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Ameliorating Effect of Vitamin E on Testicular Toxicity Induced by Endosulphan in *Capra hircus in vitro*

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ABSTRACT

During the present investigation ameliorating effect of vitamin E on endosulphan induced testicular toxicity has been analyzed in Capra hircus *in vitro*. Vitamin E exhibited the protective role against the damage induced by endosulphan in the testicular tissue. Small pieces (approximately 1 mm^3) of testicular tissue were divided into three groups (One control and two experimental groups). One experimental group was treated with 100 nmol mL^{-1} endosulphan concentration and another experimental group was treated with 100 nmol mL^{-1} endosulphan and supplemented with 100 μmol L^{-1} concentration of vitamin E (α-Tocopherol). Harvesting of the testicular tissue was carried out after 1, 4 and 8 h of exposure durations *in vitro*. Hyalinization and fragmentation was observed in the endosulphan treated group. Chromolysis was observed in spermatogonia, Sertoli cells and spermatids. As the exposure duration enhanced from 4 to 8 h there was elevation in number of pycnotic nuclei, fragmented nuclei, chromolysis of germ cells and somatic cells present in the testis. Endosulphan exposure induced the number of atretic spermatogonia from 24% in control group to 68% after 1 h, from 30 to 76% after 4 h and from 36 to 84% after 8 h of exposure duration. In the experimental group treated with endosulphan and supplemented with vitamin E there was decline in number of pycnotic nuclei, fragmented nuclei and chromolysis as compared with the endosulphan exposed group. There was decline in atretic spermatogonia from 68 to 36% at 1 h, from 76 to 44% after 4 h and from 84 to 58% after 8 h of supplementation duration.

Key words: Vitamin E, endosulphan, *Capra hircus*, testis

INTRODUCTION

The tremendous increase in the plant protection chemicals like insecticides and fungicides has resulted in environmental contamination and ill effects to human and living organisms (Mishra *et al.*, 1998). Endosulphan is a highly toxic pesticide [toxicity class 1 in the EPA (Extension Toxicology Network, 2000)]. Endosulphan is also an established environmental endocrine disrupter (Rose *et al.*, 1999) having genotoxic effects on HeoG2 cells (Lu *et al.*, 2000) and has been reported to inhibit testicular function in pubertal rats (Chitra *et al.*, 1999). Pesticides may induce oxidative stress, leading to generation of free radicals and alteration in antioxidants, oxygen free radicals, the scavenging enzyme system and lipid peroxidation (Banerjee *et al.*, 1999; Etemadi-Aleagha *et al.*, 2002). Insecticides are capable of binding to lipid component of

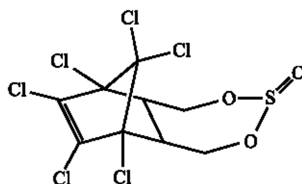
mitochondrial membrane resulting in to the change in mitochondrial function (Sitkiewick and Zalewska, 1975). The capacity of pesticides to induce oxidative stress in different organs of mammals have been observed (Bagchi *et al.*, 1995; Lemaire and Livingstone, 1993). Endosulphan and malathion exposure increase malondialdehyde (MDA) levels in ovarian tissues of female rats which is an indicator of free radicals and their lipid peroxide damages during these insecticides metabolism (Koc *et al.*, 2009). Histopathological effect of endosulphan (100 nmol mL^{-1}), on spermatogenic cells of goat (*Capra hircus*) was reported by Sharma and Chauhan (2009). The study indicated that endosulphan affected the testicular structure and induced severe atrophy of seminiferous tubules (Sharma and Chauhan, 2009). A few toxicological studies have addressed the possible relationship between reproductive toxicity and exposure to chemicals that generate Reactive Oxygen Species (ROS) (Sally, 1997). Production of free radicals/reactive oxygen species (ROS) by sperm (Iwasaki and Gagnon, 1992) and the adverse effect of excess ROS and peroxidation on sperm motility and viability were also reported (De Lamirande and Gagnon, 1992; Kim and Parthasarathy, 1998). Declines in human and animal male fertility have been observed in recent years by several researchers (Carlsen *et al.*, 1992; Auger *et al.*, 1995; Irvine *et al.*, 1996; Fisch *et al.*, 1996; Swan *et al.*, 1997; Andersen *et al.*, 2000). One of the mechanisms of male infertility is the excess production of Reactive Oxygen Species (ROS) in sperm, which can induce nuclear DNA fragmentation, lipid peroxidation and protein-protein cross links (Aitken *et al.*, 1998; Sharma and Agarwal, 1996; Jones *et al.*, 1979). It has been recognized since the 1940s that vitamin E (α-tocopherol) is a powerful lipophilic antioxidant that is absolutely vital for the maintenance of mammalian spermatogenesis (Johnson, 1979). Single i.p., injections of PCB or PCN mixtures resulted in decreases in testicular SOD activity 1 day after the exposures (-14%, $p<0.05$ and -51%, $p<0.01$, respectively) (Peltola *et al.*, 1994). Catalase activity also decreased after both exposures (-30 to -42%, $p<0.05$, at days 1-7 after PCB exposure and -37 to -43%, $p<0.05$, at days 3-7 after PCN exposure) (Peltola *et al.*, 1994). The effects of supplementation of ascorbic acid, vitamin E (Vit. E) and their combination in drinking water on sperm characteristics, lipid peroxidation (LPO) and seminal plasma enzymes of mature male rabbits have been analyzed and the results from this study indicated that supplementation of drinking water with antioxidant ascorbic acid, Vitamin E and their combination reduced the production of free radicals and can improve rabbit semen quality, but the greater improvement seemed to be from vitamin E (Yousef *et al.*, 2003).

Hence, the long-term hazard of pesticides on animals cannot be ignored and it is therefore highly desirable to search for protective measures to minimize their harmful effects. In the light of the above background information protecting effect of vitamin E against the endosulphan *in vitro* in goat testis have been analyzed.

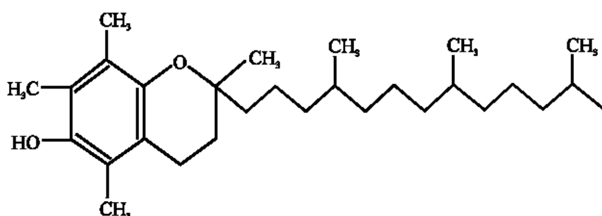
MATERIALS AND METHODS

Testis of mature goat (*Capra hircus*) were procured from slaughter houses around Kurukshetra (29°6'N, 76°50'E), Haryana, India. The material was brought to the laboratory at 4°C in normal saline during year 2009.

After decapsulation, the testis was cut into small pieces (approximately 1 mm^3) for culture.



Endosulphan
(Structure-1)
Molecular formula: $C_9H_6Cl_6O_3S$
Molar mass: 406.95



α-Tocopherol
(Structure-2)
Chemical formula : $C_{29}H_{50}O_2$
Molecular Mass: 430.69 g mol^{-1}

After washing three times with TCM-199, small pieces of testicular tissue were immediately placed on nucleopore filter and floated on medium. The medium was prepared by mixing TCM-199 and antibiotics (200 unit penicillin 100 1 U mL^{-1} and streptomycin 100 g mL^{-1}). The tissue was divided into three groups (1 control group +2 experimental groups). Experimental group (A) was supplemented with 1 nmol mL^{-1} endosulphan (Structure-1) concentration and Experimental group (B) was supplemented with 100 nmol mL^{-1} endosulphan and 100 μmol L^{-1} concentration of vitamin E (α-Tocopherol) (Structure-2) and harvesting was carried out after 1, 4 and 8 h of exposure. The culture petri plates were kept at 39°C for the specified duration in an asceptic oven. Tissue from all the groups was processed for the histomorphological studies. Paraffin embedded tissue from all experimental and control was cut at 5 μm thickness and after dewaxing in xylene, the sections were passed through decreasing grades of alcohol and stained with haematoxylene. After that the sections were gradually dehydrated up to the 70% alcohol and stained with eosin, after further dehydration up to absolute alcohol the sections were cleared with clearing agent (xylene) and finally mounted with DPX (Pearse, 1968).

RESULTS AND DISCUSSION

During the present investigation 100 μmol L^{-1} concentration of vitamin E (α-Tocopherol) induced protective role against the testicular damage induced by the endosulphan at dose level 100 nmol mL^{-1}. Histopathological study under light microscope, testicular sections stained with Hematoxylin-Eosin (HE) showed the normal seminiferous tubules with orderly arrangement of germ cells and somatic cells in the control group (Fig. 1a). In the experimental group (A) treated with endosulphan there was disorganization in the structure of the seminiferous tubules. Seminiferous tubules were atrophied, seminiferous epithelial cells disintegrated and shed in endosulphan treated groups. At one hour of exposure duration vacuolization was observed and slight detachment of basement membrane from underlying cells was noticed. Pycnotic nuclei were observed due to the endosulphan exposure (Fig. 1b). As the exposure duration increased from 1 to 4 h there was increase in number and size of vacuole. Number of pycnotic nuclei were increased after 4 h of exposure duration. Hyalinization and fragmentation were observed in the endosulphan treated group. Chromolysis was observed in spermatogonia, Sertoli cells and spermetids. As the exposure duration enhanced from 4 to 8 h there was elevation in number of pycnotic nuclei, fragmented nuclei, chromolysis of germ cells and somatic cells present in the testis (Fig. 1c). There was increase in number of atretic spermatogenic cells and somatic cells. Endosulphan exposure induced increase in number of atretic spermatogonia from 24% in control group to 68% after 1 h, from 30 to 76% after 4 h and from 36 to 84% after 8 h of exposure duration. Chi-square values between control and endoulphan treated group [experimental group (A)] were analyzed after 1, 4 and 8 h of exposure durations and all the variations recorded were statistically significant (χ^2 0.05) (Table 1).

There was also elevation in number of atretic Sertoli cells after exposure of endosulphan from 18% in control to 62%, from 24 to 70 and 32 to 78% after 1, 4 and 8 h, respectively. Chi-square

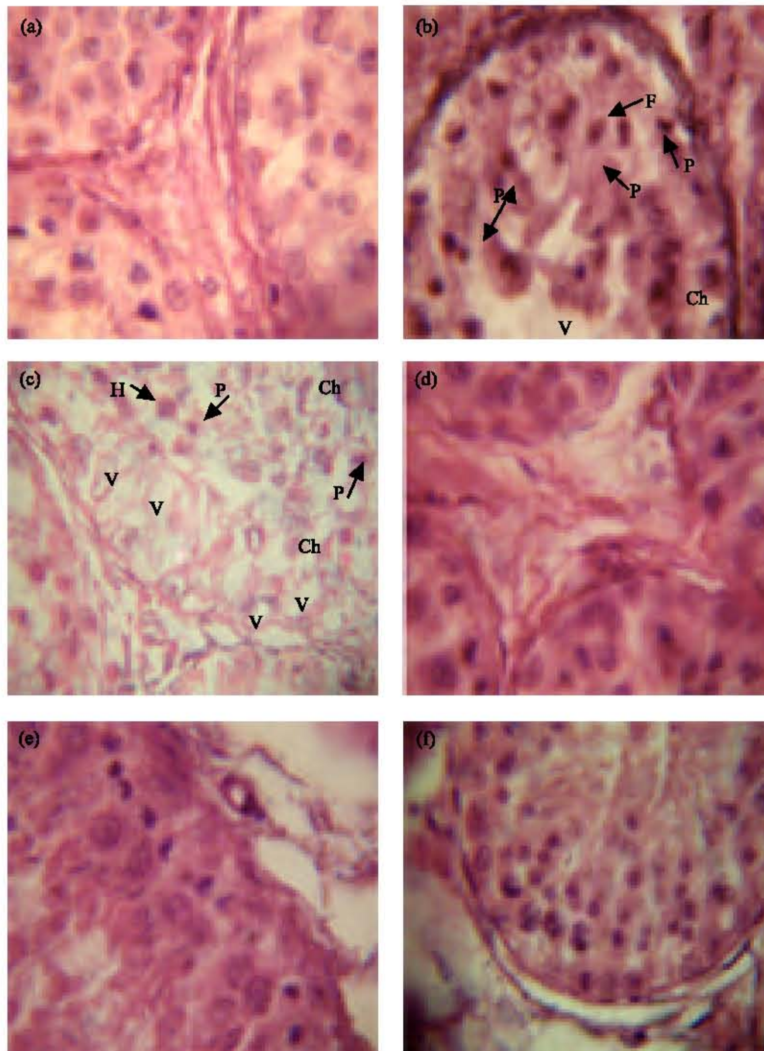


Fig. 1: (a) Light micrograph of control testicular tissue showing normal arrangement of different types of germ cells (spermatogonia, spermatocytes, spermatid, sperms) and somatic cells (Sertoli cells) in sexually mature goat characterized by the presence of well defined cellular associations and stages (X1000) (b) A portion of testicular tissue exposed to endosulphan (100 nmol mL^{-1}) for 1 h showing large number of pycnotic nuclei (P), fragmented nuclei (F), chromolysis (Ch) in spermatogonia, Sertoli cells and having large sized vacuoles (V) (intra nuclear and intra cytoplasmic). (X1000) (c) Light micrograph of testicular tissue treated with endosulphan (100 nmol mL^{-1}) for 8 h showing large number of pycnotic nuclei (P), vacuolization (V) and chromolysis (Ch)(X 1000). Note the hyalinization (H) and large spaces between the cells. (X 1000) (d) Microphotograph of testicular tissue treated with endosulphan (100 nmol mL^{-1}) and supplemented with vitamin E for 1 h showing improvement in cellular damage (X 1000) (e) A portion of testicular tissue treated with endosulphan (100 nmol mL^{-1}) and supplemented with vitamin E for 4 h showing reduction in number of atreatic germ cells and somatic cells. (X1000) and (f) Testicular tissue treated with endosulphan (100 nmol mL^{-1}) and supplemented with vitamin E for 8 h showing protection against endosulphan exposure. (X1000)

Table 1: The comparison of a number of atretic spermatogonia between control versus endosulphan (100 nmol mL^{-1}) group (A) and endosulphan (100 nmol mL^{-1}) treated group (A) versus endosulphan supplemented with vitamin E group (B) showing Chi-square values after 1, 4 and 8 h of exposure durations

Parameters	Endosulphan	Endosulphan+Vitamin E
1 h	19.4847*	7.8905*
4 h	21.2364*	5.31868*
8 h	24.00*	4.320*

*Statistically significant difference ($p \le 0.05$)

Table 2: Chi-square values between atretic Sertoli cells observed in control versus endosulphan (100 nmol mL^{-1}) treated group (A) and endosulphan (100 nmol mL^{-1}) treated group (A) versus endosulphan supplemented with vitamin E group (B) after 1, 4 and 8 h of exposure durations

Parameters	Endosulphan	Endosulphan+Vitamin E
1 h	20.1667*	5.76*
4 h	21.2364*	5.002*
8 h	21.3738*	6.4171*

*Statistically significant difference ($p \le 0.05$)

values between control and endoulphan treated group [experimental group (A)] were analyzed after 1, 4 and 8 h of exposure durations and all the variations recorded were statistically significant (χ^2 0.05) (Table 2).

Endosulphan destroys the testicular structure and function in adult testis by inducing oxidative stress and this damage was partially reversed by vitamin E antioxidant defense system. Vitamin E supplementation improved the tubular architecture in experimental group (B) (Fig. 1d, e). In the experimental group (B) treated with endosulphan and also supplemented with vitamin E there was decline in number of pycnotic nuclei, fragmented nuclei and chromolysis as compared with the endosulphan exposed group [experimental group (A)]. There was decline in atretic spermatogonia from 68 to 40% at 1 h, from 76 to 54% after 4 h and from 84 to 66% after 8 h of supplementation duration and Chi-square values were 7.8905, 5.31868 and 4.30 after 1, 4 and 8 h of exposure durations. All the values recorded were statistically significant (χ^2 0.05) (Table 1). All these atretogenic changes significantly improved by the treatment with vitamin E (Fig. 1f). There was decline in atretic Sertoli cells from 62 to 38% at 1 h, from 70 to 48% after 4 h and from 78 to 54% after 8 h of supplementation duration and Chi-square values between endosulphan treated [experimental group (A)] and endosulphan supplemented with vitamin E [experimental group (B)] were 5.76, 5.002 and 6.4171 after 1, 4 and 8 h of exposure durations, respectively. Chi-square values revealed that all the variations recorded were statistically significant (χ^2 0.05) (Table 2).

During the present investigation supplementation of vitamin E (α-Tocopherol) 100 μmol L^{-1} concentration induced protective role against the testicular damage induced by the endosulphan at dose level 100 nmol mL^{-1}. Vitamin E supplementation improved the tubular architecture in experimental group. The results of the present study strongly supports the findings of Latchoumycandane and Mathur (2002) who observed that administration of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) induces oxidative stress in testis and vitamin E could impart a protective effect against TCDD-induced oxidative stress. There was a significant decline in the activities of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase with concomitant increased levels of hydrogen peroxide and lipid peroxidation. Co-administration of TCDD and vitamin E did not show any significant changes in the weights of the testis, epididymis,

seminal vesicles and ventral prostate (Latchoumycandane and Mathur, 2002). The results of our investigation that Vitamin E (antioxidant) produced ameliorating effect in testicular toxicity induced by endosulphan. There was decline in percentage of atretic cells percentage after the supplementation of vitamin E. The observations of present study strongly advocate the findings of Lucesoli and Fraga (1999) who suggests that chronic iron overload produced a mild oxidative damage in rat testes that was partially prevented by alpha-tocopherol supplementation. Present findings that vitamin E improves the testicular damage induced by the endosulphan supports the findings of Ghosh *et al.* (2002) who suggested that cyclophosphamide treatment at its clinical dose is associated with antigonadal activities as well as induction of oxidative stress in gonad that can be ameliorated significantly by alpha-tocopherol succinate co-administration and data have some potential clinical implications. In the present study experimental group treated with endosulphan which was also supplemented with vitamin E, there was a decline in number of pycnotic nuclei, fragmented nuclei and chromolysis as compared with the endosulphan exposed group. Vitamin E induced decrease in atretic spermatogonia from 68 to 36% at 1 h, from 76 to 44% after 4 h and from 84 to 58% after 8 h of supplementation duration. The results of the present findings strongly supports the findings of Zhou *et al.* (2006) who observed that vitamin E showed protective role againsed the testicular damage induced by formaldehyde (FA) in the adult rat. The testicular weight, the quantity and quality of sperm, the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and glutathione (GSH) were significantly decreased in testes of rats in FA treated group compared with those in the control group. Vitamin E treatment restored these parameters in FA+VE group (Zhou *et al.*, 2006). In the present investigation Vitamin E treatment improved the tubular architecture in experimental group endorse the findings by Chen *et al.* (2005) in which Vitamin E found to suppress Fe^{2+}/sodium ascorbate-induced lipid peroxidation in Leydig cells. Our findings strongly advocate the findings by Assayed *et al.* (2008) who observed that the supplementation of garlic extract and L-ascorbic acid (antioxidant); with Cypermethrin (CYP) gavage; highly significantly increased the percentage of live spermatozoa as compared with corresponding values in (CYP) group.

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