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The Effect of Vitamin C Pretreatment on Acute Nickel Nephrotoxicity in Male Mice

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Abstract

This study was conducted to observe the preventive effect of using vitamin C and its effects on impaired kidney function caused by the element nickel. The study included 60 laboratory rats weighing from 29 to 33 grams Among the 60 male mice, 48 adapted mice were selected from them after weighing. These 48 mice were divided into six experimental groups, each group containing eight individuals. The treatment of laboratory mice continued for 30 days. The study showed that there was a significant increase in the weights of the kidneys of rats that were injected with nickel chloride when compared to the control group and the group that was given vitamin C orally, but there were no significant differences in the weights of the kidneys of mice that were given vitamin C previously., but at the same time it occurred an increase in the concentration of uric acid, urea and creatinine in the blood of mice, and this was confirmed by changes in the tissues of the kidneys of mice but the treatment of vitamin C can protect the kidneys from these toxins substance of nickel chloride. The study concluded that treatment with vitamin C can act as antioxidant that protect kidney and hematological elements from damage.

Keywords: nickel, vitamin C, nephrotoxicity, mice

Introduction:

Nickel (Ni (II)) is a heavy metal that is an environmental carcinogen, nephrotoxic, and hepatotoxic (1). It is also used in the production of alloys consisting of steel, iron, steel and components of electronic devices, as its presence increases the strength, hardness and resistance of these metals to corrosion and bear high temperatures (2). Many diseases that affect the kidneys or liver in humans or animals as a result of exposure to Nickel(II) chloride directly in the work environment or indirectly through the environment contaminated with these elements and that these diseases that affect the kidneys or liver lead to tissue changes in these organs of the body (3). This type of element accumulates in some organs of the living body, especially the kidneys (4). Thus, it will lead to an imbalance in the ROS (reactive oxygen species) and the process of lipid peroxidation, which in turn leads to damage to the DNA in the kidneys (5). Oxidative toxicity induced by the presence of nickel accumulation is associated with the depletion of some compounds such as glutathione as well as some endogenously antioxidant enzymes such as CAT (catalase), GST (glutathione transferase), SOD (superoxide dismutase) and GPX (glutathione peroxidase) (6). The harmful effect of some nitrogenous compounds as well as reactive oxidation can be prevented by treatment

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with some anti-oxidant supplements may help to prevent the genotoxic effects of nickel [7]. The use of some vitamins, such as vitamin C and E, has an effective anti- and sweeping role against oxidation and oxidative stress, whose increase may lead to some diseases through the production of free radicals such as hydroxide and free radioactive oxygen, although its role in many functions of the immune system and its regulation in living organisms as well as helping other immune cells to do a more effective job (8,9). These types of vitamins have the ability to dissolve. Vitamin C in ascorbic acid has the ability to dissolve in water components, while vitamin E is known to be soluble in fat elements (alpha-tocopherol) (10). Vitamin C has been shown to reduce toxic metal-induced hepatotoxicity (11, 12) and nephrotoxicity (13, 14). As a result, many studies have recorded the protective role of tocopherol, as well as ascorbic acid, against the toxic effects of nickel on some living tissues of humans and animals (15, 16,). But the dual effects of nickel on liver injury and disorders that may occur liver enzymes need many studies, because many of them remain unknown until now (17). Although the use of synthetic antioxidant supplements in the treatment of acute kidney injuries and other kidney diseases is still under study, vitamin C (ascorbic acid) is a non-protein antioxidant and has a low molecular weight that has the ability to enter the cell may be the treatment in the treatment of kidney injuries Acute and other toxic diseases that may affect the kidneys and some other parts of the body. Vitamin C works to protect the kidneys due to the antioxidant process it provides or by maintaining some necessary enzymes such as monooxygenase and hydroxylase. This is proven in some studies on patients with kidney disease caused by a lack of vitamin C levels (18).

Materials and Methods

1. Chemicals

Sigma Chemical Inc. provided the nickel sulphate (NiSO4 7H2O), vitamin C, and all other chemicals used in the experiment (St. Louis, MO, USA).

Animals

This study included 60 male rats of the Swiss albino type, and the weight of these male rats ranged between 29 to 33 grams. These rats were placed in plastic cages equipped with water and food so that the mice could access them easily and freely. The conditions in which lived consisted of a cycle consisting of 12 hours in the light and 12 hours in the dark in a humid atmosphere of 40% and a temperature of 222 degrees Celsius. All humane and ethical guidelines and practices were followed in all experiments conducted (AFRO. 123, 2009). The chemicals used in this study are from Sigma Chemical Company (St Louis, France).

Experimental procedure

Among the 60 male mice, 48 adapted mice were selected from them after weighing. These 48 mice were divided into six experimental groups, each group containing eight individuals.

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Group(A) is the control group and contains mice with free movement, food and standard water.

Group(B) VC consists of mice that were given vitamin C orally for seven days at a dose of 16.6 grams per kilogram of mouse weight.

Group(C) Ni3 included rats injected I.P(intraperitoneal) of nickel chloride at a dose of 3 mg per kg of body weight.

Group(D)Ni5 included rats injected I.P of nickel chloride at a dose of 5 mg per kg of body weight.

Group(E)Ni6 consisted of rats injected I.P with chloride Nickel at a dose of 10 mg per kg of body weight. After 24 hours of nickel injection some from these mice were killed in (C, D, E) groups.

Group(F) included mice from group B and C(VC+Ni3)

Group(G) included mice from group B and C(VC+Ni5)

Group(H) included mice from group B and C(VC+Ni10)

The three last groups injected with I.P vitamin C for 7 days respectively. On the eighth day, these last three groups were injected with a dose of nickel chloride via IP in doses of 3, 5 and 10 milligrams per kilogram of the mouse's body weight. Also, 24 hours after the nickel injection process, mice were killed using chloroform, then blood was drawn from these killed mice in tubes containing lithium heparin, then placed directly in an ice bath to be centrifuged for ten minutes at a speed of 3000 revolutions per minute at a temperature of 4 ° C. For the purpose of obtaining the serum in order to measure the percentage of uric acid, urea and creatinine in the blood, then the killed mice were dissected for the purpose of obtaining the kidneys and conducting a histological examination on them. Blood elements were measured such as hemoglobin in the blood, the number of white blood cells, the total number of red blood cells, and the package volume of red blood cells according (29).

Determination the concentration of nickel

Depending on the source (25), the concentration of nickel present in the kidney tissue of mice is determined. One gram of fresh tissue is weighed for the kidney and placed in a tissue homogenizer cold KCl (SCILOGEX D160)., then 3 ml of an acidic solution is placed from a mixture of (HClO4/HNO3, 1:3, v/v), then the homogenizer is mixed. Using the microwave, the homogeneous samples are

digested, then the homogeneous extract is taken and the nickel concentration is measured by using an automatic absorption spectrometer.

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Biochemical assays Serum biochemical markers

As functional markers for nephrotoxicity using TOTAL PROTEIN BIURET Method from biolabo company.

Histopathological analysis

Kidneys taken from killed mice are placed in the experiment and fixed for 24 hours in saline concentration consisting of formalin with 10% salt. Using a rotary slicer, the tissue eye is cut into sections with a section thickness of five to six millimeters, then stained with hematoma-xylene and eosin, before being placed in a medium composed of paraffin-free xylene in order to be ready for microscopic examination.

Table (1): The concentration of nickel(Ni) and the concentration of vitamin C (Vit C) with it is a concentration together (Ni +Vit C) and its effect on kidney weight and food and water intake.

Group	Body weight(g)		Kidney weigh (g)		Food intake g/100gbwt	
	initial	final	Absolute	Relative		
Control mean	0.6±30	1.11±41	0.05±1.2	0.03±0.33	0.34±6.62	0.37±7.81
Vit C mean	0.7±31	0.22±38	0.02±1.19	0.01±0.34	0.26±6.09	0.24±7.06
Ni mean	1.05±29	0.13±18	0.07±18	0.02±1.17	0.15±3.52	0.17±4.46
Ni +Vit C mean	0.9±32	1.21±26	0.02±1.4	0.04±0.7	1.2±4.7	0.5±6.75

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Table (2): mice blood parameter (WBC ,RBCs ,Hb ,PCV) in control group ,Vit C ,Ni ,and Ni+Vit C group.

Parameter	Control	VitC	Ni	Ni+VitC
WBC (10^3/mm^3)	**5.99 ±0.34**	**5.76 ±0.64**	**11.23 ±0.11**	**5.82 ±0.45**
RBCs (10^6/mm^3)	**6.82 ±0.02**	**6.37 ±0.31**	**4.01 ±0.22**	**6.44 ±0.05**
Hb (g/100 ml)	**14.901 ±0.323**	**14.863 ±0.440**	**8.992 ±0.51**	**14.770 ±0.114**
PCV (fI)	**56.27 ±0.02**	**55.89 ±0.12**	**31.91 ±0.62**	**54.99 ±0.54**

The results of the current study in group B showed that the rats did not change in their behavior or feeding, although the level of vitamin C was high and of significant importance($p<0.01$). The results showed that mice in group C, E, and D showed many signs, including reduced movement and decreased desire for food and drink, which led to weight loss 24 hours after the nickel injection compared to control. Also increased level of urea and uric acid as well as creatinine in group C, E, and D compared to the group of F, G and H which did not show such clinical signs. A study found that the concentration of nickel in the C, D and E groups was high and it was significant importance($p<0.01$) that its accumulation increased in the kidneys of mice but compared to the results in the F, G and H groups, notice there is a significant importance($p<0.01$) decrease in the concentration of nickel and the concentration of vitamin C and mice have normal behavior.

The process of water and food consumption decreased significantly in mice of the C, D and E group when compared with the control group, but comparing the C, D and E group with the F, G and H groups, we find that there is a significant increase in food intake in the F, G and H groups and these last groups do not there are significant statistically significant differences ($p = 0.05$) when compared with the control group.

The results of blood elements showed a decrease in their levels in C, D and E groups when compared to the control group and the F, G and H groups, while the last three groups had no statistically significant differences between them.

Histological analyzes of the kidneys after dyeing with dye hematoxylin and eosin showed positive results in the structural form of the brain and the structure of the renal cortex in each of the control group A and group B, while the results in the C, D and E group were changes in the renal tissue, which included necrosis and tubular degeneration with interstitisum hemorrhagic as a result of inflammation also Observed perivascular pickling of spots of mononuclear cells.

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In contrast to the above results, the study found no improvement and restoration of renal tissue building after treatment with vitamin C, which works to reduce the level of nickel in the kidneys.

Discussion

The irregular release and use of heavy metals and materials in the environment causes severe consequences for many parts of the body, including the kidneys (19) And the components of the blood as well (20). Exposure to nickel or nickel chloride causes an increase in the weight of the kidneys as a result of its deposition, which leads to less eating and drinking, and as a result, it will lead to a decrease in the patient's weight, this decrease in the patient's body weight is the result of an abnormal increase in the degeneration of proteins and fats, which can be interpreted as occurring due to the increased accumulation of nickel in the kidneys (21,22). Treatment of nickel-injected mice with vitamin C leads to a protective action against the physiological changes that may be caused by the presence of nickel in the kidneys of injected mice (23). The presence of this kind of metal ions as well as the minerals related to heme leads to an increase in the level of ROS in the kidneys, which leads to the production of hydroxide and oxidized fats, which were found at a high level in cases of kidney injury (24). The effectiveness of vitamin C is high in the treatment of acute renal failure, as it works to reduce the effect of inflammation. As well as diseases that may affect the kidneys (26). Also, vitamin C (ascorbic acid) is important in maintaining the reduction of active sites in minerals and is important in the process of formation and production of functional collagen. Any deficiency in vitamin C leads to a delay in the wound healing process and has a negative effect on the walls of blood vessels. Ascorbic Acid acts as a cofactor for some hormones such as cytoplasmic hydroxylase, which controls the regulation of pro-survival genes as well as angiogenic genes (27). There is a lot of laboratory evidence that ascorbic acid has an inhibitory activity against the oxidation of biomolecules (28). In addition, ascorbic acid has the ability to protect against the effects that may be caused by some types of reactive oxidation, which may lead to damage to lipids and proteins inside and outside the cell and thus lead to damage to nucleic acids (27). Mortality rates increase in patients with renal insufficiency, especially chronic ones, despite the development and progress in medical sciences, but the use of antioxidants has a significant effect in reducing kidney injuries, as well as improving the functions performed by the kidneys by treating or reducing inflammation or reactive oxidation, so the use of vitamin C appears Successful treatment of kidney failure (18). The current study showed that there is impairment in kidney function as a result of high levels of urea, uric acid, as well as creatinine in the blood of mice treated with nickel, in addition to histological changes seen in the histological analysis of the kidneys. This type of histological and functional impairment may be associated with increased production or increased breakdown of amino acids, or due to the inability of the kidneys to excrete (25). When the level of hemoglobin in the blood is generally low, this means that the individual is anemic, so we note in the current study a decrease in the level of hemoglobin in mice that were treated with nickel chloride and this corresponds to(30,31) Where they found in their

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study of minerals that inhibit the production of hemoglobin pigment in the body, as these minerals affect the enzymatic pathways of the heme-building process and thus these minerals reduce the biosynthesis of the heme-building process in the body, which may lead to anemia, whose effects are reflected in the survival of red blood cells alive. Also, the treatment of mice with nickel chloride causes an increase in the rate of white blood cells in these mice than the normal limit, and this means that the metal may cause an increase and stimulate the production of white blood cells as a result of inflammation in the kidneys, and this is consistent with (32,33). This means from the current study and found that exposure to this type of heavy metals leads to damage and changes in the blood picture as well as in other components of the blood system in the laboratory animal as well as in humans this agree with (34,35).

Conclusion

The current study concluded that vitamin C has an effective and resistant effect against the toxicity resulting from the accumulation of nickel in the kidneys of mice. Therefore, it is considered a biological anti-oxidant material resulting from toxic elements such as nickel.

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