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Protective Effect of Silymarin Extracted from *Silybum marianum* Seeds upon Nickel-Induced Hepatotoxicity in Albino Wistar Rats

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Abstract

Nickel cytotoxicity results in high oxygen species production, which leads to continuous injury and dysfunction of different organs including liver. Thus, the present study was conducted to investigate the protective effects of silymarin on nickel-induced hepatotoxicity. Male rats were divided into four groups of seven each: Control, silymarin (Sil), nickel (Ni), and nickel plus silymarin (Ni+Sil). Nickel as nickel sulfate (NiSO4 6H20) (20 mg/kg b.wt.) was given intraperitoneally on alternate days until the tenth dose, methanolic extract of milk thistle (100 mg/kg) was given orally to rats for 21 days. The administration of nickel caused a significant decrease in body weight with a significant increase in liver weight. Nickel treatment also produced oxidative liver injury characterized by an increase in serum glucose concentration, levels of hepatic markers enzymes (GPT, GOT, LDH and ALP) and bilirubin. Furthermore, the exposure to nickel significantly increased malondialdehyde level and decreased reduced glutathione concentration and superoxide dismutase, catalase and glutathione peroxidase activities. These results are substantiated with marked changes in the histopathology. However, the treatment with silymarin significantly lowered the level of lipid peroxidation and enhanced the antioxidant status, and resulted a reduction of the necrotic damage caused by nickel and thereby restored the previous biochemical parameters. The results of this investigation showed that nickel induced hepatotoxicity due to the excess generation of free radicals and impairment anti-oxidant defenses, but the use of silymarin extract countered the adverse effects of nickel to a major extent, suggesting their anti-oxidant and immunity powerful.

Keywords: Nickel; *Silybum marianum*; Silymarin; Hepatotoxicity; Oxidative stress

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Introduction

Nickel is a widespread occupational and environmental pollutant, which has many industrial and commercial uses; the general population is exposed to nickel from food, water and air produced from different sources, such as industrial waste, mining, refining, and metal plating [1]. It is used in a wide variety of applications including metallurgical processes such as catalysis, coinage, foundry plating and electrical components [2]. High concentrations of nickel are harmful to humans and animals. It is a haematotoxic, immunotoxic, neurotoxic, genotoxic, pulmonary toxic, nephrotoxic, hepatotoxic and carcinogenic agent [3]. The production of Reactive Oxygen Species (ROS) is involved in the molecular mechanism of nickel toxicity and carcinogenicity [4], typically leads to the peroxidation of lipids, oxidation of protein and DNA, inactivation of antioxidant defense system, resulting in cell damage and organ dysfunction [5,6]. Recently, antioxidants supplements have attracted much attention do to their antioxidant activity and a free radical scavenging potential to quench Reactive Oxygen Species (ROS) and thus prevent the disease progression. Recent reports have identified the mitigative action of herbal antioxidants in a few hepatic failures caused by free radicals and other reactive species. Silymarin is a flavonoid antioxidant isolated from the milk thistle plant (*Silybum marianum L.*) has been used for centuries as an herbal medicine for the treating liver disease, including cirrhosis, acute or chronic viral hepatitis and necrosis [7]. It offers protection against drugs and chemicals-induced hepatotoxicity [8,9] and has been also indicated that having multiple pharmacological activities including antioxidant, hepatoprotectant, anti-inflammatory agent, antibacterial, antiallergic, antiviral, antimutagenic, antineoplastic, and antithrombotic agent [10]. Additionally, silymarin has an ability to modulate hepatocyte transport and increase hepatic protein synthesis [11]. Their antioxidant activity results from the scavenging of free radicals and other oxidizing intermediates, from the chelation of iron or copper ions, and from inhibition of oxidase

[12]. This property is shown by the membrane stabilization, protects glutathione depletion and increasing the intracellular concentration of the antioxidant potential. Thus, the present investigation was undertaken to determine the protective effects of concurrent use of silymarin extracted from milk thistle against oxidative stress which could be induced by nickel in rats.

Materials and Methods

Chemicals

Nickel sulfate, 2-Thiobarbituric Acid (TBA), Butylated Hydroxytoluene (BHT), 5, 5'-Dithiobis-2-Nitrobenzoic Acid (DTNB), Trichloroacetic Acid (TCA), Nitrobluetetrazolium (NBT), 1-Chloro 2,4-Dintrobenzene (CDNB) were obtained from Sigma Chemical Co. (St. Louis, France) and all other chemicals were of analytical grade.

Plant extracts preparation

The seeds of *Silybum marianum* were collected from North-East of Algeria (Guelma Province) during summer. Plant was identified by the Department of Botany. The fruits were manually separated from the heads and freed of the pappus, and finely powdered. Milk thistle seeds (100g) were defatted with n-hexane by maceration at room temperature for overnight, then this defatted powder was extracted with 80% aqueous methanol for 72 h at room temperature. The extracts were filtered, concentrated in rotary evaporator at a temperature not exceeding 50°C under reduced pressure and stored in refrigerator [13].

Experiment design

Male Wistar rats (180–220 g) were purchased from Pasteur Institute, Algiers. Animals were maintained under standard conditions of temperature and humidity with 12 h light/dark cycle and fed standard pellet diet and water *ad-libitum* for two weeks as an adaptation period. Then rats were randomly divided into four groups of seven animals each: Group I, rats was served as controls. Group II, rats orally administered with silymarin extract (100 mg/kg, b.wt.) for 21 days. Group III received intraperitoneally nickel sulfate (20 mg/kg b.wt.) on alternate days for three weeks. Group IV, rats treated also in the same way with both nickel sulfate and the extract of silymarin simultaneously. 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. At the end of the experimental period, animals were sacrificed by cervical decapitation after overnight fasting. Serum was separated by centrifugation for 10 minutes at 3000 rpm and stored at -20°C for the biochemical analysis. The livers were removed immediately, rinsed with ice cold saline 0.9%. Then, one part was homogenized in 2 ml ice cold Tris Buffered Saline TBS (50 mM Tris, 150 mM NaCl, pH 7.4). The homogenates were centrifuged at 10.000 g for 15 min at 4°C and the resultant supernatant was frozen at -20°C for oxidative parameters determination. The other part was fixed in 10% neutral formalin and used for the histological examination.

Analytical methods

Determination of biochemical parameters: Glucose, transaminases (Glutamic Pyruvic Transaminase: GPT, Glutamic Oxaloacetic Transaminase: GOT), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), 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, LDH-1001260, total bilirubin-1001044, total proteins-100129.

Lipid peroxidation (LPO) level: The liver tissue malondialdehyde concentration was determined according to Buege et al. [14]. The process is determined in supernatant of homogenate liver tissue by the Thiobarbituric Acid (TBA) method. The absorbance of the resulting TBA-MDA complex was measured at 530 nm and the level of hepatic MDA was expressed as nmol/mg protein.

Reduced glutathione (GSH) concentration: Liver GSH content was estimated using a colorimetric technique, as mentioned by Jollow et al. [15] based on the development of yellow color when 5,5-Dithiobis-(2-Nitrobenzoic Acid) (DTNB) is added to compounds containing sulfhydryl groups. The absorbance was recorded at 412 nm. Total GSH content was expressed as nmol GSH/mg protein.

Antioxidant enzymes assays: Glutathione peroxidase activity (GSH-Px) was assayed by the method based on the reaction between glutathione remaining after the action of GSH-Px and 5, 5-Dithiobis (2-nitrobenzoic acid) DTNB to form a complex that absorbs maximally at 412 nm [16]. Catalase (CAT) activity was assayed spectrophotometrically as described by Aebi et al. [17]; the H_2O_2 decomposition rate was followed by monitoring absorption at 240 nm. Superoxide Dismutase (SOD) activity was determined by measuring of its ability to inhibit the photo reduction of nitroblue tetrazolium NBT [18]. A single unit of enzyme is defined as the quantity of superoxide dismutase required to produce 50% inhibition of photochemical reduction of NBT. The absorbance was read at 560 nm

Hepatic proteins content: Protein was measured by the method of Bradford [19], using bovine serum albumin as a standard.

Liver histopathology examination

Histological evaluation was performed on a lobe of the liver and portion of specimen fixed in 10% formalin and embedded in paraffin wax. Then sections were cut at 4 µm in thickness, stained with hematoxylin and eosin and viewed under light microscope for histological changes [20].

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 $p<0.05$.

Results

Effect of treatment on body, absolute and relative liver weights

Body, absolute and relative liver weights of animals subjected to different treatments were shown in Table 1. The body weight was decreased significantly ($p<0.01$), with a significant increase in liver weight ($P<0.05$) of Ni-treated animals compared to their controls. Conversely, the administration of Sil extract increased both total body weight and decreased liver weight.

Effects of treatments on plasma biochemical parameters

As seen from Table 2, treatment with nickel caused highly significant ($p<0.001$) increase of glucose, GOT, LDH and total bilirubin, a significant increase of GPT ($p<0.01$) and ALP ($p< 0.05$). Meanwhile, the concentration of serum total protein was diminished ($p<0.05$). Whereas, the supplementation of Sil extract produced a

Table 1: Body weight, absolute and relative liver weights of control and experimental rats after three weeks of treatment.

	Initial body weight (g)	Final body weight (g)	Absolute liver weight (g)	Relative liver weight (g/100 g body weight)
Control	201.33±6.5	250.3± 6,8	7.39 ± 0.38	2.84±0.156
Sil	205.83±3,3	251.7±4,5	7.55±0.41	3±0.17
Ni	202.33±6.9	181.7±11.3^{a1}	8.94± 0.46	5.21±0.56^{a}
Ni+Sil	203± 5.94	228.7± 6.6^{b}	6.91 ± 0.4^{b2}	3.03±0.2^{b1}

Values are given as mean ± SEM of seven rats each group. Statistically Significantly differences from control: $^{a}p<0.05$, $^{a1}p < 0.01$; from Ni: $^{b}p< 0.05$, $^{b1}p < 0.01$, $^{b2}p < 0.001$.

Table 2: Changes of biochemical parameters in serum of control and experimental rats after three weeks of treatment.

	Glucose (mg/dl)	GOT (U/L)	GPT (U/L)	ALP (U/L)	LDH (U/L)	Total proteins (g/dl)	Total bilirubin (mg/dl)
Control	113.2±2.5	145.9±5.5	63.7±2.62	129±1.9	318.3 ± 8.5	8.02±0.36	0.68±0.06
Sil	115.27±10.1	127 ±10.1	58.88±2.8	124.4±2.6	305.4±9.4	7.27±0.56	0.62±0.03
Ni	157.4±4^{a2}	224.2±12.12^{a2}	84.13±2.5^{a1}	164.62±9.27^{a}	397.1±14.7^{a2}	5.55±0.44^{a}	1,225±0.08^{a2}
Ni+Sil	112.72±6.5^{b2}	126±4.7^{b2}	56.8±2.23^{b2}	120.5±1.75^{b1}	287.3±12.1^{b1}	8.27±0.5^{b}	0.795±0.08^{b2}

Values are given as mean ± SEM of seven rats each group. Statistically differences from control: $^{a}p<0.05$, $^{a1}p < 0.01$, $^{a2}p < 0.001$; from Ni: $^{b}p< 0.05$, $^{b1}p < 0.01$, $^{b2}p < 0.001$.

Table 3: Effect of methanolic extract of S. marianum on the antioxidant defense systems and lipid peroxidation levels in liver tissue of nickel treated rats after three weeks of treatment.

	MDA (nmol/mg protein)	GSH (nmol/mg protein)	GPx (nmolGSH/mg protein)	CAT ($\mu molH_2O_2$/min/mg protein)	SOD (U/mg protein)
Control	0.48±0.02	108.7±2.9	0.68±0.03	157.55±3	49.95 ±2.6
Sil	0.52±0.03	106.2±4.25	0.66±0.02	155.28± 3.1	47.98±2.61
Ni	0.96±0.05^{a2}	82.35±3.34^{a2}	0.58±0.02^{a}	122.7±4.95^{a2}	29.4±1.74^{a2}
Ni+Sil	0.46±0.02^{b2}	109.6 ±2.7^{b1}	0.65±0.03	148.2±2.14^{b2}	41.62±2.2^{b}

Values are given as mean ± SEM of seven rats each group. Significantly differences from control: $^{a}p<0.05$, $^{a2}p < 0.01$, $^{a2}p < 0.001$; from Ni: $^{b}p< 0.05$, $^{b1}p < 0.01$, $^{b2}p < 0.001$.

diminution in the above mentioned biochemical parameters [Ni+Sil: serum glucose and the activities of GOT, GPT: ($p<0.001$); ALP and LDH ($p<0.01$), total bilirubin ($p<0.001$). Meanwhile Sil raised total protein ($p<0.05$)].

Effects of treatments on hepatic oxidative stress parameters

As shown in Table 3, the exposure to nickel produced a high significant augmentation ($p<0.001$) in MDA level accompanied by a reduction ($p<0.001$) in GSH concentration in liver compared to control group. However, nickel animals treated with Sil extract showed a reduction ($p<0.001$) in MDA and an increase ($p<0.01$) in GSH. Exposure to Ni produced also significant adverse effects on hepatic antioxidant enzymatic system, which was indicated by significant depletion in CAT and SOD activities ($p<0.001$, $p<0.05$). The coadministration of Sil extract produced recovery in hepatic enzymes of antioxidant defense system and modulated the GSH-Px, CAT and SOD activities.

Histopathology results

Figure 1 demonstrates the histopathological examination of the liver sections of control and the experimental rats. The liver sections of control rats showed normal architecture with no damage in the central vein and no change in sinusoids and hepatocytes architecture (Figure 1A). Almost, the same histology structure was shown in silymarin treatments (Figure 1B). In toxic nickel group, the liver sections indicated hepatic cell necrosis along; a few of the hepatocytes were vacuolated with severe damage associated with central vein (Figure 1C). In toxic group treated with silymarin, the liver section showed regeneration of hepatocytes and reduced severity of damage and minimal necrosis (Figure 1D).

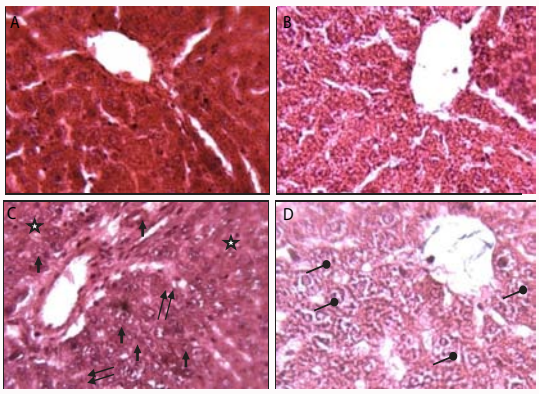

Figure 1: Light photomicrograph of liver sectional histology from control rats (A), silymarin (B), nickel (D) and Ni+Sil (E) (H&E. X 400).
: Indicate a presence of cellular degeneration, Nécrose hépatocytaire. ★: Multifocal inflammatory of hepatoytes. Hepatocytevacuolization. Regenerative cells.

Discussions

Nickel is definitely regarded as a potent real human toxicant, having the ability to form carcinomas, nickel-induced oxidative stress and can influence cellular antioxidant defense potential, this can be a hepatotoxic, hematotoxic and nephrotoxic agent. Therefore, there exists constant demand for exogenous antioxidants to be able to avoid this oxidative stress. The mean body weight of nickel subjected group decreased along with the increase in absolute and relative liver organ weight, which is contract with the studies of Adeyemi et al.[21]; Amudha et al. [22]. This decrease is an indication of the overall health-status deterioration of rats and of tissue damage and the decrease in their functions. It could possibly be related to the entire

increased degeneration of lipids and protein [23]. Findings suggested also a rise of absolute and relative liver organ weight. This might be as a result of hypertrophy and the selective deposition of nickel in the liver organ [24]. The altered body weight and liver weight parameters were retrieved to near normal levels because of the antioxidant effects of silymarin. Many studies have reported that silymarin showed significant protective effect against damage induced by heavy metals such as arsenic and cadmium [25,26]. In the present investigation, an augmentation of blood glucose was noticed in nickel animals. This may be linked with inhibition of insulin secretion from Langerhans' islets and a block of glucose utilization by cells [27], or the high glycogen breakdown and new supply of glucose production from other non-carbohydrate sources such as proteins [28]. Meanwhile, there was an amelioration of blood glucose concentration in nickel animals treated either with silymarin extract. it was suggested that the protective effect of silymarin could be due either to its antioxidant properties or to an increase of plasma and pancreatic glutathione concentrations, or both [29]. In the present study, higher activities of serum transaminases, LDH 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 by the induction of oxidative stress [30,31]. This finding was consistent with those previously recorded [21,1]. Serum total protein and bilirubin levels may signify the state of the liver and the type of damage [25]. The reduction in the proteins concentration of Ni-treated rats probably due to changes in protein synthesis and/or metabolism [32]. Furthermore, the discovered hyperbilirubinemia might be due to excessive heme destruction and blockage of biliary tract in nickel-treated rats. This obstruction may have resulted to mass inhibition of conjugation effect and release of unconjugated bilirubin from broken and dead hepatocytes [25]. Significant repair of hepatic serum marker enzymes, total proteins and bilirubin were observed in the animals treated with silymarin offering protection against nickel toxicity in rats. This means that silymarin will lessen liver damage by maintaining the integrity of the plasma membrane. Also, silymarin aids regenerating injured liver cells, results from stimulation of RNA polymerase I enzyme, thus leading to an increase of ribosomal protein synthesis which really helps to regenerate hepatocytes [33]. In this context, the membrane protecting effect of silymarin was already reported by Al-Anati et al. and Jalali et al. [34,7] they confirmed that milk thistle (silymarin) repair damage caused by toxic chemicals and medications. Nickel is well known to produce oxidative damage in liver by enhancing lipid peroxidation [35]. Lipid peroxidation is supposed to cause the destruction and damage to cell membranes, lead to changes in membrane permeability and fluidity and enhance the protein degradation [36]. 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 nickel resulted in the accumulation of iron, which in turn generate ROS via Haber–Weiss and Fenton's reaction [37,38]. The significant decrease in GSH in nickel treated group was in accordance with previous reports [4,39]. The results showed also that nickel administration induced a significant decrease GSH-Px, CAT and SOD activities, which confirms the work of Boulila et al. [40], Hfaïedh et al. [41] and Misra et al. [35]. 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 active sites of these enzymes [42,6]. In other words SOD was inhibited by hydrogen peroxide, while GSH-Px and catalase were inhibited by an excess of superoxide radical [43]. After administration of the silymarin, the activity of most assayed enzymes and GSH was normalized to the level near the control group, indicating the ability of these substances to restore homeostasis. Similar results were reported with silymarin in a model of cisplatin-induced oxidative stress in liver, and against poisoning from chemicals and environmental toxins [44]. Therefore, it suggests that silymarin scavenges free radical generation by nickel; these effects may reflect the ability of silymarin to improve the scavenging and inactivation of H_2O_2 and hydroxyl radicals, to chelate with redox metals, and to reduce lipid peroxidation by induction of enzymatic and non-enzymatic antioxidants [45].

Conclusion

This study demonstrated that nickel is capable of causing marked oxidative stress in addition to deplete the antioxidants and inhibiting the activities of antioxidant enzymes. Additionally, the treatment with methanolic extract of S. marianum could significantly attenuate the nickel induced oxidative hepatotoxicity through its antioxidant properties. Silymarin has shown more promise when it comes to supporting hepatic injury trough raising the immunity.

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