80±6.10***  |
| Direct bilirubin (mg/dl) | 0.4±0.18 | 0.4±0.12   | 0.80±0.04* | 0.55±0.12*  |
| Total lipids (mg/dl) | 330±39   | 328.16±17.45 | 415±18.00*** | 370±11.38*** |
| Total protein (mg/dl) | 7.3±0.44 | 7.6±0.35    | 5.49±0.2**  | 6.16±0.25**  |
| GOT (U/L)          | 75.05±1.74 | 73.95±1.83 | 137.72±9.11*** | 92.07±5.24*** |
| GPT (U/L)          | 44.33±5.67 | 45.10±2.60 | 65.32±6.88** | 45.01±2.5**  |
| PAL (U/L)          | 115±1.57  | 115.5±0.42 | 269.66±3.53** | 214.16±4.99*** |

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<0.001; from nickel group: #p<0.05, ###p<0.001.

Effect of treatment on liver damage parameters

Treatment with Ni caused a significant increase (p<0.05) of serum glucose and direct bilirubin; highly significant (p<0.01) for triglycerides, GPT and ALP; and very high significant (p<0.001) for cholesterol and total lipids. Meanwhile, the concentration of serum total protein was diminished (p<0.01). Nevertheless, the supplementation of ginger in combination with Ni produced a significant recovery in the above-mentioned biochemical parameters (p<0.05) for total protein, total lipids, triglycerides, and direct bilirubin; p<0.01 for GPT; and p<0.001 for glucose, cholesterol, GOT, and ALP (Table 2).
loss of functional integrity of the hepatic cell membranes implying hepatocellular destruction, which gives a sign on the hepatotoxic effect of this metal by the induction of oxidative stress [51]. In general, serum total protein and bilirubin concentrations signify the state of the liver damage [52]. In that case, the reduction in the proteins concentration of Ni-treated rats probably due to changes in protein synthesis and/or metabolism [53]. In addition, the discovered hyperbilirubinemia might be due to excessive heme destruction and blockage of biliary tract in Ni-treated rats. This obstruction may have resulted to mass inhibition of conjugation effect and release of unconjugated bilirubin from broken and dead hepatocytes [52]. 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. Significant restoration of hepatic serum marker enzymes, total proteins, and bilirubin was noticed in the animals treated with ginger offering protection against Ni toxicity in rats. This means that ginger will reduce liver damage by maintaining the integrity of the cell membrane. Otherwise, the hepatoprotective activity of ginger may be due to its direct radical scavenging activity [54]. Nickel is well known to induce oxidative damage in liver by enhancing LPO [55]. LPO is supposed to cause the destruction and damage cells membranes, leads to changes in membrane permeability and fluidity [56]. Corroborate with these findings, treatment with Ni resulted in a significant increase in LPO as indicated by the significant increase in MDA. It has been reported that administration of Ni resulted in the accumulation of iron, which, in turn, generates ROS through Haber–Weiss and Fenton’s reaction [4]. The significant decrease in GSH in Ni-treated group was in accordance with the previous reports [41,57]. The findings showed also that Ni administration induced a significant decrease GSH-Px, CAT, and SOD activities, which confirm the work of Boulla et al. [58], Hfaïedh et al. [59], and Misra et al. [55]. This might be due to their increased utilization in scavenging free radicals induced by the metal, thus causing irreversible inhibition in their activities or due to direct binding of the metal to the
Fish graph: Multifocal inflammatory hepatocyte vacuolization. Effects of 2. Sengupta P. Environmental and occupational exposure of metals
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shown that consumption of nutrient-rich antioxidants such as ginger decreased heavy metals toxicity and diabetic complications [6,63]. In
brief, ginger has an ability to increase the intracellular activities of SOD, CAT, and GSH enzymes and has synergistically combats oxidative stress by scavenging free radicals and augmenting endogenous antioxidant
activities [64]. This may be due to ginger contain many antioxidants compounds that may modulate the antioxidants enzymes in Ni hepatotoxicity. Gingerol is one among these antioxidant compounds which are responsible for the inhibition of LPO.

CONCLUSION
The investigation demonstrated that Ni is capable of causing marked oxidative stress in addition to deplete the antioxidants and inhibiting antioxidant enzyme activities. However, the treatment with ginger attenuated the Ni-induced oxidative hepatotoxicity through its antioxidant properties. Hence, the study indicated that ginger could be regarded as a potential candidate for the therapeutic intervention in hepatotoxicity.

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AUTHORS’ CONTRIBUTIONS
Zine Kechrid formulated the present hypothesis. Zine Kechrid was also responsible for writing the report. Sara Derbal was responsible for the analysis of the data.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

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