Research & Reviews in Biotechnology & Biosciences **ISSN No: 2321-8681**
Website: www.biotechjournal.in **Research Paper**
Volume: 12, Issue: 1, Year: 2025 **PP: 64-67**
DOI: https://doi.org/10.5281/zenodo.15662436 **Peer Reviewed Refereed Journal**

HISTOCHEMICAL STUDIES OF NUCLEIC ACIDS IN THE OVARY OF MONCROTOPHOS INTOXICATED RATS

[1]Kavita Arora

[1]Department of Zoology, D.A.V. College, Abohar, Punjab, India

Article History

Received: 27/04/2025
Accepted: 07/06/2025

Article ID: 10_2025

Corresponding Author:
E-Mail:
kavitaaroraabh@gmail.com

ABSTRACT

The present study was designed to evaluate the toxic effects of monocrotophos, an organophosphate, on the ovary of female albino rats. Six groups of female albino rats (each of 125-150 gms) were taken for experimental work. 1/5th of LD50 dose (14 mg/kg body weight) of monocrotophos was administered by intragastric intubation to groups TI, TII and R for 15 days, 30 days, 30 days with recovery period of 15 days respectively. Other three groups were kept as corresponding controls for all the treated groups and were fed on normal diet. Paired ovaries in proestrous phase of estrus cycle were removed and fixed in Zenker and then processed for staining with Feulgen and MG/PG for studies of nucleic acids.

In rats of control group, the sections of ovaries revealed abundant DNA in nuclei of pre-antral follicles and antral follicles including graffian follicles. In groups TI and TII, significant alteration in the intensity of nucleic acids was observed as compared to their corresponding controls. In rats of R group, recovery was observed at histochemical level.

Keywords: Organophosphate, Female albino rats, Ovary, Pesticide, Monocrotophos

INTRODUCTION

Survival of any species depends on the integrity of its reproductive system. The toxic effects of drugs and environmental chemicals on the human reproductive system have become a major health concern. Scott[1] has reported that some of the insecticides reduced the fertility and cause sterility in animals. Many factors, both environmental and endogenous, can have detrimental effects on the female reproductive cycle and on the outcome of pregnancy. Environmental chemicals accidentally introduced into the human food chain have the potential of altering the endocrine system, if chemically present in the diet[2-4]. Therefore, it is important to examine the acute effect of environmental chemicals on the reproduction potential of the animals. Present study was designed to see the histochemical effect of monocrotophos on nucleic acids of ovaries of female albino rats in proestrous phase of estrus cycle.

MATERIALS AND METHODS

LD^{50} of monocrotophos was standardized on the basis of the dose calculated by Janardhan *et al*.[5] and was found to be 14 mg/kg body weight. Adult female albino rats of Wistar strain in proestrous phase of estrus cycle weighing 100-150 gms were obtained and divided into three groups TI, TII and R (8 rats in each group). $1/5^{th}$ of LD50 value of monocrotophos i.e. 2.8 mg/kg body weight was administered for 15 days to TI group and for 30

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days to TII group. To the rats of R group, the same dose was given for 30 days and then the rats were kept on normal conditions i.e. without monocrotphos for 15 days. Another three groups CI, CII and CIII (8 rats in same phase of estrous cycle in each group) were kept as controls for all the treatment groups. All the animals were kept on the commercial standard diet and tap water *ad libitum*. The weight of animals was recorded weekly in all the groups.

At the end of the treatment period, six rats from each of the treated and control rats lying in the same phase were sacrificed by cervical dislocation. Both ovaries were dissected out, weighed and fixed in Zenker fluid ; and processed for histochemical examination [6]. Thin serial sections (5 µm) of the ovary were cut and stained for Feulgen reagent [7] and MG/PG stain to study the nucleic acids in the follicles of different developing stages.

RESULTS AND DISCUSSION

In control, in pre- antral follicles, the nulcei of the oocytes took a dark stain after Feugen test showing the presence of abundant DNA. In the antral follicles including graffian follicles as the nucleus enlarged into germinal vesicle, there was feeble staining after Feulgen due to the diffused DNA (Pmgs 1 & 5). The nuclei of the theca externa (Te) cells, took dark stain showing the abundance of DNA confirming the presence of DNA (Pmg. 9). The nuclei of the hypertrophied cells of theca interna (Ti) showed lesser staining than that of theca externa, (Pmg. 9) which may be due to the diffusion of DNA as nuclei were vesicular as compared to spindle shaped nuclei of Te. In granulosa cells, there were two types of nuclei, some were showing dark staining and some were showing some light stain for DNA, (Pmgs. 9 & 5). The difference seems to be due to the mitotic divisions taking place in the cells.

In the cytoplasm of the control oocyte of the pre-antral follicles and in its nucleolus in the nucleus, there was moderate *pyroninophilia* due to RNA after MG/PG stain but it took more stain than that of pre-antral follicles. In the cytoplasm of theca interna, theca externa and granulosa cells, there was abundant pyroninophilia due to RNA in MG/PG stain. In the developing antral follicles, the pyroninophilic granules were observed in the cytoplasm of the oocyte showing the presence of RNA. The nucleolus in the nucleus of the oocyte did not take homogenous pink stain in MG/PG as there were some vacuoles which might be due to release of RNA in the cytoplasm. Cytoplasm of Te, Ti and granulosa cells showing number of pink granules in MG/PG stain showing the presence of abundant RNA in them. There was pyroninophilia in the cytoplasm of the various cells in stroma due to pink color for RNA by MG/PG. The nuclei of the cells of stroma, the nuclei of the fibroblasts and endothelial cells of blood vessels stain pink color in Feulgen (Pmg. 1) and green in MG/PG showing the presence of DNA.

In TI group, the sites of DNA observed in the nuclei and RNA in the cytoplasm and nucleoli of the necrotic follicles after treatment with monocrotophos showed some depletion in staining in Feulgen for DNA (Pmgs. 2 & 10) and MG/PG for DNA and RNA showing a decrease in both nucleic acids. The decrease in RNA can be due to pyknosis of some nuclei in the different layers of granulosa cells, theca externa and theca interna surroundings the oocyte which took dark stain in Feulgen (Pmg. 10) and dark green stain in MG/PG for DNA due to clumping of chromatin material.

In both TI & TII, there was decrease in staining due to pyroninophilia showing decrease in RNA. Pyroninophilic granules were found scattered in between the granulosa cells which may be due to damage of granulosa cells. Some of the nuclei of granulosa cells seem to show some necrosis as the DNA positive material scattered in between the granulosa cells (Pmgs. 10, 6 & 7, 11). These DNA granules have also been observed in the zona pellucida. Some nuclei of the fibroblasts of stroma showed pyknosis as they took dark pink stain and dark green color in Feulgen (Pmgs. 3 & 7) and MG/PG respectively. In TII, depletion of DNA and RNA was much more than that of TI, as

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observe after Feulgen for DNA (Pmg. 3) and MG/PG for DNA and RNA. The number of pyknotic nuclei also increased in the various layers of granulosa cells, Ti and Te. The pyknosis was more conspicuous in the granulosa cells. These changes in the nuclei of granulosa cells may be responsible for the degeneration of the follicles.

In R group, there were some signs of recovery in DNA and RNA as the number of pyknotic nuclei stained in Feulgen and MG/PG decreased. The granules stained after Feulgen for DNA lying between granulosa cells were also absent (Pmgs. 8 & 12). There was increase in the number of normal granulosa cells and there was also some increase in the nuclei and DNA in their chromatin and RNA in their cytoplasm as observed after Feulgen for DNA (Pmgs. 4, 8 & 12) and MG/PG for DNA and RNA. Moreover, these observations are further supported by the studies made by Murphy[8] that organophosphate insecticides commonly used are rapidly metabolized and excreted and subacute poisoning by virtue of accumulation of compounds in the body does not occur and cessation of exposure normally results in the complete recovery.

ACKNOWLEDGEMENTS

The author is grateful to the Principal, Department of Zoology, D.A.V. College, Abohar (India) for providing necessary laboratory facilities and to University Grants Commission, New Delhi, for financial help.

EXPAINATION TO PMGS.

Pmg. 1. T.S. control ovary showing presence of DNA in nuclei of oocyte, granulosa cells, theca interna and cells of stroma (S). Z/F.

Pmg. 2. T.S. ovary of TI group showing the clumping of DNA in some nuclei of granulosa cells and cells of stroma. (arrow). T.S. control ovary showing presence of DNA in nuclei of oocyte, granulosa cells, theca interna and cells of stroma (S)

Pmg. 3. T.S. ovary of TII group showing depletion at the sites of DNA in some nuclei of granulosa cell, theca interna cells and cells of stroma of necrotic follicle. Note the presence of pyknotic nuclei of lutein cells of regressing corpus luteum (arrow). Z/F.

Pmg. 4. T.S. ovary of R group showing increase in staining at the sites of DNA in most of the granulosa cells. Z/F.

Pmg. 5. T.S. control ovary showing presence of DNA in nucleus of oocyte (N) and some nuclei of granulosa cells. Z/F.

Pmg. 6. T.S. ovary of TI group showing the depletion in staining for DNA in some nuclei of granulosa cells. Granules of DNA also visible among granulosa cells (arrow). Z/F.

Pmg. 7. T.S. ovary of TII group showing the depletion at the sites of DNA in some nuclei of granulosa cells. Note the presence of granules of DNA among granulosa cells of necrotic follicles. Z/F.

Pmg. 8. T.S. ovary of R group showing increase in staining at the sites of DNA in most of the granulosa cells. Z/F.

Pmg. 9. T.S. control ovary showing presence of DNA in nuclei of granulosa cells (arrow), theca interna and theca externa. Z/F.

Pmg. 10. T.S. ovary of TI group showing the depletion of staining for DNA in some nuclei of granulosa cells and theca interna (arrow). Note the presence of granules among granulosa cells and in antral cavity. Z/F.

Pmg. 11. T.S. ovary of TII group showing the depletion of DNA in some nuclei of granulosa cells and theca interna cells. Z/F.

Pmg. 12. T.S. ovary of R group showing increase in staining at the sites of DNA in granulosa cells (arrow) and theca cells. Z/F.

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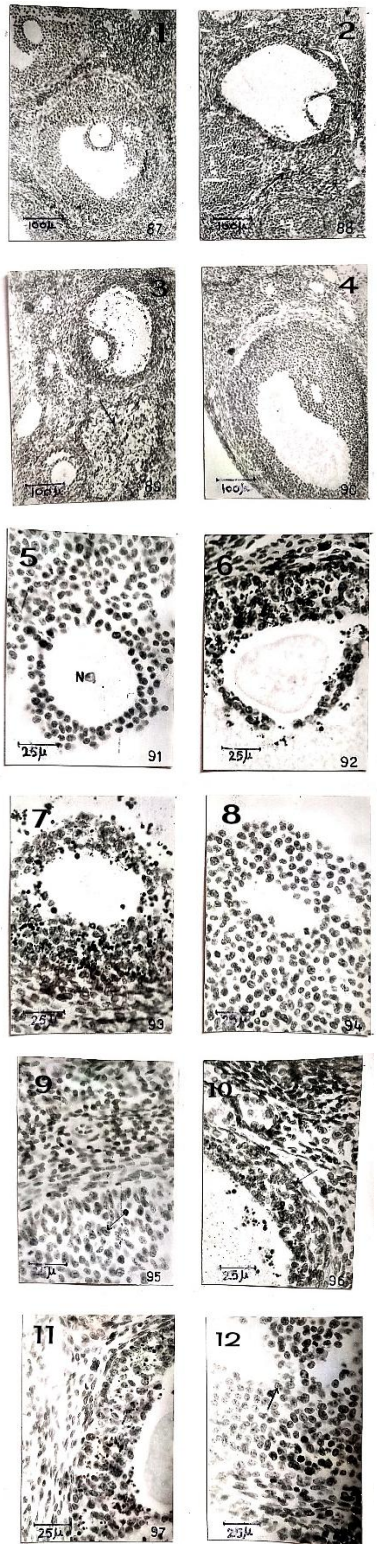

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