# CHAPTER 5

# Chapter 5

## Hepatic oxidative stress and toxicity due to Nickel exposure: Duration dependent study using realistic dosage and protective effect of melatonin.

Nickel (Ni) is considered an industrial hazard but not environmental hazard. There are occupational dangers associated with from nickel salts. Evidences suggest that nickel may be an essential trace element for mammals (Goyer, 1991). Various health effects are seen when exposed to nickel (Denkhaus and Salnikow, 2002; Lu *et al.,* 2005). Based on the epidemiological evidence that showed high incidence of nasal and lung cancers in occupationally exposed workers (Coogan *et al.,* 1989; Haber *et al.,* 2000a) and its potency to induce tumors in a variety of mammalian species (Sunderman 1977, 1989), nickel has been classified as human carcinogen. Although the toxicity and carcinogenicity of nickel compounds in human and experimental animals are well demonstrated (Sundermann *et al.,* 1985; Stohs and Bagchi, 1995), the mechanism of their action remain unclear. Nickel salts are shown to enhance lipid peroxidation (LPO) in various somatic tissues like, blood, muscle, liver and kidney of rats (Athar *et al.,* 1987; Kasprzak and Hernandez, 1989; Misra *et al.,* 1990; Chen *et al.,* 1998). Administration of nickel sulfate decreases hepatic glutathione (GSH) (Das *et al.,* 2001) and also alters the enzyme activities of glutathione-S-transferase (GST) and glutathione reductase (GR) (Mohammed *et al.,* 1987) and glutathione peroxidase (GPx) (Carnata *et al.,* 1992). Intraperitoneal (ip) injection of nickel for 28 days induces degenerative effects in the hepatic tissue with disturbed hepatic architecture and swollen hepatocytes, vacuolization and focal necrosis as shown by Das *et al.,* (2001). Nickel can elicit an oxidative stress through the generation of reactive oxygen species (ROS) (Salnikow *et al.,* 2000; Huang *et al.,* 2001) probably involving

Fenton/Haber-Weiss reactions, leading to lipid peroxidation and protein oxidation and DNA damage (Chakrabarti and Bai, 1999; Chen *et al.,* 2003).

The dominant source of nickel exposure in the non-smoking, non-occupationally exposed population is through food and water. Most of the studies evaluating oxidative stress induced by nickel is, either through intraperitoneal (ip) or subcutaneous (sc) administration but only very few studies have attempted oral exposure, which is the natural mode of exposure. There are very few long term exposure studies of nickel and hence, it becomes pertinent to understand the possible Ni toxicity on long term systemic entry into humans through diet and water. Ni has been identified as a major environmental pollutant present in high amounts in vegetables, cereals, pulses and grass grown in the highly industrialized city of Vadodara, Gujarat (Blacksmith Institute Report,1999; Labunska *et al.,* 1999; Ramachandran, 2003) and hence the present study was initiated to evaluate Ni induced hepatic oxidative stress and toxicity on male *Wistar* rats. A realistic dosage has been worked out based on the Ni content in vegetables and food grains and an average daily food intake with a conversion factor of 6.2 (OECD,2005) for extrapolation of dosage from human to rat. Using such a dosage, a duration dependent (15, 30 and 60 days) hepatic oxidative stress and toxicity have been evaluated.

As entry of metal toxicants into the body is unavoidable due to industrialization, there is need to evaluate the role of agents which can be used as antioxidant therapeutants. Since melatonin is recognized as a powerful natural antioxidant of the body, the efficacy of the same has been tested as a protectant by co-administration along with nickel.

**Material and Methods**: Same as in Chapter 1 but the metal given is Nickel (Ni) and the dose given is 200 mg/kg/BW/day.

## Results:

**Lipid Peroxidation (LPO)**: Changes in the hepatic LPO levels following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 5.1; table 5.1. An age dependent gradual increase in LPO was seen in control (Con) rats. There was significant increase in LPO content in Ni treated group when compared to Con animals. There was significant decrease in LPO in Con and Ni treated rats treated with melatonin. Ni treatment showed duration dependent decrement in LPO with maximal level being at 15 days and minimal at 60 days. Irrespective of duration of Ni exposure, melatonin afforded same degree of protection.

**Glutathione (GSH):** Changes in hepatic glutathione levels following Ni treatment for 15, 30 and 60 days are shown in figure 5.2; table 5.2. A gradual age dependent decrease in GSH level was the feature of Con rats. There was significant duration dependent decrease in the levels of GSH in Ni exposed animals compared to the Con group of animals and significant protection in Mel group of animals. Correspondingly, progressively decreasing protection effect of melatonin was discernable on co-administration with Ni.

**Ascorbic Acid (Vit C)**: Changes in hepatic Vit C levels following nickel treatment for 15, 30 and 60 days are shown in figure 5.3; table 5.3. Liver being the storage organ of Vit C in rodents, has a much higher content than the synthetic organ, the kidney. There was significant progressive decrease in Vit C levels in the Ni group when compared to Con group of animals. There was duration dependent protection by melatonin with maximal protection being seen at 60 days. There was duration

dependent degree of protection by melatonin with the maximal protection being at 60 and 15 days in that order.

**Superoxide Dismutase (SOD)**: Changes in hepatic SOD activity following Ni treatment for 15, 30 and 60 days are shown in figure 5.4; table 5.4. Control animals showed an age dependent decrease in SOD activity. There was significant decrease in SOD activity in the Ni group of animals compared to the Con group of animals with, relatively and significantly higher decrement in the short term duration of Ni exposure groups. There was significant increase in SOD activity in Mel and Ni+Mel group of animals compared to Con and Ni group of animals. The corresponding degree of protection with melatonin was also less in the 60 day Ni exposure group.

**Catalase (CAT):** Changes in hepatic CAT activity following Ni treatment for 15, 30 and 60 days are shown in figure 5.5; table 5.5. Hepatic CAT activity tended to show an age dependent decrement. There was significant decrease in CAT activity in Ni group of animals. Catalase activity was significantly decreased in a duration dependent manner in Ni exposed rats irrespective of duration of exposure. Degree of protective effect of melatonin was also found to be duration dependent with increase in protection on increase in duration.

**Glutathione Peroxidase (GPx)**: Changes in hepatic glutathione peroxidase (GPx) following Ni treatment for 15, 30 and 60 days are shown in figure 5.6; table 5.6. An age dependent decrement in GPx activity was the feature of Con group of rats. The Ni group of animals showed significant decrease in GPx activity with duration dependent positive inhibition. The degree of protective effect with melatonin was found to be duration independent.

**Metal Load:** Changes in hepatic accumulation of Ni following Ni treatment for 15, 30 and 60 days are shown in figure 5.7; table 5.7. Ni treated rats showed significant increase in hepatic Ni load with the load tending to show statistically insignificant reduction wit increasing duration. There was significant decrement in Ni accumulation in animals treated with melatonin alone or in combination with Ni. Both, Ni induced hepatic load as well as the degree of protection by melatonin were duration dependent.

**Serum Parameters:** Changes in serum glucose, insulin, melatonin and hepatotoxicity parameters like alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are shown in tables 5.8, 5.9 and 5.10. There is an age dependent decrease in melatonin level in the Con group of animals. There was significant decrease in melatonin levels in Ni treated group. There was a significant increase in the blood glucose level and significant decrease in insulin titer in Ni treated groups compared to Con group of animals with melatonin being able to decrease the blood glucose level to near normal values with corresponding changes in insulin level. The hepatic toxicity parameters (ALT and ALP) showed significant increase in Ni treated groups and significant protective effect was seen in the animals co-administered with melatonin. There was significant decrease in hemoglobin content on longer duration of Ni exposure.

**Histology:** Changes in hepatic histology following Ni treatment for 15, 30 and 60 days are shown in figure 5.8 to 5.10. Ni induced histological changes like hypertrophy and degeneration of hepatocytes, dilation of sinusoids, disturbed arrangement of hepatic cords, disruption of central vein and vacuolization and necrotic cells with fibrous periportal area could be seen clearly in all the duration period. These changes

were resisted and near normal histoarchitecture weas observed by co-administration with melatonin.

**Figure 5.1: Graph showing levels of hepatic lipid peroxidation (LPO) in animals exposed to Ni for 15, 30 or 60 days**

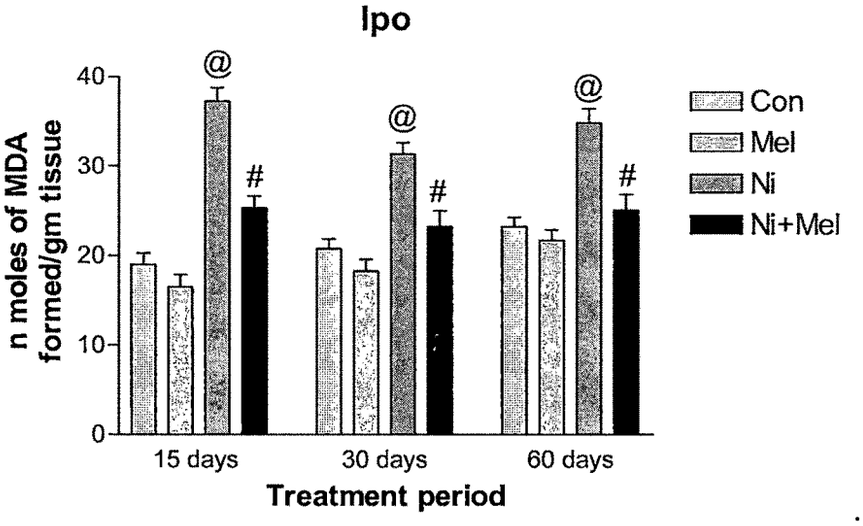


**Table: 5.1 Levels of hepatic lipid peroxidation (LPO) following 15, 30 and 60 days of Ni exposure**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 19.02 ± 1.26 | 20.79 ± 1.080 | 23.30 ± 1.02 |
| Mel | 16.490 ± 1.39 | 18.280 ± 1.290 | 21.76 ± 1.17 |
| NI | 37.29±1.49@ | 31.37±1.29@ | 34.84±1.56@ |
| Ni+Mel | 25.35±1.29# | 23.28±1.72# | 25.10±1.78# |

**@ p<0.05 between Con vs Ni**
**# p<0.05 between Ni vs Ni+mel**
***p<0.05 between Con vs Mel**

**Figure 5.2: Graph showing levels of hepatic glutathione (GSH) content in animals exposed to Ni for 15, 30 or 60 days**

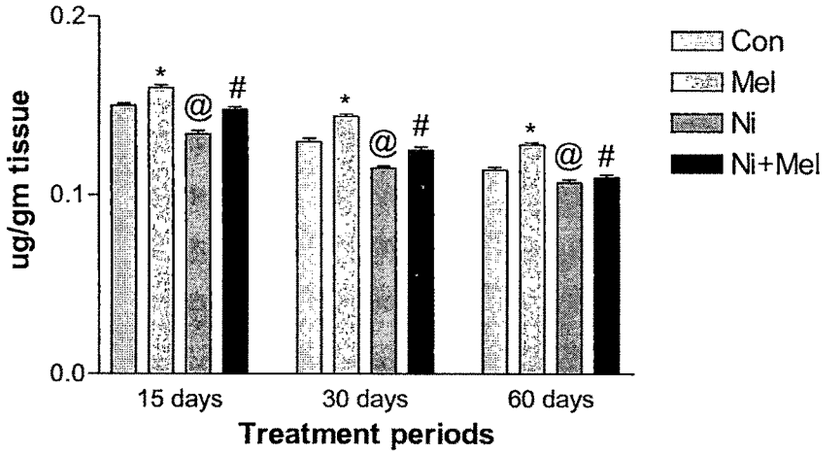


**Table 5.2. Contents of hepatic glutathione (GSH) contents following exposure to Ni for 15, 30 or 60 days**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 0.150 ± 0.0012 | 0.130± 0.0017 | 0.114±0.0015 |
| Mel | 0.160±0.0017* | 0.144±0.0012* | 0.128±0.0014* |
| Ni | 0.134±0.0021@ | 0.115±0.0013 @ | 0.107±0.0018 @ |
| Ni+Mel | 0.148 ±0.0015# | 0.125±0.0019 # | 0.110±0.0014 # |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Figure 5.3. Graph showing levels of hepatic Ascorbic Acid (Vit C) content in animals exposed to Ni for 15, 30 or 60 days**

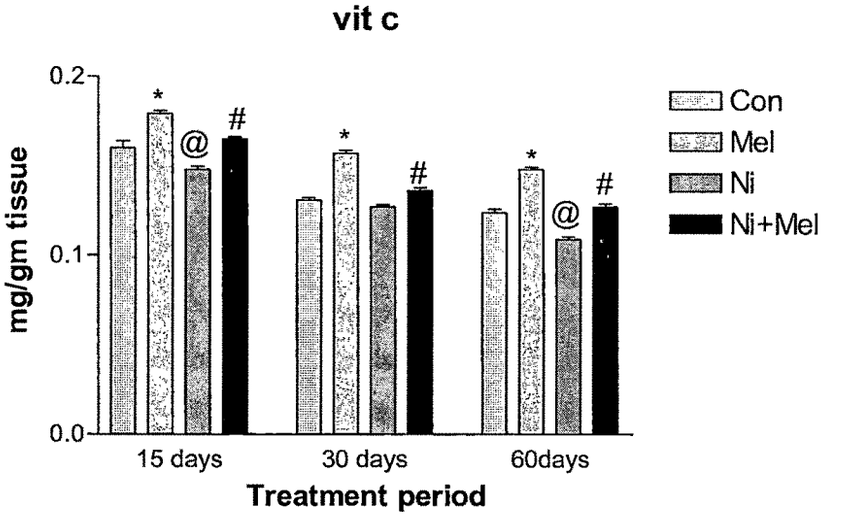


**Table 5.3. Contents of hepatic Ascorbic Acid (Vit C) following 15, 30 or 60 days treatment with Nickel**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 0.160±0.0014 | 0.131± 0.0013 | 0.124±0.0019 |
| Mel | 0.179±0.0018* | 0.157± 0.0018* | 0.148±0.0013* |
| Ni | 0.148±0.0018@ | 0.127± 0.0013@ | 0.109±0.0015 @ |
| Ni+Mel | 0.165±0.0012# | 0.136±0.0019# | 0.127±0.0017 # |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Figure 5.4. Graph showing hepatic Superoxide Dismutase (SOD) activity following 15, 30 or 60 days exposure with chromium.**

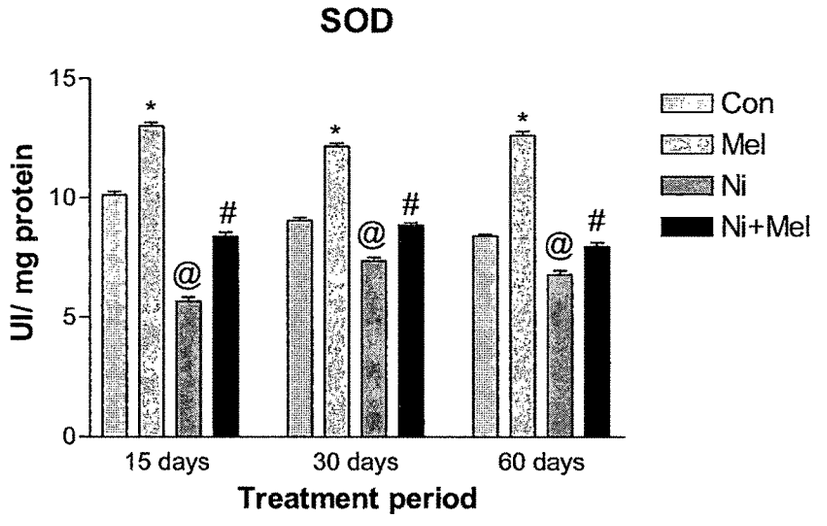


**Table 5.4. Levels of hepatic Superoxide Dismutase (SOD) activity following 15, 30 and 60 days exposure with Nickel**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 13.8700 ± 0.1300 | 12.0300 ± 0.1600 | 10.5200 ± 0.1270 |
| Mel | 16.0100 ± 0.1600* | 14.2700 ± 0.1100 * | 12.3700 ± 0.1610 * |
| Ni | 5.67 ± 0.170 @ | 7.37 ± 0.15 @ | 6.79 ± 0.18 @ |
| Ni+Mel | 8.38 ± 0.190 # | 8.87 ± 0.098 # | 7.98 ± 0.16 # |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Figure 5.5. Graph showing hepatic catalase (CAT) activity following 15, 30 or 60 days exposure to nickel.**

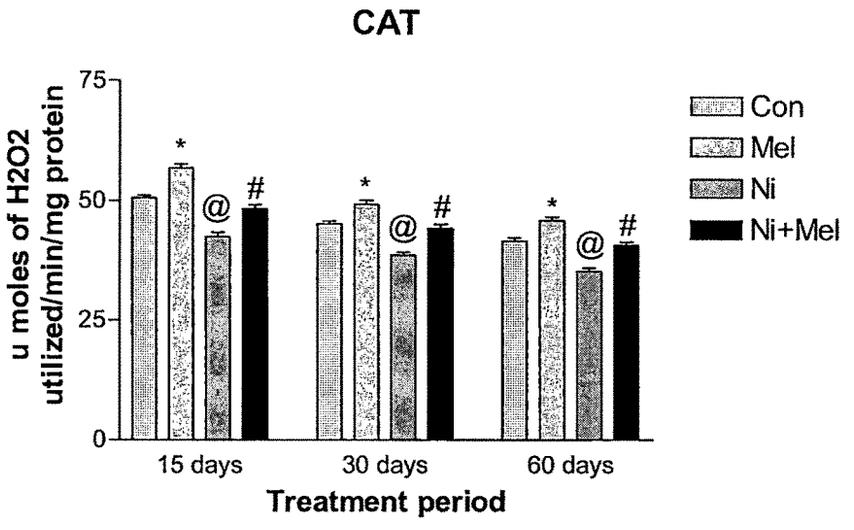


**Table 5.5. Levels of hepatic catalase (CAT) activity following 15, 30 or 60 days exposure with Nickel**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 50.600 ± 1.100 | 45.190 ± 1.390 | 41.630 ± 1.730 |
| Mel | 56.80 ± 1.780* | 49.29 ± 1.820* | 45.97 ± 1.620* |
| Ni | 42.52±1.98 @ | 38.64±1.39@ | 35.32±1.62@ |
| Ni+Mel | 48.35±1.74 # | 44.29 ±1.98# | 40.72± 1.63# |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Figure 5.6. Graph showing hepatic Glutathione Peroxidase (GPx) activity following 15, 30 and 60 days exposure to nickel.**

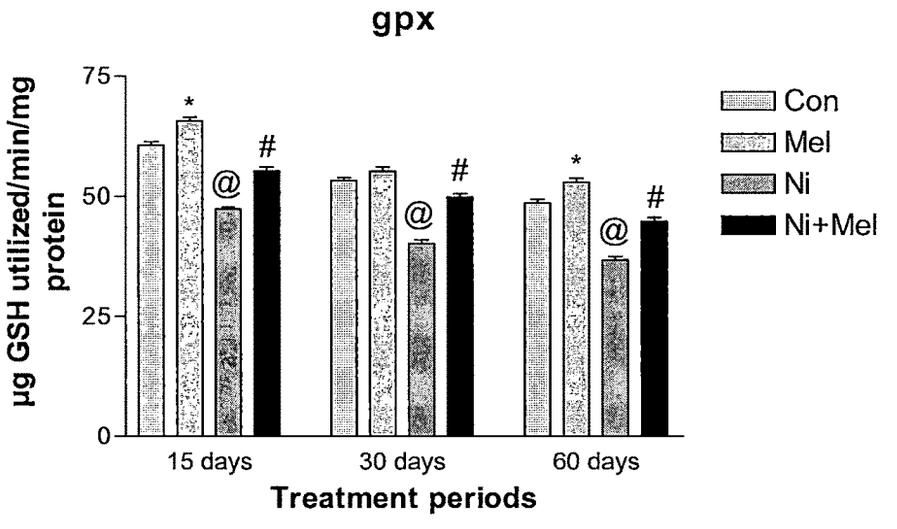


**Table 5.6. Levels of hepatic Glutathione Peroxidase (GPx) activity following 15, 30 and 60 days exposure with Nickel**

| | 15 days | 30 days | 60 days |
|---|---|---|---|
| Con | 60.730 ± 1.5 | 53.38 ± 1.15 | 48.72 ± 1.62 |
| Mel | 65.80 ± 1.7 | 55.28 ± 1.91 | 53.02 ± 1.85 |
| Ni | 47.40±1.04 @ | 40.20±1.79 @ | 36.82±1.58 @ |
| Ni+Mel | 55.35±1.84 # | 49.91±1.72 # | 44.82±2.04 # |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Figure 5.7. Graph showing % accumulation of Ni in hepatic tissue following 15, 30 and 60 days treatment with Nickel**

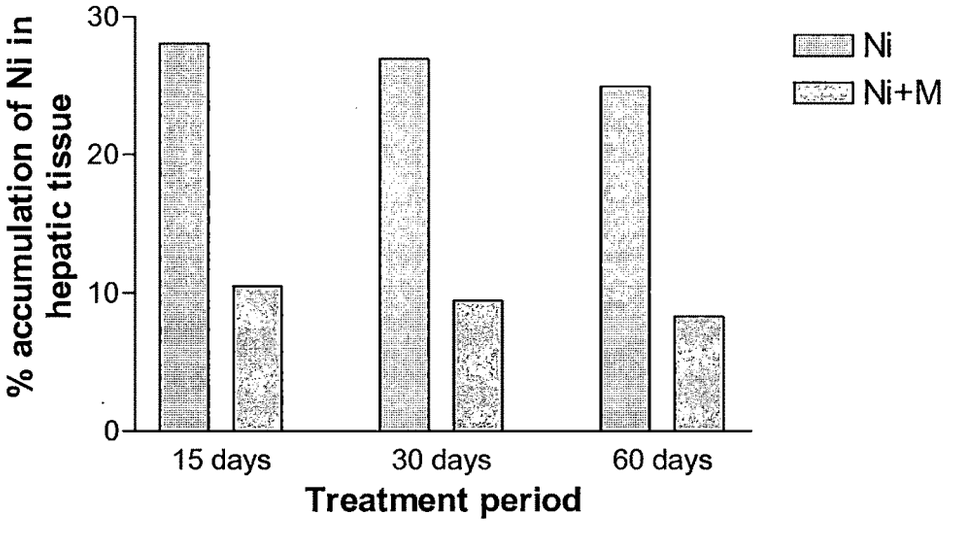


**Table 2.7. % accumulation of Ni in hepatic tissue following 15, 30 and 60 days treatment with Nickel**

| Treatment Period | Cd | Cd+M |
|---|---|---|
| **15 days** | 28.09 | 10.48 |
| **30 days** | 26.95 | 9.48 |
| **60 days** | 25 | 8.33 |

**Table 5.8. Changes in serum glucose, insulin and melatonin levels following 15, 30 or 60 days treatment with nickel**

| | 15 Days | | | 30 Days | | | 60 Days | | |
|---|---|---|---|---|---|---|---|---|---|
| | Serum glucose(mg/dl) | Insulin (µg/l) | Melatonin (pg/ml) | Serum glucose(mg/dl) | Insulin (µg/l) | Melatonin (pg/ml) | Serum glucose(mg/dl) | Insulin (µg/l) | Melatonin (pg/ml) |
| Control (Con) | 112.5±2.51 | 1.7±0.06 | 121±1.65 | 104.25±2.85 | 1.58±0.09 | 112±2.08 | 115.75±3.85 | 1.79±0.01 | 93±4.41 |
| Melatonin (Mel) | 125±3.11 | 0.6±0.09* | 140±2.65* | 115.50±1.65 | 0.95±0.01* | 153±6.65* | 120.5±2.65 | 0.81±0.05* | 126±5.65* |
| Chromium (Cr) | 148.5±1.24@ | 0.61±0.01@ | 71±5.91@ | 129.50±1.35@ | 1.08±0.09@ | 78±7.15@ | 137.6±2.85@ | 1.25±0.01 | 31±8.01@ |
| Cr+Melatonin (Cr+Mel) | 129.60±1.06# | 1.20±0.13# | 116±7.91# | 116.75±1.84# | 1.30±0.01 | 104±2.65 | 118.78±2.11# | 0.53±0.06# | 84±5.85# |

`@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Table5.9 Changes in serum ALP and ALT following 15, 30 and 60 treatment with Ni**

| | 15 days | | 30 days | | 60days | |
|---|---|---|---|---|---|---|
| | **ALP(U/I)** | **ALT(U/ml)** | **ALP(U/I)** | **ALT(U/ml)** | **ALP(U/I)** | **ALT(U/ml)** |
| **Control (Con)** | 154.0.5±3.98 | 45.2±1.25 | 161±4.6 | 48.5±2.10 | 159.8±3.45 | 49.9±1.90 |
| **Melatonin (Mel)** | 119±4.80* | 43.1±3.40 | 134.6±3.92* | 47.8±2.90 | 129.6±2.5* | 51.1±3.10 |
| **Nickel(Ni)** | 189.5±2.75@ | 54.71±1.98@ | 202.56±3.15@ | 59.5±2.90@ | 209.9±2.50@ | 84.5±3.20@ |
| **Ni+Melatonin (Ni+Mel)** | 165.79±3.15# | 47.25±2.50# | 165.35±3.76# | 50.57±2.98# | 165.51±3.15# | 56.6±2.59# |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

**Table 5.10 Changes in hematological parameters following treatment with Ni following 15, 30 and 60 days**

| | 15 Days | | | 30 Days | | | 60 Days | | |
|---|---|---|---|---|---|---|---|---|---|
| | **Hemoglobin** | **Erythrocyte count** | **Packed Cell Volume (PCV)** | **Hemoglobin** | **Erythrocyte count** | **Packed Cell Volume (PCV)** | **Hemoglobin** | **Erythrocyte count** | **Packed Cell Volume (PCV)** |
| **Control (Con)** | 15.05±0.06 | 9.66±0.01 | 51.75±0.85 | 16.75±0.48 | 10.27±0.01 | 51.75±1.32 | 15.75±0.13 | 10.68±0.09 | 60.25±1.25 |
| **Melatonin (Mel)** | 15.60±0.15 | 10.13±0.01 | 56.00±0.91 | 16.00±1.08 | 9.92±0.01 | 56.25±1.11 | 15.75±0.85 | 10.72±0.01 | 54.00±1.83 |
| **Nickel(Ni)** | 16.28±0.09 | 10.43±0.01 | 55.50±1.04 | 13.45±0.06@ | 8.38±0.01@ | 42.75±0.85 | 11.52±0.05@ | 9.87±0.01 | 47.25±0.85@ |
| **Ni+Melatonin (Ni+Mel)** | 15.55±0.06 | 10.06±0.01 | 58.00±0.91 | 14.60±0.09 | 9.97±0.01# | 53.25±0.85 | 14.40±0.15# | 9.57±0.01 | 50.25±0.63# |

@ p<0.05 between Con vs Ni
# p<0.05 between Ni vs Ni+mel
*p<0.05 between Con vs Mel

## PLATE I

**Fig 5.8** Photomicrograph of control liver(400X) showing a hepatic lobule. Note the cord like organization of hepatocytes and a central vein. CV- Central Vein, S- Sinusoid

**Fig 5.9** Photomicrograph of 15 day melatonin treated liver (400X). Note the robust appearance of hepatic cords. S- Sinusoid

**Fig 5.10** Photomicrograph of 15 day nickel exposed liver (400X). Note the endothelial disruptions in the central vein (arrows).

**Fig 5.11** Photomicrograph of liver exposed to Ni+Mel for 15 day (400X). note the near normal organization of hepatic lobule and intact central vein.

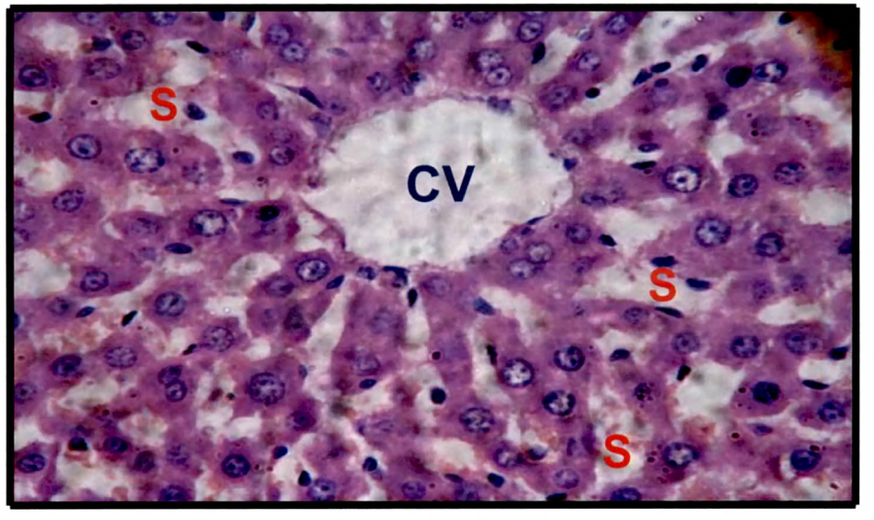


Fig 5.8 Control

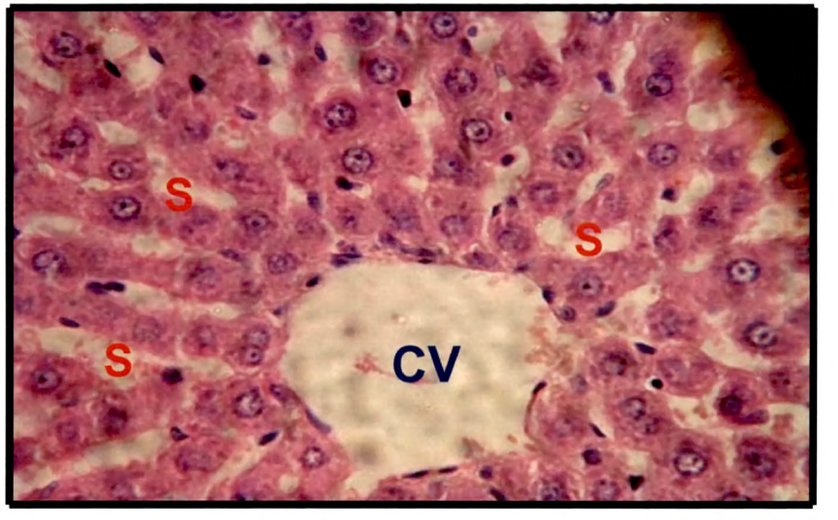


Fig 5.9 Melatonin

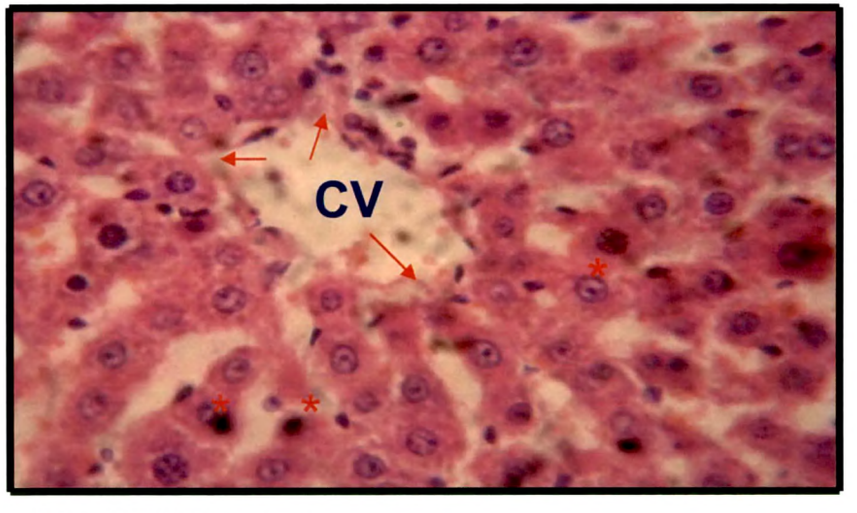


Fig 5.10 Nickel

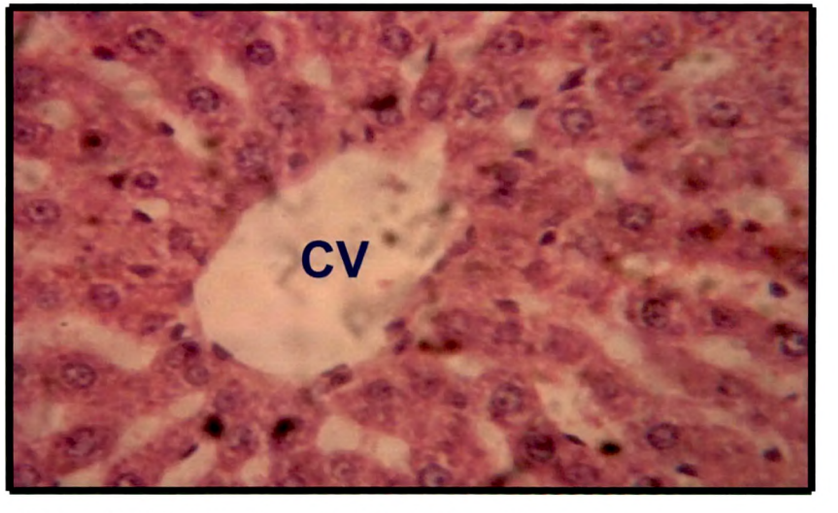


Fig 5.11 Nickel + Melatonin

## PLATE II

**Fig 5.8(a)** Photomicrograph of control liver showing periportal area (400X) PPA-Periportal Area

**Fig 5.9(a)** Photomicrograph of melatonin treated liver showing PPA area (400X); PPA-Periportal Area

**Fig 5.10(a)**Photomicrograph of 15 day nickel treated liver showing periportal area (400X). Note the congestion in portal vein and glassy degeneration of hepatocytes. PV-Portal vein

**Fig 5.11(a)** Photomicrograph of liver exposed to Ni+Mel for 15 day showing periportal area (400X). Note the minimal effect of Ni.

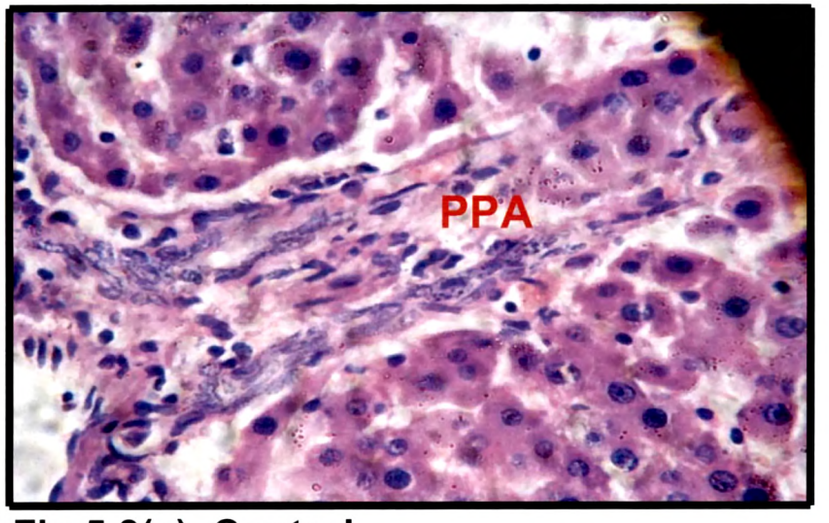


Fig 5.8(a) Control

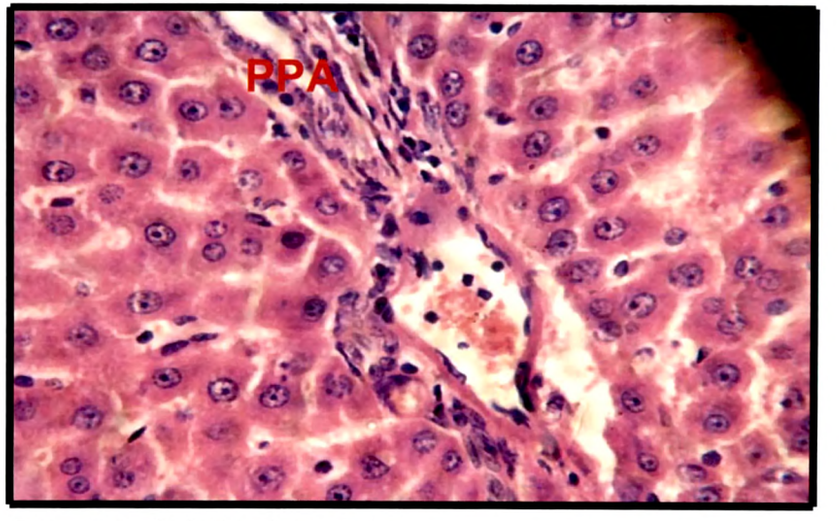


Fig 5.9 (a) Melatonin

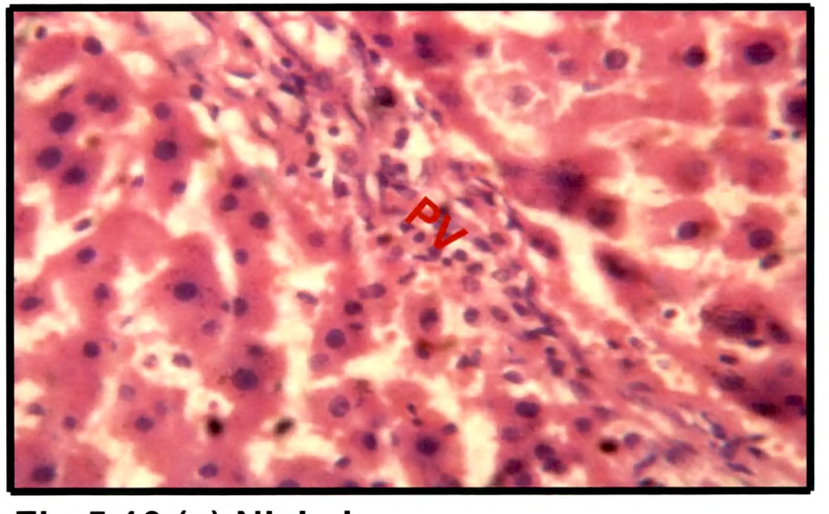


Fig 5.10 (a) Nickel

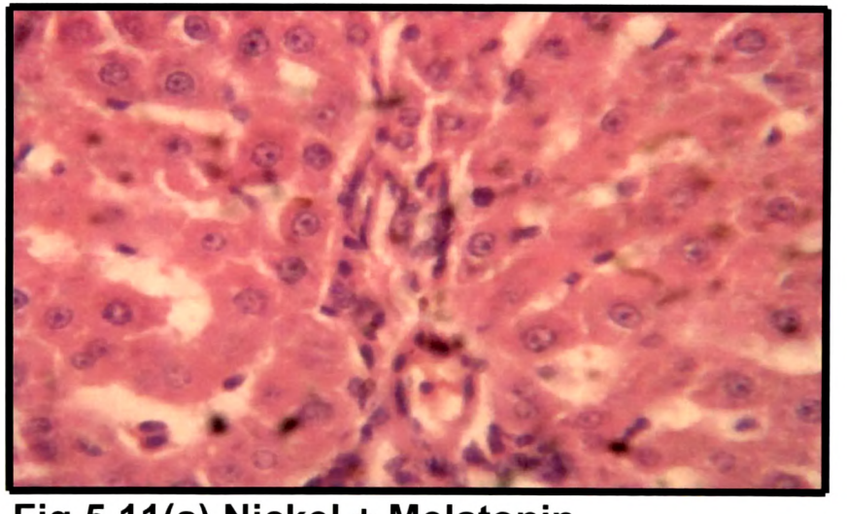

Fig 5.11(a) Nickel + Melatonin

## PLATE III

**Fig 5.12** Photomicrograph of control liver showing a hepatic lobule (400X). CV Central Vein; S Sinusoids.

**Fig 5.13** Photomicrograph of 30 day melatonin treated liver (400X). Showing central vein and hepatic cords.

**Fig 5.14** Photomicrograph of 30 day nickel treated liver showing a hepatic lobule (400X). Note the complete disruption of the endothelium of central vein (arrow) confluencing with distended sinusoid (s). Degenerating hepatocytes are clearly seen.

**Fig 5.15** Photomicrograph of liver exposed to Ni+Mel for 30 days showing a hepatic lobule (400X). Note the intact central vein but some degenerative changes in hepatocytes can be seen.

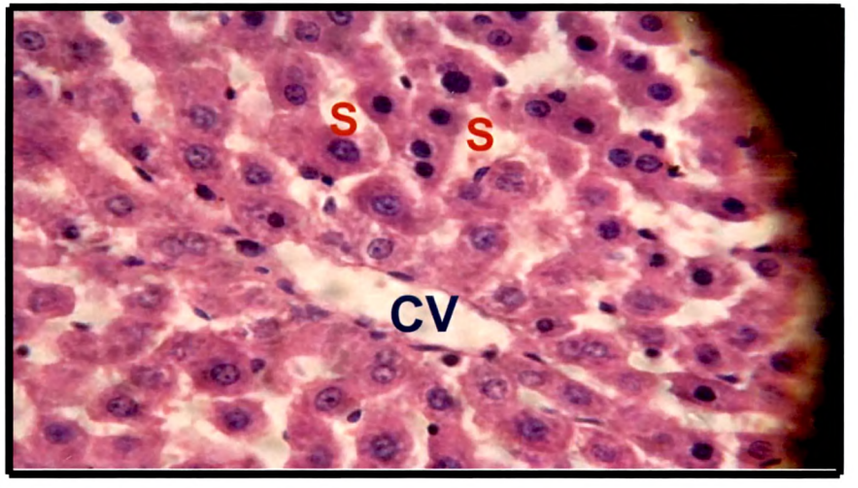


Fig 5.12 Control

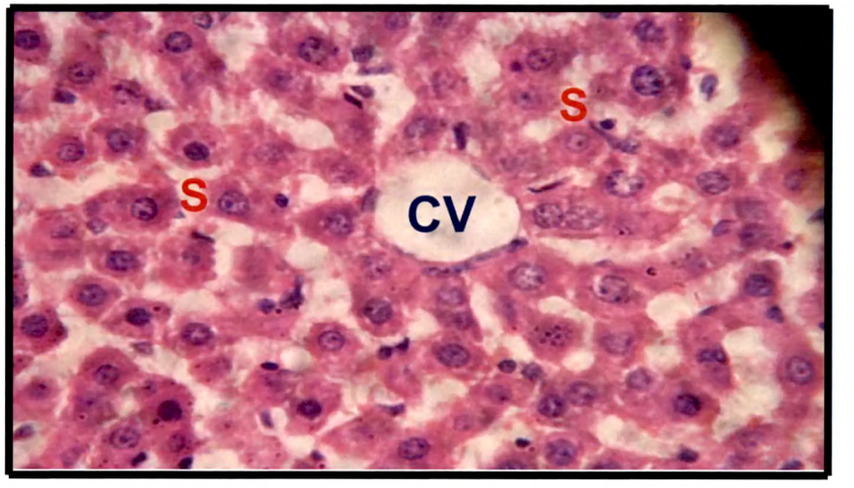


Fig 5.13 Melatonin

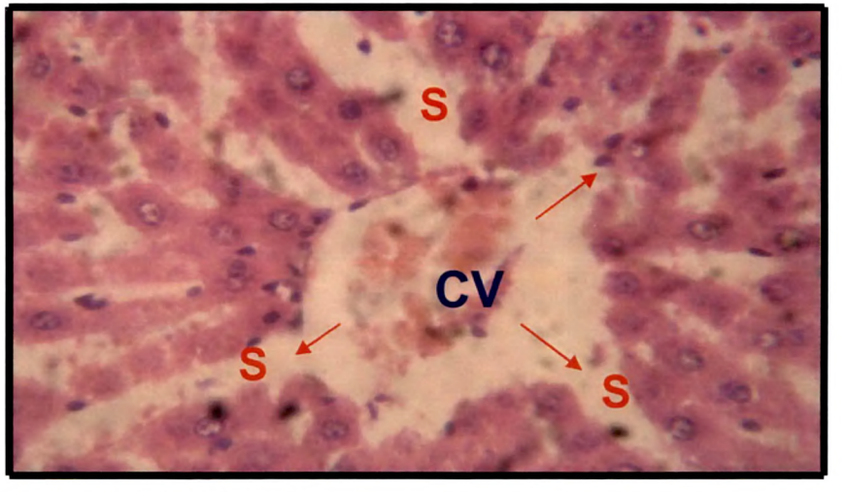


Fig 5.14 Nickel

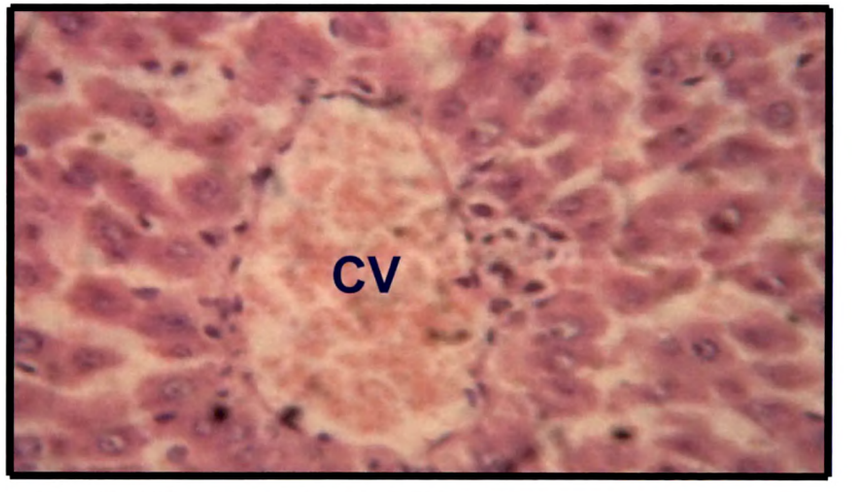


Fig 5.15 Nickel + Melatonin

## PLATE IV

**Fig 5.12(a)** Photomicrograph of control liver showing periportal area (400X).PPA-Periportal Area

**Fig 5.13(a)** Photomicrograph of 30 day melatonin treated liver showing PPA (400X)

**Fig 5.14(a)** Photomicrograph of liver exposed to Ni for 30 days showing PPA area (400X). Note the distended periportal vein (PV) with leucocyte influx. Degenerating hepatocytes can also be seen (arrow).

**Fig 5.15(a)** Photomicrograph of liver exposed to Ni+Mel for 30 days showing PPA area(400X). Note the near normal appearance but leucocytic influx in the portal vein (PV).

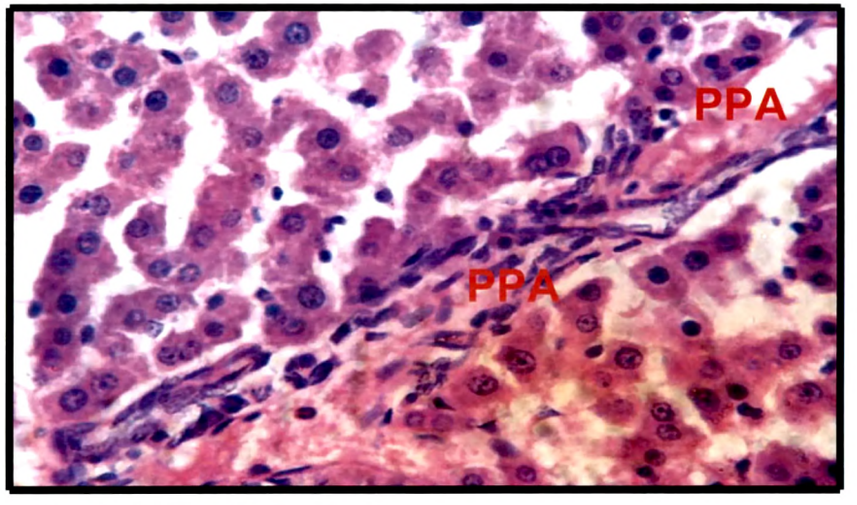


Fig 5.12 (a) Control

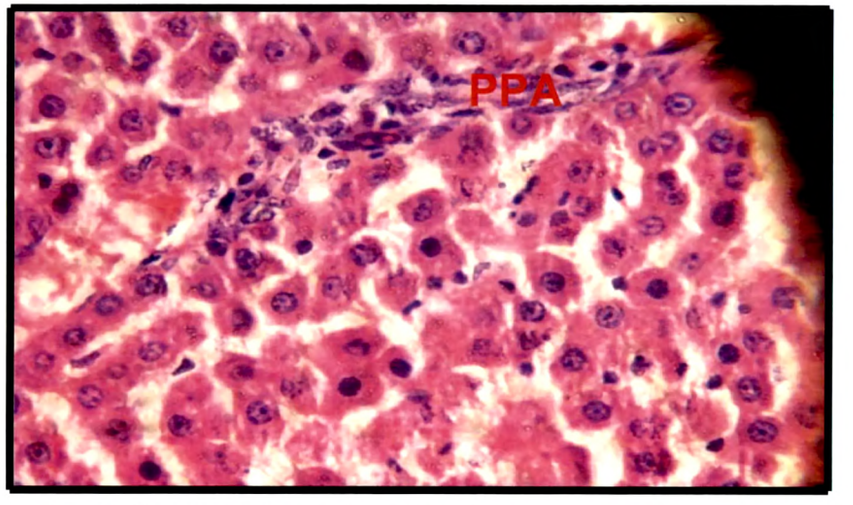


Fig 5.13 (a) Melatonin

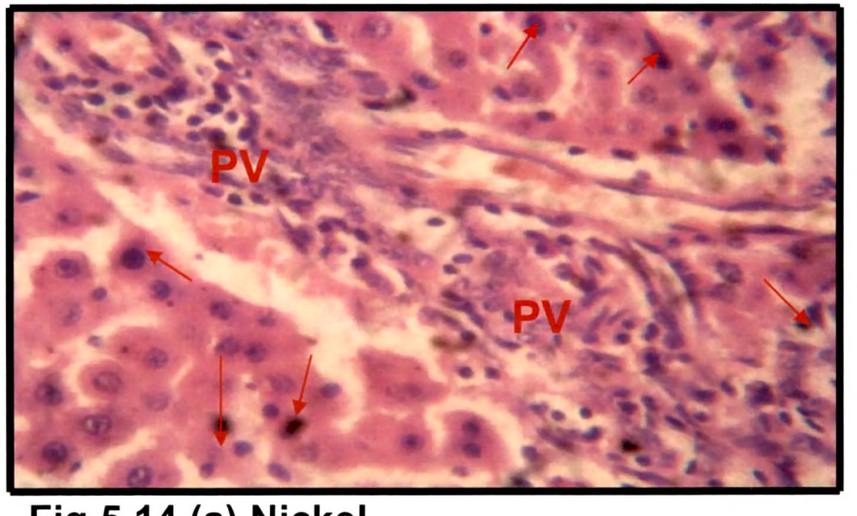


Fig 5.14 (a) Nickel

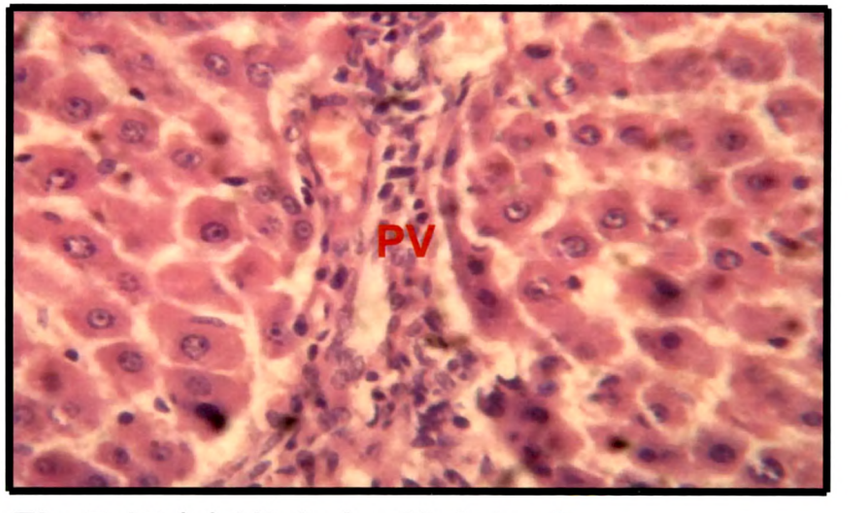


Fig 5.15 (a) Nickel + Melatonin

## PLATE V

**Fig 5.16** Photomicrograph of 60 day control liver (400X) showing a hepatic lobule. Note the cord like organization of hepatocytes and a central vein. CV- Central Vein, S- Sinusoid

**Fig 5.17** Photomicrograph of 60 day melatonin treated liver (400X). Note the robustness of hepatocytes.

**Fig 5.18** Photomicrograph of 60 days nickel exposed liver (400X). Note the breaches in the central vein (arrow) and its confluencing with sinusoids. Hepatocyte degeneration also evident.

**Fig 5.19** Photomicrograph of liver exposed to Ni+Mel for 60 days (400X). Near normal but minor disruption in endothelium of central vein (arrows) and some degenerated hepatocytes can be seen.

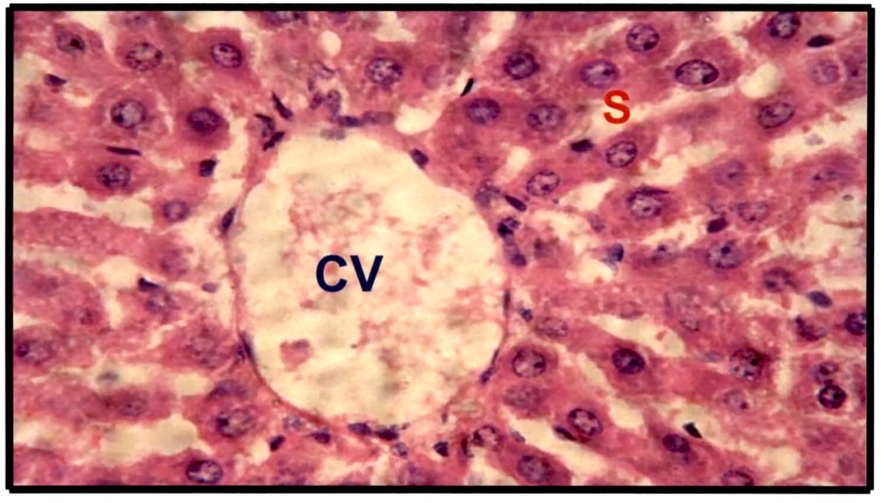


Fig 5.16 Control

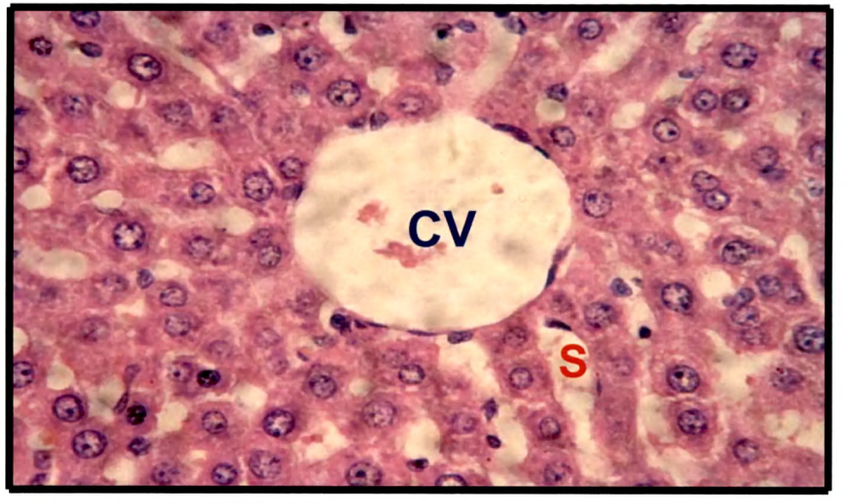


Fig 5.17 Melatonin

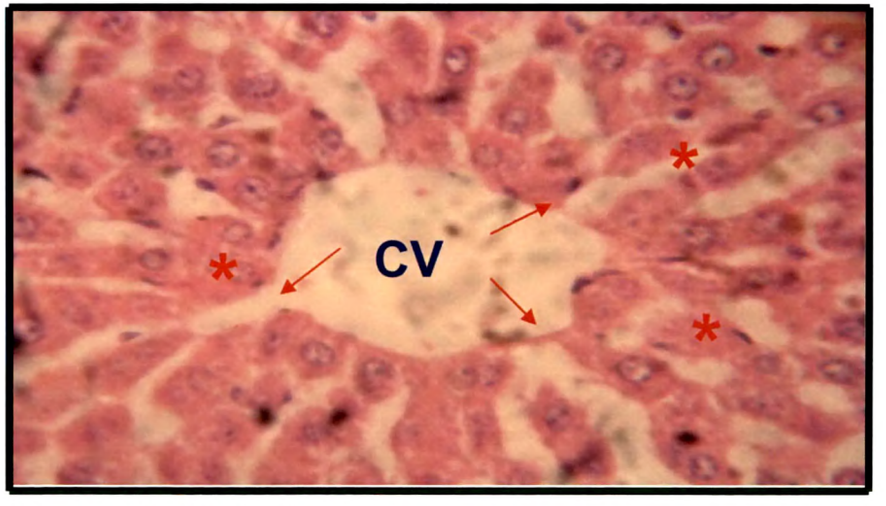


Fig 5.18 Nickel

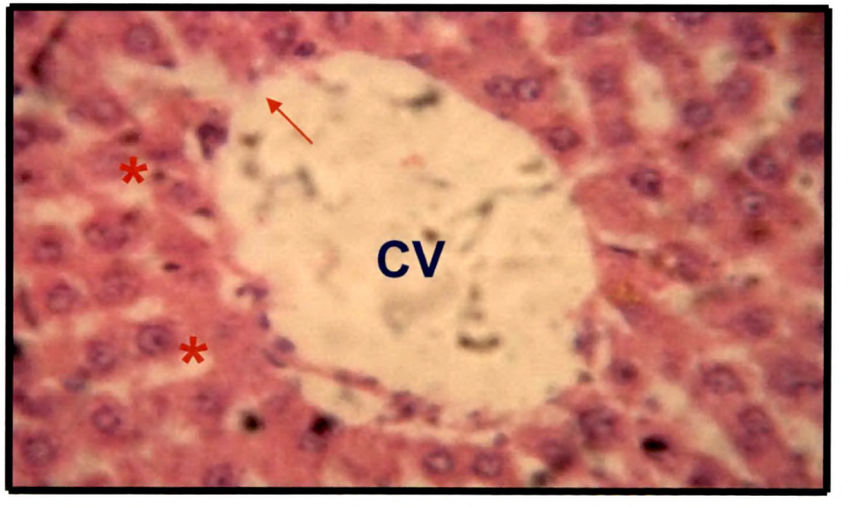


Fig 5.19 Nickel + Melatonin

## PLATE VI

**Fig 5.16(a)** Photomicrograph of central vein showing periportal area (400X). PPA-periportal area

**Fig 5.17(a)** Photomicrograph of liver treated with melatonin for 60 days showing periportal area (400X)

**Fig 5.18(a)**Photomicrograph of liver exposed to nickel for 60 days showing disintegrated periportal area (400X). Note the distorted periportal area and degenerated hepatocytes leading to formation of empty spaces(*).

**Fig 5.19(a)** Photomicrograph of liver exposed to Ni+Mel for 60 days showing periportal area (400X). hepatocytes appear normal appearance. The effect of nickel is not seen but slight congestion of portal vein is observed(PV).

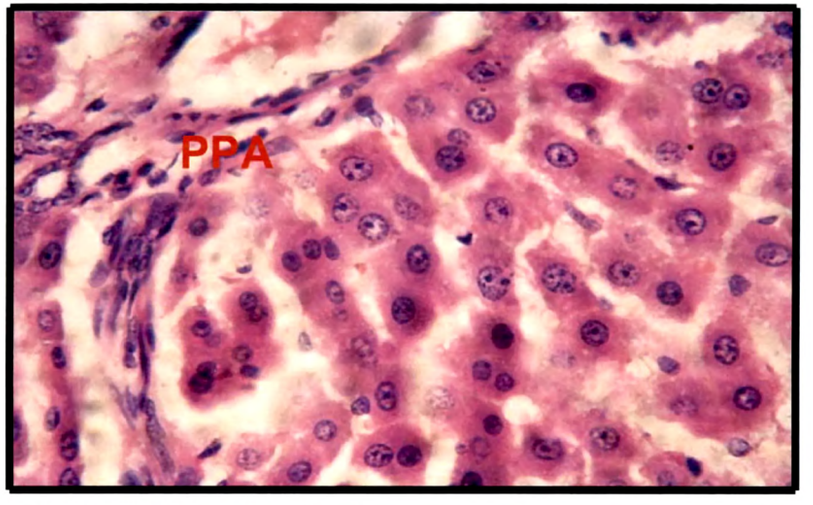


Fig 5.16(a) Control

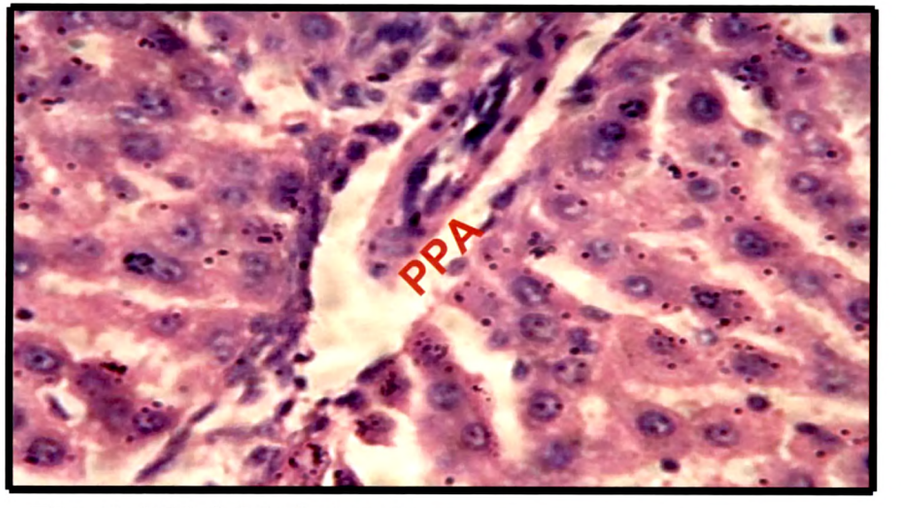


Fig 5.17(a) Melatonin

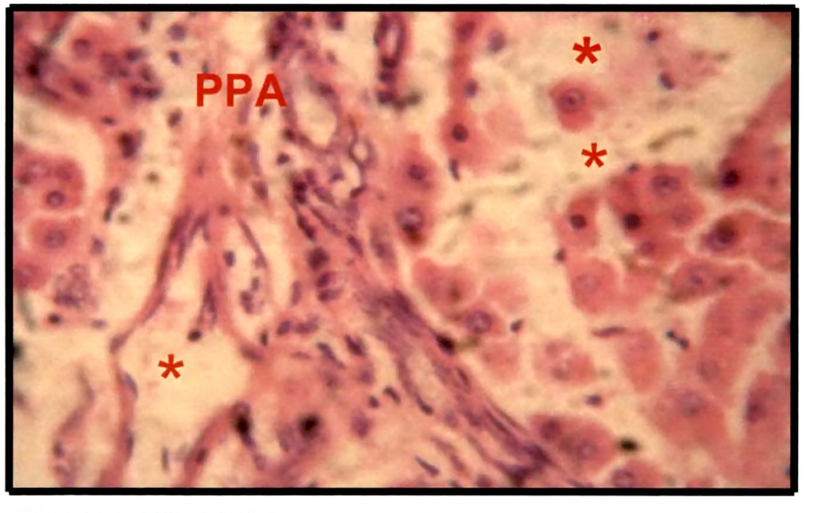


Fig 5.18(a)Nickel

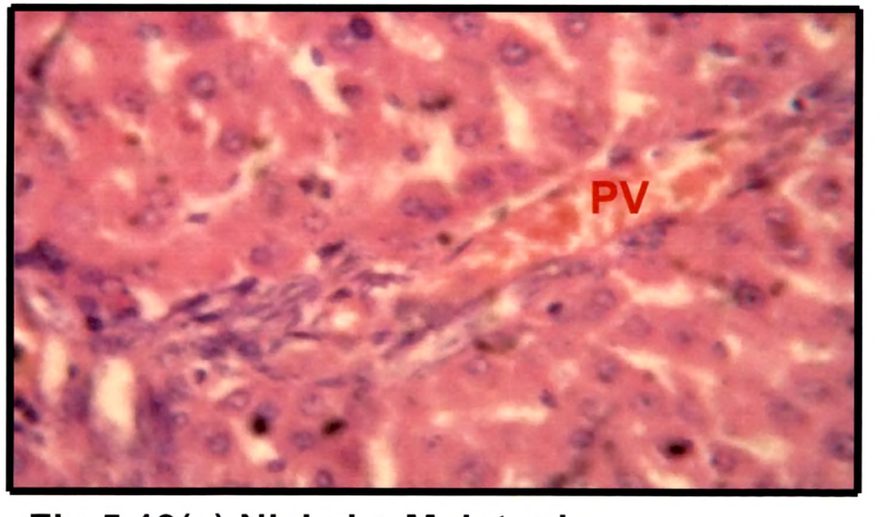


Fig 5.19(a) Nickel + Melatonin

**Discussion:**

Nickel is another metal toxicant which causes tissue damage by generation of oxidative stress (OS) but unlike Cd, Cr and Hg, the mechanism of action of Ni in generating ROS and OS is not clearly elucidated .Differing mechanisms like depletion of GSH by binding to –SH groups, NO mediated CAT inhibition ,direct generation of minor amounts of free radicals and Ni mediated Fe displacement from membrane proteins and the consequent Fe mediated Fenton reaction have all been hypothesized as all possible mechanism of Ni induced oxidative stress (Das *et al.*, 2008).Almost all the above studies involve acute or single dose exposure to Ni and the response is studied within hours or days of exposure and hence not comparable with chronic studies. Even chronic studies need to be on an environmentally relevant realistic dosage worthy of human simulation. Unfortunately much background noises in the form of metal induced responses have been generated by unthoughtful and irrational toxicological evaluations which adds to quite a bit of confusion. In this background, the present study using an environmentally realistic dosage simulatable to human situation has revealed the following salient points.

1. There is significantly elevated LPO in a short duration exposure of 15 days which tends to decrease a prolonged exposure through 30-60 days.

2. There is steady progressive depletion in GSH and Vitamin C.

3. There is steady increased inhibition of CAT and GPx.

4. There is maximal inhibition of SOD at 15 days followed by decreasing inhibition by 30 and 60 days.

5. Maximal hepatic Ni load occurs at 15 days which tends to decrease by 30 and 60 days.

These observations have been taken to suggest heightened oxidative stress on a chronic short duration exposure and the probable setting in of an adaptive mechanism thereafter to control the oxidative stress. The mechanism of elevated oxidative stress at 15 days is thought to be due to primarily Ni induced NO generation leading to inhibition of CAT by binding of NO to heme group in the CAT. This accounts for increased accumulation of H2O2.Concurrently due to the depletion of GSH and Vitamin C by Ni, there is a build up of superoxide radical normally generated by the oxidative metabolic activity of the tissue. This leads to overindulgence of SOD in trying to neutralize the elevated superoxide radical contributing to observed higher degree of inhibition of SOD at 15 days. Simultaneously interaction between superoxide anion and $H_2O_2$ leads to generation of hydroxyl radical. This process may be further aided and abetted directly by Ni and also indirectly by Fe that is released from membrane proteins by Ni as reports are available to show decreased Fe in tissue (Roy and Akhtar, 2002).Such a mechanism of changes appear to be the explanation for the high oxidative stress seen at 15 days of Ni exposure.

The possible explanation for the recorded decrease in oxidative stress through 30-60 days of Ni exposure is apparently the commissioning of adaptive mechanism by hepatic tissue to counter the cascading oxidative stress. This counter antioxidant mechanism could essentially involve induction of MT by Ni in the liver. This is well supported by reports suggesting the ability of Ni to induce MT (Misra *et al.*, 1988). The so induced MT can neutralize Ni by forming a complex and being released into circulation .It is also likely that melatonin might also aid in removal of Ni by forming

a complex with it as is known for Cd (Carolina *et al.*, 2008) which could corroborate by herein recorded decrease in serum melatonin level at the longest duration of Ni exposure. The observed check in hepatic Ni accumulation at 30 and 60 days agrees quite well with this contention. The subdued level of free radicals now seem to be adequately handled by the endogenous antioxidant machinery as noted by the increased depletion of non enzymatic antioxidants and increased inhibition of enzymatic antioxidants i.e. CAT and GPx. The decreased titer of serum melatonin in Ni treated animals can also be related with its ability to directly quench free radicals, specially hydroxyl radicals as it can saturate all intracellular compartments.

Nickel by itself does not cause efficient free radical generation but can generate free radicals directly from molecular oxygen in a two step process to produce superoxide anion. In the presence of Ni, the superoxide anions formed can then combine with protons in the dismutation reaction generating hydrogen peroxide in the process. Studies by Misra *et al.* (1990) and Huang *et al.* (2001) have shown that Ni induces $H_2O_2$ in tissues. Catalase and GPx are the enzymes which are actively involved in removing $H_2O_2$ from the tissues. With the decrement of GSH and inactivation of GR the activity levels of GPx also is decreased in the Ni treated groups. Nickel is known to induce nitric oxide production (Gupta *et al.,* 2000; Joshi *et al.,* 2004) and the catalase inhibition is possibly due to the binding of NO to the heme group (Brown 1995; Machiavelli *et al.,* 2007). Thus inhibition of catalase activity leads to H2O2 accumulation that can itself react with O2- to generate OH via Haber-Weiss raction which is the initiator of LPO. There is a reduction in the activity of CAT in the hepatic tissue of rats treated with Ni for the all the time periods. The enzyme SOD catalyses dismutation of superoxide radical, leading to formation of hydrogen

peroxide which in turn is detoxified by CAT. The decreased activity of both these enzymes lead to the increased formation of hydroxyl radical which has been reported to be increased in the hepatic tissue with Ni intoxication (Chakrabarti and Bali, 1999; Salnikow *et al.,* 2000; Huang *et al.,* 2001; Chen *et al.,* 2003).

In the present study, Ni exposure has been shown to cause hyperglycemia and decreased serum insulin level in short duration exposure of all the three treatment periods. Nickel is known to decrease serum insulin levels leading to hypoinsulinemia and increase in glucagon level leading to concomitant hyperglucagonemia with an overall increase in blood glucose level. Hepatic fructose-2,6-bisphosphate is also reported to be drastically reduced with nickel administration suggesting gluconeogenesis and not glycogenolysis to be responsible for enhanced blood glucose levels (Cartana and Arola, 1992). The increasing serum insulin level and decreasing hyperglycemia seen during the longer duration of Ni exposed can be accredited to possible countering of Ni stress by pancreas by induction of metallothionein and the inactivation of Ni by forming Ni-MT complex.

Nickel intoxication also seems to bring about differential duration dependent alterations in haematological parameters related to iron metabolism. Accordingly, the short duration exposure period at 15 days tended to show increased RBC count, hemoglobin content and packed cell volume. The only likely explanation that could be extended for the hematological observations during short duration exposure to Ni in the light of interesting observations of polycythemia, increased hemoglobin content and packed cell volume in experiments involving intrarenal administration of Ni (Das *et al.*, 2007) is, the possible Ni induced elevation in erythropoietin secretion from kidney leading to the herein observed changes. The renal accumulation of Ni recorded

during the course of this study and the recorded increase in erythropoietin in the above cited studies by intrarenal administration of Ni support the above contention. Interestingly, this hematological response was reversed and, decreased erythrocyte count, hemoglobin content and packed cell volume were the features when Ni exposure was prolonged to one month or two months. Obviously, increasing Ni renal load together with iron induced oxidative stress somehow interferes with optimal erythropoietin production which thereby contributes to the above changes. Support in this context is provided by reports of chronic Ni exposure induced reduction of erythrocyte count, hemoglobin count and packed cell volume (Das *et al.*, 2007).

Ni induced cytotoxicity is clearly indicated by the histopathological alterations seen in the hepatic tissue during all the treatment periods. Progressive deterioration of the organization of the hepatic cords with disruption of the endothelial lining of central vein, dilation of sinusoids and presence of necrotic/apoptotic cells along with vacoulization of hepatocytes are the characteristic features. These changes are very prominently manifested in the 60 day liver section indicating the increasing cytotoxicity due to Ni exposure. Similar results were observed when rats were treated with 800 mg/l nickel sulphate through drinking water for 8 weeks by Sidhu *et al.* (2004). The overall changes in histoarchitecture of liver could be explained on the basis that, nickel manifests its toxic effects primarily by the generation of reactive oxygen species (ROS). The significant induction of LPO due to of the toxic effects of ROS inflict damage to various membranous components of the cell. These cytotoxic manifestations indicating Ni induced hepatotoxictity find biochemical correlation in the recorded increase in serum ALP and ALT, marker enzymes of hepatic damage. These serum toxicity parameters are known to increase significantly

in Ni exposed rats (Sidhu *et al.,* 2004). One of the mechanisms for the increased serum levels of hepatotoxicity parameters is attributed to hepatic damage causing increased release of functional enzymes from membranes (Misra *et al.*, 1990). Another purported mechanism is due to leakage of the enzymes caused by tissue damage and altered membrane permeability (Srivatav *et al.*, 1993)

Melatonin, a powerful natural antioxidant, showed significant protection against LPO and endogenous levels of antioxidants in Ni intoxicated animals when co-administered. Melatonin affects antioxidant status directly by scavenging free radicals (Hardeland *et al.,* 1993; Allegre *et al.,* 2003) and indirectly by enhancing the antioxidant status (Reiter *et al.,* 2000c; Rodriquez *et al.,* 2004). The observed increase in the level of GSH in animals treated with melatonin alone or in combination with Ni is due to stimulation of GSH synthesis by melatonin. Melatonin is known to bring about transcriptional activation of SOD and CAT (Rodriquez *et al.,* 2004) and also of GPx (Pablos *et al.,* 1995) leading to upregulated expression of these enzymes. Such upregulation can combat oxidative stress induced by nickel on a prolonged insult. The upregulation of CAT and GPx can help decrease the amount of $H_2O_2$ while increased activity of SOD can decrease superoxide radical. The decreased level of hydroxyl radical can in turn reduce MDA formation and hence oxidative stress. The direct effect of melatonin in scavenging of free radicals during Ni intoxication is correlated with the observed decrease of serum melatonin titres. The increasing decrement of serum melatonin levels with increasing duration of Ni exposure suggests increased oxidative stress generated by Ni. Melatonin is also able to significantly decrease hepatic nickel load as seen in animals treated with melatonin alone or in combination with Ni. Though there are reports of melatonin as a powerful

antioxidant, even better than other free radical scavengers like Vit C, E and A against oxidative stress generated by many chemical and environmental agents including metals (Kirishmu, 1998; Flora, 2008; Fess, 2009), there is no report on the ability of melatonin to resist Ni toxicity. So, this study provides evidence for melatonin as an effective and powerful protectant against Ni toxicity manifestions.

Overall, the present study suggests a duration dependent Ni induced oxidative stress and the ability of hepatic tissue to activate counteractive measures to minimize the same on prolonged exposure to the metal. Duration dependent alterations in serum insulin and glucoregulation mechanisms as well as alterations associated with erythopoiesis are also effectively counteracted by melatonin. Further, there is also significant lowering of serum melatonin titre and this indole hormone is very effective in combating Ni induced oxidative stress and hence worthy of consideration as a therapeutic/protective agent against metal toxicity.

## Summary of Chapter 5

The present study using an environmentally realistic dosage of Ni (200mg/kgBW/day) simulatable to human situation provided through drinking water for 15, 30 and 60 days have revealed significantly elevated LPO at the short duration period of exposure, which tended to decrease on prolonged exposure. There was steady progressive depletion in GSH and Vit C contents with increased inhibition of CAT and GPx. Maximal inhibition was seen at 15 days followed by decreased inhibition by 30 and 60 days. Maximal hepatic Ni load was seen at 15 days which tended to decrease by 30 and 60 days of exposure. Nickel exposure lead to hypoinsulinemia and an overall increase in blood glucose level with increased RBC count, hemoglobin content and packed cell volume. Cytotoxicity is clearly indicated by the histopathological manifestations seen in the hepatic tissue during all the treatment periods. The cytotoxic manifestations indicating Ni induced hepatotoxicity find biochemical co-relation in the recorded increase in serum ALP and ALT, marker enzymes of hepatic damage. Melatonin showed significant protection against LPO and endogenous levels of antioxidants in Ni intoxicated animals when co-administered. Overall, the present study suggests a duration dependent Ni induced oxidative stress and the ability of hepatic tissue to activate counteractive measures to minimize the same on prolonged exposure to the metal. Melatonin was very effective in combating Ni induced oxidative stress ad hence worthy of consideration as a therapeutic/protective agent against metal toxicity.