ORIGINAL ARTICLE

HEPATOTOXIC EFFECT OF NICKEL SULPHATE ON MICE

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ABSTRACT

Background and Objective: Nickel is an important alloying element in metallic implants used in dentistry as well as in orthopedic surgery. The objective of this study was to see the effect of nickel sulphate on mice liver and to observe if there was any reversibility of pathological changes after cessation of nickel sulphate.

Methods: Sixty male adult mice were randomly divided into three groups (n = 20). These groups were further subdivided (n = 10) into C_1, C_2 (Receive 0.25 ml sterile distilled water intraperitoneally) A_1, A_2 received 1 mg/kg nickel sulphate (high dose) intraperitoneally for 14 days. The animals from group C_1 and A_1 were sacrificed on day 15th. Liver was examined for gross, chemical and histopathological changes. After 14 day the injections of normal saline and nickel sulphate were stopped and animals were sacrificed at day 30th. Liver was removed and gross, chemical and histopathological changes were observed to see any reversibility of histopathological changes. Group C_2 Receive 0.25 ml sterile distilled water intraperitoneally. GroupB_1, B_2 received 0.5 mg/ml (low dose) nickel sulphate intraperitoneally as a single dose for 14 days. The animals of groupC_1 and B_1 were sacrificed at day 15th. Liver was removed and examined. At day 30ththe animals of C_2 and B_2 group were sacrificed and gross and histopathological changes were compared to see any reversible change as compared to day 15th.

Results: There was a significant change in size and weight of liver. There was elevation of serum enzymes activities (ALT, AST and bilirubin) in both experimental groups at day 30th and it was irreversible. The swelling of hepatocytes was present in 70% of animals in experimental groups at 15th day. There was 10% increase in swelling of hepatocytes in group A_2 and 10% decrease in swelling of hepatocytes in group B_2 at 30th day. Acute hepatitis (ballooning degeneration) was present in 30% of mice liver in group A_1 at 15th day and only 10% in group B_2 at 30th day. Histological and gross findings were reversed 10% to 20% but biochemical parameters (ALT, AST and bilirubin) were not reversed after stoppage of nickel sulphate in both high and low dose groups.

Conclusion: It was concluded that nickel toxicity in experimental groups resulted in alterations in hepatocytes as change in size, weight and colour of liver, necrosis and acute hepatitis. The biochemical analysis revealed significant increased ALT AST and bilirubin, which supported the histological findings that nickel sulphate, is hepatotoxic. ALT and AST activities were not reversed at 30th day. The pathological changes as size, weight of liver, focal necrosis, zonal necrosis and acute hepatitis were ten to twenty percent reversible in experimental groups at 30th day.

Key Words: Nickel sulphate, hepatotoxicity, cast partial denture, corrosion.

INTRODUCTION

Biocompatibility is the capability of a material to remain biologically safe during its functional period.[1] It was observed that material can become toxic when there is release of chemical constituents which can induce local, systemic or allergic responses as well as carcinogenic and mutagenic effects. Nickel is present in many alloys used in dental practice e.g. in operative dentistry as a metallic filling material, in crown and bridge work, in prosthetics in cast partial dentures, in orthodontics in the form of brackets and wires. It is also noted that approximately 6% to 12% nickel and 15% to 22% chromium is present in most of stainless steel materials used in dentistry.[2,3]

In dentistry, metallic materials are frequently used as direct or indirect restorative materials. Most of them have corrosion resistance due to formation of protective oxide film. The stability of this layer can be

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damaged. Most commonly released elements from noble and base metals are nickel, chromium and copper. Metals or dental alloys can cause inflammatory reactions due to toxic or irritant effect. If toxicants are absorbed and distributed to other parts of the body then systemic effects can be manifestated. The physicochemical form, duration, route and biotransformation determine the biocompatibility of the material.[4]

Stainless steel, cobalt-chromium and titanium alloys have been used in different dental implants. They are associated with increased metal concentration around implant, in blood tissue and all body organs. Increase in metal ions concentration has been reported after heavy metal implantation.[5] Different exogenous and endogenous toxic metals are metabolized in liver which has limited capacity to detoxify them. Damage to this organ may lead to various disorders.[6] Corrosion releases the metallic metabolites into the parenteral circulation which causes acute hepatotoxicity. [7]In vitro studies of toxicity and metabolism by using human hepatocytes resulted in excellent correlation between in vitro data and in vivo situations.[8] Keeping this observation in view, this present study was planned to determine the in vivo performance of nickel sulphate. All previous studies described the nickel toxicity by different biological methodologies but adequate data regarding hepatotoxic effect of nickel sulphate was not available. Since nickel is the most common metal used in different dental alloys, orthodontic appliances and maxillofacial surgery, hence the present study was designed to get the exact lytic effect of nickel sulphate on gross, histological as well as biochemical basis.

METHODS

It was an experimentalstudy which was conducted at Post Graduate Medical Institute Lahore (Affiliated with University of Health Sciences. The duration of this study was six months. Inclusion criterion was healthy mice.Sixty BALB/c male mice, weighing 20 – 30 grams were kept in animal house of Post Graduate Medical Institute, Lahore, in iron cages under hygienic conditions. The mice were kept under usual laboratory light conditions and fed wit rat chow and water at libitum. The study was approved by the ethical committee of PGMI Lahore.

The animals were randomly divided into three groups (C, A and B) of twenty animals each. These groups were further subdivided into C_1 (day 15), C_2 (day 30th) A_1 (day 15), A_2 (day 30th) B_1 (day 15) and B_2 (day 30th).

Control Group (C_1, C_2): The control group was injected, intraperitonealy, 0.25 mL sterile distilled water once a day for 14 days.

Experimental group (A_1, A_2): High dose (1 mg/kg) of nickel sulphate was injected, intraperitonealy, once a day for 14 days.

Experimental Group (B_1, B_2): Low dose (0.5 mg/kg) of nickel sulphate was injected, intraperitonealy, once a day for 14 days.

The animals were sacrificed at day 15th after receiving Nickel sulphate and after withholding Nickel sulphate at day 30th.At day 15th and 30th blood samples were taken by cardiac puncture method under anesthesia with diethyl ether.[9,10] Plasma specimens were obtained and used for determination of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum bilirubin by using commercially available kit (DiaSys, Germany).

Gross and Histological Examination of Liver

Detailed examination of external and cut surface of liver was carried out by necked eye (macroscopically) to see any change in colour, nodules or necrosis etc. The weight of the liver of each animal (grams) was recorded on electronic balance (Want, Model No. WT6002-D) after removal from abdomen at day 15th and 30th in all three groups.The size of liver (density) was determined according to formula of weight/ volume.[11] The size of liver was expressed as mean and ± SD at day 15th and 30th in all groups (Table 1).The change in colour of liver and surface texture (smooth, shiny or nodular) was observed macroscopically.

Liver was removed and fixed in 10% buffered formalin (Sigma – Aldrich, Germany) for 48 hours. Tissues were cut at 6 μm thickness with rotary microtome. (Leica Jung Histocut, Model 820, China). Slides were stained with H&E staining and observed under light microscope. Photomicrograph were taken from camera (Nickon, Japan) mounted microscope.[12,13] Histological examination showed that there was swelling of hepatocytes, focal necrosis, zonal necrosis and ballooning degenerations. There was accumulation of bile in hepatic parenchyma and microvesicular steatosis.

All the collected data was entered in Statistical Package for Social Sciences (SPSS) version 18. The qualitative data was presented in form of tables. Mean ± standard deviation was applied to see any significance of nickel sulphate as compared to the control group. Multiple comparison test (LSD: Least significant difference) was performed for qualitative data (ALT, AST and bilirubin). A p-value less than 0.05 was considered as statistically significant.

RESULTS

The result of gross findings of Table 1 revealed that there was insignificant change in size of liver in both experimental groups at day 15th and 30th. There was significant increase in size of liver as compared to control group at day 15th and significant increase in size of liver at day 30th. There was significant change in morphology and colour of liver of experimental groups as compared to control group. Surface texture of liver was smooth in both experimental groups.

The findings of biochemical test revealed that there was significant increase in ALT, AST and bilirubin level in both experimental groups at day 15th and

30th (Table 2).

The result of histological findings showed that there was insignificant presence of cholestatic hepatitis in experimental groups as compared to control groups. There was significance presence of ballooning of hepatocytes in all groups. There was significant presence of swelling of hepatocytes and focal necrosis. There was insignificant presence of zone-1 necrosis and significant presence of zone-2 and zone-3 necrosis in all experimental groups as compared to control group. There was significant presence of Microvascular (Hepatic stenosis) in both study groups as compared to control groups (Table 3).

Histological and gross findings were reversed 10% to 20% but biochemical parameters (ALT, AST and bilirubin) were not reversed after stoppage of nickel sulphate in both high and low dose groups.

DISCUSSION

This study was carried out to see the hepatotoxic effect and reversibility of changes on gross, biochemical and histological basis. The gross examination of group A_1 (1 mg/kg), at 15th day, revealed that there was decrease in mean size and increase in mean weight of liver. At day 30th, there was increase in mean size and weight of liver (Table 1). This decrease in mean size may be due to degenerative effect of nickel sulphate, because it is a major site for metabolism and increase in liver weight may be due to cellular proliferation of hepatocytes and inflammatory reaction.[14,15] In group B_1 (0.5 mg/kg), there was increase in mean size and weight of liver at day 15th (Table 1). This increase in size and weight may be due to inflammatory reaction of hepatocytes.[16] There was decrease in size and decrease in mean weight of liver, as compared to control, at 30th day. This decrease in mean size and weight of liver, after stop page of

Table 1: *Comparison of Gross findings of liver between control and experimental groups before and after administration of nickel sulfate at days 15th and 30th.*

Gross Findings		*Control Group*		*Experimental Groups*				
		C1	*C2*	*A1*	*A2*	*B1*	*B2*	*p-value*
	Size of liver mg/c3	0 .7310 ± 0.28	0.8420 ± 0.32	0.6010 ± 0.30	0.5050 ± 0.16	0.9060 ± 0.80	0.7770 ± 0.31	0.286
	Weight of liver mg	1.3570 ± 0.09	1.2950 ± 0.10	1.5170 ± 0.11	1.8280 ± 0.22	1.6790 ± 0.34	1.2090 ± 0.25	< 0.001
Morphology	Normal	10 (100%)	10 (100%)	3 (30%)	3 (3%)	5 (5%)	2 (2%)	< 0.001
	Mild change	0 (0%)	0 (0%)	7 (70%)	7 (70%)	5 (5%)	8 (80%)	
Colour	Dark Reddish	10 (100%)	10 (100%)	3 (30%)	3 (3%)	5 (5%)	2 (2%)	< 0.001
	Reddish Brown	0 (0%)	0 (0%)	7 (70%)	7 (70%)	5 (5%)	8 (80%)	
Surface Texture	Smooth Shiny	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	--

a. Mean ± S.D = Mean ± standard deviation
b. mg/dl = milligram per deciliter
c. P-value < 0.05 is significant

Table 2: *Comparison of biochemical findings between control and experimental groups at days 15th and 30th.*

Biochemical Findings		*Control Groups*		*Experimental Groups*				
		C1	*C2*	*A1*	*A2*	*B1*	*B2*	*p-value*
Mean ± S.D	ALT	41.50 ± 4.83	34.40 ± 4.16	53.30 ± 27.80	53.90 ± 23.86	41.00 ± 8.90	56.00 ± 12.61	0.021
	AST	141.70 ± 19.83	130.60 ± 12.09	188.30 ± 70.71	215.80 ± 45.67	144.40 ± 33.66	190.60 ± 61.54	< 0.001
	Bilirubin (mg/dl)	0.5100 ±0.0567	0.4900 ±0.0567	0.6200 ± 0.063	0.6400 ± 0.05	0.6100 ± 0.11	0.6400 ± 0.17	< 0.001

Table 3: *Comparison of histological findings of control and experimental groups at days 15th and 30th.*

Histological changes		*Study Groups*		*Experimental Groups*				*p-value*
		C1	*C2*	*A1*	*A2*	*B1*	*B2*	
Cholestatic Hepatitis	Normal	10 (100%)	10 (100%)	10 (100%)	10 (100%)	9 (90%)	10 (100%)	0.406
	Mild change	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	
Bland [a]	Normal	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	--
Ballooning [b]	Mild change	10 (100%)	10 (100%)	7 (70%)	9 (90%)	10 (100%)	10 (100%)	0.038
	Normal	0 (0%)	0 (0%)	3 (30%)	1 (10%)	0 (0%)	0 (0%)	
Swelling of hepatocytes (Acute hepatitis)	Normal Morphology	10 (100%)	10 (100%)	3 (30%)	2 (20%)	3 (30%)	4 (40%)	< 0.001
	Mild change	0 (0%)	0 (0%)	4 (40%)	4 (40%)	7 (70%)	4 (40%)	
	Moderate changes	0 (0%)	0 (0%)	3 (30%)	4 (40%)	0 (0%)	2 (20%)	
Focal necrosis (Hepatocellular necrosis)	Normal Morphology	10 (100%)	10 (100%)	0 (0%)	0 (0%)	0 (0%)	4 (40%)	< 0.001
	Mild change	0 (0%)	0 (0%)	7 (70%)	6 (60%)	10 (100%)	4 (40%)	
	Moderate changes	0 (0%)	0 (0%)	3 (30%)	4 (40%)	0 (0%)	2 (20%)	
Massive[c]	Normal morphology	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	--
Pan lobular[d]	Normal morphology	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	--
Zone 1[e]	Normal Morphology	10 (100%)	10 (100%)	5 (50%)	8 (80%)	8 (80%)	8 (80%)	0.065
	Mild change	0 (0%)	5 (50%)	5 (50%)	2 (20%)	2 (20%)	1 (10%)	
	Moderate changes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	1 (10%)	
Zone 2[e]	Normal Morphology	10 (100%)	10 (100%)	7 (70%)	9 (90%)	8 (80%)	10 (100%)	0.103
	Mild change	0 (0%)	1 (10%)	1 (10%)	2 (20%)	2 (20%)	0 (0%)	
	Moderate changes	0 (0%)	0 (0%)	2 (20%)	0 (0%)	0 (0%)	0 (0%)	
Zone 3[e]	Normal morphology	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	10 (100%)	--
Microvesicular (Hepatic stenosis)	Normal Morphology	10 (100%)	10 (100%)	6 (60%)	6 (60%)	7 (70%)	6 (6%)	0.026
	Mild change	0 (0%)	0 (0%)	4 (40%)	4 (40%)	3 (30%)	2 (20%)	
	Moderate changes	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	2 (20%)	

a. Bland (without any parenchymal inflammation)
b. Ballooning /degeneration of hepatocytes (Acute hepatitis)
c. Massive (Hepatocellular necrosis)
d. Groups C_1, A_1 and B_1 sacrificed at 15th day
e. Groups C_2, A_2 and B_2 sacrificed at 30th day

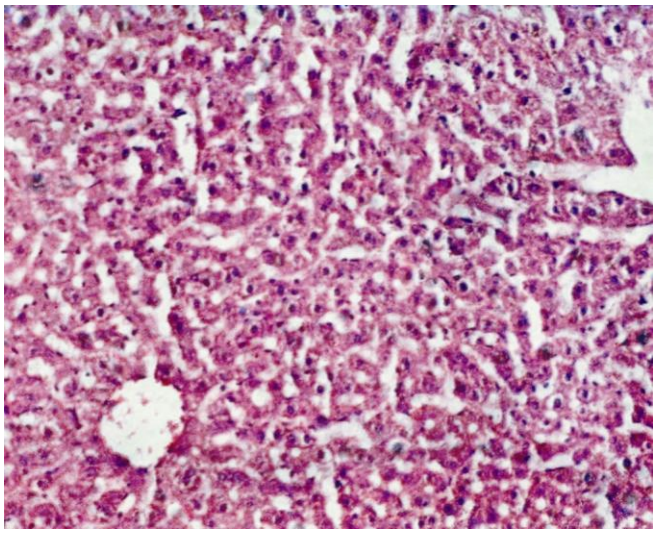

Fig. 1: *Photomicrograph of liver of control group showing: Normal morphology of hepatocytes. 2) Hepatic cords (H&E, 40X).*

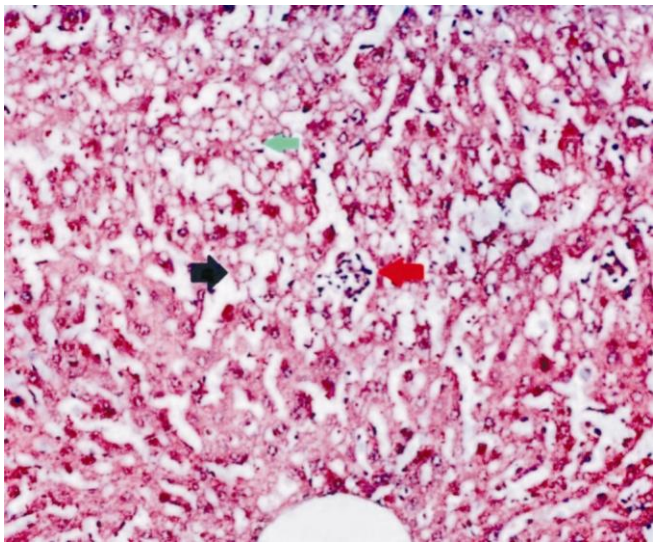

Fig. 2: *Photomicrograph of liver of group A_1 at day 15^{th} of sacrifice: Black arrow showing vacuolization 2) red arrow showing area of Zone 1 necrosis 3) Green arrow showing microvesicular steatosis (H&E, 40X).*

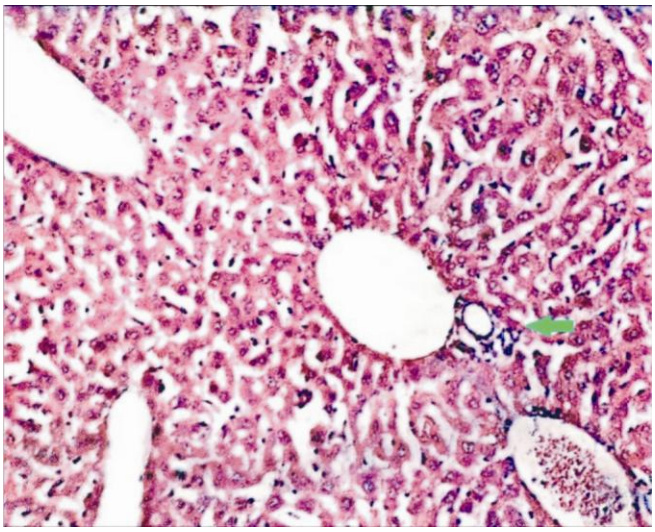

Fig. 3: *Photomicrograph of liver of group A_2 at day 30^{th} of sacrifice: Green arrow showing acute hepatitis near portal tract. (H&E, 40X).*

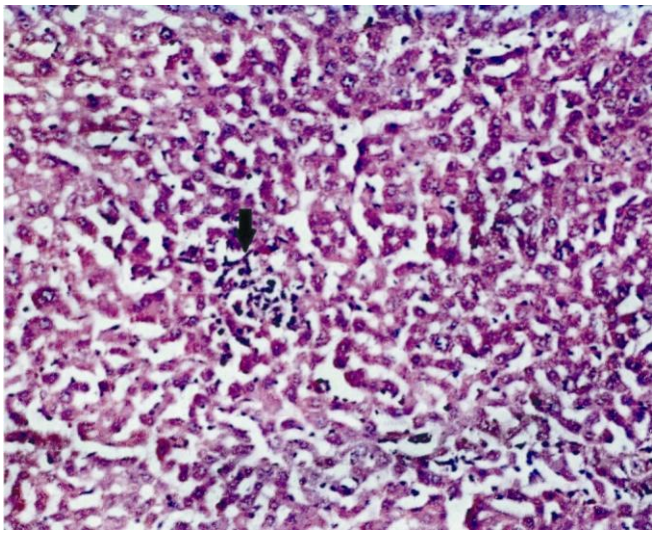

Fig. 4: *Photomicrograph of group B_1 at day 15^{th} of sacrifice: Black arrow showing focal area of necrosis (H&E, 40X).*

in mean size and weight of liver, after stop page of nickel sulphate, may be due to resolution of inflammatory changes and excellent regeneration ability of liver.

Normal color of mouse liver is dark reddish.[17] There was significant ($p < 0.05$) change in color (dark reddish to reddish brown) of both experimental groups as compared to control at day 15^{th} and 30^{th} (Table 1) which may be due inflammation of liver. The surface texture of liver was smooth and shiny in all groups.

The liver uses transaminase [alanine aminotransferase (ALT) and aspartate aminotransferase (AST)] to synthesize and break down amino acids and to convert energy storage molecules. Elevated levels are indicator of liver injury. ALT is usually found only in the liver. AST is most commonly found in the liver, but also in significant amounts in heart (cardiac) and skeletal muscle.[18]

The results of biochemical analysis of this study showed that there was non-significant increase of ALT and AST level in high and low dose groups as compared to control at day 15^{th} (Table 2). At day 30^{th}, there was significant increase in ALT and AST of high and low dose groups (Table 2) as compared to control. It could be attributed to hepatic damage resulting in increased release of functional enzymes from memb-

rane.[19] These findings coincide with our gross examination of liver which showed the effect of inflammation and necrosis on liver.

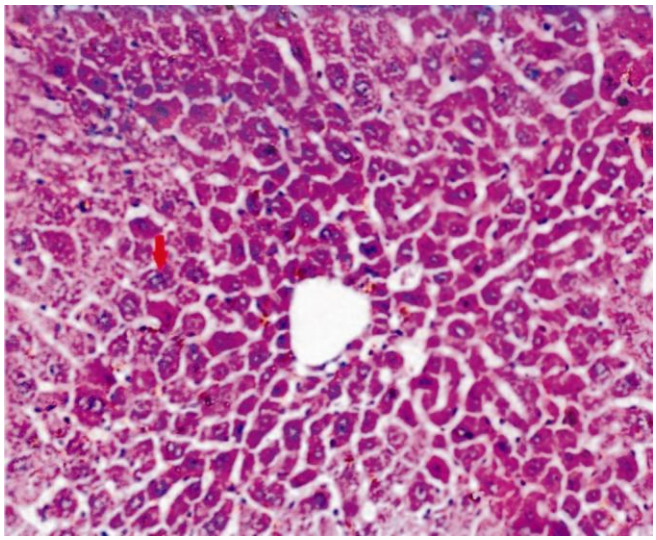

Fig. 5: *Photomicrograph of group B_2 at day 30th of sacrifice red arrow showing swelling of hepatocytes (H&E, 40X).*

There was significant increase in mean bilirubin level in high and low dose groups as compared to control at day 15th and 30th (Table 2). The abnormal increase in serum bilirubin level may be due to defect in their metabolism and excretion, retaining it in hepatic tissues.[19] These findings prove that exposure to nickel sulphate causes hepatocytes cell membrane damage, leading to increased serum ALT and Bilirubin levels. These finding are in agreement with Tikare et al.[15] who observed similar findings at dose of 2 mg / 100g of nickel sulphate intraperitonealy. These results are supportive of gross findings, which showed inflammatory changes in liver. The same times similar biochemical results were shown by various scientists that, Nickel salt caused increase level of aspartate amino transferases, alanine aminotransferase and gamma glutamyl trans peptidase in liver.[20]

The histological findings of control group revealed normal histological features with a characteristic pattern of hexagonal lobules separated by interlobular septa and normal hepatocytes structures (Figure 1). Detailed review of liver slides showed mild degeneration of hepatocytes (Figure 2) appeared to be most significant ($p < 0.05$) in both high and low dose groups at 15th day. Other significant features identified were acute hepatitis, swelling of hepatocytes and focal necrosis (Figures 3, 4 and 5; Table 3) in both high and low dose groups which were of mild to moderate type. These were statistically significant at 15th and 30th days as compared to control. This could be attributed to toxic effect of nickel sulphate on liver. It was confirmed by significant elevation of ALT and AST in present study.

Another interesting observation was regarding the pattern of severity of zonal necrosis on day 15th as compared to 30th day. There was significant association ($p < 0.05$) of zone-1 necrosis between A_1, A_2, B_1, B_2 and control groups at days 15th and 30th (Table 3). Zonal necrosis may manifest as an increase in ALT level.[21] This finding is supportive of our biochemical results, which showed increased ALT level at day 30th (Table 2).

These findings are in agreement with similar results obtained in other studies. Knight et al.[22] reported presence of necrotic hepatocytes in rats following subacute parenteral injection of 125 micro mol/kg nickel sulphate. Hepatocytes of mice receiving subcutaneous injection of metallic nickel solution were swollen with clear cytoplasm. Histological studies by Prera and Sousa[23] indicated hepatic parenchymal vacuolization and periportal changes after 10 days of 0.5 ml of metallic nickel solution administration.

The histological findings of acute hepatitis and focal necrosis and zonal necrosis were reversed 10% to 20% in groups A_2 and B_2 at 30th days. This can be explained by the fact that liver has excellent ability to regenerate.[24] Biochemical parameters (ALT, AST and bilirubin) increased significantly at day 30th because their level in blood was raised both in high and low dose groups.

Reversibility of nickel induced hepatotoxicity was observed after intramuscular administration of 10 mg/100g body weight of alpha-tocopherol by Tikare et al.[15] There is no evidence regarding the reversibility of hepatotoxicity, in literature, after withholding nickel sulphate.

It is ***concluded*** that:

1. Nickel induced hepatotoxicity was observed in the experimental animals at both high (1 mg/kg) and low (0.5 mg/kg) doses.
2. Gross, histological and biochemical analysis confirmed the toxic effects on liver. However; only histological and gross findings were reversed 10% to 20% but biochemical parameters were not reversed after stoppage of nickel sulphate in both high and low dose groups.

The release of nickel ions in dental prosthesis can cause transient hepatotoxicity on insertion of appliance; therefore nickel free dental alloys should be preferred. Therefore the release of nickel ions, their distribution and effect on vital organs may be re- evaluated.

ACKNOWLEDGMENTS

I would like to record my deepest gratitude. The department of Pathology, Morbid Anatomy and Pharmacology and the administration of Post Graduate Medical Institute, and the entire related person are

highly acknowledged for their moral, logistic and financial support.

Conflicts of Interest

Authors have no Conflicts of interest.

Author's Contribution

AQM: Author. NM and NI: Helped in write-up. AH: Helped in statistical analysis. AK: Research supervisor and mentor.

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