CHAPTER 2

Chapter 2

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

Hexavalent chromium compounds are used extensively in diverse industries like steel, alloy cast iron, chorme, paints, and metal finishes. It is a general environmental toxicant contributing to neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity (Von Burg and Liu, 1993; Barceloux, 1999). Exposure to chromium has been shown to cause adverse effects on renal and hepatic tissues (Love, 1983; Verschoor *et al.*, 1990). Long term exposure to Cr has shown to cause functional disruption in several organs due to accumulation of the metal (Nieboer and Jusys, 1988). The exact mechanism of action of chromium compounds on tissues is not extensively studied but, it is observed that, chromium can generate massive amount of reactive oxygen species (ROS) during its reduction in successive oxidation states from Cr (VI) to Cr (III), which are well known to produce toxic effects (Shi and Dalai, 1990; Sugden *et al.*, 1992; Luo *et al.*, 1996; Shi *et al.*, 1999; O'Brien *et al.*, 2003). Such excessive production of ROS leading to a state of oxidative stress can affect the functional integrity of organs by causing injury to cellular proteins, lipids and DNA (Nordberg and Arner, 2001).

Studies in mammals have suggested harmful effects of Cr (VI). Kidney being the main target tissue for Cr accumulation, is therefore more sensitive than other tissues (Dartch *et al.*, 1998). Nephrotoxicity as well as hepatotoxicity have been reported in human and experimental animals following exposure to Cr (Sugiyama, 1992).

Subcutaneous administration of Cr (VI) in rats has been shown to result in progressive proteinuria, increased urea nitrogen and creatinine along with elevated serum activity level of alanine aminotransferase and renal lipid peroxidation (Kim and Na, 1991). Studies on the mechanism of Cr excretion by kidneys indicate glomerular filtration followed by tubular reabsorption of up to about 60% of filtered amount (Langard and Norseth, 1986). Therefore, acute tubular necrosis, marked interstitial changes, and renal failure are not unexpected following massive exposure to hexavalent Cr (Barceloux, 1999).

The major route of Cr entry into humans is through food and water. There are only few toxicity studies involving oral as well as long term administration of Cr as, most of the studies have evaluated Cr toxicity by intraperitoneal or subcutaneous administration. This becomes pertinent in the local context as Cr 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, 2003). This has necessitated the present study on Cr induced renal oxidative stress and toxicity in male *Wistar* rats. In this context, since the study is aimed at understanding the possible Cr toxicity on long term systemic entry into humans though diet and water, a realistic dosage has been worked out based on the Cr content in vegetables and food grains and an average daily food intake. Conversation 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.

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 chromium.

Material and Methods: Same as in chapter 1.

Results:

Lipid Peroxidation (LPO): Changes in the renal LPO levels following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 2.1; table 2.1. There was an age dependent increase in LPO level in the control rats. Oral exposure to Cr showed significant increment in LPO. Chromium showed a duration dependent increase in LPO with a maximal level at 60 days and minimal at 15 days. Melatonin afforded similar degree of protection in Cr treated animals irrespective duration of exposure.

Glutathione (GSH): Changes in the renal GSH levels following Cr treatment for 15, 30 and 60 days are shown in fig 2.2; table 2.2. An age dependent gradual decrease in the GSH level was seen in control (Con) rats. There was significant duration dependent decrease in GSH content in animals treated with Cr. There was significant increase in GSH level in animals administered with melatonin alone or with Cr. Melatonin showed a similar degree of protection in the Cr treated animals at all the time periods.

Ascorbic Acid (Vit C): Changes in the renal Ascorbic Acid (Vit C) contents following Cr treatment for 15, 30 and 60 days are shown in fig 2.3; table 2.3. An age dependent gradual decrease in the Vit C level was seen in control (Con) rats. There was significant decrease in the Vit C levels in the animals treated with Cr with the decrement being maximal in the short term treatment period. The level of Vit C was significantly increased in animals treated with melatonin alone or in combination with Cr. Maximum protective effect was seen in animals treated for 60 days.

Superoxide Dismutase (SOD): Changes in the renal superoxide dismutase (SOD) activity level following Cr treatment for 15, 30 and 60 days are shown in fig 2.4; table 2.4. A gradual age dependent decrease in enzyme activity was seen in the Con group

of animals. The activity of SOD showed significant inhibition in the Cr group of animals with progressively increasing inhibition from 15 to 60 days of Cr exposure. There was significant increase in SOD activity in melatonin treated control animals. Co-administration of melatonin along with Cr showed significant protective effect against metal induced decrease in SOD activity.

Catalase (CAT): Changes in the renal catalase activity following Cr treatment for 15, 30 and 60 days are shown in fig 2.5; table 2.5. A gradual age dependent decrease was the feature in the Con group of animals. There was significant duration dependent decrease in the activity of CAT in the Cr treated group. Significant increase in the activity of CAT was recorded on administration of melatonin. In the Cr+Mel group of rats there was significant resistance against CAT inhibition.

Glutathione Peroxidase (GPx): Changes in the renal Glutathione Peroxidase (GPx) activity following Cr treatment for 15, 30 and 60 days are shown in fig 2.6; table 2.6. A gradual age dependent decrease in GPx activity could be seen in Con group of animals. There was significant duration dependent decrease in GPx activity in the Cr treated group. The protective effect of melatonin was similar in all the treatment groups independent of duration of Cr of exposure.

Metal Load: Changes in renal accumulation following Cr treatment for 15, 30 and 60 days are shown in figure 2.7; table 2.7. Cr treated rats showed increased renal Cr accumulation but the increment was significant only in the 30 and 60 day treatment groups. There was significant protection against Cr accumulation in the melatonin treated groups. Both, Cr 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 table 2.11. They showed an increase on Cr exposure. The increase was found to be gradual and duration dependent in relation to Cr exposure. There was significant decrease in serum urea and creatinine levels in melatonin administered control rats and in the metal administered animals co-administered with melatonin group, the increase was minimal.

Histology: Changes in renal histology following Cr treatment for 15, 30 and 60 days are shown in figure 2.8 to 2.10. Cr induced histological changes in the renal tissue could be seen clearly at all periods of Cr exposure. The visible histological lesions due to Cr exposure is, progressively increasing disruption of Bowmann's epithelium and even glomerular shrinkage and endothelial disintegrity. There is also progressive dilation, cellular vacuolization, hypertrophy, nuclear pyknosis with reduced basophilia and apoptosis of proximal tubules. Overall, Cr seems to cause extensive renal histopathological alterations. These changes were reversed and near normal histoarchitecture was observed on co-administration with melatonin.

Hematological parameters: Changes in hematology following Cr treatment for 15, 30 and 60 days are shown in table 2.8 to 2.10. There was a decrease in leucocyte count with diminished neutrophil count.

Figure 2.1. Graph showing levels of renal lipid peroxidation (LPO) in animals exposed to Cr for 15, 30 or 60 days

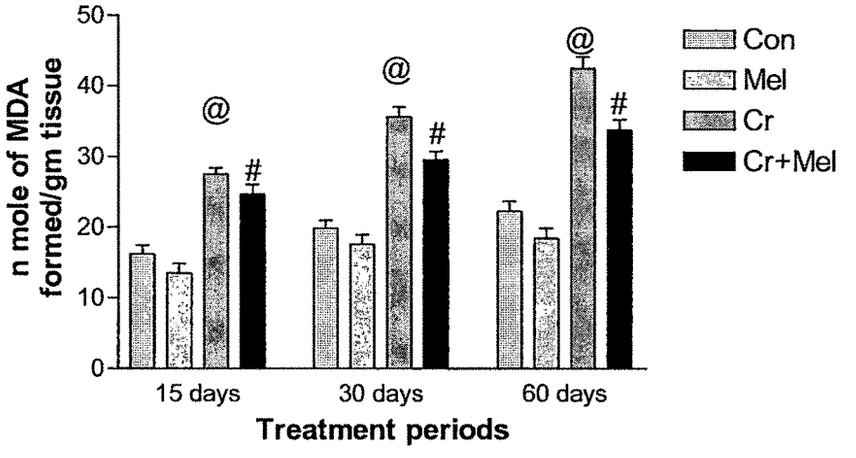

Table 2.1. Contents of renal lipid peroxidation (LPO) following exposure to Cr for 15, 30 or 60 days

	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
Cr	56.23±1.52 @	60.25± 1.29 @	70.52± 1.59@
Cr+Mel	48.56± 1.98 #	52.35±1.04 #	59.46± 1.25 #

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

Figure 2.2. Graph showing levels of renal glutathione (GSH) content in animals exposed to Cr for 15, 30 or 60 days

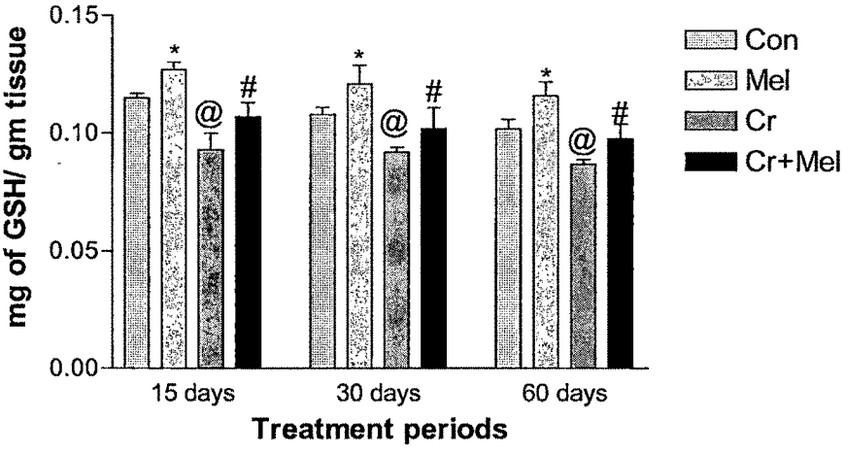

Table 2.2. Contents of renal glutathione (GSH) following 15, 30 or 60 days treatment with Chromium

	15 days	30 days	60 days
Con	0.115±0.002	0.108±0.003	0.102±0.004
Mel	0.127±0.003*	0.121±0.008*	0.116±0.006 *
Cr	0.095±0.007@	0.092±0.002@	0.087±0.002@
Cr+Mel	0.107±0.006#	0.102±0.009#	0.098± 0.007 #

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

Figure 2.3. Graph showing renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with chromium

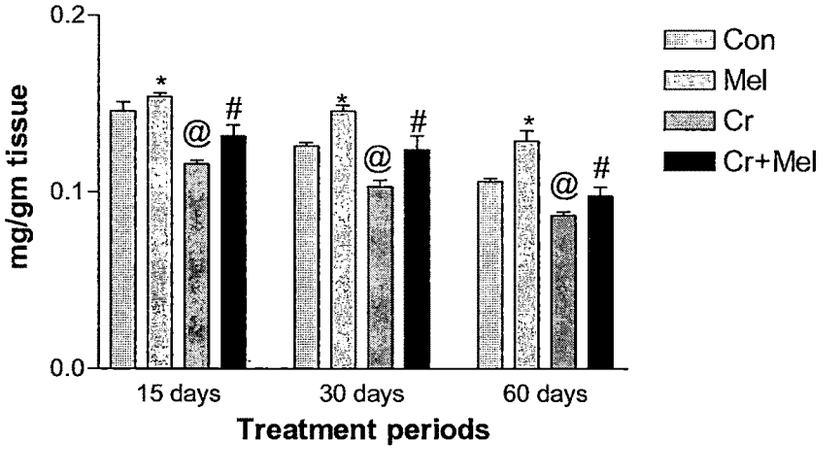

Table 2.3. Levels of renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with chromium

	15 days	30 days	60 days
Con	140.41±1.25	126.41±1.12	106.28±1.20
Mel	154.39±1.52*	146.36±1.63*	129.15±1.68*
Cr	116.79±1.82@	103.42±1.54@	87.51±1.26@
Cr+Mel	132.19±1.36#	124.14±1.82#	98.75±1.57 #

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

Figure 2.4. Graph showing renal Superoxide Dismutase (SOD) activity following 15, 30 or 60 days exposure with chromium.

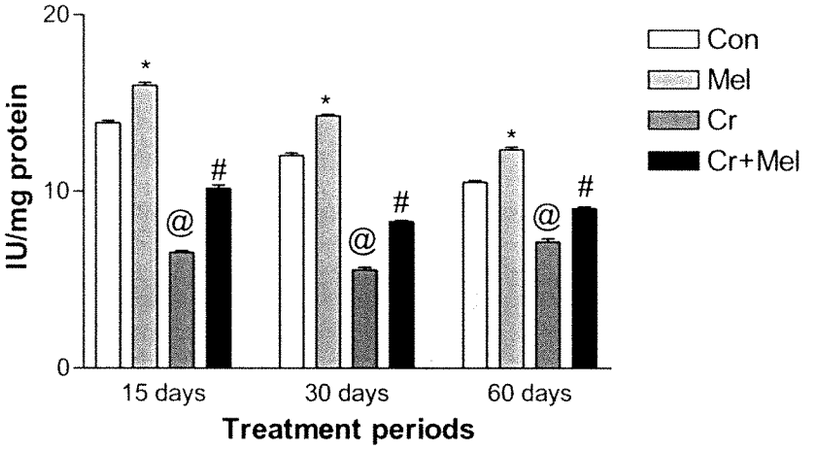

Table 2.4. Levels of renal Superoxide Dismutase (SOD) activity following 15, 30 and 60 days exposure with Chromium

	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*
Cr	5.61±0.09@	6.10±0.095@	5.83±0.11@
Cr+Mel	9.52±0.25#	8.15±0.01#	7.59±0.136#

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

Figure 2.5. Graph showing renal catalase (CAT) activity following 15, 30 or 60 days exposure to chromium.

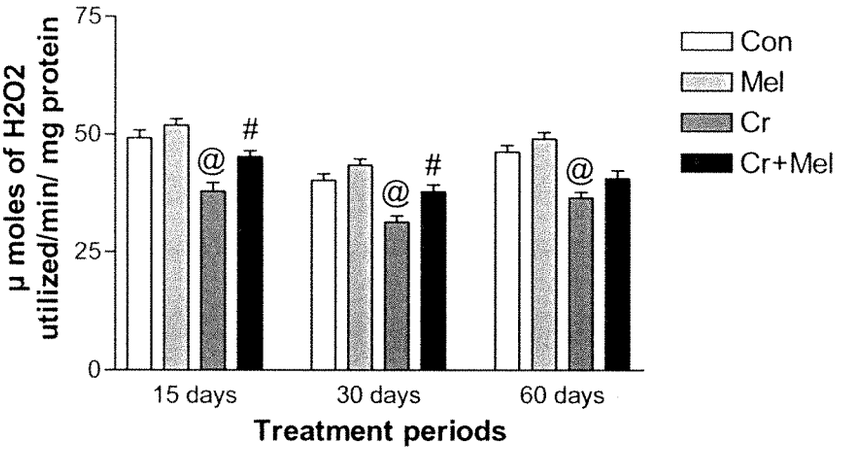

Table 2.5. Levels of renal catalase (CAT) activity following 15, 30 or 60 days exposure with Chromium

	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
Cr	35.41±1.82@	30.21±1.29@	27.35±1.24@
Cr+Mel	42.92±1.36#	37.82±1.48	35.75±1.66

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

Figure 2.6. Graph showing renal Glutathione Peroxidase (GPx) activity following 15, 30 and 60 days exposure to chromium.

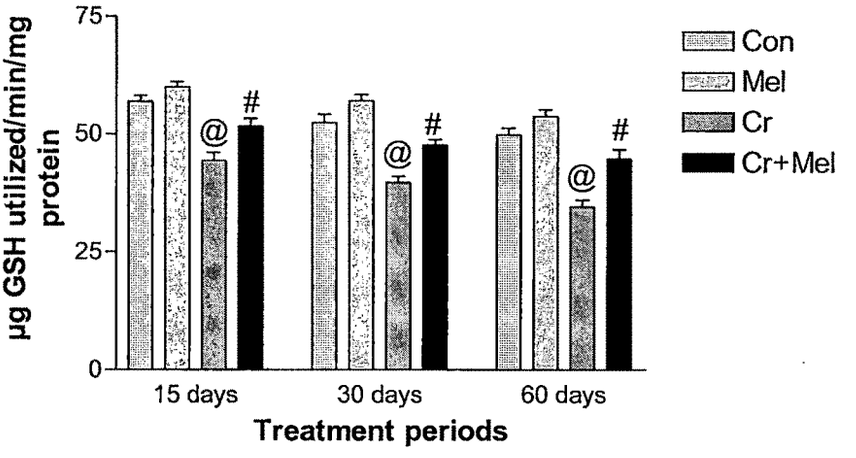

Table 2.6. Levels of renal Glutathione Peroxidase (GPx)following 15, 30 and 60 days treatment with Cr

	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
Cr	44.38±1.71@	39.73±1.69@	34.58±1.52@
Cr+Mel	51.67±1.63#	47.74±1.96#	44.48±1.93#

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

Figure 2.7. Graph showing % accumulation of Cr in renal tissue following 15, 30 and 60 days treatment with chromium.

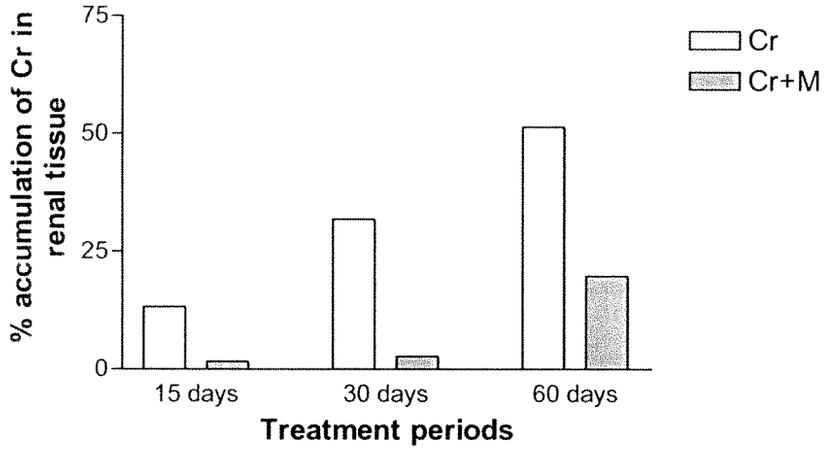

Table 2.7. % accumulation of Cr in renal tissue following 15, 30 and 60 days treatment with Chromium

Treatment Period	Cr(VI)	Cr+M
15 days	13.21	1.67
30 days	31.76	2.70
60 days	51.4	19.81

Table 2.8 Changes in hematological parameters after 15 days of Cr(VI) treatment

	Hemoglobin	Erythrocyte Count	Leucocyte Count	Packed Cell Volume(PCV)	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Con	15.05±0.17	9.66±0.01	8275.00±85.39	51.75±0.85	1016100.00±70.83	19.00±0.91	76.00±0.41	1.25±0.25	2.00±0.00
Mel	15.60±0.15	10.13±0.11	8475.00±110.87	56.00±0.91	1025500.00±104.83	21.25±1.25	79.25±0.25	1.25±0.25	2.25±0.25
Cr	14.70±0.10	9.47±0.13	6801.75±2.79@	51.00±0.91	967125.00±188.75	27.00±1.56@	74.25±0.85	1.00±0.00	2.00±0.00
Cr+M	15.27±0.09	9.75±0.11	7650.00±104.08	54.00±1.47	1094150.00±95.87	23.50±0.91	71.50±1.04	1.25±0.25	1.75±0.25

Table 2.9 Changes in hematological parameters after 30 days of Cr(VI) treatment

	Hemoglobin	Erythrocyte Count	Leucocyte Count	Packed Cell Volume(PCV)	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Con	16.75±0.48	10.27±0.19	7175.00±125.00	51.50±1.32	1302075.00±75.08	19.25±0.63	79.00±0.91	1.50±0.29	2.00±0.00
Mel	15.00±1.08	9.92±0.16	7450.00±132.29	56.25±1.11	1423150.00±64.55	23±1.25	79.25±0.85	1.50±0.29	1.75±0.25
Cr	15.08±0.09	10.20±0.12	6700.00±125.00@	48.50±1.85@	1322100.00±70.83	25±0.85@	81.25±1.11	1.50±0.29	1.50±0.29
Cr+M	14.00±0.91	10.38±0.11	9100.00±91.29#	53.00±1.47	111000.00±91.87	25±1.5	83.00±1.30	1.25±0.25	2.00±0.00

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

Table 2.10 Changes in hematological parameters after 60 days of Cr(VI) treatment

	Hemoglobin	Erythrocyte Count	Leucocyte Count	Packed Cell Volume(PVC	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Con	15.75±0.13	10.68±0.19	8500.00±91.29	54.25±1.25	1213075.00±85.39	18±0.85	78.75±1.25	1.00±0.00	2.00±0.00
Mel	16.75±0.85	10.72±0.20	8800.00±110.7	54.00±1.82	1429250.00±162.02	22.00±0.91	74.50±1.04	2.00±0.40	2.00±0.00
Cr	14.40±0.09	10.38±0.16	6525.00±103@	43.00±1.08@	845099.80±70.83@	24.25±0.8@	63.00±1.08@	1.00±0.00	1.75±0.25
Cr+M	14.70±0.09	10.14±0.21	9425.00±165.2#	54.50±1.76#	1078125.00±94.76	21.25±1.31	76.25±0.85#	1.00±0.00	2.25±0.25

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

Table 2.11 Changes in serum Urea and Creatinine following treatment of Cr(VI) 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
Chromium (Cr)	41.00±0.91@	0.62±0.05@	38.50±0.65	0.43±0.09	42.50±1.04@	0.68±0.09
Cr+Melatonin (Cr+Mel)	33.00±1.08#	0.43±0.05#	36.75±0.85	0.50±0.04	39.25±0.85	0.65±0.07

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

PLATE I

Fig 2.8 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 2.9 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 2.10 Photomicrograph of 15 day chromium treated kidney showing Bowman's capsule with glomerulus and distal tubules (400X). Note the breaches in the Bowman's capsular epithelium (arrows), widened distal tubules due to degeneration of epithelial cells can be seen. BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 2.11 Photomicrograph of 15 day chromium+melatonin treated kidney(400X). Some distended distal tubules can be seen. The Bowman's capsular epithelium is intact. BC-Bowman's Capsule; DT- Distal tubule; G-Glomerulus

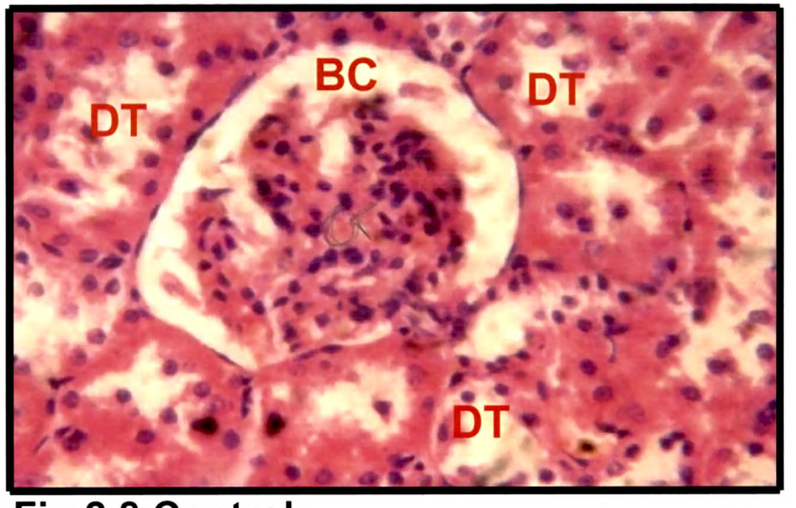

Fig 2.8 Control

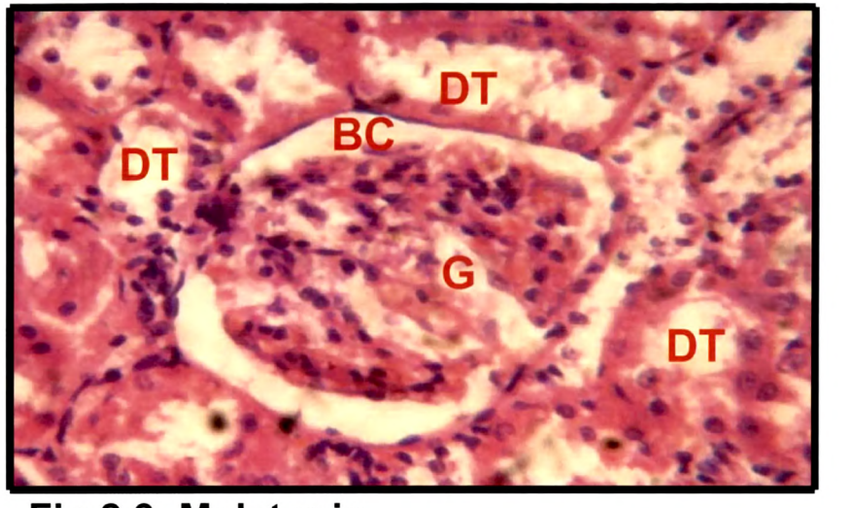

Fig 2.9 Melatonin

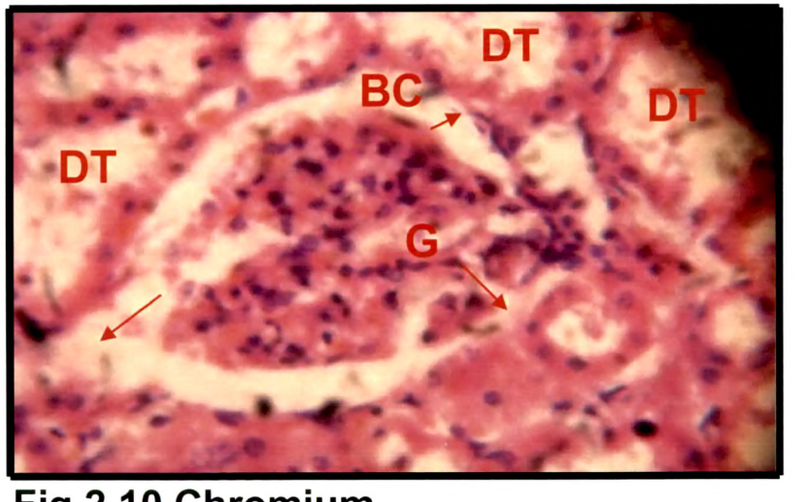

Fig 2.10 Chromium

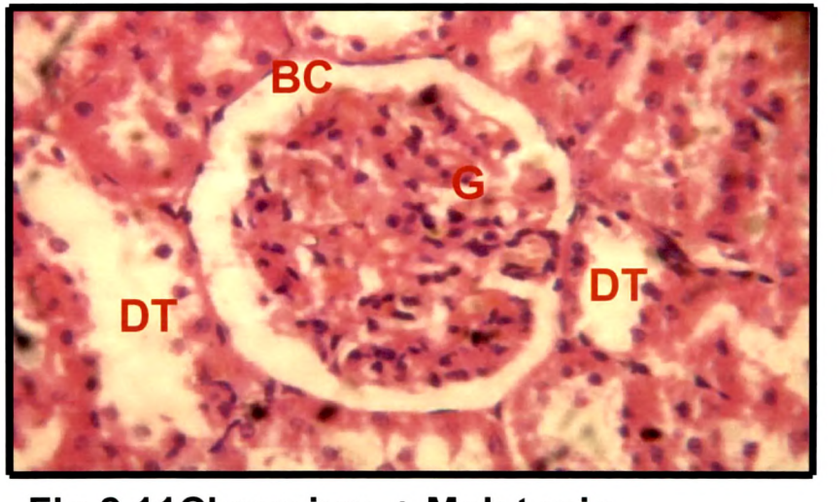

Fig 2.11Chromium + Melatonin

PLATE II

Fig 2.12 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule

Fig 2.13 Photomicrograph of kidney exposed to melatonin for 15 days showing proximal tubules (400X). PT- Proximal tubule

Fig 2.14 Photomicrograph of kidney exposed to chromium for 15 days showing proximal tubules (400X). Note the widened and distended PT due to apoptotic or necrotic degeneration of epithelial cells.

Fig 2.15 Photomicrograph of kidney exposed to chromium + melatonin for 15 days showing normal looking PT (400X).

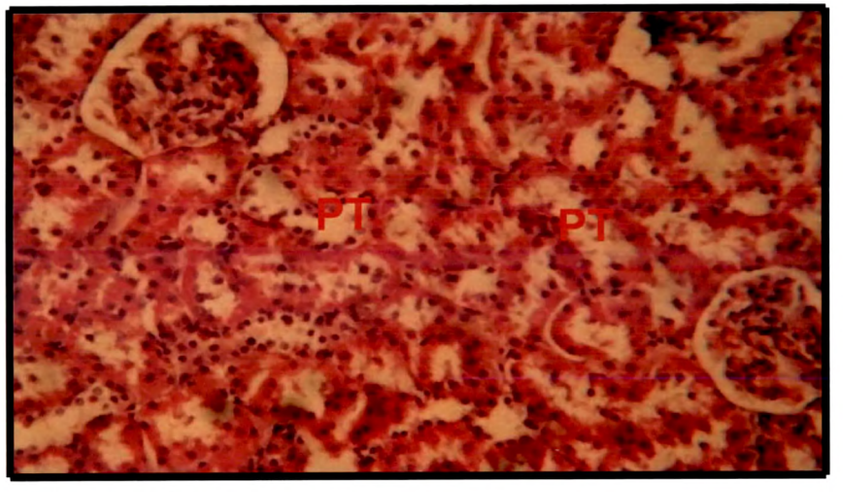

Fig 2.12 Control

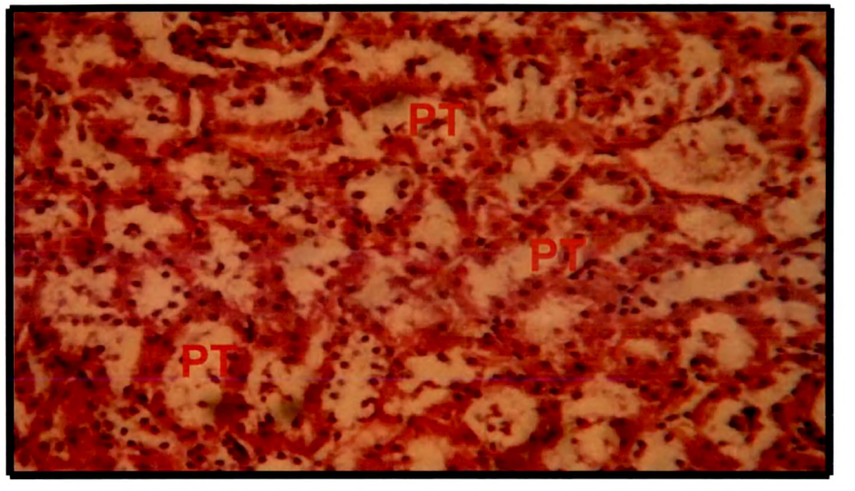

Fig 2.13 Melatonin

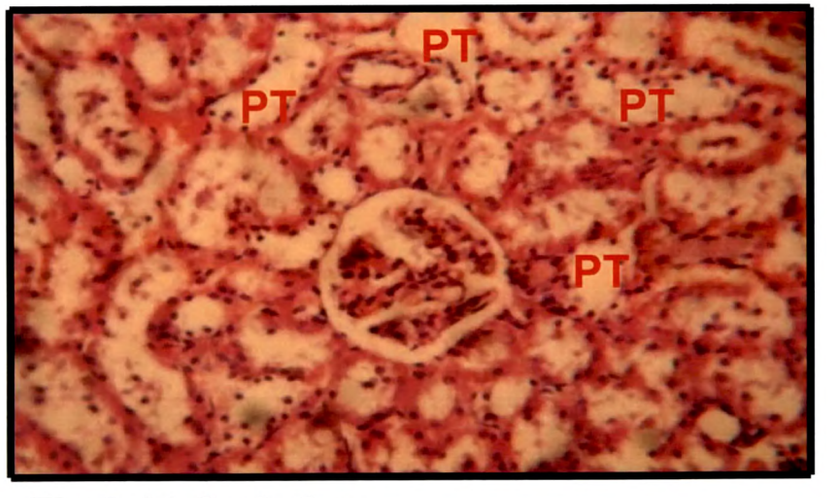

Fig 2.14 Cadmium

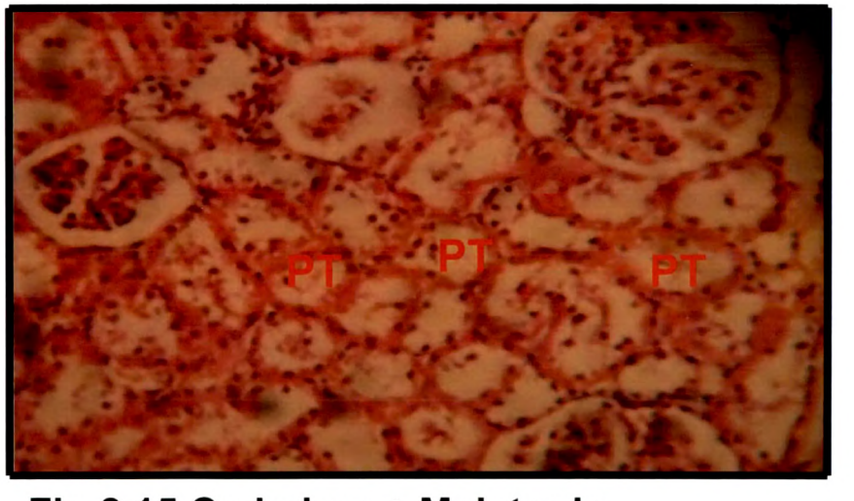

Fig 2.15 Cadmium + Melatonin

PLATE III

Fig 2.16 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 2.17 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 2.18 Photomicrograph of kidney exposed to chromium for 30 days showing Bowman's capsule with glomerulus and distal tubules (400X). Note the discontinuities in the Bowman's capsular epithelium (arrow). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 2.19 Photomicrograph of kidney exposed to chromium + melatonin for 30 days showing Bowman's capsule with glomerulus (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

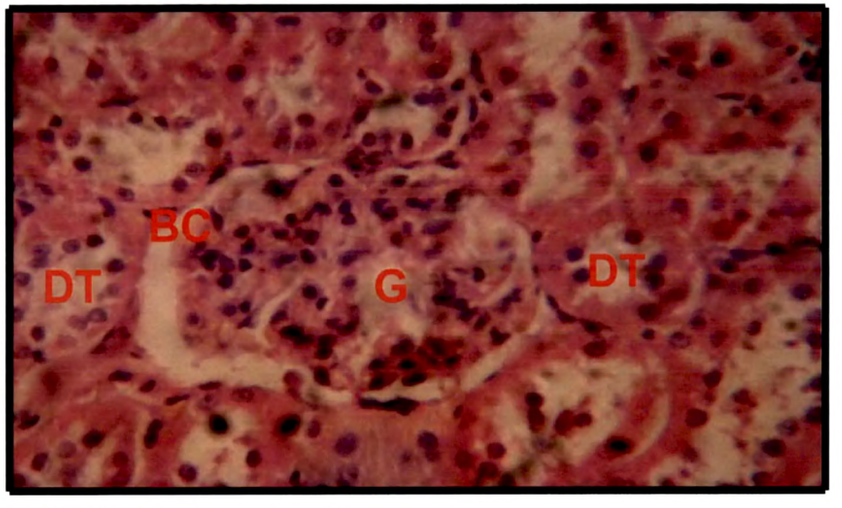

Fig 2.16 Control

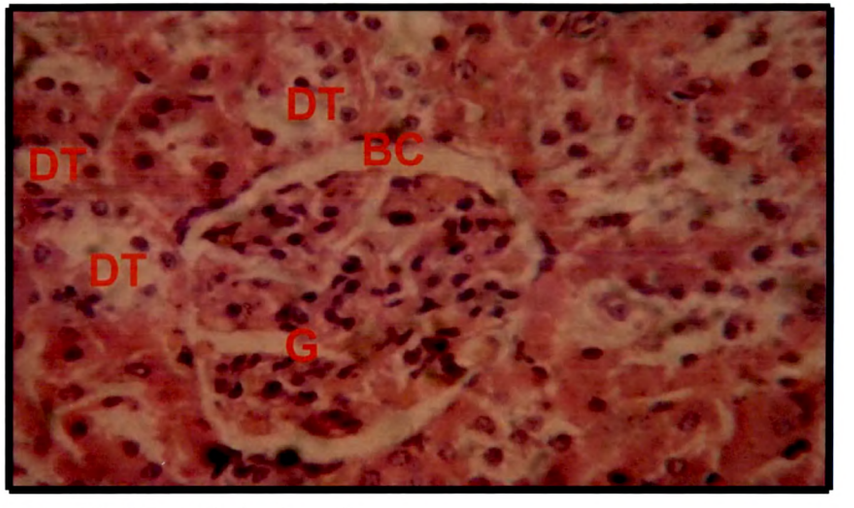

Fig 2.17 Melatonin

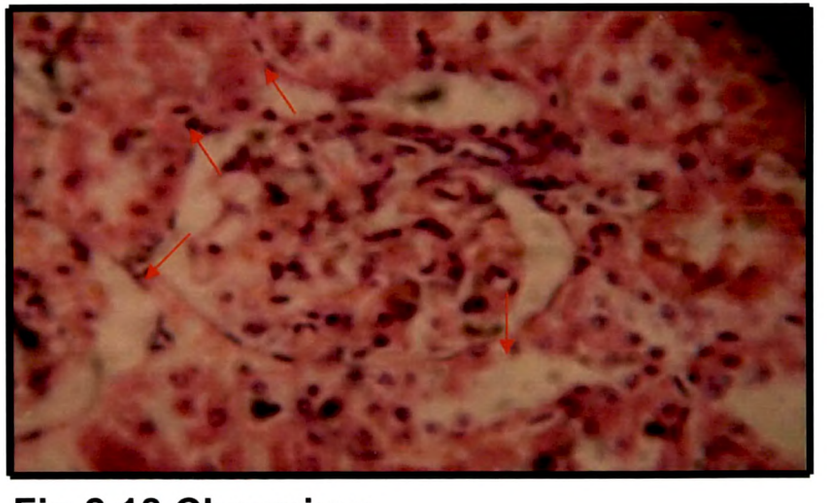

Fig 2.18 Chromium

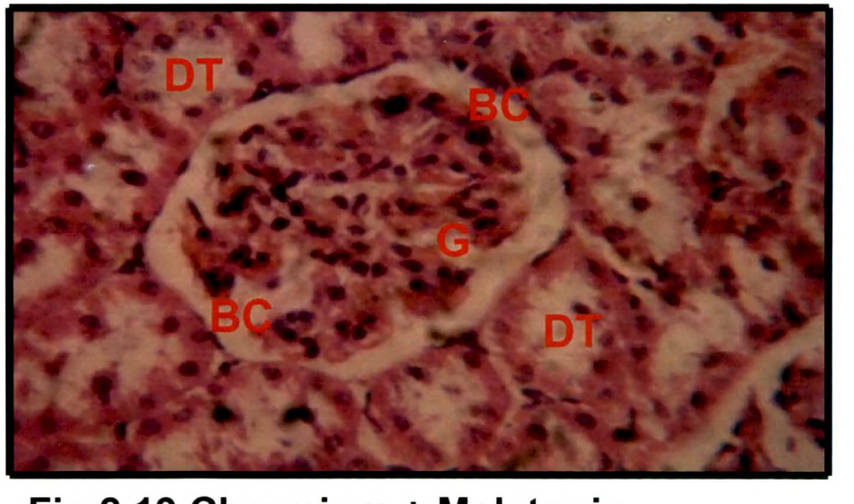

Fig 2.19 Chromium + Melatonin

PLATE IV

Fig 2.20 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule.

Fig 2.21 Photomicrograph of kidney exposed to melatonin for 30 days showing proximal tubules (400X). PT- Proximal tubule

Fig 2.22 Photomicrograph of kidney exposed to chromium for 30 days showing proximal tubules (400X). PT- Proximal tubule

Fig 2.23 Photomicrograph of kidney exposed to chromium + melatonin for 30 days showing proximal tubules (400X). PT- Proximal tubule

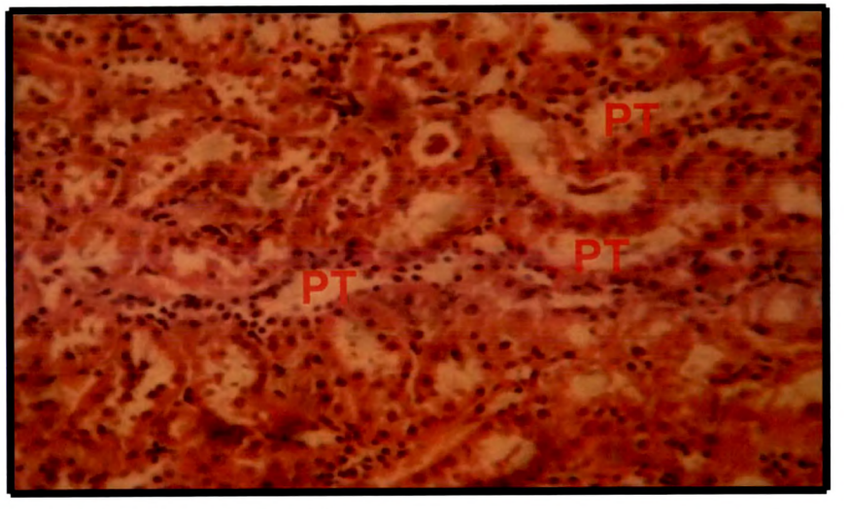

Fig 2.20 Control

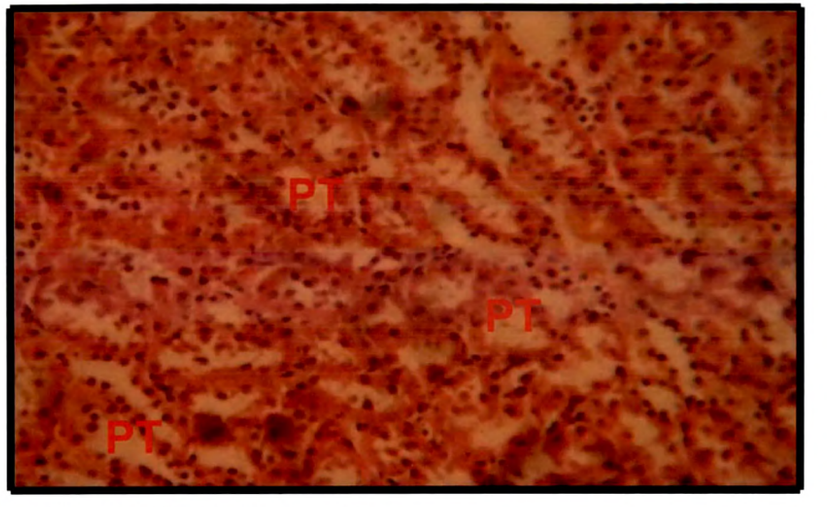

Fig 2.21 Melatonin

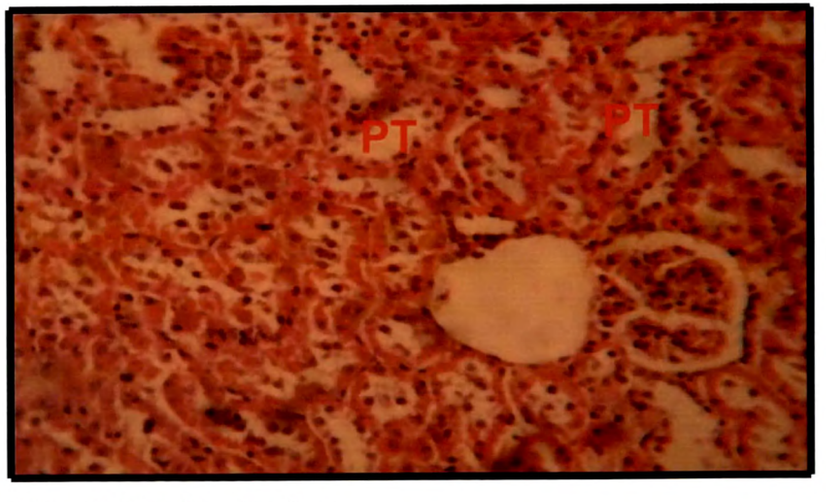

Fig 2.22 Cadmium

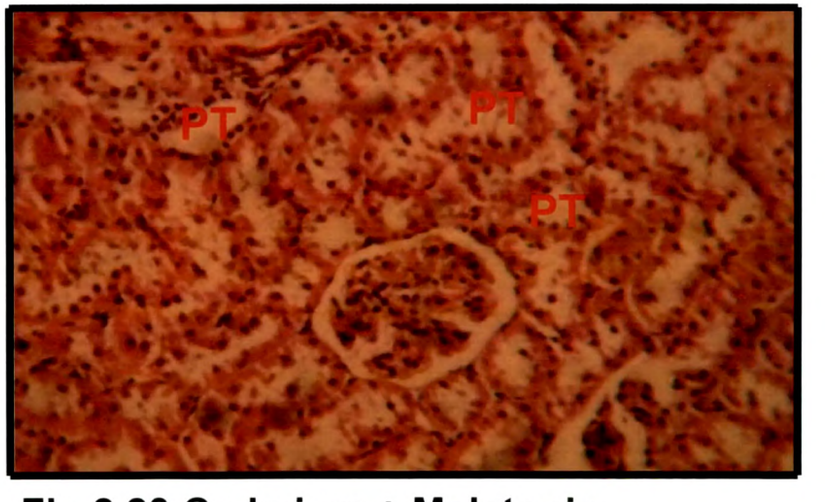

Fig 2.23 Cadmium + Melatonin

PLATE V

Fig 2.24 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 2.25 Photomicrograph of kidney exposed to melatonin for 60 days showing Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 2.26 Photomicrograph of kidney exposed to chromium for 60 days showing Bowman's capsule with glomerulus and distal tubules (400X). Note the more or less denuded Bowman's capsule (arrow) and glomerular disruptions. BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 2.27 Photomicrograph of kidney exposed to chromium+melatonin for 60 days showing normal looking Bowman's capsule (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

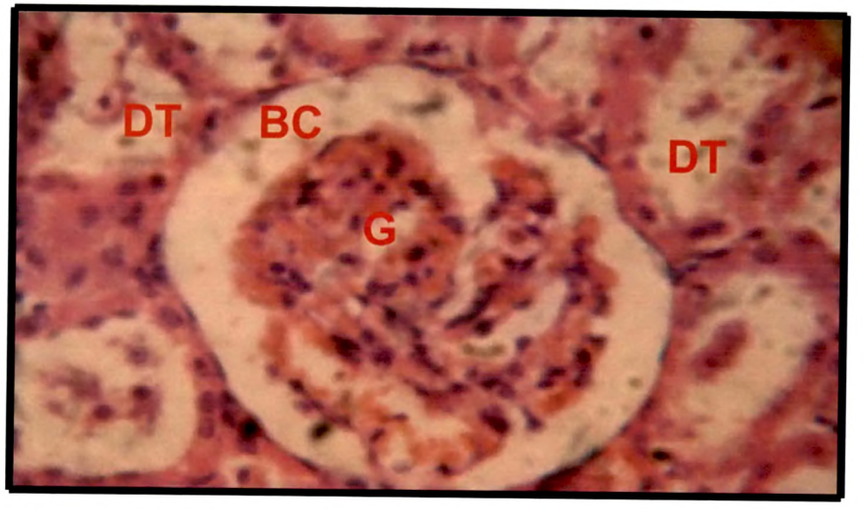

Fig 2.24 Control

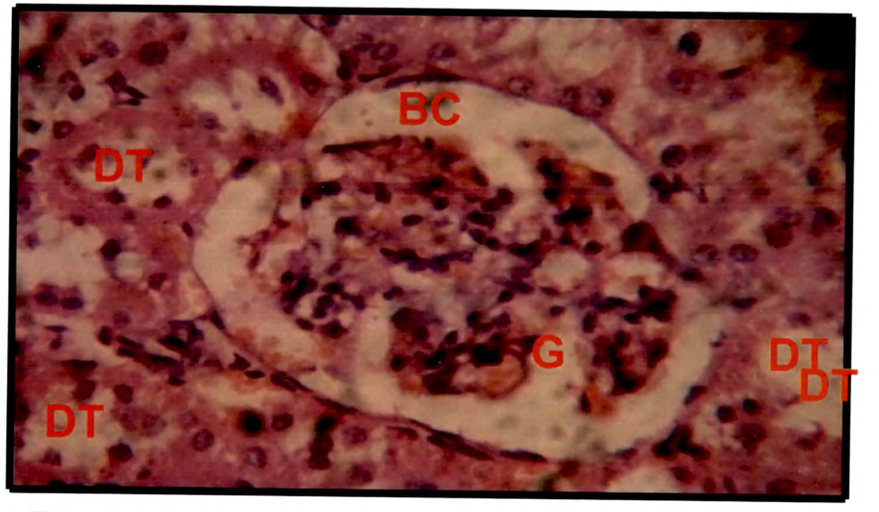

Fig 2.25 Melatonin

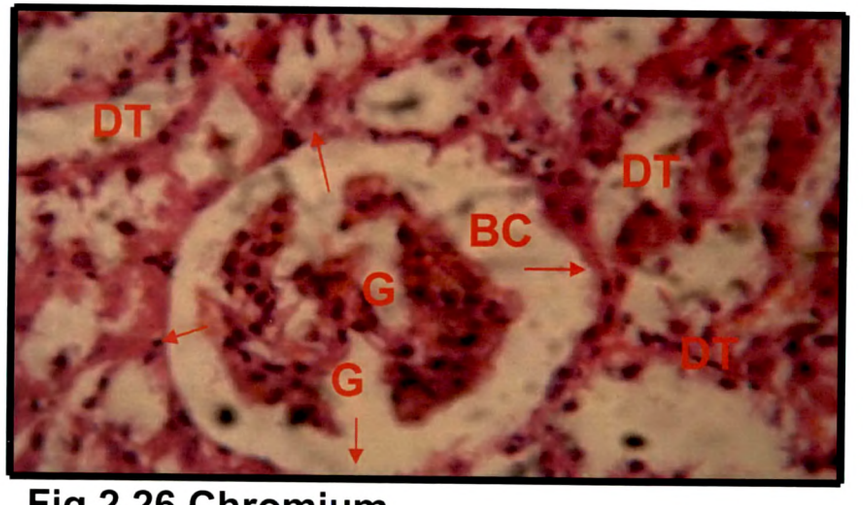

Fig 2.26 Chromium

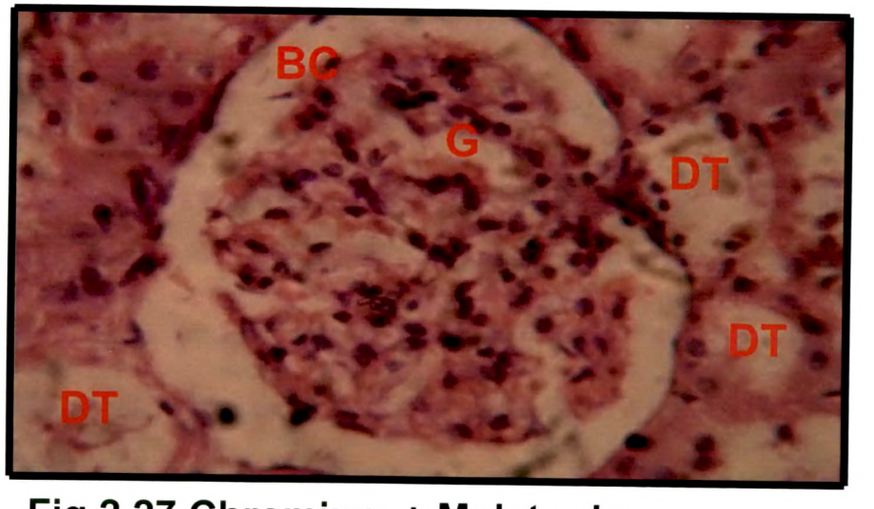

Fig 2.27 Chromium + Melatonin

PLATE VI

Fig 2.28 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule.

Fig 2.29 Photomicrograph of kidney exposed to melatonin for 60 days showing proximal tubules (400X). PT- Proximal tubule

Fig 2.30 Photomicrograph of kidney exposed to chromium for 60 days showing proximal tubules (400X). Note the apoptotic/necrotic degeneration of proximal tubular epithelial cells. PT- Proximal tubule

Fig 2.31 Photomicrograph of kidney exposed to chromium + melatonin for 60 days showing normal proximal tubules (400X). PT- Proximal tubule

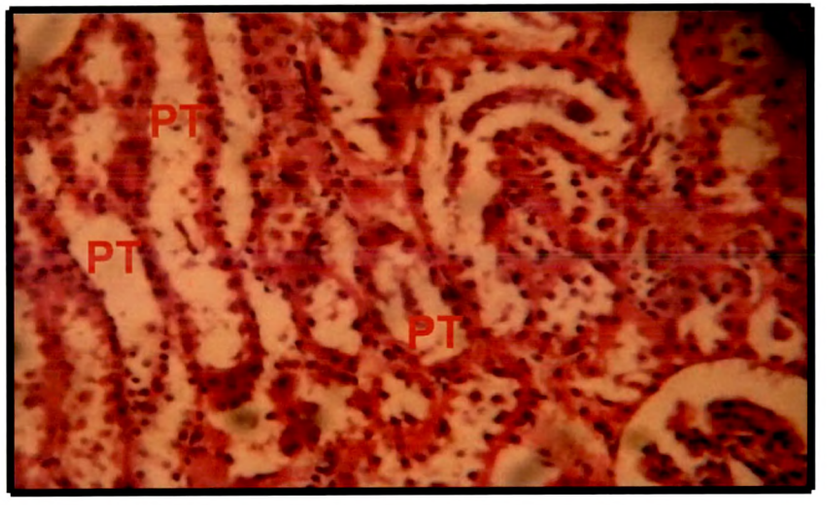

Fig 2.28 Control

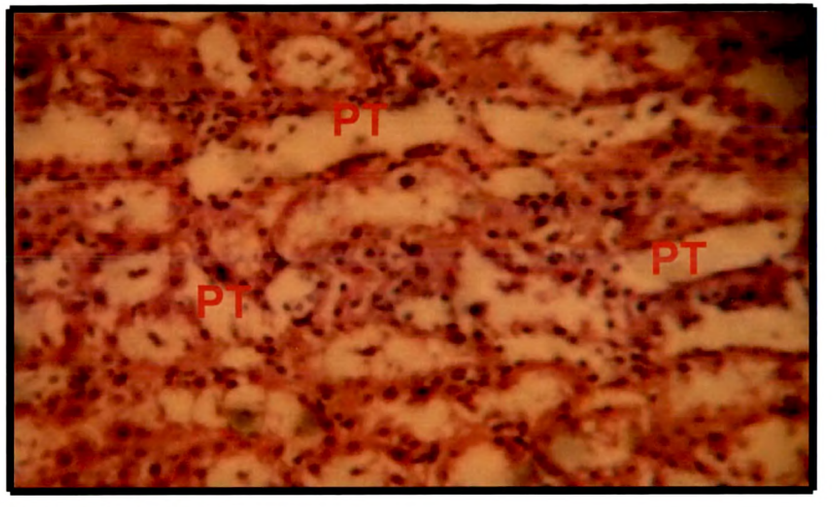

Fig 2.29 Melatonin

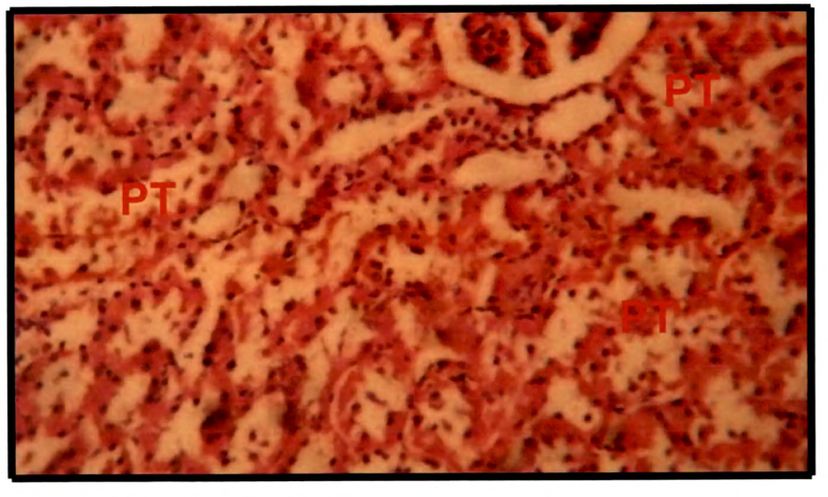

Fig 2.30 Cadmium

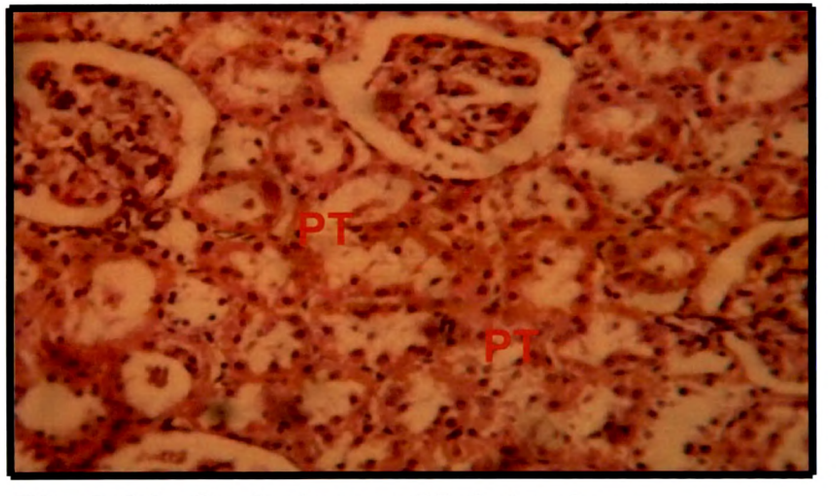

Fig 2.31 Cadmium + Melatonin

Discussion:

Renal response to a chronic realistic dose of Cr (VI) assessed in adult rats has shown increasing oxidative stress and toxicity manifestations. Development of renal oxidative stress is marked by the induction of significantly high level of LPO and decreased contents of non-enzymatic (GSH and Vit C) as well as enzymatic antioxidants (SOD, CAT, GPx). Significantly elevated LPO seen at 15 days seemed to increase through 30 days to reach a maximal level at 60 days. The increasing level of LPO with duration suggests the lack of any adaptive/protective mechanism to control oxidative stress as is characteristic of hepatic response (Chapter 1). The progressive increase in LPO recorded herein well parallels the increase in renal metal load from 15 to 60 days. A steady decrease in the levels of GSH and Vit C along with decreased activity levels of enzymatic antioxidants has been seen. It has been shown that liver has a more robust antioxidant system than kidney to combat Cr (VI) toxicity (Patlolla *et al.*, 2008).

The fact that CAT, GPx and SOD are showing increasing inhibition with duration of Cr exposure suggests increasing oxidative stress which is well reflected in the recorded degree of LPO. Increasing inhibition of the enzymatic antioxidants can be looked upon in the context of Reactive Oxygen Species (ROS) being generated by Cr (VI) during its successive reduction from Cr (VI) to Cr (III). Both superoxide and hydroxyl radicals are usually generated by Cr (VI) and Cr (III) though the latter predominate ultimately (Bagchi *et al.*, 2001, 2002; Dong *et al.*, 2006). It is likely that during the initial period of Cr exposure (15 days), successive oxidation of Cr (VI) to Cr (III) and depletion in GSH and Vit C, both contribute to an increase in superoxide radicals (Bagchi *et al.*, 2001). The neutralization of superoxide radical is under the purview of SOD and as such the recorded maximal inhibition of SOD at 15 days

attests to this. Most of the studies on chromium toxicity and even LPS toxicity (Leech *et al.*, 1998) have reported decreased SOD activity. The progressively improvement in SOD activity seen in the present study on prolonged exposure to Cr, seems to suggest either decreased generation of superoxide radicals or increased mechanism of direct superoxide scavenging. The improvement in renal GSH content from 15 to 60 days and continuing depletion of Vit C are indicative of bettered GSH production by the NADPH pathway and conversion of dehydroascorbic acid to ascorbic acid by GSH. However, the depletion in ascorbic acid seen herein despite the purported conversion of dehydroascorbic acid to ascorbic acid could be due to the increased scavenging of superoxide anion by ascorbic acid. Simultaneously, the purported recycling of GSSG to GSH by NADPH is expected to be due to increased operation of G-6-PDH catalyzed shunt pathway. The upregulated shunt pathway may in turn along with as yet unknown factors of Cr toxicity may lead to reduced oxidative metabolism and milder hypoxic conditions. Both, ascorbic acid mediated superoxide scavenging, as well as hypoxic condition, could contribute to the improvement in SOD activity (Wang *et al.*, 2003). Moreover, it is likely that most of the Cr that is reaching renal tissue secondarily from liver will be in Cr (III) state which is known to generate less superoxide (Wang *et al.*, 2006), further providing support to the observed favorable change in SOD activity.

Persistent and increasing degree of inhibition of CAT and GPx to the same extent suggests equal participation of both these enzymes in the removal of hydrogen peroxide (H_2O_2). These changes in CAT and GPx activity compared to the improving SOD activity clearly document the increasing exigency of H_2O_2 decomposition as opposed to superoxide dismutation. The increasing oxidative stress and lipid peroxidation seen in the renal tissue on prolonged exposure to Cr can therefore be

attributed to greater generation of peroxyl and hydroxyl radicals. These Cr induced renal biochemistry of oxidative stress also finds its impact on the microscopic anatomy of the renal tissue. The observed histopathological alterations affecting the structural integrity and functioning of the Malphigian body (Bownmann's capsule + glomerulus) and proximal tubules are all indicative of histopathological lesions due to the action of free radicals, as well as accrediatable to the induced membrane lipid peroxidation. Compromised functional integrity of the renal tubules and other renal damage are well reflected in the significantly elevated serum levels of urea and creatinine, the biochemical makers of kidney damage. Apparently, over a prolonged period of exposure, Cr does, inflict kidney damage and cause toxicity manifestations.

Hematological alterations evaluated during Cr toxicity have failed to show any remarkable effect except for decreased leucocyte count. The general decrease in leucocyte count seems to be mainly a reflection of the change in polymorph/neutrophil number. The diminished neutrophil count could be related to chromium induced mild tissue inflammation and efflux of neutrophils from blood. Though there are no direct studies on Cr induced alterations in circulating blood cells, a recent study on Cr induced lung injury and inflammation has shown influx of neutrophils. This provides support to our current observation and indicates the possibility of some degree of tissue inflammation as part of Cr toxicity and tissue damage.

It is interesting to note that, all the alterations induced by chronic Cr exposure including oxidative stress, lipid peroxidation, structural lesions in relation to renal response and, even the reduced neutrophil count in the blood, could be totally prevented or checked substantially on co-administration of melatonin, a known powerful natural antioxidant of the body. Its effectiveness in scavenging hydroxyl and

peroxyl radicals is clearly shown by the marked protective effect on CAT and GPx. Not so efficient effect on SOD suggests, its relative ineffectiveness in neutralizing superoxide radical. However, its general ability to directly quench free radicals is reflected in the protective effect in conserving the non-enzymatic antioxidants. It is also observed from histological observations that, melatonin has potential to maintain the structural integrity of tissues and that, the Cr induced renal damage can be effectively checked and minimized. There are no *in vivo* studies todate except for the present one detailing the role of melatonin as a potent protectant against Cr induced renal toxicity manifestations.

In conclusion, the present study has successfully documented Cr induced increasing renal toxicity on a realistic chronic Cr exposure. Increasing LPO as marker of oxidative stress is suggested to be due to an initial onslaught of superoxide radicals followed later by peroxyl and hydroxyl radicals. Further, renal cytotoxicity has also been demonstrated with possible induction of mild inflammation. All these plethora of Cr induced alterations are also shown to be effectively combated by melatonin providing a base for developing the indoleamine as a possible therapeutic agent against metal toxicity alone or in combination with other agents.

Summary of Chapter 2

Nephrotoxicity induced by Cr(VI) in male *Wistar* rats exposed to a realistic dosage (20 mg/kgBW/day) via drinking water for 15, 30 and 60 days has been evaluated in the present study. Melatonin as a protectant has also been tested. Development of oxidative stress, marked by significantly high levels of lipid peroxidation (LPO) and decreased contents of non-enzymatic (GSH and AA) as well as enzymatic antioxidants (SOD, CAT, GPx) was the feature in Cr (VI) exposed animals. The degree of LPO progressively increased from 15 to 60 days. The progressive increase in LPO was well paralleled by the concurrent increase in renal metal load. A steady decrease in the levels of GSH and AA together with decreased activity levels of enzymatic antioxidants have been noted. The duration dependent increase in LPO suggests the lack of any adaptive/protective mechanism to control the oxidative stress. The histopathological alterations affecting the integrity and functioning of Malphigian tubules and proximal tubules are indicative of cytological lesions due to Cr (VI) exposure. Dysfunctional renal tubules and other renal damages are well reflected in the significantly elevated serum levels of urea and creatinine. Hematological alterations were marked by decreased leucocyte count mainly due to change in polymorph/neutrophil number. It is interesting to note that all alterations induced by chronic Cr exposure including oxidative stress, lipid peroxidation, structural lesions in relation to renal response and even the reduced neutrophil count in the blood were all checked substantially on co-administration with melatonin. In conclusion, the present study has successfully documented Cr induced increasing renal toxicity towards a realistic dosage as well as the efficacy of melatonin to combat all the negative effects of Cr.