CHAPTER 6

Chapter 6

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

Nickel (Ni) salts are considered as industrial health hazard since they are used extensively in electroplating, in the manufacture of steel and other alloys, and in the manufacture of batteries and electronic devices. Nickel and its compounds have been reported to be potent carcinogenic and/or toxic agents in humans and experimental animals. Exposure to nickel can lead to multitudes of health effects. Kidney is the principal organ for Ni toxicity in terms of toxicokinetics and accumulation (Sundermann, 1988). Acute intraperitoneal (*i.p*) injections of nickel chloride in rats have shown the highest accumulation of the metal in kidney (Sarkar, 1980). Rats injected with 65-85 μmoles of $NiCl_2$/kg bw given intraperitoneally have shown toxic nephropathy with proteinuria, aminouria and reduced renal clearance with regard to nephrotoxicity (Gitlitz *et al.,* 1975). Vyskocil *et al.* (1994) showed accumulation of Ni in kidney where, Ni might damage glomerular function resulting in proteinuria. The exact mechanism of action of nickel induced oxidative stress and toxicity is unclear. Ni compounds can induce LPO and modify the cellular antioxidant system as shown by many studies. Rats treated subcutaneously with $NiCl_2$ have increased LPO in liver and kidney (Sundermann *et al.,* 1984). Treatment of rats with Ni compounds caused a decrease in the GSH level as well as a decrease in glucose-6-phosphate dehydrogenase and glutathione reductase activity (Cartana *et al.,* 1992). Nickel acetate administered to rats i.p at a dose of 107 μmol increased LPO in kidneys with concomitant decrease in GSH content and activity levels of antioxidant

enzymes such as catalase, glutathione peroxidase, glutathione reductase and superoxide dismutase (Misra *et al.*, 1990).

Generally, humans are exposed to Ni through oral route via food or water. Most of the studies have tended to evaluate Ni toxicity by intraperitoneal (*i.p*) or subcutaneous (*sc*) administration and there are hardly any toxicity studies involving oral administration of the metal. Further, there is again lacuna in term of long duration exposure to Ni. The present study was in this context undertaken to understand possible Ni toxicity on long term systemic entry into humans through diet and water. In the local context, as Ni has been identified as a major environmental pollutant present in high amounts in vegetables, cereals, pulses and grass in the highly industrialized city of Vadodara, Gujarat (Blacksmith Institute Report, 1999; Labunska *et al.*, 1999; Ramachandran 2002), a realistic dosage has been worked out based on the Ni content in vegetables and food grains. Conversion factor of 6.2 (OECD, 2005) has been used for extrapolation of dosage from human to rat. Using such a dosage, a duration dependent (15, 30 and 60 days) renal oxidative stress and toxicity have been evaluated in male *Wistar* rats.

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 5.

Results:

Lipid Peroxidation (LPO): Changes in the renal LPO levels following nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.1; table 6.1. An age dependent gradual increase in LPO was seen in control (Con) rats. There was significant progressive increase in LPO levels of Ni treated animals. There was significant decrease in the LPO levels in animals treated with melatonin. Melatonin did not show any duration dependent protective effect with more or less same efficacy for all exposure durations.

Glutathione (GSH): Changes in the renal GSH levels following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.2; table 6.2. An age dependent gradual decrease in GSH levels was the feature in control (Con) rats. There was significant progressive decrease in GSH level in animals treated with Ni. There was significant increase in GSH level when the animals were administered with melatonin alone or in combination with Ni with, increasing duration tending to show slightly lesser efficacy.

Ascorbic Acid (Vit C): Changes in the renal Ascorbic Acid (Vit C) contents following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.3; table 6.3. An age dependent gradual decrease in Vit C content was seen in control (Con) rats. There was significant progressive decrease in Vit C content in the Ni treated rats. The Vit C contents were significantly increased in animals treated with melatonin alone or in combination with Ni and had a tendency to show a gradual increasing effect with time.

Superoxide Dismutase (SOD): Changes in the renal superoxide dismutase (SOD) levels following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.4; table 6.4. A gradual age dependent decrease in SOD activity could be seen in the Con group of animals. The enzyme activity showed significant inhibition in Ni exposed animals with increasing degree of inhibition paralleling duration of exposure. There was significant protective effect in the activity level of SOD in Mel and Mel+Ni group of animals compared to Con and Ni groups respectively. The degree of protection with melatonin was duration dependent with maximal at 15 days, gradually decreasing thereafter.

Catalase (CAT): Changes in the renal catalase activity following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.5; table 6.5. A gradual age dependent decrease in the enzyme activity was seen in the Con group of animals. There was significantly decreased CAT activity in the Ni treated group compared to Con group. Melatonin treatment showed a tendency to increase the activity of enzyme with increasing duration of Ni exposure and the protective effect of melatonin was found to be steady.

Glutathione Peroxidase (GPx): Changes in renal Glutathione Peroxidase (GPx) activity following Nickel (Ni) treatment for 15, 30 and 60 days are shown in fig 6.6; table 6.6. Control animals tended to show a gradual age dependent decrement in GPx activity. Ni exposure resulted in duration dependent progressive decrease in enzyme activity. Melatonin administration tended to increase GPx activity in control rats. The protective effect of melatonin on administration with Ni was found to be almost to the same degree irrespective of duration.

Metal Load: Changes in renal accumulation of Ni following Ni treatment for 15, 30 and 60 days are shown in figure 6.7; table 6.7. Ni treated rats showed increased renal Ni accumulation but the increment was significant in all the treatment groups. There was significant decrease in Ni accumulation in the melatonin treated groups. Both, Ni induced renal load and protection by melatonin were duration dependent.

Serum Parameters: Changes in serum renal toxicity parameters like urea and creatinine are shown in tables 6.8. The renal toxicity parameters (Urea and Creatinine) showed an increase with Ni exposure. There was a gradual dose dependent increase in both the parameters with Ni exposure but the increase was significant in all the treatment groups. There was a decrease in the renal toxicity parameters in the Mel and Ni+Mel groups of animals but, the decrease was not significant.

Figure 6.1. Graph showing levels of renal lipid peroxidation (LPO) in animals exposed to Ni for 15, 30 or 60 days

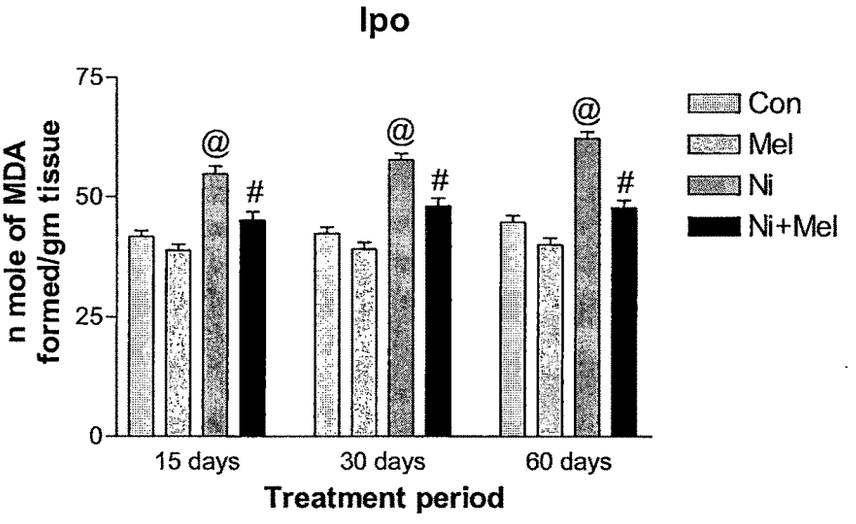

Table 6.1. Contents of renal lipid peroxidation (LPO) following exposure to Ni for 15, 30 or 60 days

	15 days	30 days	60 days
Con	41.78 ± 1.26	42.49± 1.20	44.89±1.41
Mel	38.92 ± 1.32	39.20± 1.290	40.21 ±1.38
Ni	54.89±1.56@	57.92± 1.29@	62.45± 1.42@
Ni+Mel	45.19± 1.78 #	48.21±1.72#	47.89± 1.58 #

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

Figure 6.2. Graph showing levels of renal glutathione (GSH) content in animals exposed to Ni for 15, 30 or 60 days

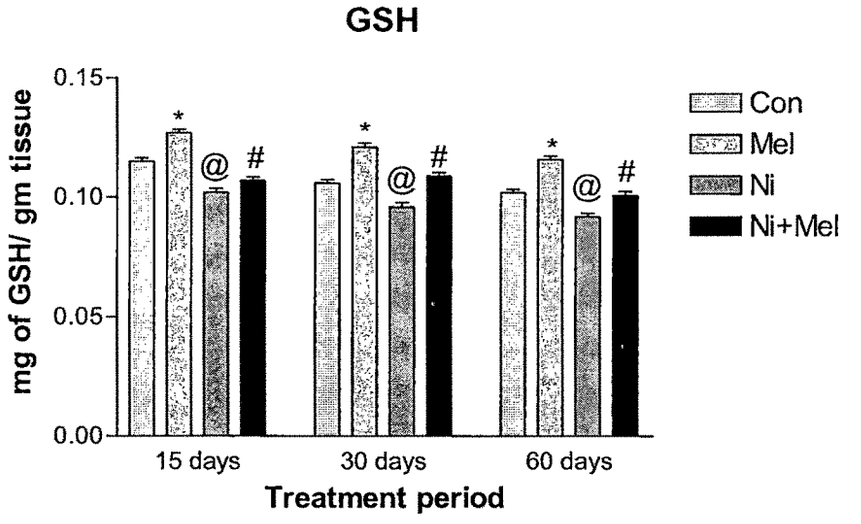

Table 6.2. Contents of renal glutathione (GSH) following 15, 30 or 60 days treatment with Ni

	15 days	30 days	60 days
Con	0.115±0.0013	0.108±0.0014	0.102±0.0013
Mel	0.127±0.0014*	0.121±0.0018*	0.116±0.0013*
Ni	0.102±0.0017@	0.096±0.0015@	0.086±0.0015@
Ni+Mel	0.107±0.0014#	0.103±0.0015#	0.101± 0.0016#

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

Figure 6.3. Graph showing renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with nickel

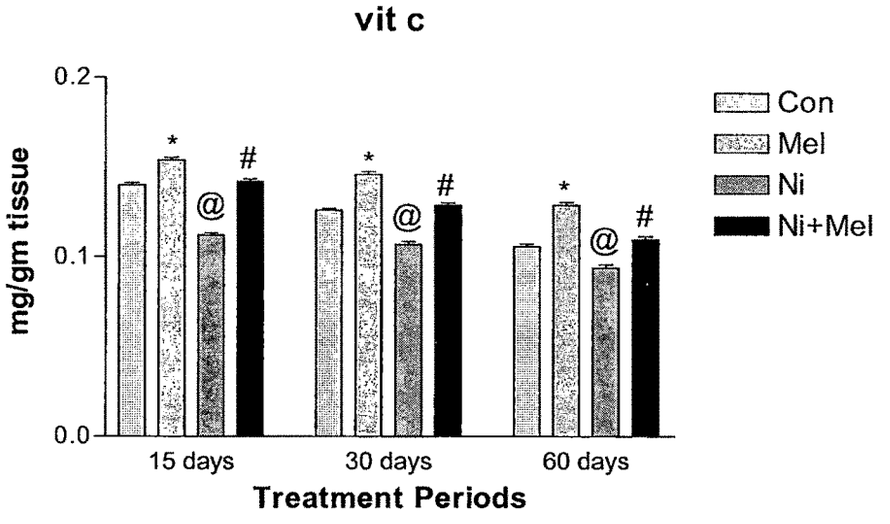

Table 6.3. Levels of renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with Ni

	15 days	30 days	60 days
Con	0.140±0.0012	0.126±0.0011	0.106±0.0012
Mel	0.154±0.0015*	0.146±0.0016	0.129±0.0018*
Ni	0.115±0.0013@	0.101±0.0018@	0.081±0.0019@
Ni+Mel	0.142±0.0015#	0.129±0.0013#	0.110±0.0016 #

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

Figure 6.4. Graph showing renal Superoxide Dismutase (SOD) activity following 15, 30 or 60 days exposure with nickel

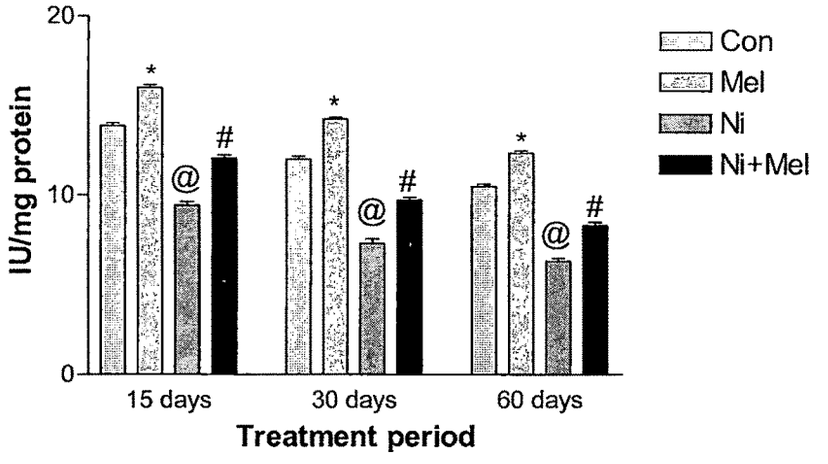

Table 6.4. Levels of renal Superoxide Dismutase (SOD) activity following 15, 30 and 60 days exposure with nickel

	15 days	30 days	60 days
Con	13.87±0.13	12.03±0.16	10.52±0.12
Mel	16.01±0.16*	14.27±0.11*	12.37±0.16*
Ni	9.45±0.19@	8.10±0.27@	6.32±0.189@
Ni+Mel	12.05±0.21#	9.75±0.15#	8.32±0.210#

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

Figure 6.5. Graph showing renal catalase (CAT) activity following 15, 30 or 60 days exposure to nickel

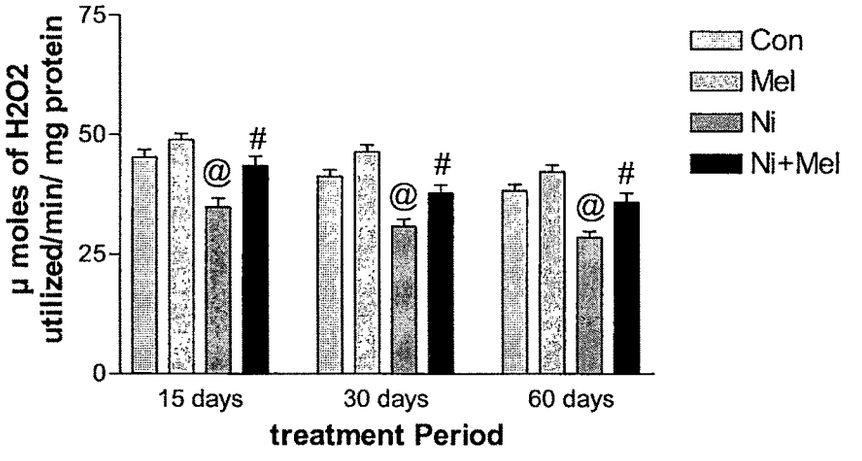

Table 6.5. Levels of renal catalase (CAT) activity following 15, 30 or 60 days exposure with nickel

	15 days	30 days	60 days
Con	45.26±1.63	41.27±1.39	38.32±1.39
Mel	48.98±1.29	46.47±1.45	42.35±1.37
Ni	34.85±1.95@	30.75±1.58@	25.54±1.32@
Ni+Mel	43.50±2.01#	37.85±1.75#	35.80±1.36

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

Figure 6.6. Graph showing renal Glutathione Peroxidase (GPx) activity following 15, 30 and 60 days exposure to nickel

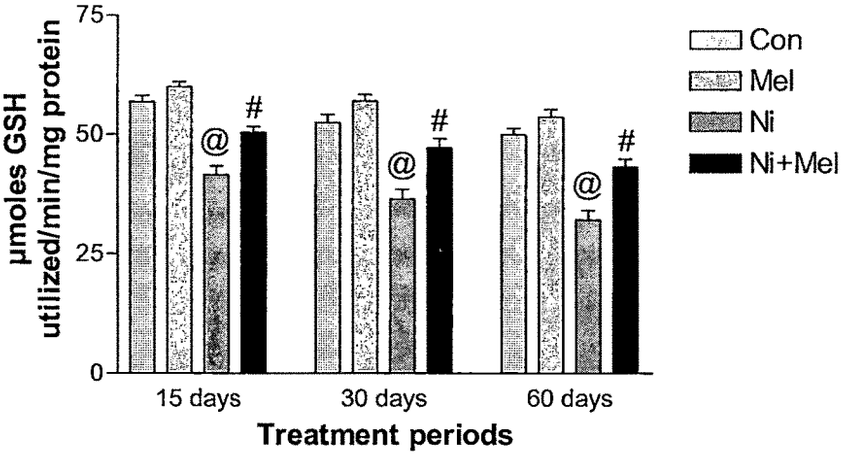

Table 6.6. Levels of renal Glutathione Peroxidase (GPx) following 15, 30 and 60 days treatment with nickel

	15 days	30 days	60 days
Con	56.87±1.28	52.38±1.82	49.92±1.43
Mel	59.98±1.09	57.04±1.39	53.76±1.52
Ni	41.56±1.85@	36.49±2.01@	32.18±1.98@
Ni+Mel	50.41±1.25#	47.21±1.95#	43.29±1.59#

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

Figure 6.7. Graph showing % accumulation of Ni in renal tissue following 15, 30 and 60 days treatment with Nickel.

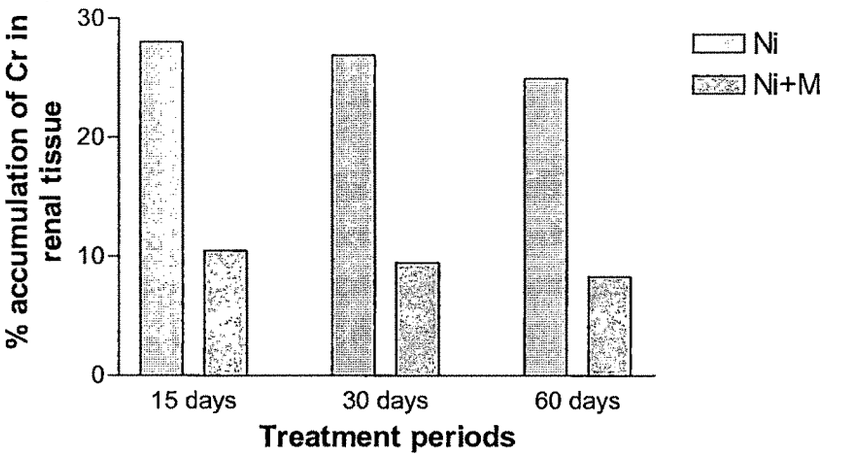

Table 6.7. % accumulation of Ni in renal tissue following 15, 30 and 60 days treatment with Nickel.

Treatment Period	Ni	Ni+M
15 days	28.06	10.48
30 days	26.95	9.48
60 days	25	8.33

TABLE 6.8 Levels of Serum Urea and Creatinine following Ni exposure for 15, 30 and 60 days

	15 Days		30 Days		60 Days	
	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)
Control (Con)	38.25±0.85	0.55±0.07	38.75±0.85	0.55±0.05	31.50±0.65	0.60±0.00
Melatonin (Mel)	35.25±0.63	0.68±0.09*	37.50±0.65	0.58±0.03	29.25±0.85	0.68±0.05
Nickel(Ni)	42.25±1.25@	0.45±0.07@	44.78±2.01@	0.705±0.03@	50.49±1.95@	0.700±0.05
Ni+Melatonin (Ni+Mel)	37.25±0.85#	0.80±0.11#	35.19±1.73#	0.650±0.09	38.50±1.27#	0.65±0.07

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

Table 6.9 Changes in serum lipid profile following 15 days of exposure with nickel (Units expressed as mg/dl)

	Cholesterol	Triglyceride	H DL	VLDL	LDL
Control (Con)	103.50±1.04	119.25±0.85	64.25±0.85	24.25±0.85	16.25±1.11
Melatonin (Mel)	91.75±0.62*	132.00±1.08*	55.00±1.47*	26.00±1.08	11.75±0.85*
Nickel(Ni)	99.50±0.64@	136.75±0.85@	51.00±0.91@	25.50±0.65	23.75±0.85@
Ni+Melatonin (Ni+Mel)	116.00±0.91#	139.25±0.48	65.50±1.04#	27.25±0.85	21.75±0.85

Table 6.10 Changes in serum lipid profile following 15 days of exposure with nickel (Units expressed as mg/dl)

	Cholesterol	Triglyceride	H DL	VLDL	LDL
Control (Con)	122.75±0.85	97.75±1.11	62.50±1.04	28.50±1.19	33.75±0.85
Melatonin (Mel)	90.25±0.48*	134.50±0.65*	39.50±1.04*	19.50±0.65*	33.00±1.08
Nickel(Ni)	119.25±0.85	140.75±0.85@	61.75±0.85	21.50±0.65@	37.25±1.25@
Ni+Melatonin (Ni+Mel)	121.75±0.85	137.75±0.25#	59.50±0.64	28.00±0.41#	30.50±0.65

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

Table 6.11 Changes in serum lipid profile following 15 days of exposure with chromium (Units expressed as mg/dl)

	Cholesterol	Triglyceride	H DL	VLDL	LDL
Control (Con)	117.75±1.11	139.25±0.85	57.50±1.04	29.00±1.30	32.00±1.08
Melatonin (Mel)	111.75±0.85	135.75±0.25	59.00±1.08	27.25±0.85	25.25±0.85
Nickel(Ni)	127.75±1.69@	146.43±1.05@	63.50±0.58@	27.75±1.11	37.25±0.63
Ni+Melatonin (Ni+Mel)	100.75±2.95#	147.32±1.39	47.50±1.79#	23.50±0.65#	29.75±0.85

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

Table 6.12 Changes in hematological parameters after 15 days of Ni treatment

	Leucocyte Count	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Control (Con)	8275.00±85.39	1016100.00±70.83	19.00±0.91	76.00±0.41	1.25±0.25	2.25±0.25
Melatonin (Mel)	8475.00±110.87	1025500.00±1040.83	21.25±1.25	79.25±0.25	1.25±0.25	2.00±0.00
Nickel(Ni)	8099.75±70.83	1235120.00±96.95@	29.75±0.85@	67.25±1.11@	1.25±0.25	2.25±0.25
Ni+Melatonin (Ni+Mel)	7212.25±12.59	1132925.00±249.58	15.00±1.08#	82.25±0.85#	1.25±0.25	1.25±0.48

Table 6.13 Changes in hematological parameters after 30 days of Ni treatment

	Leucocyte Count	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Control (Con)	7175.00±125.00	1302075.00±75.08	19.25±0.63	79.00±0.91	1.50±0.29	1.75±0.25
Melatonin (Mel)	7450.00±132.29	1423150.00±64.55@	18.25±1.25	79.25±0.85	1.50±0.29	1.50±0.29
Nickel(Ni)	6225.00±110.8@	703349.80±218.42	22.75±1.1@	75.50±0.65	1.00±0.00	2.00±0.00
Ni+Melatonin (Ni+Mel)	7614.50±22.59#	1115125.00±149.30	20.00±0.91	80.25±0.85#	0.25±0.25	1.00±0.00

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

Table 6.14 Changes in hematological parameters after 60 days of Ni treatment

	Leucocyte Count	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Control (Con)	8500.00±91.29	1213075.00±85.39	37.75±0.85	74.75±1.25	1.00±0.00	2.00±0.00
Melatonin (Mel)	8800.00±10.87	1429250.00±1652.02	52.00±0.91*	78.50±1.04	2.00±0.41	1.75±0.25
Nickel(Ni)	4605.00±10.41@	988500.00±1190.24@	35.75±0.63	63.00±1.08@	0.75±0.25	2.00±0.00
Ni+Melatonin (Ni+Mel)	6200.00±10.80#	1307500.00±8539.13#	18.00±1.08#	81.75±1.38#	1.00±0.00	0.75±0.25

@ 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 6.8 Photomicrograph of control kidney showing a Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 6.9 Photomicrograph of 15 day melatonin treated kidney showing Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 6.10 Photomicrograph of 15 day nickel treated kidney showing Bowman's capsule with glomerulus and distal tubules (400X).Note the shrinkage and degeneration of glomerulus also noted. Apoptotic distal tubular cells and Bowman's capsular epithelial disruption (arrow) can be seen. G- Glomerulus

Fig 6.11 Photomicrograph of 15 day nickel treated kidney(400X). Near normal appearance of the Bowmann 's capsule.

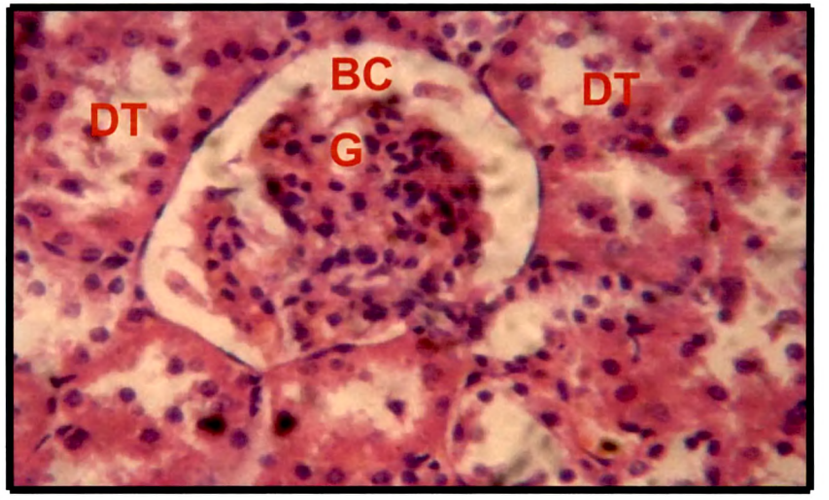

Fig 6.8 Control

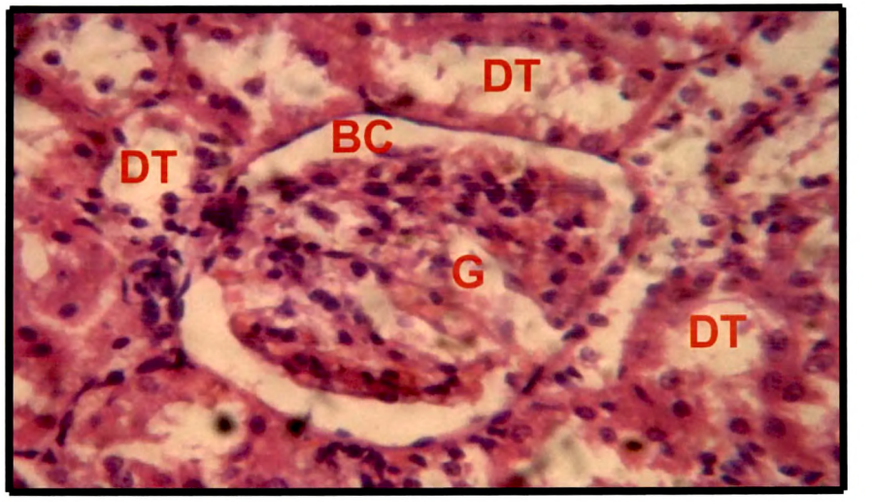

Fig 6.9 Melatonin

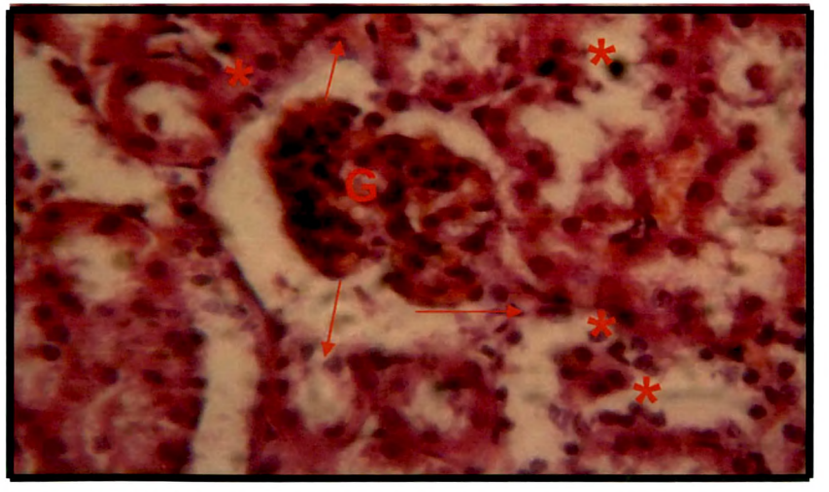

Fig 6.10 Ni

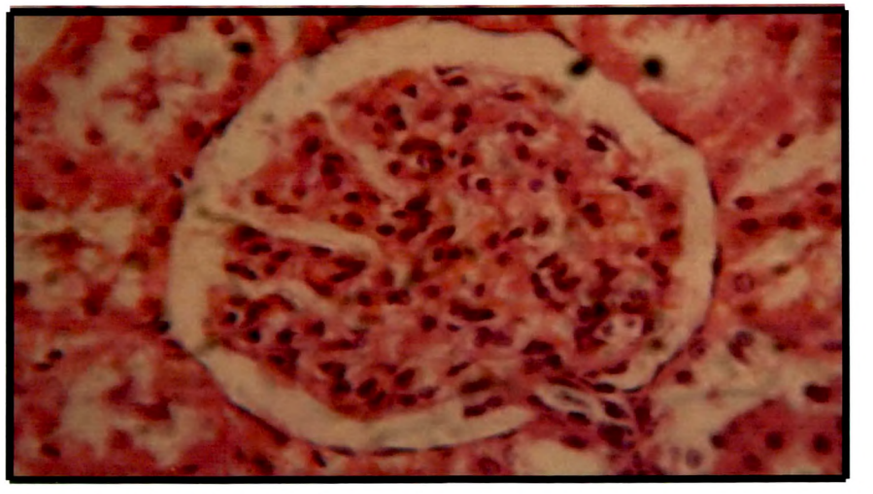

Fig 6.11 Ni + Melatonin

PLATE II

Fig 6.12 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule

Fig 6.13 Photomicrograph of kidney exposed to melatonin for 15 days showing proximal tubules (400X). PT- Proximal tubule

Fig 6.14 Photomicrograph of kidney exposed to nickel for 15 days showing proximal tubules (400X). Note perituular epithelial cell degeneration.

Fig 6.15 Photomicrograph of kidney exposed to nickel+melatonin for 15 days showing near normal appearance of PT (400X).

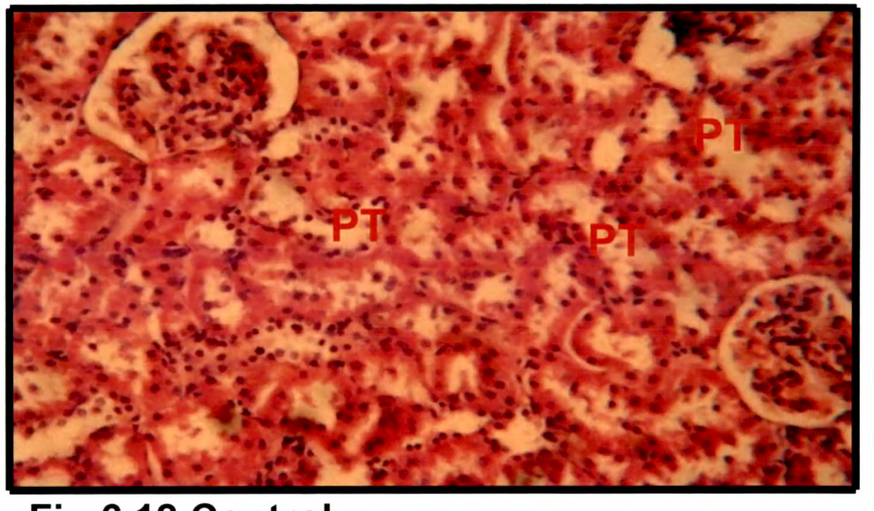

Fig 6.12 Control

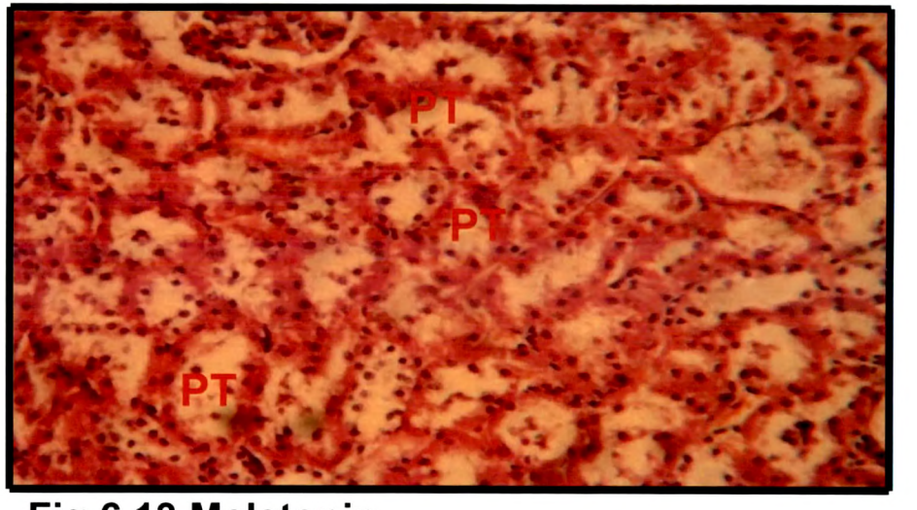

Fig 6.13 Melatonin

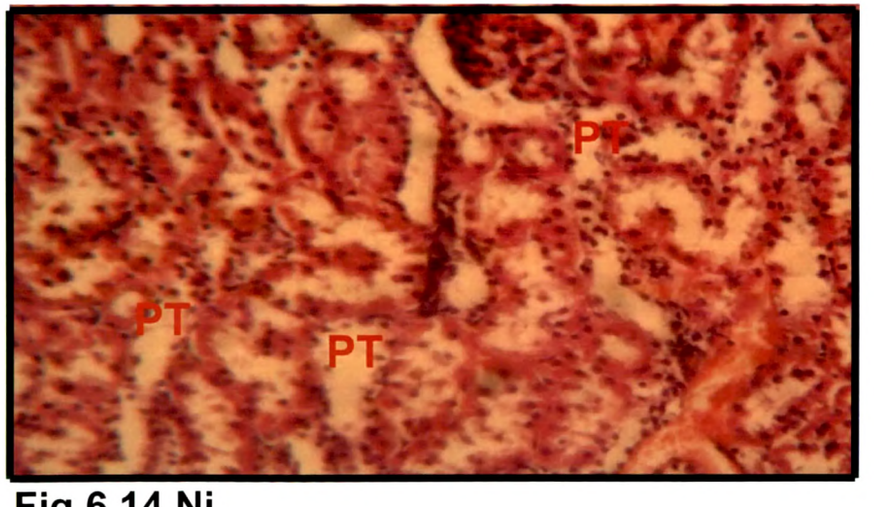

Fig 6.14 Ni

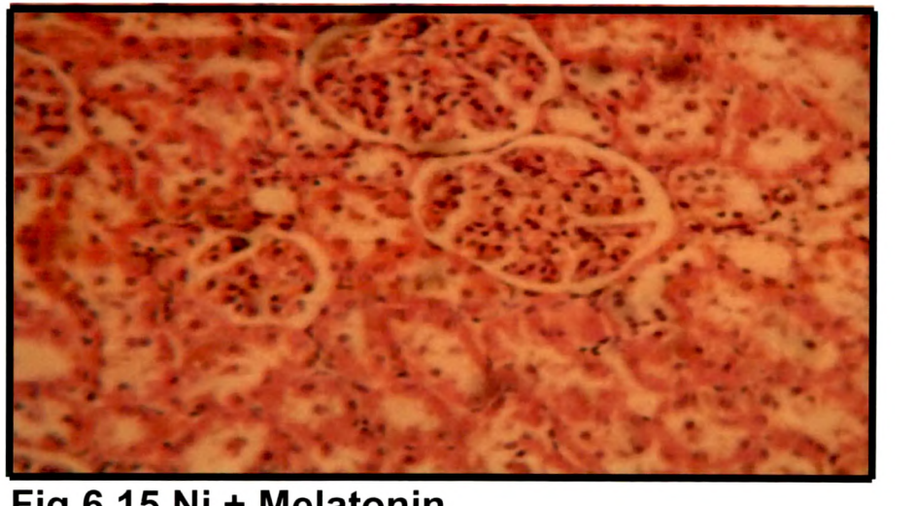

Fig 6.15 Ni + Melatonin

PLATE III

Fig 6.16 Photomicrograph of control kidney showing a Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 6.17 Photomicrograph of kidney exposed to melatonin for 30 days showing Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 6.18 Photomicrograph of kidney exposed to nickel for 30 days showing Bowman's capsule with glomerulus and distal tubules (400X). Note the shrinkage and degeneration of glomerulus, Bowman's capsular epithelial degeneration (arrow) and widened distal tubules as well as apoptotic/necrotic death of epithelial cells. DT- Distal tubule

Fig 6.19 Photomicrograph of kidney exposed to nickel+melatonin for 30 days showing normal looking PT (400X).

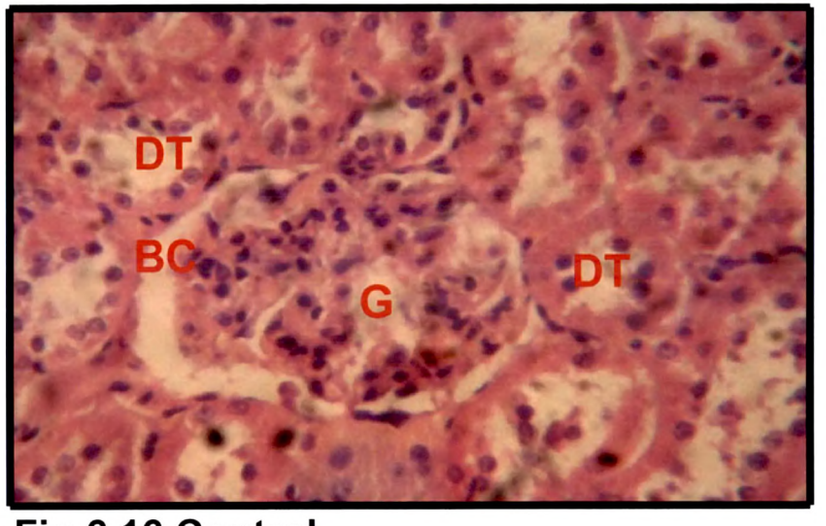

Fig 6.16 Control

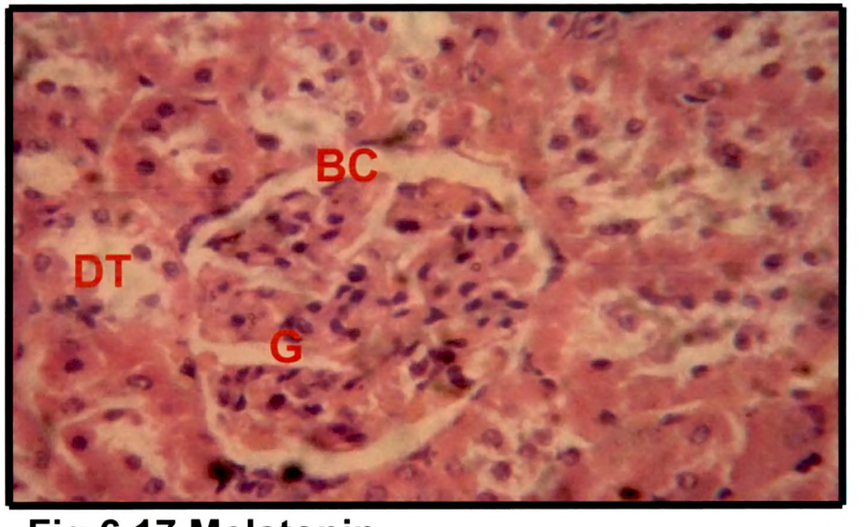

Fig 6.17 Melatonin

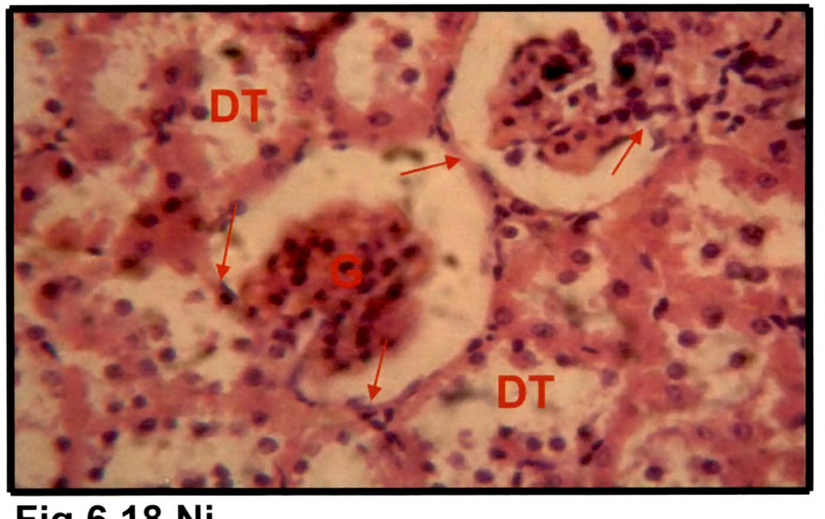

Fig 6.18 Ni

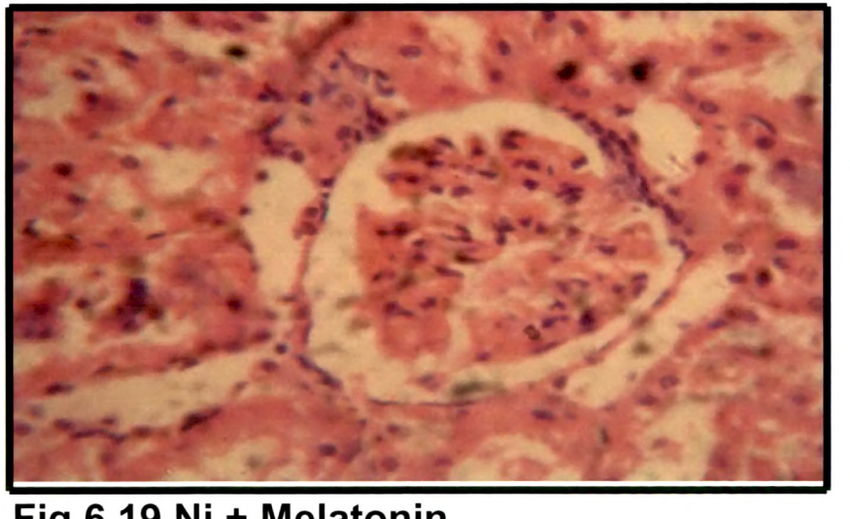

Fig 6.19 Ni + Melatonin

PLATE IV

Fig 6.20 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule.

Fig 6.21 Photomicrograph of kidney exposed to melatonin for 30 days showing proximal tubules (400X). PT- Proximal tubule

Fig 6.22 Photomicrograph of kidney exposed to chromium for 30 days showing proximal tubules (400X).Note the wide spread proximal tubular cell damage and distended proximal tubule.PT- Proximal tubule

Fig 6.23 Photomicrograph of kidney exposed to nickel+melatonin for 30 days showing near normal proximal tubules (400X).

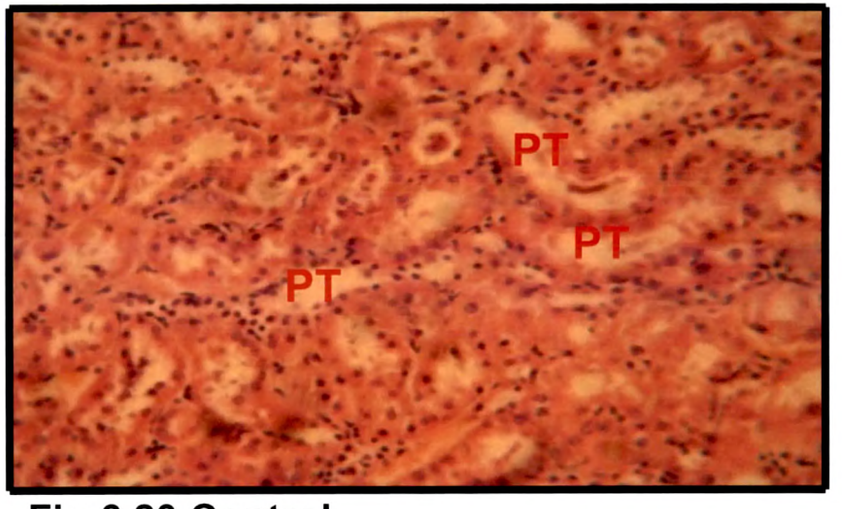

Fig 6.20 Control

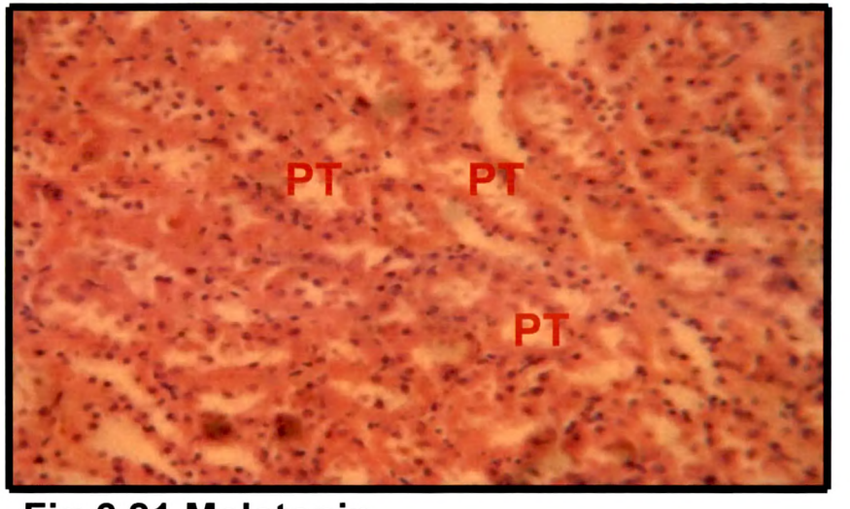

Fig 6.21 Melatonin

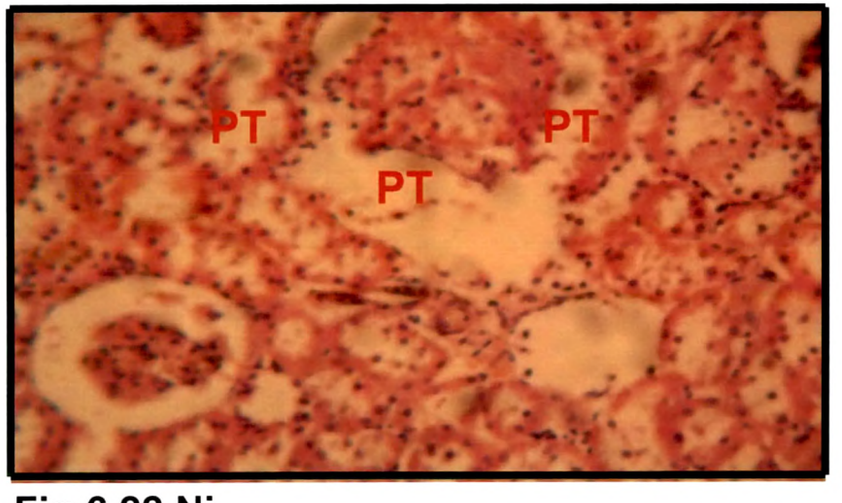

Fig 6.22 Ni

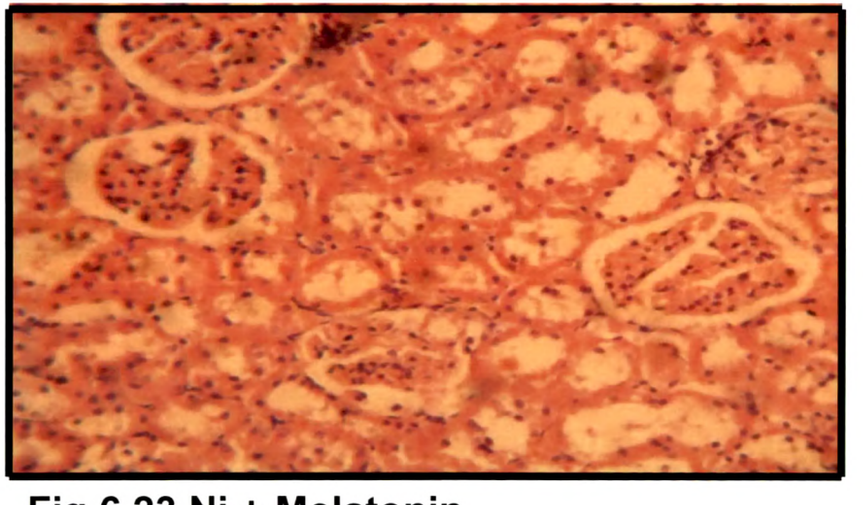

Fig 6.23 Ni + Melatonin

PLATE V

Fig 6.24 Photomicrograph of control kidney showing a Bowman's capsule with glomerulus and distal tubules (400X). DT- Distal tubule; G- Glomerulus

Fig 6.25 Photomicrograph of kidney exposed to melatonin for 60 days showing Bowman's capsule with glomerulus and distal tubules (400X). DT- Distal tubule; G- Glomerulus

Fig 6.26 Photomicrograph of kidney exposed to nickel for 60 days showing Bowman's capsule with glomerulus and distal tubules (400X). Note the glomerular degeneration and shrinkage and degeneration and disruptions in Bowman's capsular epithelium (arrow). DT- Distal tubule; G- Glomerulus

Fig 6.27 Photomicrograph of kidney exposed to nickel+melatonin for 60 days showing normal looking Bowman's capsule and minor effects on glomerulus(400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

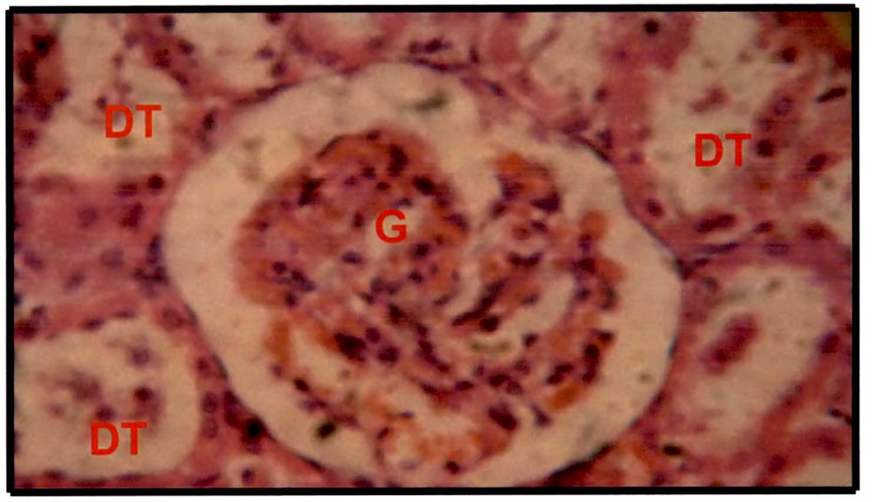

Fig 6.24 Control

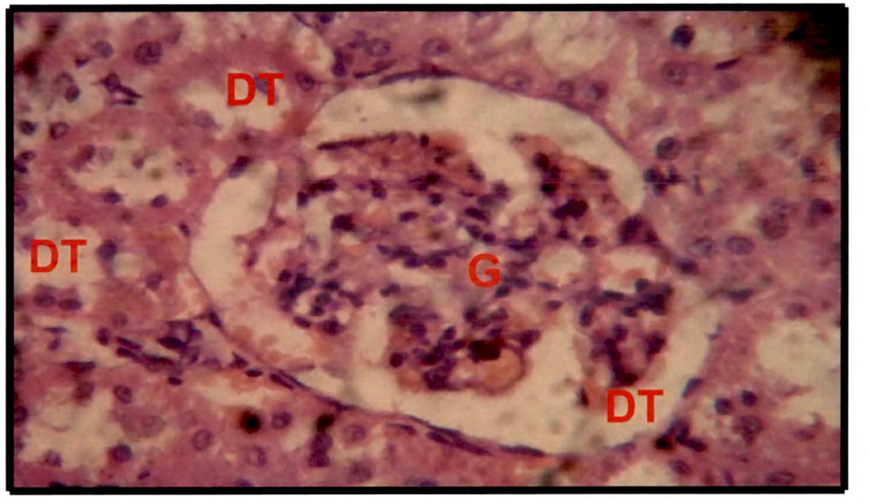

Fig 6.25 Melatonin

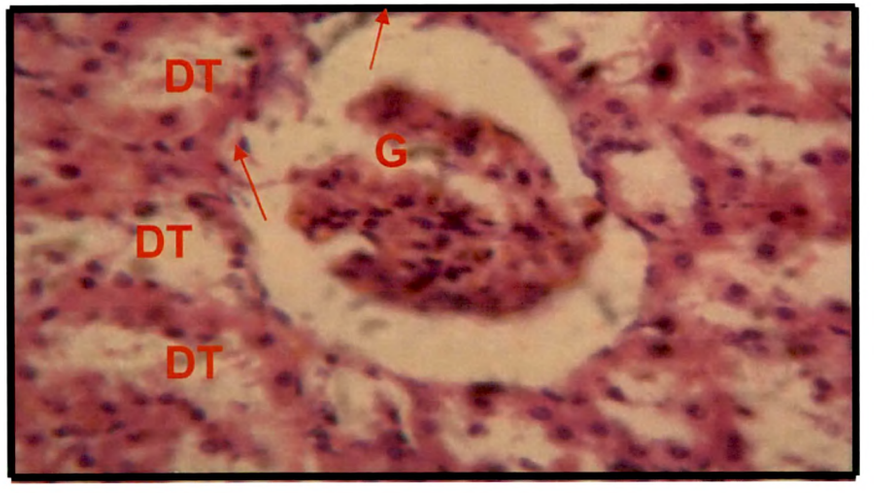

Fig 6.26 Ni

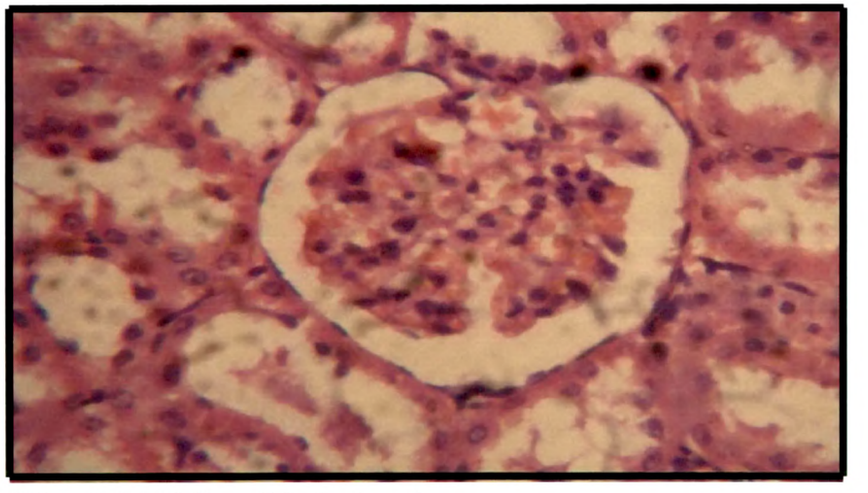

Fig 6.27 Ni + Melatonin

PLATE VI

Fig 6.28 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule.

Fig 6.29 Photomicrograph of kidney exposed to melatonin for 60 days showing proximal tubules (400X). PT- Proximal tubule

Fig 6.30 Photomicrograph of kidney exposed to chromium for 60 days showing proximal tubules (400X). Note the generalized proximal tubular epithelial cell degeneration. PT- Proximal tubule

Fig 6.31 Photomicrograph of kidney exposed to nickel+melatonin for 60 days showing normal proximal tubules (400X). PT- Proximal tubule

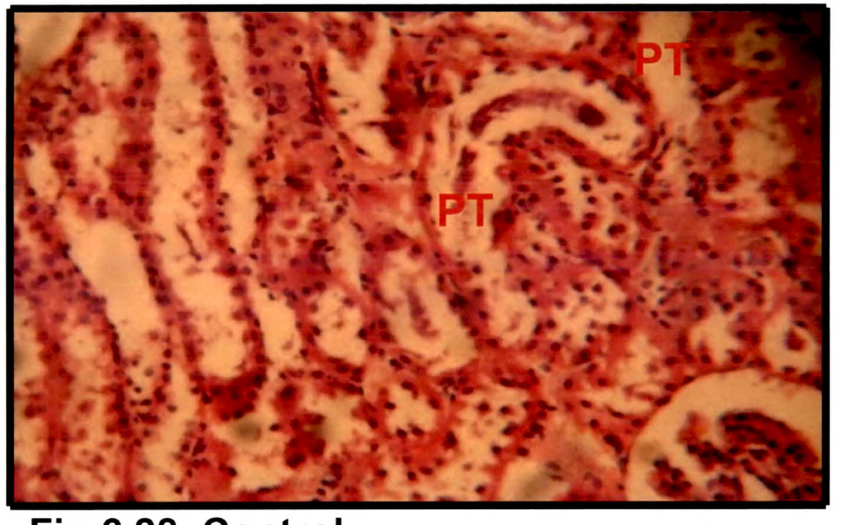

Fig 6.28 Control

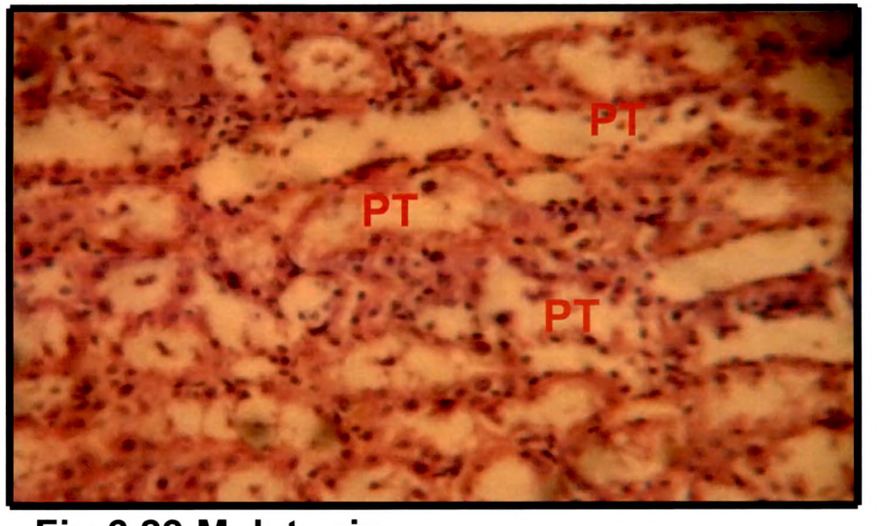

Fig 6.29 Melatonin

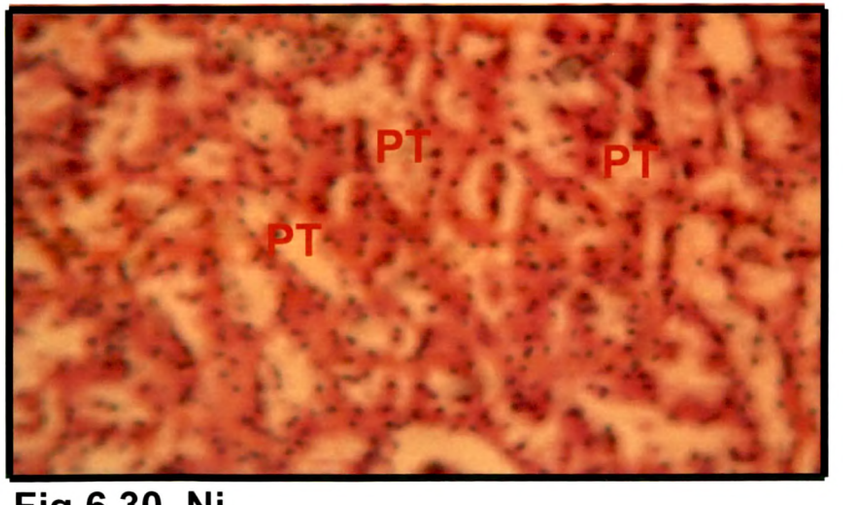

Fig 6.30 Ni

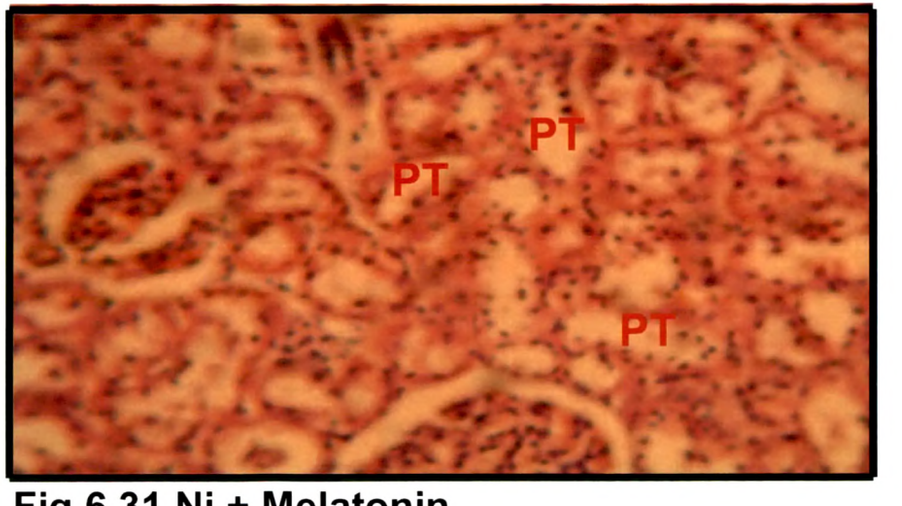

Fig 6.31 Ni + Melatonin

Discussion:

Kidney is known to accumulate Ni in greatest proportion compared to other organs and, this present study on subchronic exposure to a realistic dosage has revealed both increased oxidative stress and lipid peroxidation. Both these aspects of metal toxicity have shown progressive increase with prolonging duration of Ni exposure well paralleled by temporally increasing renal Ni load. While elevation in LPO was marked by increased TBARS as measured by the amount of MDA formed, heightened oxidative stress was marked by equally potent depletion in GSH and Vitamin C and inhibition of SOD, CAT and GPx. Though there are no studies on subchronic testing of Ni on a realistic dosage, few studies on acute toxicological evaluation of Ni on renal toxicity have reported similar trend of changes (Sundermann., 1995; Misra *et al.,* 1990; Das and Dasgupta, 1998; Das *et al.,* 2006; Das *et al.,* 2008). Apparently, a subchronic exposure leads to persistent but gradual increase in peroxidation induced damages and oxidative stress which can result in tissue damage. It is generally believed that LPO and oxidative stress go hand in hand with a probable relationship between the two. However, Ni induced LPO and generation of oxidative stress seems to be mutually exclusive events and LPO may be an effect rather than cause of oxidative stress. This has been clearly brought out by a review of available literature and the study on role of oxidative stress in Ni induced cell injury in rat renal tissue by Chakravarty and Bai (1999) and by the reported relationship between LPO with GSH, GPx and GR but not with CAT, SOD or GST as reviewed by Das *et al.* (2008). Based on the discussion on the subject matter of nickel toxicity to tissues, in the above cited references, it is surmisable that whereas LPO can be related with thiol metabolism and non-enzymatic antioxidants, increased oxidative stress can be related with quantitative

or qualitative inhibition of SOD and CAT. The presently observed Ni induced persistent depletion of GSH and vitamin C and inhibition of GPx can in this context be related with the observed increase in LPO while, the steady inhibition of CAT and SOD activities can be related with increased oxidative stress.

In the present study, Ni induced renal cytotoxic manifestations involving renal tubular damage is marked by structural alterations as seen by microscopic anatomy. These changes principally involve glomerular shrinkage, disruption of endothelial lining, rupturing of Bowmann's capsular epithelium and proximal tubular damage varying from cellular vacuolization, necrosis and dilation and, overall structural disorganization, which are all accreditable to increasing oxidative stress generated by Ni. These cytotoxical changes affect renal physiology which is clearly shown by the herein elevated serum urea and creatinine levels. It is also likely that there should be proteinurea and glycosurea etc. In this perspective, superoxide anion, hydrogen peroxide and hydroxyl radical can be considered as the potent culprits. An overall increased production of free radicals under metal exposure is conceivable as *in vitro* exposure has been shown to generate greater amount of free radicals by two hepatic cell lines (chapter 9). There are different mechanisms by which Ni can generate free radicals. One possible mode is generation directly from molecular oxygen of superoxide anion in a two step process followed by interaction of superoxide anions with protons in the dismutation reaction generating H_2O_2 (Das *et al.*,2008). Free radical generation from the reaction of Ni-thiol complexes and molecular oxygen, and/or lipid hydroperoxide could yet be another mechanism of Ni toxicity (Das and Buchner, 2007; Das *et al.*, 2008). Production of superoxide anion, hydroxyl radical and singlet oxygen from H_2O_2 reacting with Ni complex of glycine-glycine histidine

has also been envisaged (Chakravarty and Bai,1999). Enhanced generation of hydroxyl radical is an essential feature of Ni induced oxidative stress (Chakravarty and Bai, 1999; Salnikow *et al.,* 2000; Chen *et al.,* 2003; Das *et al.,* 2008; Hfeih *et al.,*2008). Generation of these three classes of free radicals i.e. superoxide anion, H_2O_2 and hydroxyl radical, more profusely the latter, is facilitated by the inhibition of SOD and CAT activity. Though it is widely recorded that Ni toxicity results in marked SOD inhibition, the mechanism of inhibition is not yet clearly understood. However, a purported mechanism involving Cu metallochaperonine and SOD1 has been hypothesized in a related study of reproductive toxicity on Ni during the course of the study (Joshi,2009). The mechanism of inhibition of CAT by Ni is envisaged to be due to generation of NO by Ni (Gupta *et al.,* 2001; Joshi *et al.,* 2004) and the binding of NO to the heme group of CAT(Brown, 1995; Mashiavelli *et al.,* 2007). Obviously, the accumulation of H_2O_2 due to inhibition of CAT can in turn lead to generation of hydroxyl radical by reaction with superoxide anion being accumulated by the inhibition of SOD (Haber-Weiss reaction). The histopathological alterations of the renal tissue detailed above could be a consequence of the hydroxyl radical (primarily) and superoxide anion (secondarily) induced cytotoxicity. Even other workers have suggested similar possibility for Ni induced cytotoxicity and tissue damage providing corroborative and compelling evidences to the concept. Despite the suggested mechanism of LPO and oxidative stress, the currently observed gradual increase of LPO and oxidative stress rather than a dramatic increase on a longer duration of Ni exposure is probably correlatable with the ability of melatonin to quench the free radical being generated as, a significant temporal reduction in serum titre of melatonin had been recorded in the study.

Nickel intoxication in the present study has also been shown a tendency of hypertriglyceridemia and hypercholesterolemia with decreased HDL and increased LDL and VLDL cholesterols. These observations suggest possible disturbance in lipid metabolism during Ni intoxication and need to be studied in detail to establish a possible link between Ni and metabolic alterations. In the absence of any relevant information in this context it is well neigh impossible to make meaningful discussion on the observed alterations at this junction. It is also interesting that Ni exposure has resulted in some subtle hematological alterations marked by decreased leucocyte count essentially correlatable with lymphocyte number and a transcient increase of platelet count at 15 days followed by decrease during longer periods of exposure to Ni. There are reports of both increase and decrease of leukocyte and platelet counts and, the present observations are in agreement with that of Das *et al.,* (2007). The discrepancy in the form of increased leucocyte and platelet counts noted by some workers (Das *et al.,* 2008) is probably due to usage of low dose of Ni in their studies. Perhaps, this highlights the fact that Ni induced hemotoxicity may depend on dosage and duration of exposure to Ni.

The positive aspect of the study is the observation of the potential ability of melatonin, an indole amine hormone of the body, to counter the effects of Ni on renal LPO, oxidative stress as well as histopathological alterations when used as a protectant by co-administration with Ni. From the results it is clear that, melatonin is able to significantly minimize the Ni induced effects on many fronts and even normalize certain parameters. The study therefore gives compelling evidences for melatonin as powerful antioxidant for resisting Ni induced renal toxicity manifestations. Though the antioxidant properties of melatonin have received

attention, there are no studies related to its utility as an agent against metal toxicity in general and Ni toxicity in particular. The lipophilic and hydrophilic property therefore gain access to various cellular compartments and its competence to scavenge free radicals directly, are very interesting propositions for its usage as a metal antidote. Further, its ability to form complex with metals (and hence aid in the inactivation and/or elimination of metal) and its lipophilic and hydrophilic known role in transcriptional induction of antioxidants as well as combat metal induced alterrations on sub-chronic to chronic metal intoxication. Clinical evaluations are needed to validate its possible usage as a metal therapeutant.

Overall, the present study has revealed Ni induced increase renal LPO and oxidative stress along with microscopic structural changes leading to alterations in renal physiology and hematological modulaltion on sub-chronic prolonged exposure to Ni. Some disturbance in lipid metabolism is also seen. Melatonin has also been shown to be successful in negating most of the major Ni induced toxic manifestations.

Summary of Chapter 6

The present study investigates renal oxidative stress due to exposure to a realistic dosage of nickel (Ni) provided through drinking water for 15, 30 and 60 days. Renal oxidative stress and lipid peroxidation due to metal toxicity have shown progressive increase with prolonging duration of Ni exposure, well paralleled by temporally increasing renal Ni load. Elevation in LPO and heightened oxidative stress were marked by equally potent depletion of GSH and Vit C and inhibition of SOD, CAT and GPx. Nickel induced renal cytotoxic manifestations involving renal tubular damage are marked by structural alterations. The overall structural disorganization marked by these changes is accreditable to increasing oxidative stress generated by Ni. Nickel intoxication has shown a tendency for hypertriglyceridemia and hypercholesterolemia with decreased HDL and increased LDL and VLDL cholesterol. Exposure to Ni resulted in subtle hematological alterations marked by decreased leukocyte count, essentially corelatable with lymphocyte number, and a transcient increase in platelet count at 15 days followed by decrease during longer periods. Melatonin was able to counter the effects of Ni on renal LPO, oxidative stress as well as histopathological alterations when used as a protectant. It was able to significantly minimize the Ni induced effects on many fronts and even normalize certain parameters. Overall, the present study has revealed significant Ni induced renal LPO and oxidative stress along with structural changes and hematological modulations on exposure to Ni. Melatonin was successful in negating most of the major Ni induced toxic manifestions.