Chapter: 1 Chromium (VI) induced oxidative stress in Testes and Epididymis: Realistic dosage and duration dependent study and, role of Melatonin

Introduction:

Chromium, one of the heavy metals, exist in many oxidation states ranging from $Cr(II^-)$ to $Cr(VI^+)$, making its chemistry more complicated. Chromium mainly occurs in the environment as two valence states *i.e.* Cr(III) and Cr(VI) (Bagchi *et al.*, 2002; Chandra *et al.*, 2007). Compared to Cr(VI), Cr(III) is relatively less toxic and also an essential metal, influencing glucose and fat metabolism, while Cr(VI) is more toxic and carcinogenic (Anderson *et al.*, 1997; Qi *et al.*, 2000). The deleterious effects of hexavalent chromium are attributed to active cellular uptake via SO_4^{2-} and HPO_4^{2-} channels while Cr(III) fails to enter into cells. (Nobuyuki *et al.*, 1997; Codd *et al.*, 2001).

Cr(VI) is extensively used in more than 50 different industries and is the major form of chromium pollutant due to anthropogenic activities (Barcelous *et al.*, 1999). Occupational exposure to Cr(VI) occurs in workers engaged in industries such as welding, chromate production, chrome plating, ferrochrome, cement, rubber, candle, battery, paints, pigments etc. (ATSDR, 2000). Moreover, in general, heavy metals are devoid of biodegradable properties and can remain in the ecosystem for a long time (Kelly *et al.*, 2004). Man and animals could be exposed to hexavalent chromium

compounds via air, drinking water, food, through dermal contact or by occupational exposure and are the major environmental contaminants (Anon, 1990).

After entering into the cell, Cr(VI) gets reduced to stable oxidation state Cr(III) via two intermediate oxidation states *i.e.* Cr(V) and Cr(IV) (De Flora *et al.*, 1990). This process of reduction of Cr(VI) to Cr(III) is carried out by intracellular reductants like thiols(GSH), ascorbate, cystein, NADPH, fructose, ribose, lipoic acid etc.(Rao *et al.*, 1993; Chen *et al.*, 1997; Liu *et al.*, 2001; Obrien *et al.*, 2001; Kasprazak, 2002). Further, these intermediates react with other intracellular molecules, like H_2O_2, to add more hydroxyl free radicals to the pool of reactive oxygen species (Stearns *et al.*, 1997). Even, Cr(III) complexes can act as cyclic electron donors in Fenton chemistry and generate hydroxyl radical, the most notorious of all the ROS (Sugden *et al.*, 1992; Hong Li *et al.*, 2001). Above findings are in agreement with the work of Stohs *et al.* (2001) suggesting generation of heavy bulk of reactive oxygen species (ROS) from Cr(VI) leading to significant oxidative stress.

Excess ROS production leading to increased oxidative stress could cause damage to intracellular molecules such as proteins, carbohydrates and lipids including enzymes and hereditary material (Bindu and Annamali, 2004). Relatively high content of polyunsaturated fatty acids(PUFA) makes testicular tissue a prime target for metal induced oxidative stress leading to lipid peroxidation (Acharya *et al.*, 2004). Cells have a pool of enzymatic and non-enzymatic antioxidants to protect themselves against oxidative stress (Gibanananda and Syed, 2002), and to maintain equilibrium between pro-oxidants and antioxidants (Jamieson, 1989). Non-enzymatic antioxidants like low molecular weight peptide GSH, Vit. A, Vit. C, Vit. E and, enzymatic

antioxidants like Superoxide Dismutase (SOD), Catalase (CAT), Glutathione Peroxidase (GPx), Glutathione Reductase (GR), etc. plays key roles to counteract the deleterious effects caused by excessive generation of free radicals by environmental pollutants, UV-radiation or due to any pathological disorder. Elevated levels of reactive oxygen species and subnormal levels of antioxidants may cause damage to cell membrane, cellular protein and nucleic acids which may lead to cell death, followed by tissue/ organ dysfunction (Maritim *et al.*, 2003) ultimately resulting in under performance of organ systems.

Melatonin, a pineal hormone, until 1993, was known as a molecule responsible for maintaining the circadian and circannula rhythms of the animal body. Tan *et al.* (1993) using a combination of spin trapping and electron resonance spectroscopy (ESR) established the role of pineal hormone, melatonin, as a powerful hydroxyl radical scavenger (Pandi-Perumal *et al.*, 2006) and a number of *In Vivo* and *In Vitro* studies have demonstrated it to be a powerful antioxidant. Moreover, it is more effective than vitamin E in protecting against the damage caused due to the peroxyl radical ($LOO^{\bullet}$) (Pieri *et al.*, 1994). The highly lipophilic and somewhat hydrophilic properties of this indole facilitate its penetration into all tissues and subcellular compartments. It is also well documented that melatonin can increase the activity of antioxidant enzymes (Reiter *et al.*, 2000).

Deleterious effect of Cr(VI) on different organ system in different animal models has been suggested by many studies (ATSDR, 2000). However, only few studies are available regarding male reproductive toxicity of hexavalent chromium compounds. Occurrence of Cr in the testis and its conversion from hexavalent to

trivalent oxidation state is well reported (Sipowicz *et al.*, 1997; Sutherland *et al.*, 2000). Studies on Swiss mice have shown increased lipid peroxidation, oxidative stress and reduced antioxidant titre in the testes by CrO_3 treatment (Acharya *et al.*, 2006). Chromium(VI) has also been shown to decrease antioxidant enzyme activity of seminal plasma reflecting the disturbed functions of male accessory sex glands (Li *et al.*, 2001).

Since, chromium is a common pollutant present in significant amount in the environment of Vadodara, we have tried to assess duration dependent reproductive toxicity of Cr(VI) by assessing (i.e. 15, 30 and 60 Days of treatment) oxidative stress status of testes and epididymis. Concurrently, the possible protective effect of melatonin against Cr(VI) induced oxidative stress has also been studied.

Material and Method:

For treatment, methodology employed and protocols refer material and methods section (Page no. 17)

Results:

Chromium(VI) treatment showed duration dependent increase in testicular lipid peroxidation when compared with the control. Simultaneous administration of melatonin significantly minimized lipid peroxidation (Figure: 1.1, Table: 1.1) without any duration dependent effect.

Epididymis also showed increased lipid peroxidation (Figure: 1.2, Table: 1.2) with chromium treatment without any duration dependent effect. However, co-administration of melatonin along with Cr(VI) significantly reduced lipid peroxidation levels with largest duration chromium exposure having minimal protective effect.

In the testes of rats treated with Cr(VI), there was significant duration dependent decrease in GSH (Figure: 1.3, Table: 1.3) and ascorbic acid contents. Simultaneous treatment with melatonin exerted significant protective effect on the depletion of the both non enzymatic antioxidants. Though there was a duration dependent increment in protective effect on testis ascorbic acid content (Figure: 1.5, Table: 1.5) the longer duration treatment tended to show a decreased protective effect on GSH content.

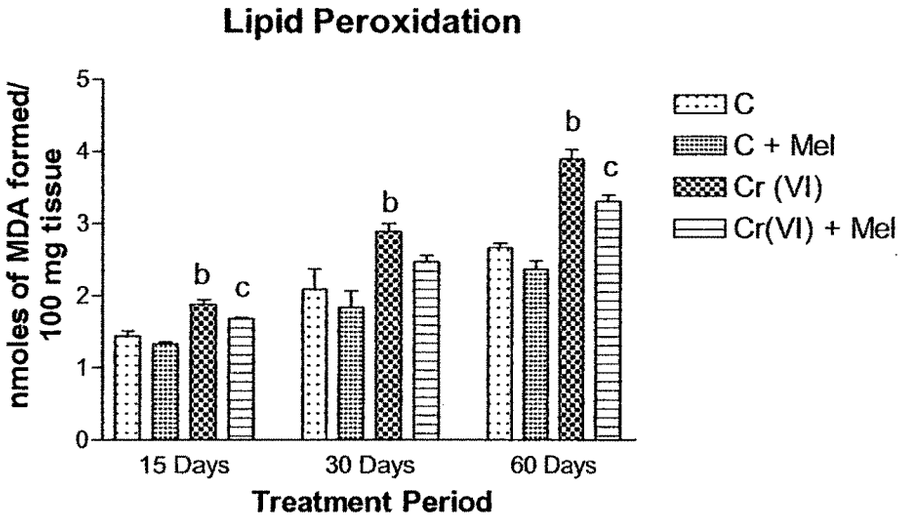
Though the epididymal ascorbic acid content showed a similar duration dependent decrement with Cr treatment and increment in protective effect with

melatonin (Figure: 1.6, Table: 1.6), there was no significant duration dependent effect on epididymal GSH content (Figure: 1.4, Table: 1.4).

Exposure to chromium showed significant non-linear decrease in both testis (Figure: 1.9, Table: 1.9) and epididymal (Figure: 1.10, Table: 1.10) SOD and catalase activity. Concurrent melatonin administration also showed significant non-linear protective effect.

Decrement in GPx activity of testis was minimal with chromium exposure (Figure: 1.11, Table: 1.11), though the longer duration treatment had a significantly greater effect. Protective effect of concurrent administration was also significantly lower on a preventive basis. The epididymal GPx activity was however significantly affected by chromium exposure and melatonin also showed better protective effect (Figure: 1.12, Table: 1.12), though both these effects were non-linear with the three duration of treatment. In contrast, GR activity of both testis (Figure: 1.13, Table: 1.13) and epididymis (Figure: 1.14, Table: 1.14) depicted a duration dependent linear decrement with chromium exposure, more pronounced in epididymis, and protective effect with simultaneous administration of melatonin.

Figure 1.1: Chromium(VI) induced Lipid peroxidation (LPO) levels in testis with or without Melatonin.



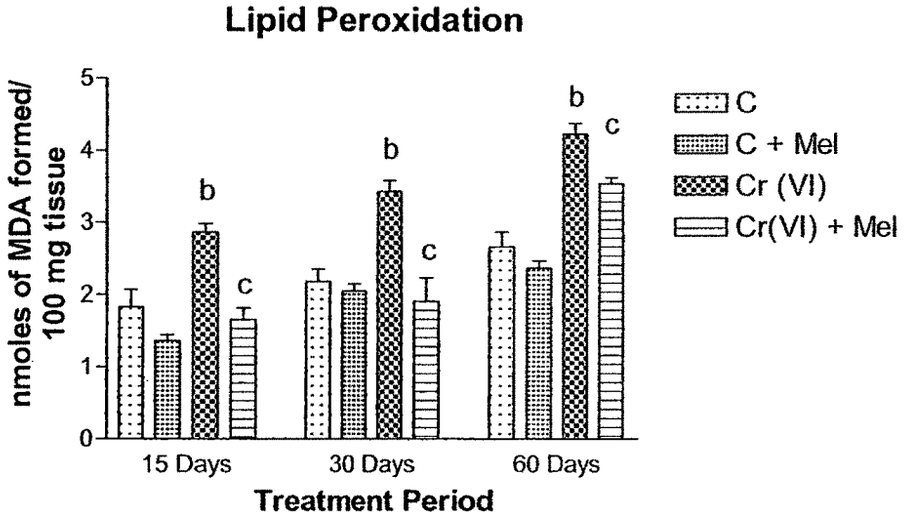
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.1: Chromium(VI) induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	1.44 ± 0.07	1.33 ± 0.03	1.88 ± 0.06[b]	1.68 ± 0.02[c]
30 Days	2.09 ± 0.16	1.84 ± 0.22	2.89 ± 0.11[b]	2.47 ± 0.09
60 Days	2.67 ± 0.06	2.37 ± 0.11	3.90 ± 0.13[b]	3.31 ±0.09[c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.2: Effect of Chromium(VI) induced Lipid peroxidation (LPO) levels in epididymis with or without Melatonin.



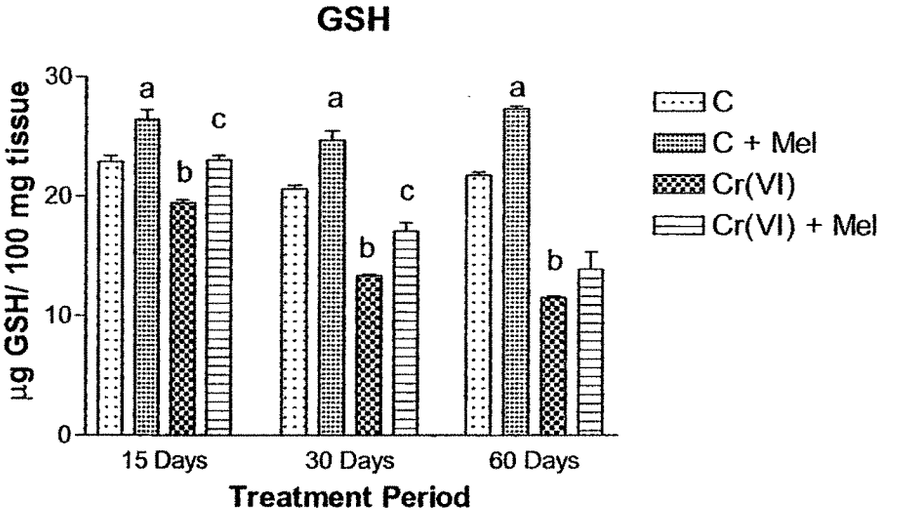
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.2: Chromium(VI) induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	1.83 ± 0.24	1.37 ± 0.08	2.87 ± 0.11 [b]	1.66 ± 0.16 [c]
30 Days	2.18 ± 0.17	2.05 ± 0.10	3.43 ± 0.15 [b]	1.91 ± 0.33 [c]
60 Days	2.66 ± 0.21	2.37 ± 0.10	4.23 ± 0.14 [b]	3.54 ± 0.09 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.3: Chromium(VI) induced Glutathione (GSH) levels in testis with or without Melatonin.



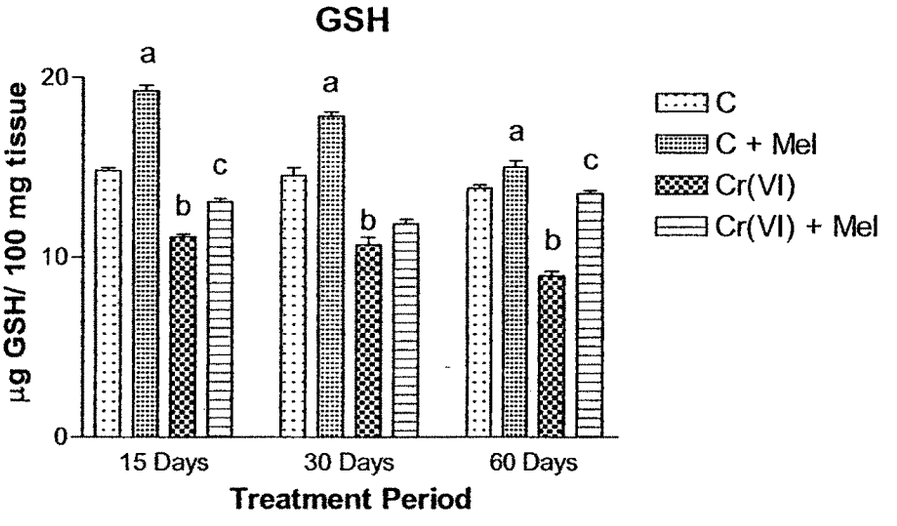
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.3: Chromium(VI) induced Glutathione (GSH) levels (µg GSH/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	22.89 ± 0.51	26.45 ± 0.80[a]	19.46 ± 0.22[b]	23.07 ± 0.38[c]
30 Days	20.66 ± 0.31	24.71 ± 0.81[a]	13.38 ± 0.14[b]	17.10 ± 0.72[c]
60 Days	21.80 ± 0.28	27.39 ± 0.23[a]	11.56 ± 0.12[b]	13.95 ± 1.46

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.4: Chromium(VI) induced Glutathione (GSH) levels in epididymis with or without Melatonin.



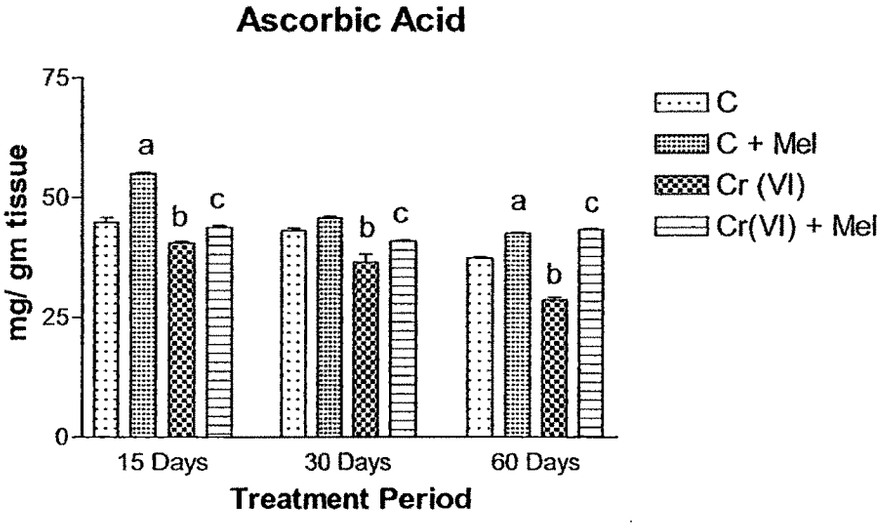
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.4: Chromium(VI) induced Glutathione (GSH) levels (µg GSH/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	14.83 ± 0.14	19.27 ± 0.33 [a]	11.14 ± 0.15 [b]	13.09 ± 0.19 [c]
30 Days	14.56 ± 0.46	17.87 ± 0.26 [a]	10.73 ± 0.39 [b]	11.87 ± 0.27
60 Days	13.86 ± 0.20	15.04 ± 0.37 [a]	8.98 ± 0.27 [b]	13.57 ± 0.17 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.5: Chromium(VI) induced Ascorbic Acid levels in testis with or without Melatonin.



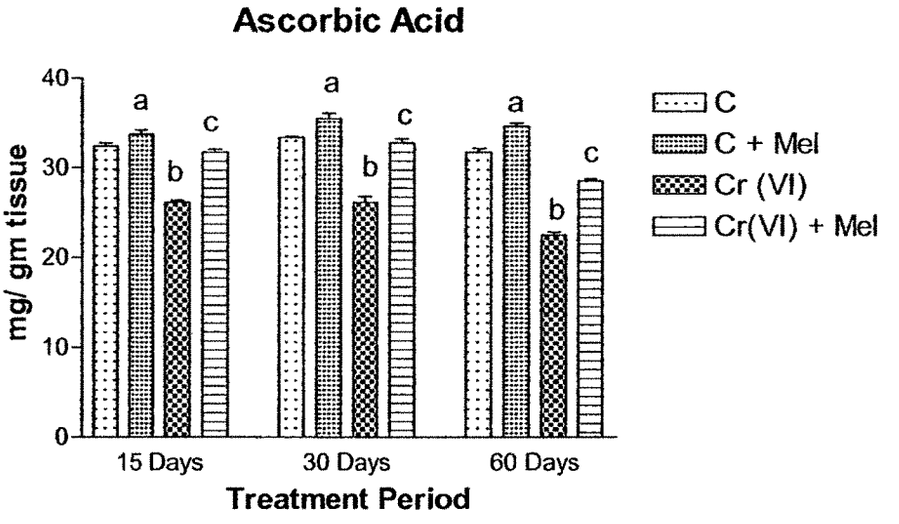
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.5: Chromium(VI) induced Ascorbic Acid levels (µg/ 100mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	44.87 ± 0.97	54.99 ± 0.26 [a]	40.53 ± 0.30 [b]	43.78 ± 0.46 [c]
30 Days	43.08 ± 0.51	45.73 ± 0.35	36.53 ± 1.69 [b]	40.94 ± 0.22 [c]
60 Days	37.41 ± 0.21	42.54 ± 0.14 [a]	28.64 ± 0.56 [b]	43.40 ± 0.19 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.6: Chromium(VI) induced Ascorbic Acid levels in epididymis with or without Melatonin.



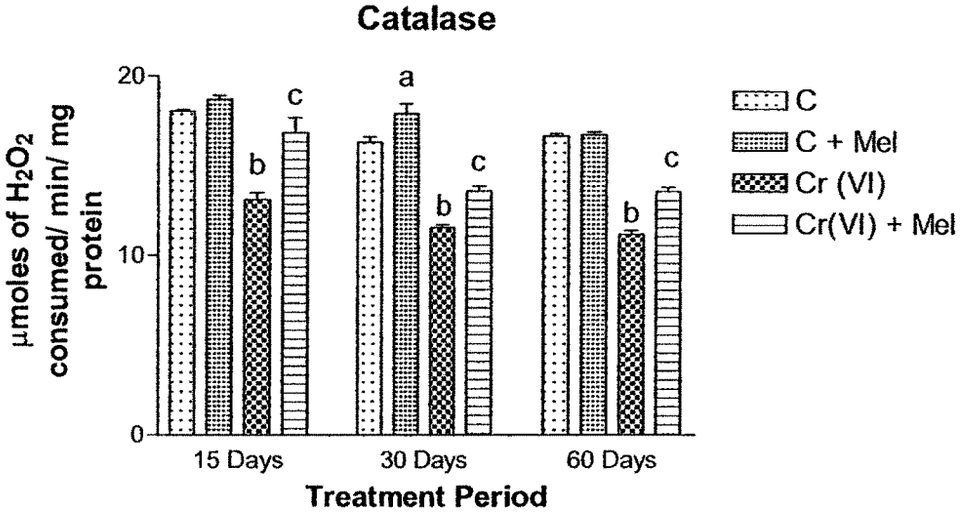
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.6: Chromium(VI) induced Ascorbic Acid levels (µg/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	32.38 ± 0.37	33.76 ± 0.44 [a]	26.15 ± 0.23 [b]	31.75 ± 0.34 [c]
30 Days	33.42 ± 0.10	35.47 ± 0.66 [a]	26.14 ± 0.68 [b]	32.77 ± 0.52 [c]
60 Days	31.76 ± 0.42	34.67 ± 0.34 [a]	22.50 ± 0.35 [b]	28.56 ± 0.24 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.7: Chromium(VI) induced Catalase (CAT) activity in testis with or without Melatonin.



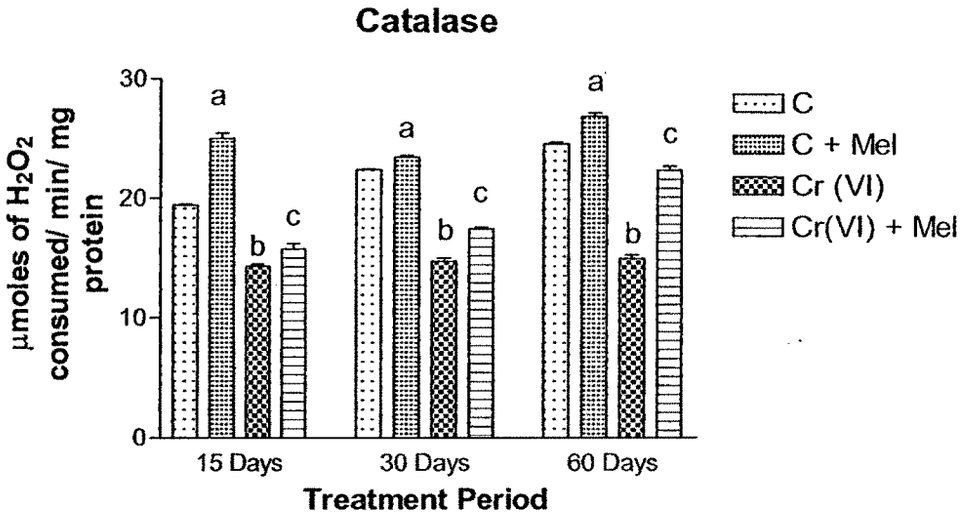
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.7: Chromium(VI) induced Catalase (CAT) activity (µmoles of H_2O_2 consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	18.05 ± 0.07	18.70 ± 0.25	13.12 ± 0.39 [b]	16.86 ± 0.83 [c]
30 Days	16.33 ± 0.28	17.93 ± 0.54 [a]	11.56 ± 0.17 [b]	13.61 ± 0.28 [c]
60 Days	16.68 ± 0.13	16.74 ± 0.16	11.20 ± 0.23 [b]	13.58 ± 0.23 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.8: Chromium(VI) induced Catalase (CAT) activity in epididymis with or without Melatonin.



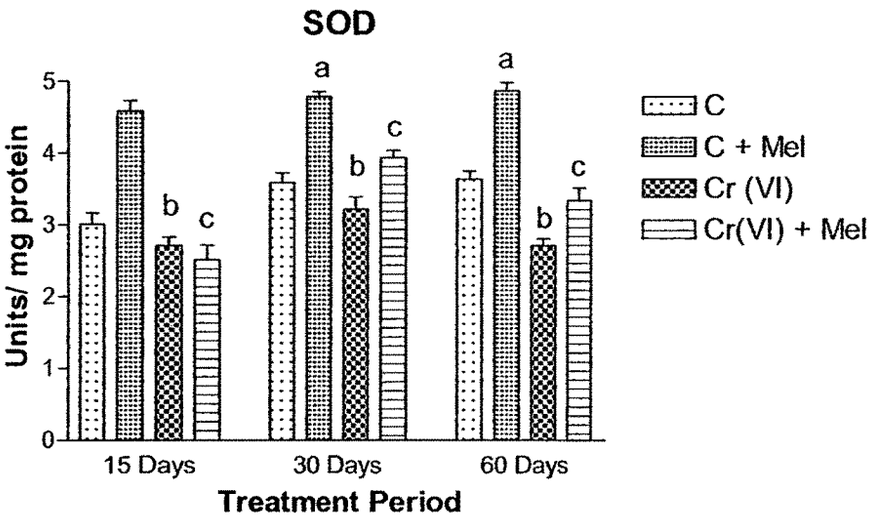
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.8: Chromium(VI) induced Catalase (CAT) activity (µmoles of H_2O_2 consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	19.43 ± 0.08	25.02 ± 0.44 [a]	14.33 ± 0.17 [b]	15.78 ± 0.44 [c]
30 Days	22.42 ± 0.07	23.46 ± 0.16 [a]	14.77 ± 0.26 [b]	17.46 ± 0.15 [c]
60 Days	24.57 ± 0.15	26.89 ± 0.27 [a]	14.98 ± 0.32 [b]	22.40 ± 0.33 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.9: Chromium(VI) induced Superoxide dismutase (SOD) activity in testis with or without Melatonin.



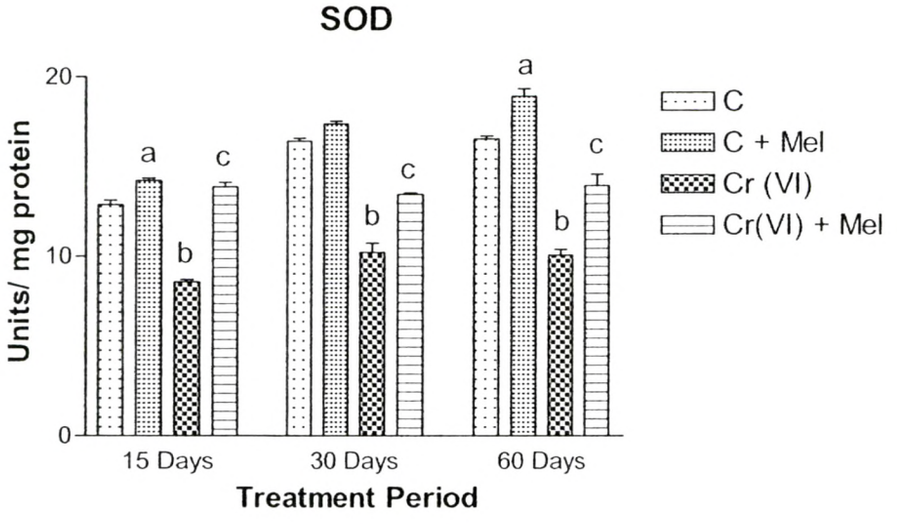
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.9: Chromium(VI) induced Superoxide dismutase (SOD) activity (units/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	3.01 ± 0.15	4.59 ± 0.14	2.71 ± 0.12 [b]	2.51 ± 0.20 [c]
30 Days	3.59 ± 0.14	4.79 ± 0.07 [a]	3.22 ± 0.17 [b]	3.94 ± 0.09 [c]
60 Days	3.64 ± 0.11	4.87 ± 0.11 [a]	2.71 ± 0.10 [b]	3.34 ± 0.17 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.10: Chromium(VI) induced Superoxide dismutase (SOD) activity in epididymis with or without Melatonin.



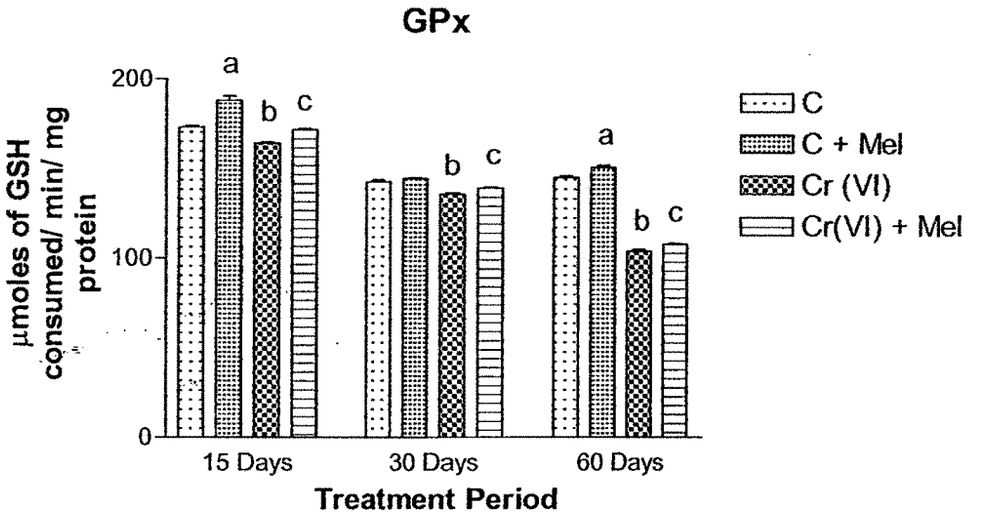
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.10: Chromium(VI) induced Superoxide dismutase (SOD) activity (units/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	12.87 ± 0.25	14.23 ± 0.13 [a]	8.57 ± 0.14 [b]	13.88 ± 0.25 [c]
30 Days	16.42 ± 0.17	17.39 ± 0.16	10.23 ± 0.51 [b]	13.46 ± 0.08 [c]
60 Days	16.56 ± 0.17	18.94 ± 0.41 [a]	10.07 ± 0.32 [b]	13.96 ± 0.64 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.11: Chromium(VI) induced Glutathione peroxidase (GPx) activity in testis with or without Melatonin.



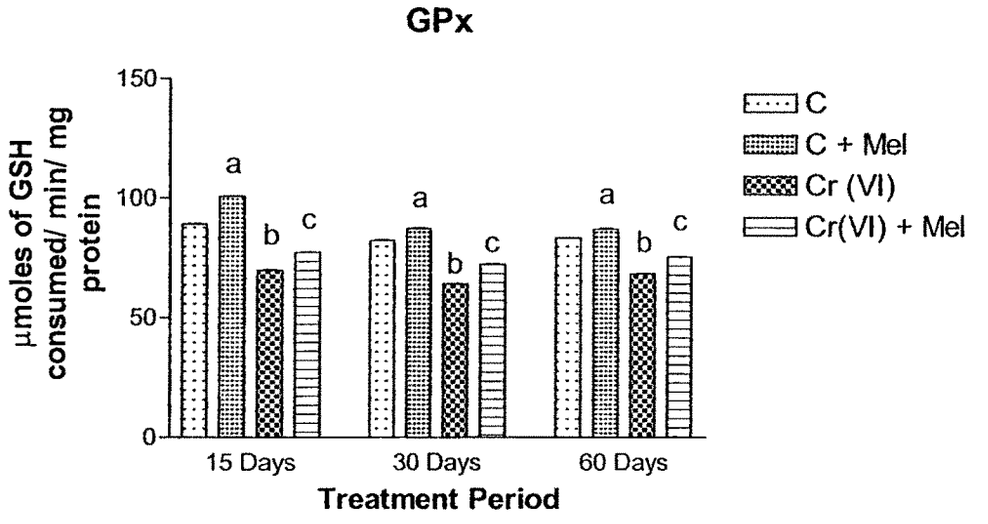
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.11: Chromium(VI) induced Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	173.02 ± 1.10	188.09 ± 2.28 [a]	164.15 ± 0.53 [b]	171.60 ± 0.66 [c]
30 Days	142.64 ± 0.93	144.35 ± 0.40	135.79 ± 0.76 [b]	139.03 ± 0.86 [c]
60 Days	144.68 ± 1.21	150.38 ± 1.15 [a]	103.67 ± 1.04 [b]	107.75 ± 0.45 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.12: Chromium(VI) induced Glutathione peroxidase (GPx) activity in epididymis with or without Melatonin.



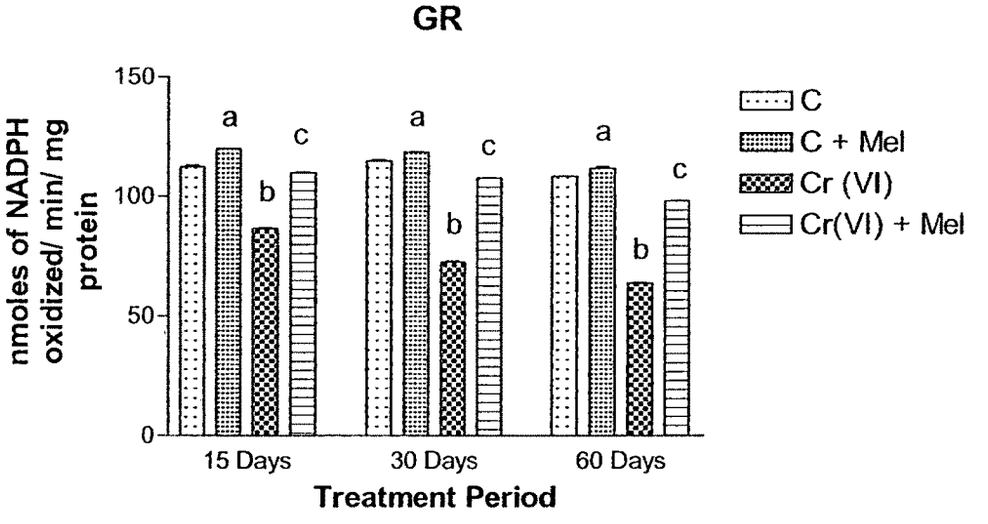
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.12: Chromium(VI) induced Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	89.17 ± 0.24	100.77 ± 0.18 [a]	69.80 ± 0.24 [b]	77.37 ± 0.20 [c]
30 Days	82.26 ± 0.37	87.32 ± 0.18 [a]	64.12 ± 0.24 [b]	72.36 ± 0.18 [c]
60 Days	83.43 ± 0.19	87.12 ± 0.32 [a]	68.49 ± 0.14 [b]	75.43 ± 0.15 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.13: Chromium(VI) induced Glutathione reductase (GR) activity in testis with or without Melatonin.



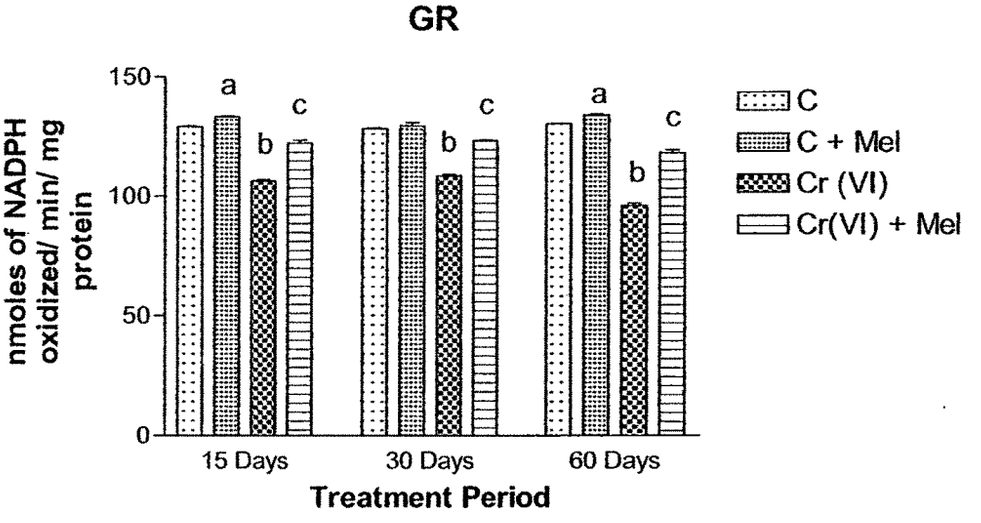
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.13: Chromium(VI) induced Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	112.35 ± 0.63	119.90 ± 0.21 [a]	86.46 ± 0.25 [b]	109.75 ± 0.40 [c]
30 Days	114.87 ± 0.58	118.47 ± 0.09 [a]	72.39 ± 0.40 [b]	107.55 ± 0.18 [c]
60 Days	108.34 ± 0.13	111.72 ± 0.71 [a]	63.71 ± 0.25 [b]	98.03 ± 0.30 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Figure 1.14: Chromium(VI) induced Glutathione reductase (GR) activity in epididymis with or without Melatonin.



Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.14: Chromium(VI) induced Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Cr (VI)	Cr(VI) + Mel
15 Days	129.21 ± 0.36	133.45 ± 0.37 [a]	106.42 ± 0.68 [b]	122.45 ± 1.25 [c]
30 Days	128.45 ± 0.39	129.53 ± 1.39	108.56 ± 0.63 [b]	123.45 ± 0.20 [c]
60 Days	130.43 ± 0.13	134.09 ± 0.53 [a]	96.17 ± 1.05 [b]	118.47 ± 1.09 [c]

Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium(VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium(VI);
Cr(VI) + Mel – Chromium(VI) + Melatonin

Table 1.15: Chromium(VI) induced percentage changes in chromium content of testis and epididymis with or without Melatonin.

Treatment	Testis		Epididymis	
	Cr(VI)	Cr(VI) + Mel	Cr(VI)	Cr(VI) + Mel
15 Days	57.50	33.33	9.59	10.32
30 Days	50.00	26.19	13.33	12.21
60 Days	106.98	88.89	10.90	15.20

C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium;
Cr(VI) + Mel – Chromium + Melatonin

Table 1.16: Chromium(VI) induced changes in the serum titre of Melatonin(pg/ ml) with or with out Melatonin.

Treatment	C	C + Mel	Cr(VI)	Cr(VI) + Mel
15 Days	121	140	67.00	113.00
30 Days	112	143	75.00	109.00
60 Days	93	126	26.00	83.00

Values expressed as Mean ± SEM of 4 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Chromium (VI)
C – Control; C + Mel – Control + Melatonin; Cr(VI) – Chromium;
Cr(VI) + Mel – Chromium + Melatonin

Discussion:

Results of the current study demonstrate the potential of hexavalent chromium to generate excessive ROS leading to impaired oxidative status of male reproductive organs. The present observations find identity with observations of earlier studies (Acharya *et al.*, 2004, 2006). Adult rat testis, an active steroidogenic tissue, is known to generate ROS and undergo lipid peroxidation during normal process of spermatogenesis and steroidogenesis (Sivasankaran *et al.*, 2007; Hanukoglu *et al.*, 1993). Under normal conditions, testis is able to manage the so generated ROS by means of enzymatic and non-enzymatic antioxidants. However, it is also known that increased ROS generated due to intoxication by any toxicant, can attack cellular membranes and decrease their fluidity, alter membrane functions and lead to loss of membrane bound enzymes (Gutteridge *et al.*, 2000; Chandra *et al.*, 2007).

Decreased levels and availability of non-enzymatic (Ascorbic acid and GSH) and enzymatic (SOD, CAT, GPx and GR) antioxidants respectively in testis and epididymis of chromium treated rats could be held responsible for the increased LPO levels recorded in the present study by way of increased ROS formation. SOD plays a central role in antioxidative reactions as, this enzyme dismutates superoxide anion to hydrogen peroxide and oxygen molecule (Junichi *et al.*, 2003). Down stream to this particular reaction, highly toxic H_2O_2 is detoxified by CAT and GPx. In the present study, decreased level of SOD activity seen in testis and epididymis of Cr(VI) exposed rats infers relatively high level of superoxide radical production. Gibanananda and Syed (2002) had listed the various fates of the notorious $O^{\bullet}_2^-$ in the cell; it can start the peroxidation of unsaturated lipids, cause oxidation of thiols and

can directly affect the activities of many enzymes such as catalase, peroxidase, aconitase, 6-phosphogluconate dehydratase, etc. leading to altered cellular function leading to cancer.

All the three enzymes (SOD, CAT and GPx) function in a well coordinated manner to form the first line of defense against the $O^{\bullet}_2^-$ and H_2O_2 mediated injury to the cell. Cells become highly vulnerable to oxidative stress if any change occurs in their activity (Sivasankaran *et al.*, 2007). Chandra *et al.* (2007) reported a marked decrease in testicular SOD activity of the Sprague Dawley rats when exposed to Cr(VI) for 26 days. In a study related to Cr-picolinate induced hepatic and renal toxicity in rats, Mahboob *et al.* (2002) reported increased LPO and reduced GSH, SOD, GPx, GR and CAT activity. Results of the present study with reference to SOD activity are in agreement with the above reports despite the dissimilarity in the rat species used, treatment schedule and dosage of chromium.

Similar to the hepatic tissue, testis also maintains a high level of GSH compared to other tissues. This may be attributed to the fact that GSH plays a pivotal role in the proliferation and differentiation of spermatogenic cells apart from protecting them from ROS mediated damage, specifically by quenching toxic hydroperoxides in a reaction catalyzed by GPx (Teaf *et al.*, 1985). The recorded decrease in the levels of GSH of both testis and epididymis of rats, subjected to Cr(VI) exposure in the present study could adversely affect the activities of GSH dependent enzymes. Concurrent reduced activity of the GSH recycling enzyme, glutathione reductase (GR), seen in the present experiment could be the possible explanation for the marked reduction in GSH levels. In contrast, a study conducted on

non-human primate (*Macaca radiate* Geoffroy) has reported increased GSH concentration in the testis of animals exposed to hexavalent chromium for 180 days (Aruldhas *et al.*, 2005). This discrepancy in the level of GSH may be attributed to animal model used or more plausibly to the duration of chromium exposure. Decreased content of GSH in tissues can lead to improper ascorbate recycling process as, one of the mechanisms of this process is GSH dependent (May *et al.*, 2001). The present study has indeed in this context recorded decreased testicular and epididymal ascorbic acid contents. The depletion of ascorbic acid may in turn favour ROS generations as ascorbic acid is a known scavenger of many types of free radicals (superoxide, hydrogen peroxide, hydroxyl radical, singlet oxygen and reactive nitrogen species). Further, the decreased level of testis ascorbic acid may also result in hampered steroidogenesis as the importance of it is well documented with reference to its role in steroidogenesis (Sengupta *et al.*, 2004). Altered steroidogenesis may result in decreased intra-testicular as well as serum testosterone titres, adversely affecting spermatogenesis and functional capacity of the androgen dependent reproductive organs such as epididymis, prostate and seminal vesicle. The proposed adverse effects on the functioning of testis and epididymis are clearly documented (chapter – 5).

There is a generalized decrement in the activity of antioxidant enzymes on exposure to Chromium which could be related with the increased ROS generation by Chromium as, Chromium *per se* is not known to directly interact and/ or inactivate proteins. An interesting observation of the present study is that while the non-enzymatic antioxidants (GSH and Ascorbic acid) showed a duration (chromium exposure) dependent linear decrease, there was no such linear effect on the enzymatic antioxidants (CAT, SOD, GPx and GR). The duration dependent increment in ROS

generation by chromium seems to be effectively and continuously quenched by GSH and ascorbic acid (as marked by their progressive and linear decrement) thereby sparing linear or progressively greater inactivation of enzymatic antioxidants, recovery of which would require upregulation of transcriptional activity. Along with the endogenous tissue GSH and ascorbic acid, melatonin also seems to play a contributing role in lessening the impact of Cr(VI) induced oxidative stress as marked by the significant reduction in serum titre of the indole in a progressive duration dependant manner. There is no report so far on reduction in serum melatonin on metal exposure. It is also of interest to note that, of all the antioxidant enzymes, except for GPx which has higher activity in testes, all other enzymes show significantly higher activity levels in epididymis compared to testis. This may be relevant in terms of epididymal functions related to maturation, decapacitation and integrity of sperms. Another significant observation is the resistance of testicular GPx activity to chromium toxicity and may be looked upon as an adaptive mechanism to protect testis against peroxide radicals which could probably interfere with the vital processes of spermatogenesis and steroidogenesis.

The findings of the present study suggest that, co-administration of melatonin along with chromium can exert marked protection against chromium induced oxidative insult. Lipid peroxidation is significantly lesser in testis and epididymis of rats treated with melatonin and this effect is purportedly due to its potential capacity to tackle notorious hydroxyl radical and superoxide anion which are the main culprits for initiating lipid peroxidation of biological membranes. Importantly, co-administration of melatonin shows well maintained pool of enzymatic (SOD, CAT, GPx, GR) and non-enzymatic (GSH, Ascorbic acid) antioxidants, reflecting the

relatively healthy antioxidant *milieu* of testis and epididymis compared to animals treated with Cr(VI) alone.

In a study related to sepsis induced multiple organ failure, Sener *et al.* (2005) reported that melatonin can protect organs against oxidative stress leading to organ injury (Liver, Kidney, Lung, Diaphragm, Heart and Brain) in a rat model of sepsis and supported the clinical use of melatonin as an antioxidant to cure the conditions in which risk of organ failure due to oxidative damage is involved. It is well established that oxidative stress plays a key role in carcinogenesis and, as a powerful antioxidant, this indole may help the cellular defense machinery to defend against cancer (Karbownik *et al.*, 2001). The present study adequately supports the protective role of melatonin against the hexavalent chromium induced oxidative damage leading to impaired reproductive organ functions. It is also clear from the present study that though melatonin exerts significant protective effect on both non-enzymatic and enzymatic antioxidantsm, its ability is slightly decreased on long duration exposure to chromium.

Current findings, in the light of previous results, suggest increased oxidative stress due to ROS generation to be responsible for increased lipid peroxidation and impaired antioxidant cellular defense in epididymis and testis of hexavalent chromium treated animals in a duration dependent manner. It is of interest that, hexavalent chromium induced oxidative stress of testis and epididymis is relatively milder compared to that generated by Cd (Chapter-6) and neither was there a duration dependent effect on the modulation of antioxidant enzymes. The duration dependent effect was essentially restricted to non-enzymatic antioxidants. It may be inferred that

environmental and/or occupational exposure to Cr(VI) compounds, and excessive ROS generation resulting in oxidative stress, can lead to poor male reproductive health/ male infertility. These effects may be protected totally or partially by the supplementation of a powerful antioxidant, as already reported by Irvine (1996) of antioxidant treatment to help infertile men. WHY NOT MELATONIN? Results of the present study, in agreement with the previously available data, advocate the use of melatonin as a potential antioxidant therapeutic. However, proper human trials need to be conducted for extrapolation of these findings.