In mammals the process of spermatogenesis is divided into two distinct phases, namely, spermatocytogenesis and spermiogenesis. Spermiogenesis is the final phase of differentiation of spermatozoa. During this process spermatids undergo enormously complex morphological, biochemical and physiological changes that result in the formation of highly asymmetrical and flagellated spermatozoa. These unique events are essential for the production of fertile sperm.

Spermiogenesis therefore, offers the potential for targeted intervention in fertility control. Acrosome formation by Golgi bodies is one of the transforming events during spermiogenesis, which plays an important role during the process of fertilization. Pharmacological agents have proven useful to explore the functional mechanisms of Golgi apparatus. The chemical agents that interfere in Golgi function can result in the failure of germ cell maturation or can also result in a defective acrosomal system rendering non-functional spermatozoa. Disturbance of Golgi function by a drug is recognized as a pleiotropic effect of the compound (Dinter and Berger, 1998). These authors reviewed the effects of a number of drugs like
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bafilomycin, brefeldin A, illimaquinone, nigericin, nocodazole, okadaic acid, retinoic acid and monensin, which are known for their Golgi disturbing nature. The molecular targets of monensin, brefeldin A, illimaquinone and retinoic acid remain to be elucidated whereas those of bafilomycin, okadaic acid and nocodazole are reasonably well understood.

Monensin is a carboxylic polyether ionophore produced by Streptomyces cinnamoneus (Mollenhauer et al., 1990). Monensin is an open chain molecule that is capable of ion complexation through a cyclic form stabilized by hydrogen bonding between the carboxyl and hydroxyl groups. Charge transfer bonding within the cavity formed is responsible for ion binding. Because of the affinity of monensin for Na⁺ is 10 times that for K⁺, which is its nearest competitor in biological systems, monensin mediates primarily a Na⁺/H⁺ exchange. Monensin like other carboxylic ionophore, binds metal ions through liganding sites such that the ions become centrally oriented and masked from the extracellular environment. The outer surface of the ionophore – ion complex is composed largely of non-polar hydrocarbon, which imparts a high solubility to the complexes in non-polar solvents. In biological systems, these complexes are freely soluble in the lipid components of the membrane. Once the ion traverses the membrane as a monensin – ion complex, it is released and monensin molecule picks up a proton to form an undissociated molecule which then re-traverses the membrane to release the proton to the outside of the cell, vesicle, organelle or other subcellular compartments (Mollenhauer et al., 1990).
Although mechanisms for ion transfer through a bimolecular leaflet (membrane) have been proposed, question still remains as to how this action is related to the known effects of monensin on cell function and what relationships these may have in turn, on biochemical mechanisms leading to animal toxicity.

Monensin has been known for many years in poultry industry for its useful effect as food additive. At the biochemical level it is a well-known Na⁺/H⁺ exchanger across biological and model membranes. Monensin's main action consists of exchanging protons for Na⁺, which leads to the osmotic swelling of post-Golgi endosomal structures and Golgi sub-compartments by virtue of its membrane associated effect as cationophore. It leads to the transformation of Golgi cisternae increasingly from cis to trans and forms peripheral swollen vesicles apposed to the Golgi region. However, the Golgi functions are disturbed non-specifically depending upon the predominant impact of monensin.

The swelling of cisternae is easily visible at light microscopic level (Tartakoff and Vassalli, 1977), this effect seems to be ubiquitous as it is observed in plant cells also (Morre et al., 1983). Berger et al. (1993), showed the effect of monensin on the resident enzymes of Golgi bodies. Monensin affects the specific Golgi retention mechanism of galactosyl transferase (Gal T). Monensin has also been reported to inhibit the efflux of cholesterol from cholesterol-loaded fibroblasts (Orci et al., 1981). This effect also supports the possible impact of monensin on the post Golgi structure by disrupting the vesicular cholesterol transfer from the trans Golgi site to the plasma membrane (Mendez, 1995). These

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Observations clearly support the interference of monensin with the structural and functional properties of Golgi apparatus. In the Golgi apparatus, late processing events such as the terminal glycosylation and proteolytic cleavage are most susceptible to the inhibition by monensin. Yet, many incompletely processed molecules may still be secreted via poorly understood mechanisms that appear to bypass the Golgi apparatus. Cellular effects of monensin may vary markedly depending upon the organism and the route of administration.

Monensin is known to exert stoichiometric Na⁺/H⁺ exchange into biological membrane by virtue of its hydrophobicity. This Na⁺/H⁺ antiport regulates the multiple cellular phenomena including the regulation of intracellular pH (pHᵢ), cell volume, transport of salt, water and acid base equivalents (Grinstein et al., 1989; Zhu and Loh, 1995).

Atef et al. (1986) studied the effect of monensin on the fertility status of rats. Prolonged administration of monensin at two dose levels (1.75 and 3.50 mg/kg b. wt.) in male rats for 60 days decreased the conception rate in the untreated female rats. There was a marked decrease noticed in the weight of testicles, epididymes and seminal vesicles after the drug treatment. Monensin has also been found to induce oligospermia and azoospermia, and decrease the activity of spermatogenic epithelium and degeneration of germ cells.

Being an ionophoric antibiotic, monensin is able to form lipophilic complexes with monovalent cations, and hence causes
cation imbalances, which are known to cause different biochemical and histological alterations (Mollenhauer et al., 1990). Monensin is also known for its acute toxicity and membrane effects (Gad et al., 1985). The toxicological studies regarding monensin have been studied in several species. The main toxic signs include, anorexia, muscle weakness, diarrhea, ataxia and weight loss (Todd et al., 1984). However, the interactions between monensin and the tissues of the host animal are not well understood although the severe toxicological manifestation of monensin poisoning is well known.

Although, it seems that the Golgi disturbing nature of monensin may account for its antispermatogenic effects, some other factors like oxidative stress and DNA damage are required to be investigated to understand the effects of monensin on male fertility. Therefore, many studies have reported relationship between the drug effect, production of DNA damage, disruption of cell cycle and initiation of apoptosis (Reynolds, 1999 and Parez et al., 1997). Also, there is a close relationship between male fertility, oxidative damage and apoptosis. Many studies have correlated the higher DNA fragmentation in infertile subjects. In contrast to other cells, sperms are partially deficient in defense mechanisms against the oxygen metabolites; hence make them sensitive to peroxidative attack. The other factor is the higher composition of polyunsaturated fatty acids, which makes the spermatozoa more vulnerable to oxidative attack. Oxidative damage is the key regulator in chemical induced apoptosis and
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DNA damage while the protein inactivation and cell membrane instability are the main resultant events of such oxidative damage.

The overproduction of reactive oxygen species may cause the disruption of the plasma membrane, leakage of lysosomal enzymes and DNA damage resulting into apoptosis. It is reasonable to believe that the drug/chemical induced infertility is closely related to the onset of oxidative stress, which may cause apoptosis. It had been seen that there is increased frequency in the number of spermatozoa with apoptotic DNA in the ejaculates of infertile men in comparison to the fertile ones (Gorczyca et al., 1993 and Baccetti et al., 1996).

Since, the cellular effects of monensin includes, disturbance of Golgi bodies, toxic effects and induction of apoptosis as reported by various studies, the present study has been planned to investigate the effect of monensin on male reproductive system in rats and the molecular understanding of the potential role in contraceptive intervention.
AIMS AND OBJECTIVES

1) The effect of monensin on male reproductive system was studied by carrying out in vivo, intratesticular and in vitro experiments in rats.

2) The biochemical estimations of lactate dehydrogenase, ATPase and acid phosphatase were done to find out the effect of monensin on testicular functions.

3) To assess the oxidative damage produced by monensin, the estimations of glutathione (reduced), glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase, glutathione-S-transferase and lipid peroxidation were performed in rat testis in various experiments.

4) To monitor the fertility status of the animals, sperm motility, sperm concentration and litter size analysis were performed.

5) DNA fragmentation studies were carried out to observe the effect of monensin on DNA.

6) To observe the structural changes in rat testis after exposure of monensin, histological studies were done.

7) The change in the Golgi apparatus after monensin treatment was studied by employing transmission electron microscopy. The biochemical and cytochemical assay for Golgi body marker enzyme, thiamine pyrophosphatase was performed to evaluate the alterations in Golgi functioning.