International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 7 (8): 821-828, 2011
ISSN 1811-7775 / DOI: 10.3923/ijp.2011.821.828

Vitamin E: An Antioxidant Therapy to Protect Endosulphan Induced Follicular Toxicity

R.K. Sharma, P.K. Chauhan and A. Fulia
Reproductive Physiology Laboratory, Department of Zoology,
Kurukshetra University, Kurukshetra-136119, India

Abstract: Endosulphan is a xenoestrogen that imitates the effect of estrogens, causing reproductive and developmental abnormalities in mammals. The aim of the present investigation was to analyze the effect of vitamin E in rescue of degenerating changes induced by endosulphan in granulosa cells of goat *Capra hircus in vitro*. On the basis of colour and texture normal follicles (3-5 mm in diameter) were selected for the tissue culture. The follicles were divided into two groups (one control+two experimental groups). Experimental group (A) was exposed with 100 nmol mL^{-1} endosulphan concentration. Experimental group (B) was exposed with 100 nmol mL^{-1} endosulphan as well as supplemented with 100 μmol L^{-1} concentration of vitamin E (α-Tocopherol). Harvesting was carried out after 1 h, 4 h and 8 h of exposure. Control was run simultaneously along with all the experimental groups. Endosulphan at dose level 100 nmol mL^{-1} induced a decline in cell diameter from 7.5±0.0456 in control to 4.5±0.1024, 3.7±0.1001 and 3.2±0.1154 μm after exposure of 1, 4 and 8 h, respectively but in case of endosulphan supplemented with vitamin E, there was less decline in cell diameter that was 6.4±0.1235, 4.8±0.1809 and 4.1±0.0809 μm after exposure of 1, 4 and 8 h, respectively. Endosulphan induced atretogenic changes like hyalinization of granulosa cells, crinkled and wavy membranes and pycnosis and thus affects the functions in adult goat due to the oxidative stress. Vitamin E treatment at dose level 100 μmol L^{-1} in experimental group (B) these atretogenic changes were milder and restore the normal structure of granulosa cells.

Key words: Pesticide, endosulphan, vitamin E, follicle, granulosa cells, goat, *Capra hircus*

INTRODUCTION

Pesticides have unique status of all food residues because of their use in agricultural fields to meet the increasing worldwide food demands and affect human and wild life (Harvey, 2010). Pesticides such as bisphenol A (BPA), phthalates and certain pesticides (e.g., vinclozolin, dicofol, atrazine, endosulphan) alter estrogen, androgen and thyroid signaling, essential for normal embryonic development and reproductive activity in mammals (McLachlan, 2001; Zoeller *et al*., 2002; Gray *et al*., 2006). Organophosphorus pesticides such as chlorpyrifos methyl, diazinon and profenofos resulted in significant decrease in testosterone levels of male rats (Zidan, 2009). Blood testosterone levels indicate that leydig cell steroidogenesis is acutely and deeply damaged by diazinon in males (Alahyary *et al*., 2008). *In vitro* effects of pesticide on spermatogenesis and sperm motility of maturing and mature fish have been investigated (Masouleh *et al*., 2011). Among all pesticides organochlorine compounds used in the agricultural fields are mostly persistent in the environment (Biswas *et al.*, 2010). Many organochlorine pesticides have been associated with estrogenic activity both *in vivo* and *in vitro*. Kepone, an organochlorine pesticide produces persistent vaginal estrous and an ovulation in rats treated neonatally. Kepone has been banned in part for its estrogenic activity (Gellert, 1978). Cryptorchidism, hypospadias, oligospermia and testicular cancer in males are proposed to be linked as the Testicular Dysgenesis Syndrome (TDS) resulting from disturbed prenatal testicular development (Bay *et al*., 2006; Skakkebaek *et al*., 2001). Endosulphan (6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-methano-2,4,3-benzodioxathiepin-3-oxide) is an organochlorine insecticide and acaricide that imitates or enhances the effect of estrogens, causing reproductive and developmental abnormalities in both animals and humans (Varayoud *et al*., 2008). Endosulphan induced ultrastructural changes in goat spermatogonia and induced male infertility (Sharma *et al*., 2010a). During recent years it has been observed that oxidative stress play a fundamental role in the regulation of apoptosis (Buttke and Sandstrom, 1994). Antioxidant supplementation improves the endogenous antioxidant defense systems within cells and protects the cell from

Corresponding Author: R.K. Sharma, Reproductive Physiology Laboratory, Department of Zoology, Kurukshetra University, Kurukshetra-136119, India

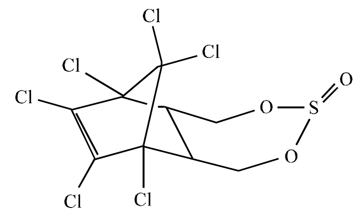
oxidative stress by inhibiting a variety of apoptotic pathways (Verhaegen *et al.*, 1995; Tilly and Tilly, 1995; Gorman *et al.*, 1997). Free radicals and reactive oxygen species play an important role in a wide range of physiological processes, including sex and reproduction (Fujii *et al.*, 2005; Aitken and Baker, 2006; Halliwell and Gutteridge, 2007). Lindane, an organochlorine pesticide, induced reproductive abnormalities in male rats and the induction of oxidative stress due to lindane exposure is considered to play an important role in the toxicity caused by lindane (Saradha *et al.*, 2008). The data suggest that chronic iron overload produced a mild oxidative damage in rat testes that was partially prevented by α-tocopherol supplementation (Lucesoli and Fraga, 1999). It has been reported that vitamin E, having cardioprotective and hypolipidemic potential and also reduce the risk of pregnancy complications involving oxidative stress, such as pre-eclampsia (Iribhogbe *et al.*, 2011). Supplementation of vitamin E in goat granulosa cells induced decline in atratogenic changes *in vitro* due to scavenging the free radicals produced in culture medium (Sharma and Fulia, 2009). Protective effect of ascorbic acid has been observed in minimizing the testicular toxicity induced by endosulphan in male goat *Capra hircus* (Sharma *et al.*, 2010b). Less attention has been paid in the study of effect of vitamin E amelioration against pesticide induced toxicity in female reproductive organs. Endosulphan (6,7,8,9,10-hexachloro-1,5,5a,6, 9,9a-hexahydro,6-9-methano 2,3,4,benzodioxathiepin-3-oxide) is a member of the cyclodiene group of organochlorine insecticide used worldwide in agriculture and is absorbed by both humans and animals through the intestinal tract, lungs and skin (Vale *et al.*, 2003).

Keeping in view the effect of pesticides on female reproduction and seeking for the preventions of the hazardous effects of pesticides present in the environment, the present study was designed to investigate the effect of vitamin E (α-tocopherol) in rescue of degenerative changes induced by the endosulphan in granulosa cells of goat (*Capra hircus*).

MATERIALS AND METHODS

The mature goat (*Capra hircus*) ovaries were procured from slaughter houses around Kurukshetra (29°6'N, 76°5'E) and Chandigarh (30°30' N to 30°45' N, 76°45' E to 76°55' E) during year 2010. The material was brought to the Reproductive Physiology Laboratory at 4°C in normal saline. Twelve Goats, 24 ovaries and 5 follicles in each group were used during this experiment. Follicles were separated manually with the help of fine pair of forceps. On the basis of colour and texture, normal follicles (3-5 mm in diameter) were selected for the tissue culture.

Tissue culture: After washing with normal saline the follicles were cultured by Sharma and Fulia (2009) method. The follicles were divided into two groups (one control group+two experimental groups).



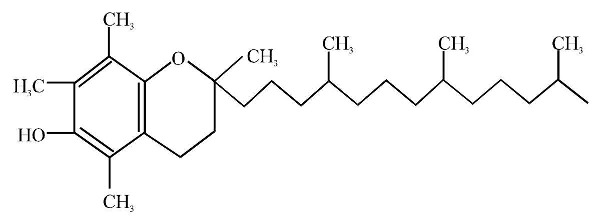
Endosulphan

(Structure 1)

IUPAC name: 6,7,8,9,10,10-hexachloro-1,5,5a,6,9,9a-hexahydro-6,9-methano-2,4,3-benzodioxathiepine-3-oxide

Chemical formula: $C_9H_6Cl_6O_3S$

Molecular mass: 406.95



α-Tocopherol

(Structure 2)

Chemical formula: $C_{29}\ H_{50}\ O_2$

Molecular mass: 430.69 g moL^{-1}

Experimental group (A) was supplemented with 100 nmol mL^{-1} (endosulphan) (Structure-1) concentration (used 35% endosulphan present in market). Experimental group (B) was supplemented with 100 nmol mL^{-1} endosulphan and 100 μmol L^{-1} concentration of vitamin E (α-tocopherol) (Structure-2). Culture petri plates were kept at 39°C for the specified duration in an asceptic oven. Harvesting of the follicles was carried out after 1, 4 and 8 h of exposure. Follicles from all the groups were processed for the histomorphological studies. Paraffin embedded follicles 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).

Statistical analysis: During the present investigation the under mentioned statistical formulas were used for statistical analysis of the data according to Zar (1984):

- Mean, Standard deviation and Standard error
- Student "t" test

RESULTS

During the present investigation 100 μmol L^{-1} concentration of vitamin E (α-Tocopherol) induced protective role against the atretogenic changes generated by the endosulphan at dose level 100 nmol mL^{-1}. Histopathological study under light microscope, granulosa cells stained with Hematoxylin-eosin (HE) showed the normal contour in the control group. In the control group, nucleus was spherical in shape and stained darkly with haematoxylin. Nuclear sap was clear and two to three heterochromatin bodies linked by a fine fibrillar chromatin were visible. The cytoplasm was dense and stained pink with eosin (Fig. 1). In the experimental group (A) treated with endosulphan at the dose level 100 nmol mL^{-1} revealed increasing morphological alterations in granulosa cells. Condensation of the granulosa cell nuclei was noticed after 1 h of endosulphan exposure and these atretic nuclei were darkly stained with haematoxylin (Fig. 2). Fragmentations of nucleus were also observed in most of the cells when exposure duration was increased up to 4 h. Bilobed, multilobed and crescent shaped nuclei were observed due to the endosulphan treatment. Hyalinization of cytoplasm became evident after the exposure durations of 4 h. As the exposure duration further enhanced up to 8 h there was increase in number of degenerating cells. The pyknoic nuclei were more frequently observed as the exposure duration increased. Fragmentation and crescent shaped nuclei number increased. Chromolysis and hyalinization were observed after 8 h in endosulphan treated group (Fig. 3).

Endosulphan destroys the granulosa cells structure and function in follicle by inducing oxidative stress and this damage was partially reversed by vitamin E antioxidant defense system. Vitamin E supplementation improved the cellular architecture in experimental group (B). In the experimental group (B) treated with endosulphan and also supplemented with vitamin E, histologically, cells acquired spherical contour; nucleus was vesicular and filled with nucleosol. The atretogenic

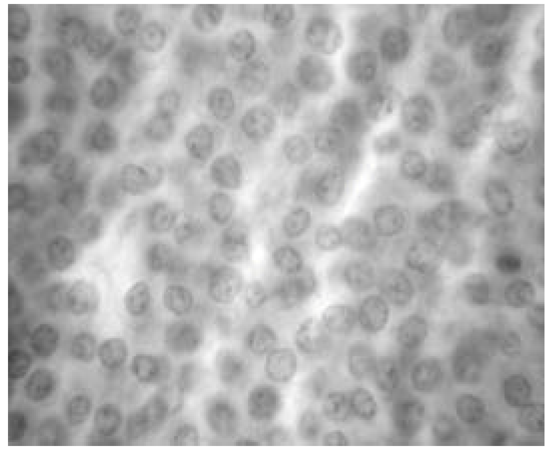


Fig. 1: Microphotograph of granulosa cells showing clarity of cells having normal contour and their general morphology in control group (X 1000)

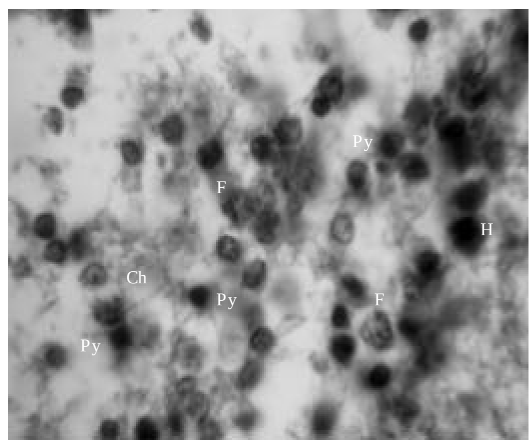


Fig. 2: Granulosa cells treated with endoulphan (100 nmol mL^{-1}) after 1 h of exposure duration showing pycnotic nuclei (Py), hyalinization (H), chromolysis (Ch) and fragmented nuclei (F). Pinching off of nuclear material was also observed. (X1000)

changes like pycnosis, chromolysis, condensation, fragmentation, hyalinization and crinkled and wavy membranes were drastically reduced in frequency as compared with the endosulphan exposed group [experimental group (A)]. In the granulosa cells treated with endosulphan and also supplemented with vitamin E [experimental group (B)], changes in cellular architecture were also observed but these changes were milder as

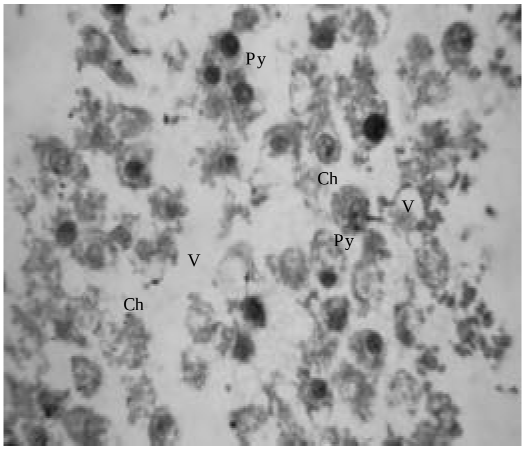


Fig. 3: Light micrograph of granulosa cells exposed with endoulphan (100 nmol mL^{-1}) showing increasing atretogenic changes, pycnotic nuclei (Py), chromolysis (Ch) were clearly noticed. Degenerating cytoplasm and vacuolization (V) was observed after 8 h of exposure duration. (1000 X)

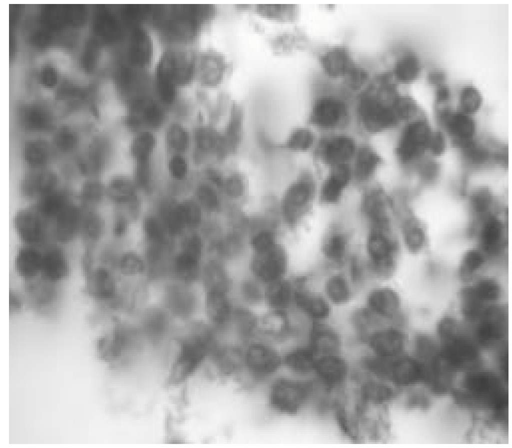


Fig. 5: Granulosa cells treated with endoulphan (100 nmol mL^{-1}) and supplemented with vitamin E (100 μmol L^{-1}) showing decline in number of pycnotic and fragmented nuclei after 4 h of exposure durations. (X1000)

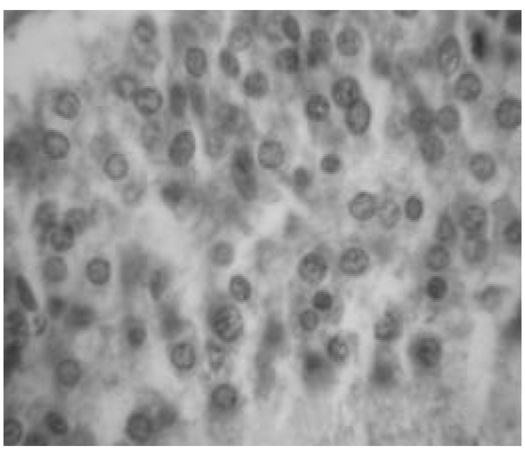


Fig. 4: Microphotograph of granulosa cells treated with endoulphan (100 nmol mL^{-1}) and supplemented with vitamin E (100 μmol L^{-1}) showing decline in atretogenic changes induced by endosulphan after 1 h of exposure duration. (X1000)

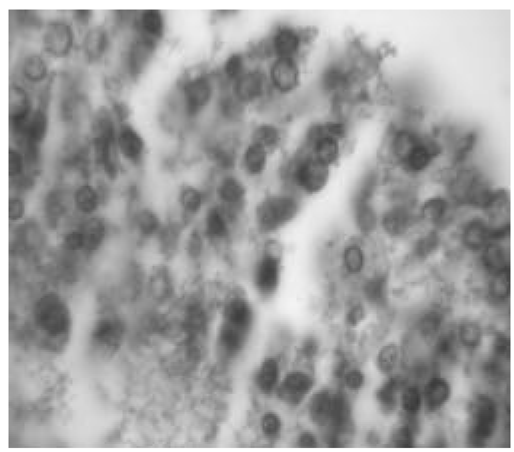


Fig. 6: Light micrograph of granulosa cells treated with endoulphan (100 nmol mL^{-1}) and supplemented with vitamin E (100 μmol L^{-1}) showing reduction in atreatic granulosa cells as compared with endosulphan treated granulose cells after 8 h of exposure duration. (X1000)

compared with the endosulphan exposure without vitamin E [experimental group (A)] (Fig. 4-6).

Endosulphan at dose level 100 nmol mL^{-1} [experimental group (A)] induced a decline in cell diameter from 7.5±0.0456 in control to 4.5±0.1024, 3.7±0.1001 and 3.2±0.1154 μm after exposure of 1, 4 and 8 h, respectively (Fig. 7) but in case of endosulphan supplemented with vitamin E [experimental group (B)], there was less decline in cell diameter that was 6.4±0.1235, 4.8±0.1809 and 4.1±0.0809 μm after exposure of 1, 4 and 8 h, respectively (Fig. 7). All the variations recorded were statistically

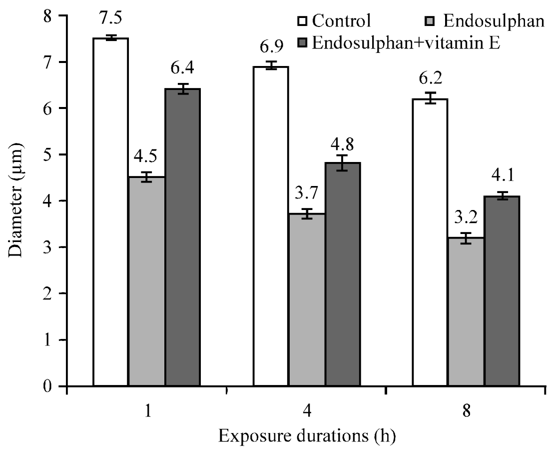


Fig. 7: Effect of vitamin E supplementation on diameter of granulosa cell after varying exposure durations

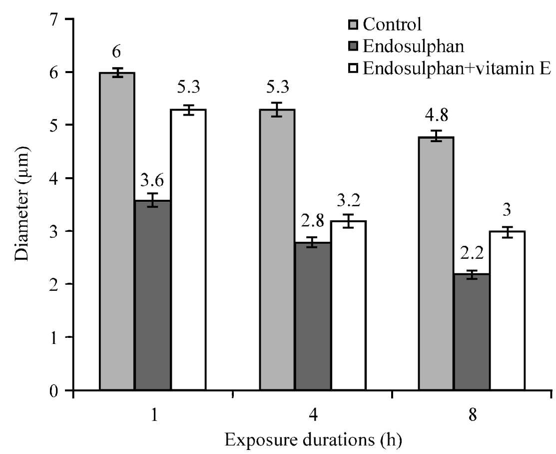


Fig. 8: Effect of vitamin E supplementation on nuclear diameter of granulosa cell after varying exposure durations

significant ($p \leq 0.05$). There was also decline in nuclear diameter of granulosa cells from 6.0±0.0872 in control group to 3.6±0.1252, 2.8±0.0954 and 2.2±0.0634 μm after exposure of endosulphan [experimental group (A)] at 1, 4 and 8 h, respectively, whereas in case of endosulphan with vitamin E supplementation [experimental group (B)], there was less decline in nuclear diameter that was 5.3±0.0966, 3.2±0.1397 and 3.0±0.0970 μm after exposure duration of 1, 4 and 8 h, respectively (Fig. 8). The variations recorded were statistically significant ($p \leq 0.05$).

In endosulphan treated group [experimental group (A)], atretic granulosa cells were enhanced from 24% in control to 53% after 1 h, 29 to 72% after 4 h and 42 to 88% after 8 h of exposure duration (Fig. 9). Chi-square values between control and endosulphan treated groups were

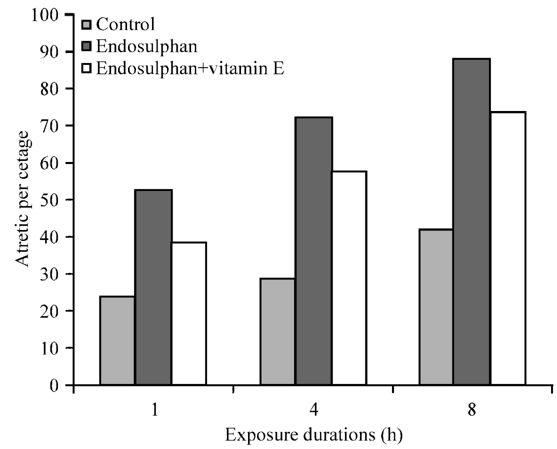


Fig. 9: Effect of vitamin E supplementation on atretic percentage of granulosa

Table 1: The comparison of a number of atretic granulosa cells 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 (h)	Endosulphan	Endosulphan+Vitamin E
1	17.76*	3.96*
4	36.98*	4.30*
8	65.22*	6.36*

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

17.76, 36.98 and 65.22 after 1, 4 and 8 h of exposure durations and all the variations recorded were statistically significant (χ^2 0.05) (Table 1). In the experimental group treated with endosulphan and simultaneously supplemented with vitamin E [experimental group (B)] there was decline in atretic cell percentage as compared with granulosa cells treated with endosulphan without antioxidant [experimental group (A)]. This decline in atretic granulosa cells was from 53% in endosulphan treated group [experimental group (A)] to 39% in endosulphan supplemented with vitamin E [experimental group (B)] was observed after 1 h of exposure duration, from 72 to 58% after 4 h of exposure duration and from 88 to 74% after exposure duration of 8 h (Fig. 9). Chi-square values were 3.96, 4.30 and 6.36 after 1, 4 and 8 h of exposure duration, respectively (Table 1).

DISCUSSION

During the present investigation effect of vitamin E (α-tocopherol) in rescue of degenerative changes induced by the endosulphan in granulosa cells of goat *Capra hircus* have been analyzed *in vitro*. The results of the present experimental study revealed that antioxidant vitamin E have exerted the protective effect in atretogenic changes generated by the endosulphan in granulosa cells *in vitro*. During the present investigation endosulphan exposure induced decline in granulosa cells diameter and also resulted in reduction in nuclear diameter of granulosa cells in the time dependent manner. Supplementation of vitamin E with the exposure of endosulphan prevents the decline in cell and nuclear diameter of granulosa cells and thus provides defense system against the endosulphan induced degenerating effects. Results of the present investigation strongly support the earlier studies involving the antioxidants amelioration against different toxicants (Evans and Bishop, 1922; El-Demerdash *et al*., 2004; Guney *et al*., 2007; Fulia *et al*., 2011). Guney *et al*. (2007) have investigated the effect of subchronic administration of Methidathion (MD) on ovary and evaluated ameliorating effects of vitamins E and C against methidathion toxicity. Present studies are in agreement with the results of Evans and Bishop (1922) who have reported that vitamin E prevents loss of spermatogenesis in males and the failure to retain zygotes in female rats. There is not so much literature available related to the amelioration of antioxidants in females, lots of work have been done by the earlier researchers in male organisms exhibiting the rescue of degeneration induced by different toxicants including pesticides. The present observations that vitamin E supplementation prevents the fast increase in number of atreatic cells in culture due to the endosulphan toxicity and thus resulted in decline in atretic granulosa cell percentage. This decline in atretic granulosa cells was from 53% in endosulphan treated group to 39% in endosulphan supplemented with vitamin E after 1 h of exposure duration, from 72 to 58% after 4 h of exposure duration and from 88 to 74% after exposure duration of 8 h. The results of the present investigation suggested that antioxidant exerts ameliorating effects and improves the female fertility by declining the atretogenic changes induced by the exposure of endosulphan, these results are in accordance with the findings of Akmal *et al*. (2006) in which antioxidant enhance the reproductive potentials in males. Antioxidant vitamin C supplementation in infertile men improves sperm count, sperm motility and sperm morphology and might have a place as an additional supplement to improve the semen quality towards conception (Akmal *et al*., 2006). The present study showed that vitamin E can protect the tissue from damage due to the toxicant strongly supports the findings of El-Demerdash *et al*. (2004) who have observed the role of vitamin E and selenium in alleviating the negative effects induced by aluminium in male rats. Keskes-Ammar *et al*. (2003) have reported that vitamin E and selenium supplementation produced a significant decrease in MDA concentrations and an improvement of sperm motility. The results confirm the protective and beneficial effects of vitamin E and selenium on semen quality and advocate their use in infertility treatment. Esterbauer *et al*. (1991) also observed that vitamin E have beneficial effects in improving the fertility in which they indicate that α-tocopherol results in oxidation resistance of Low-density Lipoproteins (LDL) and also the oxidative resistance increased nearly linearly with increasing α-tocopherol content.

CONCLUSION

Nano-molar concentrations of endosulphan severely impaired the granulosa cell contour and hence affect their function. Despite lower doses of exposure, prolonged duration in pesticide lashed environment leads to serious endocrine problems associated with the reproduction in particular. Vitamin E supplementation provides protection against oxidative stress induced by exposure of pesticide endosulphan. The information generated will help in formulating policies and strategies to circumvent the toxic affects of endosulphan.

ACKNOWLEDGMENT

Authors are thankful to Department of Zoology, Kurukshetra University, Kurukshetra for providing all the facilities throughout the study.

REFERENCES

Aitken, R.J. and M.A. Baker, 2006. Oxidative stress, sperm survival and fertility control. Mol. Cell. Endocrinol., 250: 66-69.

Akmal, M., J.Q. Qadri, N.S. Al-Waili, S. Thangal, A. Haq and K.Y. Saloom, 2006. Improvement in human semen quality after oral supplementation of vitamin C. J. Med. Food, 9: 440-442.

Alahyary, P., M.I. Poor, F.F. Azarbaijani and V. Nejati, 2008. The potential toxicity of diazinon on physiological factors in male rat. Pak. J. Biol. Sci., 11: 127-130.

Bay, K., C. Asklund, N.E. Skakkebaek and A.M. Andersson, 2006. Testicular dysgenesis syndrome: Possible role of endocrine disrupters. Best Pract. Res. Clin. Endocrinol. Metab., 20: 77-90.

Biswas, A.K., N. Kondaiah, A.S.R. Anjaneyulu and P.K. Mandal, 2010. Food safety concerns of pesticides, veterinary drug residues and mycotoxins in meat and meat products. Asian J. Anim. Sci., 4: 46-55.

Buttke, T.M. and P.A. Sandstrom, 1994. Oxidative stress as a mediator of apoptosis. Immunol. Today, 15: 7-10.

El-Demerdash, F.M., M.I. Yousef, F.S. Kedwanyand and H.H. Baghdadi, 2004. Role of alpha-tocopherol and beta-carotene in ameliorating the fenvalerate-induced changes in oxidative stress, hemato-biochemical parameters and semen quality of male rats. J. Environ. Sci. Health B, 93: 443-459.

Esterbauer, H., M. Dieber-Rotheneder, G. Striegl and G. Waeg, 1991. Role of vitamin E in preventing the oxidation of low-density lipoprotein. Am. J. Clin. Nutr., 53: 314S-321S.

Evans, H.M. and K.S. Bishop, 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science, 56: 650-651.

Fujii, J., Y. Iuchi and F. Okada, 2005. Fundamental roles of reactive oxygen species and protective mechanisms in the female reproductive system. Reprod. Biol. Endocrinol., 3: 43-52.

Fulia, A., P.K. Chauhan and R.K. Sharma, 2011. Ameliorating effect of vitamin E on testicular toxicity induced by endosulphan in *Capra hircus in vitro*. J. Pharmacol. Toxicol., 6: 133-140.

Gellert, R.J., 1978. Kepone, mirex, dieldrin and aldrin: Estrogenic activity and the induction of persistent vaginal estrus and anovulation in rats following neonatal treatment. Environ. Res., 16: 131-138.

Gorman, A., A. McGowan and T.G. Cotter, 1997. Role of peroxide and superoxide anion during tumour cell apoptosis. FEBS Lett., 404: 27-33.

Gray, L.E. Jr., V.S. Wilson, T. Stoker, C. Lambright and J. Furr *et al*., 2006. Adverse effects of environmental antiandrogens and androgens on reproductive development in mammals. Int. J. Androl., 29: 96-104.

Guney, M., H. Demirin, B. Oral, M. Ozguner, G. Bayhan and I. Altuntas, 2007. Ovarian toxicity in rats caused by methidathion and ameliorating effect of vitamins E and C. Hum. Exp. Toxicol., 26: 491-498.

Halliwell, B. and J.M.C. Gutteridge, 2007. Free Radicals in Biology and Medicine. 2nd Edn., Oxford University Press, New York.

Harvey, F., 2010. Agricultural pollution: Inputs that place huge pressure on the land. Environ. Correspondent, 16: 56-56.

Iribhogbe, O.I., A. Aigbiremolen, U. Akpamu, J.E. Emordi and E.O. Nwoke, 2011. Effects of selected antioxidant vitamins on lipid profile in early pregnancy. Asian J. Sci. Res., 4: 62-70.

Keskes-Ammar, L., N. Feki-Chakroun, T. Rebai, Z. Sahnoun and H. Ghozzi *et al*., 2003. Sperm oxidative stress and the effect of an oral vitamin E and selenium supplement on semen quality in infertile men. Arch. Androl., 49: 83-94.

Lucesoli, F. and C.G. Fraga, 1999. Oxidative stress in testes of rats subjected to chronic iron intoxication and alpha-tocopherol supplementation. Toxicology, 132: 179-186.

Masouleh, F.F., B.M. Amiri, A.R. Mirvaghefi and M.A. Nemtollahi, 2011. *In vitro* effects of diazinon on male reproductive tissue and sperm motility of caspian kutum (*Rutilus frisii kutum*). Res. J. Environ. Toxicol., 5: 108-116.

McLachlan, J.A., 2001. Environmental signaling: What embryos and evolution teach us about endocrine disrupting chemicals. Endoc. Rev., 22: 319-341.

Pearse, A.G.E., 1968. Histochemistry: Theoritical and Applied. Churchill, London..

Saradha, B., S. Vaithinathan and P.P. Mathur, 2008. Lindane alters the levels of HSP70 and clusterin in adult rat testis. Toxicology, 243: 116-123.

Sharma, R.K. and A. Fulia, 2009. Influence of tocopherol in rescue of granulosa cell atresia in goat ovary. Adv. Pharmacol. Toxicol., 10: 119-126.

Sharma, R.K., P.K. Chauhan and A. Fulia, 2010a. Endosulphan induced changes in fine morphology of goat spermatogonia *in vitro*. Res. J. Environ. Toxicol., 4: 214-222.

Sharma, R.K., A. Fulia and P.K. Chauhan, 2010b. Protective effect of ascorbic acid against endosulphan induced testicular toxicity in goat *in vitro*. J. Biol. Sci., 10: 624-630.

Skakkebaek, N.E., E.R. De Meyts and K.M. Main, 2001. Testicular dysgenesis syndrome: An increasingly common developmental disorder with environmental aspects: Opinion. Human Reprod., 16: 972-978.

Tilly, J.L. and K.I. Tilly, 1995. Inhibitors of oxidative stress mimic the ability of follicle stimulating hormone to suppress apoptosis in culture rat ovarian follicles. Endocrinology, 136: 242-252.

Vale, C., E. Fonfria, J. Bujons, A. Messeguer, E. Rodriguez-Farre and C. Sunol, 2003. The organochlorine pesticides γ-hexachlorocyclohexane (lindane), α-endosulfan and dieldrin differentially interact with GABAA and glycine-gated chloride channels in primary cultures of cerebellar granule cells. Neuroscience, 117: 397-403.

Varayoud, J., L. Monje, T. Bernhardt, M. Munoz-de-Toro, E.H. Luque and J.G. Ramos, 2008. Endosulfan modulates estrogen-dependent genes like a non-uterotrophic dose of 17 beta-estradiol. Reproductive Toxicol., 26: 138-145.

Verhaegen, S., A.J. McGowan, A.R. Brophy, R.S. Fernandes and T.G. Cotter, 1995. Inhibition of apoptosis by antioxidants in the human HL-60 leukemia cell line. Biochem. Pharmacol., 50: 1021-1029.

Zar, J.H., 1984. Biostatistical Analysis. 4th Edn., Prentice Hall, Englewoods Cliffs, New Jersey.

Zidan, N.E.H.A., 2009. Evaluation of the reproductive toxicity of chlorpyrifos methyl, diazinon and profenofos pesticides in male rats. Int. J. Pharmacol., 5: 51-57.

Zoeller, R.T., A.L.S. Dowling, C.T.A. Herzig, E.A. Iannacone, K.J. Gauger and R. Bansal, 2002. Thyroid hormone, brain development and the environment. Environ. Health Perspect, 110: 355-361.