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Karyotypes of *Chironomus* spp. (Diptera: Chironomidae) from the Bongshi River of Bangladesh †

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Abstract: Cytological studies of *Chironomus* spp. belonging to the salivary gland polytene chromosomes have been carried out for the first time in Bangladesh. Among Diptera, *Chironomus* is considered as valuable insect because of polytene chromosomes and it is distributed in a broad range of ecological conditions. The objective was to investigate cytogenetic variation of the different *Chironomus* species, collected from the Bongshi River, Dhaka, Bangladesh. Salivary glands of forth instar larvae were used for chromosome preparation following the aceto-orcein method. Each cell contained four pairs (2n=8) of polytene chromosomes with precious banded structures, puffs, Balbiani rings (BR) and nucleolar organizers (NOR). Four polytene chromosomes are numbered with AB (1st), CD (2nd), EF (3rd) and G (4th). Initially, seven types of polytene chromosomes (Type 1-7) were identified that most likely refers to seven *Chironomus* species. In case of Arm G, Type 1 contains one BR and one NOR; Type 2 has flattened funnel shaped structure; Type 3 possess BR, NOR and inversion; Type 4 has branching structure; Type 5 stands with BR and NOR; Type 6 has triangle shape with three dotted bands and other end possess a deeply stained band that likely to be the centromere; Type 7 composed of one linear arm. These cryptic polytene chromosome banding patterns and their arrangements provide significant cytotaxonomic information for identifying *Chironomus* species. Further cytogenetic study of natural populations based on long-term sampling strategy from different ecological conditions could be useful for testing the hypothesis of the role of chromosome inversion on local adaptation.

Keywords: Chironomid larvae; aquatic insect; polytene chromosomes; salivary gland; cytotaxonomy

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1. Introduction

Chironomids are the most widely distributed and frequently one of the most abundant insects in freshwater substantial components of true flies belonging to the order Diptera (Class- Insecta). They can tolerate and develop in polluted waters such as waste stabilization ponds where they become a dominant macro-invertebrate. The family Chironomidae is a cosmopolitan group of insect's members of which occur in all zoogeographical regions of the world [1,2]. This group is of great interest from both the biological and practical point of view as they are found in all types of inland water and are central to freshwater ecosystems. They are recognized as an important food for many fishes and cultured crustaceans, and are very popular in aquarium fish trade. Larvae are

an excellent source of protein, lipid, vitamins and minerals with high energy content, and highly digestible [3].

Species identification within this family can be done with the help of polytene chromosome karyotypes. It is the most important taxonomic feature for species identification in *Chironomus* larvae, because most of the *Chironomus* larvae have very similar morphology. Generally, they possess good quality giant polytene chromosomes, which can be used for study of both taxonomy and phylogeny [4,5]. Chironomids have well characterized banding structures in their polytene chromosomes that permits precise cytogenetic analyses [6]. The bands of polytene chromosome arms form specific banding sequences, which are considered as genetic markers to analyze divergence patterns of the linear genome structure during evolution [7].

Present study investigated polytene chromosome karyotypes of *Chironomus* species, collected from different sites of the Bongshi River of Bangladesh and describe their cytogenetic variation based on the polytene chromosome banding patterns. The information could be used to confirm the presence of different species in the Bongshi River and to study genetic diversity in species with a wide range.

2. Materials and Methods

Larval materials were collected from three different sites- Pathaliya (23°55'26.9"N 90°13'34.7"E), Ghughudia (23°53'42.6"N 90°13'55.8"E) and Sinduria (23°52'51.6"N 90°13'58.6"E) of the Bongshi River, Savar, Dhaka during December 2020 to March 2021. Identification to the genus *Chironomus* has been done using the external larval morphology through standard keys [8,9]. The whole experiments were carried out at the Cytology and Biocontrol Research (CBR), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka.

Fourth instar larvae were used for the salivary gland polytene chromosome preparations using the routine aceto- orcein staining method [10]. Larvae were dissected in saline solution; salivary glands were isolated and fixed in 96% ethanol and glacial acetic acid (3:1). The glands were then stained in aceto- orcein for about 15- 30 min. After staining, salivary glands were squashed for polytene chromosome preparations.

Polytene chromosomes were observed under Olympus Microscope (CX41RF) using objective lens 40X. Photographs were taken from well- spread polytene nuclei under 400x.

3. Results

Each polytene cell contains the chromosome set 2n= 8, with the chromosome arm combinations: the 1st (AB), the 2nd (CD), the 3rd (EF), and the 4th (G). They are numbered according to the descending order in size, the 1st chromosome is the longest and the 4th chromosome is the shortest in length. Puffs, Balbiani Rings (BR), Nucleolar Organizers (NOR) and the major landmarks were observed in the polytene chromosomes of the sampled *Chironomus* spp. Initially, seven different types (Types 1-7) of polytene chromosome banding structures were identified in the present study. Clear banding sequences are shown by brackets in the figures (Figures 1-7).

Type 1 (Figure 1) has a sequence of banding in arms A-F. Standard banding sequences have been observed in arms D and E with a NOR in arm B. Three potential banding constrictions also found in arm E, at the terminal region of arms C and G. Arm G contains one BR in the middle with two different forms (BR1 and BR2) and one NOR at its both ends. Banding inversion is also observed in this arm. Flattened tip with a constriction and adjacent twelve dark bands are the landmark of arm C. Square tip with three bands along with lightly stained region and two darkly stained bands are the landmarks of arm D (shows in closed bracket). Pointed tip with a series of ten dark bands

and a constriction with seven dark bands are the identifying landmarks of arm E (shows in closed bracket).

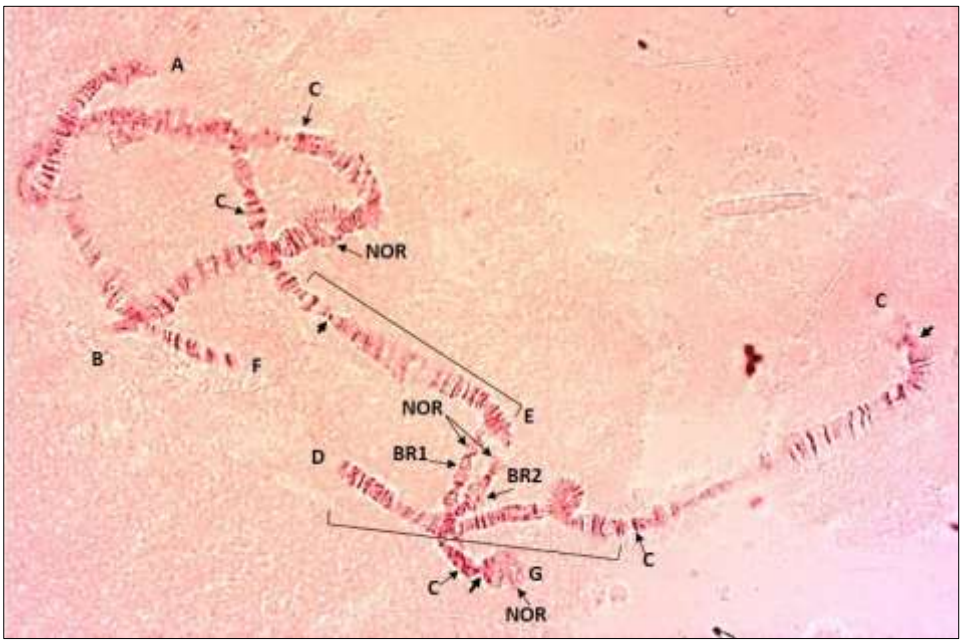

Figure 1. Polytene chromosomes of *Chironomus* Type 1; A-G = Chromosomal arms, C= Centromere, NOR = Nucleolar Organizer Region, BR = Balbiani Ring, Bold arrows indicate banding constrictions, brackets indicate standard banding sequences in arms.

Clear banding sequences are found in the chromosomes of Type 2 (Figure 2). Arms C and E possess good banding structures with two constrictions in arm C, one in arm E near its centromere regions and one in arm A at its terminal region. Short arm G has a remarkable flattened funnel shaped banding structure at one terminal end and the other end contains a deep dark band, which could be considered as centromere. NOR and BR were not observed in the G arm. Rounded tip with a dark band, along with a faint region and five dark bands followed by another faint region with six dark bands and a constriction are the landmarks of arm C (shows in closed bracket). Flattened tip with a constriction and four dark bands are the characteristic landmarks of arm A. Arms B, D and E possess prominent deeply stained bands.

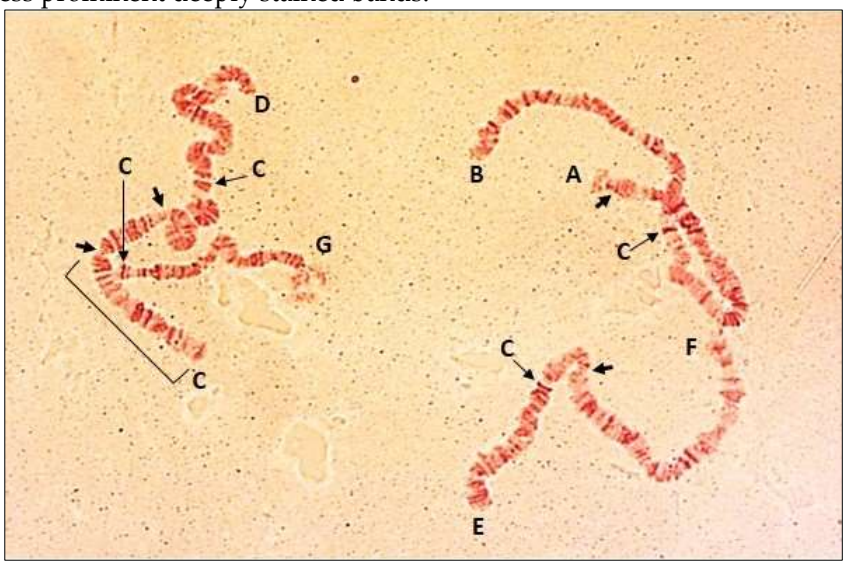

Figure 2. Polytene chromosomes of *Chironomus* Type 2; A-G = Chromosomal arms, C= Centromere, Bold arrows indicate banding constrictions, bracket indicate standard banding sequences in arm.

Type 3 (Figure 3) polytene chromosomes comprise of long arm AB along with standard banding structure in arms B and D. A prominent puff with a banding constriction was found in arm C. Arm G has adorned with BR and NOR with banding inversion in the middle. Arm B has fused banding tip and arm D possess a flattened tip with one deeply stained band. Both arms contain several prominent dark bands along with their tips (shows in closed bracket).

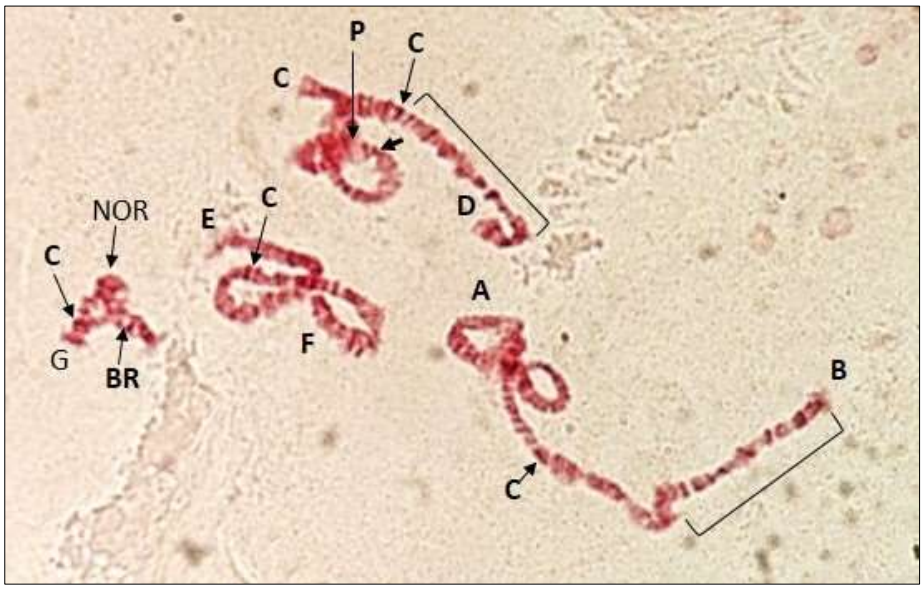

Figure 3. Polytene chromosomes of *Chironomus* Type 3; A-G = Chromosomal arms, C= Centromere, Bold arrow indicate banding constriction, brackets indicate standard banding sequences in arms.

A banding sequence with three dark bands and a puff near the tip of arm D are the landmarks of polytene chromosome in Type 4 (Figure 4). Arm B has standard banding structures and Arm G has branching but not very clearly observed. Rounded tip with at least eight dark bands of arm A and straight tip with fused banding followed by five deeply stained bands of arm B are their landmarks (shows in closed bracket). Rounded tip with four narrow and dark bands along with a puff and another three dark bands are the characteristic zone of arm D (shows in closed bracket). Arm tip C and F have fused banding compare with arm E.

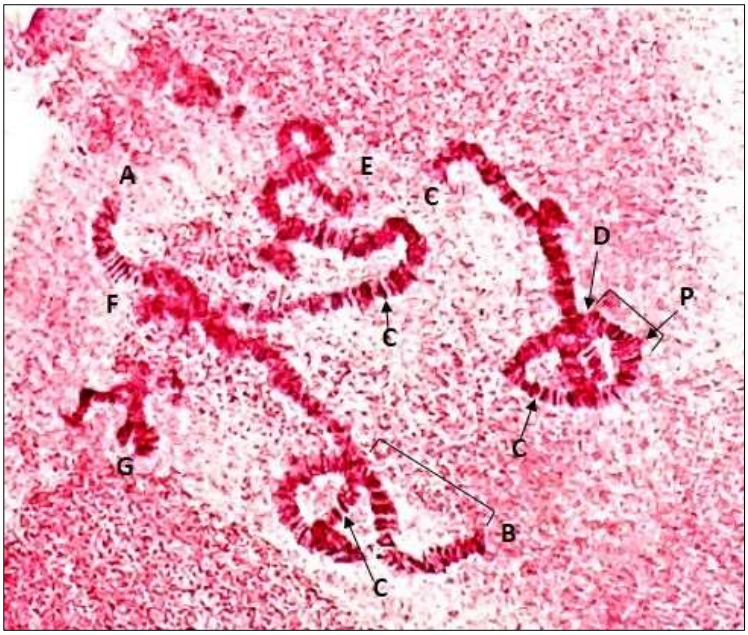

Figure 4. Polytene chromosomes of *Chironomus* Type 4; A-G = Chromosomal arms, C= Centromere, brackets indicate standard banding sequences in arms.

Type 5 (Figure 5) has long polytene arms (A-F) with well-defined banding sequences in comparison with other six types. Chromosome arms AB, CD and EF have one puff each with a banding constriction in arm D. Arms B, D and F have standard banding patterns. Arm G has BR, NOR and banding inversion in the middle. Fused banding tip followed by another at least seven dark bands are the identifying mark of arm B (shows in closed bracket). Flattened tip with very closed six dark bands followed by faint region with another four dark bands are the characteristic landmarks of arm F. Tips of arm C, D and E composed of fused bands.

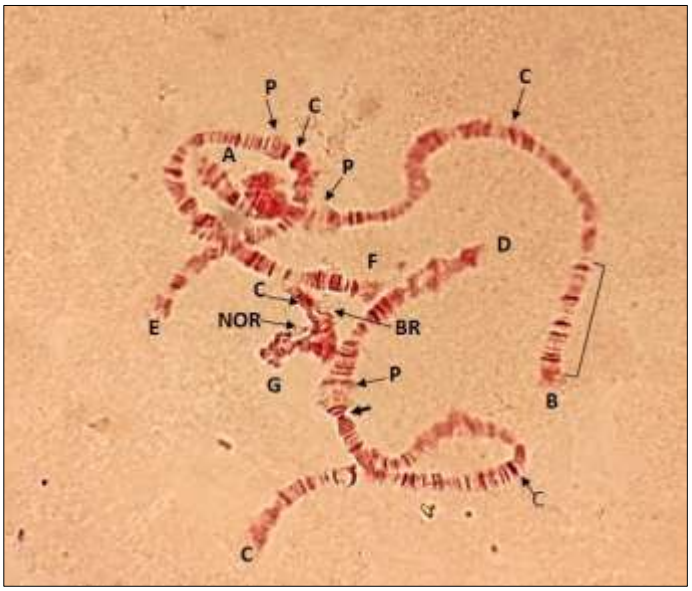

Figure 5. Polytene chromosomes of *Chironomus* Type 5; A-G = Chromosomal arms, C= Centromere, Bold arrow indicate banding constriction, bracket indicate standard banding sequences in arm.

Specific and regular banding structures are observed in arms A-G of Type 6 (Figure 6), among them arms B, D and E have very good banding characters. Arm G of Type 6 has a specific triangle shape with three dotted bands at one end and other end possess a

deeply stained thick band that likely to be the centromere of this arm. A banding constriction found in Arm E. Fused banding tip with three dark bands followed by a faint region with another five dark bands are the identifying mark of arm B. Flattened tip with eleven dark bands continued with a banding constriction are the land mark of arm E.

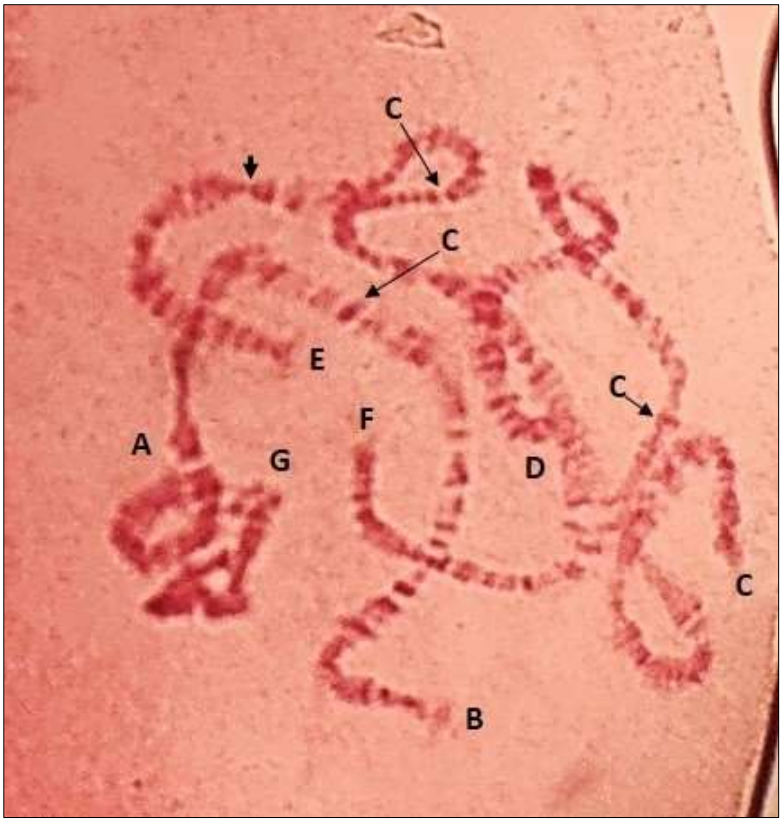

Figure 6. Polytene chromosomes of *Chironomus* Type 6; A-G = Chromosomal arms, C= Centromere, Bold arrow indicate banding constriction.

Polytene chromosomes of Type 7 (Figure 7) has clear banding sequences in all arms with regular dark and faint bands. Here, arm G composed of one linear arm, no branching observed. Pointed tip with three dark bands followed by a faint region with six dark bands are the landmark of arm A, while arm B has many darkly stained banding almost the full arm (shows in bracket). Chromosome arms C and D contains clear banding pattern with dark and light bands. Arm E has several dark bands compare to arm F that possess light bands.

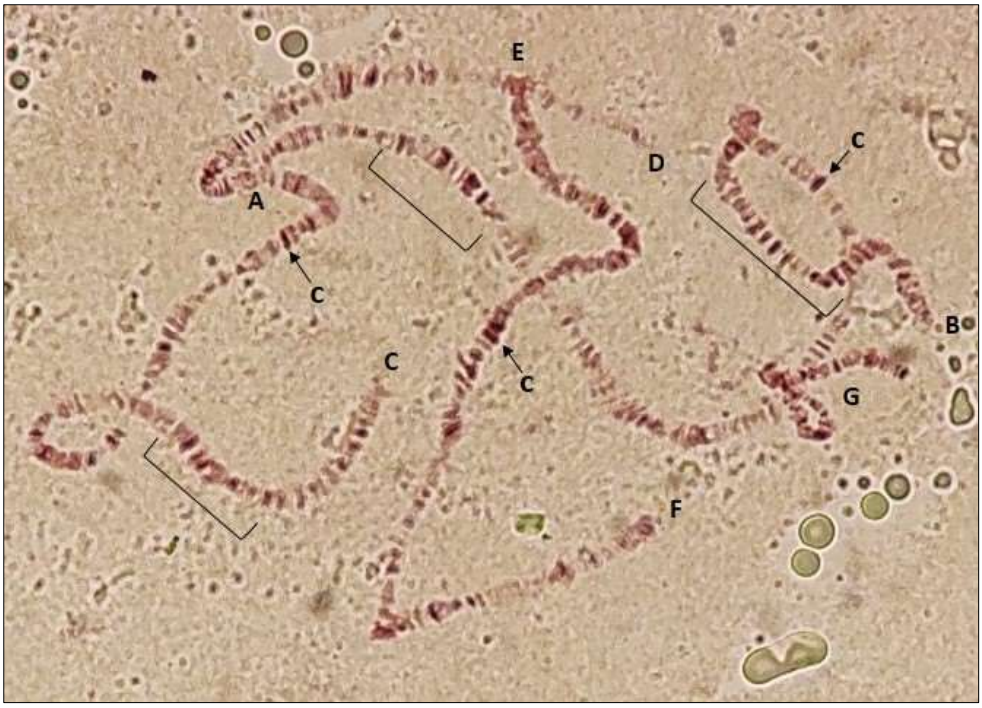

Figure 7. Polytene chromosomes of *Chironomus* Type 7; A-G = Chromosomal arms, C= Centromere, brackets indicate standard banding sequences in arms.

4. Discussion

Chironomid larvae are prospective subjects for cytogenetic studies because of the large polytene chromosomes with good banding structures. Some structural aberrations (deletions, inversions and deficiencies) and functional alterations (decrease in the level of activity of BR and the NOR) also help to mark up the level of genotoxicity [6]. In this study, we have identified seven different types of polytene chromosomes banding structures/sequences in *Chironomus* larvae, collected from three sampling spots in the Bongshi River of Bangladesh. Each type has four polytene chromosomes with seven arms (A-G), which is consistent with the previous results of *Chironomus* species [4,5,7]. Arm combinations of all chromosomal types were considered as AB, CD, EF and G which follows that of 'thummi' cytocomplex [11]. Arm G of the chromosomal type 1, 3 and 5 contained Balbiani rings (BRs) and Nucleolar organizer region (NORs) and it displayed telocentric morphology. It agrees with the chromosome combination of the 'thummi' cytocomplex and the chromosomal characteristics of *Chironomus* species in Palearctic and Holarctic regions [11,12]. Puffs with banding sequences have found in different locations in polytene chromosome arms AB, CD and EF, especially in chromosomal types 3-5. It consistent with the results of *C. circumdatus* [1]. In our previous study, larval materials were collected from the Jahangirnagar University Campus Lake, Savar area. Here, we found one type of *Chironomus* species containing four giant polytene chromosomes with seven band sequences (A-G). The chromosome arm combinations were 1st (AB) the longest, 2nd (CD), 3rd (EF) and 4th (G)- the shortest. The homologues of arm G are paired and it is decorated with one Balbiani ring (BR) in the middle and two nucleolar organizers (NOR) [13]. These results of chromosome arm combinations are similar with the present study in all types (Type 1-7).

5. Conclusions

For a complete understanding of the adaptation mechanism of Chironomids to various environmental conditions, data on the cytogenetic structure of populations living in poorly studied regions are of obvious interest. Since the cytological study of

Chironomids have been carried out for the first time in Bangladesh, it would be facilitated the cytotaxonomy and the future comparative karyological analysis of *Chironomus* species worldwide. Further experiments are in progress for the cytotaxonomic analysis of the *Chironomus* species.

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Data Availability Statement: The experiments were carried out at the Cytology and Biocontrol Research (CBR), Institute of Food and Radiation Biology (IFRB), Atomic Energy Research Establishment, Savar, Dhaka. Bangladesh. All data and materials were collected and assessed from our own experiments and are available from the corressponding author, if required. All data related to the current study are included in the manuscript.

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

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