Chapter 3: Nickel induced oxidative stress in Testes and Epididymis: Realistic dosage and duration dependent effect and, role of Melatonin

Introduction:

Nickel, a heavy metal, is mainly used for production of stainless steel though it has wide applications in various industries like electroplating, polishing, production of Ni-Cd batteries, food processing, refining, etc. (Das and Dasgupta, 2002). This leads to occupational exposure of nickel in workers employed in above mentioned industries, causing hypernickelemia in such subjects if exposed for long duration of time. Due to its wide industrial usage it is difficult to avoid presence of nickel in our environment and is considered as a potent pollutant and industrial health hazard. Humans are exposed to nickel via food, air, dermal contact (jewellrey and artificial body parts), drinking water and smoking (Das and Buchner, 2007). Dietary source is the primary route of nickel entry into the body of non-smoking and non-occupationally exposed humans and, following its entry into GI tract, absorption of this metal greatly depends on the type of nickel species present in the food and its absorptive potential. Generally, around 1-2% of ingested Ni is absorbed by the intestine (Valko *et al.*, 2005).

Toxic effects of this metal are well documented following entry into on various organs in different animal models and humans including the mutagenic and carcinogenic properties of many nickel compounds (Kakela *et al.*, 1999). Though the deleterious effects of various nickel species on male reproductive system are well documented with reference to testis, there is limited amount of literature available

with regard to the epididymis and even the few studies have mainly focused on cauda epididymal sperm analysis. There is no study related with induction of oxidative stress in epididymis (store house of mature sperms in mammals). Nickel produces measurable amount of free radicals directly or indirectly by depleting the enzymatic and non-enzymatic antioxidant pools of the cell (Salnikow *et al.*, 2000; Bal and Kasprzak, 2002). Though it is well established that oxidative stress plays a major role in testicular toxicity leading to poor reproductive performance of males, the exact mechanism of nickel induced male reproductive toxicity is not well understood (Doreswamy *et al.*, 2004; Turner and Lsiak, 2008). Moreover, most of the available studies are physiologically irrelevant with reference to route, dosage and duration of nickel exposure to humans.

In the present study an attempt is made to assess oxidative stress generated by Ni in testis and epididymis. To this end, a duration dependent evaluation has been made employing a realistic dosage in keeping with the natural concentration of Ni found in vegetables and food grains grown in and around Vadodara and to which the local population is exposed to through dietary intake (Sharma, 1996). Apparently, most of the toxicological studies are based on dosages derived on the basis of LD_{50} values and that too acute or single dose exposure studies employing intraperitoneal or subcutaneous administration. These are totally unrelated to environmental levels and not simulatable or extrapolatable to an human situation. Hence, the present study in this context, is an environmentally relevant one (based on data obtained for Vadodara city) and physiologically simulatable to humans, employing a realistic dosage.

Entry of the toxicant into the human body is unavoidable due to food habits, occupational compulsions and life style. In this context, need of the hour is to assess the possible use of efficacious natural antioxidants which can counter the ill effects of environmental pollutants. Melatonin, one of the natural antioxidants as understood in recent times is considered in the present investigation as a possible potent therapeutic agent to protect against Ni induced oxidative insult on testis and epididymis.

Material and Method:

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

Results:

Lipid peroxidation (LPO):

Both testis (Figure 3.1, Table 3.1) and epididymis (Figure 3.2, Table 3.2) showed a duration dependent linear increase in LPO, with the increase in epididymis being more pronounced. Melatonin exerted significant protection when co-administered with Ni in both the organs with the protective effect being more pronounced at 15 days.

Reduced Glutathione (GSH):

Significant depletion was observed in testicular (Figure 3.3, Table 3.3) and epididymal (Figure 3.4, Table 3.4) GSH content of Ni administered animals without exhibiting steady significant duration dependent effect though, these was a tendency for increasing depletion in testis and decreasing depletion in epididymis. Relatively, the depletion in epididymis was double than in testis. The protective effect of melatonin was also quite significant without much statistical significance in terms of duration of exposure.

Ascorbic acid (AA):

In control animals there was an age dependent decrease in testis ascorbic acid content (Figure 3.5, Table 3.5), though the epididymal ascorbic acid content tended to remain steady (Figure 3.6, Table 3.6). Both testicular and epididymal ascorbic acid contents showed a duration dependent linear decrease in Ni administered animals with the change at each time period being more pronounced in epididymis. Though, melatonin had a generalized influence in increasing ascorbic acid content in control

rats more clearly in testis, the protective effect of melatonin when co-administrative with Ni was found to be progressively lesser in both the organs.

Catalase (CAT) activity:

Testis CAT activity was steady throughout (Figure 3.7, Table 3.7) whereas, the epididymal enzyme activity tended to show increase with increasing age in control animals (Figure 3.8, Table 3.8). Ni treatment significantly decreased CAT activity in a duration dependent manner and co-administration of melatonin prevented the inhibition of enzyme activity in both the testis and epididymis. Though, Ni induced inhibition in CAT activity was more or less identically similar at 30 and 60 days, at the short duration exposure of 15 days the inhibition in testis CAT activity was much greater than is epididymis.

Superoxide dismutase (SOD) activity:

Both testis (Figure 3.9, Table 3.9) and epididymis (Figure 3.10, Table 3.10) showed a duration dependant linear increase in SOD inhibition by Ni exposure. Relatively epididymis tended to show more SOD inhibition than testis. Further, the inhibition seen at 15 days was much greater in epididymis compared to testis. Melatonin administration showed significant protective effect against SOD inhibition by Ni in both the organs. This effect of melatonin was more potent in testis and in fact at 15 days, melatonin tended to increase SOD activity above normal level despite Ni treatment.

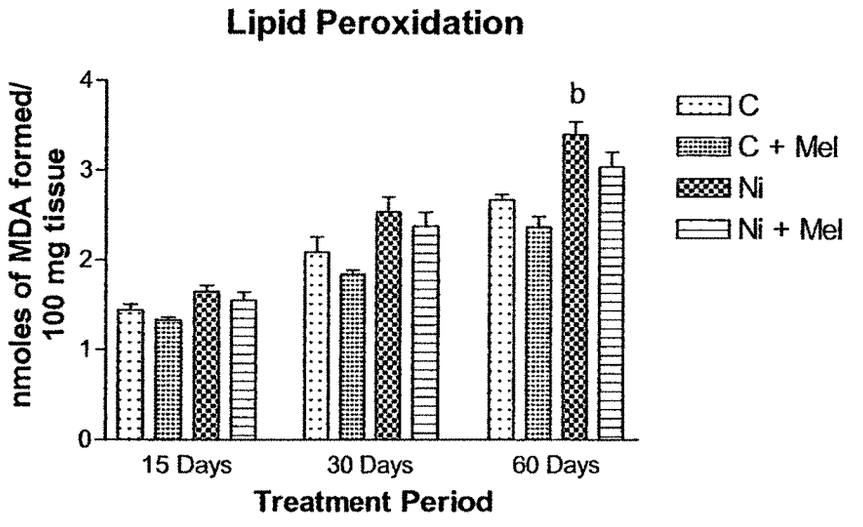
Glutathione peroxidase (GPx):

There was tendency for age dependent decrement in GPx activity in both testis (Figure 3.11, Table 3.11) and epididymis (Figure 3.12, Table 3.12) of control rats. Ni administration inhibited testicular GPx activity in a duration dependent manner with maximum inhibition on longer exposure in both the organs. Though, the percentage inhibition of enzyme activity was higher in epididymis at 30 and 60 days, the percentage inhibition at 15 days was much higher in testis. Concurrently, melatonin co-administration showed marked protection against inhibition of GPx activity in testis and epididymis, with tendency for decreasing protection with increasing duration. Interestingly, the degree of protection on co-administration with melatonin was found to be more effective in short term treatment in both testis and epididymis.

Glutathione reductase (GR) activity:

Though there was no age dependent difference in enzyme activity in either testis (Figure 3.13, Table 3.13) or epididymis (Figure 3.14, Table 3.14) of control animals, there was a duration dependent increasing inhibition in GR activity in both testis and epididymis of Ni exposed animals. Though, the inhibition of enzyme activity was equally high at 60 days, at 15 and 60 days the inhibition in enzyme activity was significantly greater in epididymis. Co-administration of melatonin showed marked protection against the Ni induced inhibition of testicular and epididymal GR activity.

Figure 3.1: Nickel induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in testis with or without Melatonin in testis with or without Melatonin.



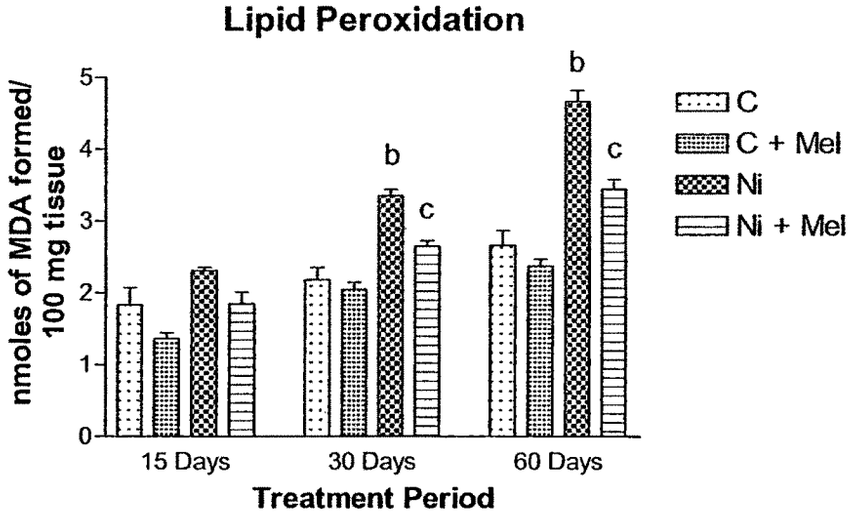
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.1: Nickel induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	1.44 ± 0.06	1.33 ± 0.03	1.65 ± 0.06	1.55 ± 0.09
30 Days	2.09 ± 0.16	1.84 ± 0.04	2.54 ± 0.17	2.38 ± 0.15
60 Days	2.67 ± 0.06	2.37 ± 0.11	3.40 ± 0.14[b]	3.04 ±0.17

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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.2: Nickel induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in epididymis with or without Melatonin.



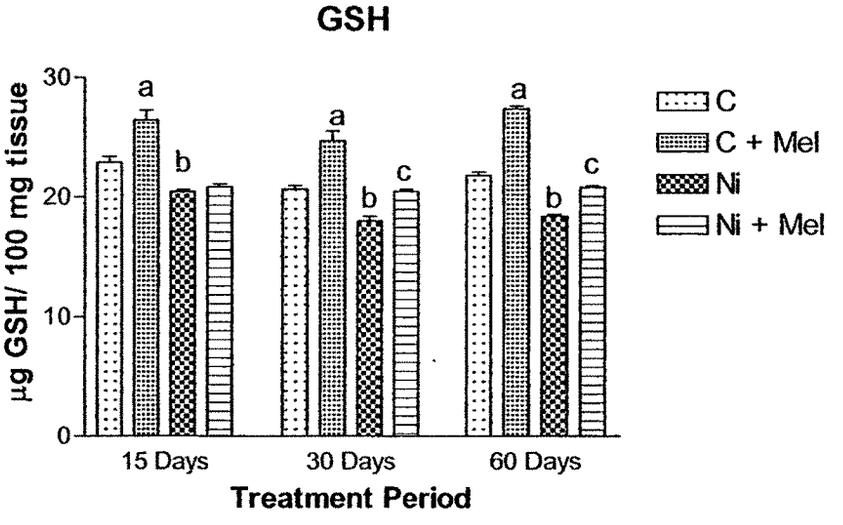
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.2: Nickel induced Lipid peroxidation (LPO) levels (nmoles of MDA formed/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	1.83 ± 0.24	1.37 ± 0.08	2.31 ± 0.04	1.85 ± 0.15
30 Days	2.18 ± 0.17	2.05 ± 0.10	3.35 ± 0.08 [b]	2.65 ± 0.08 [c]
60 Days	2.66 ± 0.21	2.37 ± 0.10	4.67 ± 0.15 [b]	3.45 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.3: Nickel induced alterations in Glutathione (GSH) levels in testis with or without Melatonin.



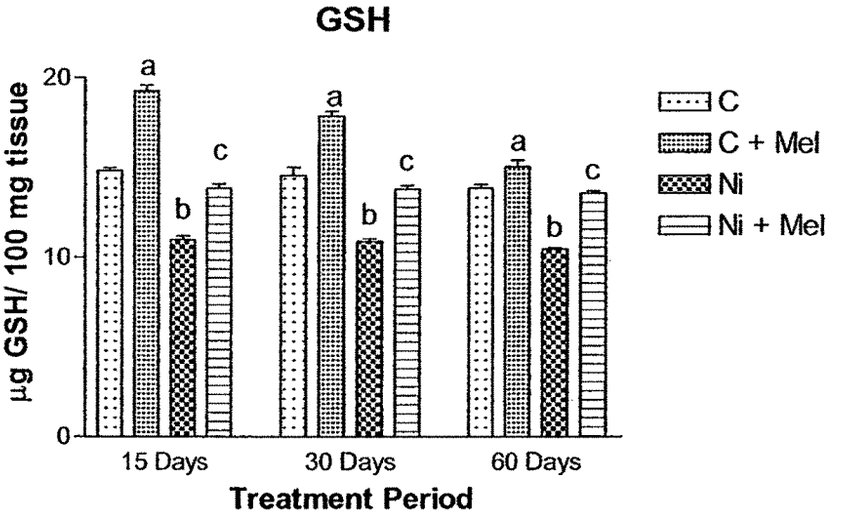
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.3: Nickel induced alterations in Glutathione (GSH) levels (µg GSH/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	22.89 ± 0.51	26.45 ± 0.80[a]	20.46 ± 0.18[b]	20.83 ± 0.25
30 Days	20.66 ± 0.31	24.71 ± 0.81[a]	17.98 ± 0.40[b]	20.47 ± 0.16[c]
60 Days	21.80 ± 0.28	27.39 ± 0.23[a]	18.39 ± 0.16[b]	20.78 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.4: Nickel induced alterations Glutathione (GSH) levels in epididymis with or without Melatonin.



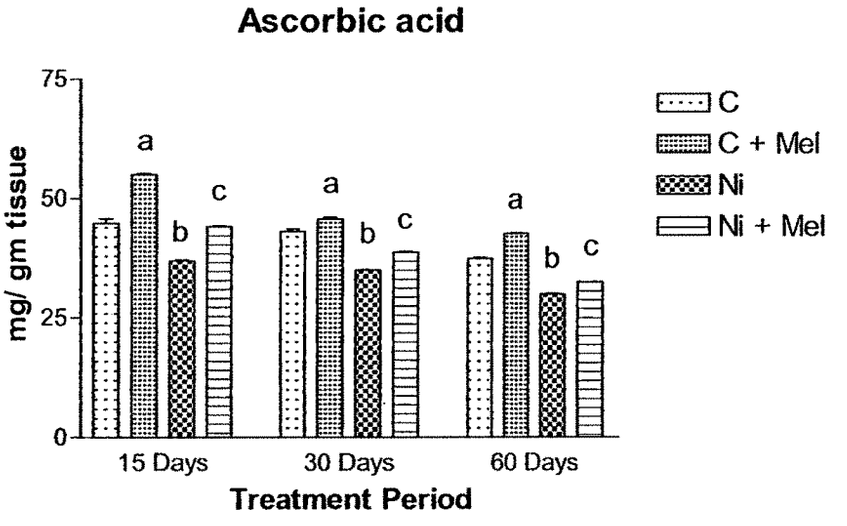
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.4: Nickel induced alterations in Glutathione (GSH) levels (µg GSH/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	14.83 ± 0.14	19.27 ± 0.33[a]	10.97 ± 0.21[b]	13.86 ± 0.22[c]
30 Days	14.56 ± 0.46	17.87 ± 0.26[a]	10.87 ± 0.17[b]	13.79 ± 0.22[c]
60 Days	13.86 ± 0.20	15.04 ± 0.37[a]	10.45 ± 0.07[b]	13.56 ± 0.13[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.5: Nickel induced changes in Ascorbic Acid levels in testis with or without Melatonin.



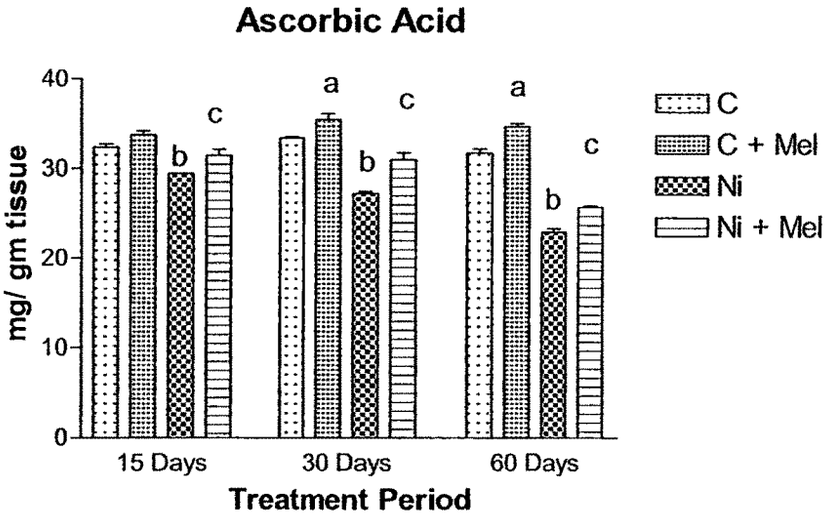
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.5: Nickel induced changes in Ascorbic Acid levels (µg/ 100 mg tissue) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	44.87 ± 0.97	54.99 ± 0.26[a]	36.88 ± 0.12[b]	44.08 ± 0.19[c]
30 Days	43.08 ± 0.51	45.73 ± 0.35[a]	34.98 ± 0.10[b]	38.71 ± 0.20[c]
60 Days	37.41 ± 0.21	42.54 ± 0.14[a]	29.86 ± 0.16[b]	32.44 ± 0.05[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.6: Nickel induced changes in Ascorbic Acid levels in epididymis with or without Melatonin.



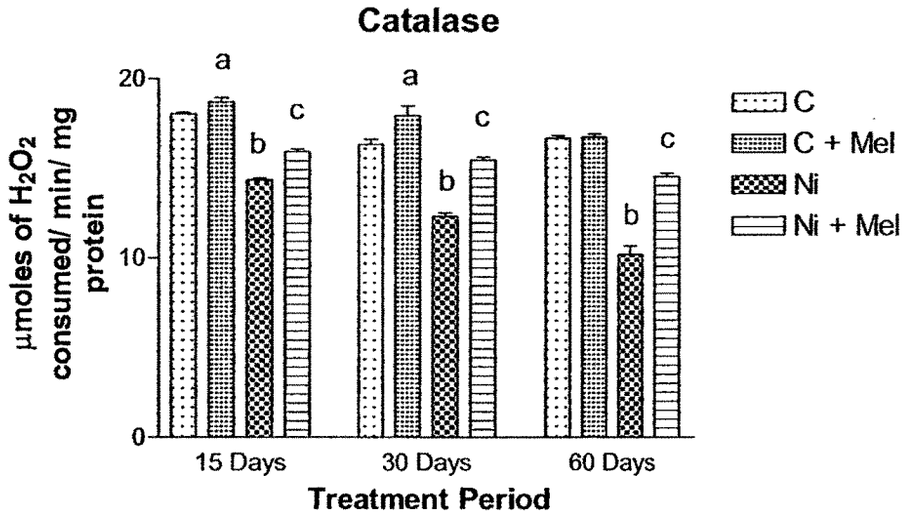
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.6: Nickel induced changes in Ascorbic Acid levels (µg/ 100 mg tissue) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	32.38 ± 0.37	33.76 ± 0.44	29.46 ± 0.02^{b}	31.48 ± 0.65^{c}
30 Days	33.42 ± 0.10	35.47 ± 0.66^{a}	27.21 ± 0.25^{b}	30.97 ± 0.82^{c}
60 Days	31.76 ± 0.42	34.67 ± 0.34^{a}	22.92 ± 0.36^{b}	25.69 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.7: Nickel induced changes in Catalase (CAT) activity in testis with or without Melatonin.



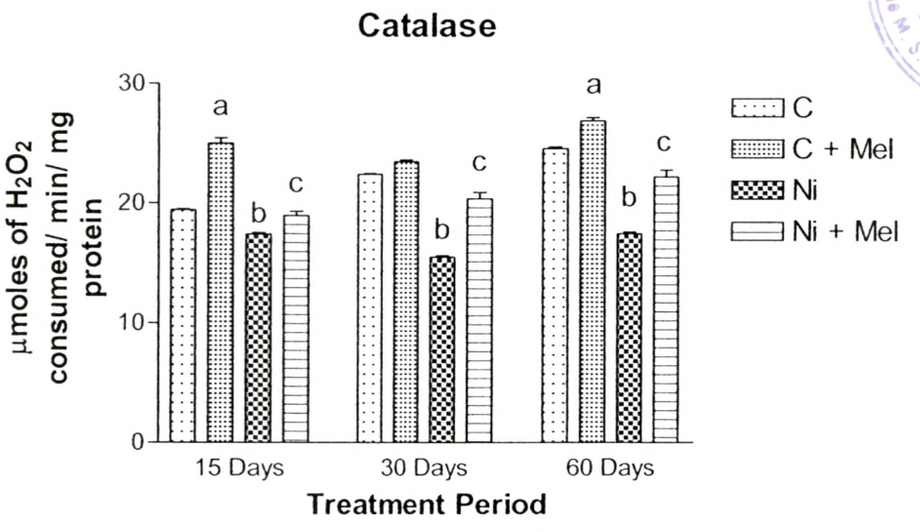
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.7: Nickel induced changes in Catalase (CAT) activity (μmoles of H_2O_2 consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	18.05 ± 0.07	18.70 ± 0.25[a]	14.35 ± 0.08[b]	15.92 ± 0.15[c]
30 Days	16.33 ± 0.29	17.93 ± 0.54[a]	12.29 ± 0.20[b]	15.44 ± 0.18[c]
60 Days	16.68 ± 0.13	16.74 ± 0.16	10.18 ± 0.49[b]	14.53 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.8: Nickel induced changes in Catalase (CAT) activity in epididymis with or without Melatonin.



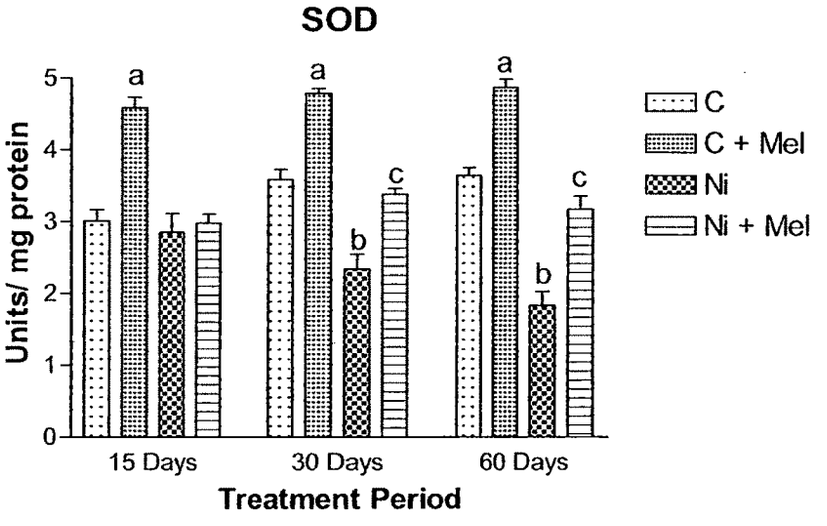
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.8: Nickel induced changes in Catalase (CAT) activity (µmoles of H_2O_2 consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	19.43 ± 0.08	25.02 ± 0.44[a]	17.41 ± 0.13[b]	18.97 ± 0.34[c]
30 Days	22.42 ± 0.07	23.46 ± 0.16	15.48 ± 0.14[b]	20.35 ± 0.55[c]
60 Days	24.57 ± 0.15	26.89 ± 0.27[a]	17.45 ± 0.15[b]	22.19 ± 0.58[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + MelatoninNi – Nickel; Ni + M – Nickel + Melatonin

Figure 3.9: Nickel induced changes in Superoxide dismutase (SOD) activity in testis with or without Melatonin



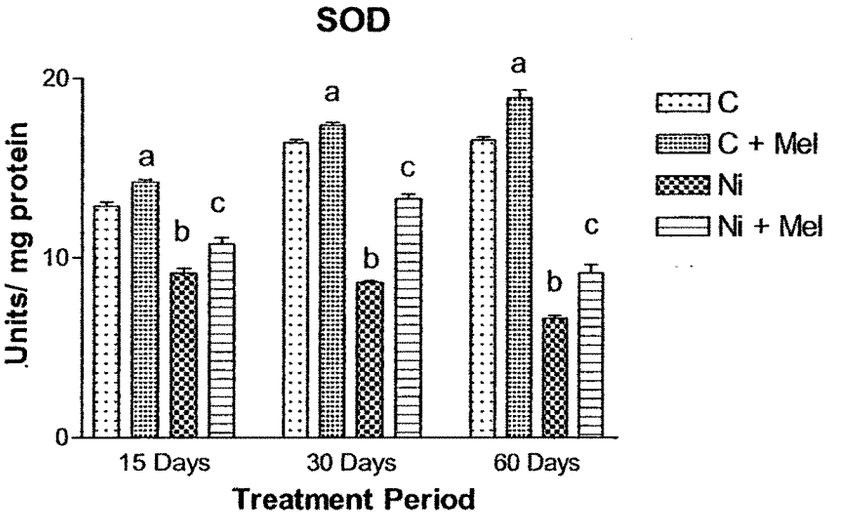
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.9: Nickel induced changes in Superoxide dismutase (SOD) activity (units/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	3.01 ± 0.15	4.59 ± 0.14[a]	2.85 ± 0.26	2.98 ± 0.12
30 Days	3.59 ± 0.14	4.79 ± 0.07[a]	2.34 ± 0.21[b]	3.38 ± 0.07[c]
60 Days	3.64 ± 0.11	4.87 ± 0.11[a]	1.83 ± 0.20[b]	3.17 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + MelatoninNi – Nickel; Ni + M – Nickel + Melatonin

Figure 3.10: Nickel induced changes in Superoxide dismutase (SOD) activity in epididymis with or without Melatonin.



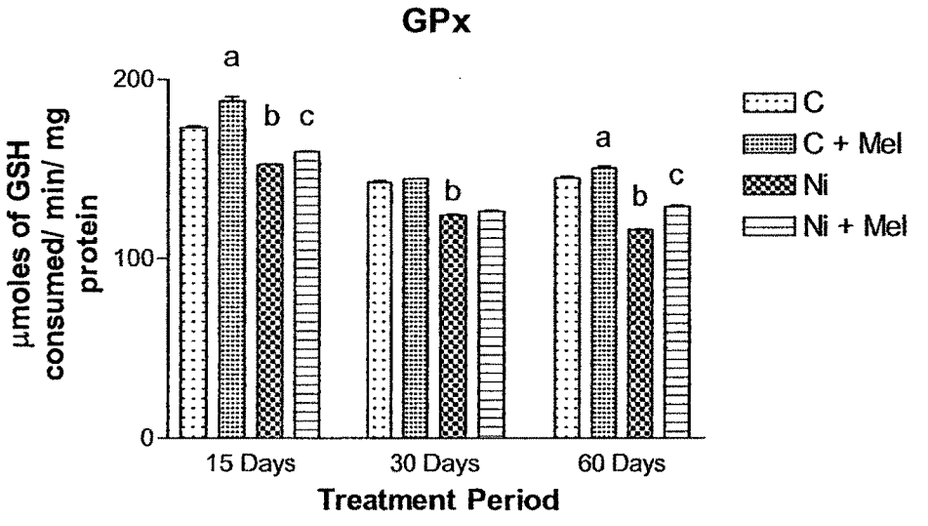
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.10: Nickel induced changes in Superoxide dismutase (SOD) activity (units/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	12.87 ± 0.25	14.23 ± 0.13[a]	9.16 ± 0.25[b]	10.78 ± 0.37[c]
30 Days	16.42 ± 0.17	17.39 ± 0.16[a]	8.64 ± 0.08[b]	13.29 ± 0.26[c]
60 Days	16.56 ± 0.17	18.94 ± 0.41[a]	6.64 ± 0.15[b]	9.17 ± 0.47[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.11: Nickel induced changes in Glutathione peroxidase (GPx) activity in testis with or without Melatonin.



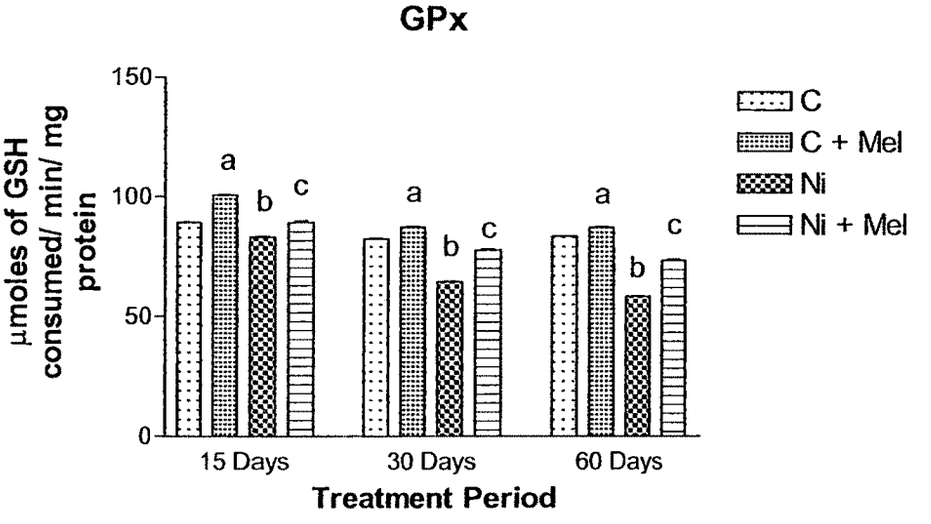
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.11: Nickel induced changes in Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	173.02 ± 1.10	188.09 ± 2.28[a]	152.54 ± 0.30[b]	159.83 ± 0.32[c]
30 Days	142.64 ± 0.93	144.35 ± 0.40	124.09 ± 0.56[b]	126.45 ± 0.72
60 Days	144.68 ± 1.21	150.38 ± 1.1[a]	116.15 ± 0.11[b]	128.93 ±0.66[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.12: Nickel induced changes in Glutathione peroxidase (GPx) activity in epididymis with or without Melatonin.



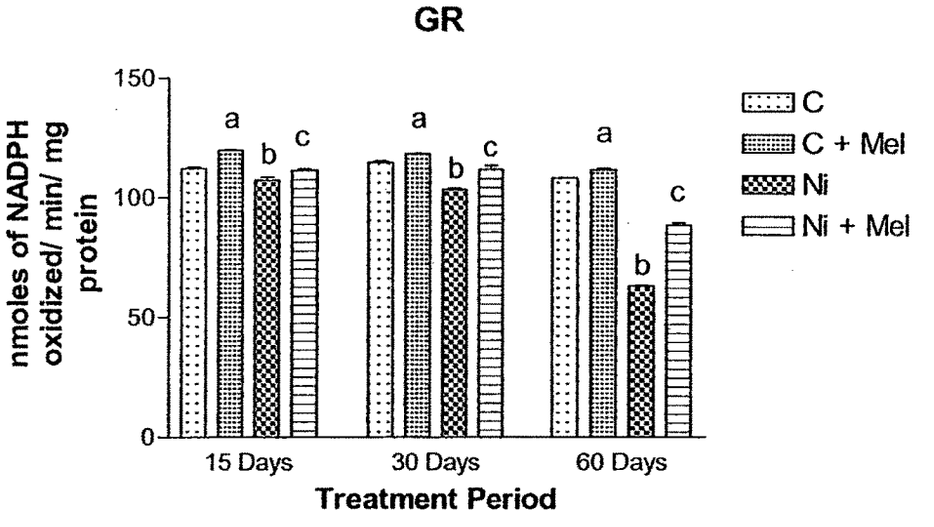
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.12: Nickel induced changes in Glutathione peroxidase (GPx) activity (µmoles of GSH consumed/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	89.17 ± 0.24	100.77 ± 0.18[a]	83.10 ± 0.39[b]	89.14 ± 0.81[c]
30 Days	82.26 ± 0.37	87.32 ± 0.18[a]	64.54 ± 0.16[b]	77.72 ± 0.36[c]
60 Days	83.43 ± 0.19	87.12 ± 0.32[a]	58.33 ± 0.08[b]	73.28 ± 0.53[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.13: Nickel induced changes in Glutathione reductase (GR) activity in testis with or without Melatonin.



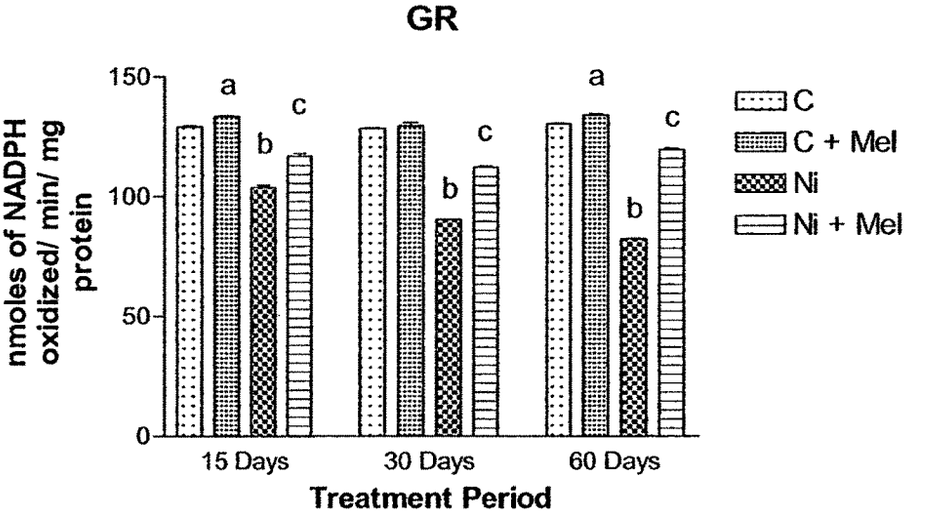
Values expressed as Mean ± SEM of 6 animals per group.
a $p<0.05$, compared with the control; **b** $p<0.05$, compared with the Control;
c $p<0.05$, compared with Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.13: Nickel induced changes in Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in testis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	112.35 ± 0.63	119.90 ± 0.21[a]	107.43 ± 1.23[b]	111.48 ± 0.70[c]
30 Days	114.87 ± 0.58	118.47 ± 0.09[a]	103.45 ± 0.59[b]	111.79 ± 1.72[c]
60 Days	108.34 ± 0.13	111.72 ± 0.71[a]	63.24 ± 0.23[b]	88.39 ± 1.04[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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Figure 3.14: Nickel 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.14: Nickel induced changes in Glutathione reductase (GR) activity (nmoles of NADPH oxidized/ min/ mg protein) in epididymis with or without Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	129.21 ± 0.36	133.45 ± 0.37[a]	103.78 ± 1.02[b]	116.87 ± 1.05[c]
30 Days	128.45 ± 0.39	129.53 ± 1.39	90.46 ± 0.07[b]	112.13 ± 0.57[c]
60 Days	130.43 ± 0.13	134.09 ± 0.53[a]	82.26 ± 0.26[b]	119.70 ± 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 Nickel
C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.15: Nickel induced percentage changes in nickel content of testis and epididymis with or without Melatonin.

Treatment	Testis		Epididymis	
	Ni	Ni + Mel	Ni	Ni + Mel
15 Days	20.41	7.75	37.79	35.63
30 Days	16.56	5.92	20.72	32.41
60 Days	19.90	25.00	31.35	34.68

C – Control; C + Mel – Control + Melatonin; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Table 3.16: Nickel induced changes in the serum titre of Melatonin(pg/ ml) with or with out Melatonin.

Treatment	C	C + Mel	Ni	Ni + Mel
15 Days	121	140	71	116
30 Days	112	143	78	104
60 Days	93	126	31	84

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; Ni – Nickel;
Ni + Mel – Nickel + Melatonin

Discussion:

There are only very few studies on male reproductive toxicology of Ni and most of them pertain to histopathological alterations and spermatogenic effects or testicular ascorbic acid concentration under protein deprivation (Pandey *et al.*, 1999; Das and Dasgupta, 2000). Though accumulation of Ni in testis has been demonstrated, the mechanism of its toxic effect on reproductive organs has not yet been elucidated (Kakela *et al.*, 1999; Obone *et al.*, 1999). Except for a single dose administration or short term multiple exposure of 3-5 days on certain aspects of oxidative stress in mice (Doreswamy *et al.*, 2004), the involvement of oxidative stress has not been evaluated at all. The present study not only addresses this lacunae but also tries to gain a realistic understanding of the effects of physiologically reluctant Ni exposure for human extrapolation by, designing a dosage and route of exposure keeping in with environmental load and mode of systemic entry. Accordingly, the study has revealed pronounced Ni induced oxidative stress in both testis and epididymis marked by duration dependent increase in LPO and depletion and inhibition of endogenous non-enzymatic and enzymatic antioxidants respectively. In a study dealing with DNA damage and genotoxic effects of Ni in mouse testis, Doreswamy *et al.* (2004) had shown a dose dependent increase in LPO by 25% and GPx activity by 27% on short term administration of nickel for 3-5 days. Based on their observations, they had concluded that Ni induced testicular dysfunction at lower sub-lethal doses is mediated through oxidative damage to macromolecules including DNA and apoptotic cell death. A recent study on the role of ascorbic acid supplementation on Ni induced lipid peroxidation; Gupta *et al.* (2007) have also shown decreased activity of all antioxidant enzymes and GSH concentration. This is

in keeping with the present observations and suggests the susceptibility of testis and epididymis to Ni induced oxidative stress.

It is of relevance that the present study using a realistic dosage simulatable to human situation has provided compelling evidence towards increasing oxidative stress on continuing exposure to nickel. Apparently, the vital interrelated steroidogenic and spermatogenic functions of testis are likely to be disrupted on persistent oxidative stress contributing in all probability to more infertility. It is known that Ni can generate oxidative stress like Cd, Cr and Hg but, the mechanism of Ni mediated generation of free radicals and oxidative stress is still obscure. Different hypotheses like non functioning of GSH by binding to –SH group, catalase inhibition by Ni induced NO, direct generation of minor amount of free radicals and iron displacement from membrane protein leading to Fenton reactions, are all suggested by different workers (Joshi *et al.*, 2004, Valko *et al.*, 2005)

One of the most common mechanisms by which Ni can induce oxidative stress is by rendering GSH molecules non-functional by binding to the –SH group and, a reduction in GSH content of both testis and epididymis recorded herein attests to this aspect. Simultaneous depletion of ascorbic acid suggests its participation in attempting to quell the increasing oxidative stress. The relatively higher levels of GSH, GPx and GR in testis compared to epididymis seem to offer a greater buffering capacity to resist oxidative stress in this organ, as it is involved in vital processes of spermatogenesis and steroidogenesis, so essential for male fertility. Generally, oxidative stress is generated by an imbalance between the degree of ROS generation and efficiency of the antioxidant system for its disposal. ROS generation occurs

naturally in all tissues during the normal oxidative metabolic reactions and a xenobiotic can cause oxidative stress by either increasing the rate of ROS generation or by decreasing the ability of antioxidant system or even by both. In the light of the known inefficiency of Ni to generate ROS by itself, the most likely mechanism of Ni induced oxidative stress has to be by interfering with the antioxidant machinery. One such interference as mentioned above is inactivation of GSH moiety by –SH binding; this is likely to affect the GPx enzyme thereby compromising the handling of H_2O_2. The milder depletion in GSH content as well as GR activity of testis suggest not only inefficient recycling of GSH by NADPH mechanism but also relative minor participation of GSH in the molecular ecology of Ni induced oxidative stress. The higher depletion of testicular ascorbic acid tends to suggest its relatively greater importance in nullifying Ni induced oxidative stress.

The markedly pronounced inhibition of SOD activity in both testis and epididymis compared to all other antioxidant enzymes indicates its greater vulnerability under Ni intoxication. As of now there is no known mechanism of Ni induced inhibition of SOD. However, an attempt is made here to make a far-fetching hypothesis of SOD inhibition by way of Ni binding to the Cu-Chaperonin for superoxide dismutase (CCS) which would prevent Cu loading to Cu-Zn SOD. This hypothesis presumes that CCS may have some homology with prokaryotic Ni-SOD and the presence of Cys-His residues permitting Ni binding to it which could alter its conformation and thereby prevent dimerization with SOD1 and affect the process of Cu loading. Whatever be the mechanism of inhibition of SOD, the consequence is inefficient handling of superoxide radical. Normally, hydrogen peroxide generated in cells is acted upon by CAT or GPx. Thus, greater inhibition of CAT than GPx in testis

provides evidence for CAT mediated decomposition of H_2O_2. Increased H_2O_2 generation can be considered feasible in reproductive organs by activation of enzymes like Xanthine oxidase, NADPH oxidase, etc. (Beauchamp and Fridovich, Kellogg and Fridovich, 1975) under Ni stress as, lower or depleted GSH levels of tissues (testis and epididymis) can lead to increased Ni load by altering the toxicokinetics of and/ or the type of Ni bio-ligand complex formed (Srivastava *et al.*, 1990). The stage is now well set for the generation of more rowdy $OH^{\bullet-}$ radical by the interaction of $O_2^{\bullet-}$ and H_2O_2. Increased accumulation of hydrogen peroxide can not only be related with upregulation of hydrogen peroxide generating enzymes, but also with the inhibition of catalase. The mechanism of inhibition of CAT by Ni is indirect by way of production of NO and binding to heme group of the enzyme (Joshi *et al.*, 2004; Gupta *et al.*, 2007). Since it is known that Ni can upregulate eNOS, nNOS and iNOS in different compartments of testis, Ni induced NO generation becomes very pertinent.

A conglomerate of these events together seems to decrease the antioxidant machinery of reproductive organs and thereby contribute to a heightened oxidative stress, which is reflected in a duration dependent increase in both testis and epididymis. On a comparative note based on the observations of this study, epididymis seems to be more dependant on GSH and GPx rather than ascorbic acid as in testis. Another point of relevance is that the Ni induced inhibition of antioxidant machinery seems to be of greater severity in epididymis which is well reflected in the recorded higher levels of LPO and, this might bespeak of nickel mediated interference in epididymal functions related to sperm maturation and motility.

In the present study, melatonin has been co-administered along with Ni to test its competence as a protectant against nickel induced oxidative stress. The results obtained suggest that melatonin has a highly potent ability not only to upregulate the antioxidant compounds when administered alone but also to protect and/ or nullify Ni induced inactivation/ inhibition of antioxidant machinery when co-administered with the metal. It is also clear that melatonin is efficient in nullifying duration dependent effect of Ni. Though different vitamins have been tried out as protectants or ameliorative agents in Ni toxicity, melatonin seems to be a more meaningful alternative as a therapeutic agent against Ni induced oxidative stress as, usage of higher doses of vitamins can have toxic manifestations of hypervitaminosis.

Overall, the present study records Ni induced oxidative stress in testis and epididymis with slightly differential adaptive mechanisms and relatively greater sensitivity of epididymis. The role of melatonin as an effective protectant against Ni induced oxidative stress is also brought out.