Histo-morphological alterations in testis of goat *(Capra hircus)* induced by atrazine in vitro: evaluation of ameliorating effect of vitamin E

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SUMMARY

The aim of this study was to investigate the histomorphological alterations in goat testis which are induced by atrazine. Atrazine [2-chloro-4 (ethylamino)-6 (isopropylamino) -s-triazine] is an agricultural herbicide that are used to prevent pre and post emergence broadleaf weeds in crops such as maize and sugarcane but it has been proved that it interferes with hormonal activity of the humans and animal at extremely low doses. It has now been recognized as endocrine disruptor and thus affects the reproductive performance by reducing fertility. For this purpose, the testis from slaughtered mature goat (*Capra hircus*) were collected and the restoration effect of α-tocopherol (100 mol L-1) on degenerative alterations generated by atrazine have been evaluated in vitro on sampled tissue. The seminiferous tubules in the experimental group treated with atrazine at dose levels of 1 nM or 100 nM showed histomorphological changes. Small vacuoles were found in the cytoplasm of spermatogonia after 4 hours of exposure to atrazine at a concentration of 1 nM. Chromolysis were also noticed in the spermatogonia and spermatids. The change detected in seminiferous tubules by 1 nM and 100 nM atrazine concentrations was decreased to some extent in the experimental group treated with 1 nM and 100 nM atrazine doses combined with 0.1 nM Vitamin E (Vitamin E is best known for its antioxidant properties). In seminiferous tubules, vacuolization was considerably decreased, the basal lamina remained intact, and nucleus fragmentation was minimized as compared to tubules treated with single pesticide dosage. The results gathered in the current study suggest that Vitamin E therapy was able to reverse atrazine-induced derangements through the improvement of antioxidant capacity and endocrine function.

KEY WORDS

α-tocopherol; atrazine; seminiferous tubules; goats; vacuolization; vacuoles.

INTRODUCTION

In the process of development of agriculture, pesticides have become an important tool in livestock farming, cropping, horticulture, forestry, home gardening, homes, hospitals, kitchens, roadsides, recreational and industrial areas (Kent, 1991)[1]. A vast majority of the population in India is engaged in agriculture and is, therefore, exposed to the pesticides due to their high persistence in the environment. These pesticides can be fungicide, herbicide, weedicide or insecticide. The 6-chloro-s-triazine herbicides, including atrazine, cyanazine, propazine and simazine, which inhibit photosynthesis (the Hill reaction, PSII) in plants, are the most heavily used pesticides in the world (Gammon, 2005)[2].

Atrazine [2-chloro-4 (ethylamino)-6 (isopropylamino) -s-triazine] is an agricultural herbicide that are used to prevent pre and post emergence broadleaf weeds in crops such as maize and sugarcane but it has been proved that it interferes with hormonal activity of the humans and animal at extremely low doses. It has now been recognized as endocrine disruptor and thus affects the reproductive performance by reducing fertility. It can act within the brain to disrupt the cascade of hormonal signal need to initiate ovulation. Atrazine maintains high blood levels of 17 beta-estradiol (E2) and prolactin by suppressing the luteinizing hormone surge throughout the estrous cycle. Increase in testicular weight, degenerative changes in seminiferous tubules and abnormality in reproductive functions in rat have been recorded due to exposure of pesticides (Lee et al., 1978; Mishra et. al., 1993)[3,4]. Chloropyrifos-methyl, diazinon and profenofos induced decline sperm count associated with increased number of abnormal spermatozoa (Berseth et al., 2009)[5]. The pesticides used in the crop field have adverse effects on livestock (Hambidge, 1986)[6]. While most of this special issue is devoted to the testis, which is where most drug and chemically induced toxicity of the male reproductive tract is identified, being able to recognize and understand the potential effects of toxicants on the epididymis is immensely important and an area that is often overlooked (Kempinas and Klinefelter, 2014)[7]. The epididymis is the duct of male reproductive system where the post-testicular sperm differentiation occurs that provides microenvironment that is pivotal for testicular sperm to mature

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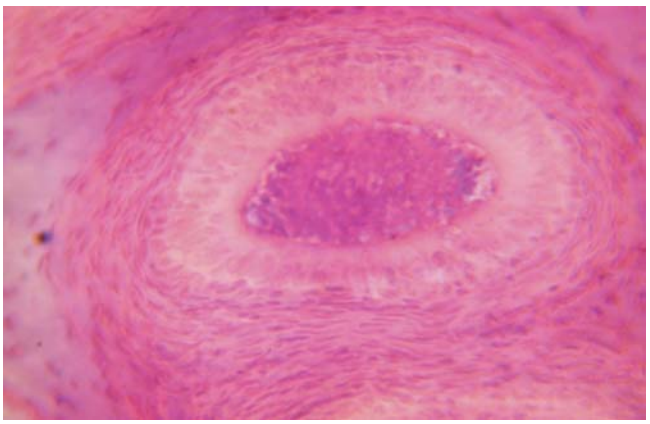

Figure 1 - Microphotograph of goat caput showing principle cells and luminal space filled with spermatozoa in control (without treated) stained with Hematoxylin and Eosin at 400X.

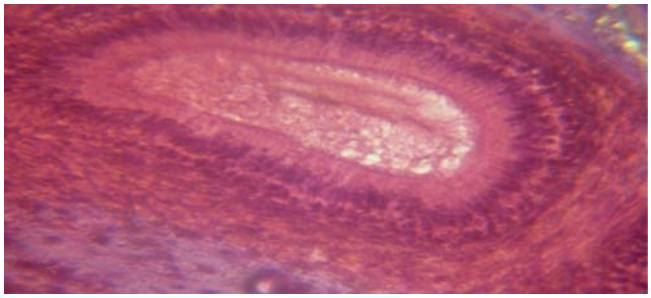

Figure 2 - Microphotograph of goat caput showing intact basal and principle cells and luminal space is filled with speramatozoa in control (without treated) (H&E 400X).

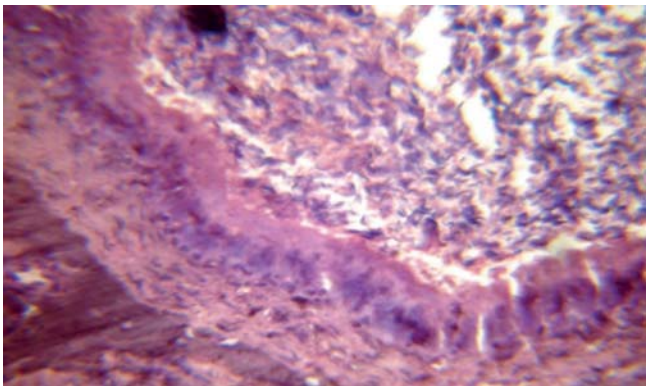

Figure 3 - Microphotograph of goat caput after exposure with 1 nM concentration of atrazine for 4 h showing intact seminiferous tubules with spermatogonia having vacuolization in cytoplasm (arrow) (H&E 400X).

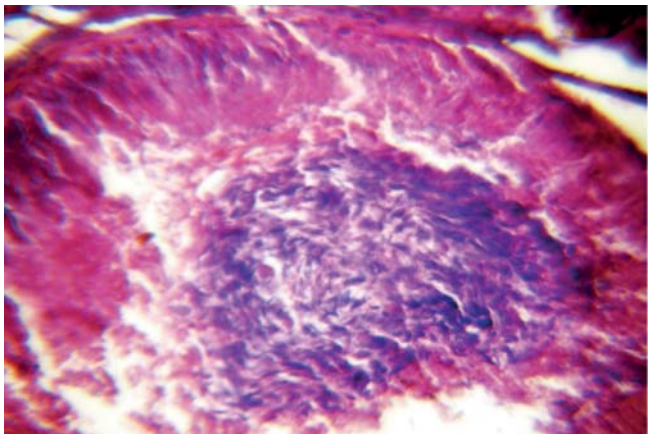

Figure 4 - Microphotograph of goat caput treated with 1 nM Atrazine concentration after exposure for 6 hours showing high density of spermatozoa (solid arrow) (H&E 400X).

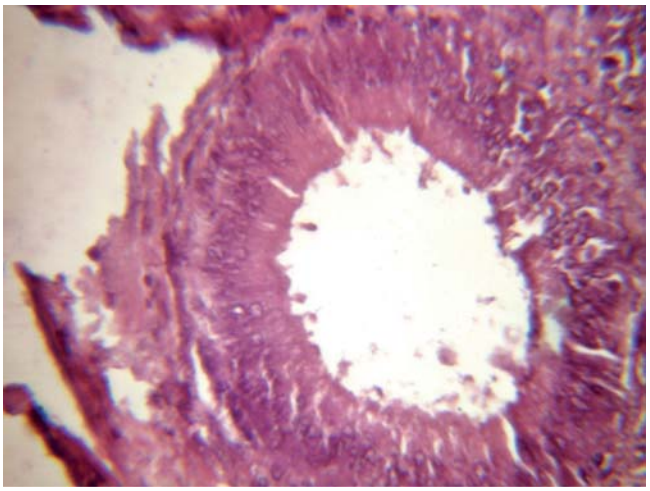

Figure 5 - Microphotograph of goat caput treated with 100 nM Atrazine concentration after exposure for 4 hours showing increase in luminal diameter (double headed arrow) with scanty spermatozoa (H&E 400X).

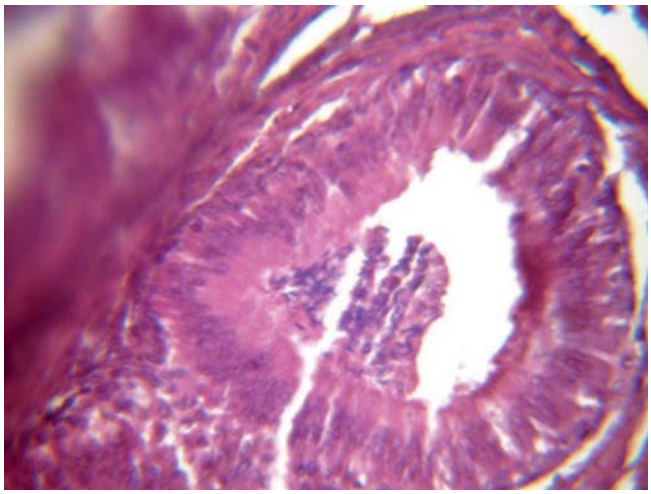

Figure 6 - Microphotograph of goat caput treated with 100 nM Atrazine concentration after exposure for 6 hours showing detachment from basal lamina (solid arrow), reduction in thickness of epithelium (dashed double headed arrow), increase in diameter of lumen (H&E 400X).

cells (Kempinas and Klinefelter, 2014)[7].

Various environmental stresses lead to excessive production of ROS causing progressive oxidative damage and ultimately cell death. Oxidative stress can cause a host of serious health problems, including neurodegenerative disease. Scavenging or detoxification of excess ROS is achieved by an efficient antioxidative system comprising of the non-enzymatic as well as enzymatic antioxidants (Pallavi et al., 2012)[8]. Antioxidants counteract free radicals and free radicals cause oxidative stress. Antioxidants have various biological activities, including the abil-

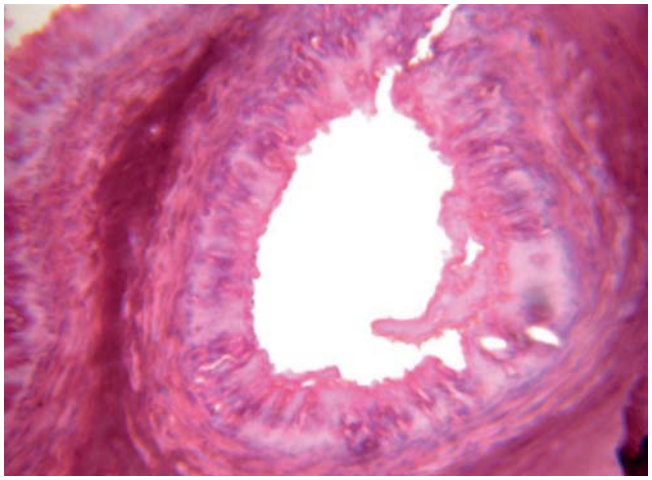

Figure 7 - Microphotograph of goat caput after exposure with 1 nM concentration of atrazine with 0.1 nM Vitamin E after exposure for 4 h showing different cell types in epithelium viz. principal cells (solid arrow), basal cells (dashed arrow) and apical cells (hollow arrow) (H&E 400X).

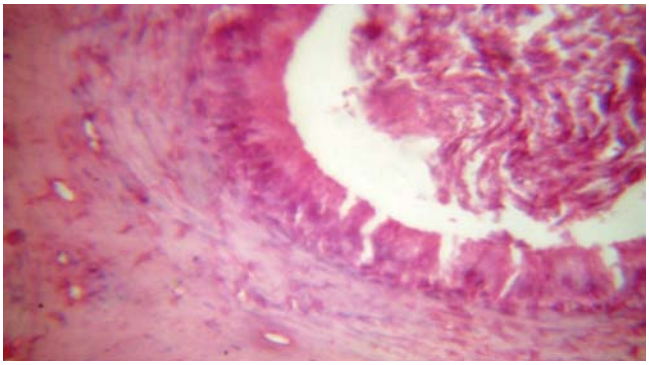

Figure 8 - Microphotograph of goat caput treated with 100 nM Atrazine concentration with 0.1 nM vitamin E after exposure for 4 hours showing recovery in lumen and high sperm density (H&E 400X).

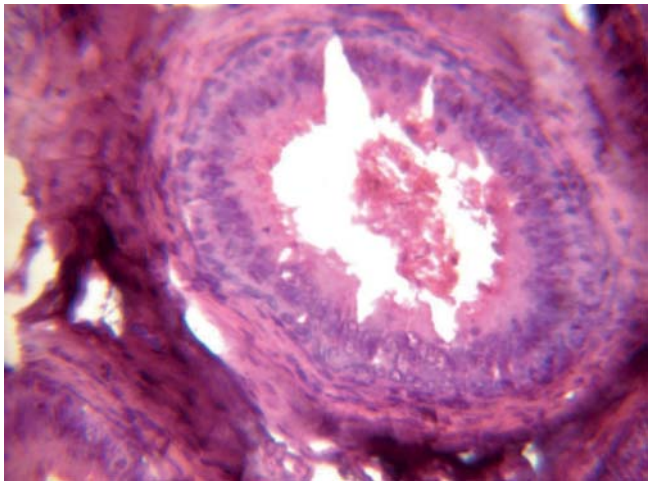

Figure 9 - Microphotograph of goat caput treated with 1 nM Atrazine concentration and 0.1 nM Vitamin E after exposure of 6 hours showing decrease in luminal diameter and some clear appearance of cells in epithelium (H&E 400X).

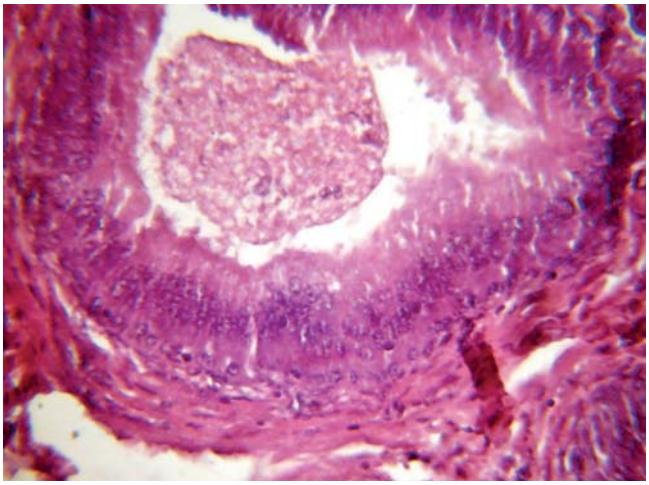

Figure 10 - Microphotograph of goat caput after exposure with 100 nM concentration of atrazine and 0.1 nM Vitamin E for 6 hours showing increase in thickness of epithelium (double headed arrow), decrease in luminal diameter along with increased density of spermatozoa (solid arrow) (H&E 400X).

ity to counteract pesticide toxicity. Some experimental studies have demonstrated that antioxidants like vitamin C and E can be used to nullify pesticide toxicity (Yousef et al., 2006)[9]. Vitamin E is probably best known for its antioxidant properties. It helps to stabilize cell membranes and protect the tissues of the skin, eyes, liver, breast, and testes; as "In particular, Vitamin E, thanks to its antioxidant properties, is of paramount importance in the stabilizing the cell membrane and protecting the lungs from oxidative damage from environmental substances; helps heart and muscle cell respiration by improving function with less oxygen. Vitamin E may improve stamina and endurance and reduce cardiovascular disease. Vitamin E reduces platelet aggregation and platelet adhesiveness to collagen, even more than aspirin (Celestini et al., 2002)[10].

So, the aim of the study was to determine the histomorphological alterations in goat testis which are induced by atrazine. It is in the light of this information, the present study has been focused on the effect of atrazine and its reversal by Vitamin E in caput of goat (*Capra hircus*).

MATERIALS AND METHODS

Collection of Materials

Goat Testis: The testis of mature goat (*Capra hircus*) was procured from slaughter house near Kurukshetra latitude 29.97oN, 76.84o E. The material was brought to the laboratory in the Department of Zoology, Kurukshetra University, Kurukshetra, in ice cold 0.9% normal saline.

Pesticide Atrazine: Atrazine was collected from the pesticide/fertilizer distributor near the Kurukshetra latitude 29.97oN, 76.84oE and stored in dry conditions.

Vitamin E: it was collected from the wholesale pharmaceutical distributor in Kurukshetra and brought to the laboratory in the Department of Zoology, Kurukshetra University, Kurukshetra, and stored at 10 °C.

Tissue Processing

The tissue was harvested and processed for histological slide preparation. The caput sections were fixed in the Bouins fix-

Table 1 - Experimental design of *in vitro* culture of testis treated with nanomolar concentration of Atrazine and Vitamin E.

Control (Group A)	Exposure duration	Treatment (Group B)	Treatment (Group C)	Treatment + Ameliorate (Group D)	Treatment + Ameliorate (Group E)
Group A1	4 Hours	Group B1 (1nM : Atrazine)	Group C1 (100nM : Atrazine)	Group D1 (1nM : Atrazine + 0.1nM : Vitamin E)	Group E1 (100nM : Atrazine + o.1nM : Vitamin E)
Group A2	6 Hours	Group B2 (1nM : Atrazine)	Group C2 (100nM : Atrazine)	Group D2 (1nM : Atrazine + 0.1nM : Vitamin E)	Group E2 (100nM : Atrazine + 0.1nM : Vitamin E)

ative for 24 hours. Then the testis tissues were washed in running tap water for 2 hours. These were then dehydrated in upgrade alcohol series. After proper dehydration the tissue were embedded in paraffin wax at 58-60 °C. Blocks were made, trimmed and ribbons of wax having sections of tissue were cut using microtome at 5-micron thickness. These sections were placed on slides and were stretched to remove any folds in the tissue section. The stretched slides were stained with hematoxylin and eosin.

Staining procedure for Hematoxylin-Eosin Stain

Stretched slides were de-waxed in xylene for 15 minutes and were then passed through downgrades of alcohols 100% to 30% for 5 minutes each. After keeping in water for 2-3 minutes, the slides were kept in haematoxylin stain for 2 minutes. The excess of stain was removed by giving dip in acid water. The slides were then placed under slow running tap water for another 2-3 minutes for stain to develop.

Further upgrading of slides was done from 30% to 70% alcohol. Then these were double stained by giving a dip in eosin stain, then processed through 70% to 100% alcohol grades. After placing xylene for 10 minutes, the slides were mounted in DPX.

RESULTS

Testicular tissue sections (5 m) stained with haematoxylene and eosin revealed normal histoarchitecture of the seminiferous tubules in control group. In the control group, the epididymis revealed the normal arrangement of different types of tissue (Figure 1 and 2).

In the experimental group treated with atrazine with dose level 1 nM or 100 nM revealed histomorphological alterations in the seminiferous tubules. Atrazine of 1 nM concentration after 4 hours of exposure duration revealed small vacuoles in the cytoplasm of spermatogonia (Figure 3). Chromolysis were also noticed in the spermatogonia and spermatids. Pycnosis were also visible in some of the germ cells present in seminiferous tubule.

As the exposure duration of atrazine increased up to 6 hours the atretogenic changes in different germ cells and somatic cells were enhanced in a time dependent manner (Figure 4). Large vacuoles of varied sizes and shapes were clearly visible in the seminiferous tubules. Various small vesicles were also noticed in nuclei of various germ cells and somatic cells.

Atrazine of 100 nM concentration after 4 hours of exposure duration revealed hyalinization and fragmented nuclei. Basal lamina was also detached from the underlying cells in few portions of seminiferous tubules, were frequently observed. Vacuoles were of very large size and dislodging of germ cells in seminiferous tubules was observed (Figure 5). Frequency of fragmented nuclei was enhanced with increased exposure duration of 6 hours of atrazine at dose level of 100 nM concentration. It also induced a decline in diameter of spermatocyte. Chromatin gets condensed and apoptosis in spermatogonial cells were also observed (Figure 6).

When experimental group treated with 1 nM and 100 nM atrazine dose along with 0.1 nM Vitamin E, the alteration observed in seminiferous tubules by 1 nM and 100 nM atrazine concentrations were reduced to some extent. Vacuolization reduced greatly, basal lamina remained intact and decrease in fragmentation of nucleus was observed in seminiferous tubules as compared to the tubules treated with only pesticide dose (Figures 7, 8, 9 and 10). Thus we can say that administration of vitamin E was able to revert the atrazine induced derangements through promoting antioxidant capacity and endocrine function.

DISCUSSION

Effects of atrazine at different dose level of testicular tissue of goat have been analyzed. The results of present investigation demonstrated that degenerative changes in spermatogonial germ cells were induced by nanomolar concentration of atrazine *in vitro* in dose and time dependent manner. It also demonstrated the ameliorating effect of Vitamin E against atrazine toxicity on caput epididymis of goat.

Light microscopic analyses revealed increased thickness of tunica albuginea, atrophied seminiferous tubules, arrested spermatogenesis, decreased Leydig cells/mm^2 of interstitial tissue (2.0 ± 0.7/mm^2 in high dose received rats), vasodilatation and thrombosis (Dehkhargani et al., 2011)[11]. The results of present finding are in agreement with the above findings.

DMMP (dimethyl methyl phosphonate) altered reproductive function at all dose levels, while histological abnormalities of the testis were seen only in the high-dose group. Changes in the testes of the high-dose animals were characterized by lack of spermatogenesis or by degeneration, vacuolization, and necrosis of cells in the spermatogenic tubules (Dunnick et al., 1984)[12]. The results of present finding are in agreement with the above findings.

Histological examination revealed significant alteration in the testis including focal mild testicular damages, blood hemorrhage and vascular congestion, hypospermatogenesis, dilation and tubular deformity, cellular vacuolated degeneration (necrosis), aspermatogenesis and tubular destruction and atrophy (Al-Jahdali, 2007)[13]. Results of present study demonstrated that there was increase in atretic percentage of spermatogonial

germ cells as the exposure duration increased. This study supports the findings of Al-Jahdali (2007)[13].

The alteration observed in seminiferous tubules by 1 nM and 100 nM atrazine concentrations was reduced to some extent when treated with 0.1 nM Vitamin E. In seminiferous tubules, vacuolization was greatly reduced, the basal lamina remained intact, and nucleus fragmentation was reduced when compared to tubules treated with only pesticide dose. Similar types of degenerating changes were reported by Sharma et al. (2012)[8] upon supplementation of Vitamin E at dose level 100 umol L^{-1} along with exposure of atrazine.

The results demonstrated that atrazine induced atretogenic changes like chromolysis, pycnosis, fragmenataton, hyalinization and condensation were dramatically diminished when the atrazine culture was supplemented with 100 umol L^{-1} concentrations of vitamin E. Thereby indicating protective effect of Vitamin E on atrazine damage induced potential on the cells. Our results are in agreement with the observations of Mediratta et al. (2008)[14] who have reported the sub chronic lindane exposure increased MDA level in serum indicative of an elevation in free oxygen radical generation pesticides. Antioxidant supplementation with ascorbic acid produced significant amelioration up to the control level in morphological degeneration due to pesticides. Ascorbic acid exhibited beneficial effects in detoxifying the effects of atrazine in the spermatogonial parameters in rabbits and other mammals.

CONCLUSIONS

In conclusion, the results gathered in the current study showed that atrazine-organochloride compound, even at low concentration, has damaging effects on goat testicular tissue as fine morphology and severely impaired spermatozoa formation with negative repercussion on the reproductive performance of the goat. According to the findings herein obtained, the use of antioxidants such as Vitamin E could be a represent a strategy useful in ameliorating testicular histological alteration induced by pesticides.

This finding will be of great value in assessing the health hazards of pesticides in domestic animals and in providing a baseline study regarding the reproductive toxicity induced by exposure of pesticides in reproductive tissue altering structural and functional integrity and directly affecting the reproductive potential of target and non-target organism. But this kind of toxic effect can be cured by uptake of these antioxidants in diet.

Declarations of Competing Interest

The authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

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