Chapter: 2 Cadmium induced oxidative stress in Testes and Epididymis: Realistic dosage and duration dependent study and, role of Melatonin

Introduction:

Cadmium is a common environmental pollutant finding use in various industries such as production of Ni-Cd batteries, chemical stabilizers, metal coatings, pigments, alloys etc (Valko *et al.*, 2005). The most common oxidation state of this transition metal is Cd^{+2}, though however also rarely found as Cd^{+1}, and is without any beneficial biological role except for the Cd dependent carbonic anhydrase very recently discovered in marine diatoms (Lane *et al.*, 2005). According to the report of International Agency for Research on Cancer (IARC, 1993), Worldwide release of Cd in the environment due to anthropogenic activity is ten times higher than the natural one. Cadmium released due to anthropogenic activity contaminates air, water, food and soil. However, humans are primarily exposed to cadmium by food they consume, mainly in the form of leafy vegetables and meat of animals including fishes. Agency for Toxic Substances and Disease Registry (ATSDR, 2000) has ranked cadmium as the seventh toxic compound in their priority list. This toxic metal is known to cause wide range of injury and damage to many tissues and organs and is also considered as a potent carcinogen (IARC, 1993).

The main route of Cd exposure in humans is dietary; however Cd is very poorly absorbed by the gastro-intestinal tract(around 5-10%). Once inside the body,

due to its high volume of distribution it can invade into different tissues rapidly (Monsefi *et al.*, 2009). Studies have confirmed the presence of Cd in various organs including testis and male reproductive tract following cadmium administration and are stored therein (Danielsson *et al.*, 1984; Oldereid, 1993; Kusakabe *et al.*, 2007; Monsefi *et al.*, 2009). Cadmium, due to its long biological half-life (15-30 years in case of human and 6 months in case rats) and low rate of excretion leads to its significant accumulation in soft tissues making it more hazardous to humans and animals (Yan *et al.*, 2003, Benoff *et al.*, 2008).

Male reproductive toxicity of cadmium was reported way back (Alsber and Schwarze, 1919); however, it was not taken as a serious problem at that time. Identification of *Itai-itai* disease, caused due to Cd intoxication through polluted water in Japan around 1949 ignited great concern and initiated research related to the toxic manifestation of Cd with reference to human health. Testicular Cd toxicity is suggested to be mediated via the formation of reactive oxygen species (ROS) (Oteiza *et al.*, 1999). Though Cd is not able to generate ROS by itself, indirect generation of notorious radicals like superoxide, hydroxyl and nitric oxide has already been reported (Galan *et al.*, 2001). Recently, Liu *et al.* (2008) using *In Vivo* spin trapping studies claimed that cadmium generates reactive oxygen- and carbon- centered radical species in rats. Many studies reported deleterious effect of Cd with reference to testicular damage and male infertility in different animal models like guinea pigs, mice, dogs, hamsters, rats, rabbits, etc. (Allanson and Deanesly, 1962; Johnson, 1970; Nordberg, 1971; Aoki and Hoffer, 1978; Hew, *et al* 1993; Xu, 1996). However, except for the study by Sacerdote *et al.*, (2008) there are hardly any report available related to effect of Cd on

epididymis. Going through the literature, no study was found related to cadmium induced oxidative stress in epididymis.

Although there are many studies available with reference to testicular insult following the Cd administration, such studies are mainly based on acute effect of a single high dose or short term exposure. Further, the routes of exposure in most of these studies are intravenous, intramuscular, subcutaneous or intraperitoneal, much contrary to the known oral route of natural entry of the metal in humans. In order to understand the effect of the toxicants present in our hostile environment, animal model, dosage, route of administration and duration should all be in keeping with natural physiological relevance to humans.

A previous study from our laboratory has already reported the presence of cadmium as a common pollutant in significant amounts in the environs of Vadodara. In the present study, we have tried to understand duration dependent (i.e. 15, 30 and 60 Days of treatment) reproductive toxicity of Cd by assessing oxidative stress status of testes and epididymis using a realistically relevant dosage in keeping with natural levels in the environment and in dietary components.

Since entry of toxic metals into human body is unavoidable in the context of industrialization and related activity, there is pertinent need to evaluate the possible use of natural antioxidants as protectants. Melatonin has gained scientific validity in the recent past as a powerful antioxidant and hence, melatonin has been studied in the present context as a possible therapeutic agent against Cd induced oxidative stress.

Material and Method:

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

Results:

Lipid peroxidation (LPO):

Both testis (Figure 2.1, Table 2.1) and epididymis (Figure 2.2, Table 2.2) have tended to show a duration dependent linear increment in lipid peroxidation on Cd exposure with epididymis being more vulnerable than testis. Cadmium exposed animals co-administered with melatonin exhibited significant protection with testis showing a duration dependent negative effect and epididymis a positive effect.

Reduced Glutathione content (GSH):

There was significant depletion of GSH content in both testis (Figure 2.3, Table 2.3) and epididymis (Figure 2.4, Table 2.4) of cadmium exposed animals. The degree of depletion was more pronounced in testis and positive with increasing duration. However, the degree of depletion of epididymal GSH content was negatively correlated with increasing duration of cadmium exposure. Interestingly, though melatonin afforded protection against cadmium induced GSH depletion, this effect was much pronounced in epididymis as the GSH level increased beyond the control levels and had a positive correlation with duration. However, the degree of protection in testis was negatively correlatable with duration.

Ascorbic acid (AA):

Whereas there was an age dependent decrease in testis ascorbic acid content (Figure 2.5, Table 2.5), the epididymal ascorbic acid content tended to remain steady (Figure 2.6, Table 2.6). The testis ascorbic acid content showed a duration independent decrease in Cd exposed rats while the epididymal ascorbic acid content showed a duration dependent increasing decrement. Though melatonin had a generalized influence in increasing ascorbic acid content in control rats, the protective effect of melatonin when co-administrative with Cd was found to be progressively better with increasing duration in the case of testis ascorbic acid content and lesser in the case of epididymal ascorbic acid.

Catalase (CAT) activity:

Testis CAT activity was steady throughout (Figure 2.7, Table 2.7), whereas, the epididymal enzyme activity tended to show increase with increasing age (Figure 2.8, Table 2.8). Cadmium exposure brought about decreasing inhibition of enzyme activity and increasing protective effect with co-administered melatonin on testis CAT activity. On the other hand, epididymal CAT activity showed progressively increasing inhibition with increasing duration of Cd exposure and decreasing protective effect on co-administration with melatonin.

Superoxide dismutase (SOD) activity:

There was neither age dependent nor duration dependent effect on either testis or epididymis SOD activity, though the epididymal (Figure 2.9, Table 2.9) SOD activity seemed to be relatively higher than that of testis (Figure 2.10, Table 2.10). Similarly, the degree of inhibition in enzyme activity on exposure to Cd and protective effect

with melatonin were also duration independent though the protective effect of melatonin tended to be mainly favorable with increasing duration on testis SOD activity.

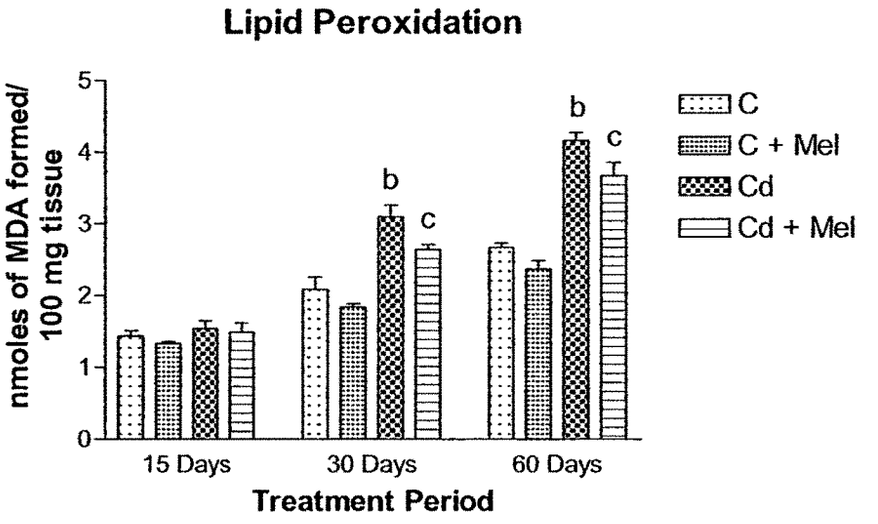
Glutathione peroxidase (GPx) activity:

There was tendency for age dependent decrement in GPx activity with the enzyme activity on testis (Figure 2.11, Table 2.11) being more than double in relation to epididymal (Figure 2.12, Table 2.12) GPx activity. Cadmium induced decrement in GPx activity was duration independent in both testis and epididymis. Interestingly, the degree of protection on co-administration with melatonin was found to decrease with duration in testis and, increase in epididymis.

Glutathione reductase (GR) activity:

The enzyme activity was more or less identical in both testis (Figure 2.13, Table 2.13) and epididymis (Figure 2.14, Table 2.14) though marginally higher in the latter. Though there was no age dependent difference in enzyme activity in either testis or epididymis, Cd exposure was seen to progressively increase the inhibition in GR activity in both the organs almost to the same degree. Conversely, the protective effect on co-administration with melatonin was found to be progressively better on increasing duration in both testis and epididymis.

Figure 2.1: Cadmium induced Lipid peroxidation (LPO) levels in testis with or without Melatonin.



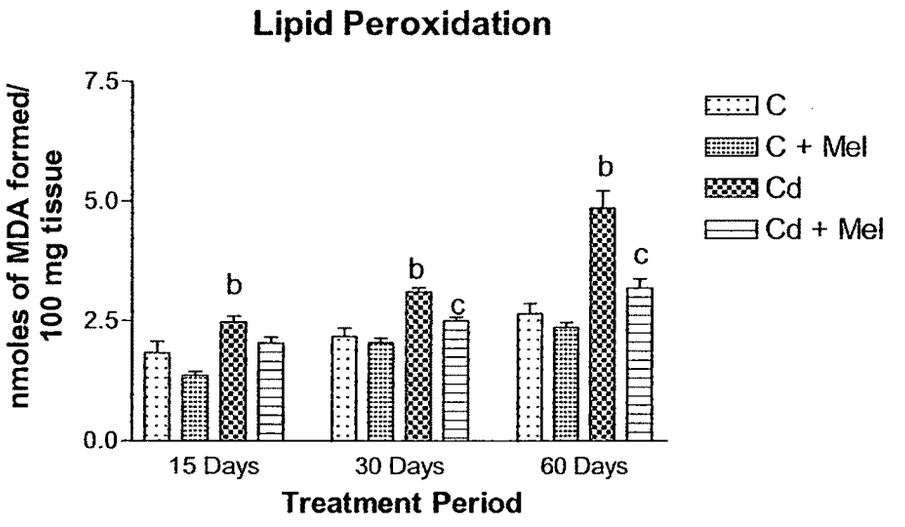
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.1: Cadmium induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	1.44 ± 0.06	1.33 ± 0.032	1.54 ± 0.10	1.49 ± 0.13
30 Days	2.09 ± 0.16	1.84 ± 0.047	3.10 ± 0.16^{b}	2.64 ± 0.07^{c}
60 Days	2.67 ± 0.06	2.37 ± 0.116	4.16 ± 0.11^{b}	3.67 ± 0.18^{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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.2: Cadmium induced Lipid peroxidation (LPO) levels in epididymis with or without Melatonin.



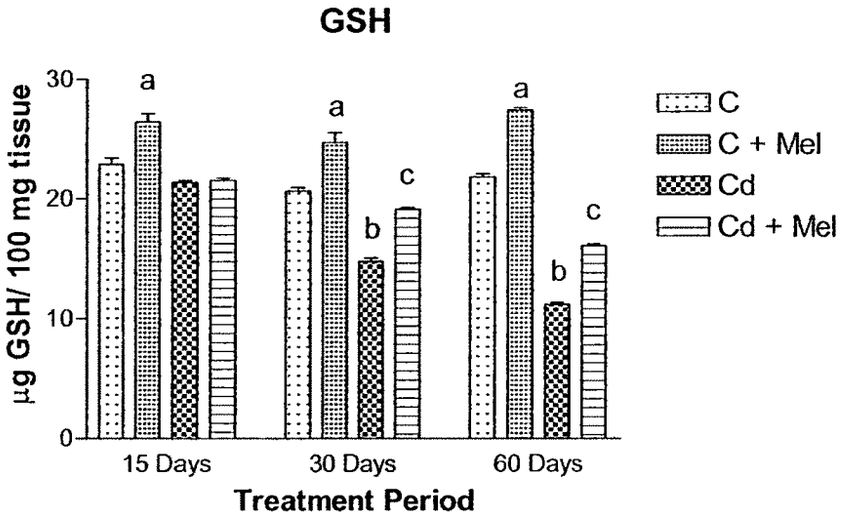
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.2: Cadmium induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	1.83 ± 0.24	1.37 ± 0.08	2.48 ± 0.13^{b}	2.04 ± 0.12
30 Days	2.18 ± 0.17	2.05 ± 0.10	3.11 ± 0.08^{b}	2.50 ± 0.08^{c}
60 Days	2.66 ± 0.21	2.37 ± 0.10	4.86 ± 0.36^{b}	3.19 ± 0.20^{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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.3: Cadmium induced alterations in Glutathione (GSH) levels in testis with or without Melatonin.



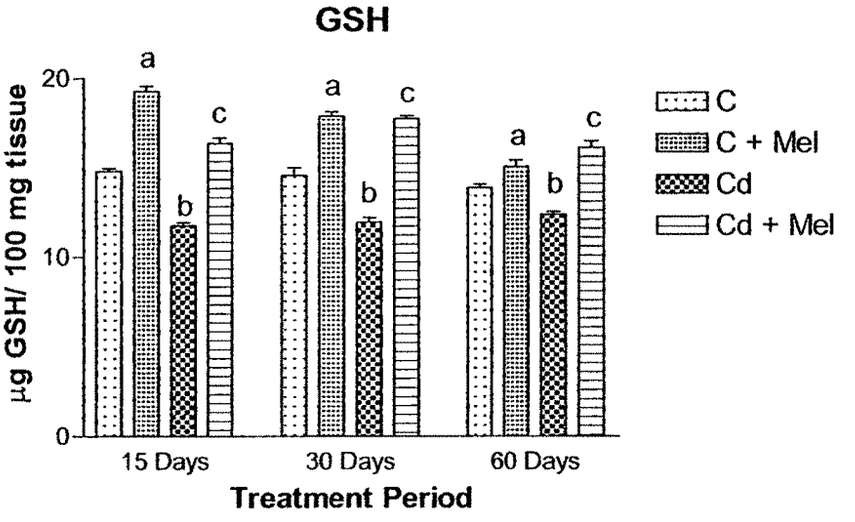
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.3: Cadmium induced alterations in Glutathione (GSH) levels (µg GSH/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	22.89 ± 0.51	26.45 ± 0.68[a]	21.37 ± 0.15	21.53 ± 0.21
30 Days	20.66 ± 0.31	24.71 ± 0.81[a]	14.79 ± 0.29[b]	19.13 ± 0.13[c]
60 Days	21.80 ± 0.28	27.39 ± 0.23[a]	11.16 ± 0.18[b]	16.05 ±0.16[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.4: Cadmium induced alterations Glutathione (GSH) levels in epididymis with or without Melatonin.



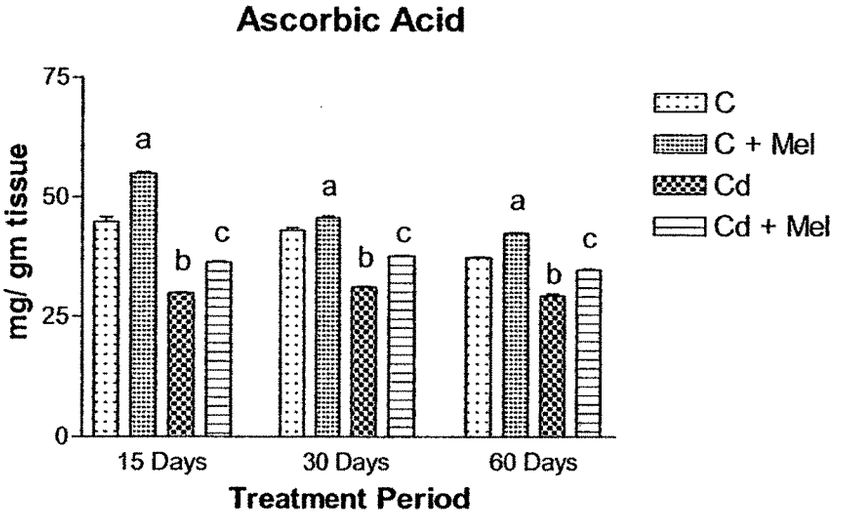
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.4: Cadmium induced alterations in Glutathione (GSH) levels (μg GSH/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	14.83 ± 0.14	19.27 ± 0.33[a]	11.76 ± 0.18[b]	16.35 ± 0.33[c]
30 Days	14.56 ± 0.46	17.87 ± 0.26[a]	11.94 ± 0.26[b]	17.72 ± 0.18[c]
60 Days	13.86 ± 0.20	15.04 ± 0.37[a]	12.37 ± 0.16[b]	16.11 ± 0.35[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.5: Cadmium induced changes in Ascorbic Acid levels in testis with or without Melatonin.



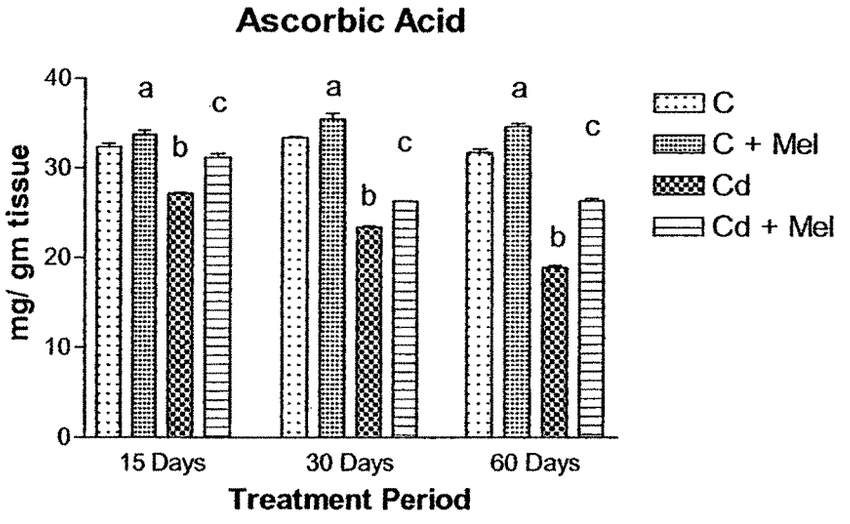
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.5: Cadmium induced changes in Ascorbic Acid levels (mg/ gm tissue) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	44.87 ± 0.97	54.99 ± 0.26[a]	29.97 ± 0.12[b]	36.43 ± 0.14[c]
30 Days	43.08 ± 0.51	45.73 ± 0.35[a]	31.28 ± 0.17[b]	37.73 ± 0.16[c]
60 Days	37.41 ± 0.21	42.54 ± 0.14[a]	29.40 ± 0.42[b]	34.89 ± 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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.6: Cadmium induced changes in Ascorbic Acid levels in epididymis with or without Melatonin.



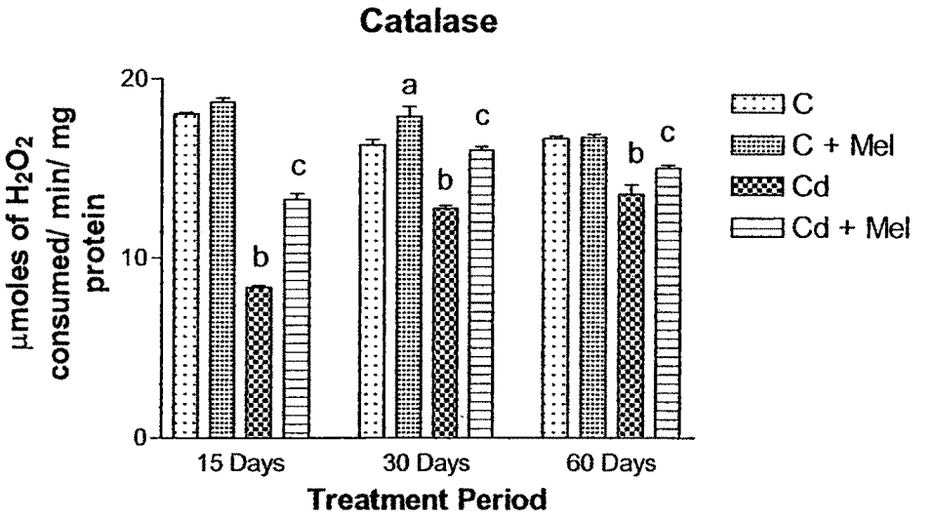
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.6: Cadmium induced changes in Ascorbic Acid levels (mg/ gm tissue) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	32.38 ± 0.37	33.76 ± 0.44[a]	27.19 ± 0.09[b]	31.17 ± 0.44[c]
30 Days	33.42 ± 0.10	35.47 ± 0.66[a]	23.45 ± 0.11[b]	26.30 ± 0.08[c]
60 Days	31.76 ± 0.42	34.67 ± 0.34[a]	18.96 ± 0.20[b]	26.40 ± 0.25[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.7: Cadmium induced changes in Catalase (CAT) activity in testis with or without Melatonin.



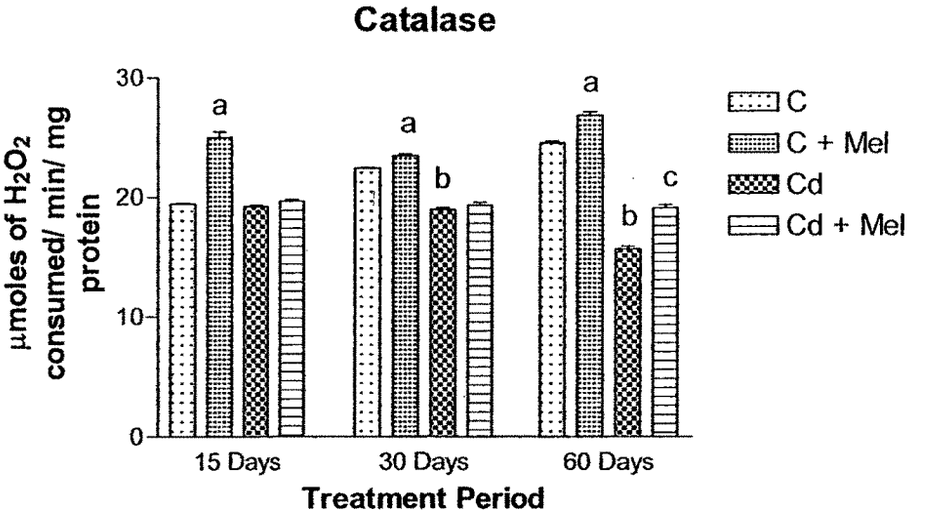
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.7: Cadmium induced changes in Catalase (CAT) activity (µmoles of H_2O_2 consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	18.05 ± 0.07	18.70 ± 0.25	8.37 ± 0.12^{b}	13.27 ± 0.33^{c}
30 Days	16.33 ± 0.29	17.93 ± 0.54^{a}	12.77 ± 0.18^{b}	16.03 ± 0.20^{c}
60 Days	16.68 ± 0.13	16.74 ± 0.16	13.58 ± 0.52^{b}	15.03 ± 0.16^{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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.8: Cadmium induced changes in Catalase (CAT) activity in epididymis with or without Melatonin.



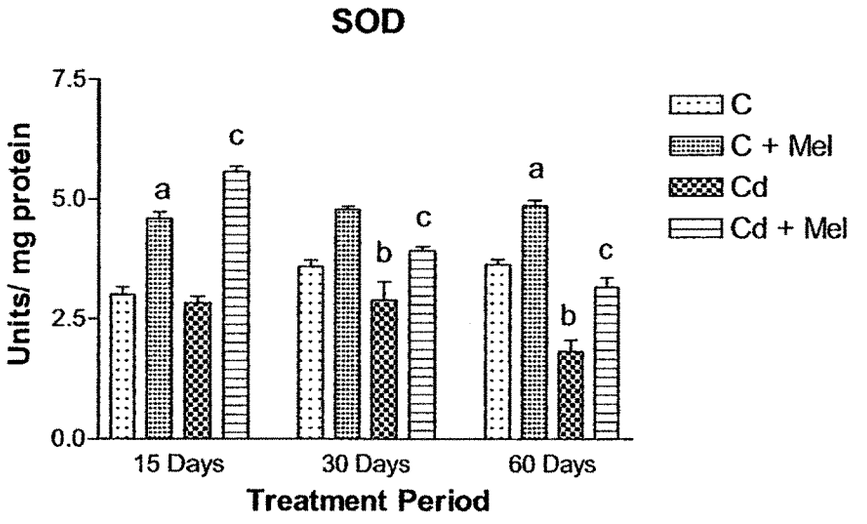
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.8: Cadmium induced changes in Catalase (CAT) activity (µmoles of H_2O_2 consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	19.43 ± 0.08	25.02 ± 0.44[a]	19.22 ± 0.11	19.68 ± 0.16
30 Days	22.42 ± 0.07	23.46 ± 0.16[a]	18.96 ± 0.18[b]	19.34 ± 0.22
60 Days	24.57 ± 0.15	26.89 ± 0.27[a]	15.70 ± 0.21[b]	19.13 ± 0.26[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.9: Cadmium induced changes in Superoxide dismutase (SOD) activity in testis with or without Melatonin.



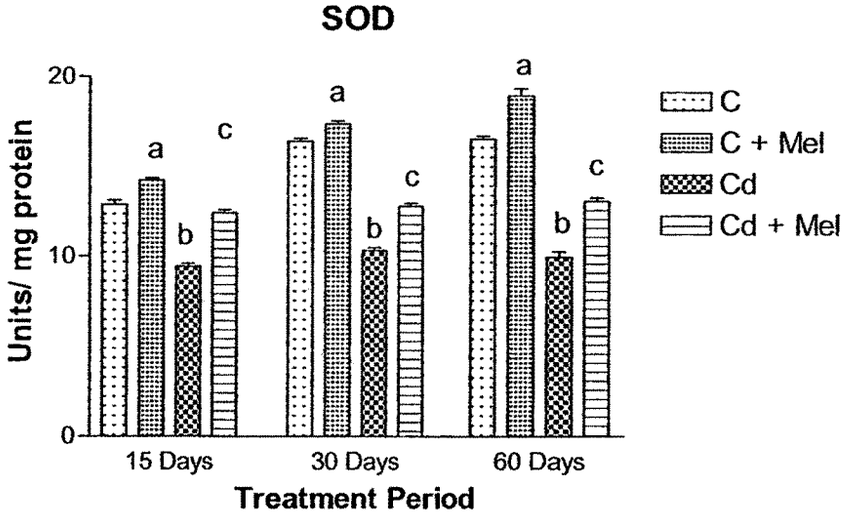
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.9: Cadmium induced changes in Superoxide dismutase (SOD) activity (units/ mg protein) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	3.01 ± 0.15	4.59 ± 0.14^{a}	2.83 ± 0.14	5.57 ± 0.12^{c}
30 Days	3.59 ± 0.14	4.79 ± 0.07	2.89 ± 0.39^{b}	3.93 ± 0.09^{c}
60 Days	3.64 ± 0.11	4.87 ± 0.11^{a}	1.83 ± 0.24^{b}	3.17 ± 0.20^{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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.10: Cadmium induced changes in Superoxide dismutase (SOD) activity in epididymis with or without Melatonin.



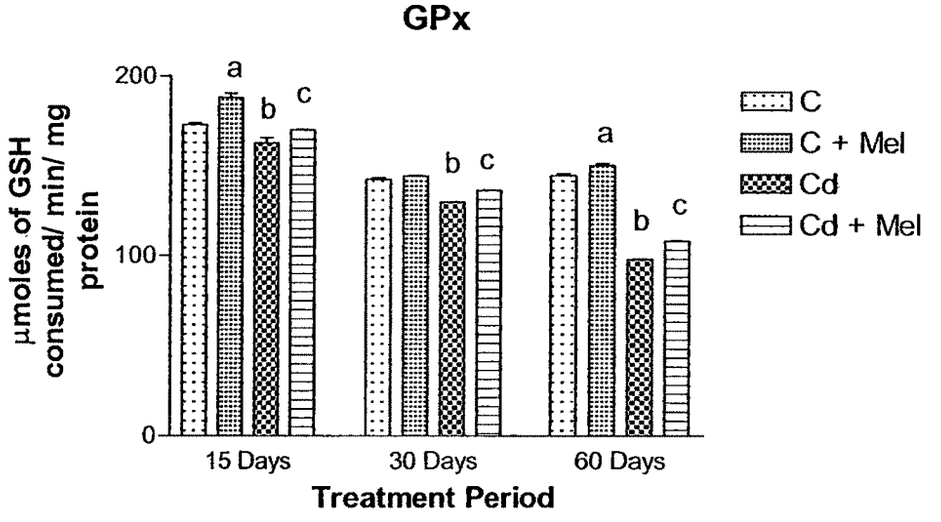
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.10: Cadmium induced changes in Superoxide dismutase (SOD) activity (units/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	12.87 ± 0.25	14.23 ± 0.13[a]	9.44 ± 0.15[b]	12.42 ± 0.17[c]
30 Days	16.42 ± 0.17	17.39 ± 0.16[a]	10.32 ± 0.16[b]	12.79 ± 0.17[c]
60 Days	16.56 ± 0.17	18.94 ± 0.41[a]	9.98 ± 0.28[b]	13.09 ± 0.21[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.11: Cadmium induced changes in Glutathione peroxidase (GPx) activity in testis with or without Melatonin.



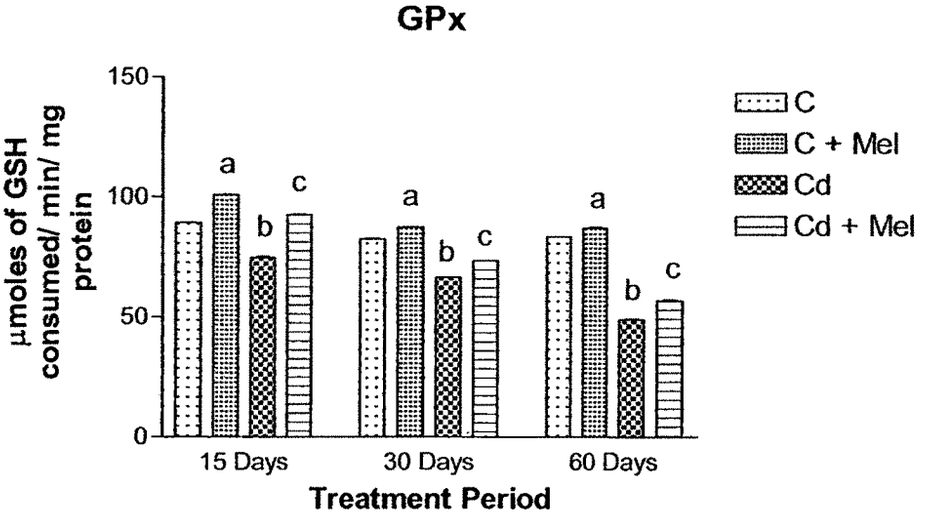
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.11: Cadmium induced changes in Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	173.02 ± 1.10	188.09 ± 2.28[a]	162.79 ± 3.17[b]	170.07 ± 0.27[c]
30 Days	142.64 ± 0.93	144.35 ± 0.40	129.87 ± 0.20[b]	136.45 ± 0.34[c]
60 Days	144.68 ± 1.21	150.38 ± 1.1[a]	98.16 ± 0.30[b]	108.49 ±0.14[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.12: Cadmium induced changes in Glutathione peroxidase (GPx) activity in epididymis with or without Melatonin.



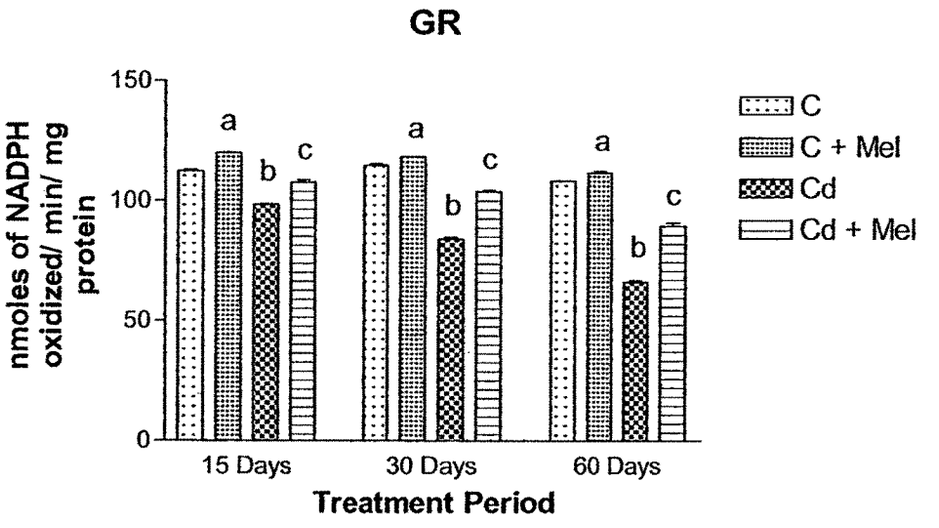
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.12: Cadmium induced changes in Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	89.17 ± 0.24	100.77 ± 0.18[a]	74.55 ± 0.49[b]	92.47 ± 0.16[c]
30 Days	82.26 ± 0.37	87.32 ± 0.18[a]	66.42 ± 0.32[b]	73.39 ± 0.08[c]
60 Days	83.43 ± 0.19	87.12 ± 0.32[a]	49.01 ± 0.17[b]	56.77 ± 0.36[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.13: Cadmium induced changes in Glutathione reductase (GR) activity in testis with or without Melatonin.



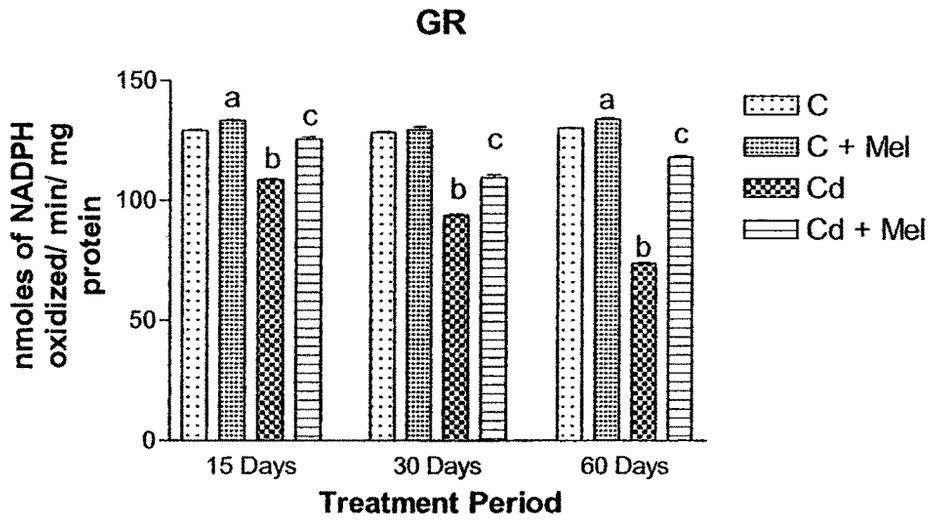
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.13: Cadmium induced changes in Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	112.35 ± 0.62	119.9 ± 0.21[a]	98.43 ± 0.27[b]	107.64 ± 1.09[c]
30 Days	114.87 ± 0.57	118.47 ± 0.08[a]	84.07 ± 0.80[b]	103.87 ± 0.54[c]
60 Days	108.34 ± 0.13	111.72 ± 0.70[a]	66.13 ± 0.73[b]	89.58 ±1.35[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Figure 2.14: Cadmium induced changes in 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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.14: Cadmium induced changes in Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + M	Cd	Cd + M
15 Days	129.21 ± 0.35	133.45 ± 0.36[a]	108.65 ± 0.40[b]	125.67 ± 1.05[c]
30 Days	128.45 ± 0.39	129.53 ± 1.39	93.97 ± 0.38[b]	109.71 ± 1.20[c]
60 Days	130.43 ± 0.13	134.09 ± 0.52[a]	73.86 ± 0.50[b]	118.31 ± 0.5[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 Cadmium
C – Control; C + Mel – Control + Melatonin; Cd – Cadmium; Cd + Mel – Cadmium + Melatonin

Table 2.15: Cadmium induced percentage changes in cadmium content of testis and epididymis with or without melatonin.

Treatment	Testis		Epididymis	
	Cd	**Cd + Mel**	**Cd**	**Cd + Mel**
15 Days	26.32	7.14	23.92	48.39
30 Days	15.56	-4.26	21.33	27.39
60 Days	30.33	21.85	16.13	18.99

C – Control; C + Mel – Control + Melatonin; Cd – Cadmium;
Cd + Mel – Cadmium + Melatonin

Table 2.16: Cadmium induced changes in the serum titre of melatonin(pg/ ml) in control and experimental rats.

Treatment	C	C + Mel	Cd	Cd + Mel
15 Days	121	140	59	104
30 Days	112	143	52	106
60 Days	93	126	26	88

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; Cd – Cadmium;
Cd + Mel – Cadmium + Melatonin

Discussion:

The present study was undertaken, essentially to make an experimental evaluation as physiologically relevant to humans as possible in terms of administration of Cd, keeping in mind the natural mode of exposure in humans and an environmentally relevant realistic dosage based on the metal content in vegetables and good grains in and around Vadodara. This is particularly important as, most of the current understanding of Cd insult on testis is based on studies on acute effects of a single high dose well above the typical chronic low level environmental exposure. A literature survey amply justifies the present study as, there are only few studies addressing the effects of chronic exposure or involving oral administration (Passia *et al.*, 1985; Caflisch., 1994; Ohta *et al.*, 1997; Brozoska *et al.*, 2003; Zhang *et al.*, 2004; Benoff *et al.*, 2008). Further, epididymis has only received passing attention as compared to testis. Accordingly, this study clearly indicates duration dependent increase in oxidative stress in both testis and epididymis by chronic exposure to Cd through the oral route. Significantly, duration dependent increase in lipid peroxidation to the tune of more than 50% in the case of testis and more than 80% in the case of epididymis provides validity.

The duration dependent significant increment in LPO in both testis and epididymis is likely to affect the functional competence of both these organs. The observed marked increase in LPO could be related with induction of oxidative stress due to compromised antioxidant status. This is clearly indicated by the significant reduction in the tissue contents of non-enzymatic antioxidants (GSH and ascorbic acid) as well as inhibited activities of enzymatic antioxidants (SOD, CAT, GPx and

GR). Though Cd is not responsible for forming ROS by the classical pathway of their generation, a pertinent mechanism that is considered relevant in the case of Cd is its ability to replace important microelements from the active sites of enzymes. In this context, the inhibition of antioxidant enzymes, SOD and GPx, via Cd mediated replacement of Zn from Cu-Zn-SOD to form Cu-Cd-SOD (inactive form) and release of Se from GPx could be considered the potent modes of development of oxidative stress in testis and epididymis. This finds validity in the reported observation of even other workers in Cd induced testicular toxicity (Amara *et al.*, 2008). Yet other mechanisms of Cd induced oxidative stress in reproductive organs could be its ability to cause depletion of GSH and protein bound -SH group (Bagchi *et al.*, 1997) and/ or its ability to replace iron and copper from various cytoplasmic and membrane proteins (ferritin, apoferritin) leading to generation of unbound free or chelated copper and iron ions precipitating oxidative stress via Fenton reactions (Valko *et al.*, 2005).

A comparison of duration dependent effects of Cd in testis and epididymis clearly reveals much higher lipid peroxidation in epididymis. Though there is minimal LPO in testis during short duration exposure to Cd, increasing oxidative stress is nevertheless indicated by the significantly inhibited activities of SOD and CAT and marked depletion of ascorbate. However, with increased duration of exposure to Cd, observed progressive increase in LPO in testis could be accredited to highly inhibited activities of GPx and GR and significant depletion in the GSH content. The maximal effect seen on SOD, CAT and ascorbate is indicative of the flux of superoxide and peroxyl radicals. The increasing lipid peroxidation on long duration exposure to Cd seems to be due to the significant inhibitory effects on GPx and GR and highly depleted GSH content. On the other hand, noticeably higher lipid peroxidation seen in

the epididymis on short duration exposure to Cd along with significant depletion in GSH content might suggest depletion of –SH groups and release of copper and iron from membrane proteins and enzymes and the resultant ROS formation as suggested above as the causes of oxidative stress. In this context, the high mitochondrial ferritin content in epididymis (Santambrogio *et al.*, 2007) could be an ideal candidate for the Cd mediated iron release and Fenton reaction. The maximal LPO in epididymis on long duration exposure appears to be due to a greater derangement of the antioxidant machinery involving GR, CAT, SOD and ascorbic acid. The greater utilization of GSH in testis as against ascorbic acid in epididymis on long duration exposure to cadmium is probably suggestive of the resistance to testicular ascorbic acid participation in antioxidative activities due to its other functional importance in testis (steroidogenesis).

In recent time melatonin has been projected as a vary effective antioxidant and free radical scavenger (Kim *et al.*, 1998; Tan *et al.*, 2002; Tsia and Lu, 2003). Though the roles of many antioxidants have been studied with reference to metal toxicity, the role of melatonin has not been given its due status. There are only a couple of studies which has tried to test the efficacy of melatonin in Cd induced toxicity to tissues (Noda *et al.*, 1999; Karbownik *et al.*, 2001). The present study in this context, intended to evaluate the protective effect rather than ameliorative effect, has definitely shown a remarkable ability of melatonin to protect against Cd induced toxicity to testis and epididymis. Clearly, co-administration of melatonin along with Cd could effectively prevent and/ or minimize the alteration in endogenous antioxidants caused by cadmium. This is further well supported by our observation of significantly reduced serum melatonin titre in cadmium intoxicated animals. The ability of

melatonin to offset the cadmium mediated shift in antioxidative to pro-oxidative state can be due to the plurality of its function in the form of direct and indirect free radical scavenging as well as transcriptional activation of antioxidant genes. It is likely that the former function may be of primary importance in short term exposure to Cd and the latter function then comes into play on long term metal exposure.

Overall, the present study brings out (I) a generalize Cd induced reproductive toxicity by oxidative stress, (II) differential mechanism of Cd toxicity in testis and epididymis on short term verses long term exposure and (III) the significant role of melatonin as a protectant and a possible future therapeutic agent worthy of testing against metal toxicity.