Indian J. L. Sci. 6 (1): 01-05, 2016
ISSN: 2277-1743 (Print)
ISSN: 2278-7879 (Online)

BERENIL-INDUCED UNDERCONDENSATION OF MITOTIC CHROMOSOMES IN *Sarcophaga* (DIPTERA)

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ABSTRACT

Berenil (diminazene aceturate), an AT-specific DNA-binding drug, has been widely used in cytogenetic studies to investigate chromatin organization and stability. This study evaluates the genotoxic effects of Berenil on mitotic chromosomes in the dipteran fly Sarcophaga, with a focus on chromatin condensation patterns. Larval brain ganglia were treated with Berenil, and mitotic spreads were prepared using standard squashing techniques. Cytological analysis revealed a dose-dependent increase in undercondensation, particularly in heterochromatic regions. At 30 µM of Berenil, metaphase spreads exhibited visible undercondensation, compared to controls. These findings suggest that Berenil disrupts chromatin integrity by targeting AT-rich regions, with heterochromatin being especially susceptible. This study provides insights into the utility of Berenil as a probe for assessing chromatin dynamics and highlights the vulnerability of heterochromatin to DNA-binding genotoxicants.

KEYWORDS: Berenil*, Sarcophaga,* Heterochromatin, Chromosome, Condensation, Cytogenetics

Genome stability is crucial for proper cellular function, development, and organismal survival. Heterochromatin, especially constitutive heterochromatin, is known for preserving chromosomal integrity. It is highly enriched with repetitive DNA sequences and AT-rich regions, susceptible to external chemical and physical genotoxic stressors. Many environmental pollutants, pesticides, and pharmaceutical compounds induce genotoxic effects through interactions with DNA. Among these, DNA intercalators and groove-binders like Berenil (diminazene aceturate) target AT-rich sequences, destabilizing chromatin and interfering with DNA replication and transcription (Poot et al., 1990; González et al., 1997; Molina and Galetti, 2007; Kuriakose and Uzonna, 2014). Its action involves inhibition of DNA-dependent enzymes, leading to replication errors and structural alterations in chromatin (Heng et al., 2004). Chromosome condensation is a critical feature of mitosis, ensuring proper segregation of genetic material. The role of chromatin structure, particularly heterochromatin, in this process is not fully understood. In cytogenetic studies, Berenil is known to cause visible undercondensation in heterochromatic regions of polytene and mitotic chromosomes, serving as a marker of chromatin stress (Haaf et al., 1989; Poot et al., 1990; Wegner and Grummt, 1990; Tsuji et al., 1991; Gandhi et al., 1994; Sumner and Mitchell, 1994; Haaf and Schmid, 2000; Donya, 2006). Studying such compounds helps assess potential genotoxic risks in non-target organisms, including beneficial insects and aquatic species (Rosefort et al., 2004; Srivastava et al., 2016).

In the present study, we investigate the effects of Berenil, a diamidine-based DNA-binding drug, on mitotic chromosome structure in the dipteran fly Sarcophaga. Berenil is known to preferentially bind to AT-rich regions of DNA, which are commonly found in heterochromatin. Our findings reveal that Berenil treatment induces significant undercondensation in specific regions of *Sarcophaga* mitotic chromosomes, particularly within heterochromatic domains. This phenomenon supports the hypothesis that Berenil disrupts histone-DNA interactions or chromatin remodeling factors, leading to an open chromatin conformation.

MATERIALS AND METHODS

Fly species and their breeding

The present work has used two species of fleshflies belonging to the genus *Sarcophaga*. Laboratory colonies of these species were started from the progenies of wild inseminated females in insect rearing cages maintained at 27 ± 2 °C according to the fly rearing procedures (Srivastava et al., 2012). Third instar larvae of *Sarcophaga* spp. (flesh fly) were collected from decomposing organic matter. The larvae were maintained under laboratory conditions at 25 ± 2 °C and 70% relative humidity with a natural photoperiod.

Chromosome Preparation

Mitotic chromosomes were prepared from the neural ganglia of third-instar larvae (Srivastava et al., 2012). Larvae were dissected in insect saline (0.9% NaCl), and cerebral ganglia were isolated. Tissues were pre-treated with 0.05% colchicine for 10–15 minutes to arrest cells in metaphase. Following colchicine treatment, tissues were exposed to hypotonic solution (0.075 M KCl) for 10–15 minutes. Samples were fixed in Carnoy's fixative (methanol: acetic acid, 3:1) for 4–5 minutes with multiple changes. Fixed tissues were macerated in a 60% acetic acid drop on pre-cleaned glass

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slides. The cell suspension was spread using the flame-drying method to ensure chromosome spreading. Slides were stained with 4% Giemsa solution (pH 6.8) for 20 minutes.

Berenil Exposure Treatment

Sterile distilled water was used to create a 1 mM stock solution of Berenil, an AT-specific DNA minor groove-binding agent, which was then kept at 4°C. For experimental treatments, the desired working concentration of 30 µM of berenil was freshly prepared. For in vitro treatment, isolated ganglia were incubated in 30 µM of Berenil solution for 20 minutes before hypotonic and fixation steps. Control groups were treated with distilled water only.

Microscopy and Image Analysis

Slides were observed under a light photo microscope (Nikon Eclipse series) at 1000x magnification using oil immersion. Chromosomal undercondensation was scored based on puffed or loosened regions, particularly at pericentromeric and telomeric domains. A minimum of 50 well-spread metaphase plates per treatment group were analyzed. Images were captured using a high-resolution CCD camera and processed using Microsoft software.

RESULTS

Berenil treatment led to marked undercondensation in several chromosomal regions, characterized by diffuse, extended chromatin in contrast to the tightly packed structure of untreated controls. These under-condensed areas likely correspond to heterochromatic regions, suggesting that Berenil disrupts normal condensation by targeting AT-rich repetitive sequences. This altered chromatin structure suggests that Berenil interferes with chromosomal DNA's normal folding and compaction during cell division. Under light microscopy, the undercondensation was visually evident and involved extended, less compact regions along certain chromosome arms. The six pairs of diploid mitotic chromosomes of *Sarcophaga* larvae exposed to Berenil show clear undercondensation in pericentromeric and telomeric regions (Figure 1). These changes reflect a loss of chromatin compaction, particularly in heterochromatic domains. Microscopic analysis reveals puffing and disorganization, implicating heterochromatin as a hotspot for chemical sensitivity.

In the control group, mitotic chromosomes of *Sarcophaga* displayed well-condensed, clearly defined structures with dense chromatin packing in pericentromeric and telomeric regions. No visible puffing or condensation was observed. In contrast, Berenil-treated larvae exhibited notable undercondensation in mitotic chromosomes. The effect was dose-dependent, with increasing Berenil concentrations resulting in progressively more pronounced chromatin decondensation (Table 1 and Figure 2). These alterations were evident as diffuse, less compact chromosomal regions, especially within AT-rich heterochromatic domains.

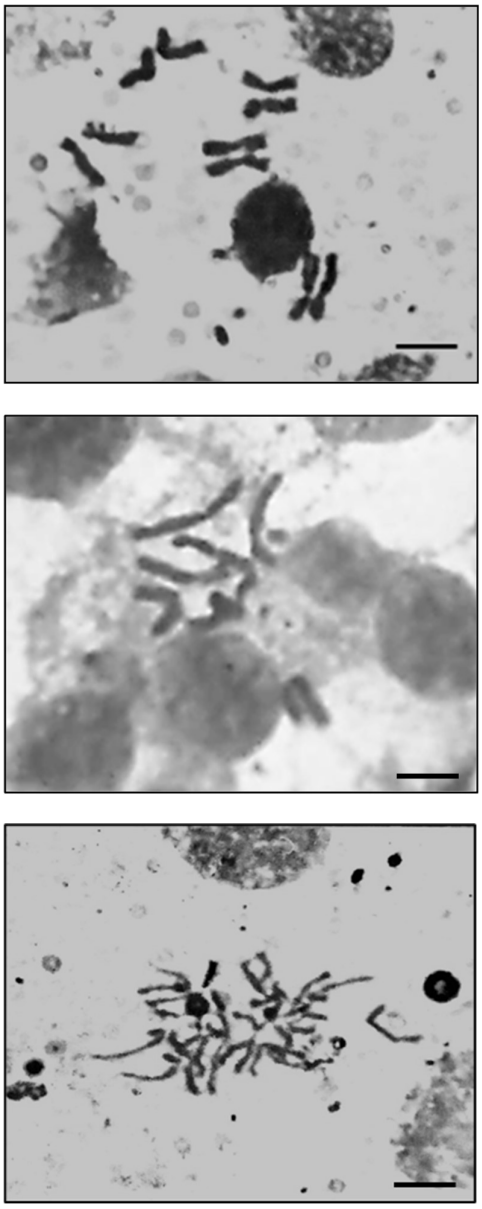

Figure 1: (A) Normal metaphase spread used as control. (B–C) Berenil-induced under-condensation in mitotic chromosomes of the dipteran fly *Sarcophaga* sp. at Berenil concentrations of 10 µM (B) and 30 µM (C), (Scale bar: 10 µm.).

Table 1: Effect of Berenil on the condensation of mitotic chromosomes at different concentrations.

Berenil Concentration (µM)	Percentage Under condensation (%)	Mean Under Condensation Score*	Intensity of Under Condensation
0 (Control)	4	0.2 ± 0.1	-
10	18	0.6 ± 0.2	+
30	48	1.4 ± 0.3	+++

'-'No undercondensation; '+' Undercondensation; '+++' High Undercondensation

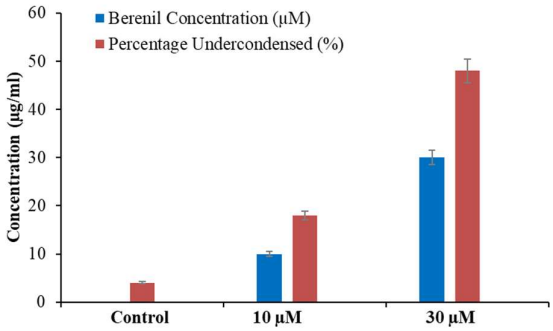

Figure 2: Berenil induced under condensation in the mitotic chromosomes of *Sarcophaga* species

DISCUSSION

Undercondensation is a visual marker of genotoxic stress and is particularly indicative of heterochromatin vulnerability (Hatzi et al., 2006). The observed increase in the frequency and severity of undercondensation with higher Berenil concentrations supports the hypothesis that this drug preferentially targets AT-rich repetitive sequences, which are abundant in constitutive heterochromatin (e.g., centromeric and pericentromeric regions) (Haaf and Schmid, 2000). This dose-dependent pattern further validates the use of undercondensation as a cytological biomarker in ecotoxicology and chromatin research, especially for detecting subtle alterations in genome architecture due to chemical exposure (Baccarelli and Bollati, 2009). The results imply a role for heterochromatin in maintaining structural integrity during mitosis. Berenil's ability to induce undercondensation provides a valuable cytogenetic tool for identifying heterochromatic regions and studying their organization and behavior (Bhasin, 2005). This model may also serve in comparative studies across dipteran species. These results offer valuable insights into the organization and regulation of chromatin, particularly the role of heterochromatin in chromosome compaction. Undercondensed regions induced by Berenil may serve as markers to identify heterochromatic zones or structural abnormalities in insect chromosomes. The study opens avenues for using DNA-binding ligands like Berenil as tools to study chromosome structure-function relationships, transcriptional regulation, and epigenetic modifications (Srivastava and Gaur, 2015). The results can be compared across different Dipteran species to understand the evolution and function of heterochromatin.

Heterochromatin is typically transcriptionally silent, highly condensed, and composed of repetitive sequences. It localizes primarily to centromeres, telomeres, and pericentromeric regions. Its integrity is essential for genome architecture, replication timing, and suppression of transposable elements (Srivastava et al., 2012; Srivastava et al., 2013). Disruption in heterochromatic regions has been linked to genomic instability, aberrant gene expression, and tumorigenesis (Srivastava et al., 2016). The chromosomal effects of Berenil observed in *Sarcophaga* will provide insight into the broader impact of genotoxic pollutants on insect biodiversity and genome regulation. Since heterochromatin harbors essential regulatory elements, its perturbation can lead to long-term genetic consequences, including mutation accumulation and reduced reproductive fitness (Zoghbi and Beaudet, 2016). Assessing chromatin integrity in sentinel species like *Sarcophaga* can serve as an effective biomonitoring tool. Chromosomal undercondensation, especially in heterochromatin, may be used as a cytogenetic biomarker to evaluate environmental genotoxicity. Coupling cytogenetic assays with molecular tools like qPCR and epigenetic profiling can strengthen toxicological assessments (Chappell, 2016).

Genetic studies on the genetic makeup of *Sarcophaga* species have primarily focused on genome sequencing and electrophoretic characterization (Singh and Thakur, 2012). Studies on *Sarcophaga dux* have utilized electrophoretic techniques to analyze enzyme polymorphisms, revealing genetic variability and aiding in species identification (Singh and Thakur, 2012). While few studies enhance our understanding of *Sarcophaga* genetics, they do not specifically address the impact of Berenil on their chromosomal structures. Given Berenil's known DNA-binding properties and its cytogenetic effects in other organisms, further research is necessary to explore its impact on *Sarcophaga* chromosomes by examining chromosomal changes following Berenil exposure using techniques like karyotyping and fluorescence in situ hybridization. Comparing the genomes of treated and untreated *Sarcophaga* specimens to detect potential mutations or structural variations induced by Berenil would contribute to a more

comprehensive understanding of Berenil's genotoxic potential and its implications for insect genetics and pest management strategies. The prospects of the current findings accentuate the importance of chromatin-level studies in ecotoxicology and genome-environment interactions, contributing to our understanding of genome stability in both model and non-model organisms under chemical stress.

Conflicts of Interest: The authors declare no conflicts of interest.

Acknowledgments

Rashmi Srivastava gratefully acknowledges the University Grants Commission (UGC), New Delhi, Government of India, for awarding a Ph.D. Research Fellowship to pursue research in the Department of Zoology, University of Allahabad.

REFERENCES

Baccarelli A, Bollati V (2009) Epigenetics and environmental chemicals. Curr Opin Pediatr **21**: 243-251

Bhasin M (2005) Human population cytogenetics: a review. International Journal of Human Genetics **5**: 83-152

Chappell GA (2016) Alterations to the epigenome represent critical mechanisms of chemically induced carcinogenesis. The University of North Carolina at Chapel Hill

Donya SM (2006) Cytogenetic studies of Berenil in mice. Cytologia **71**: 41-48

Gandhi P, Sengupta LK, Sengupta S, Nicholls EM (1994) Chromosomal ‘Puffing’ and Undercondensed Segments-additional Indicators of Chromosome Fragility. CYTOLOGIA **59**: 393-398

González VM, Pérez J, Alonso C (1997) The berenil ligand directs the DNA binding of the cytotoxic drug Pt-berenil. Journal of inorganic biochemistry **68**: 283-287

Haaf T, Feichtinger W, Guttenbach M, Sanchez L, Müller C, Schmid M (1989) Berenil-induced undercondensation in human heterochromatin. Cytogenetic and Genome Research **50**: 27-33

Haaf T, Schmid M (2000) Experimental condensation inhibition in constitutive and facultative heterochromatin of mammalian chromosomes. Cytogenetics and cell genetics **91**: 113-123

Hatzi V, Terzoudi G, Paraskevopoulou C, Makropoulos V, Matthopoulos D, Pantelias G (2006) The use of premature chromosome condensation to study in interphase cells the influence of environmental factors on human genetic material. The Scientific World Journal **6**: 1174-1190

Heng HH, Stevens JB, Liu G, Bremer SW, Ye CJ (2004) Imaging genome abnormalities in cancer research. Cell & Chromosome **3**: 1

Kuriakose S, Uzonna J (2014) Diminazene aceturate (Berenil), a new use for an old compound? International immunopharmacology **21**

Molina WF, Galetti PM (2007) Early replication banding in Leporinus species (Osteichthyes, Characiformes) bearing differentiated sex chromosomes (ZW). Genetica **130**: 153-160

Poot M, Kausch K, Köhler J, Haaf T, Hoehn H (1990) The minor-groove binding DNA-ligands netropsin, distamycin A and berenil cause polyploidisation via impairment of the G2 phase of the cell cycle. Cell structure and function **15**: 151-157

Rosefort C, Fauth E, Zankl H (2004) Micronuclei induced by aneugens and clastogens in mononucleate and binucleate cells using the cytokinesis block assay. Mutagenesis **19**: 277-284

Singh K, Thakur S (2012) Electrophoretic Characterization of Sanitarily and Medically Important Flesh Fly, Sarcophagadux (Diptera, Sarcophagidae) Using Isozymes. National Academy Science Letters **35**: 339-342

Srivastava, R., Dabas, A., Srivastava, R., & Gaur, P (2012). Qualitative analysis of chromosomes from neural ganglia of flesh-flies Sarcopahga. Biochem. Cell. Arch., **12** (1), 149-152.

Srivastava R, Gaur P (2015) Revelation of heterochromatin heterogeneity in Sarcophagid chromosomes using DNA ligand Mithramycin. Caryologia **68**: 55-60

Srivastava R, Gaur P, Mishra N (2013) Photo-oxidation and quinacrine fluorescence response in the mitotic chromosomes of the flesh-flies Sarcophaga. International Journal of Pharma and Bio Sciences **4**: 250-255

Srivastava R, Mishra N, Singh UM, Srivastava R (2016) Genotoxicity: Mechanisms and its impact on human diseases. Octa Journal of Biosciences **4:** 67-70

Srivastava R, Srivastava R, Ahn SH (2016) The Epigenetic Pathways to Ribosomal DNA Silencing. Microbiology and Molecular Biology Reviews **80:** 545-563

Sumner AT, Mitchell AR (1994) Inhibition of chromosome condensation. Chromosome Analysis Protocols: 113-121

Tsuji H, Hitomi A, Takahashi E-i, Murata M, Ikeuchi T, Yamamoto K, Tsuji S, Hori T-a (1991) Induction of distamycin A-inducible rare fragile sites and increased sister chromatid exchanges at the fragile site. Human genetics **87**: 254-260

Wegner M, Grummt F (1990) Netropsin, distamycin and berenil interact differentially with a high-affinity binding site for the high mobility group protein HMG-I. Biochem Biophys Res Commun **166**: 1110-1117

Zoghbi HY, Beaudet AL (2016) Epigenetics and human disease. Cold Spring Harbor perspectives in biology **8**: a019497