

EFFECTS OF DIETARY SELENIUM (SE) ON MORPHOLOGY OF TESTIS AND CAUDA EPIDIDYMIS IN RATS

R. KAUR* AND K. KAUR

Department of Zoology,
Punjab Agricultural University,
Ludhiana - 141 004

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Abstract : Selenium is an essential micronutrient for animals. To determine whether its excess in diet induces morphological changes within the male reproductive system, a detailed qualitative and quantitative evaluation of the changes in the histology of the testis and cauda epididymis was undertaken in male rats. Adult male albino rats were fed 6 and 8 ppm Se in diet for 6 and 9 weeks. Each male consuming 6 ppm Se was mated with two untreated females, their offsprings were allowed to mature upto 12 weeks of age. The testes and cauda epididymes of male rats were prepared for light microscopy. Excess of dietary Se caused dose--time-dependent reduction in body weight and reproductive organ weights but increase in number of morphologically abnormal spermatozoa. Histopathological studies of the testes and cauda epididymis have revealed that Se-rich diets cause dose-time-dependent reduction in tubular diameter, epithelial height, number of spermatogenic cells and disintegration of cellular associations in the seminiferous tubules of testes along with reduction in the diameter of cauda epididymal tubules and pseudostratification of their epithelial lining. Progeny (feeding on normal diet) of paternally treated rats has shown retarded growth.

Key words : testes
selenium

cauda epididymis
paternal offsprings

INTRODUCTION

Se is a micronutrient essential for animals (1) and plays a crucial role in maintenance of human health and reproduction (2). This is because of the fact that Se is an essential component of the enzyme glutathione peroxidase (3) required for the functional integration of the cell membranes and plays an important role in maintaining spermatogenesis and sperm morphology (4).

Investigations have shown that the testis is one of the main target organs for Se and plays an important role in the male reproductive system. Se concentration in testis is the highest among the tissues of adult rats (5). Se in the testis is regulated by gonadotrophic hormones (6) and its concentration increases considerably during puberty with onset of spermatogenesis (7). Se is also necessary for testosterone biosynthesis and normal development of spermatozoa (8, 9). A greater amount of the

*Corresponding Author

mineral is taken by testicular interstitial tissue than by developing spermatozoa (10). The administration of radioactive Se (^{75}Se) has resulted in its accumulation in the midpiece of spermatozoa (11) in the outer membrane of sperm mitochondria in the form of a specific selenoprotein (12). Rats fed 4 ppm Se containing diet for over 5 weeks produced spermatozoa with impaired motility and morphological abnormalities that frequently occurred in the midpiece region of flagella (13). In human, a high Se level in the diets of both the partners and high semen Se levels ($<80\text{ ng/ml}$) are associated with a high rate of abortion and female reproductive failures but the pregnancy rate is not affected (2). *In vitro* studies on rat embryos indicate that postimplantation embryos are susceptible to Se teratogenicity (14).

Animal studies from Se rich areas of Punjab and Haryana (India) have shown that Se poisoning or chronic exposure to otherwise non-toxic doses provoke decreased productivity, anoestrus or delayed onset of oestrus, failure of conception, abortion and ultimately death of animals (15–17). The plant species of crops, vegetables and fodder of this area accumulate Se ($6.55 - 244.4\text{ mg/kg}^{-1}$) which is more than the upper toxic limits of 5 mg Se kg^{-1} (16).

There are some indications that ingestion of excess of Se effects the testicular morphology (8, 18) but the detailed information on the nature and severity of these lesions in testis and cauda epididymis is scarce. Therefore, present investigations were aimed to study the effects of excess of Se ingestion on the morphology of reproductive organs of male rats.

METHODS

Adult male and female albino rats were acclimatized for 7–10 days in the laboratory. During the period of acclimatization and experiments, all rats were offered pellets of rat feed in crushed form (CPF) supplied by Lipton India Ltd., Calcutta and water *ad libitum*. The pellets were crushed to ensure proper mixing of the required amount of Se in the food. Male rats were divided into three groups with twelve rats in each group. First group of male rats was fed CPF without addition of sodium selenite and those of second and third groups were offered CPF containing 6 and 8 ppm sodium selenite for 6 and 9 weeks. Gain and loss in body weight of animals was measured at weekly intervals. Morphological changes in their appearance and behaviour were observed daily. On day 43 and 64, rats were killed by using chloroform vapours. Their testes and cauda epididymes were dissected out and weighed. Testis and cauda epididymes of one side were fixed in aqueous Bouin's fluid, dehydrated, cleared, embedded, sectioned ($5\text{ }\mu\text{m}$) and counter stained with haematoxylin and eosin (19). These slides were studied under light microscope. The diameters of seminiferous tubules and thickness of seminiferous epithelium were measured using an ocular micrometer from twenty five stained sections of seminiferous tubules selected at random from three slides of each rat. Number of different spermatogenic cells were counted at stage 8a (20) and number of elongated spermatids embedded in Sertoli cells at stage 7 (21) of the seminiferous epithelial cycle. Changes in the morphological characteristics of the cellular associations of seminiferous tubular

epithelium were noticed. The diameters of the cauda epididymal tubules, their epithelial heights and lumen diameters were measured. Structural changes in their epithelium were also observed.

The cauda epididymal fluid was stained with eosin-nigrosin solution and smears were drawn on clean grease free slides, air dried and mounted in DPX. Three slides of each rat were studied for morphological abnormalities in different regions of spermatozoa under the light microscope, from each slide atleast 100 spermatozoa were examined, that is, about 300 spermatozoa/rat.

Males treated with 6 ppm Se containing diet for 6 and 9 weeks were mated with untreated cyclic females for one week. Cohabitated females were allowed to complete their gestation period. Offsprings of these parents were allowed to feed on CPF without addition of sodium selenite upto 12 weeks. Birth weight and gain in body weight of male pups were recorded at weekly intervals upto 12 weeks.

The data collected were subjected to statistical analysis by two-way ANOVA test. The significance between variants was determined by Student's t-test and was considered significant when P values were ≤ 0.05 .

RESULTS

The rats feeding on diets containing 6 and 8 ppm Se showed loss of body fur at certain points and reddening of claws started after 6 weeks of dietary Se

treatment and became more prominent as the duration of feeding was prolonged to 9 weeks and loss of vision in one animal.

The gain/loss in body weight recorded at the end of each week has been depicted in Fig. 1. Ingestion of 6 and 8 ppm Se caused a significant reduction in body weight of rats as compared to controls. However, control rats continued to gain weight. Progeny of paternally treated rats and control rats did not show a significant difference in their birth weights but from week 7 to 12, the

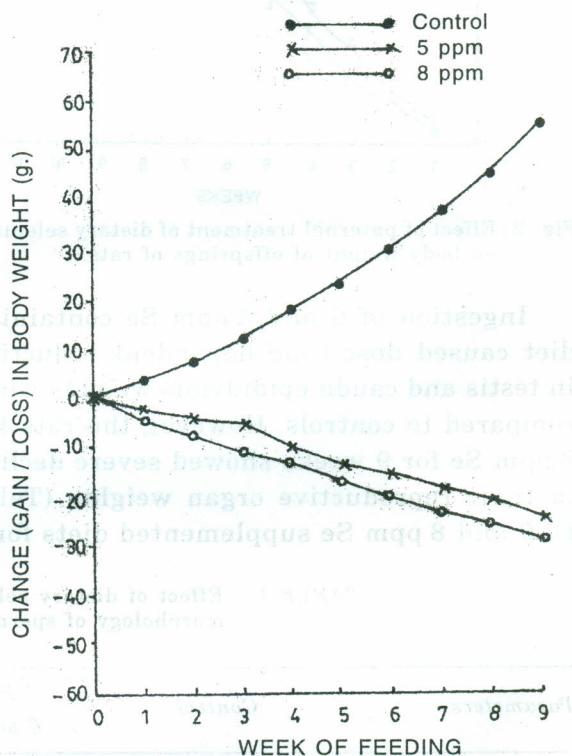


Fig. 1: Change in body weight (g.) due to consumption of selenium in rats.

difference became significant (Fig. 2) showing retarded growth in offsprings. However, the offsprings of untreated male parents had normal growth.

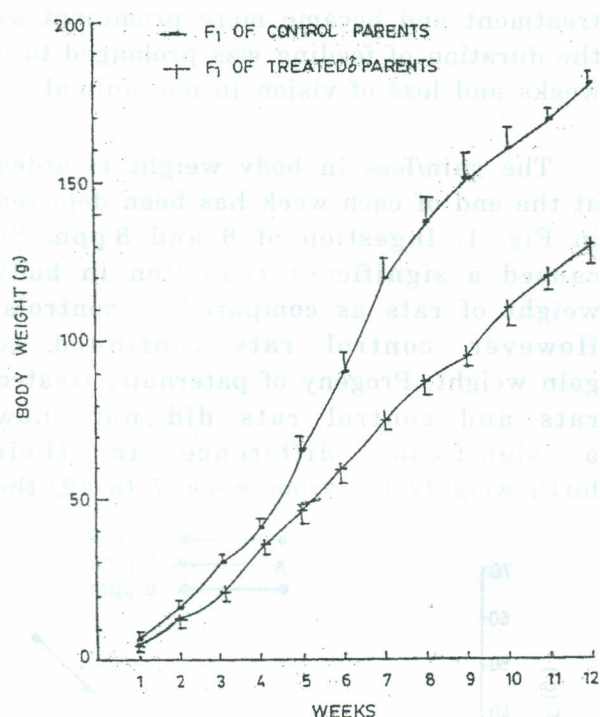


Fig. 2 : Effect of paternal treatment of dietary selenium on body weight of offsprings of rats.

Ingestion of 6 and 8 ppm Se containing diet caused dose-time dependent reduction in testis and cauda epididymis weights when compared to controls. However, the rats fed 8 ppm Se for 9 weeks showed severe decline in their reproductive organ weights (Table I). 6 and 8 ppm Se supplemented diets for 9

weeks severely affected the semen quality as the number of abnormal forms of spermatozoa increased 4 and 5 times respectively (Table I).

The pathomorphological changes in the seminiferous tubules and interstitium are depicted in Fig. 3. A considerable decrease in the seminiferous tubule diameter, their epithelial heights and reduction in number of spermatogenic cells have been observed in Se-treated rats whereas 8 ppm Se for 9 weeks caused severe pathomorphological changes. (Table II and Fig. 3). The degenerative changes in the testis were not uniform. These tubules with degenerative activities were in patches with varied degree of degeneration and the seminiferous tubules adjacent to the necrosed ones were apparently normal and severely affected tubules showed the presence of only preleptotene cells (Fig. 3B). Sloughing off the gametogenic epithelial elements started from elongated spermatids and ended with pachytene spermatocytes (Fig. 3C-D). The interstitial tissue appeared relatively more widened and a dose-time-dependent reduction in the size of the Leydig cells was observed in treated rats (Fig. 3F).

TABLE I: Effect of dietary selenium on reproductive organ weights and morphology of spermatozoa in rats (Mean \pm S.E. of 6 rats).

| Parameters | Control | 6 ppm seleniferous feed | | 8 ppm seleniferous feed | |
|--|-----------------|------------------------------|--------------------------------|-------------------------|---------------------------------|
| | | 6 weeks | 9 weeks | 6 weeks | 9 weeks |
| Testicular weight (g/100 g, b.wt.) | 0.57 \pm 0.03 | 0.54 \pm 0.01 | 0.41 \pm 0.05 ^{ab} | 0.47 \pm 0.07 | 0.27 \pm 0.04 ^{abcd} |
| Cauda epididymis weight (g/100 g, b.wt.) | 0.10 \pm 0.00 | 0.08 \pm 0.00 ^a | 0.07 \pm 0.01 ^a | 0.07 \pm 0.01 | 0.04 \pm 0.01 ^{abcd} |
| Abnormal spermatozoa (%) | 1.62 \pm 0.13 | 3.46 \pm 0.57 ^a | 7.05 \pm 0.67 ^{abd} | 4.91 \pm 0.63 | 8.91 \pm 0.42 ^{abd} |

Significantly different from ^acontrol; ^b6 ppm 6 weeks; ^c6 ppm 9 weeks; ^d8 ppm 6 weeks at P \leq 0.05

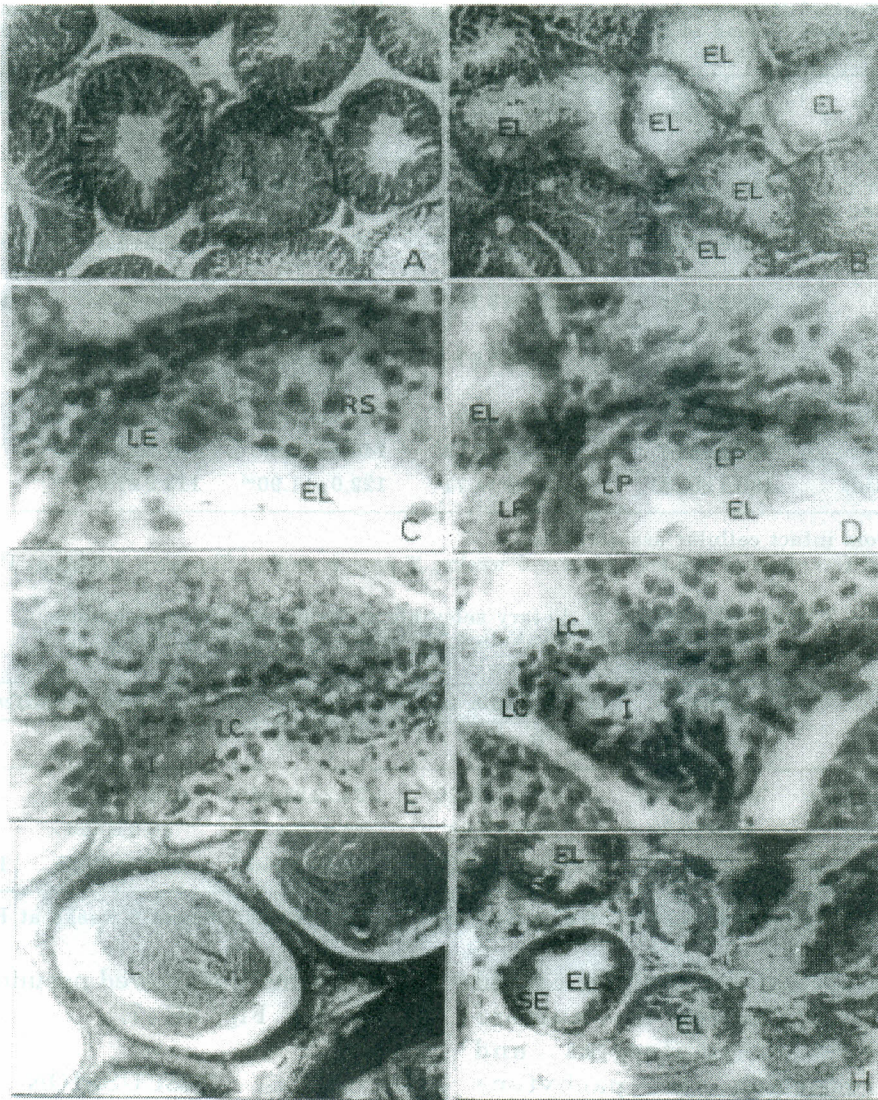


Fig. 3 : Morphological changes in the testis and cauda epididymis of rat

- A - Normal seminiferous tubules of control rat with lumen full of spermatozoa.
- B - Seminiferous tubules of Se treated rats showing reduction in tubular diameters and disappearance of spermatogenic cells.
- C - Seminiferous tubular epithelium of treated rat without elongated spermatids and few round spermatids.
- D - Seminiferous tubular epithelium of treated rats showing presence of only preleptotene spermatocytes and empty lumen.

- E - Interstitium showing normal Leydig's cells in control rat.
 - F - Interstitium relatively widened with reduced size of Leydig's cells in treated rats.
 - G - Normal cauda epididymal tubules of control rat.
 - H - Tubules showing reduction in diameter pseudostratified epithelium and tubular lumen without spermatozoa in selenium treated rats.
- Abbreviations: L - lumen, E - empty lumen, PS - pachytene spermatocyte, RS - round spermatid, LP - preleptotene spermatocyte, LE - epithelial lesions, LC - Leydig's cell, I - interstitium, SE - pseudostratified epithelium.

TABLE II: Effect of dietary selenium on seminiferous tubules (μm) and number of spermatogenic cells per cross section of the tubule in the testis of rats (values are mean \pm S.E. of 6 rats).

| Parameters | Control (plain feed) | 6 ppm seleniferous feed | | 8 ppm seleniferous feed | |
|---|-------------------------|--------------------------------|---------------------------------|---------------------------------|-----------------------------------|
| | | 6 weeks | 9 weeks [†] | 6 weeks [†] | 9 weeks [†] |
| Testicular diameter of seminiferous tubules | 245.4 \pm 1.65 | 240.8 \pm 2.62 | 202.0 \pm 5.51 ^{ab} | 212.7 \pm 13.71 ^{ab} | 185.34 \pm 12.43 ^{abc} |
| Height of seminiferous epithelium | 60.6 \pm 0.60 | 54.8 \pm 2.01 ^a | 45.1 \pm 0.61 ^{ab} | 52.0 \pm 2.67 ^{ac} | 40.51 \pm 2.63 ^{ab} |
| Lumen diameter Spermatogenic cells | 125.2 \pm 1.48 | 121.6 \pm 3.11 | 119.4 \pm 2.46 | 108.3 \pm 8.94 ^{ab} | 104.32 \pm 2.30 ^{abc} |
| Preleptotene spermatocyte | 39.0 \pm 1.31 | 36.6 \pm 5.01 | 34.8 \pm 0.90 | 40.3 \pm 3.53 | 32.4 \pm 3.20 |
| Pachytene spermatocytes | 61.6 \pm 2.13 | 57.10 \pm 1.50 | 51.8 \pm 0.49 | 48.2 \pm 8.24 ^a | 42.1 \pm 2.33 ^{ab} |
| Round spermatids | 202.2 \pm 8.87 | 184.8 \pm 22.79 | 140.3 \pm 2.16 ^a | 131.2 \pm 24.36 ^{ab} | 123.9 \pm 2.12 ^{ab} |
| Elongated spermatids | 198.1 \pm 6.65 | 159.9 \pm 14.71 ^a | 122.0 \pm 11.90 ^{ab} | 113.9 \pm 7.93 ^{abc} | 93.3 \pm 3.15 ^{abcd} |

[†]Values are from intact cellular associations.

Significantly different from ^acontrol; ^b6 ppm 6 weeks; ^c6 ppm 9 weeks; ^d8 ppm 6 weeks at P \leq 0.05

TABLE III: Effect of dietary selenium on cauda epididymis tubules (μm) of rats (Values are mean \pm S.E. of 6 animals).

| Parameters | Control | 6 ppm seleniferous feed | | 8 ppm seleniferous feed | |
|------------------------|-------------------|--------------------------------|--------------------------------|-------------------------|-----------------------------------|
| | | 6 weeks | 9 weeks | 6 weeks | 9 weeks |
| Cauda tubular diameter | 332.4 \pm 12.87 | 273.3 \pm 19.20 ^a | 263.2 \pm 27.06 ^a | 278.4 \pm 19.79 | 182.9 \pm 17.47 ^{abcd} |
| Epithelial height | 14.9 \pm 0.39 | 15.2 \pm 0.83 | 12.9 \pm 0.67 ^a | 15.0 \pm 0.50 | 14.8 \pm 0.47 |
| Tubular lumen diameter | 302.6 \pm 12.23 | 242.9 \pm 20.22 ^a | 237.4 \pm 26.06 ^a | 248.3 \pm 20.65 | 152.6 \pm 17.78 ^{abcd} |

Significantly different from ^acontrol; ^b6 ppm 6 weeks; ^c6 ppm 9 weeks; ^d8 ppm 6 weeks at P \leq 0.05

Ingestion of excessive Se in diet caused non-significant reduction in number of preleptotene spermatocytes and significant reduction in pachytene spermatocytes, round spermatids and elongated spermatids (Table II). Therefore, quantitative analysis has shown that the early and most affected cells are the post-meiotic cells (round and elongated spermatids) and the least affected cells are preleptotene cells.

As compared to controls, the diameter of cauda epididymal tubules and their lumen were significantly reduced after 6 and 8 ppm Se consumption for 6 and 9 weeks whereas

their epithelium showed pseudostratification (Table III, Fig. 3G, H).

DISCUSSION

A dose-time-dependent decrease in body weight, shedding of fur and nails occurred in all groups of rats ingesting seleniferous diets. Unlike the control rats, treated rats did not grow. One of the treated rats was blind. Progeny of paternally treated rats has also shown retarded growth. These results provide an indication that the general health of the treated rats was impaired which might be due to the increase in the Se content in the body organs. Progressive

deterioration in the general health of fifty per cent of human living in the seleniferous areas of Punjab (India) have been attributed to Se poisoning (17). Se content in the blood, hair and hooves of cattle and buffalo of this region was found to be 47, 211 and 47 times higher than that of normal animals and bilateral cataract has also been observed in one buffalo (22). Loss of hair, malformation of fingers and nails, skin lesions followed by ulceration and nervous disorders in human residing in seleniferous areas are considered common signs of Se poisoning (23, 17). Large doses of Se reduced the serum levels of somatotrophic hormones leading to growth retardation in rats (24).

The results of present study reveal that 6 and 8 ppm Se in diet manifested two principal impacts on the male reproductive organs of rats which are antispermatogenic and antiandrogenic. The antispermatogenic effect is reflected in the cessation of spermatogenesis, sloughing off of the germ cells, absence of spermatozoa in the seminiferous tubules and presence of empty lumen in some of the testicular and epididymal tubules. Interference of excessive Se in gametogenic activities of testis may probably be mediated via gonadotropins. Accumulation of Se in anterior pituitary of rats exposed to sodium selenite has already been reported (25, 26).

The antiandrogenic activity of Se exposure is reflected in regression in size of Leydig cells, the regressive and degenerative changes in the cauda epididymis and morphometric studies of testicular tissue showing reduction in the number of pachytene spermatocytes, round and elongated spermatids because such changes are attributed to androgen deprivation (27). Studies in our laboratory (26) have

shown that excessive Se in diet caused disturbance in metabolic processes of testis which includes a marked increase in its cholesterol content which implies inhibition of steroidogenesis in Leydig cells.

In the Leydig cells, glutathione peroxidase (GPX) has been localized immunocytochemically in the cytoplasm in close relationship to the smooth endoplasmic reticulum (28) and it is suggested that the metabolic pathway of testosterone biosynthesis requires protection against peroxidation and is thus affected by a decrease in the activity of this enzyme (9). It may be possible that the testicular morphology was affected only indirectly via the decrease in testosterone production. In addition to GPX, several other Se-containing proteins have been found in the male gonads (29, 30) and it may be possible that testicular lesions observed in selenosis (toxicity due to excessive Se consumption) are due to the decrease in the biological activity of more than one Se-compound. Similar type of testicular lesions have also been reported in Se deficient rats (9) which shows that its excess and deficiency results in the same type of abnormalities in the morphology of testis and spermatozoa but the mechanisms responsible for the appearance of abnormalities in male gonads need further clarification.

The effect of dietary Se on sperm morphology was found to be time and dose dependent. An appreciable increase in the sperm head abnormalities may be due to genetic alterations induced by Se. Topham (31) stated that changes in sperm head morphology is the indication of alterations in the testicular DNA and genetic damage in the whole animal.

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