The evaluation of vitamin C effect on nickel induced hepatotoxicity in albino-Wistar rats

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ABSTRACT (10 PT)

The nickel is an environmental harmful metal, which is able to induce severe health problems and cancergenic effects. Vitamin C is a naturally occurring organic compound with antioxidant properties and it can reduce or neutralize reactive oxygen species. Thus, this study was aimed to search the probability protective effect of this vitamin on certain physiological and biochemical hepatic parameters in rats exposed to nickel toxicity. Rats were divided into four groups (six each). Group 1 served as control group and the other three experimental groups received vitamin C (1g/L), nickel (800mg/L) and vitamin C with nickel in their drinking water respectively. Body weight, and food intake and water consumption were measured and the treatment lasted for three weeks. According to the results, a decrease in body weight and a rise in liver weight were observed. These findings indicated also variation in biochemical hepatic parameters including an increase in bilirubin concentration and the activities of AST, ALT and Alkaline phosphatase, whereas, the administration of vitamin C brought all the previous parameters to their normal values. This research indicated that nickel led to hepatotoxicity via oxidative stress. However, the supplementation of vitamin C treated the previous parameters. Therefore, the findings confirmed that vitamin C had a powerful effect against nickel toxicity through its antioxidant capacity.

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Introduction

There are many types of heavy metals such as copper (Cu), nickel (Ni), cobalt (Co), Cadmium (Cd) and iron (Fe). Both natural and manufactured sources, including volcanos, pollution, mining, industrial discharge, vehicle gas exhausts release such metallic particles into the environment. Heavy metals are not recyclable, unlike organic pollutants, and therefore tend to accumulate in organisms. So, most of them are understood to be carcinogenic. Due to extended and ongoing exposure to heavy metals, a number of negative health risks are known [1]. Humans are exposed to heavy metals through cutaneous contact, ingestion through the skin, inhalation, food intake and drinking water [2].

Nickel, a known heavy metal, is found at very low levels in the environment due to industrial activities, like the production of batteries, paints and artificial medical implant. Therefore, it is considered an important material in industry, which can cause public health problems. Exposure to nickel compounds is inevitable; this exposure might be via contaminated water and food [3]. The metal cannot be metabolized and in a high level, it deposits in certain organ especially the liver and the kidney causing serious health effects [4]. Furthermore, nickel is cytotoxic metal that induces kidney cell damages [5]. The most possible mechanism in Ni toxicity is the generation of reactive oxygen species (ROS) which initiates lipid peroxidation, causing damage to macromolecules like proteins and DNA [6]. Recently, todays antioxidants have received high attention due to their capacity to remove reactive oxygen species that provokes oxidative tissue injuries and cellular death [7].

Ascorbic acid is water-soluble micronutrient required for multiple biological functions. It is necessary for normal growth and development. Some animals have not the capacity to produce ascorbic acid; therefore, their need is dependent upon diet to maintain sufficient vitamin C levels, necessary for their

metabolism and oxidative protection. Ascorbic acid is an antioxidant agent, which plays a role in neutralizing reactive oxygen species. The vitamin has also roles in different physiological processes, such as immune stimulation, synthesis of collagen, hormones, neurotransmitters, iron absorption, and detoxifying the body from heavy metals. On the other hand, vitamin C can act sometimes as a pro-oxidant, in the presence of transition metals, such as iron and copper [**8**]**.**

Therefore, this study is intended to examine the toxic impact of nickel and evaluating the protective function of vitamin C against the hepatotoxicity in female rats. In other words, this study seeks to explore the effects of nickel on certain physiological and hepatic biochemical parameters, and to evaluate the protective effect of vitamin C against the hepatic oxidative damage induced by nickel.

2. Materials and methods

2.1. Biological materials

Twenty-four female albino-Wistar rats weighing 210–228 g, were obtained from Pasteur Institute (Algiers, Algeria). Prior to experiments, the animals were allowed to acclimate to their surroundings for two weeks. Rats were housed in plastic cages for 21 days. Standard diet (table 1) and water tape were available *ad-libitum*. The environment conditions were characterized by temperature and photoperiod normal.

Table 01: Components of the diet (ONAB EL-Harouch, Skikda)

Ingredients	**Quantity (g/kg)**	**Percentage (% in 1 kg)**
Corn starch	300	30 %
Sucrose	210	21 %
Soja	200	20 %
Fiber	60	6 %
Mineral mix	100	10 %
Vitamin mix	30	3 %
Calcium	100	10 %

2.2 Experimental design

Rats were divided into 4 groups; 6 each:
Group 1 (control group): received tap water.
Group 2: received vitamin C dissolved in drinking water 1g/l [**9**]**.**
Group 3: received 800 mg/l as $NiSO_4$6H2O dissolved in drinking water [**10**]**.**
Group 4: received vitamin C associated with nickel dissolved in drinking water.

2.3-Measurement of body weight, food intake and water consumption

Body weight was measured every three days. While, food intake and water consumption were recorded every day.

2.4- Collection of blood samples and liver

After 21 day of treatment, animals were sacrificed by decapitation without anesthesia. The blood was collected immediately into heparin tubes and were taken to the analysis laboratory to determine various hepatic parameters AST, ALT, alkaline phosphatase and total bilirubin. After incision of the abdominal wall, the liver was collected, rinsed in NaCl 0.9% and weighted**.**

2.5-Biochemical analysis

Aspartate aminotransferase (AST), alanine aminotranferase (ALT), total bilirubin (TB) and alkaline phosphatase (ALP) were measured depending on reference methods, which are as follows AST (**11**), ALT (**11**), TB· (**12**) and ALP (**13**) respectively.

2.6-Statistical analysis

All the results were expressed as mean values ± SEM. Comparisons between the groups were performed by one-way ANOVA followed by student's t-test. Statistical analysis was realized using origin 6 software. The level of significance was limited at $p < 0.05$

3. Findings

3-1- Effect of treatments on physiological parameters

3-1-1- Effect of treatments on body weights

This study showed the effect of nickel on body weight of animals. Nickel supplementation led to a very highly significant decrease ($p<0.001$) in body weight of rats, however the treatment with ascorbic acid ameliorated the body weight ($p<0.05$). The treatment with vitamin C had no significant effect on body weight (fig.1).

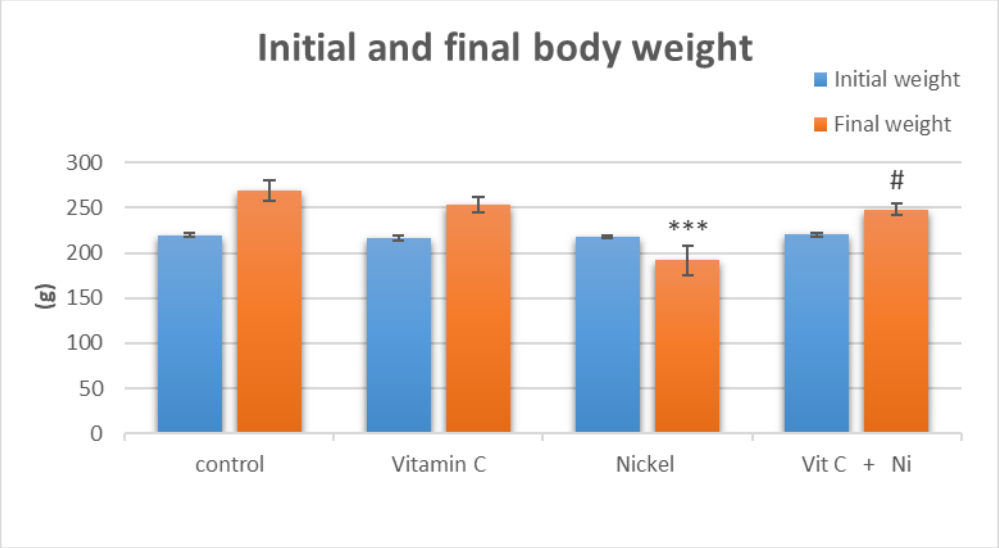

Figure 1: Initial and final body weights of controls, vitamin C, nickel and nickel +vitamin C.
***p<0.001: nickel group compared to control group.
#p<0.05: nickel + vitamin C group compared to nickel group.

3-1-2- Effect of treatments on liver weight

The results illustrated in figure 2 demonstrated that liver weight of rats treated with nickel was slightly increased as compared to controls, but the addition of vitamin C to nickel decreased the liver weight ($p<0.01$). Vitamin C alone treatment have not any effect on liver weight.

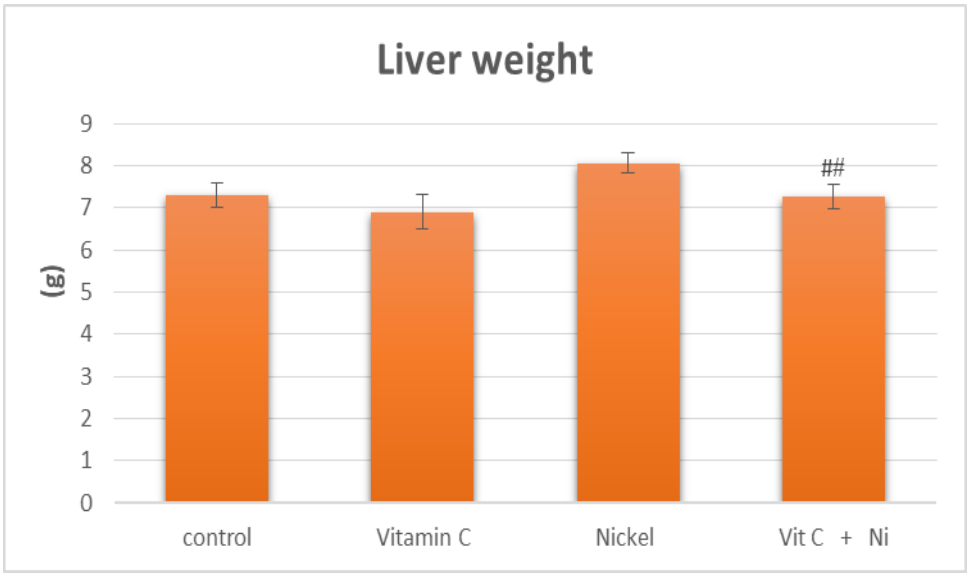

Figure 2: Liver weight of controls, vitamin C, nickel, and nickel + vitamin C.
##p<0.01: nickel + vitamin C group compared to nickel group.

3-1-3- Effect of treatments on food intake

The group affected by nickel showed a significant decrease ($p<0.05$) in food intake in comparison with control group. However, the food intake was slightly increased of rats treated with nickel and vitamin C but not significant different (fig. 3).

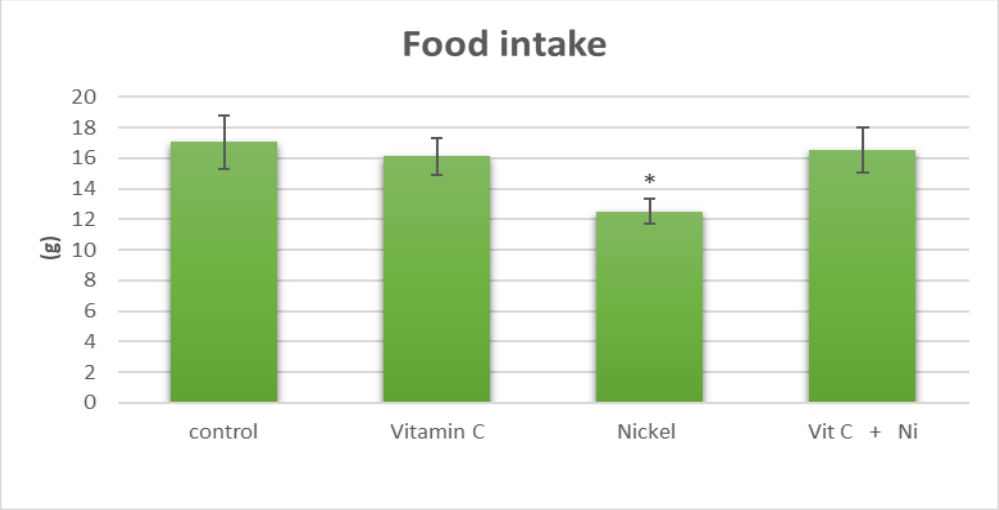

Figure 3: Food intake of controls, vitamin C, nickel and vitamin C + nickel.
*p<0.05: nickel group compared to control group.

3-1-4- Effect of treatments on water consumption

As shown in figure 4, the consumption of water by nickel group was very low ($p<0.001$) as compared to controls. In addition, supplementation of vitamin c with nickel caused a slightly increase of water consumption but not significantly different.

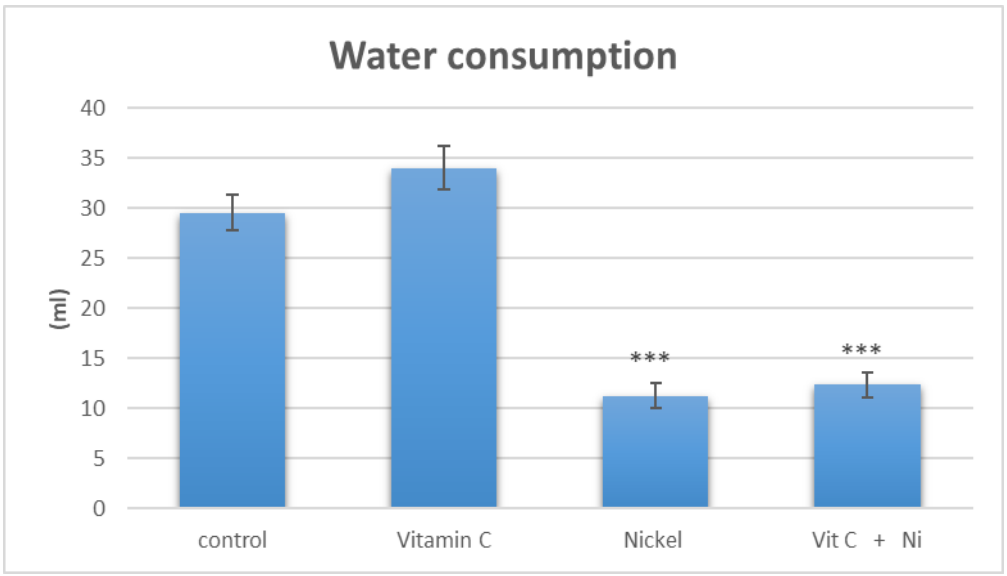

Figure 4: Water consumption of controls, vitamin C, nickel and vitamin C + nickel.
***p<0.001: nickel and vitamin C + nickel compared to control group.

3-2- Effect of treatments on hepatic biochemical parameters

3-2-1- Effect of treatments on aspartate aminotransferase (AST) activity

According to the results obtained, there was high significant increase ($p<0.01$) of AST activity in-group treated with nickel compared to controls. However, the administration of vitamin C with nickel decreased AST activity ($p<0.01$). Vitamin C alone does not affect AST activity (fig. 5).

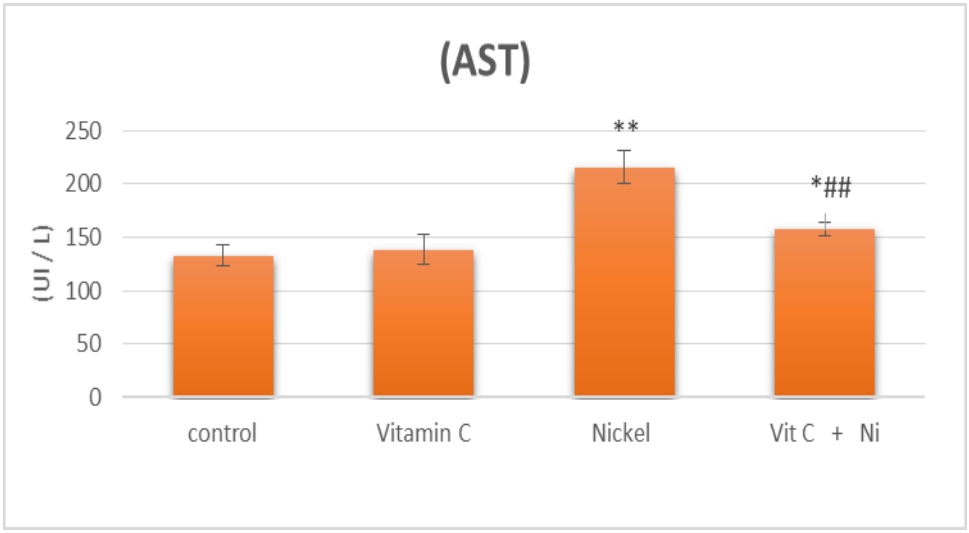

Figure 5: Aspartate aminotransferase (AST) activity of controls, vitamin C, nickel, and nickel +vitamin C.
**p<0.01, *p<0.05: nickel and vitamin C + nickel groups compared to control group.
##p<0.01: nickel + vitamin C group compared to nickel group.

3-2-2- Effect of treatments on alanine aminotransferase (ALT) activity

As showed in figure 6, the metal induced a very significant increase (p<0.01) in ALT activity. Whereas, the supplementation of vitamin C combined with nickel provoked a diminution of ALT activity (p<0.01). No changement of ALT activity in vitamin C group was observed .

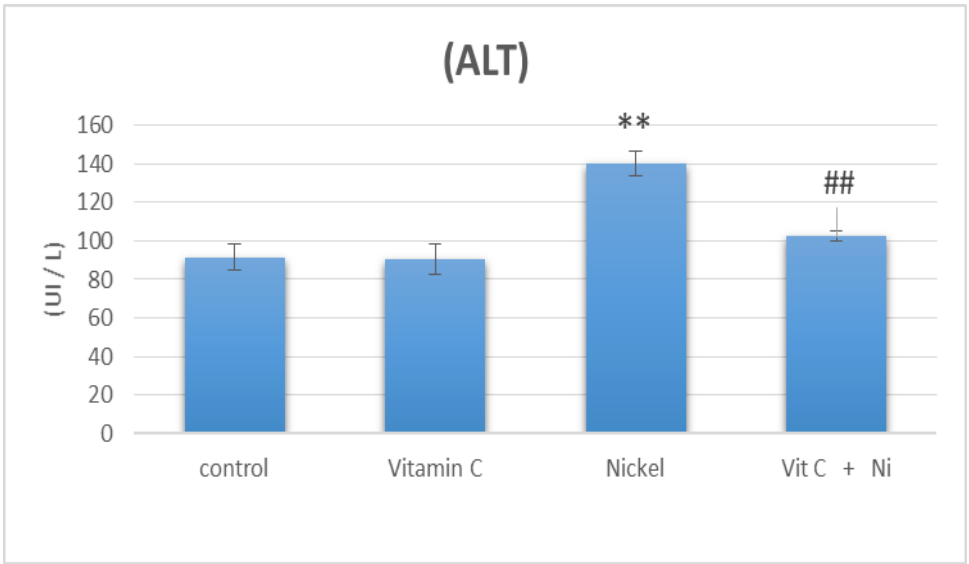

Figure 6: Alanine aminotransferase (ALT) activity of controls, vitamin C, nickel, and nickel +vitamin C.
**p<0.01: nickel group compared to control group.
##p<0.01 : nickel + vitamin C group versus nickel group.

3-2-3- Effect of treatments on total bilirubin

The level of total bilirubin was very high significant (p<0.001) in nickel group. While, the supplementation of vitamin C with nickel restored the level of bilirubin concentration (p<0.05).
The concentration of total bilirubin in vitamin C group does not changed.

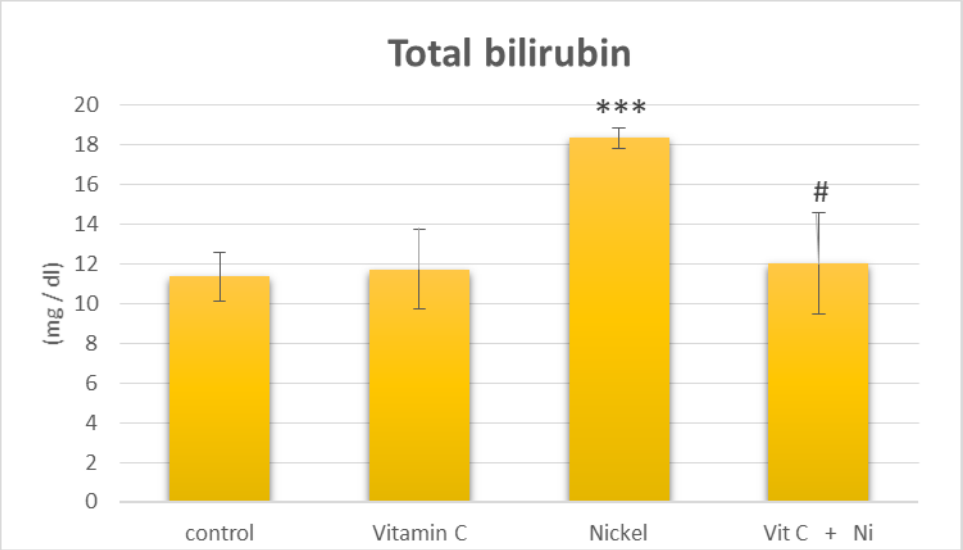

Figure 7: Total bilirubin of controls, vitamin C, nickel and vitamin C + nickel.
***p<0.001: nickel group compared to control group.
#p<0.05: vitamin C + nickel group compared to nickel group.

3-2-4- Effect of treatments on alkaline phosphatase activity

Alkaline phosphatase activity was very significantly increased (p<0.01) in nickel group as compared to controls. While, the administration of vitamin C with nickel decreased alkaline phosphatase activity (p<0.01). The addition of vitamin C separately did not cause any variation of alkaline phosphatase activity.

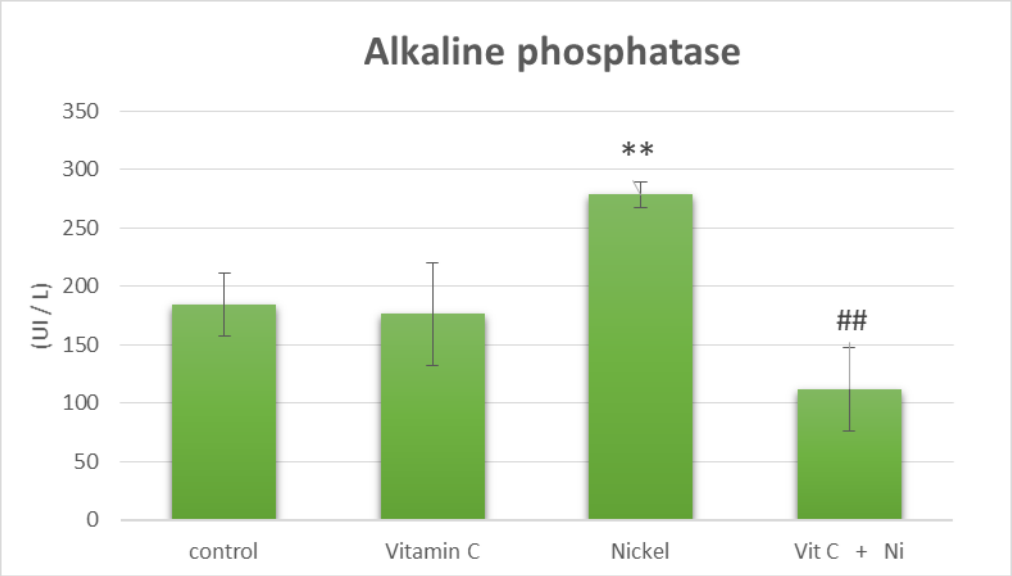

Figure 8 : Alkaline phosphatase activity of controls, vitamin C, nickel and vitamin C + nickel.
**p<0.01 : nickel group compared to control group.
##p<0.01 : vitamin C + nickel group compared to nickel group.

4- Discussion

Nickel is an earth rare metal that was discovered in the 18th century. Metallic nickel and its compounds are utilized in industrial field. The metal is ubiquitously element that found in rocks, soil, plants and water [14). The liver is the principal organ exposed to toxicity through its detoxification function [15]. The nickel generates the reactive oxygen species (ROS), which produce the lipid peroxidation and the later cause damage to proteins, lipids and DNA [6]. It is known that vitamin C is an antioxidant and free radical scavenger [16). So this research

was undertaken to investigated whether vitamin C supplementation have a capacity protection effect on nickel induced oxidative liver damage in rats.

The findings got in the present investigation showed that the administration of nickel induced a remarkable decrease in final body weight. Numerous studies have shown that heavy metals, including nickel, are capable to induce physiological developmental disturbances. These disturbances are due to inhibition of intestinal absorption and transport of nutrients (amino acids, glucose and certain other essential minerals such as zinc, iron, magnesium…etc….) resulting in poor intake of food as confirmed by the present results and previous reports [17]. In addition, previous studies have shown that the loss of body weight can be explained by the increased catabolism of body lipids and proteins due to the toxic effect of nickel [18]. Furthermore, nickel caused a significant increase of liver weight (hepatomegaly) [19]. This might be related to tissue hypertrophy as a result of high accumulation of this metal in this target organ [20]. However, supplementation of vitamin C to nickel treated rats showed a remarkable improvement in body weight and liver weigh due to increased daily food intake and water consumption. This may be might be to the reduction in free radical accumulation under the protective action of vitamin C [21].

As illustrated in hepatic parameters results, nickel intoxication caused a significant increase in AST, ALT and alkaline phosphatase activities. This is certainly due to membrane cells damage resulting in a rise liberation out of these enzymes from the liver into the blood circulation, which gives an indication on the hepatotoxic effect of this metal [22]. The increase in bilirubin concentration shown in this investigation can be explained by the hepatotoxic effect of nickel, inducing hemolysis of erythrocytes and catabolism of hemoglobin, which are responsible for hyperbilirubinemia or is it a result of a diminution of albumin biosynthesis in the liver, which it is known that the albumin is a responsible for bilirubin transport. However, vitamin C minimized the toxic effect of Ni on the transaminase (AST, ALT), alkaline phosphatase and bilirubin. This antioxidant factor can stabilize the liver cell membrane and protect hepatocytes from the damaging effects of the metal [23].

In that case, the administration of vitamin C had protected liver function from nickel harmful as indicated by the significant restoration of the previous parameters [22].

In conclusion, the obtained findings demonstrated that nickel contamination is responsible for the appearance of major alterations caused by oxidative stress, which affected the enzymatic detoxification systems and defense mechanisms. In other words, this metal caused disturbances in the physiological and hepatic biochemical parameters and these alterations were ameliorated by the administration of vitamin C. In that case, the supplementation of vitamin C attenuated these disturbances caused by nickel, which were undoubtedly through its antioxidant properties and inhibiting ROS generation.

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