Impairment of normal spermatogenesis and sperm function are the most common cause of male infertility. Potential role of various kind of stresses generated by industrial and environmental pollutants on the male reproductive capacity has been a topic of great concern for the past few years. Oxidative stress has been also implicated by various researchers as a major causative factor of male infertility.

Selenium is an essential micronutrient and its physiological role was established by Rotruck et al., (1973). Selenium is essential for the normal functioning of male reproductive system and its deficiency has been associated with the testicular dysfunction and reduced sperm motility. Selenium is known to be involved in antioxidant defense mechanism via glutathione redox cycle (Tarp, 1994) and has direct role in the activity of selenoenzyme glutathione peroxidase. However, selenium at high concentration is extremely toxic also.

Considering above, the aim of the present study was to establish the role of selenium status in oxidative stress response in mice testis. Selenium status mice in three groups viz. selenium deficient, selenium adequate and selenium excess were created by feeding the respective diet for eight weeks.

The animals in selenium deficient group received 0.02ppm of selenium (group I) which is well below the nutritionally adequate level of 0.2ppm given to the animals in selenium adequate group (group II). Animals in the selenium excess group (group III) received 1ppm selenium diet which is considered to be in the supranutritional range but is well below the toxic levels (3-4ppm).
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No significant change in the body weight of the animals from the three groups was observed. Decrease in the glutathione peroxidase activity was seen in group I which is a direct result of selenium deficiency created in this group of animals. The glutathione peroxidase levels were unaltered in group III suggesting that incorporation of selenium in the enzyme is under the homeostatic control. Increased lipid peroxidation was seen in the selenium deficient group which substantiates the fact that glutathione peroxidase plays an important role in the free radical scavenging and also increased oxidative stress in the group I animals. Increase in lipid peroxidation was also observed in the group III animals, despite of no change in the glutathione peroxidase activity.

Other antioxidant enzymes in the testis were also analysed to study the oxidative stress status in the different groups. Increased activity of glutathione-S-transferase in the selenium deficient group suggested an adaptive response of the germ cells to stress. The enzyme activity was found to be unaltered in group III.

The glutathione redox ratio was altered in group I with the reduced GSH levels and increase in the oxidized glutathione. The redox ratio was also altered in group III with the increase in GSSG levels. The glutathione reductase, SOD and catalase activity were decreased in the selenium deficient condition. However the increased activity of glutathione reductase and SOD were observed in group III whereas there was no change in the catalase activity. Thus the present study indicates that selenium deficiency exerts oxidative stress in the testis, however, the excess selenium may also cause oxidative stress to some extent due to the generation of selenols. Further studies were designed to evaluate the influence of selenium and in turn the
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oxidative status on the reproductive activity of the animals in different groups.

The levels of serum FSH, LH and testosterone were significantly decreased in the group I suggesting the effect of selenium deficiency at the level of gonadal steroid biosynthesis or at the level of the hypothalamic pituitary unit resulting in the testicular pathology. No change in the various hormone levels in serum was seen in group III.

The direct effect of different selenium status on the reproductive activity was determined in terms of sperm motility, sperm concentration and fertility status of the treated male mice in three groups. Sperm motility and concentration were found to be severely reduced in selenium deficient diet fed group I, also a significant decrease in the percent fertility as well as the litter size was observed when treated males were mated with normal females. These results demonstrate the functional significance of the oxidative stress as generated by the selenium deficiency in group I and its role in the development of the male infertility. No significant change was observed in the sperm motility, litter size as well as the percent fertility in group III in comparison to group II.

Histopathological changes observed in the selenium deficient group I included shrinkage of seminiferous tubules, enlarged lumen and cell kinetic studies also revealed appreciable decrease in the number of pachytene spermatocytes suggesting a disruption in the meiotic process. A significant decrease in the spermatid and mature spermatozoa further suggest the effect of selenium deficiency generated oxidative stress at the maturation level of the germ cells. In group III also shrinkage of the seminiferous tubule was observed.
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along with the decrease in the central lumen. Displacement of the germ cell population observed in this group might be due to excessive selenite accumulation in the germ cells which may further affect the cell division and proliferation.

LDH-X is a specific marker for the testis and LDH-X levels in the testis have been linked directly to the germ cell status in the seminiferous epithelium. A marked decrease in the LDH-X levels was observed in group I both with the ELISA and western immunoblot analysis. Immunohistochemical localization for LDH-X also revealed a faint expression of the enzyme around the central lumen whereas negligible staining was observed in other regions of the seminiferous tubule. Thus suggesting that oxidative stress generated due to deficient selenium condition in group I is affecting the process of spermatogenesis which in turn is being reflected in terms of reduced LDH-X levels. In the selenium excess group III, the LDH-X level was found to be comparable to group II and the immunohistochemical localization demonstrated the presence of LDH-X in the seminiferous tubule from pachytene spermatocytes onward to spermatozoa suggesting that 1ppm dose of selenium as sodium selenite was not sufficient to cause the change in the LDH-X levels in the testis.

To evaluate the level of stress in the mice testis on various selenium diet feeding, the stress proteins or the heat shock proteins were analyzed by ELISA, western immunoblot analysis and immunohistochemical localization. Western immunoblot analysis for hsp70 revealed a single band of 70kDa in all the three treatment groups. The direct quantitation of hsp70 by ELISA revealed a higher level of hsp70 in group I animals in comparison to group II suggesting excessive stress. The immunohistochemical analysis revealed very intense expression of hsp70 in the spermatid and the sperm head
region of the seminiferous tubules whereas the expression in the other
regions of the tubule was very less suggesting a stage specific
expression of hsp70 in the seminiferous tubule and the greater
vulnerability of the spermatids and the spermatozoa to the oxidative
stress. Group III animals did not show any significant change in the
hsp70 levels when compared with the selenium adequate diet fed
group II animals. Immunohistochemical localization also suggested
expression comparable to the group II animals.

Immunohistochemical analysis for hsp60 in both selenium
deficient diet fed group I and selenium excess group III animals
revealed increased expression of heat shock protein in mice testis. The
hsp90 expression was found to be enhanced in the seminiferous
tubules in group I in comparison to group II which may be due to the
oxidative stress. The stress protein expression was also increased in
selenium excess diet fed group III animals which may be attributed to
the excessive accumulation of the selenite in the cell and this may
indirectly interfere with the various biochemical and metabolic
pathways. The oxidative stress generated as a result of redox cycling
of selenide with oxygen leading to the oxidation of thiols may also be
responsible for the increased expression of stress proteins in this
group.

The genotoxic damage caused to the germ cells in response to
the various diet treatments, in term of DNA damage was studied by
DNA fragmentation analysis. The genomic DNA was found to be
intact in the selenium adequate diet fed group II animals. DNA
fragmentation analysis in group I revealed that oxidative stress
conditions as created by selenium deficiency might have a detrimental
effect on the genomic DNA. The DNA fragmentation was also
observed in the selenium excess group which is the result of the
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activity of catalytic selenols generated as a result of excessive selenium supplementation.

In conclusion, the results of the present study clearly demonstrate the role of the different selenium status in the generation of the oxidative stress in the testis and consequently its effect on the antioxidant defense mechanism, histoarchitecture of the testis, the stress protein expression and the reproductive ability of the animals.

Significant alteration in the antioxidant enzyme status was seen in both the group I and III resulting in the different level of oxidative stress in the different group. The stress response in terms of heat shock protein expression suggested a stage specific expression of the hsp70 and that certain cell stages of the spermatogenic process are more vulnerable to the oxidative stress as compared with other stages in the seminiferous tubule.

Since male germ cells carry the paternal half of the genetic material, the genotoxic damage caused to the germ cells in the group I and group III as a consequence of oxidative stress might in turn effect the reproductive ability and result in birth defects in progeny.

The results of the present study clearly demonstrate that selenium at different levels regulates the oxidative stress status which in turn influence the male reproductive activity.