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TRICLOSAN INDUCED OXIDATIVE STRESS IN EPIDIDYMAL SPERM OF GOAT, *CAPRA HIRCUS* L. (1758) *IN VITRO*

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ABSTRACT

Triclosan is a widely used antimicrobial agent in pharmaceuticals and personal care products. The present study was undertaken to evaluate the influence of triclosan on the induction of oxidative stress in goat epididymal sperm *in vitro*. Sperm collected from mature fertile domestic male goat (*Capra hircus* L.) weighing 16 ± 2 Kg were incubated with triclosan in Ringer's phosphate solution (RPS medium). Experiments were carried out in three groups, such as Group I: Single duration at different concentrations (50, 100, 200 and 300 µM/L for 5 h), Group II and III: Two different single concentrations (200 and 300 µM/L, respectively) at different durations (1, 2, 3, 4 and 5 h). Assay of antioxidant enzymes as superoxide dismutase, catalase and glutathione reductase were done in all groups. In addition, the levels of hydrogen peroxide generation and lipid peroxidation were also determined in all groups. In Group I, when epididymal sperm incubated for 5 h with triclosan, at 200 and 300 µM concentrations significantly decreased the activities of antioxidant enzymes and increased hydrogen peroxide generation and lipid peroxidation. In Group II, significant reduction in antioxidant enzymes with concomitant increase in lipid peroxidation were observed only after 4 h of incubation of sperm with triclosan. However, in Group III, the activities of antioxidant enzymes decreased significantly immediately after 1 h of exposure to triclosan with time-dependent significant increase in the levels of hydrogen peroxide and lipid peroxidation. The present results suggest that triclosan induced oxidative stress in epididymal sperm of goat *in vitro*.

KEYWORDS: Triclosan, epididymal sperm, oxidative stress, antioxidant enzymes, goat.

INTRODUCTION

Exposure to toxic man-made environmental contaminants has profound and lasting effects on reproductive health of humans and other animals. The evidences that links the exposure of environmental contaminants to that of adverse reproductive health effects has become a critical area of intervention to biologists in the field of reproductive toxicology. Organisms when exposed to environmental contaminants at any point of time, either prenatal or postnatal exposure, can lead to harmful reproductive health outcomes, particularly to humans. Sometimes, it can disrupt or interfere with the physiology of cell, tissue or organ and may be passed down to future generations.

Triclosan, 5-chloro-2-(2, 4-dichlorophenoxy) phenol or 2, 4, 4′-trichloro-2′-hydroxydiphenyl ether is a widely used antibacterial or antimicrobial agent in a variety of pharmaceuticals, personal care and household products.[1] Its efficacy and safety in use have led to its widespread use in personal hygiene products and in cosmetics. Pharmacokinetics and toxicological effects of triclosan have been extensively studied in humans as it is known to be absorbed from the gastrointestinal tract and across the skin in humans.[2] Numerous reports have noted its occurrence in body fluids such as plasma, breast milk and urine.[3,4] Triclosan has also been detected in maternal and cord blood at higher concentrations and are likely to be retained in human cells.[5] Although the biological half-life of triclosan in human plasma and urine is 21 and 11 h, respectively, data from biological studies have demonstrated unambiguous evidence for bioaccumulation of triclosan resulting from its daily use.[6]

Triclosan, an endocrine disruptor has been shown to disrupt normal function of androgens in males and estrogen binding in females of rats.[7] Triclosan has been shown to reduce sperm count and produced degenerative damage to male reproductive tissues such as the testes, vas deferens and prostate, and also disrupted the level of androgen, a potent male hormone in rats.[8] Therefore, there is clear evidence that triclosan impairs male reproductive functions in rats. Epididymis, an important male accessory sex organ located between the efferent ducts and vas deferens protect, store and mature the spermatozoa before its release. The predominance of polyunsaturated fatty acids (PUFA) in the plasma membrane of the spermatozoa renders them highly susceptible to lipid peroxidation because of attacks by reactive oxygen species (ROS).[9]

Gametes are susceptible to the attack of ROS when exposed to exogenous toxicants and that can be analyzed *in vitro* by using valid reproductive toxicological parameters. The imbalance between the production of ROS and a biological system's ability to readily detoxify the reactive intermediates or easily repair the resulting damage is known as oxidative stress, which is ultimately harmful to spermatozoa. In the present study the effect of triclosan on the activities of antioxidant parameters was evaluated using oxidative stress as biomarker of triclosan toxicity in epididymal sperm of goat *in vitro*. The study, therefore, provides baseline knowledge of triclosan toxicity by the induction of oxidative stress which may further influence on male infertility of goat.

MATERIALS AND METHODS

Chemicals

Triclosan (5-chloro-2-(2,4-dichlorophenoxy)phenol) of 97% purity, malondialdehyde, pyrogallol, EDTA, NAD and NADPH were obtained from HiMedia Laboratories, Mumbai, India. All other chemicals were of analytical grade and obtained from local commercial sources.

Collection and incubation of epididymal sperm

Testes of adult fertile goat were obtained from local slaughter house, from which epididymal sperm were collected as described by Gangadharan et al.[10] Briefly, epididymis was chopped into several pieces with a sharp razor blade and dispersed in a modified Ringer's phosphate solution (RPS) at pH 6.9, with gentle stirring. Sperm samples dispersed in media were adjusted to final concentration of 10^8 sperm in 2 ml of buffer and incubated with triclosan.

Experimental design

Group I: Single duration at different concentrations

Triclosan at 20 mM/ L concentration was prepared as stock dissolved in 100% DMSO. From the stock solution, different concentrations of Triclosan were prepared as 50, 100, 200 and 300 µM and suspended in culture media for incubation at 32ºC for 5 h. The culture medium consists of Triclosan at respective concentrations (10 µl) and epididymal sperm sample maintaining 10^8 sperm cells in 2 ml of modified RPS medium. Toxicant-free controls namely, epididymal sperm in modified RPS medium, and epididymal sperm in modified RPS medium with DMSO were maintained along with treatment groups at 32ºC for 5 h.

Group II: Single concentration (200 µM/ L) at different durations

From the stock solution, 200 µM/ L concentration of Triclosan was treated in culture media containing epididymal sperm suspension and RPS medium which was incubated at 32ºC for different time intervals as 1, 2, 3, 4 and 5 h. Two control groups such as toxicant-free epididymal sperm in modified RPS medium, and epididymal sperm in modified RPS medium with DMSO were maintained along with treatment groups at 32ºC for 1, 2, 3, 4 and 5 h.

Group III: Single concentration (300 µM/ L) at different durations

In this experiment, culture media containing epididymal sperm suspension in RPS medium were treated with 300 µM/ L concentration of Triclosan was incubated at 32ºC for 1, 2, 3, 4 and 5 h. Two control groups such as toxicant-free and solvent-free group, and toxicant free but with solvent (DMSO) were maintained separately along with treatment groups at 32ºC for 1, 2, 3, 4 and 5 h.

At the end of respective hour of incubation in all experiments, the sperm cell suspensions, after washing with Ringer's phosphate medium, were homogenized in a glass teflon homogenizer for ten seconds and centrifuged at 800g for 10 minutes and the supernatant was used for biochemical assays.

Biochemical analysis

Superoxide dismutase,[11] catalase,[12] glutathione reductase[13] were assayed. Hydrogen peroxide generation[14] and the level of lipid peroxidation was measured using thiobarbituric acid color reaction for malondialdehyde, according to the method of Ohkawa et al.[15] using UV-Visible spectrophotometer.

Statistical analyses

Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test using statistical package SPSS 17.0. Differences were considered to be significant at $p<0.05$ against control groups. Data are presented as mean ± SD for n=6. All biochemical estimations were carried out in triplicates.

RESULTS

Group I: Single duration at different concentrations

Incubation of sperm with triclosan for 5 h at 50 and 100 µM/ L concentrations showed no significant changes in the activities of antioxidant enzymes as superoxide dismutase, catalase

and glutathione reductase in epididymal sperm of goat when compared to the corresponding control groups (Figures 1-3). Similarly the levels of hydrogen peroxide generation and lipid peroxidation showed no significant changes at 50 µM/ L concentration than the control groups, whereas lipid peroxidation increased significantly at 100 µM/ L concentration without changes in the level of hydrogen peroxide generation (Figures 4 and 5). Epididymal sperm when incubated with triclosan for 5 h at 200 and 300 µM/ L concentrations showed significant ($P<0.05$) decrease in the activities of superoxide dismutase, catalase and glutathione reductase with concomitant significant ($P<0.05$) increase in the levels of hydrogen peroxide generation and lipid peroxidation than that of corresponding control groups (Figures 1-5).

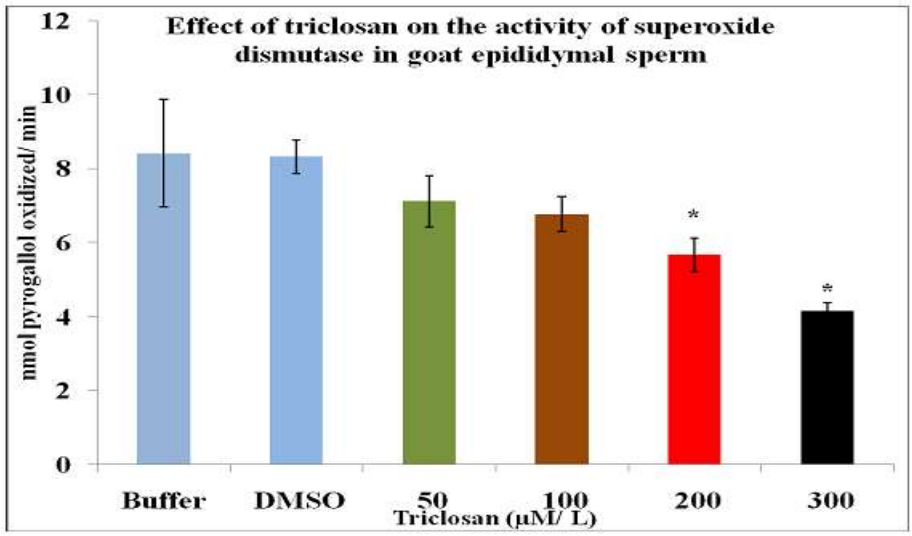
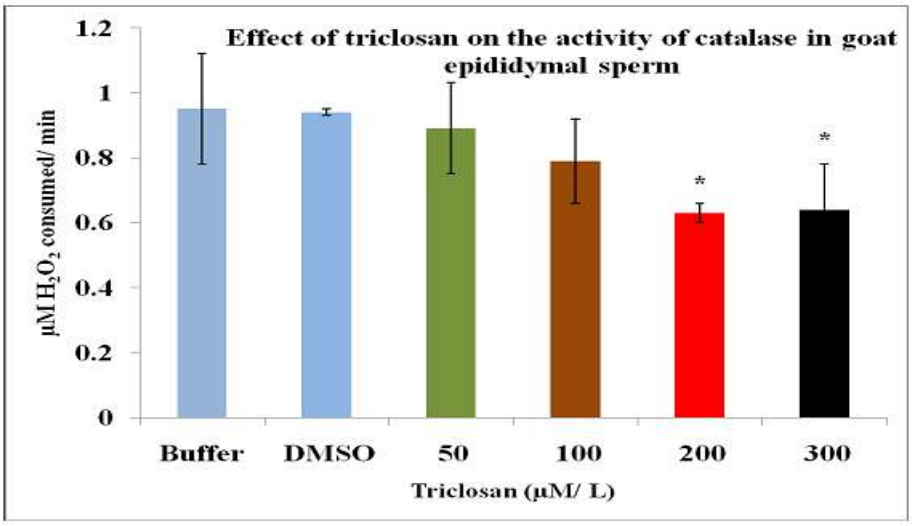


Fig. 1.



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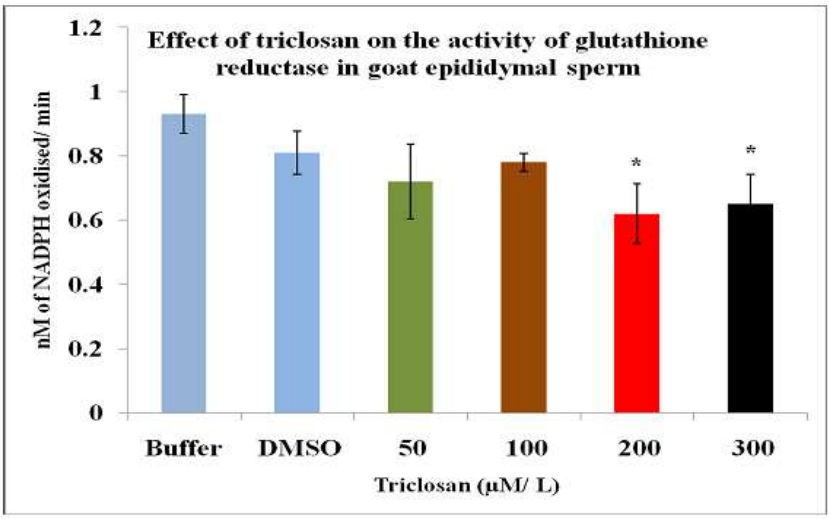

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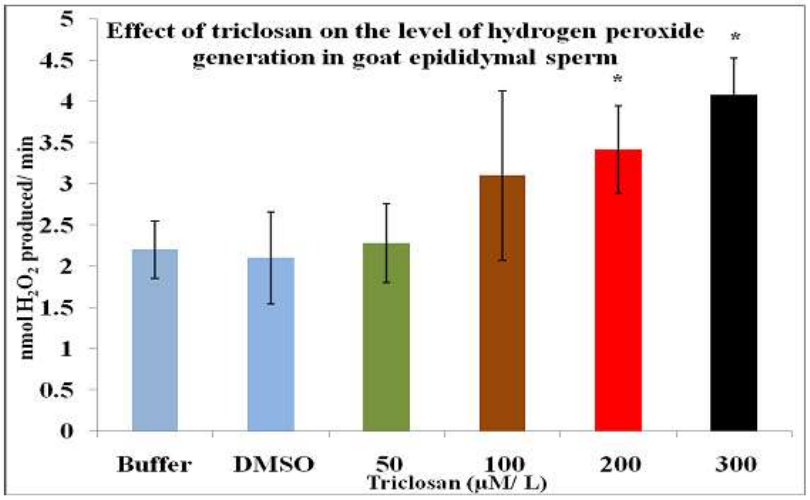

Fig. 4.

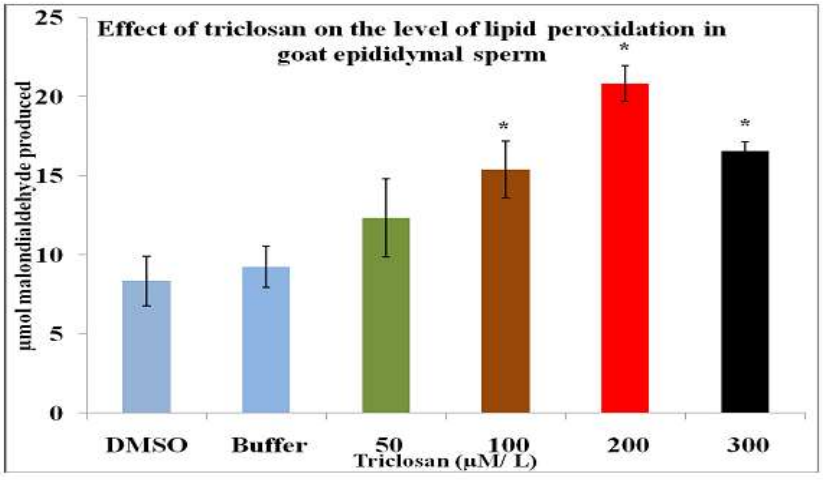

Fig. 5.

Group II: Single concentration (200 µM/ L) at different durations

Triclosan at 200 µM/ L concentration when incubated for 1, 2, 3, 4 and 5 h durations showed significant ($P<0.05$) decrease in the activity of superoxide dismutase (Figure 6). But the activities of catalase and glutathione reductase decreased significantly only after 4th h of triclosan incubation in epididymal sperm of goat when compared to the corresponding control groups (Figures 7 and 8). Similarly, the level of hydrogen peroxide generation increased significantly ($P<0.05$) only after 4 h of incubation (Figure 9), whereas the level of lipid peroxidation showed significant increase immediately after 1 h of triclosan incubation (Figure 10).

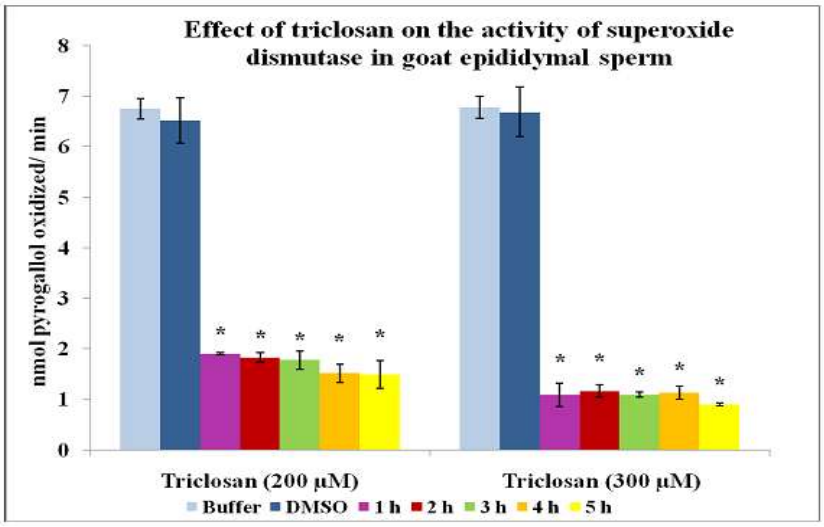

Fig. 6.

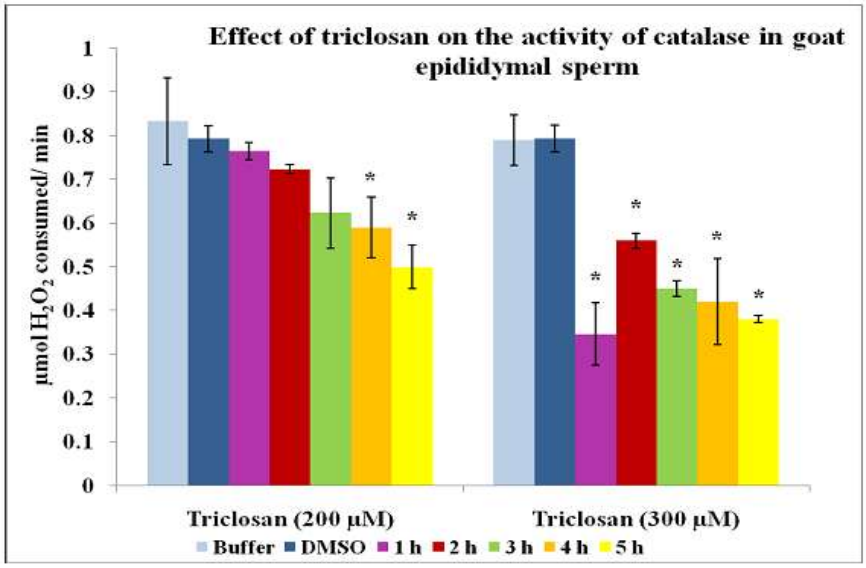

Fig. 7.

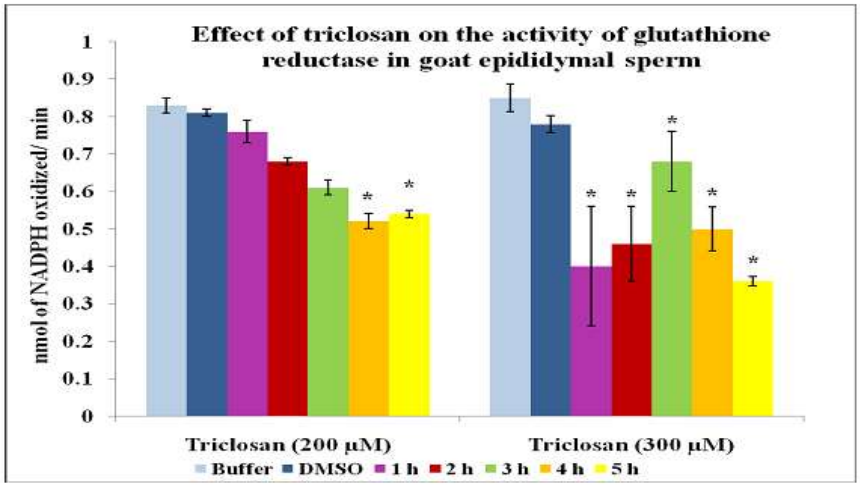

Fig. 8.

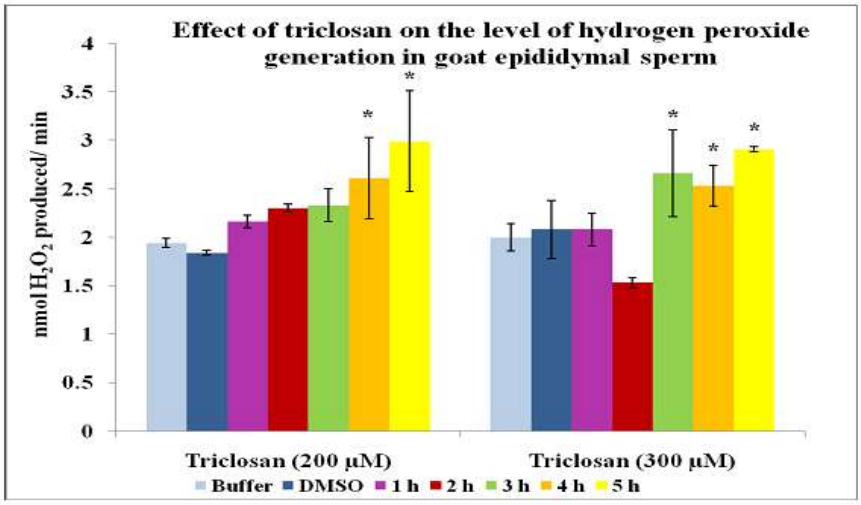

Fig. 9.

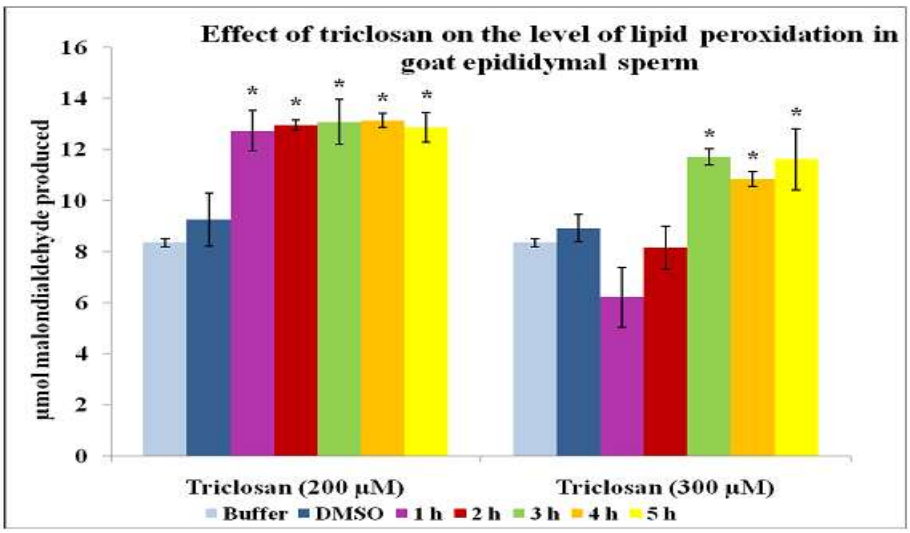

Fig. 10.

Group III: Single concentration (300 µM/ L) at different durations

Sperm when incubated with triclosan at 300 µM/ L concentration for 1, 2, 3, 4 and 5 h durations showed significant ($P<0.05$) decrease in the activities of superoxide dismutase, catalase and glutathione reductase in epididymal sperm of goat when compared to that of control groups (Figures 6-8). However, the levels of hydrogen peroxide generation and lipid peroxidation showed significant ($P<0.05$) increase only after 3 h of triclosan incubation (Figures 9 and 10).

DISCUSSION

Triclosan is a most common chlorinated aromatic compound with antibacterial, antifungal and antiviral properties. Its popular use in consumer products is known to have extensive contamination in environment, particularly affecting humans. The bioaccumulation of triclosan is more prominent as the compound is lipophilic in nature. Owing to its continuous threat to human population, the present study was focused mainly on the effect of triclosan on oxidative stress in epididymal sperm and thereby affects male fertility of goat.

There has been an increased concern regarding the male infertility and environmental pollutants. One of the major causes of impaired sperm functions and male infertility is oxidative stress. Variety of adverse environmental factors can initiate oxidative stress in male germ cells. Oxidative stress is caused by an excess of reactive oxygen species (ROS) generation that damages proteins, lipids and DNA in human spermatozoa and is considered as a major cause of impaired sperm function.[16]

Spermatozoa have been considered to be highly susceptible to the damage induced by ROS because of their high content of polyunsaturated fatty acids. In spermatozoa several antioxidant defence systems, namely, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase are known to operate in order to prevent possible cellular damage.[17] Oxygen is an essential element for aerobic metabolism, since it is the terminal acceptor of electrons in oxidative phosphorylation. ROS such as superoxide anion (O_2^-), hydroxyl radicals ($OH^\cdot$) and hydrogen peroxide (H_2O_2) can elicit widespread damage to cells, such as lipoperoxidation of polyunsaturated membrane lipids. Lipid peroxidation is a free radical-mediated chain reaction, since it is self-perpetuating and the length of the propagation depends on the chain breaking antioxidant. The most commonly generated ROS by spermatozoon are O_2^-, H_2O_2 and $OH^\cdot$. Generally, low concentrations of superoxide anion and hydrogen peroxide are needed for sperm capacitation, hyperactivation of motility, acrosome

reaction and sperm-oocyte fusion.[17,18] However, the higher concentrations lead to oxidative stress.

Decreased antioxidant enzymes and simultaneous elevation of H_2O_2 in sperm exposed to triclosan indicate a functional insufficiency of antioxidant system. SOD dismutase superoxide anion into H_2O_2 and oxygen, the H_2O_2 further neutralised by CAT and glutathione peroxidase/reductase system using reduced glutathione enzymes.[9] Also, CAT reduced exogenous and endogenous H_2O_2 at lower concentrations.[19] Decreased activity of CAT and GR is responsible for increased H_2O_2 production in sperm. Glutathione peroxidase/ reductase directly act as antioxidant enzymes to inhibit sperm lipid peroxidation.[20] However, in the present study the decrease in the activities of catalase and glutathione reductase may be responsible for the increased levels of H_2O_2 production and lipid peroxidation in sperm. High concentrations of either exogenous or endogenous H_2O_2 was responsible to cause a severe oxidative stress to sperm, resulting in a rapid decline in sperm motility and energy metabolism.[21] The decreased activity of antioxidant enzymes in sperm exposed to triclosan is may be due to inactivation, degradation or reduced synthesis of enzymes.

Lipid peroxidation is highly toxic to spermatozoa and causes an irreversible arrest of sperm motility, damage of sperm integrity and other sperm functions.[22] In the present study triclosan incubation significantly increased the level of lipid peroxidation in epididymal sperm of goat. Relatively high polyunsaturated fatty acid (PUFA) content of sperm membrane make sperm highly susceptible to oxidative stress and peroxidative attack, which leads to irreversible oxidative changes of membranes.[23] Increased lipid peroxidation may indicate an increased generation of free oxygen radicals in tissues and has been associated with mid-piece abnormalities in sperm and decline in sperm counts.[24] Lipid peroxidation has often been used as a biomarker of toxicant stress, reflecting damage to cell membranes from free radicals and is an important feature in cellular injury. The present results clearly demonstrate that triclosan induces ROS generation in epididymal sperm resulting in oxidative stress, which in turn possess negative impact on male fertility of goat.

CONCLUSION

In the present study, triclosan induced oxidative stress in epididymal sperm of goat that was known by the significant reduction in the activities of antioxidant enzymes and significant increase in the levels of hydrogen peroxide and lipid peroxidation, this could adversely affect the male reproduction of goat.

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