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Efficacy of vitamin E and vitamin C against silica induced toxicity on male reproductive organs of albino rats

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Abstract: Silica is one of the most documented workplace contaminants. Long-term occupational exposure to silica is associated with an increased risk in respiratory diseases such as silicosis, tuberculosis, chronic bronchitis, chronic obstructive pulmonary disease and lung cancer. The present study was carried out to observe the alteration in testosterone level & histopathological changes in the testis and epididymis after silica exposure, and to show whether therapeutic agents (Vitamin E + Vitamin C) used in study may provide recovery against exposure to silica. For investigations, silica was administered in albino rats as silicon dioxide at a dose of 40 mg/Kg for 28 days (*IP)* to produce toxic effects. Recovery pattern was evaluated by Vitamin E + vitamin C (50 mg/kg, *ip* + 100 mg/kg, *po*, for 5 days after silica administration. The study showed alterations in the various blood parameters after intraperitoneal intoxication of silicon dioxide. Testosterone was significantly decreased in experimental rats after 28 days of silica intoxication. Therapeutic agents *i.e*. vitamin E and vitamin C recouped the values to normal control and recoupment was also observed in histology of testis and epididymis

Keywords: Blood parameters, Intraperitoneal, Silicon di-oxide, Testosterone, Therapeutic agents

INTRODUCTION

Silica is one of the most fibrogenic material found in nature. Silicon contributes to about 28% of the earth's crust. Silicon being very reactive does not remain in the elemental form but combines either with oxygen alone and forms free silica (SiO_2) or with oxygen and other elements forming silicates, such as asbestos (Jaffrey *et al.*, 1999; Magnani *et al.*, 1998; Wilson *et al.*, 1994; Weiss 2000). Some of the occupations such as slate, pencil industry and agate grinding industry, which carry a high risk of silicosis in workers are peculiar to India (ICMR Bulletin, 1999; Karnik *et al.*, 1990). There are about three million workers at high potential risk of silica exposure (Yucesoy, 2001).

Further, increased risk by smoking and silica act synergistically in causing chronic obstructive disease in the lung (Hnizdo and Sluis-Cremer., 1991; Hnizdo 1990; Malmberg *et al.*, 1993). The LD50 of silica through intraperitoneal route is 40 mg/kg of body weight (Vanessa *et al.*, 1996).

Vitamin E a constituent of a plasma membrane is an effective anti-oxidant and is present at the site of free radical generation. It may neutralize the toxic effects of reactive oxygen species (ROS) (John *et al.*, 2001). In addition, vitamin C is also involved in the regeneration of tocopherol from tocopheroxyl radicals in the membrane. It has been reported that vitamin E and C can have interactive effects (Stoyanovsky *et al.*, 1995). Present study involves the study of toxic effects of silica on albino rats and to evaluate the therapeutic effectiveness of vitamin E and vitamin C against silica induced toxicity in reproductive organs of male albino rats.

MATERIALS AND METHODS

Male albino rats weighing 150±10 g. were selected for the study. They were housed under standard conditions (25±2 °C, 60-70% RH and 12 h photoperiod) and allowed for food and water *ad libitum*. The toxicant, SiO_2 was dissolved in normal saline, whereas vitamin E was dissolved in olive oil and vitamin C was dissolved in distilled water.

The animals were divided into three groups of five animals each. Group 1 was injected normal saline and was treated as normal control. Groups 2 and 3 were administered silicon dioxide at a dose of 40 mg/Kg for 28 days daily after toxicant exposure Group 2 was given saline for 5 days while Group 3 received therapeutic agents (combination of vitamin E + vitamin C (50 mg/kg *intraperitoneal* and 100 mg/kg, *oral* respectively for 5 days.

Blood was collected from animals by puncturing the retro-orbital sinus and centrifuged to obtain serum. The serum was stored in a refrigerator for the analysis of male hormone, testosterone, (Kit method (CHOD- PAP method, No. 1117678.0001) from Merck). Immediately after necropsy the male reproductive organs (testis and epididymis) were excised. These organs were fixed in Bouin's fluid, embedded in wax sectioned using microtome and haemotoxylin-eosin stained slides were observed for histopathological changes.

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Photomicrographs were taken for histopathological observation.

All results were analyzed statistically using the statistical Package for the Social Sciences (SPSS) packed program for Windows. Mean ± SE values were calculated. Comparisons between two independent groups were made by the Students' t-test and $P<0.05$ was considered statistically significant.

RESULTS

Biochemical assay: The present study showed alterations in the testosterone of male albino rats after intra-peritoneal intoxication of silicon dioxide. An estimated level of testosterone was significantly decreased, in experimental rats after 28 days of silica intoxication. Therapeutic agents *i.e*. Vitamin E and vitamin C recouped the values to normal control value (it is 3.9) (Table 1). Weight of body, testes and epididymis were decreased, in experimental rats after 28 days of silica intoxication. Therapeutic agents *i.e*. vitamin E and vitamin C recouped the values to normal control (Table 2).

Histopathological studies: Photomicrograph of the T. S. of testis of albino rat of control group showed a normal adult picture. The tunica propria was well organized and seminiferous tubules exhibited regular stages of spermatogenesis from spermatogonia to spermatozoa. The Leydig's cells were clearly observed in the interstitium. The vascularity of the organ was normal (Fig. 1). The exposure to silica, however caused severe testicular damage. The vascularization had increased considerably in the seminiferous tubules. The interstitium was totally devoid of Leydig's cells (Fig. 2). Treatment with vitamin E and vitamin C after silica administration improved the testicular architecture to a great extent. It showed significant regeneration in testicular damage caused by treatment. The seminiferous tubules and Leydig's cells became normal (Fig. 3).

Photomicrograph of the T. S. of caput and cauda epididymis of the control rats presented normal histological features. Both caput and cauda showed increased height in epithelial cells and wide lumen of ductules packed with spermatozoa. Well developed connective tissue was seen between intertubular spaces (Fig. 4). Silica exposure caused marked changes in histology of caput and cauda epididymis. The ductules of caput and cauda were shrunken, deformed and were devoid of spermatozoa. The epithelial cells were reduced in height and without stereocilia. . Inter ductular space showed less connective tissue (Fig. 5). Treatment with vitamin E and vitamin C after silica administration showed significant improvement in histological changes of the caput and cauda. The reduction in size of the lumen of ductules was not significant in both parts. The sperm concentration was affected in caput and cauda. Vacuoles were formed (Fig. 6).

Table 1. Changes in testosterone of albino rats.

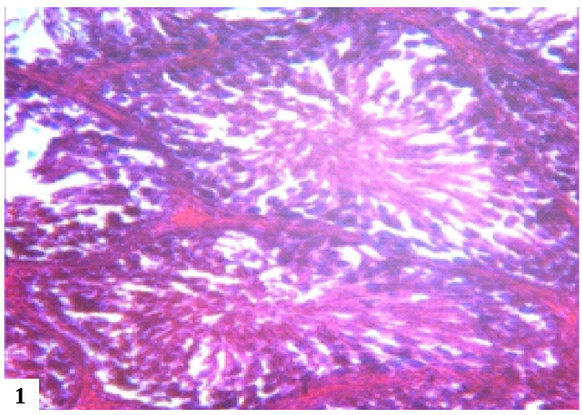
	Control	**SiO_2**	**SiO_2 +vit E + vit C**
Testosterone (ng/ml)	3.9±0.05	1.7+0.3	2.27+0.5

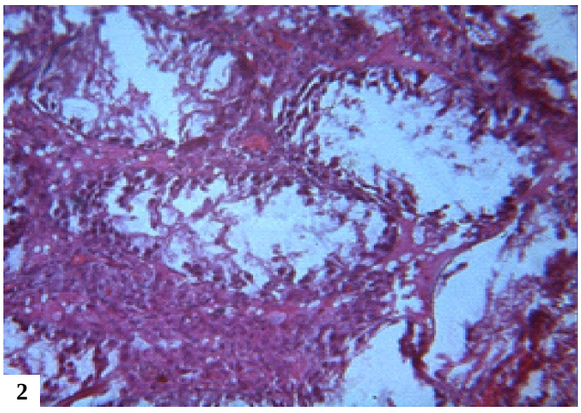
*Significant, NS Not significant P value SiO_2 Vs control at a≤ 0.05, b≤ 0.01 p value (vit. E + vit C) Vs Sio_2 at c≤ 0.05, d≤ 0.01

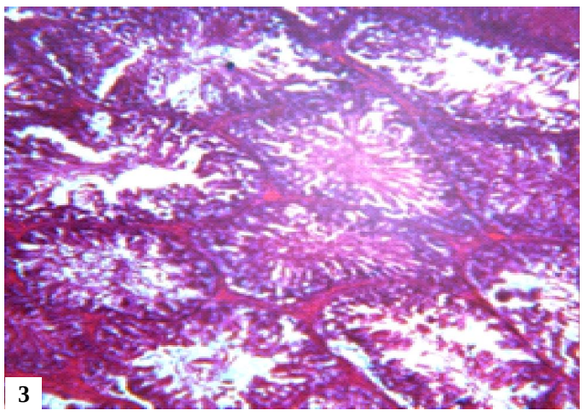
DISCUSSION

The deposition of silica particles in the lungs of man and experimental animals leads to an industrial era disease silicosis. In the present investigations, daily administration of silicon dioxide at a dose of 40 mg/Kg for 28 days showed arrest of spermatogenesis when the extracts were administrated. Varying degree of damage was caused to different tissues. Leydig's cells were mostly atrophied. The oedematous fluid filled the interstitium. The cauda ductules were devoid of spermatozoa including other histological alterations. The weight of the testes was also reduced. The damage to the testes was followed by karyolysis and karyyorrhexis of the spermatogonia and spermatocytes.

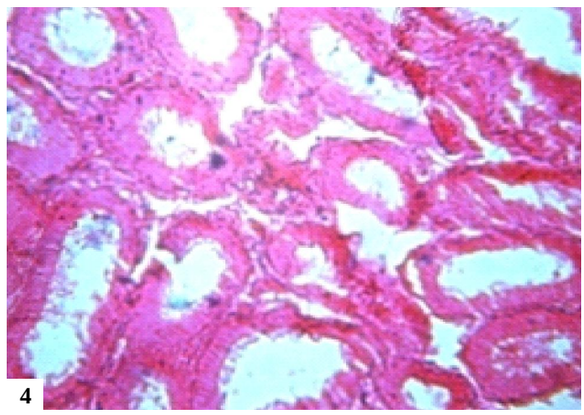
Alteration in the genital organs of male albino rats was observed after administration of alcoholic extract of *Dacus* seeds and crude powder of *Plemeria* leaves (Jacob *et al.* 1988). These plant materials caused involution of the genital structure leading to fertility control. Kaur *et al*. (1988) studied the affect of gossypol on testis and epididymis of albino rats and found necrotic changes in the seminiferous tubules in large percentage of treated rats. It seems that vitamin E and vitamin C protect the cellular membranes from oxidative degeneration caused by toxicant, as vitamin E and vitamin C are well known antioxidants. Vitamin E is lipophilic in nature so it easily penetrates the cell membrane and breaks oxidative chain reactions occurring in phospholipids of cell membrane. Similarly, due to hydrophilic nature of vitamin C, it passes into cytosol where it reduces oxidative stress occurring due to xenobiotic. Significant rise in the level of testosterone hormone were found after sub chronic exposure of silica. The weight gain by the rats exposed to cement dust was lower than the unexposed rats and this confirms that the constituents of cement dust can negatively affect growth of animals. Pollutants from cement dust have found to be toxic, mutagenic or carcinogenic to animals (Fatma *et al.*, 2001). So the growth rate of the rats exposed to cement dust can therefore be attributed to toxic substances in the cement dust which include zinc, copper, aluminium, iron and silicon. This finding confirms the report of Nigragau and Davidson (1986) who found that sulphuric and hydrochloric acids emitted from cement manufacturing plants could impede

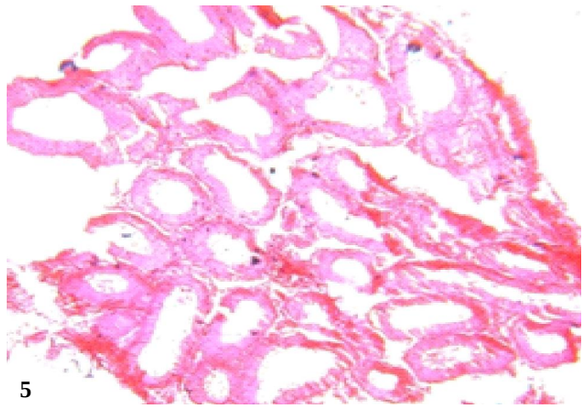


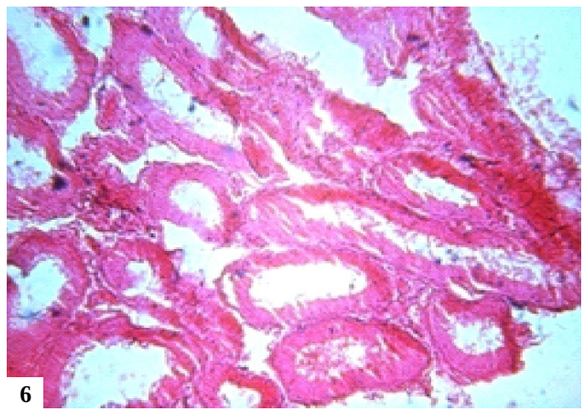




Figs. 1-3. *1. T. S. of testis of albino rat of control group, 2. T. S. of testis of albino rat after silica exposure, 3. T. S. of testis of albino rat exposed to silica and treated with vitamin E and vitamin C.*







Figs. 4-6. *4. T. S. of caput and cauda epididymis of the control rats, 5. T. S. of caput and cauda epididymis of rats after silica exposure,6. T. S. of caput and cauda epididymis after silica exposure and treatment with vitamin E and vitamin C.*

the growth of animals. Furthermore, Oleru (1984) reported pathogenic condition in histopathologal changes of heart, lungs, liver, kidney and reproductive system of due to cement dust in rats. The changes become more severe as the period of exposure increased. The histopathology changes were as a result of cytotoxic agents from the cement dust which caused multi-organ injuries which led to cellular oedama, atrophy and necrosis. Bogue (1991) reported that the exposure to cement dust can cause health effects such as eye irritation, lung allergies, and damage to the liver, kidney, gastric system and epidermal irritation.

The abnormal fatty deposition on the ovary and testis might be caused by the inability of the exposed rats to produced offspring. Joseph (1994) reported that exposure to cement dust even at lower levels may have effects on the reproductive process and foetal development. Selevan *et al.* (2000) reported that in the periods of

Table 2. Changes in weight of testes and epididymis of albino rats after treatment.

Groups	Body weight (gm)	Testes (mg)	Epididymis (mg)
Control	150±10	790± 10	290±10
SiO_2	140±10	360±10	230+10
SiO_2Vit E+vit C	145±10	760±10	260± 10

*Significant, NS Not significant P value SiO_2 Vs control at a≤ 0.05, b≤ 0.01 p value (vit. E + vit C) Vs Sio_2 at c≤ 0.05, d≤ 0.01

elevated air pollution in Teplice and Czech were significantly associated with decrements in the other semen measures including proportionately fewer motile sperm, proportionately fewer sperm with normal morphology or normal head shape and proportionately more sperm with abnormal chromatin. These results suggest that young men may experience alteration in sperm quality after exposure to periods of elevated air pollution, without changes in sperm numbers. Effect of air pollution on mouse female fertility has been investigated. These results support the concept that female reproductive health represents a target of air pollutants (Mohallem *et al*., 2005).

In the present study, significant change in weight of testis of rats was observed after silica intoxication of 28 days and recoupment have also seen when these silicotic rats were treated with a combination of vitamin C and vitamin E. The reduced production causes a significant decrease in the weight of testis and accessory organs in male rats (Dorfman *et al*., 1963). Paul *et al*., (1953) have also demonstrated the reduction in weight of testis and accessory organs in the absence of spermatids and spermatozoa

The study showed significant change in weight of epididymis of rats was observed after silica intoxication of 28 days and recoupments have also seen when these silicotic rats were treated with a combination of vitamin C and vitamin E. The significant change in the process of spermatogenesis and histological alterations in the testes were seen when rats was were treated with silicon di oxide for 28 days and recoupments were also seen when these silicotic rats were treated with a combination of vitamin C and vitamin E.

Conclusion

The study provides strong evidence that combination of vitamin E and vitamin C are useful therapeutic agents against disorders related to occupational silica exposure. Measurements of oxidative stress-related parameters and various marker enzymes also confirm the results obtained.

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