The present study was conducted to explore the antifertility potential of monensin in male Wistar rats. The experiments were mainly divided into three parts.

**IN VIVO EXPERIMENTS**

In these experiments the rats were segregated into four different groups viz., Group I (control), Group II (2.5 mg Mon/Kg b.wt.) Group III (5mg Mon/kg b.wt) and Group IV (10 mg Mon/kg b.wt). The animals were sacrificed after 67 days of the drug treatment. Some animals were kept for another 60 days after the termination of drug treatment to observe the recovery from monensin treatment.

**INTRATESTICULAR EXPERIMENTS**

In the intratesticular treatment, the animals were divided into five groups viz., Group I (Control), Group II (1 µg Mon/testis), Group III (5 µg Mon/testis), Group IV (10 µg Mon/testis) and Group V (20 µg Mon/testis). Monensin was administered intratesticularly by a single dose and the animals were killed after a period of 5 days. In some other studies, time dependent effects of monensin were studied with the lower doses of the drug.

**IN VITRO EXPERIMENTS**

In these experiments, testicular cell culture was done and the testicular cells were incubated with different concentrations of monensin.
Summary and Conclusion

After completing the treatments various investigations were undertaken in testis, which included biochemical estimations, spermatozoal concentration and motility, fertility studies and litter size, histology and electron microscopy, cytochemistry of thiamine pyrophosphatase and DNA fragmentation.

The results of various investigations obtained during the present study are summarized below:

**IN VIVO EXPERIMENTS**

Body and Organ weight

It was observed that the body weight of the control and treated animals increased progressively throughout the study period. Significant decrease in the weight of different sex organs was noticed. The changes were more significant after a recovery period of 60 days.

Oxidative stress enzymes

Marked inhibitions were noticed in the activities of SOD, catalase, GSH-PX, GR and GST after monensin treatment in various groups. However, after the recovery of 60 days no significant changes were observed in the activities of SOD and GST.

Glutathione (reduced) and Lipid peroxidation

A significant depletion in GSH content was noticed in all the treated animals. However, after the recovery of 60 days, the GSH
Summary and Conclusion

was significantly lowered in the higher doses of monensin treatment. It was observed a significant increase in the levels of lipid peroxidation in various treatments of monensin. No significant change was noticed after the recovery of 60 days from the drug treatment.

Lactate dehydrogenase and ATPase

Monensin treatment caused a significant decrease in the activities of LDH and ATPase in the treated animals, and the same trend was noticed after the recovery of 60 days.

Acid phosphatase and Thiamine pyrophosphatase

A significant increase in the activity of acid phosphatase was noticed in Group II animals. After recovery, significant elevation in all the monensin treated groups was noticed. Thiamine pyrophosphatase activity was found to be enhanced significantly in Group IV animals. However, no change was noticed after the recovery.

Sperm concentration and motility

Various monensin treatments have resulted in a significant reduction in sperm number and motility, which was more pronounced after the recovery time.

Litter size

Monensin treatment has caused a reduction in the litter size, which was found to be significant in Group IV animals.
Summary and Conclusion

Cytochemistry of Thiamine pyrophosphatase (TPPase)

The distribution of TPPase activity in the cryosections of rat testis was found to be increased in the monensin treated groups in comparison to the controls.

DNA fragmentation

A characteristic ladder formation has been observed in all the treated animals.

Histology and Electron microscopy

Histological findings point towards the marked degenerative changes in rat testis after various monensin treatments. The results from the electron microscopic study are suggestive of a disintegration of Golgi apparatus by monensin.

INTRATESTICULAR STUDIES

Oxidative stress enzymes

SOD: A significant increase was noticed in the activity of SOD in Group II animals in comparison to the controls. Catalase: The activity of catalase was found to be significantly elevated in different monensin treated groups. However, in the intratesticular treatment of monensin (5 μg/testis) for different time intervals, it was observed a significant decrease in catalase activity after 2 and 4 days. GSH-PX: Monensin at various dose levels has caused a significant increase in the activity of GSH-PX. A significant decrease was observed in the enzyme activity after 3
days of drug treatment (5 μg / testis). **GR:** A significant elevation in the enzyme activity was observed in Group IV animals. Similarly, the activity was increased significantly in monensin treated animals (1 μg Mon/testis for 35 days). The activity was found to be elevated after 4 days of drug treatment (5 μg Mon/testis). **GST:** Activity of GST was increased significantly after the intratesticular treatment of monensin (1 μg/testis) for 35 days. However, a significant inhibition in the enzyme activity was observed after 2 and 3 days of drug treatment (5 μg Mon/testis).

**Glutathione (reduced) and Lipid peroxidation**

Intratesticular treatment of monensin (1 μg /testis) for 35 days resulted into a significant depletion of GSH. Similarly, a significant fall in GSH content was observed after the interval of 2 and 3 days of drug treatment (5 μg Mon/testis).

A significant elevation in the levels of LPO was observed in various intratesticular studies.

**Lactate dehydrogenase and ATPase**

Group II and III have been observed with a significant decrease in LDH activity. The intratesticular treatment of monensin (5 μg/testis) for 2 and 4 days significantly inhibited the enzyme activity. Monensin treatment (5 μg/testis) depleted the ATPase activity significantly.
Summary and Conclusion

Acid phosphatase and Thiamine pyrophosphatase

A significant increase in the activity of acid phosphatase was noticed in Group II and IV in comparison to Group I. Treatment of monensin (5 mg / testis) for 1,2,3 and 4 days elevated the acid phosphatase activity.

Significant increase in the TPPase activity was observed in group IV and V in comparison to the control.

Cytochemistry of TPPase

The TPPase activity in the cryosections of monensin treated testis was found to be increased in comparison to the control.

DNA fragmentation

Monensin caused DNA fragmentation in the testis of treated animals in comparison to the controls.

Histology and Electron microscopy

Histologically, germ cell degeneration was noticed in all the monensin treated groups. The changes include sloughing of different cells, decrease in the number of various cell populations and increase in vacuole formation. The alterations in the Golgi apparatus and acrosomal system were noticed in the electron microscopic study.
IN VITRO EXPERIMENTS

Oxidative stress enzymes

SOD: Monensin treatment at different concentrations caused a marked inhibition in SOD activity. Catalase: A highly significant decrease in catalase activity was observed with different concentrations of monensin treatment. Glutathione peroxidase: Monensin at various concentrations has significantly inhibited the GSH-PX activity in the testicular cells. Glutathione reductase: Significant changes have been noticed in GR activity in all the monensin treatments. At the lowest dose the activity was found to be increased. However, it was significantly inhibited at the higher concentrations of monensin. Glutathione-S-transferase (GST): A marked reduction in the GST activity was observed in all the monensin treatments as compared to the control.

Glutathione (reduced) and Lipid peroxidation

It was observed an overall depletion of GSH content after treating the testicular cells with different concentrations of monensin.

Higher concentrations of monensin significantly increased the levels of lipid peroxidation in the treated cells.

Lactate dehydrogenase and ATPase

Monensin at different concentrations reduced significantly the activity of LDH.
Summary and Conclusion

ATPase activity was found to be inhibited at the highest concentrations of monensin.

Acid phosphatase and TPPase

Monensin at 20-80 µM significantly elevated the acid phosphatase activity. However, the activity of TPPase was increased by monensin at the concentration range of 20-100 µM.

DNA fragmentation

In vitro treatment of monensin induced the DNA fragmentation in the treated testicular cells in comparison to the control.

It may be concluded from the present study that monensin exerts an antifertility potential by inhibiting spermatogenesis at structural, genomic and biochemical levels. The changes in various enzyme activities, alterations in oxidative stress parameters, histopathological damage, altered sperm functions, disintegration of Golgi apparatus as evident by electron microscopy and DNA fragmentation point towards the pronounced effects of monensin in the testis.