CHAPTER 4

Chapter 4

Renal oxidative stress and toxicity and alterations in serum parameters due to Cadmium exposure: Duration dependent study using realistic dosage and protective effect of melatonin.

Cadmium (Cd) is an inorganic toxicant of great environmental and industrial pollutant. Cadmium is widely used in occupational settings such as smelting, refining of Zinc, electroplating, galvanizing, nickel-cadmium battery production, and welding. Crops grown on soil or water contaminated with Cd naturally or by emission from industries, take up Cd efficiently (Elinder, 1985). Generally, Cd enters human body via food, water, air and dust and cadmium induced hepatic, renal and pulmonary damage (Liu *et al.,* 1994) or effects on nervous system (Pal *et al.,* 1993) are dependent on duration and route of exposure. When Cd accumulates in blood, it affects renal cortex and causes renal failure (Jonah and Bhattacharyya, 1989; Oner *et al.,* 1996). A number of studies in human and experimental animals have shown kidney as the critical target organ for cadmium-induced nephrotoxicty (Renugadevi and Prabu, 2009). A variety of renal toxic effects involving proximal tubules and glomeruli such as glomerular swelling, atrophic and pyknotic nuclei, glomerular basement membrane swelling, vacuolization, apoptosis, necrosis and even development kidney stone have all been suggested in cadmium exposed rats (Ahn *et al.,* 1999; Hu, 2000; Kaur *et al.,* 2006). Oxidative stress and reactive oxygen species (ROS) formed in the presence of cadmium could be responsible for its toxic effects in many organs or cells (Wang *et al.,* 2004; Watjen and Beyermann, 2004). The mechanism responsible for the toxic effects of Cd is not completely understood. Unlike Chromium, Cd has got only one oxidation state and hence is unable to produce

free radicals directly but indirect generation of various radicals like superoxide, hydroxyl and nitric oxide has been reported (Galan *et al.,* 2001).

Early manifestation of Cd toxicity is marked by oxidative and DNA damage to tissues (Eybl *et al.,* 2006) and, shifting the prooxidant-antioxidant balance in favour of the former seems to be the pivotal mode of bringing about oxidative stress by Cd (Sies, 1985). Oxidative stress caused by inhibition of antioxidant defense system seems to be the major mode of chronic Cd induced hepatic and renal toxicity (Koyu *et al.,* 2006). Cadmium primarily binds to metallothionein (MT), a heavy metal binding low-molecular weight protein, and the Cd-metallothionein complex is then released from the damaged liver cells and gets filtered through glomerulus into the urinary space to be endocytosed by proximal tubular cells and degraded by the lysosomes resulting in the release of Cd (Morales *et al.,* 2006). This free Cadmium is then able to bring about further oxidative stress as well as toxicity manifestations.

The major route of entry of Cd in humans is the oral route through food and water. There are only few toxicity studies involving oral administration of Cd as, most of the studies have evaluated Cd toxicity by intraperitoneal (ip) or subcutaneous (sc) administration. Further, there is also dearth in studies involving long duration exposure to Cd. This becomes pertinent in the local context as, Cd 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 Cd induced renal oxidative stress and toxicity in male *Wistar* rats. In this context, since the study is aimed at understanding the possible Cd toxicity on long term systemic entry into humans though diet and water, a

realistic dosage has been worked out based on the Cd content in vegetables and food grains and an average daily food intake. 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.

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 withcadmium.

Material and Methods: Same as in chapter 3.

Results:

Lipid Peroxidation (LPO): Changes in the renal LPO levels following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.1; table 4.1. An age dependent gradual increase in LPO was seen in control (Con) rats. There was significant increase in LPO levels of Cd exposed animals with the maximal levels at 60 days. There was significant decrease in LPO levels in animals treated with melatonin. Cadmium showed a duration dependent increase in LPO level with, maximal level at 60 days and minimal at 15 days. Melatonin afforded a similar degree of protection in Cadmium treated animals irrespective of the duration of exposure.

Glutathione (GSH): Changes in the renal GSH levels following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.2; table 4.2. An age dependent gradual decrease in the GSH levels was seen in control (Con) rats. There was significant decrease of GSH levels in the animals treated with Cadmium. There was significant increase in the GSH levels when the animals were administered with melatonin alone or with cadmium. Melatonin showed a similar degree of protection in the cadmium treated animals in all the duration periods. Cadmium did not reveal any duration dependent change in GSH levels.

Ascorbic Acid (Vit C): Changes in renal Ascorbic Acid (Vit C) contents following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.3; table 4.3. An age dependent gradual decrease in Vit C content was seen in control (Con) rats. The Vit C contents were significantly increased in animals treated with melatonin alone or

in combination with cadmium. The maximum protective effect was seen in 30 days of treatment. There is significant decrease in the Vit C content in animals treated with cadmium. The decrement was found to be maximal in 30 day treatment group.

Superoxide Dismutase (SOD): Changes in the renal superoxide dismutase (SOD) levels following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.4 ; table 4.4. A gradual age dependent decrease was seen in the activity of SOD in the Con group of animals. The activity of SOD showed significant inhibition in the Cd group of animals with almost same degree of inhibition in all the three treatment periods. There was a significant increase in the activity of SOD in Mel and Cd+Mel groups compared to Con and Cd groups respectively. The degree of protection with melatonin was slightly lesser in the 60 day Cd exposure group.

Catalase (CAT): Changes in the renal catalase activity following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.5; table 4.5. A gradual age dependent decrease was seen in the Con group of animals. There was significant decrease in CAT activity in the Cd treated group compared to Con group. The activity was decreased to a similar extent in all the Cd exposed groups. There was significant increase in the activity of CAT in the Mel treated group. The protection of melatonin was duration independent.

Glutathione Peroxidase (GPx): Changes in the renal Glutathione Peroxidase (GPx) activity following Cadmium (Cd) treatment for 15, 30 and 60 days are shown in fig 4.6; table 4.6. A gradual age dependent decrease was seen in the activity of GPx in the Con group of animals. There was significant decrease in GPx activity in the Cd treated group and the degree of decrease was same in all the treatment periods. The

protective effect of melatonin was also similar in all the treatment groups and independent of duration of Cd of exposure.

Metal Load: Changes in renal accumulation of Cd following Cd treatment for 15, 30 and 60 days are shown in figure 4.7; table 4.7. Cd treated rats showed increased renal Cd accumulation but the increment was significant in the 30 and 60 day treatment groups. There was significant decrease in Cd accumulation in the melatonin treated groups. Both, Cd 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 as well as serum lipid profile are shown in table 4.8 to 4.13 The renal toxicity parameters (urea and creatinine) showed an increase with Cd exposure. There was a gradual dose dependent increase in both the parameters with Cd exposure but the increase was not significant except for urea in the 60 day treatment group. There was a decrease in renal toxicity parameters in the Mel and Cd+Mel groups of animals but the decrease was not significant.

Histology: Changes in renal histology following Cd treatment for 15, 30 and 60 days are shown in figures 4.8 to 4.10. Cd induced histological changes could be seen clearly in all the duration periods. Cadmium treatment tended to show duration dependent increase in histopathological alterations like Bowman's capsular epithelial discontinuity, glomerular shrinkage, vacuolization of tubular cells, and disorganization and cellular damage in ductal tubules. These changes were reversed and near normal histoarchitecture was observed by co-administration with melatonin.

Figure 4.1. Graph showing levels of renal lipid peroxidation (LPO) in animals exposed to Cd for 15, 30 or 60 days

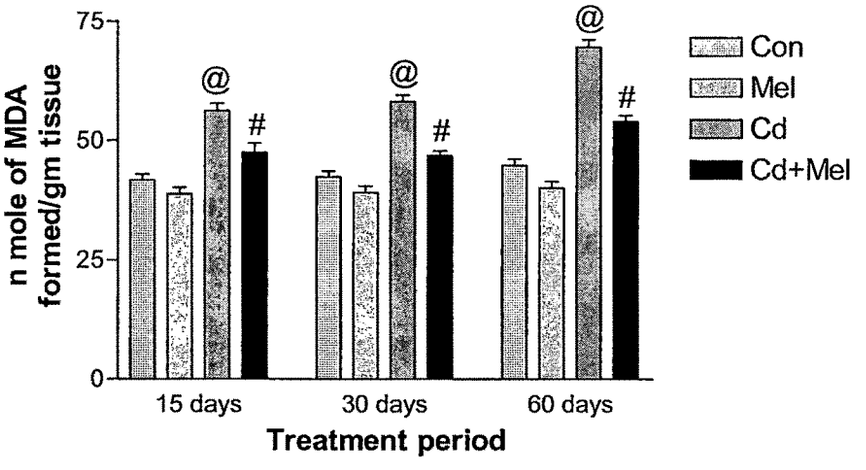

Table 4.1. Contents of renal lipid peroxidation (LPO) following exposure to Cd for 15, 30 or 60 days

	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
Cd	56.31±1.52 @	58.37± 1.29 @	69.75± 1.59@
Cd+Mel	47.52± 1.98 #	46.90±1.04 #	54.20± 1.25 #

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

Figure 4.2. Graph showing levels of renal glutathione (GSH) content in animals exposed to Cd for 15, 30 or 60 days

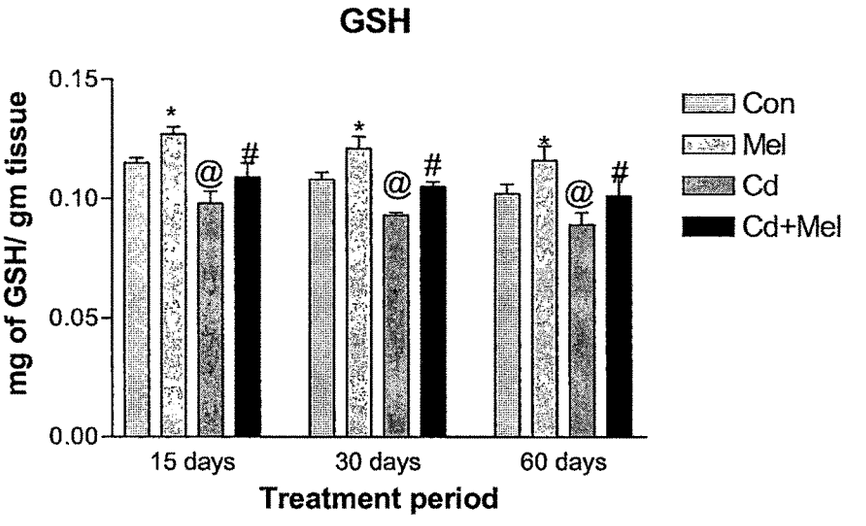

Table 4.2. Contents of renal glutathione (GSH) following 15, 30 or 60 days treatment with Cadmium

	15 days	30 days	60 days
Con	0.115±0.002	0.108±0.003	0.102±0.004
Mel	0.127±0.001*	0.121±0.003*	0.116±0.006 *
Cd	0.098±0.003@	0.093±0.001@	0.089±0.005@
Cd+Mel	0.109±0.006#	0.105±0.002#	0.101± 0.008#

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

Figure 4.3. Graph showing renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with cadmium

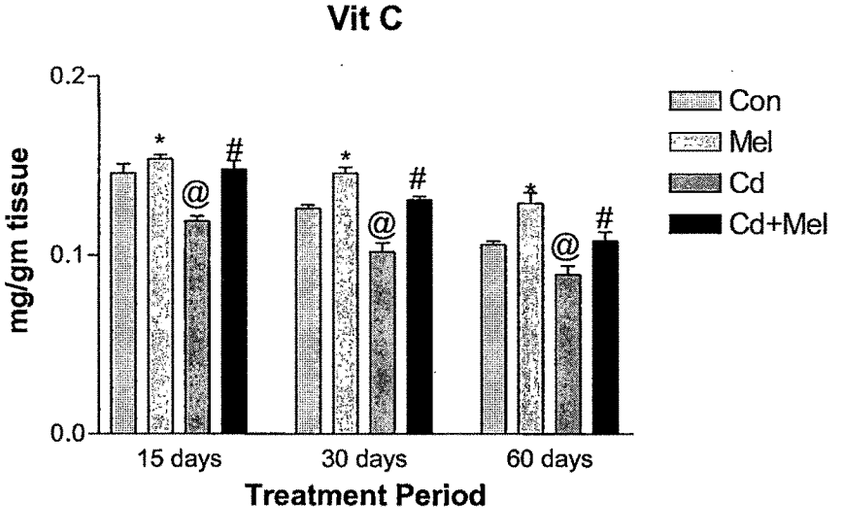

Table 4.3. Levels of renal Ascorbic Acid (Vit C) following 15, 30 and 60 days treatment with cadmium

	15 days	30 days	60 days
Con	0.140±0.005	126.41±0.002	106.28±0.002
Mel	0.154±0.002*	146.36±0.003*	129.15±0.006*
Cd	0.119±0.003@	102.45±0.005@	89.51±0.005@
Cd+Mel	0,148±0.005#	131.14±0.002#	108.75±0.005 #

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

Figure 4.4. Graph showing renal Superoxide Dismutase (SOD) activity following 15, 30 or 60 days exposure with cadmium.

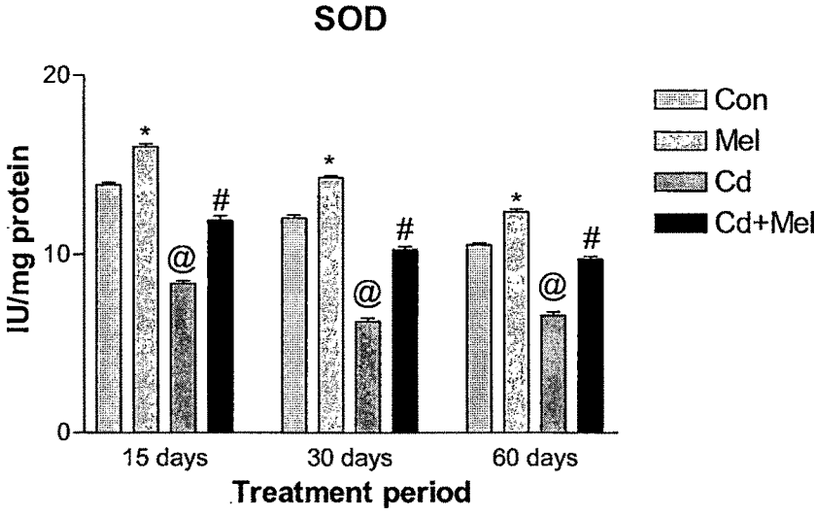

Table 4.4. Levels of renal Superoxide Dismutase (SOD) activity following 15, 30 and 60 days exposure with cadmium

	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*
Cd	8.35±018@	6.24±0.19@	6.58±0.21@
Cd+Mel	11.89±0.25#	10.24±0.2#	9.72±0.16#

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

Figure 4.5. Graph showing renal catalase (CAT) activity following 15, 30 or 60 days exposure to cadmium.

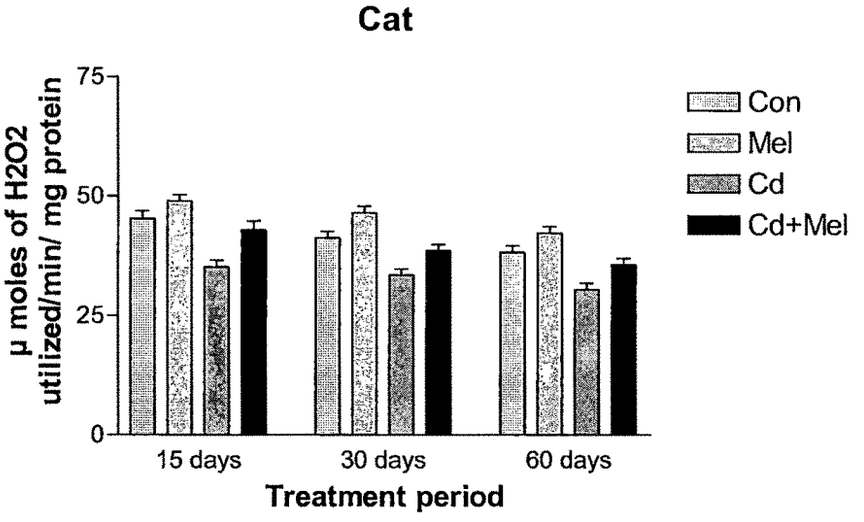

Table 4.5. Levels of renal catalase (CAT) activity following 15, 30 or 60 days exposure with cadmium

	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
Cd	35.19±1.42@	33.51±1.35@	30.50±1.32@
Cd+Mel	42.92±1.91#	38.63±1.32	35.70±1.36

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

Figure 4.6. Graph showing renal Glutathione Peroxidase (GPx) activity following 15, 30 and 60 days exposure to cadmium.

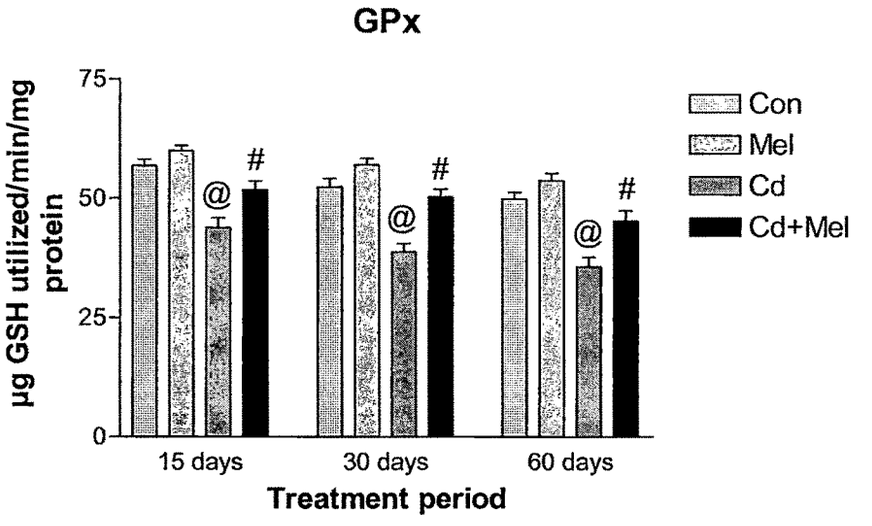

Table 4.6. Levels of renal Glutathione Peroxidase (GPx) following 15, 30 and 60 days treatment with cadmium

	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
Cd	43.93±2.03@	38.83±1.69@	35.73±1.98@
Cd+Mel	51.80±1.87#	50.27±1.96#	45.28±2.18#

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

Figure 4.7. Graph showing % accumulation of Cd in renal tissue following 15, 30 and 60 days treatment with chromium.

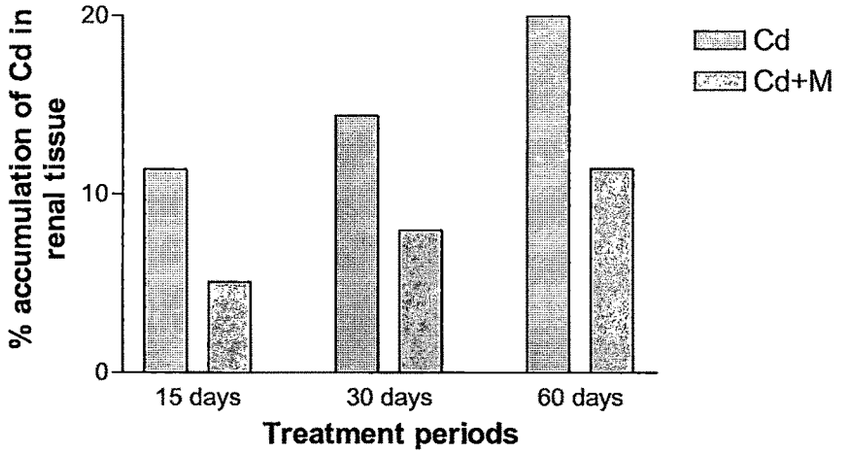

Table 4.7. % accumulation of Cd in hepatic tissue following 15, 30 and 60 days treatment with Cadmium

Treatment Period	Cd	Cd+M
15 days	11.37	5.09
30 days	14.4	7.98
60 days	20	11.42

Table 4.9 Changes in serum lipid profile following 15 days exposure with cadmium (Units: 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±0.63*	132.00±1.08*	55.00±1.47*	26.00±1.08	11.75±0.85*
Cadmium (Cd)	81.89±1.5@	92.75±1.11@	33.50±0.65@	17.75±0.85@	27.50±0.65@
Cd+Melatonin (Cd+Mel)	104.75±1.85#	137.00±0.91#	63.25±0.85#	27.25±0.85#	15.00±0.91#

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

Table 4.10 Changes in serum lipid profile following 30 days of treatment with Cadmium (Units: mg/dl)

	Cholesterol	Triglyceride	H DL	VLDL	LDL
Control (Con)	122.75±0.85	97.75±1.11	62.50±1.04	28.50±1.20	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
Cadmium (Cd)	94.75±1.11@	120.50±1.04@	39.75±0.85@	24.25±0.85@	29.50±1.04@
Cd+Melatonin (Cd+Mel)	106.00±0.41#	147.75±1.11#	55.00±1.30#	29.75±0.85	20.25±0.85#

Table 4.11 Changes in serum lipid profile following 60 days of treatment with cadmium(Units: 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*
Cadmium (Cd)	102.00±1.91@	112.50±1.04@	52.00±1.08@	22.50±1.04@	28.00±0.91@
Cd+Melatonin (Cd+Mel)	108.25±0.85	142.25±0.85#	49.25±1.55#	25.75±0.85	35.50±0.65#

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

Table 4.12 Changes in hematological parameters following Cd treatment for 15 days

	Leucocyte Count	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Control (Con)	8275.00±85.39	1025500.00±70.83	19.00±0.91	76.00±0.41	1.25±0.25	2.25±0.25
Melatonin (Mel)	8475.00±110.81	1025500.00±1040.83	21.25±1.25	79.25±0.25	1.25±0.25	2.00±0.00
Cadmium (Cd)	7317.00±12.07@	3405950.00±165.20@	18.50±0.65	78.50±0.65	1.00±0.00	2.75±0.48
Cd+Melatonin (Cd+Mel)	7807.25±4.92#	1304100.00±147.20#	25.25±1.25#	71.00±0.91#	2.00±0.41	1.75±0.25

Table 4.13 Changes in hematological parameters following Cd treatment for 30 days

	Leucocyte Count	Platelet Count	Polymorph	Lymphocyte	Eosin	Monocyte
Control (Con)	7175.00±125.00	1302075.00±70.08	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
Cadmium (Cd)	6897.75±3.33@	1196025.00±62.92@	20.50±0.65	79.00±0.85	0.25±0.25	2.00±0.00
Cd+Melatonin (Cd+Mel)	7608.75±7.74#	1003075.00±85.40#	20.50±0.65#	88.75±0.85#	0.25±0.25	1.00±0.00

Table 4.14 Changes in hematological parameters following Cd treatment for 60 days

	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
Cadmium (Cd)	6317.00±13.90@	1050000.00±195.20@	43.43±0.91@	53.50±1.05@	1.00±0.20	3.75±0.48
Cd+Melatonin (Cd+Mel)	7905.58±4.92#	1374100.00±189.20#	39.58±0.98#	57.45±0.78#	1.50±0.56	2.75±0.25

PLATE I

Fig 4.8 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 4.9 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 4.10 Photomicrograph of 15 day cadmium treated kidney showing Bowman's capsule with glomerulus and distal tubules (400X). Note the disruptions in the Bowman's capsular epithelium and glomerulus(arrows).

Fig 4.11 Photomicrograph of 15 day cadmium+melatonin treated kidney(400X). Near normal appearance of the Bowmann 's capsule. BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

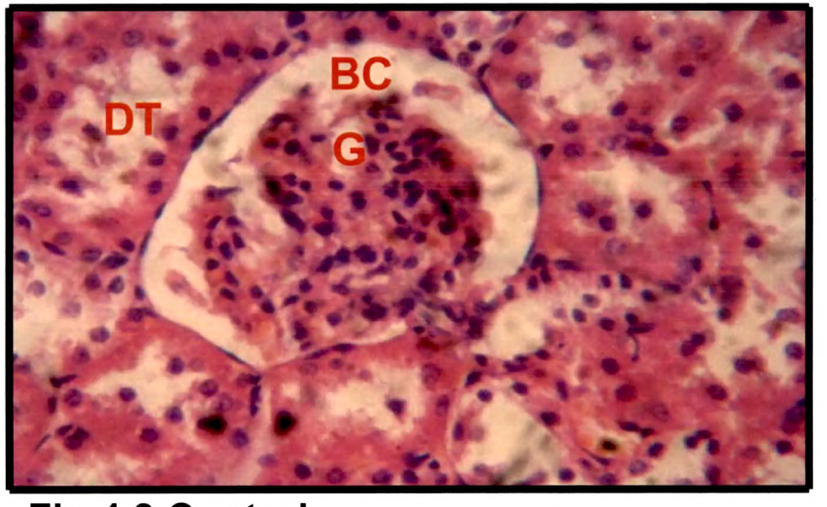

Fig 4.8 Control

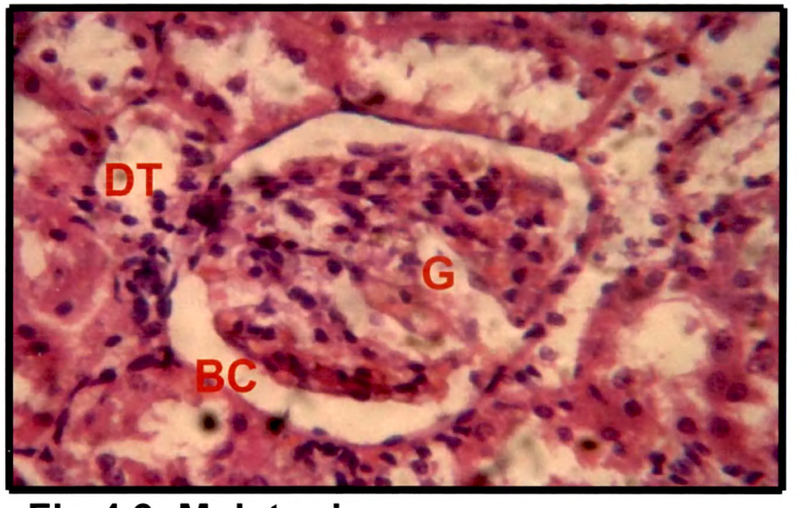

Fig 4.9 Melatonin

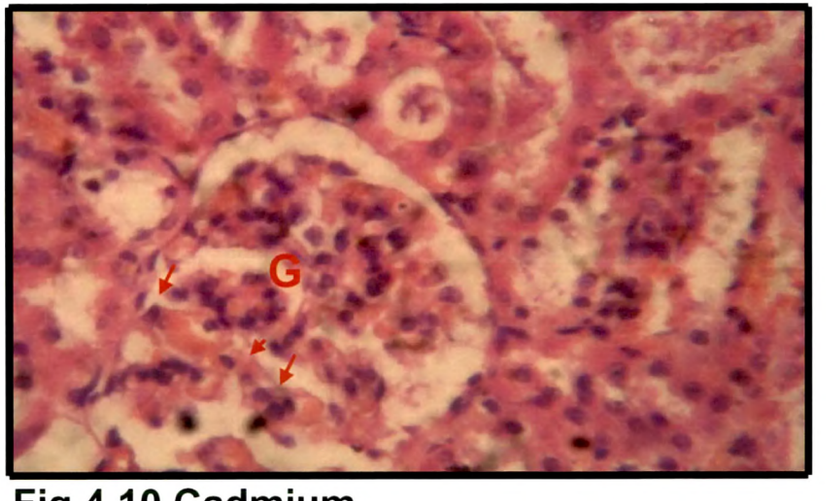

Fig 4.10 Cadmium

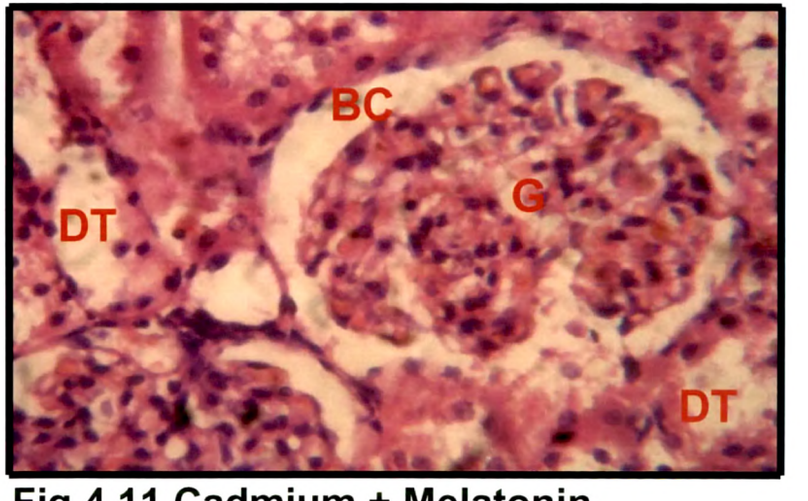

Fig 4.11 Cadmium + Melatonin

PLATE II

Fig 4.12 Photomicrograph of control kidney showing proximal tubules (400X), PT- Proximal tubule

Fig 4.13 Photomicrograph of kidney exposed to melatonin for 15 days showing proximal tubules (400X). PT- Proximal tubule

Fig 4.14 Photomicrograph of kidney exposed to cadmium for 15 days showing proximal tubules (400X).

Fig 4.15 Photomicrograph of kidney exposed to cadmium+melatonin for 15 days showing near normal appearance of PT (400X).

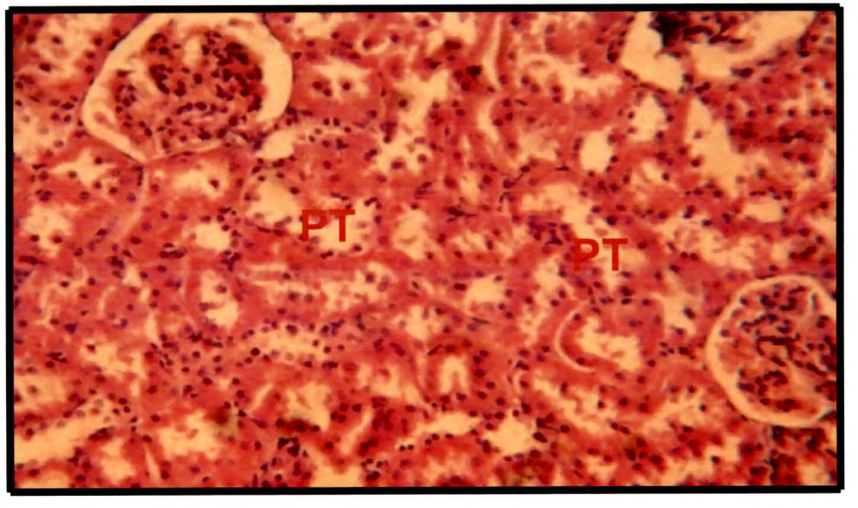

Fig 4.12 Control

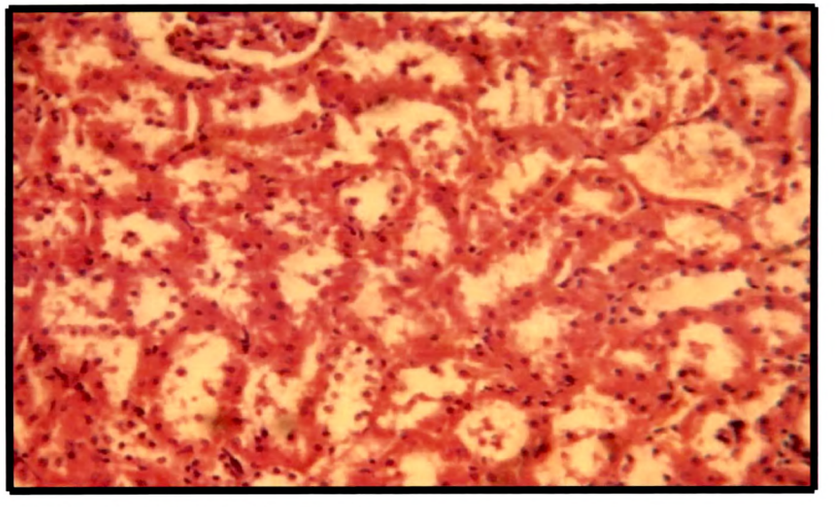

Fig 4.13 Melatonin

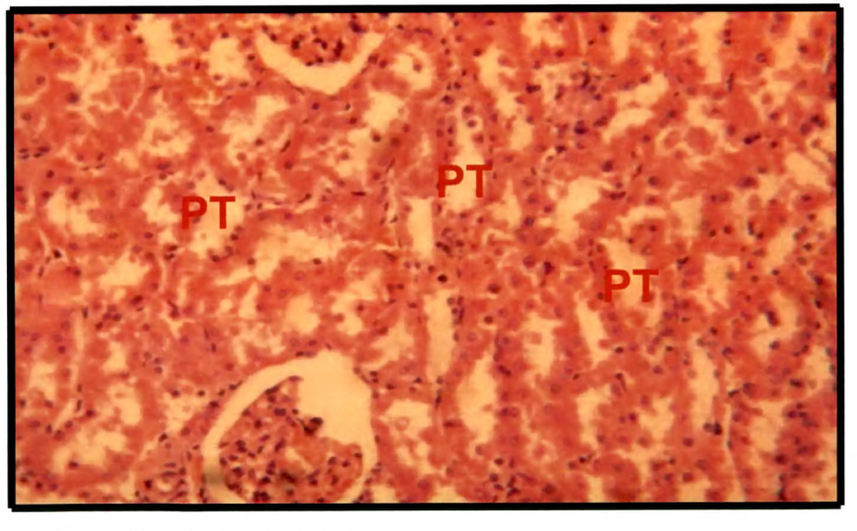

Fig 4.14 Cadmium

Fig 4.15 Cadmium + Melatonin

PLATE III

Fig 4.16 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 4.17 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 4.18 Photomicrograph of kidney exposed to cadmium for 30 days showing Bowman's capsule with glomerulus and distal tubules (400X). Note the disrupted Bowman's capsular epithelium (arrow), shrinkage and degeneration of glomerulus. BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 4.19 Photomicrograph of kidney exposed to cadmium+melatonin for 30 days showing normal looking Bowman's capsule and glomerulus (400X)

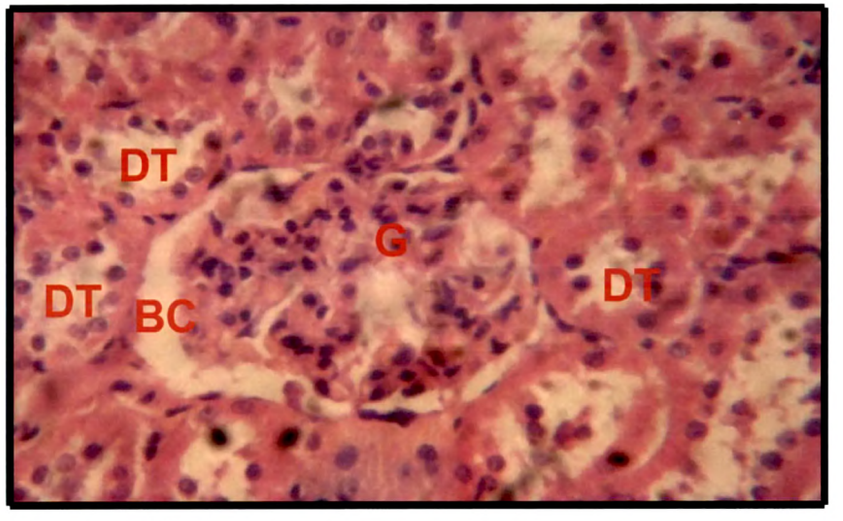

Fig 4.16 Control

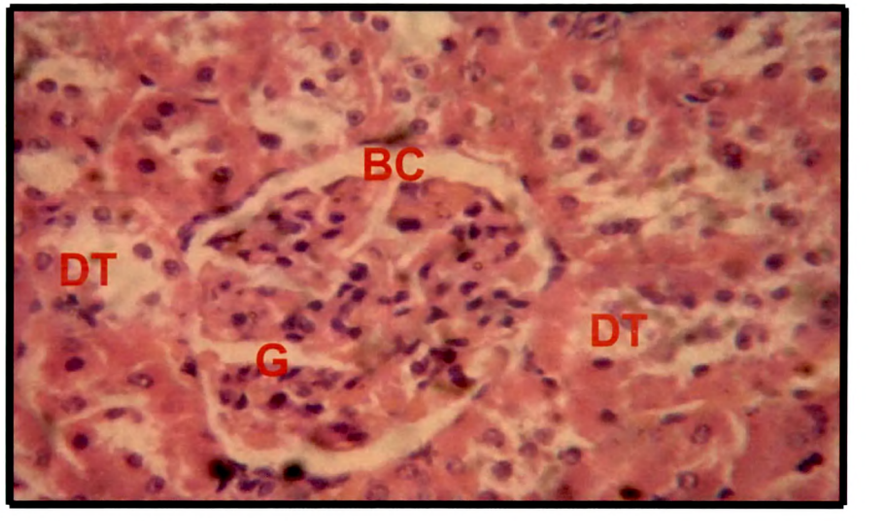

Fig 4.17 Melatonin

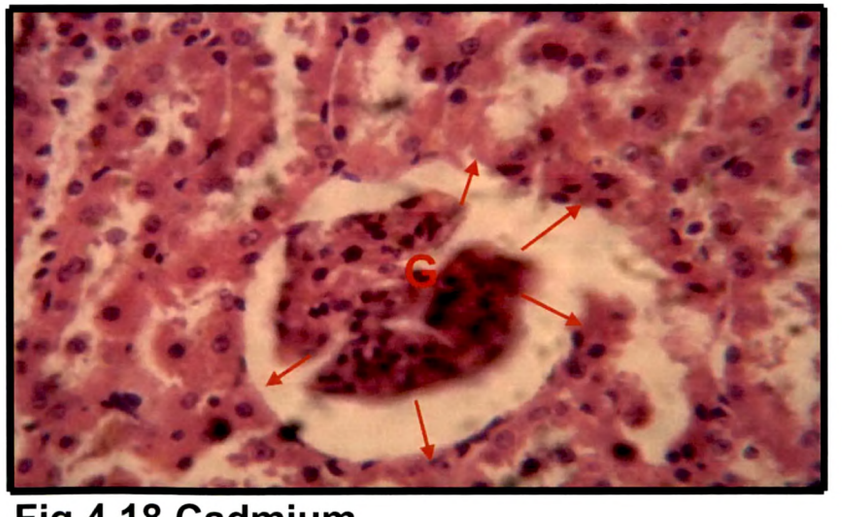

Fig 4.18 Cadmium

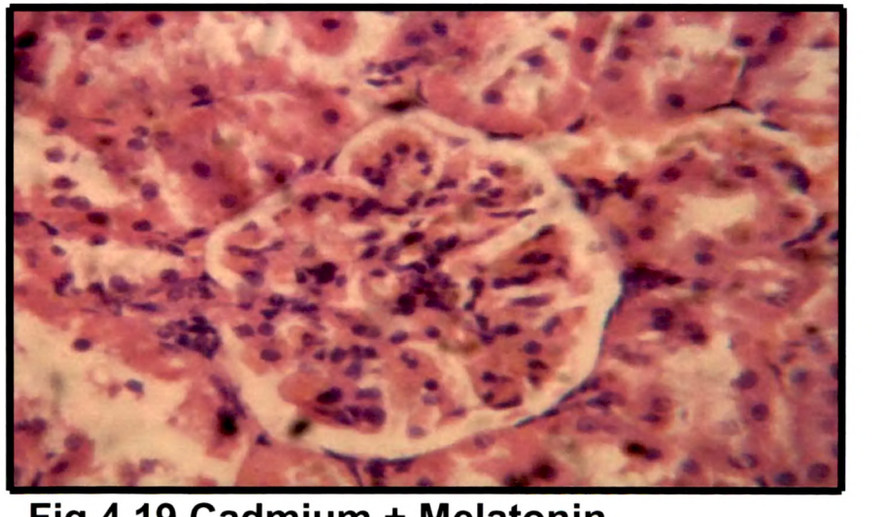

Fig 4.19 Cadmium + Melatonin

PLATE IV

Fig 4.20 Photomicrograph of 30 day control kidney showing proximal tubules (400X), PT- Proximal tubule.

Fig 4.21 Photomicrograph of kidney exposed to melatonin for 30 days showing proximal tubules (400X).

Fig 4.22 Photomicrograph of kidney exposed to cadmium for 30 days showing proximal tubules (400X).Note the disturbed proximal tubules with rampant apoptotic tubular cells PT- Proximal tubule

Fig 4.23 Photomicrograph of kidney exposed to cadmium+melatonin for 30 days showing near normal proximal tubules (400X). PT- Proximal tubule

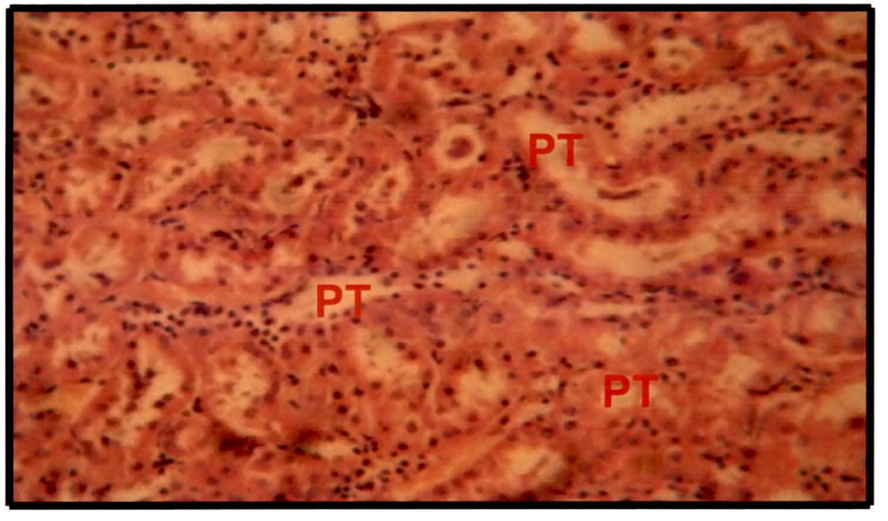

Fig 4.20 Control

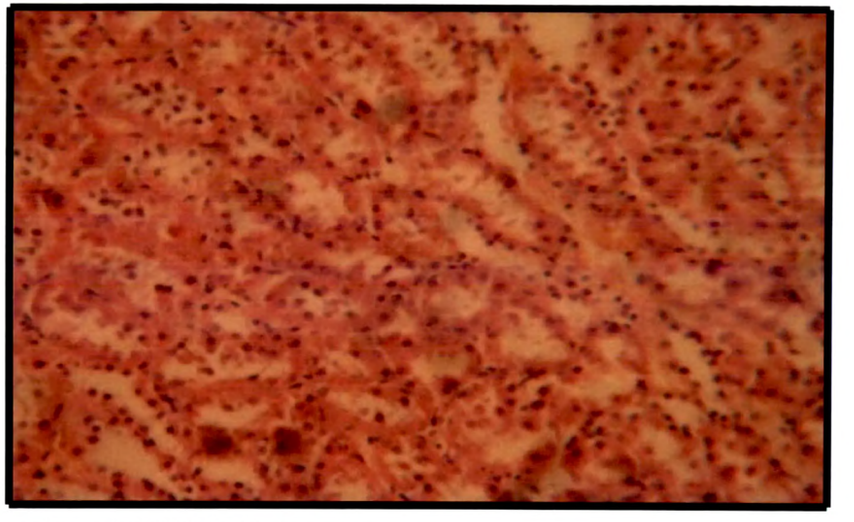

Fig 4.21 Melatonin

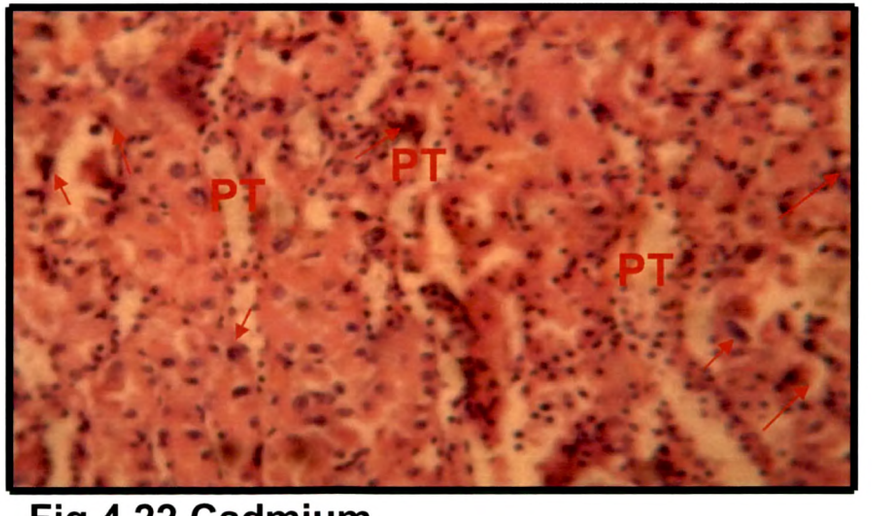

Fig 4.22 Cadmium

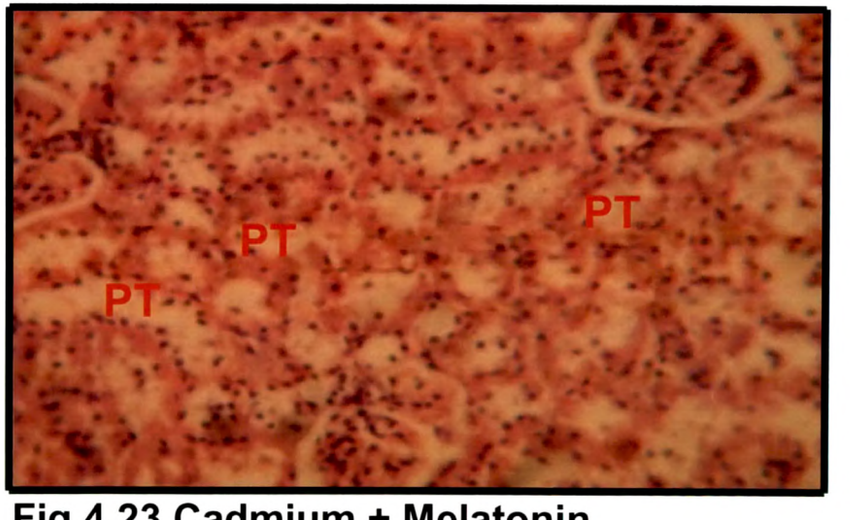

Fig 4.23 Cadmium + Melatonin

PLATE V

Fig 4.24 Photomicrograph of 60 day control kidney showing a Bowman's capsule with glomerulus and distal tubules (400X). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 4.25 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 4.26 Photomicrograph of kidney exposed to cadmium 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). BC-Bowman's Capsule; DT- Distal tubule; G- Glomerulus

Fig 4.27 Photomicrograph of kidney exposed to cadmium+melatonin for 60 days showing normal looking Bowman's capsule and minor effects on glomerulus(400X). G- Glomerulus

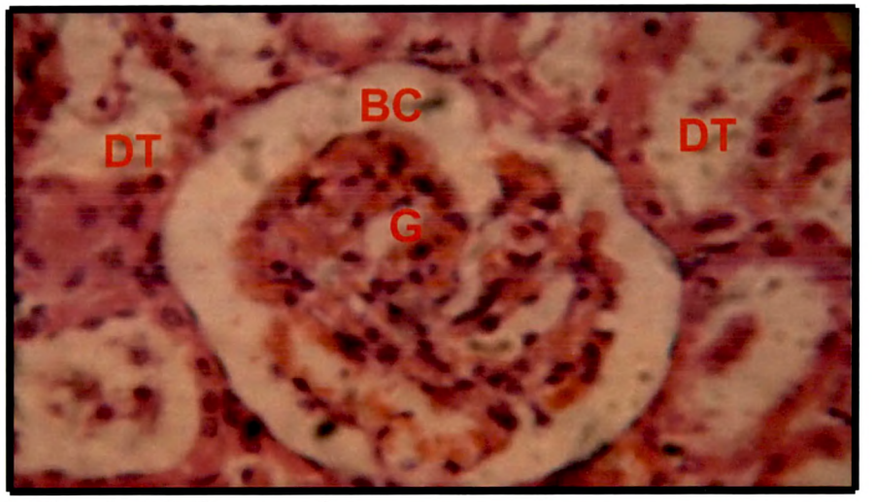

Fig 4.24 Control

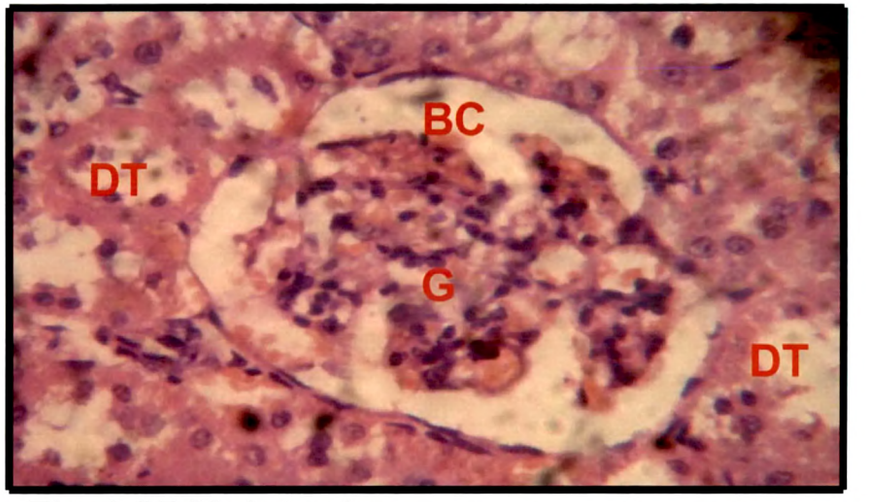

Fig 4.25 Melatonin

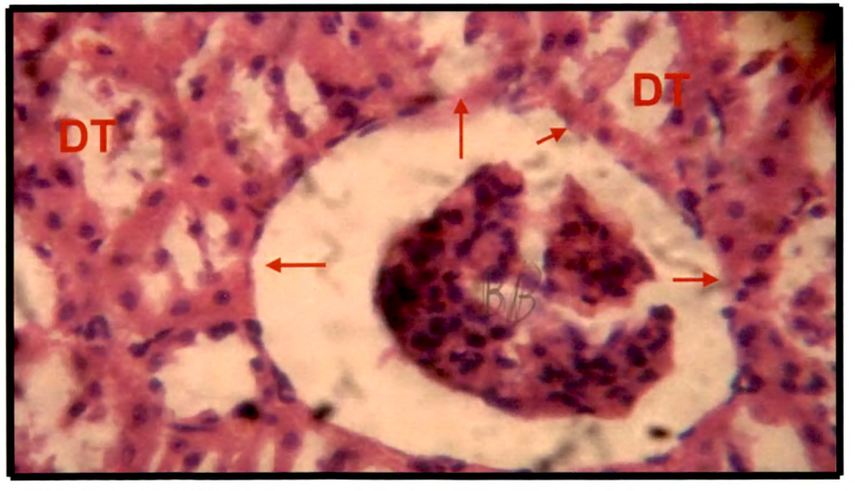

Fig 4.26 Cadmium

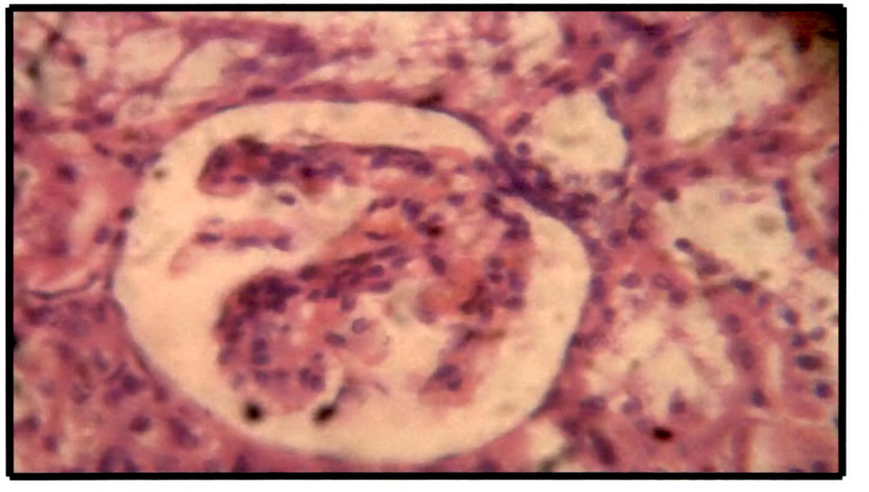

Fig 4.27 Cadmium + Melatonin

PLATE VI

Fig 4.28 Photomicrograph of control kidney showing proximal tubules (400X).

Fig 4.29 Photomicrograph of kidney exposed to melatonin for 60 days showing proximal tubules (400X).

Fig 4.30 Photomicrograph of kidney exposed to cadmium for 60 days showing proximal tubules (400X). Note the degeneration of proximal tubular epithelial cells.

Fig 4.31 Photomicrograph of kidney exposed to chromium+melatonin for 60 days showing normal proximal tubules (400X).

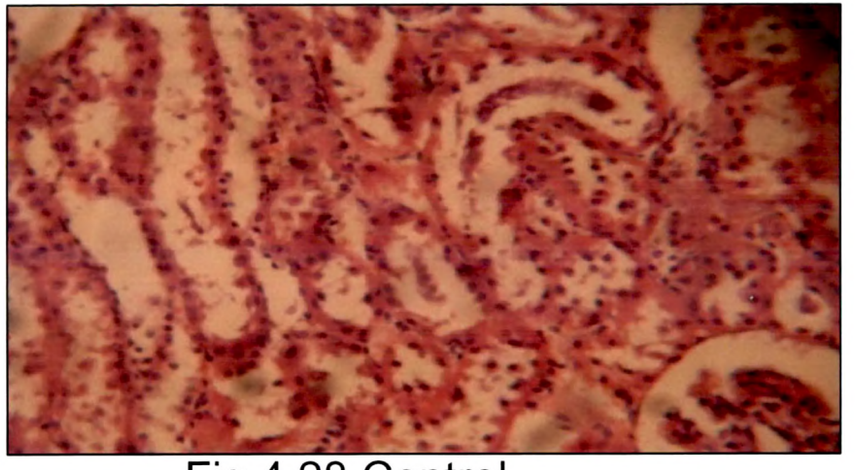

Fig 4.28 Control

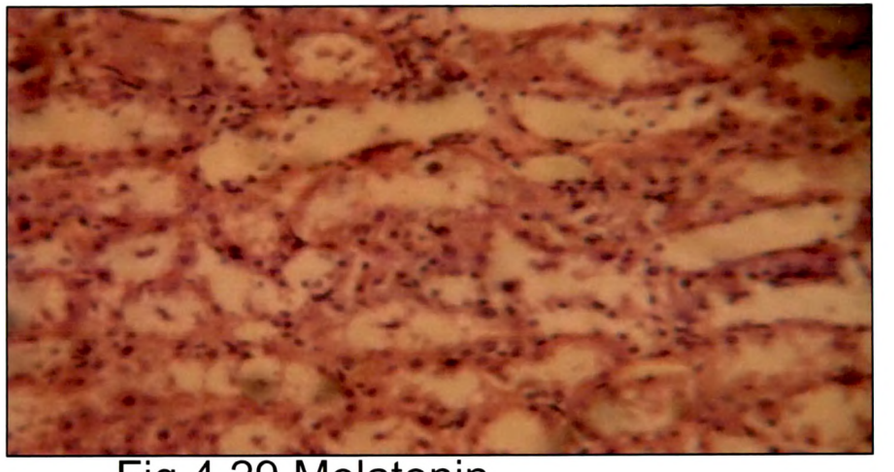

Fig 4.29 Melatonin

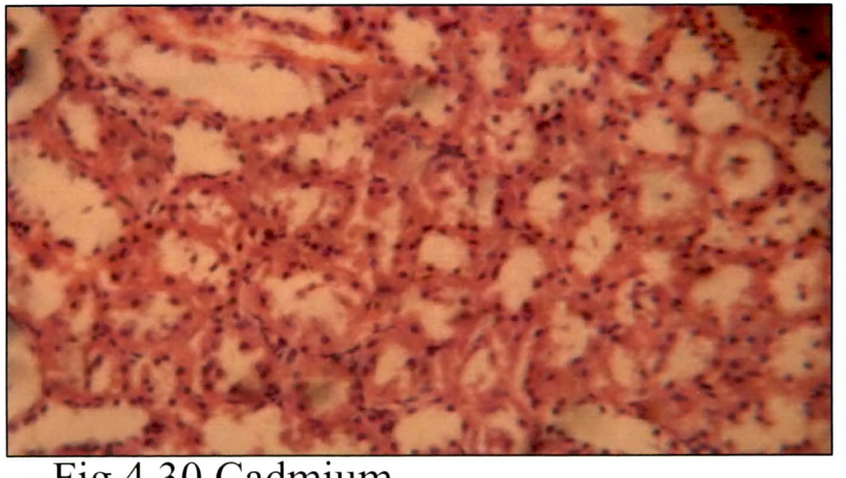

Fig 4.30 Cadmium

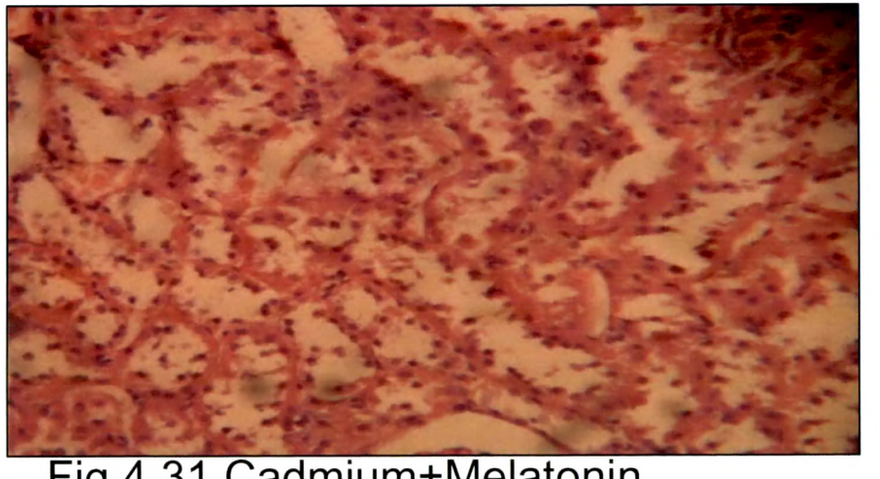

Fig 4.31 Cadmium+Melatonin

Discussion:

The present study undertaken to evaluate the renal responses to a chronic oral exposure to cadmium has shown induction of oxidative stress and toxicity manifestations. Decreased content of non-enzymatic antioxidant (GSH and Vit C) and activity of enzymatic antioxidants (SOD, CAT, GPx) with markedly increased LPO levels are indicative of Cd induced renal oxidative stress. The increasing level of LPO with duration (maximal at 60 days) suggests absence of any adaptive/protective mechanism to stem the oxidative damage unlike the hepatic tissue which showed an adaptive/protective mechanism (Chapter 3). Reduced non-enzymatic antioxidants and inhibited enzymatic antioxidants are quite in tune with the increase in LPO level. Increased LPO level and decreased endogenous antioxidant status are characteristic of Cd induced stress in a wide range of organs. Most of the studies with Cd are short term ranging from hours to days or even a single dose acute administration (El-Maraghy *et al.*, 2001; Casalino *et al.*, 2002; Eybl *et al.*, 2004, 2006). The present study involving short (15 days), medium (30 days) and long (60 days) duration of oral Cd exposure has revealed maximal oxidative stress, as marked by LPO, to be at the longest duration of exposure. The gradual increase in LPO can be correlated with the parallel increase in the renal metal load through 30 to 60 days. For the all the three periods of Cd exposure, there is a gradual depletion of GSH and Vit C along with decreased activities of enzymatic antioxidants. Clearly, the renal tissue shows greater Cd toxicity on long duration exposure unlike liver which manifests the same in the early phase of Cd exposure (Chapter 3).

Cadmium is known to be bound to metalothionein and stored as cadmium-metalothionein (Cd-MT) complex in the liver. Due to Cd intoxication, the damaged

liver cells release these complexes into circulation and are filtered through the glomerulus into the urinary space where it is endocytosed by the proximal tubular cells and degraded by the lysosomes resulting in the release of free cadmium (Morales *et al.*, 2006). This free Cd may then stimulate the production of metallothionein in proximal tubular cells (Sendelbach and Klaassen, 1988). The release of free Cd from Cd-MT complex and its uptake leads to the renal oxidative stress and toxicity. It is already deduced previously (Chapter 3) that, subsequent to increased oxidative stress by iron mediated Fenton reaction in the liver in the initial 15 days of exposure, an adaptive induction of metallothionein and iron binding proteins occurs to stem the oxidative stress as an adaptive mechanism. Obviously, this leads to formation of more Cd-MT complex which gets transported to the kidney thereby leading to increasing Cd stress of the renal tissue on continued and protracted exposure.

Cadmium has an affinity to bind to sulphydryl group and hence Cd is known as an intracellular depleter of GSH (Stohs, 2000). Decreasing GSH level from 15 to 60 days of cadmium intoxication is justifiable in this context. Significantly increasing inhibition of both SOD and GPx seen at 30 and 60 days of Cd exposure is correlatable with the ability of Cd to displace Zn from Cu-Zn SOD and Se from GPx to form Cu-Cd SOD and Se free GPx, the inactive forms of these two enzymes. Obviously, the decreasing level of GSH and GSH:GSSG ratio together with inactivated SOD and GPx compromises the efficiency of the renal endogenous antioxidant machinery. This leads to an upset in the pro-oxidant-antioxidant status of the renal tissue leading to an apparently augmented oxidative stress contributed to by the free radicals generated abnormally during oxidative metabolism and functioning of the organism. Further, the released Zn together with any iron (Fe^{3+} to Fe^{2+}) that may also be released

from bound proteins of the membrane can lead to more oxidative stress through the Fenton reaction (Valko *et al.,* 2005). Apparently, this compromised pro-oxidant antioxidant state and increased oxidative stress are also reflected in the inhibition of CAT and depletion of Vit C.

Cytotoxicity is clearly indicated by the histopathological alterations seen in the renal tissue. Progressive degeneration or shrinkage of the glomerulus, disruption in the continuity of Bowmann's epithelium, vacuolization, hemorrhage and swelling of tubules and proximal tubule cellular damage were the cytotoxic features induced by Cd treatment. These changes are prominently manifested by 60 days of Cd exposure. Lesions involving tubular necrosis, inflammatory cell infiltration, tubular degeneration, hemorrhage, swelling of tubules and vacuolization have all been shown in the kidney of rats treated with 5mg/kg BW of Cd for 4 weeks (Renugadevi, 2009). Aughey *et al.* (1984) showed tubular necrosis, interstitial fibrosis and glomerular epithelial cell hypertrophy in small areas of kidney cortex after 12 weeks of exposure to 50 mgCd/l through drinking water. There is, in the present study, progressive shrikage of glomerulus with increasing tubular necrosis and vacuolization in the sections of kidney of rats exposed to Cd for 60 days. These histopathological alterations could be due to the free radical induced damage and, as such, functional damage in the form of inhibiton Na^+K^+ATPase has (have) been documented (Stajn *et al.*, 1997).

Serum urea and creatinine used as indicators or diagnostic features of renal toxicity has shown gradual increase with increasing duration of Cd exposure. Urea is a major nitrogen-containing metabolic product of protein metabolism and it is well established that Cd inhibits amino acid incorporation into proteins leading to an increase in the

urea level (Davies, 1991). There is also an increase in serum creatinine levels in the Cd treated animals though not statistically significant. Increased creatinine levels reflect a condition of renal failure.

Other important hematological alterations occurring as a consequence of Cd toxicity are slightly reduced leucocyte count, significantly reduced platelet count and, decreased HLDL with increased LDL and VLDL. Hypertriglyceridemia is also a feature of Cd toxicity. These changes suggest that Cd can systemically alter the relative proportions of cholesterol transport proteins in duration dependent manner and, accordingly there is an increase in the so called bad cholesterol (LDL, VLDL) decreasing at the same time the good cholesterol (HDL); changes which portend development of atherosclerosis and cardiovascular problems. There are reports in this context on Cd induced hypertension and cardiovascular effects. The present observation on platelet count also tends to suggest the development of a hypercoagulatory state due to Cd intoxication and as such, there is just one report suggesting reduced thrombocytes, decrease in protein C and antithrombin activity together with shortening of prothrombin time and activated partial thromboplastin time (Kosak and Aksil, 2006).

In the light of melatonin's role as a powerful natural antioxidant, simultaneous supplementation of melatonin in Cd intoxicated animals has shown significant protection against LPO and endogenous levels of antioxidants. The possible mechanisms of action of melatonin could be in the form of direct or indirect scavenging of free radicals (due to its ability to freely saturate intracellular compartments), form a complex with Cd leading to its disposal and induce the synthesis of antioxidant enzymes (Rodriguez *et al.*, 2004; Carolina *et al.*, 2008).

Melatonin's role in preventing cell damage and apoptosis is clearly seen by the near normal histological architecture of renal tissue. Melatonin supplementation along with Cd, is able to nullify to a greater degree the cytotoxic effects of the metal and is supported by the near normal serum levels of urea and creatinine. Further, the changes in cholesterol transport proteins and platelet count are also well resisted by melatonin. This inferred role of melatonin in controlling oxidative stress and cytotoxicity is clearly emphasized by the herein observed decrement in circulating melatonin levels in Cd exposed groups of rats. The increasing degree of oxidative stress and cytotoxicity with increased duration of Cd exposure is paralleled by duration dependent significant decline in serum melatonin titre. The present observation suggests that melatonin is one of the few antioxidants that has shown ability to reduce the metal load of renal tissue while, other antioxidants have failed to show such an effect.

Overall, the present study suggests a duration dependent effect of Cd on increased renal oxidative stress and cytotoxicity along with alterations in serum urea and creatinine levels. Further, melatonin is found to be very effective in counteracting the negative effects of Cadmium.

Summary of Chapter 4

The present study was essentially undertaken to decipher the oxidative stress and toxic effect induced by Cadmium (Cd) on renal tissue in male *Wistar* rats exposed to a realistic dose of Cd through drinking water in keeping with the levels of these metals found in the environment for a duration of 15, 30 and 60 days. Decreased content of non-enzymatic antioxidants (GSH and Vit C) and activity of enzymatic antioxidants (SOD, CAT, GPx) with markedly increased LPO levels are indicative of Cd induced renal oxidative stress. The duration dependent increasing level of LPO suggests absence of any adaptive/protective mechanism. The gradual increase in LPO can be co-related with the parallel increase in the renal metal load through 30 to 60 days. For, the all three periods of Cd exposure, there is a gradual depletion of GSH and Vit C along with decreased activities of enzymatic antioxidants. Clearly, the renal tissue shows greater Cd toxicity on longer duration of exposure. Progressive degeneration/shrinkage of the glomerulus, vacuolization, hemorrhage and swelling of tubules and, proximal tubule cellular damage were the cytotoxicity features induced by Cd treatment. The histopathological alterations were maximally seen at 60 days of Cd exposure. Cadmium induced fall in leucocyte count, significantly reduced platelet count and hypertriglyceridemia ware also evident. All these changes were brought to a near normal state with co-administration of melatonin. Overall, the present study suggests a duration dependent effect of Cd on increased renal oxidative stress and cytotoxicity with melatonin being very effective in counteracting the negative effects of cadmium.