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EFFECT OF EXOGENOUS SELENIUM ON THE TESTICULAR TOXICITY INDUCED BY ETHANOL IN RATS

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Abstract : The effects of supplementation of selenium at a dose of 10 µg/kg body weight were investigated on ethanol induced testicular toxicity in rats. In the present study, four groups of male albino rats were maintained for 60 days, as follows : (1) Control group (normal diet) (2) Ethanol group (4g/kg body weight) (3) Selenium (10 µg/kg body weight) (4) Ethanol + Selenium (4g/kg body weight + 10 µg/kg body weight). Results revealed that ethanol intake caused drastic changes in the sperm count, sperm motility and sperm morphology. It also reduced the levels of testosterone and fructose. The activities of 3βHSD, 17βHSD in the testis and SDH in the seminal plasma were also reduced. Lipid peroxidation was also enhanced as the lipid peroxidation products were increased and the activities of the scavenging enzymes were reduced. But on coadministration of selenium along with alcohol all the biochemical parameters were altered to near normal levels indicating a protective effect of selenium. These results were reinforced by the histopathological studies.

Key words : selenium ethanol sperm testes lipid peroxidation fructose testosterone

INTRODUCTION

Alcohol consumption is injurious to health and it is one of the major health problems throughout the world. Increased oxidative stress is a well accepted mechanism of alcohol induced tissue injury in the heart, liver, central nervous system and particularly in the testes. (1, 2, 3, 4). Ethanol is a direct testicular toxin (5). It causes atrophy of seminiferous tubules, loss of sperm cells and an increase in abnormal sperms (6). It can also cause significant deterioration in sperm concentration, sperm output and motility (7, 8).

Selenium is an essential micronutrient which has an important role in the regulation of reproductive system. Nutritional studies indicate that selenium is essential for male fertility (9, 10). Selenium is found in the outer membrane of the sperm mitochondria in the form of specific selenoproteins (11). Selenium is

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essential for the formation of phospholipid hydroperoxide glutathione peroxidase, the enzyme that is present in the spermatids, which become a structural protein comprising over 50% of the mitochondrial capsule in the mid-piece of the mature spermatozoa. Thus a deficiency of dietary selenium leads to immotile, deformed sperms and infertility in rats (12, 13). It also functions as an antioxidant.

The detailed informations on the role of selenium on ethanol induced testicular toxicity are not known. Therefore present studies were aimed to investigate the effect of supplementation of exogenous selenium on ethanol induced testicular toxicity in rats.

MATERIALS AND METHODS

Animals

Male albino rats (Sprague Dawley) weighing between 90 and 110 g were divided into four groups of 6 rats each. Animals were housed in polypropylene cages. Cages were kept in a room that was maintained between 28 and 32°C. The light cycle was 12 h light and 12 h dark. Animals were handled using the laboratory animal welfare guidelines (14). Rats were fed with rat feed *(Lipton India Ltd.). Food and water was given *ad libitum*. Sodium selenite and ethanol was administered as detailed below. Sodium selenite was freshly dissolved in distilled water and ethanol diluted in the ratio 1:1 was given orally by gastric tube. Control rats received the same volume of water. The caloric intakes in different groups were adjusted by supplementing glucose to group I and group III. The duration of the experiment was 60 days.

Experimental design :

Group I (Control) :

Group II Ethanol treated rats (4 g ethanol/kg body weight)

Group III. Selenium treated rats (10 µg sodium selenite/kg body weight)

Group IV Ethanol + Selenium treated rats (4 g ethanol/kg body weight + 10 µg sodium selenite/kg body weight)

At the end of the experimental period the rats were fasted overnight and sacrificed. Blood and tissues were removed to ice cold containers for various estimations.

Biochemical analysis

Malondialdehyde (MDA) was estimated by the method of Hiroshi Ohkawa (15). Hydroperoxides (HP) were estimated by the method of Mair and Hall (16) and conjugated dienes (CD) were estimated by the method of Recknagel and Ghoshal (17). Tissue protein was estimated by the method of Lowry et al (18). Super oxide dismutase was estimated by the method of Kakkar et al (19). Catalase was assayed by the method of Maehly and Chance (20). The activity of glutathione reductase (GR) was determined by the method of David and Richard (21). The activity of glutathione peroxidase (GPx) was determined by the method of Lawrence and Burk (22) as modified by Agergurd and Jense (23). The tissues were extracted according to the procedure of Folch et al (24) and the cholesterol was estimated by the method of Abell's et al (25). The assay of 3β hydroxy steroid dehydrogenase (3βHSD) was

done by the method of Shivanandappa and Venkatesh (26). 17β hydroxy steroid dehydrogenase (17βHSD) was estimated by the method of Jarabak (27). Activity of sorbitol dehydrogenase (SDH) was determined by the method of by Wootten (28) in the seminal vesicle. The fructose in the seminal plasma was estimated by the method of Karvoven and Malm (29). Glutathione content was determined by the method of Patterzon and Lazarow (30). The serum testosterone was estimated by RIA method using the kit supplied by BRIT India. For hispathological study testes fixed in 10% buffered formalin was embedded in the paraffin wax and sections were taken in the microtome. Sections were stained using haematoxylin and eosin. The pathological changes were examined using a sensitive light microscope.

Statistical analysis

The results were analysed using a statistical programme SPSS/PC+, Version 5.0 (SPSS Inc., Chicago, IL, USA). A one-way ANOVA was employed for comparison among the six groups. Duncan's post-hoc multiple comparison tests of significant differences among groups were determined, $P<0.05$ was considered to be significant.

RESULTS

Body weights and testis weight of the rats (Table I) were decreased in the ethanol administered group, when compared to the control. On Selenium supplementation and in the group treated with Selenium and ethanol, the weight were increased in comparison to the ethanol group.

TABLE I: Body weight and testis weight (gms).

Groups	*Body*	*Testis*
Control	181.85±16.59	0.83±0.07
Ethanol	147.69±13.47[a]	0.44±0.04[a]
Selenium	182.69±16.66[b]	0.81±0.07[b]
Ethanol+selenium	161.45±14.72[c]	0.66±0.06[c]

[a]P<0.05 between control and ethanol groups
[b]P<0.05 between ethanol and selenium groups
[c]P<0.05 between ethanol and ethanol+selenium groups
Values expressed as mean±SD
Body weight (F-7.25)
Weight of testes (F-48.405)

The concentration of cholesterol (Table II) increased significantly in the testes of the ethanol treated group when compared to the control. Whereas in the group treated with ethanol and Selenium showed a significant decrease when compared to the ethanol group. The levels of serum testosterone (Table II) decreased significantly in the ethanol treated group compared to the control group. In the group treated with ethanol and selenium and selenium treated group the level of testosterone was increased significantly when compared to the ethanol group.

TABLE II: Concentration of cholesterol (mg/100 g tissue) and levels of serum testosterone.

Groups	*Testes (mg/100 g tissue)*	*Testosterone (ng/ml)*
Control	206.09±18.80	2.9±0.27
Ethanol	262.79±23.96[a]	1.68±0.15[a]
Selenium	205.98±18.78	3.52±0.32[b]
Ethanol+selenium	234.84±21.42[c]	2.87±0.26[c]

[a]P<0.05 between control and ethanol groups
[b]P<0.05 between ethanol and selenium groups
[c]P<0.05 between ethanol and ethanol+selenium group
Values expressed as mean±SD
Testes-cholesterol (F-10.463)
Serum testosterone (F-53.238)

The concentration of MDA, HP, and CD (Table III) were increased significantly in the testes of the ethanol treated group compared to the control. But there was a significant decrease in the group treated with ethanol and Selenium when compared to the ethanol group. On administration of selenium there was no significant change compared to the control. There was decrease in glutathione content in the alcohol group. In the group treated with ethanol and Selenium glutathione content increased in comparison with the alcohol group.

The activities of catalase, SOD and glutathione reductase (Table IV) were decreased significantly in the ethanol treated group when compared to the control. But there was a significant increase in the enzyme activity of the group in comparison with the ethanol treated group. On administration of selenium the activity of catalase, SOD and glutathione reductase increased when compared to the ethanol treated group. The activity of glutathione peroxidase (Table IV*)* increased significantly in the ethanol treated group compared to the control, while the group treated with Selenium and ethanol showed a decrease in the activity of glutathione peroxidase when compared to the ethanol group. The selenium treated group showed no significant change

TABLE III : Testicular concentrations of malondialdehyde, hydroperoxides, conjugated dienes and glutathione.

Groups	*Malondialdehyde (mmol/100 g tissue)*	*Hydroxyperoxides (mmol/100 g tissue)*	*Conjugated dienes (mmol/100 g tissue)*	*Glutathione (mmol/100 g tissue)*
Control	0.11±0.01	12.62±1.59	25.29±2.30	69.92±6.37
Ethanol	0.19±0.02[a]	26.13±2.38[a]	60.24±5.48[a]	40.38±3.69[a]
Selenium	0.11±0.01	13.21±1.19	25.87±2.36	68.23±6.21
Ethanol+selenium	0.16±0.01[b]	18.34±1.66[b]	30.70±2.78[b]	59.15±5.39[b]

[a]P<0.05 between control and ethanol groups
[b]P<0.05 between ethanol and ethanol+selenium groups
Values expressed in mean±SD
Testes MDA (F-13.368) Testes HP (F-83.54) Testes CD (F-136.790) Testes-Glutathione (F-36.120)

TABLE IV : Activities of catalase, super oxide dismutase, glutathione reductase and glutathione peroxidase in the testes.

Groups	*Catalase (Units[#]/mg protein)*	*SOD (Units*/mg protein)*	*Glutathione reductase (Units[@]/mg protein)*	*Glutathione peroxidase (Units[@]/mg protein)*
Control	9.41±0.86	0.15±0.13	1.85±0.17	0.67±0.06
Ethanol	5.92±0.54[a]	0.06±0.003[a]	1.06±0.52[a]	0.89±0.08[a]
Selenium	9.44±0.86[b]	0.18±0.02[b]	1.41±0.13	0.66±0.06
Ethanol+selenium	8.28±0.75[c]	0.12±0.009[c]	1.24±0.10[c]	0.62±0.06[c]

[a]P<0.05 between control and ethanol groups
[b]P<0.05 between ethanol and selenium groups
[c]P<0.05 between ethanol and ethanol+selenium groups
Values expressed as mean±SD
[#]Units – Velocity constant/s
*Units – Enzyme concentration required to inhibit the chromogen production by 50% in one min.
[@]Units – 1 µ mol NADPH oxidized/min.
Catalase Testis (F-28.064) SOD Testes (F-127.980)
Glutathione reductase Testes (F-42.070) & Glutathione peroxidase Testes (F-21.039)

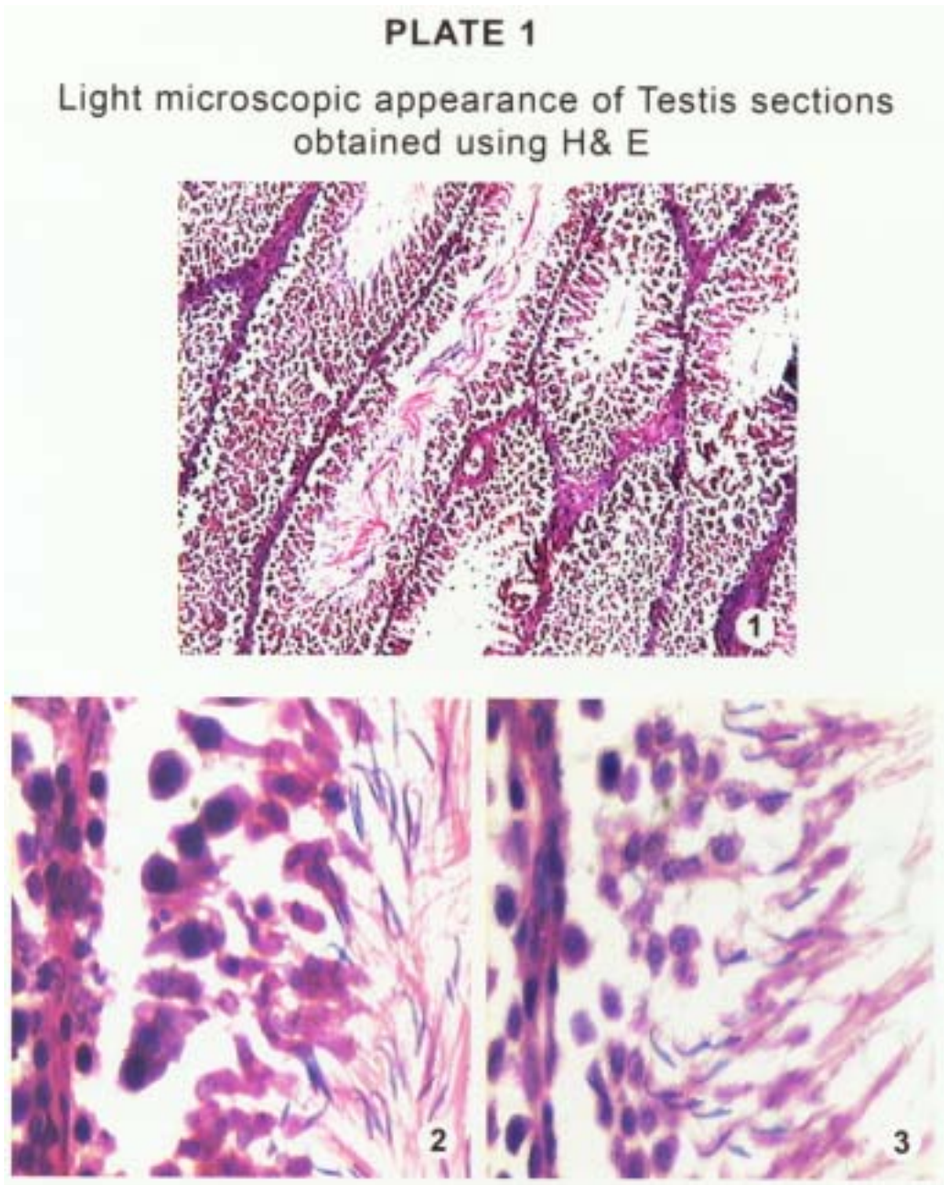

Fig. 1 : Microphotograph of testes of the Control group original magnification (60 x).
This slide shows the structure of a normal testes. Each testicular capsule is surrounded by a basement membrane. Testicular capsules consists of germ cells that mature to form sperms in the lumen.

Fig. 2 : Microphotograph of testes of the control group original magnification (100 x).
This slide shows high power image of germ cells .Germ cells in the control group have a prominent nucleus surrounded with cytoplasm. The slide shows three stages of maturation of germ cell: spermatogonia-spermatocyte-spermatozoa. The matured sperms can be seen in the lumen.

Fig. 3 : Microphotograph of testes of the Selenium group original magnification (100 x)
This slide shows high power image of germ cells in the selenium group. The cells were almost similar to that of the control.

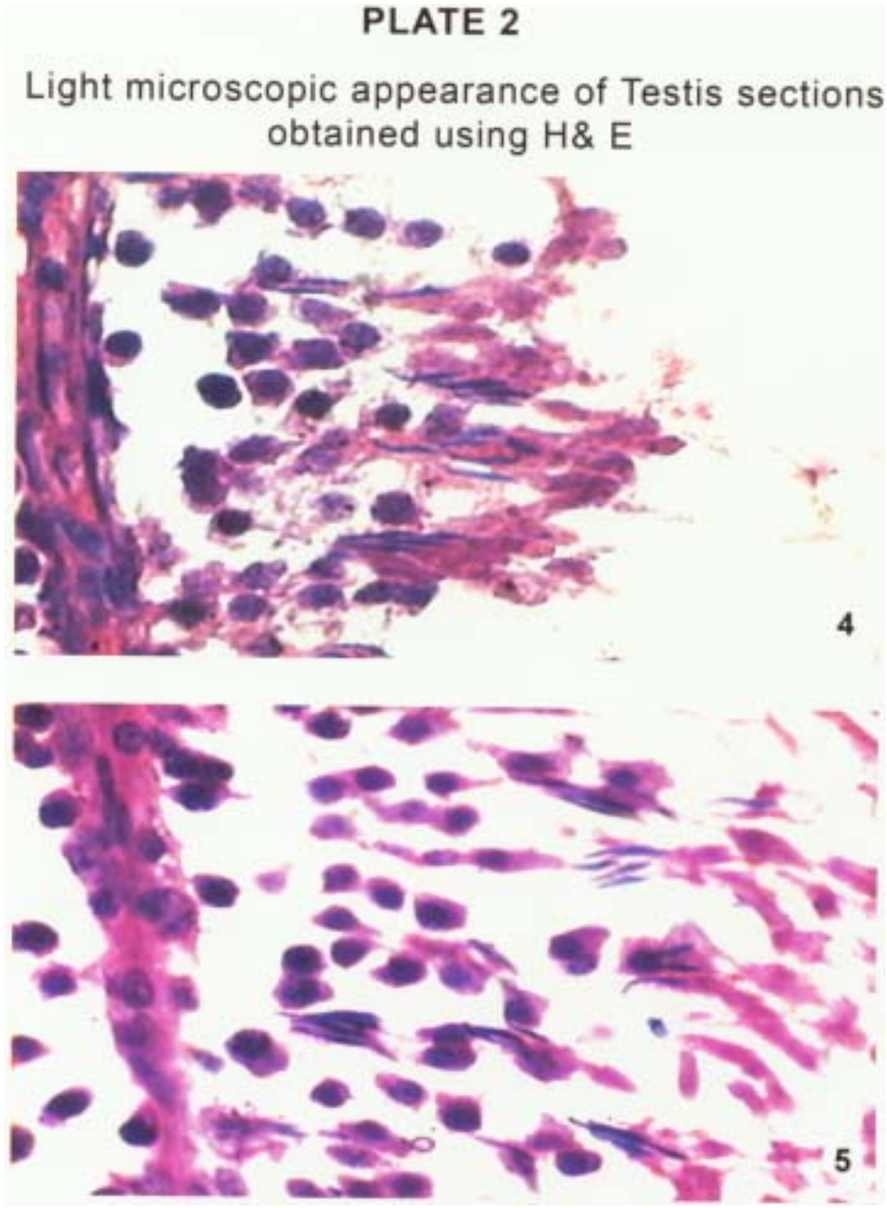

Fig. 4 : Microphotograph of testes of the Ethanol group original magnification (100 x).
Germ cells of this slide have undergone degeneration. The cells are clustered without cytoplasm. Only few cells are present near the basement membrane. Less numbers of matured sperms can be observed.

Fig. 5 : Microphotograph of testes of the Ethanol+Selenium group original magnification (100 x).
The high power image of this group shows the germ cells having prominent nucleus and cytoplasm. Although the cells are loosely packed they have the normal morphology. Spermatogenesis can also be observed.

TABLE V: Activities of 3βHSD and 17βHSD in the testes, SDH in the seminal vesicle and fructose content of seminal plasma.

Groups	*3βHSD (Units/mg protein)*	*17βHSD (Units/mg protein)*	*Sorbitol dehydrogenase (IU*/mg protein)*	*Fructose (mg/ml)*
Control	7.80±0.71	1.74±0.16	2.52±0.23	2.12±0.19
Ethanol	4.68±0.43[a]	0.75±0.06[a]	1.19±0.11[a]	0.85±0.08[a]
Selenium	7.9±0.72	1.67±0.15	2.50±0.23[b]	2.06±0.19
Ethanol+selenium	6.60±0.60[c]	1.13±0.10[c]	1.54±0.14[c]	1.87±0.17[c]

Values expressed as mean±SD
[a]P<0.05 between control and ethanol groups
[b]P<0.05 between ethanol and selenium groups
[c]P<0.05 between ethanol and ethanol+selenium groups
*µg fructose liberated/min at 37°C
Testes 3βHSD (F-34.61) & 17βHSD (F-83.847) Seminal vesicle (F-83.008)
Seminal plasma (F-77.583)

as compared to that of the ethanol treated group.

The activities of 3βHSD and 17βHSD (Table V) in the testes decreased significantly in the ethanol treated group compared to the control. But the group treated with ethanol and Selenium showed a significant increase in the activities of both these enzymes when compared to the ethanol treated group. The decline in 3βHSD and 17βHSD due to ethanol was prevented upon co-administration of Selenium. The selenium treated group showed no significant change as compared to control. The activity of SDH (Table V) in the seminal vesicle and the concentration of fructose in the seminal plasma were significantly decreased in the ethanol treated group. But the group treated with ethanol and Selenium showed a significant increase in the activities of SDH and concentration of fructose when compared to the ethanol treated group. The selenium treated group showed an increase in these parameters when compared to that of ethanol.

Histopathology of the testes of the control rats showed normal structure with germ cells having distinct nucleus and cytoplasm with spermatozoa lying in the lumen .Selenium treated group also had the testicular structure similar to that of control. In the alcohol treated group the germ cells did not have a distinct cytoplasm and cells were reduced in size and loosely arranged. In the group treated with ethanol and Selenium cells had a normal structure with a distinct nucleus and cytoplasm.

DISCUSSION

Alcohol consumption leads to oxidative stress and this causes sexual dysfunction and also impairs sperm production both in the experimental animals and humans (31). Our studies also confirmed the testicular toxicity of ethanol. This was seen in the reduction in the sperm count and morphological changes in the testes.

The drastic reduction in the weight of the testis and the body weight was altered

by the coadministration of selenium along with alcohol indicating the beneficial effects of selenium supplementation.

Imbalance of antioxidants and free radicals leads to oxidative injury. Consistent with this hypothesis we also observed enhanced levels of lipid peroxidation products in the ethanol treated groups. The activities of scavenging enzymes were lower in alcohol treated groups. Supplementation of selenium enhanced the activity of scavenging enzymes. Oztruck-Urek et al (32) had also shown that some antioxidants and trace elements such as selenium have effects on the scavenging enzymes. The activity of glutathione peroxidase was elevated in the ethanol treated group. Previous reports from our laboratory showed that chronic ethanol administration can cause an increase in glutathione peroxidase activity (33). The increased activity of glutathione peroxidase in the ethanol treated group may be due to its adaptability to detoxify the increased amounts of H_2O_2 and fatty acid derived hydroperoxides formed due to ethanol intake. The supplementation of selenium increased the activity of the enzyme than the control. But the activity of the enzyme was significantly reduced in the group treated with ethanol and Selenium in comparison with ethanol group. The glutathione content in the testes was decreased in the ethanol treated group. The reduction in glutathione content can be correlated with the reduced activities of glutathione reductase. This is in agreement with the report of Husain et al (34) that chronic ingestion of ethanol results in a significant depletion of glutathione content in testes. On co-administration of ethanol and Selenium the glutathione content was increased.

Fructose is the source of energy for the sperms. The ethanol treated rats showed a decrease in the fructose content of seminal plasma. This is due to reduced synthesis since the key enzyme of its biosynthesis, SDH had reduced activity. Hence reduction in fructose content indicates lower activity of the sperms. But the activity of sorbitol dehydrogenase and the fructose content was significantly increased in selenium treated group and also in the group treated with ethanol and selenium in comparison with the ethanol treated group. The role of selenium in this process is not clear. Many selenoproteins are formed when high doses of selenium are administered. These selenoproteins may be modulating the metabolism of fructose.

There are reports that exogenous selenium brings down the tissue lipid levels (35) and selenium deficiency can result in hypercholesterolemia and cardiovascular diseases. Consistent with the reports we also observed elevated cholesterol level in alcoholic rats and decreased levels in the administered group. Testosterone is the key hormone involved in spermatogenesis. The levels of circulating testosterone were found to be decreased in the ethanol administered group. This may be due to reduced biosynthesis since key enzymes of its biosynthesis 3β hydroxy steroid dehydrogenase and 17β hydroxy steroid dehydrogenase had reduced activities in the ethanol treated group. This is in agreement with other reports that chronic ethanol intoxification results in the diminished testosterone levels and diminished activity of these two enzymes (36) Cholesterol is the substrate for the synthesis of testosterone. Though cholesterol levels in the ethanol treated

group was found to be elevated, the concentration of testosterone was found to be decreased, this may be due to the lower activities of 3β hydroxy steroid dehydrogenase and 17β hydroxy steroid dehydrogenase which are key enzymes in testosterone synthesis. The levels of testosterone and the activity of these enzymes were elevated in the selenium group and also in the group treated with ethanol and Selenium. This is in agreement with the reports (37) that selenium supplementation ameliorated the decrement of serum testosterone levels induced by ethanol. So selenium alters the microenvironment of the testes to make it congenial for the production of sperms.

Thus it can be concluded from both biochemical and histopathological studies that the coadministration of selenium and ethanol offers protection against ethanol induced testicular toxicity. Involvement of selenium as an antioxidant and hypolipidemic agent may be reasons for the protective effect. Altered testosterone level and fructose content may be also contributing factors for the beneficial effects of selenium administration.

Our studies confirm that alcohol consumption leads to testicular toxicity as evidenced by the sperm count, histopathological alteration of the testes and reduction in the weight of testis. Selenium supplementation along with alcohol prevents alcohol related injuries to the testis.

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