

Effect of Anticancer Drug, Cisplatin on the Nucleolar Changes in the Developing Oocytes of Fresh Water bivalve, *Corbicula Striatella* (Deshayes1854).

Bhosale PA

Department of Zoology Sundarrao More Arts, Commerce, and Science (Sr.) College, Poladpur.Tal- Poladpur Dist- Raigad, Maharashtra, India. 402 303.

Email : Bhosale_popat@rediffmail.com

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ABSTRACT

Cisplatin drug has anticancer properties for chemotherapy against solid tumors. This drug exhibits effective chemoprevention in cancer therapy and most active cytotoxic agents in the treatment of cancer and also leads to several manipulations and cytotoxicity. In present toxicity studies, sub-lethal dose of cisplatin (LC50/10 for 96 hours) was given to an experimental model, the fresh water bivalve *Corbicula striatella* for 45 days. The nucleolar changes of developing oocytes from female gonads ovary were observed from control and treated bivalves by using Methyl green and Pyronin-Y stains. It was found that the chronic exposure of anticancer drug, Cisplatin (2.009 ppm) induced alterations in the structure of nucleolus and hence the nucleolus of developing oocytes showed condensation of the chromatin, aggregation of the nucleic acid such as DNA and RNA at certain locations, Overall result high dose of cisplatin the in *Corbicula Striatella* production of multiple or overgrowth and induction of increased number of nucleoli. Extra nucleoli were more prominent in cisplatin treated bivalves after 45 days of exposure.

Keywords: Cisplatin, Anticancer drug, Developing Oocytes, Nucleolus, NOR, *Corbicula Striatella*.

INTRODUCTION

The important process and strategy to control the malignancies in the cancers to develop the anticancer drug that could inhibit the DNA replication. Since the expression of all the genes through the process of replication, transcription and translation several inhibitors also developed as the anticancer drugs. In cell nucleus, nucleolus is the site of the fast replication of DNA to form tandem repeats of DNA and the site for the transcription of the rRNA. The nucleolar activities are multiplied many fold in the developing oocytes. And hence this can act as the best suitable marker to screen the anticancer drugs. Lodish et al. (2000) reported that approximately 80 % of the total RNA in rapidly growing mammalian cells is rRNA and 15 % is tRNA; protein encoding mRNA is thus constitutes very small quantity of the total RNA. During embryonic development i. e. cleavage, large quantity and number of proteins are needed.

Since the DNA contents are actively involved in the process of replication for its rapid multiplication, most of the rRNA, mRNA and ribosomes required during the cleavage are synthesized during oogenesis and are stored in the ooplasm. When the developing oocytes are exposed to replication inhibitors and the transcription inhibitors, they will show varied effects on the nucleolus. Thus, by applying single anticancer drug test, one can determine whether the drug is replication inhibitor or transcription inhibitor. The nucleolus is the most important and definitely differentiated nuclear sub component. It is very important nuclear structure, where the biosynthesis of ribosome takes place. It is also clear that the nucleolus also performs non ribosomal functions. The antitumor activities of cisplatin involves induction of inter and intra crosslinks that severely leads to distortion of the DNA helix and blocks its duplication. Repair of cisplatin-DNA adducts by mammalian excision nuclease (Raska et al., 2006).

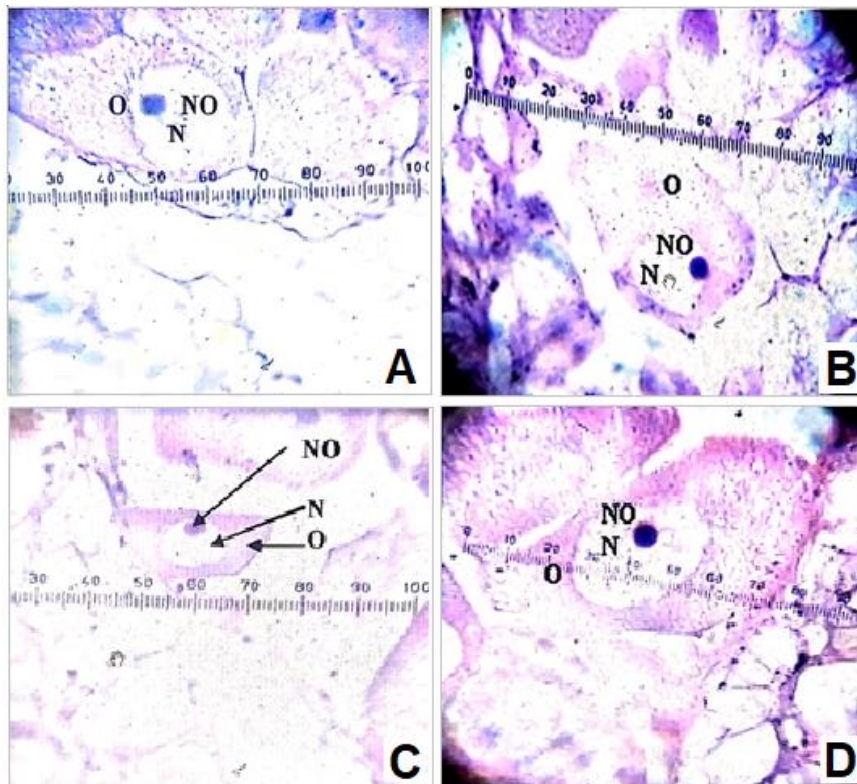
MATERIAL METHODS

The fresh water bivalves, *Corbicula Striatella* (D) were collected from Girna lake area near Jamda (Latitude 20° 33'N, Longitude 75°10'E, 352 m MSL) which is 14 km away from Chalisgaon, District Jalgaon of Maharashtra State. Bivalves were collected and brought to laboratory in aerated container. They were maintained in a glass aquarium containing dechlorinated water for 3- 4 days at 23 °C to 28 °C temperature. The PH of water was in the range of 7.0- 7.5 and well acclimatized at laboratory conditions. The water in aquarium was changed regularly after every 24 hours. After acclimatization, healthy full size bivalves of 2.8-3.00 cm height X 4.8- 5.5 cm length were selected from the aquarium and used for the experiments. The well acclimatized bivalves, *Corbicula Striatella* were divided into two groups with equal number of animals. They were kept in separate aquarium for 30 days. Bivalves from one group were maintained as a control and one group was treated by chronic concentration (LC50/10 value of 96 hours) of Cisplatin (2.009 ppm). 45th day of exposure, bivalves from control group and experimental group were sacrificed and their gonads were removed and fixed in Carnoy's fluid for 25 to 30 minutes only, as it is a rapid nuclear fixative. Then gonads were dehydrated in alcohol grades, cleared in xylene and embedded in paraffin wax (56 to 58°C). Then, prepared blocks of the gonads, trimmed and attached to microtome pegs and were then cut with the thickness of 07 μ (micron),

arranged ribbons of the section on the glass slides smeared with thin film of egg albumen and affixed for 24 hours, and stained with Methyl Green Pyronin-Y stain. So as to observe the DNA and RNA specific areas in the nucleolus, the sections were also stained by Methyl Green and Pyronin-Y stains. Among sections some oocytes were without nucleus or nucleolus on the basis of path through which the sections of oocytes were taken. The oocytes in section with prominent nucleus and nucleolus were selected for the study. The characteristic features of the nucleolus and their number were counted, measured and photographed. The photographs are presented in the plates.

RESULTS & DISCUSSION

Fresh water bivalve, *Corbicula Striatella* is hermaphrodite animal. The gonads are composed of different follicles such as male and female, Ovarian follicles with four to six developing oocytes with size measures from 225 μm to 345 μm in diameter. and in the follicles, the female follicles shows developing ova of varying sizes. The size of the oocytes measures from 40 μm to 230 μm in diameter, the size of the nucleus varies from 20 μm to 64 μm in diameter while the size of the nucleolus varies from 05 μm to 28 μm in diameter. Majority of the oocytes were between 60 μm to 180 μm in diameter. The oocytes of different stages of development such as oogonia, primary oocytes, vitellogenic oocytes, mature oocytes and degenerative oocytes are also found among female gonads. The 6 micron thick sections were stained by Methyl green-Pyronin Y stain to study the changes in nucleolar structure. But, due to high rate of transcription of rRNA copies on each gene, the staining of DNA by methyl green become poor and methyl green pyronin Y stain could not differentiate the DNA and rRNA rich areas in the nucleolus. Different photomicrographs of control and treated bivalve's oocytes are given in the Photomicrograph plates -I fig a,b,c, and d shows the normal oocytes from control bivalves, stained by Methyl green-Pyronin Y stain; Methyl green stain and Pyronin Y stain respectively. Micrometer scale measures 16 μm per ocular division at 100x magnification and 04 μm per ocular division at 400x magnifications. Each oocyte shows large nucleus and a single large nucleolus. shows the oocyte containing nucleus with single nucleolus and nucleus. Photomicrograph plates -II fig a,b,c, and d shows the oocytes stained by Methyl green-Pyronin Y stain from the bivalves exposed to chronic dose of cisplatin (2.009 ppm) for 45 days.



Figures 1: Photomicrographs of Normal histological structure of Oocytes of *Corbicula Striatella* stained by methyl green pyronin (Magnification a to d=400X). (N=Nucleus, NO-II=Nucleolus, O=Oocytes).

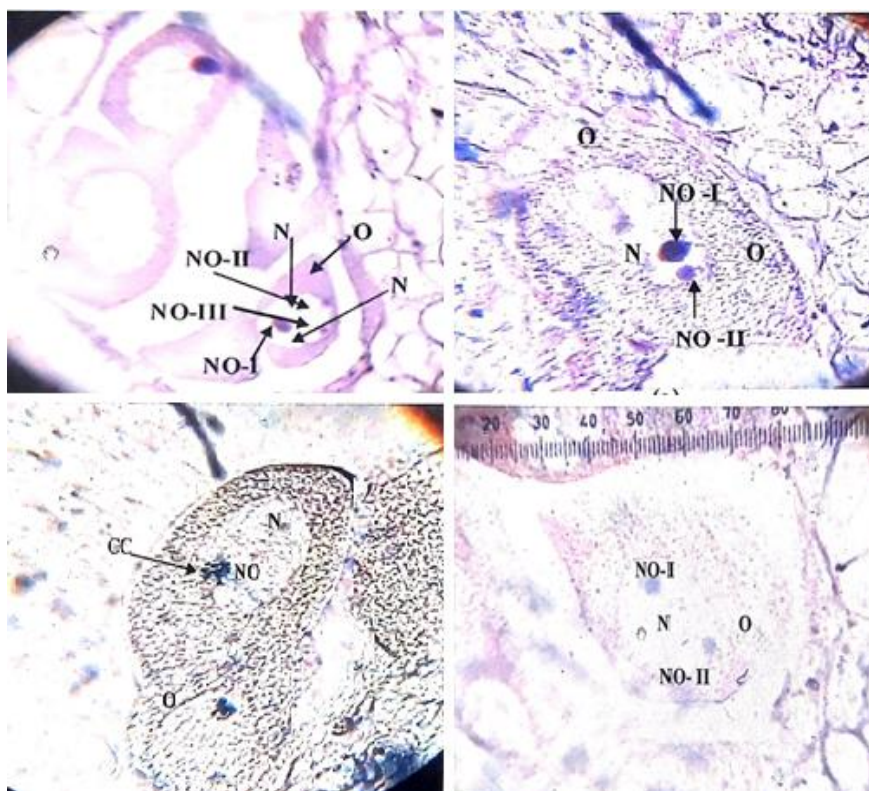


Figure 2: Photomicrographs of histological structure of Oocytes stained by Methyl green pyronin after exposure of *Corbicula Striatella* to Cisplatin for 45 days. (N=Nucleus, O=Oocytes, NO-I =Nucleus, NO-II=Nucleolus, CC=Condensed chromatin)

Plate II shows two, three and four nucleoli with extra outgrowths and condensed chromatin. shows the oocytes stained by Methyl green-Pyronin Y stain from the bivalves exposed to chronic dose of cisplatin (2.009 ppm) Most of the oocytes are large, spherical, and subspherical in shape, and their size measures from 48 μm to 224 μm in diameter, the size of the nucleus varies from 24 μm to 64 μm in diameter while the size of the nucleolus varies from 04 μm to 24 μm in diameter. Majority of the oocytes were between 56 μm to 160 μm in diameter. The present investigation study clearly indicates that the nucleolus can be used as a biomarker for the primary screening of the DNA replication and transcription inhibitors for development of new anticancer drugs. Due to high amount of nucleic acids (i.e. DNA and RNA), nucleolus is stained darkly as per the stain used.

DISCUSSION

There may not be more NOR regions in a cell or chromosomes, but the number of nucleoli is specific to the cell type and species. However, when is demand more NOR may be involved in the formation of additional nucleoli. At the time of replication and transcription inhibition in the nucleolus, due to increased need of ribosomes, additional nucleoli can be derived from other NOR, and it can thus act as a biomarker for the indication of toxicant, if it is transcription or replication inhibitor. The present work is concerned with the nucleolar changes in the vitellogenic oocytes. Since the nucleolus is the site of speedy replication and transcription, any blockage or inhibition of these mechanisms reflects on its size, as there is single large nucleolus in the oocytes of the *Corbicula striatella*. Nucleolar organizer region of the chromosomes are responsible for the development of nucleolus after mitotic phase of cell division, since nucleolus disappears during cell division. (Zambare, 1991) reported his primary studies during the reproductive cycle in *Corbicula striatella* and revealed that single nucleolus grows in size from 2.27 microns to 18.16 microns and showed differential staining, thus it is the best study material to show the intra-nucleolar organization and its interaction with the growing oocytes. It can thus act as the best biomarker for the screening of the anticancer drugs (Jordan and Carmo-Fonseca, 1998). The results shown that binding of cisplatin with the DNA molecule and inhibits the replication of the DNA from their binding sites. The results of histopathological studies to study nucleolar changes in developing oocytes of *Corbicula striatella* shows the condensation of

chromatin material in nucleus, condensation of nucleoli, change in the shape of nucleoli, extra growth of the nucleoli, induction and formation of the supernumerary nucleoli after the exposure to the anticancer drugs, Cisplatin indicates the biomarker capacity of nucleolus. (Rozenewig et al. 1977). Effect of cisplatin after chronic exposure of *Corbicula striatella* for 45 days, has showed increased number of nucleoli in developing oocytes. The results shows that the binding of cisplatin with the DNA molecule, which can inhibit the replication of the DNA from their binding sites. Since the oocytes are highly active in the process of protein, ribosome synthesis because most of the ribosomes required during cleavage, are synthesized and stored in the ooplasm. As cleavage involves repeated process of cell division, there is no time for the synthesis of required protein synthesis machinery. Increased demand of more ribosomal rRNA may leads to increased number of the tandem repeats from the nucleolar organizer region seems to be increased and hence an extra growth on some sides of the nucleoli were found. This can also be the reason for the induction and formation of the supernumerary nucleoli. It is an effective antitumor agent used in the treatment of wide variety of human cancers (Prestayko et al., 1979), Cisplatin is very effective anticancer drug widely used in the treatment of the bladder, testis, ovary and other solid tumors (Borch, 1987). The present study will be useful to develop the simple model for the screening of the anticancer drugs and their effects at the primary level. This study can also help us to compare effectiveness and side effects of various anticancer drugs.

CONCLUSION

The chronic exposure of Cisplatin (2.009 ppm) induced alterations in the structure of nucleolus and hence the nucleolus of developing oocytes showed condensation of the chromatin, aggregation of the RNA at certain locations, overgrowth of the nucleolus and induction of increased number of nucleoli. Extra nucleoli were more prominent in cisplatin treated bivalves after 45 days of exposure. The results also indicates that nucleolus of developing oocytes is the best biomarker, as it shows the changes on exposure to replication and transcription inhibitors. The nucleolus thus can be used as biomarker for the primary screening of anticancer drugs reacting at replication and transcription level. There may be signals from the ooplasm to the nucleus, more specifically to the NOR regions to replicate the rDNA genes for the formation of the nucleolus.

Conflicts of interest: The authors stated that no conflicts of interest.

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