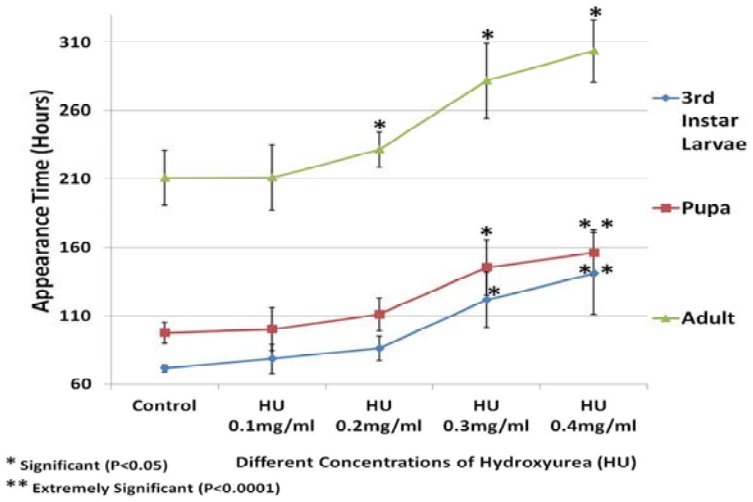
Figure 1



ii. Assessment of the number of representative individuals of pupal and adult stages:

Number (mean +/- SD) of representative individuals of pupa and adult stages generated up to 100 hours from their initial time of appearances, has been shown in Figure 2. The figure reveals that, compared to controls, significant reduction in number of pupae and emergent adults was intiated in the fly cultures exposed to HU concentration of 0.2 and 0.1 mg/L respectively. However, hallmark of developmental inhibition (P=<0.0001) was observed in cultures exposed to HU concentration of 0.4 mg/L where, compared to control, metamorphosis of developing flies into adult was reduced by approximately77% .

The percent reduction of population of pupae and emergent adults compared to controls in response to exposure to varying concentration of HU has been shown in the **Table 1.**

Figure 2: Population data of *D. melanogaster* exposed to varying concentrations of Hydroxyurea(HU). Data are represented as the number (Mean +/-SD) of pupae and young adults appeared up to 100 hours from their initial time of appearances in control and test cultures.

Figure 2

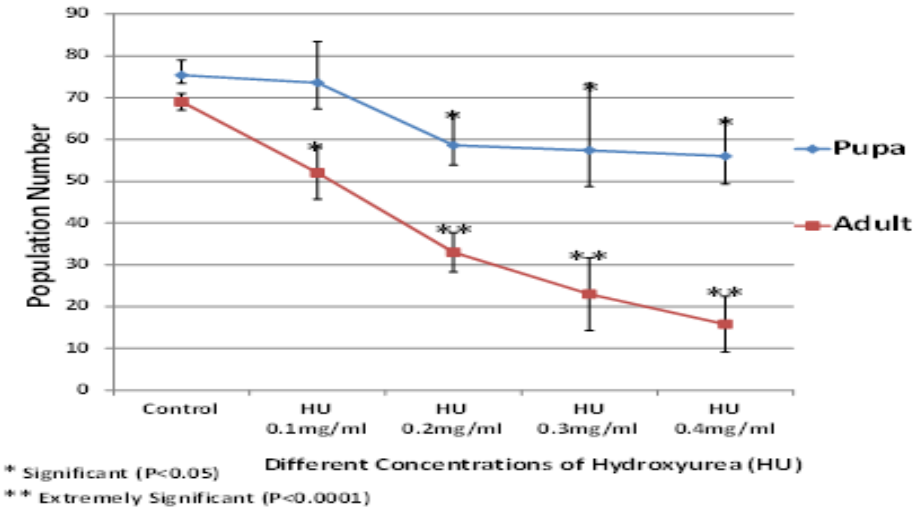


Table1. Percent reduction of population of pupae and emergent adults compared to controls in response to exposure to varying HU concentration.

Concentration of HU	Percent (%) reduction of pupa population	Percent (%) reduction of emergent adult population
0.1mg/ml	2.4	25
0.2 mg/ml	22.3	52
0.3 mg/ml	24	67
0.4 mg/ml	76	77

iii. Assessment of chromosomal abnormalities:

Polytene chromosomes prepared from the third instar larvae of the flies exposed to HU at all concentrations (i.e. 0.1mg/ml, 0.2mg/ml, 0.3mg/ml and 0.4mg/ml) exhibited a number of chromosomal rearrangements. However, occurrence of chromosomal aberrations at HU concentration of 0.1 mg/ml was very low. Chromosomal abnormalities were represented by asynapsis, ectopic pairing, constriction etc (Figures 3,4,5,6 and7). Frequency of chromosomal rearrangements in larval polytene chromosomes was highest at HU concentrations of 0.4mg/ml followed by decreasing HU concentrations.

Figure 3 Ectopic pairing in polytene chromosome in response HU concentration 0.3 mg/ml.

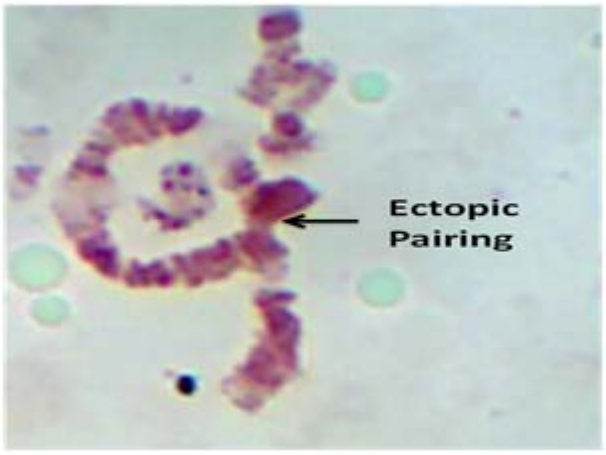


Figure 4 Asynapsis in response HU concentration 0.3 mg/ml.

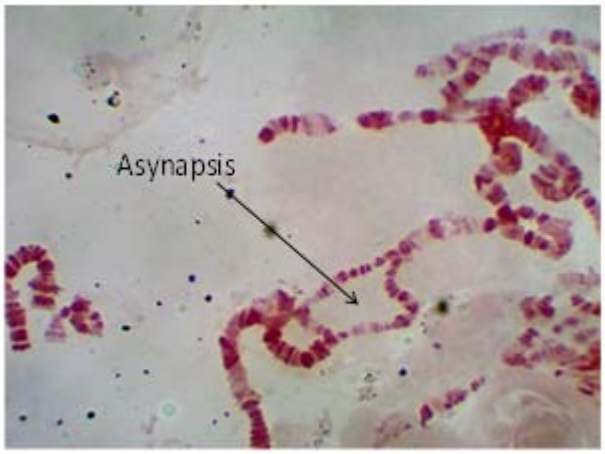


Figure 5 Ectopic pairing, constriction in response HU concentration 0.3 mg/ml.

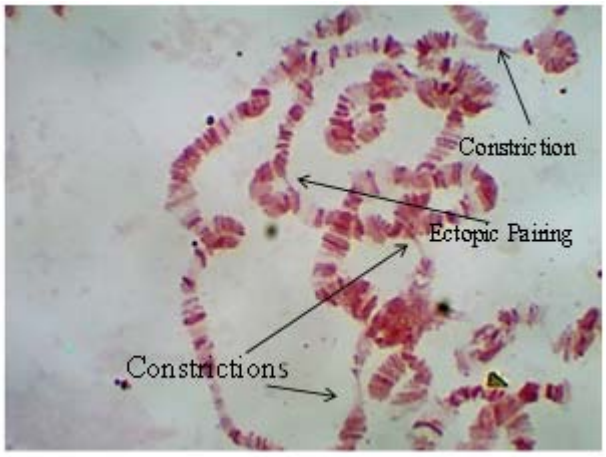


Figure 6 Ectopic pairing in response HU concentration 0.4mg/ml.

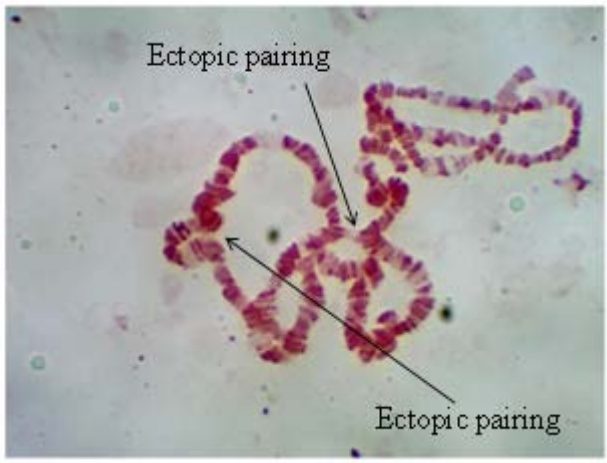
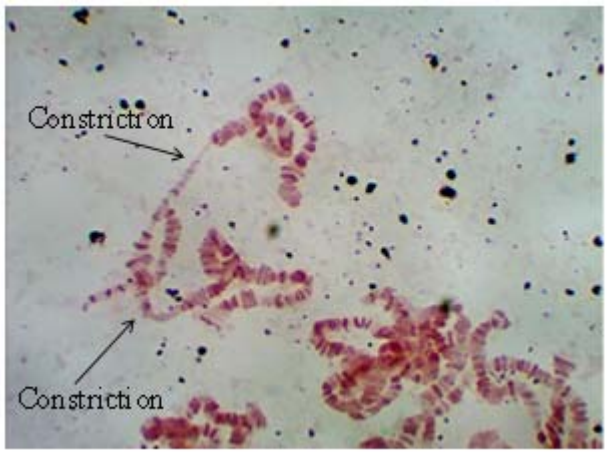


Figure 7 Chromosomal constriction in response HU concentration 0.4 mg/ml.



Discussion: In this study an assessment of dose dependent effects on development of a drug i.e. HU, which is used for treating cancer and inherited blood disorder like sickle cell anemia, was made by using the model organism *D. melanogaster*. It was observed that the drug caused dose dependent delays in metamorphosis as well as reduction in number of emergent adults of this fly. However, significant delay in the initial appearance of pupa and emergent adults was observed in cultures exposed to HU concentration of 0.3 ad 0.4 mg/ml of

which later concentration conferred highest significant impact. Although the significant delay in the initial appearance of the pupa and adult stages commenced at HU concentration of 0.3 mg/ml, significant reduction in pupa and emerging adult number were initiated by further lower HU concentrations of 0.2 and 0.1 mg/ml respectively. This indicates that, compared to controls, most of the developing flies under the exposure of HU could not tolerate even these lower concentrations of HU which caused the paucity of their metamorphosis. In addition, metamorphosis into adult stage was drastically reduced with the increasing doses of HU (Table 1). The developing flies which survived the challenge of these two HU concentration, suffered the significant delays in metamorphosis. In other words, HU exerted a distinct negative impact on the development of this fly. After extensive search we could find of only one report regarding the experimental evaluation of the impact of this drug on the development of *D.melanogaster* [23]. In that study, flies were exposed to only two concentrations of HU viz, 0.1 and 0.25 mg/ml. When we compared our result with the results of the aforesaid study, it was observed that reduction of emergent adult population in our case was many folds higher than the reduction of adult population reported in that study. We speculate that the differences in tolerance of HU by the flies might be due to the strain differences of *D. melanogaster* used by us and the above mentioned group, since it has been reported that strain differences in *Drosophila* make differences in their tolerance for chemicals[24].

Mutagenic potential of chemical and physical agents can be evaluated by the extent of chromosomal lesions induced by them and experimental observation of polytene chromosomes of dipterans especially *Drosophila* provides us an opportunity to see these effects in an magnified way due to the enormous size of this chromosome[25]. In our study, examination of the polytene chromosomes in the third instar larval stage of *D. melanogaster*, exposed to varying concentration of HU exhibited several chromosomal rearrangements in the forms of constriction, ectopic recombination, asynapsis etc. These kinds of chromosomal abnormalities were found to be of very negligible occurrence in case of controls. Although we found a few chromosomal structural abnormalities in larvae exposed to HU at 0.1 mg/ml concentration, significant occurence of these chromosomal aberrations was evident at HU concentrations 0.2 mg/ ml. that was increased further with increasing doses of HU.

One abundant rearrangement that we observed was the constriction of polytene chromosomes. Due to the inhibition of DNA replication, constrictions in chromosomes may appear and the sites become fragile that facilitate their breakages[26,27]. As it has been mentioned earlier that HU interferes with DNA replication, occurrence of such chromosomal constrictions were quite possible in our study that might ultimately repress the developmental gene expressions in *Drosophila* larvae. In asynapsis, homologs of polytene chromosome fail to pair with each other. As homologs remain attached with each other by fibrillar connectors[28] or bundles of microfilaments[29], we speculate that chemical interaction of drugs with these structures may cause their distortion rendering the separation of homologs in polytene chromosomes. This separation of homologs may provide severe impacts in larval gene expressions as it can cause the disruption of trans-interaction (transvection) of enhancer and promoter elements that occurs during their paired state [30]. Ectopic pairing, which was observed in our larval chromosomes, occur through linkages made by heterochromatin threads between regions of sequence homology present either at different regions of the same chromosomal arm or in different arms of a chromosome. If the linkage occurs between adjacent regions of sequence homology, the intermediate portion of the chromosome bulges out to form a toroidal structure. It is possible that due to this linkage, discrete homologous nucleotide sequences in a chromosome might unite to produce a new gene sequence [25, 31]. We speculate that such nascent gene sequence might code for an unusual protein not normally found in *Drosophila* larvae and proved to be detrimental for development of the flies. We speculate that HU hindered the normal expression of genes required for development of the fly by inducing deleterious rearrangement of chromosomes. Asyanapsis possibly caused inhibition of interactions of controlling elements for gene expressions in the larvae. Ectopic pairing might be responsible for creating new genetic sequences [25] the product of which was deleterious for embryonic development. Constrictions of polytene chromosomes in larvae exposed to HU might result into chromosomal breakage as they represent the fragile sites on chromosomes [26]. This might result in disruption of the expression of genes needed for fly development. Thus it seems probable that wide range of larval chromosomal abnormalities those appeared in the polytene chromosomes of *D. melanogaster*, in response to HU exposure, might cause serious inhibition of the expression of the genes needed development of the fly. *D. melanogaster* serves as an important model for developmental biology [32] and toxicological studies [33]. Furthermore, owing to extensive genetic homology between human and *D.*

melanogaster, the experimental results obtained by using this fly raises an alarm regarding the negative impacts of HU exposure during human pregnancies that may bring about chromosomal abnormalities in developing embryo leading to deleterious effect on offsprings. child births.

Conclusion

Our present study revealed that HU exerted negative impact on the development of *D. melanogaster,* a model organism, in a dose dependent manner. Although the drug is very useful in treating cancer and hereditary blood disorders, our study has proved that it exerts genotoxic effects on eukaryotic chromosomes. Chromosomal damages in turn may have deleterious effects on the expressions of genes needed for the normal development. Owing to substantial genetic homology between human and *Drosophila,* results of our study strongly indicates the possibility of obtaining analogous outcome in human due to the exposure of HU during development. Thus the need of exercising cautious use of this drug during human pregnancy is strongly recommended.

Conflict of Interest

The authors declare no conflict of interest.

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