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Histomorphological Alterations in Goat Testicular Tissues by Cyfluthrin and Its Restoration by Vitamin E

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ABSTRACT: The aim of this study was to reveal the impact of cyfluthrin on goat testicular tissue and its restoration by vitamin E. The culture of testicular tissue was done for 4 and 8 hours. Cyfluthrin was given to testicular tissues in two concentrations, i.e., 100 µg/ml and 200 µg/ml, while vitamin E (0.1 M/ml) was given to testicular tissues having the above-mentioned concentrations of cyfluthrin to study its mitigating effects. Cyfluthrin has been found to cause severe damage to testicular cells, as there was a decrease in the number of spermatozoa and spermatids, vacuolization of the lumen of the germ cells, and decreased sperm lumen in cyfluthrin-treated groups. The addition of vitamin E reduced the number of apoptotic cells, vacuolization, and desquamation of germ cells in the lumen and pyknotic cells. In comparison to the treatment group, the sperm population increased and the shape of the seminiferous tubules improved. Vitamin E, an antioxidant, prevented cyfluthrin-induced testicular damage *in vitro* and restored fertility in goat testes exposed to the drug. The findings of this study will be extremely helpful in assessing the potential health risks of cyfluthrin in domestic animals and will also be used as an *in vitro* model for research on the effects of xenobiotics on mammals and will have implications in determining the harmful effects that can cause reproductive damage. The outcomes of this study will support the use of vitamin E in the physiological treatment of negative effects brought on by cyfluthrin exposure in ruminants.

Keywords: Spermatozoa, cyfluthrin, Testis, spermatids, Vitamin E.

INTRODUCTION

Pest control and agriculture benefit greatly by the widespread use of pesticides. In addition to their beneficial effects, they also have negative effects on various biological structures of organisms that are not targeted. Due to their non-persistent and biodegradable properties, pyrethroids, a class of insecticides, seek greater fascination. Reversibly, they interact with numerous ion channels, possibly by targeting sodium channels (Davis *et al*., 2007; Narashi *et al.,* 2007; Peterson *et al.,* 2008). There are two types of pyrethroids based on the cyano group: types I and II. A common household insecticide is cyano pyrethrin, also known as cyfluthrin used for a wide range of things, like controlling pests (Jamjoom *et al*., 1993) and treating scabies; both in domestic and agricultural practices. They are extremely targeted at their targets (Wolansky *et al*., 2008). However, several studies have also shown that these pesticides harm the male reproductive systems of non-targeted species and have also been linked to deteriorating effects on the normal architecture of the mammalian testis (Omotoso *et al*., 2014).

Zhang and co-workers in 2007 too reported decreased sperm count, sperm motility, plasma testosterone concentration, and testicular testosterone concentration when these pesticides were administered orally to adult mice. A common Type II pyrethroid, cyfluthrin, is proposed for the present study. It is the first insecticide (mixture of four diastereoisomeric pairs of enantiomers) which was approved in USA for use (USEPA, 1987). In some countries, it is frequently used in agriculture, veterinary medicine to combat variety of pests and used in residential and industrial settings and for product safety and public health (Ritter and Chappel, 1997; FAO, 1999; Surgan *et al*., 2002). Cyfluthrin, a pesticide used in agricultural and household products, has recently been shown to have the ability to increase the activity of several subfamilies of the CYP1A, CYP4A, and CYP2E enzymes in Wistar rats. This information lends credence to its classification as a peroxisome proliferator-activated receptor alpha (PPARα) ligand with potential implications for oxidative stress (Anadón *et al*., 2013). Additionally, it has been reported that intraperitoneal cyfluthrin treatment alters AchE and GPx activities in the kidney and liver of Wistar rats (Yilmaz *et al*., 2015). Cyfluthrin enters the brain, accumulates significantly, and is easily absorbed orally; therefore, it stands to reason that non-target organisms' CNS function is affected by cyfluthrin (Rodrguez *et al*., 2018). There are few studies on the reproductive toxicity of cyfluthrin (Mense *et al*., 2006; Rodriguez *et al*., 2016) and it is one of the pyrethroid insecticides that is used most frequently around the world (Surgan *et al*., 2002) therefore, we selected this pesticide to reveal its toxicity on testis of goat.

Vitamin E on the other side is an antioxidant with significant lipid-soluble properties. It protects spermatozoa from peroxidative damage and mobility loss by acting as a significant membrane protector against lipid peroxidation and ROS (Ghosh *et al*., 2002). Wang and colleagues discovered that vitamin E reduced a variety of testicular alterations and increased testicular weight in rats. It was also discovered that it helps in restoring normal structure and reduce apoptosis in testicular cells (Wang *et al*., 2017). Cyfluthrin-induced abnormalities in goat testis and Vitamin E's protective effects were poorly understood in small ruminants as evident from the review of literature. The significance of the afore mentioned impacts of cyfluthrin in mammalian testis requires further research, especially using tissues taken from different region of goat testis such as corpus, caput, and cauda. The creation of appropriate animal models will be critical in gaining a better understanding of the impact of cyfluthrin on reproductive system of mammals and most of the earlier studies have been carried out on rat, mice, and pigs. Yet only fragmentary information is available on small ruminants. Considering these voids, the current study proposed to determine the useful effects of Vitamin E in cyfluthrin induced alterations in the structure of goat testicles.

MATERIALS AND METHODS

A. Chemicals

The cyano-4 fluoro-3-phenoxybenzyl cyfluthrin has the following molecular formula: C22H18Cl2FNO3 with CAS number and molar mass of 434.29 68359-37-5 was purchased from Sigma Aldrich Chemicals Pvt Ltd Plot No.12, Jigini Link Road, Bommasandra Industrial Area, Bangalore - 560099, India, with a purity of 98 percent. In dimethyl sulfoxide (DMSO, Sigma Chemical Co.), a fresh stock solution of 1 mg/ml was made. By dissolving it in tissue culture media of the appropriate concentration, additional dilutions (100µg/ml and 200µg/ml) were prepared.

B. Sample collection

The mature goat testis (*Capra hircus*) was brought from slaughter houses near Kurukshetra (N 29° 58' 34.6764", E 76° 53' 33.9") and brought to the laboratory in normal saline at 4 °C. After the testis was decapsulated, it was cut into pieces about the size of 1 mm^3. Tissues from the testicles were washed three times with TCM-199 after being floated on medium on the nucleopore filter. According to the experimental design, the medium is kept in a CO_2 incubator at 39°C, 95% humidity, and 5% CO_2 and contains TCM-199 as well as antibiotics (200 units of penicillin 100IU/ml and streptomycin 100IU/ml).

C. Experimental Design

Two groups of testicular tissues were separated. Control samples were in Group I and the experimental groups E1 and E2 were in group II. The E1 (the treated group) received two different doses of cyfluthrin, 100µ g/ml and 200 µg/ml, for 4 and 8 hours, respectively. Cyfluthrin and vitamin E (0.1 M/ml) were given to the E2 (protective) group.

Histomorphological studies [Pearse, 1968]. Testicular tissues from both the control and the experimental groups were processed following treatment for histomorphological studies using slightly modified standard methods from Pearse (1968). After being fixed in Bouin's fixative, the cultured tissues were dehydrated using a series of alcoholic grades and cleared using two xylene changes in succession. Paraffin wax was used to encase the tissues. After being serially sectioned at a thickness of 5 µm using a rotatory microtome, the tissues were stretched and dewaxed for 15 minutes using xylene. The slides were stained using the standard hematoxylin and eosin staining method after being soaked in various grades of alcohol. The slides were viewed on an Olympus CX41 equipped with a Magnus digital adapter. The photomicrographs were taken with a 400X Olympus digital camera attached to the microscope.

RESULTS

The testicular section in the control group had a normal histopathological structure during the current study. Spermatocytes, spermatogonia, spermatozoa, and spermatids were well organised in seminiferous tubules. The sertoli and leydig cells are shown within the connective tissue around the seminiferous tubules (Fig. 1 A). Cyfluthrin at a dose of 100 µg/ml altered the normal histoarchitecture in the goat testicular tissue, causing seminiferous tubule distortion, vacuolization, and germ cell desquamation in the lumen (Fig. 1 B). It was observed that the testicular sections were more damaged at a dose of 200 µg/ml of cyfluthrin. The vacuolization and dislodging of germ cells increased when compared to a dose of 100 µg/ml, while the number of spermatogonia and spermatocyte cells decreased (Fig. 1 F). The testicular sections showed pyknosis, vacuolization and damaged seminiferous epithelium at a dose level of 100 µg/ml (cyflythrin) and there was increase in the above-mentioned apoptotic characteristics with an increase in exposure duration from 4 to 8 hours and decrease in the germ cell population, spermatozoa, spermatids, spermatogonia, and spermatocytes (Fig. 1 D). After administering 200 µg/ml of dose, the seminiferous tubules separated from one another and empty spaces between them were clearly visible, resulting in an increase in testicular section alterations in a time and dose dependent manner (Fig. 1 H and F). Cyfluthrin-induced damage, including vacuolization, pyknosis, and desquamation of germ cells, was less severe in the vitamin E-supplemented tissue. The presence of an increased number of spermatozoa, spermatids, and spermatozoa in the lumen of the seminiferous tubules suggests that the germ cells present there have been restored (Fig. 1 I, C, G and E).

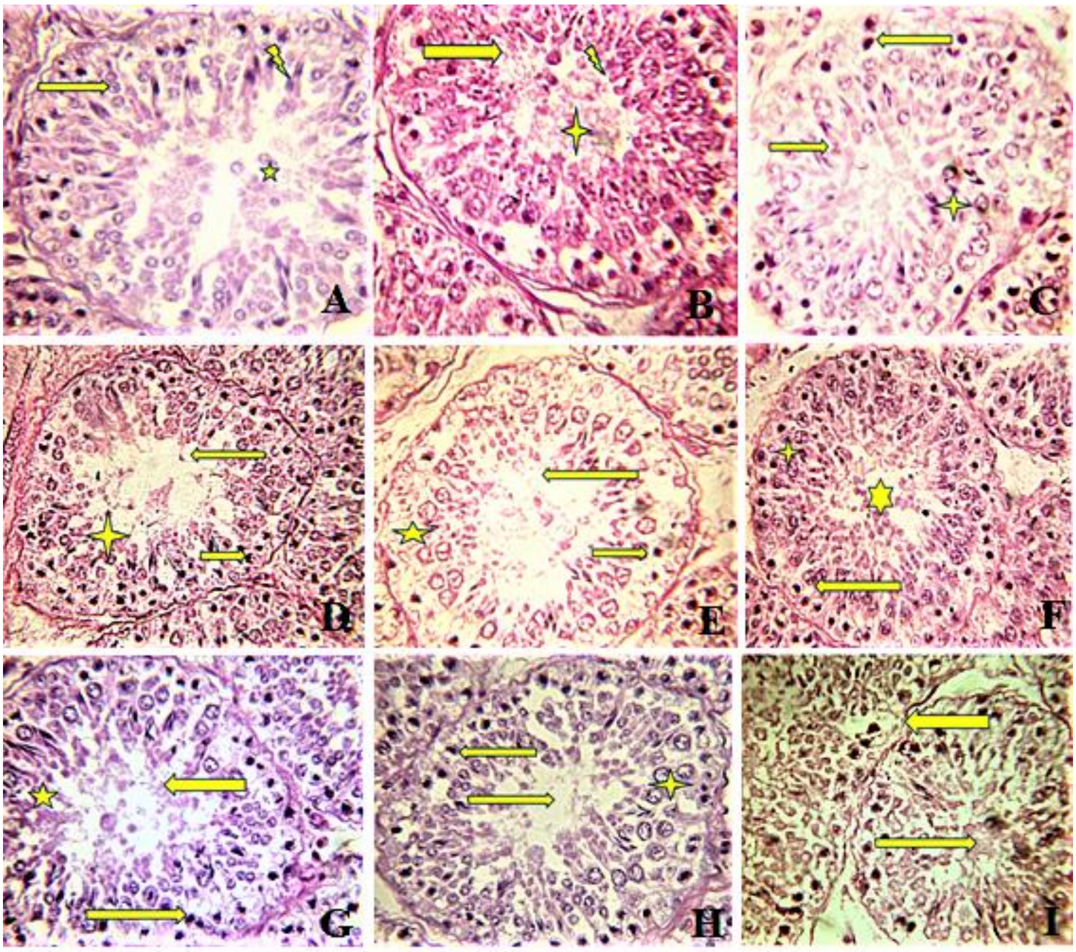

Fig. 1. A: Tranverse section of testis of control shows intact seminiferous tubules containing normal architecture of spermatocytes, spermatogonia and sperms at 4 hr of exposure duration. B: Exposure of 100 μg/ml cyfluthrin at 4hrs reveals vacuolization, detachment of germ cells from basement membrane towards lumen and decrease in number of sperms. C: Upon supplementation of Vitamin E along with 100 μg/ml cyfluthrin exhibit intact basement membrane with proper orientation of germ cells *viz.*, spermatocytes, spermatogonia and sperms decrease in vacuolisation and pyknosis. D: At 8 hrs exposure duration and dose of 100 μg/ml cyfluthrin unveil damage in seminiferous epithelium, dislodging of germ cells in lumen and decreased number of spermatozoa population. E: Seminiferous tubules are almost normal in their structure with healthy population of germ cells, less pyknotic cells and reduced vacuolization was observed after the administration of Vitamin E and 100 μg/ml cyfluthrin at 8 hrs of time duration. F: Dose of 200 μg/ml cyfluthrin at 4hrs duration displayed utmost vacuolization, increased number of damaged germ cells in the lumen and diminished spermatozoa. G: Amelioration by Vitamin E showed remarkable recovery in histological alterations of seminiferous tubules and germ cells in comparison to the treated groups. H: Maximum alterations in the histoarchitecture of testicular tissue were seen at 200 μg/ml cyfluthrin and 8 hrs of exposure duration. I: Upon administration of Vitamin E with 200 μg/ml cyfluthrin revealed increase in healthy number of germ cells population and almost structured seminiferous tubule in comparison to treated groups.

DISCUSSION

Food sources that have been contaminated with pesticides are to blame for several sexual problems in humans and animals. It has raised significant concerns among health, toxicology, and biological scientists regarding pesticides' potential to be toxic. The current study found that vitamin E protected goat testis from cyfluthrin's deteriorating effects. Cyfluthrin has been shown in previous studies to cause brief nerve impulse trains, affecting the normal function of the testis (Perger and Szadkowski 1994). The present study reported that cyfluthrin caused histopathological changes in goat testicular tissue in relation to dose and time. At 100 μg/ml of cyfluthrin, the altered characters were noticed such as vacuolization, Pyknosis, germ cell desquamation in the lumen, and decrease in the number of spermatogenic cells.

The results of the present study are in consistent with the findings of Rajawat *et al.* (2014) who also reported vacuolization, cell degeneration, and a decrease in the number of spermatids and spermatozoa in the testicular tissue of Swiss albino mice treated with cyfluthrin. The observations of the current study strongly support the earlier findings of Bhardwaj and Sharma (2017) who have also reported that permethrin induced histological alterations in goat testicular tissue and Vitamin C helps in restoration of testicular alterations. Present research work also exfoliated that dose and exposure time of cyfluthrin play important role in determining extent of testicular damage. The Present findings agree with

Gabbianelli *et al.* (2004), who have suggested that the time of treatment is the most decisive parameter in inducing damages. Present observations strongly support the earlier findings on pesticides induced various histopathological alteration in the reproductive system of male mammals (Mahgoub and El-Medany, 2001; Uzunhisarcikli *et al.*, 2007).

During the present study, the testicular structure got repaired when vitamin E was given along with respective doses of cyfluthrin. The changes recorded were increase in population of spermatocytes, spermatids and spermatogonia in the peripheral region of the seminiferous tubules and reduced vacuolization, pyknosis and dislodging. Sharma *et al*. (2012) had also observed similar morphological changes in atrazine treated goat testis after Vitamin E supplementation. The present results also support the earlier findings of Mohafrash *et al*. (2017), who reported that the germinal epithelium underwent cyfluthrin-induced atrophy and vacuolization. The results of the recent findings strongly support the observation of Leung and Wong (2013), who noticed an increase in the population of germ cells in mice exposed to amifostine. Fulia *et.al* (2011) have also reported that testicular tissues supplemented with vitamin E showed a higher degree of recovery in germ cells and majority of the testicular structure was re-established. The results of the present study strongly advocate the findings of Omran *et al.* (2017) who have demonstrated that administration of the Vitamin E along with the BPA (reproductive toxicant) showed proper orientation of the germ cells within the seminiferous tubules, less sloughing off the germ cell in the luminal compartment and increased number of the healthy spermatogonia in male rats. As Vitamin E is a potent antioxidant which helps in restoring the testicular functioning in males by reducing the level of the reactive oxygen species and lipid peroxidation. The ROS and peroxides which were produced as a result of exposure of the BPA were neutralized by Vitamin E (Fawzy *et al.*, 2018).

CONCLUSION

Cyfluthrin has a significant impact on mammals' reproductive system, impairing spermatogenesis, according to the findings of this study. The testis' normal structure significantly improved after Vitamin E administration. To lessen the harmful effects of pesticides and insecticides, vitamin E supplementation is suggested. Cyfluthrin's negative effects on domestic animals and their amelioration by antioxidants like Vitamin E will be assessed using the findings of this study, which will also be used to develop strategies and approaches to manage its negative effects.

FUTURE SCOPE

The results of present study have revealed the deleterious effects of cyfluthrin on testes. The information generated will be of great value in evaluating the health hazards of insecticides to domestic animals and their reversal by Vitamin E can provide important early evidences about the damage induced by the exposure of pyrethroids on reproductive potential of mammals and its management. The information gathered will help in devising policies and strategies to manage insecticides toxicology. The effect of vitamin E shall be analysed for their antioxidant potential in reversing cyfluthrin induced changes and can safely be recommended for further use.

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Conflict of Interest. There is no conflict of interest between the authors.

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