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Research Article

The Impact of Different Doses of Nickel Chloride on Some Biochemical and Histopathological Changes in the Liver of Rats

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ABSTRACT

The present study investigated the effects of different doses of nickel chloride ($NiCl_2$) on biochemical markers and liver histology. The present study used 21 young male rats, aged 3–4 weeks and weighing 150–200 g, who were randomly assigned to three groups (n = 7). The control group received only tap water, while the other two groups were exposed to nickel chloride at concentrations of 100 mg/kg and 150 mg/kg in their drinking water for 6 weeks. The results indicated no significant differences in alkaline phosphatase, aspartate aminotransferase, alkaline phosphatase, bilirubin, cholesterol, triglyceride, low-density lipoprotein, high-density lipoprotein, total protein, albumin, globulin, and glucose as controlled within control subjects. Histological examination of liver tissues from rats exposed to nickel chloride (100 mg/kg and 150 mg/kg) revealed significant pathological changes. Observed abnormalities included cellular swelling, nuclear pyknosis, degeneration, necrosis, and blood vessel congestion, with higher doses leading to more pronounced damage. These findings suggest that nickel chloride poses a potential risk to liver health even at low concentrations and short exposure durations.

Keywords: Rat, nickel, liver, apoptosis, degeneration

INTRODUCTION

Exposure to heavy metals, particularly nickel, leads to both critical biochemical and histological alterations in various organisms. In particular, nickel has emerged as a significant environmental concern since the 20th century, primarily due to the continually increasing levels of this metal in our surroundings, which has led to great harmful effects of these pollutants on the health of humans and animals.[1,2] Nickel chloride is a light-green inorganic compound with moderate water solubility. It exhibits notable reactivity and industrial applications in textiles, steel, glass, and galvanizing. Moreover, exposure to nickel chloride, especially without proper protective gear like gloves, poses significant health risks due to its potential release of carcinogenic nickel.[3] The most prevalent nickel compounds are nickel oxide (NiO), nickel carbonate ($NiCO_3$), nickel sulfate ($NiSO_4$), and nickel chloride ($NiCl_2$). There is a strong correlation between the toxicity of nickel in the body and the concentration of dissolved metal ions.[4] Nickel naturally exists as a silvery-white metallic element, is corrosion-resistant, and accumulates in organisms. Humans are exposed to nickel primarily through the skin, inhalation, lips, and digestive system from water, dust, gases, food, and dietary sources such as legumes, spinach, nuts, cocoa, and acidic beverages. Less than 10% of soluble nickel salts are absorbed by the digestive system, while lung absorption depends on particle size and solubility. Therefore, prolonged exposure to nickel disrupts organ function, enzyme activity, and metal ion balance, leading to dermatitis, allergic reactions, teratogenic effects, and severe conditions such as chronic bronchitis, lung fibrosis, liver, brain, kidney, respiratory damage, gastrointestinal issues, and cancer.[5] The toxicity effects of nickel are influenced by its component form and interactions with ligands; therefore, it seems further research is necessary to investigate the environmental and biological effects.[6,7]

The liver is the largest internal organ, constitutes approximately 2–5% of total body weight, and contains around 13% of the body's blood supply at any given time. It plays a crucial role in metabolism, protein synthesis,

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micronutrient storage, bile production, and the detoxification of xenobiotics.[8,9] Research has established that the liver serves as the primary organ of the mononuclear phagocyte system, capturing and retaining approximately 30–99% of administered nanoparticles from the bloodstream. As these nanomaterials infiltrate the liver, their flow rate decreases by a factor of 1,000, while their accumulation increases 7.5-fold due to uptake by Kupffer cells, immune B cells, and hepatic sinusoidal endothelial cells.[10,11] Nickel binds to albumin in the blood, and is metabolized in the liver into various forms. Moreover, it accumulates mainly in the kidneys but also deposits in the pancreas, brain, and lungs.[12] Both acute and chronic poising in the workplace often lead to hypersensitivity. Moreover, nephrotoxicity, immunotoxicity, hepatotoxicity, neurotoxicity, genotoxicity, reproductive, pulmonary issues, and carcinogenicity are the other serious complications of nickel toxicity. Nickel exposure led to a dose-dependent increase in the expression of heme oxygenase-1 and heat shock protein 70, with their levels rising after 60 weeks of treatment with 45 mg/kg.[13] Nickel exposure in rats has been shown to cause significant biochemical disturbances that interfere with several cellular functions, including transport, metabolism, signal transduction, oxidative balance, and ion regulation. It also disrupts vital processes such as cell cycle control, DNA synthesis and repair, and apoptosis, which are associated with disease progression.[14] Moreover, prolonged exposure to nickel for over 120 weeks markedly elevates blood concentrations of essential trace elements such as iron, copper, and manganese.[15-17] The current study is designed to comprehensively assess the toxic effects of nickel chloride administered at doses of 100 and 150 mg/kg on the liver of male rats, with particular attention to both structural and functional changes. Biochemical and histological analyses are performed to provide deeper insight into the compound's hepatotoxic mechanisms.

EXPERIMENTAL DESIGN

Laboratory rats (*Rattus norvegicus*) aged 3–4 weeks and weighing between 150 and 200 g were utilized in the present study. The rats were housed in plastic cages measuring 30 × 12 × 11 cm. Animals were grown in the animal house of Applied Sciences College, Cihan University- Erbil, Kurdistan region, Iraq, at a controlled temperature of 22 ± 2°C. With 12 h of light and 12 h of darkness. The total numbers of animals used in this study were (21) males, that are divided into three categories:

- Group I (Control): Seven intact male rats received only tap water and served as the control group (n = 7).
- Group II ($NiCl_2$ - 100 mg/kg): Seven intact male rats were administered nickel chloride at 100 mg/kg body weight (n = 7).
- Group III ($NiCl_2$ - 150 mg/kg): Seven intact male rats were administered nickel chloride at 150 mg/kg body weight (n = 7).

To estimate the concentration and median lethal dose (LD_{50}) of nickel chloride ($NiCl_2$), standard toxicological evaluation procedures are performed using laboratory rats. Various doses (such as 10, 50, 100, 200, and 400 mg/kg body weight) are administered to determine the toxicity threshold. The LD_{50} value, representing the dose that results in 50% mortality of the test animals, is then determined through statistical analysis using Karber's method. The experiment was conducted over 6 weeks. In the end, animals were anesthetized with chloroform, and blood was obtained directly through heart puncture and placed in a yellow tube to measure various physiological parameters.

Serum Separation Procedure

For terminal blood collection through cardiac puncture under anesthesia, approximately 3–6 mL of blood was obtained from each rat (weighing 150–200 g), yielding about 1.5–3 mL of serum (equivalent to 40–60% of total blood volume). The samples were transferred into serum separator tubes (SST, yellow-top) or plain tubes and kept upright to prevent agitation. To minimize hemolysis, blood was collected gently using an appropriate needle size. The samples were then left to clot for 30 min at room temperature (20–25°C). Following clot formation, tubes were centrifuged at 1,000–2,000 × g for 10 min—commonly achieved by running the centrifuge at approximately 3,000 rpm for 10 min in small clinical rotors, corresponding to about 1,500–2,500 × g, depending on the rotor radius. After centrifugation, the clear serum layer was carefully aspirated without disturbing the gel or red blood cell (RBC) layer and transferred into labeled cryovials. In SST tubes, the gel served as a physical barrier separating serum from the clot. The separated serum was stored at 4°C for short-term use (up to 24 h) or frozen at −20°C for several weeks and −80°C for long-term preservation. Liver organs for both control and treated groups were removed, dissected, and immediately kept in 10% formalin for 24 h, and then processed for routine histological examinations.[18] The following parameters were measured:

1. Biochemical assessments encompassed liver enzyme activity and lipid profile analysis
2. A histopathological examination was performed to evaluate structural changes in the liver.

The histological method used has been processed according to Bancroft and Stevens (1982).[18].

Statistical Analysis

The software SAS program. USA/version 9 (2004) was used to analyze the data of the present work using a complete random design, and then compared the differences between the averages using the test of least significant difference.[19]

RESULTS

Biochemical Tests

Analysis of various biochemical parameters in liver tissue revealed no statistically significant differences between the experimental groups and the control group [Tables 1-4 and Figures 1-3].

HISTOLOGICAL RESULTS

Histological examination of the liver slices from the intact control group [Figures 4 and 5] revealed a normal appearance. Several hexagonal hepatic lobules are separated from one another by a skinny layer of connective tissue known as

Table 1: The effects of different doses of nickel chloride on liver enzymes (ALT, AST, ALP, and Bilirubin) in various experimental groups of male rats. All values are mean±SD

Parameters treated	Mean±SD			
	ALT (U/L)	AST (U/L)	ALP (U/L)	Bilirubin (u mol/L)
Control	55.57±7.93	150.86±28.33	217.57±25.08	0.34±0.07
100 mg/kg of $NiCl_2$	74.71±9.71	181.29±12.28	248.57±106.20	0.25±0.06
150 mg/kg of $NiCl_2$	71.49±0.43	178.64±15.92	254.86±21.64	0.26±0.05

ALT: Alkaline phosphatase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, SD: Standard deviation

Table 2: The lipid profile levels (cholesterol, triglycerides [TRI], LDL, and HDL) in the experimental groups showed no significant differences between groups. All values are expressed as mean±SD

Parameters treated	Mean±SD			
	Cholesterol mmol/L	Triglyceride mmol/L	LDL mmol/L	HDL mmol/L
Control	44.29±3.03	53.86±2.67	26.33±0.97	29.29±1.11
100 mg/kg of $NiCl_2$	52.83±0.41	49.33±4.07	29.89±8.77	32.34±0.37
150 mg/kg of $NiCl_2$	56.57±1.7	47.43±13.3	19.58±0.38	37.43±4.75

LDL: Low-density lipoprotein, HDL: High-density lipoprotein, SD: Standard deviation

Table 3: The levels of total protein, albumin, and globulin in different experimental groups

Parameters treated	Mean±SD		
	Total protein g/L	Albumin g/L	Globulin g/L
Control	5.58±0.7	3.4±0.08	3.14±0.22
100 mg/kg of $NiCl_2$	6.32±0.28	3.55±0.21	2.94±0.25
150 mg/kg of $NiCl_2$	5.77±0.07	3.2±0.08	2.46±0.11

SD: Standard deviation

Table 4: Glucose levels in the different experimental groups showed no significant differences

Parameters treated	Mean±SD
	Glucose mg/dL
Control	214.9+27.51
100 mg/kg of $NiCl_2$	199.57+15.96
150 mg/kg of $NiCl_2$	186.7+17.54

SD: Standard deviation

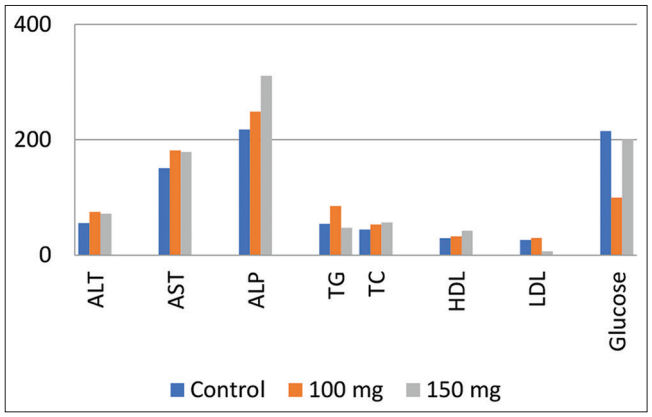


Figure 1: The levels of enzymes and other physiological parameter in liver tissue

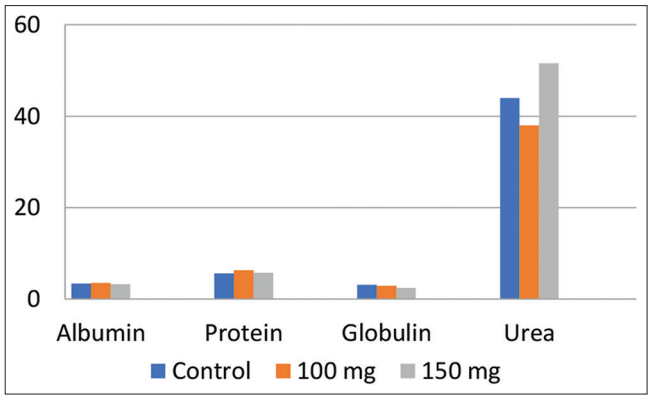


Figure 2: Levels of protein and other biochemical parameters in liver tissue are presented

interlobular septa, with each lobule containing a central vein surrounded by hepatic cords and sinusoids. The shape of hepatocytes is polyhedral with basophilic granules that contain vesicular, rounded central nuclei.

Sections of liver tissue from the treated group with 100 mg/kg $NiCl_2$ showed considerable modifications, including widespread vacuolation inside the hepatocytes and the formation of certain characteristic globular forms, which could be infiltrated lipids [Figures 6 and 7]. A few hepatocytes lost their polygonal form as they became hypertrophied. Hepatic cell membranes were observed to be thicker. Sinusoids were found to have deteriorated, resulting in bleeding within intercellular spaces. Hepatic cell degeneration was visible with Some cells exhibited enlargement, disarray, and a lack of cytoplasmic components.

Furthermore, congestion has been observed in some central veins and hemorrhage in some interstitial tissues. Some liver cells became distorted with pyknotic nuclei and lost cytoplasmic density.

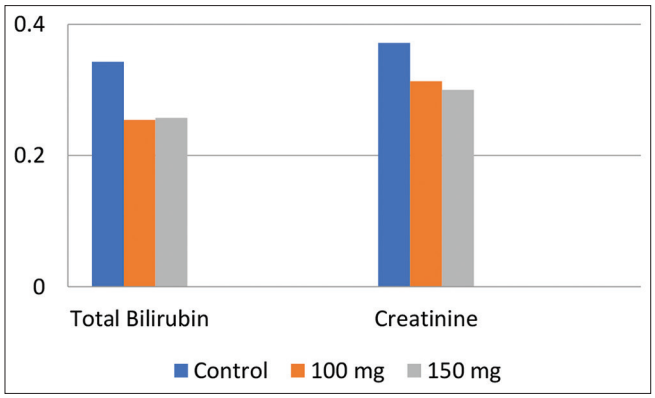
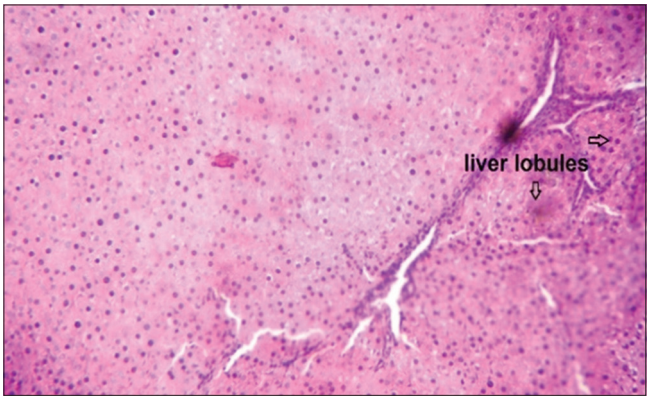
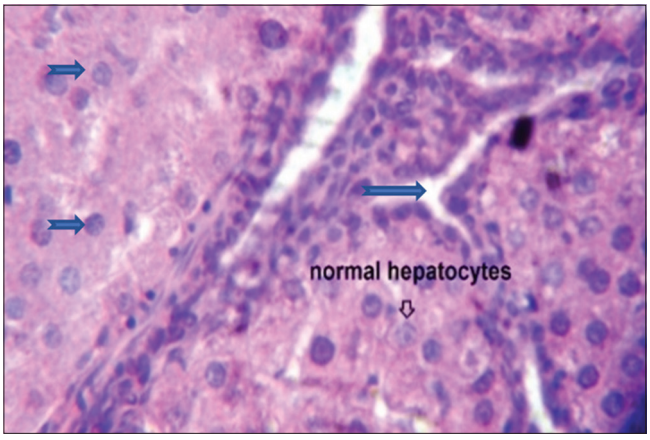


Figure 3: Levels of total bilirubin and creatinine are presented. All values are expressed as mean±standard deviation

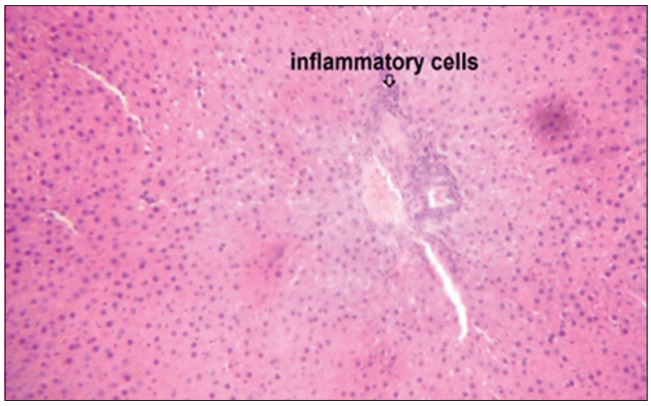


Figures 4: Histological sections of liver tissue from control rats showing normal hepatic architecture, with intact hepatocytes exhibiting prominent nuclei (arrow) and a distinct central vein (arrow) (H&E stain, ×100)

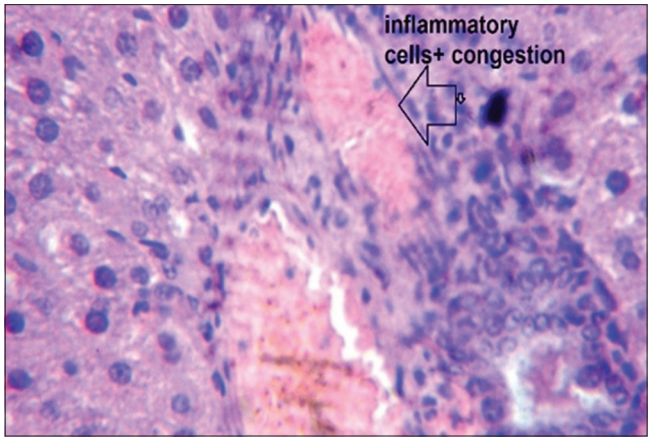


Figures 5: Histological sections of liver tissue from control rats showing normal hepatic architecture, with intact hepatocytes exhibiting prominent nuclei (arrow) and a distinct central vein (arrow) (H&E stain, ×400)

Examination of liver tissue from rats treated with 150 mg/kg of $NiCl_2$ [Figures 8, 9, and 10] revealed extensive hepatic damage, where the liver parenchyma appeared as a necrotic, spongy mass with marked sinusoidal degeneration. Most hepatocytes exhibited loss of cellular boundaries, cytoplasmic condensation, and



Figures 6: Photomicrograph showing degeneration of hepatic cells, with some cells exhibiting swelling, disorganization, and loss of cytoplasmic contents in the 100 mg/kg $NiCl_2$-treated group (×100)



Figures 7: Photomicrograph showing degeneration of hepatic cells, with some cells exhibiting swelling, disorganization, and loss of cytoplasmic contents in the 100 mg/kg $NiCl_2$-treated group (×400)

disruption of hepatic cords. Moreover, widespread degeneration was observed, with many hepatocytes showing focal necrosis, lateralized nuclei, pyknosis, and cytoplasmic disintegration. Some cells appeared nearly devoid of cytoplasmic contents. The lesions were also characterized by vascular elongation, necrosis, and degeneration.

DISCUSSION

Several studies have demonstrated the toxic impact of this heavy metal; however, information remains scarce regarding its tissue bioaccumulation and the cellular or molecular disturbances caused by nickel (Ni) exposure in living organisms. Due to its widespread industrial use, nickel has been extensively released into the environment, leading to notable pollution.[20] The findings of the current study align with those of previous researchers,[2] who observed that liver tissues of male mice exposed to different concentrations of nickel chloride and potassium dichromate exhibited marked hepatic lesions, such as vascular congestion, nuclear shrinkage (pyknosis), cellular degeneration, and necrosis. The detected alterations could be due to the buildup of chemical pollutants such as nickel (Ni) and chromium (Cr), which are well-documented for their detrimental effects on the liver's histological architecture and

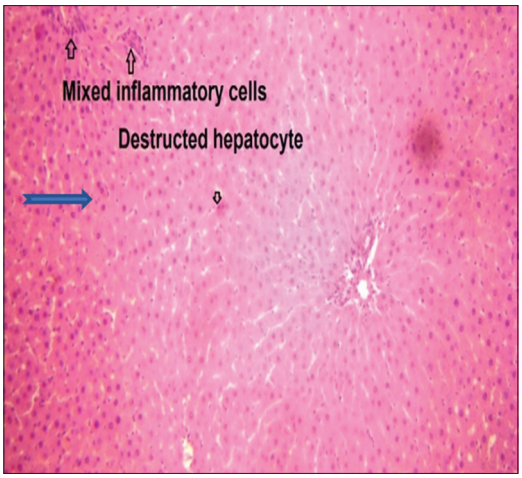


Figure 8: Photomicrograph showing the entire liver tissue as a necrotic, spongy mass with degeneration of sinusoids, loss of cell borders, accompanied by cytoplasmic condensation (mononuclear inflammatory cells infiltration predominantly lymphocytes) (×100)

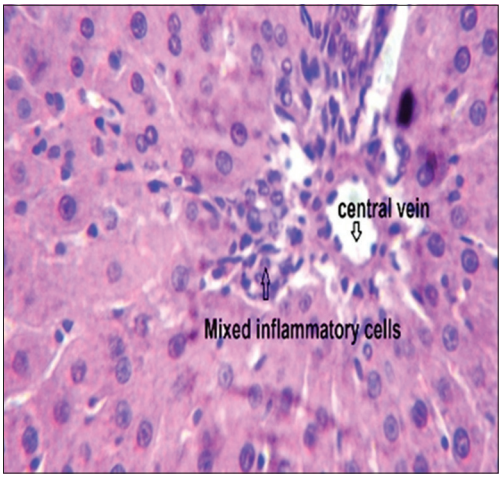
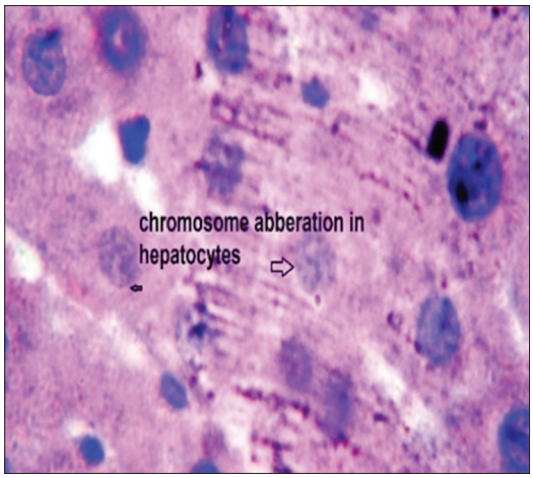


Figure 9: Photomicrograph showing the entire liver tissue as a necrotic, spongy mass with degeneration of sinusoids, loss of cell borders, accompanied by cytoplasmic condensation (mononuclear inflammatory cells infiltration predominantly lymphocytes) (×400)

physiological performance. These heavy metals function as powerful toxicants that interfere with the normal biological activities of animals.[21] The findings of the current study are in agreement with those of Shati,[22] who demonstrated that male rats receiving 8 mg/kg of potassium dichromate ($K_2Cr_2O_7$) through drinking water developed pronounced hepatic lesions, including focal necrosis, vascular congestion, marked lymphocytic infiltration surrounding blood vessels, nuclear degeneration (karyolysis and pyknosis), and activation of Kupffer cells. Such pathological alterations may result from oxidative stress, particularly affecting the plasma membrane, or through suppression of oxidative phosphorylation, which subsequently reduces the cellular energy required for protein synthesis. Chromium and nickel are essential trace elements, but also represent common occupational and environmental toxicants. With the extensive expansion of Cr and Ni mining activities, their environmental and health-related impacts have become increasingly alarming.[23] Exposure to either or both



Figures 10: Photomicrograph showing the entire liver tissue as a necrotic, spongy mass with degeneration of sinusoids, loss of cell borders, accompanied by cytoplasmic condensation (mononuclear inflammatory cells infiltration predominantly lymphocytes) (×1000)

metals can exert wide-ranging adverse effects on ecological systems and human health. Histopathological examination of liver sections from male mice exposed to varying doses of nickel chloride and potassium dichromate revealed severe hepatic damage, including vascular congestion, nuclear pyknosis, cellular degeneration, and necrotic lesions. These alterations may be attributed to the accumulation of chemical pollutants such as nickel (Ni) and chromium (Cr), which are known to adversely affect liver histology and function, as heavy metals act as potent toxic agents that disrupt the normal physiological processes of animals.[24] The current findings are consistent with those reported by Ho and Leung,[25] who observed that male rats administered 8 mg/kg of potassium dichromate ($K_2Cr_2O_7$) through drinking water exhibited marked hepatic injuries, including focal necrosis, blood vessel congestion, intense lymphocytic infiltration around blood vessels, nuclear karyolysis and pyknosis, and proliferation of Kupffer cells. These pathological alterations may result from oxidative stress, particularly targeting the plasma membrane, or from inhibition of oxidative phosphorylation, leading to reduced energy availability for protein synthesis. In this experiment, administration of $NiCl_2$ at doses of 100 mg/kg and 150 mg/kg did not produce any significant changes in biochemical indicators (alkaline phosphatase [ALT], aspartate aminotransferase [AST], alkaline phosphatase [ALP], bilirubin) or lipid profile components (cholesterol, triglycerides, low-density lipoprotein, high-density lipoprotein) in rats. These findings are consistent with previous research suggesting that the physiological and toxicological responses to heavy metals depend on several factors, including dose and duration of exposure.[26,27] The stable levels of total protein, albumin, and globulin across all experimental groups suggest that hepatic protein synthesis remained unaffected by nickel chloride treatment throughout the study. The observed stability highlights the need for further research on the long-term impacts of heavy metals, especially nickel chloride, on liver function and overall biochemical balance. In contrast, extended exposure to nickel has been shown to cause renal tubular injury, structural alterations in hepatic tissue, and adverse

effects on the reproductive system. Nickel accumulation in the kidneys has also been linked to decreased urine output and glucose concentration, accompanied by elevated β2-microglobulin levels.[28,29] Exposure to nickel chloride ($NiCl_2$) has been linked to toxic effects on both the blood and liver of rats. Studies suggest that $NiCl_2$ induces liver injury through several mechanisms, including increased oxidative stress, apoptosis, inflammatory responses, and disruption of normal hepatic structure and function.[30] Moreover, nickel exposure caused dose-dependent changes in serum albumin, glucose, cholesterol, and triglyceride levels. Alterations in plasma protein patterns indicated compromised liver function, while elevated urea and creatinine concentrations pointed to potential renal impairment.

The stability of these biomarkers indicates that the administered doses did not induce significant biochemical disturbances during the experimental period.[31-33] This absence of marked changes may be attributed to factors such as limited metal absorption, suppression of erythropoiesis, damage to bone marrow stem cells, or the destruction of erythrocytes. In support of this Topić Popović *et al*.,[34] reported reductions in white blood cells, RBCs, hemoglobin, and packed cell volume in male rats exposed to chromium and nickel, suggesting that Ni-Cr toxicity may result from impaired hematopoietic stem cell function. Furthermore, Zhang *et al*.,[35] demonstrated that elevated levels of Mn, Cd, Fe, and Ni in the livers of newborn mice from contaminated environments were associated with pronounced toxic effects. These observations are consistent with those of Yildiz Deniz *et al*.,[36] who found that prolonged exposure to high concentrations of heavy metals compromises antioxidant defense systems – such as metallothionein and superoxide dismutase – thereby promoting metal accumulation in tissues.

Histological examination of liver sections from male rats treated with different concentrations of nickel chloride revealed pronounced hepatic alterations. The observed changes included necrotic lesions, vascular congestion, and nuclear degeneration characterized by pyknosis. Additional findings comprised ballooning degeneration of hepatocytes, marked lipid accumulation indicative of macrovesicular steatosis, and prominent lobular inflammation. Moreover, evidence of hepatocyte apoptosis, hydropic degeneration consistent with microvesicular steatosis, infiltration of inflammatory lymphocytes, and the presence of Councilman bodies were also observed.[37] In agreement with the observations of Yu *et al*.,[38] Osman,[39] and Abed,[40] histopathological examination in the current study demonstrated marked hepatic injury following nickel chloride administration. Liver sections from the control group displayed normal histological organization with well-preserved hepatocytes, whereas those from rats exposed to 100 mg/kg of $NiCl_2$ revealed distinct pathological changes. These alterations were characterized by cytoplasmic vacuolation, hypertrophy of hepatocytes, lipid accumulation suggestive of steatosis, and distortion of sinusoidal architecture, occasionally accompanied by hemorrhagic areas. At a higher dose (150 mg/kg), the hepatic injury became markedly pronounced, showing widespread necrosis, cytoplasmic condensation, pyknotic nuclei, and complete disorganization of the normal hepatic cord pattern—indicative of a clear dose-dependent hepatotoxic effect of nickel chloride. These observations emphasize the liver's heightened vulnerability to $NiCl_2$-induced damage. Notably, current literature provides limited insight into the mechanistic aspects of $NiCl_2$ toxicity, particularly its influence on apoptosis and oxidative stress in vital organs such as the liver, kidneys, and testes of both animals and humans. The current results are consistent with those of Akinwumi *et al*.,[41] who reported significant hepatic injury in mice exposed to 2 mg/kg of nickel chloride through drinking water. These pathological alterations were attributed to oxidative stress, primarily impacting the plasma membrane, and potentially to interference with oxidative phosphorylation, which may redirect cellular energy toward compensatory protein synthesis.

CONCLUSION AND RECOMMENDATION

The present findings suggest that while exposure to various doses of nickel chloride did not cause significant changes in ALT, AST, ALP, creatinine, or urea serum levels, higher doses did lead to notable histopathological alterations in liver tissue. These changes included vascular dilation, extensive fatty degeneration of hepatocytes, lobular disorganization, and necrosis, indicating localized tissue damage despite stable biochemical markers. These results underscore the potential systemic toxicity of nickel chloride. Future studies should investigate its biological mechanisms, long-term health risks, environmental effects, and potential sex-related differences in metabolism and nephrotoxicity. The present study recommends studying how nickel chloride affects the body: Look deeper into how nickel chloride harms liver cells, such as whether it causes stress, cell death, or damage to cell energy centers. Also, to explore new markers in the body that can show liver damage earlier than current blood tests, as well as to compare tissue damage with Blood Markers: Find early warning signs in blood or tissues that match the damage seen in the liver, which could help in early detection.

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