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Review

An insight into the genotoxicity assessment studies in dipterans

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ABSTRACT

The dipterans have been widely utilized in genotoxicity assessment studies. Short life span, easy maintenance, production of large number of offspring in a single generation and the tissues with appropriate cell populations make these flies ideal for studies associated to developmental biology, diseases, genetics, genetic toxicology and stress biology in the group. Moreover, their cosmopolitan presence makes them suitable candidate for ecological bio-monitoring. An attempt has been made in the present review to reveal the significance of dipteran flies for assessing alterations in genetic content through various genotoxicity biomarkers and to summarize the gradual advancement in these studies. Recent studies on genotoxicity assays in dipterans have opened up a broader perspective for DNA repair related mechanistic studies, pre-screening of chemicals and environmental bio-monitoring. Studies in dipterans, other than *Drosophila* may be helpful in using them as an alternative model system for assessment of genotoxicity, especially at the gene level and further extension of these studies give a future insight to develop new strategies for maintaining environment friendly limits of the toxicants.

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1. Introduction

The genotoxicity studies identify and analyze the action of toxicants on the hereditary components of living systems, which facilitate to define the impact of these genotoxicants present in the environment which may alter the integrity of the gene pool and to detect their mechanistic actions *in vivo* [1].

Among invertebrates, insects and especially members of the order Diptera have been widely explored in genetic toxicological studies [2–5]. Some advantageous characteristics found in dipterans like short generation time, production of the large number of offspring in a single generation, small life span and occurrence of the tissues with appropriate cell populations have been ideal in such studies in the view of statistical and experimental replication. The dipteran flies also have well-defined

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karyotypes with a low number of distinguishable diploid chromosome complement possessing localized centromere [6–8] and showing characteristic somatic pairing [9]. Moreover, these flies are endowed with giant polytene chromosomes which help in illustrating minute genetic changes [10,11].

Pioneering studies on genotoxicity assessment in dipterans were carried out by Muller [12] using X-ray radiation followed by Auerbach [13] using chemicals on fruit fly *Drosophila.* These studies led the workers to study genotoxicity in two directions: (i) Physical factors like radiation, temperature and hypoxia induced genotoxicity and (ii) chemical induced genotoxicity. These studies comprehensively improved and were adopted in several families of the order Diptera.

The present review chronicles the advancement in genotoxicity studies with dipterans and deals with the importance of these flies used for assessing alterations in genetic material through various genotoxicity biomarkers like germ line mutation assays, somatic mutation assays, chromosomal aberration assay, micronucleus assay, comet assay and other DNA sequence based assays. However, it is a well-known fact that the use of dipterans does not entail any ethical issue and therefore provides a better scope for the use of these flies as an alternative model in genotoxicity assessment studies.

An overview of genotoxicity assessment studies through various assays is presented as follows:

1.1. Germ line mutation assays

In the early years of 20th century, after the rediscovery of Mendel's laws, T.H. Morgan with H.J. Muller, C. Bridges and A. H. Sturtevant initiated his work on inheritance of mutant traits of *Drosophila* flies. Later Muller started his own work on artificially induced mutations on *Drosophila* with E. Altenburg in 1912. In 1927, Muller discovered that gene mutations could be induced in *D. melanogaster* by the administration of X-rays. He improved the mutation assessment technique by introducing the *ClB*-method (C stands for long inversion on X-chromosome, l for recessive lethal and B indicates the bar eye trait used as a marker) which was able to detect all kinds of mutations on the X-chromosome and phenotypically it expressed in the mutant carrying males [14]. These studies were followed by the findings that ultraviolet rays were also capable of inducing mutations in the genes [15,16]. However, it was found that X-radiation produced many chromosome rearrangements in comparison to UV treatment, which produced comparatively a few changes of such kinds. Furthermore, in a comparative study, Timofeeff-Ressovsky [17] reported that gamma- rays are also about three times less effective than X-rays in inducing mutations.

In subsequent studies, the effect of irradiation/other physical factors on genetic mutation were assessed in terms of (i) sex-linked recessive lethals (SLRL) by *ClB*-method (ii) dominant lethal (death of fertilized egg or developing embryo due to chromosomal breakage in parent germ cells), (iii) translocations and their relation to chromosomal aberrations e.g., chromosome loss and other rearrangements as the consequence of chromosome breaks and gaps and (iv) non-disjunction during gametogenesis in *Drosophila*. These factors were X-rays [18–22], UV- rays [16,23,24], gamma rays [25] fast neutrons [26,27] and temperature [28–31]. A comparative assessment of X- rays and UV- ray [32], X-rays and near infra-red [33], temperature along with X-rays [34,35], oxygen with X-rays [36–38] and anoxia with fast neutron [39] have also been analyzed. Cytological studies were also carried out along with genetic studies to unravel mechanisms behind the genetic manifestation of genotoxicity. Muller [40] and Kaufmann [41] discussed the nature and genetic effects produced by radiation regarding sensitivity of euchromatin and heterochromatin for breaks in X- rays treated cells during spermatogenesis. Savontaus and Nokkala [42] observed the cytological aberrations including chromosome displacements resulting into non-disjunction and other unidentifiable chromosome aberrations in X-ray treated oocytes of *D. melanogaster*. They supported the concept that chromosomal breakage is the cause of a majority of X-ray-induced dominant lethals.

Chemical induced genotoxicity studies were started with the observation of abnormal chromosome segregations induced by chemicals (mustard gas and allyl isothio-cyanate or mustard oil) during gametogenesis resulting into non-disjunction, chromosome rearrangements and sex linked recessive lethal [13,43,44]. Rapoport [45,46] assessed the mutagenic effect of formaldehyde and several other chemicals on *Drosophila* larvae. Further studies, with chemicals, also included the sex linked recessive lethal, dominant lethals, translocations and non-disjunction as genetic end points of genotoxicity [47–52]. Since then, about 100 chemicals in terms of consequences of chromosome breakage, chromosome loss and translocations causing genetic mutations and more than 750 chemicals have been tested using SLRL mutation as end point in *D. melanogaster* [53].

Auerbach and Robson [54] opined that chemical substances could be as effective as X-rays in inducing mutations and chromosomal rearrangements through a comparative study. Fahmy and Fahmy [55] assessed the effects of carcinogens and tumour inhibitors in *D. melanogaster* by using mechanism of induction of dominant lethal and interpreted that the dominant lethal curves i.e., non-linear in "two hits" condition, follow the similar pattern as found in X-irradiated mature sperm and oocytes of *D. melanogaster* [18]. Several other studies related with "one hit" condition were represented that the dominant lethal curve generally show linear pattern on irradiation and chemical stress in dipterans [56–58].

Except sex linked recessive lethal study, all the other types of mutations like dominant lethals, occurrence of translocations etc. have been observed in other dipteran flies. Wagoner [59] analyzed linkage group karyotype correlation in house fly *Musca domestica* as determined by X-ray induced translocations. Radiation and chemical induced dominant lethal mutations were also observed in *M. domestica* [58,60,61]. Quantitative dose-effect relationship of the mutagenic action of X-rays on the germ cells of the malaria mosquito *Anopheles messeae* was measured by Pleshkova and Plevako [62] by observing frequency of dominant lethals. Similar results have also been observed in *Cochliomyia hominivorax* (Coquerel) after X-rays, UV-rays, chemicals and temperature exposure [63–66] and in *Dacus oleae* Gmel after γ-rays' exposure [67]. In a recent study, the toxic potential of organophosphate pesticides acephate and chlorpyrifos has been evaluated by dominant lethal test on *Culex quinquefasciatus* [68].

Wendell and co-workers [69] in case of *Aedes aegypti* suggested that the molecular understanding of a gene can be facilitated by analyzing the phenotypes of mutants for that gene and developed both methylmethane sulphonate and gamma-rays mutagenesis for *Ae. aegypti* using the white (w) gene (sex linked gene) as an assay.

1.2. Chromosomal aberration assay

1.2.1. Mitotic chromosomes

This assay allows the examination of those mutations that do not go beyond either gametogenesis or the development of zygote and surpasses the limitation of germ line mutation studies in which mutations are analysed only in subsequent generations [70]. The direct studies were started with the use of neuroganglial tissue of third instar larvae, which remain in the state of division, to quantitate the type and relative frequency of chromosome aberrations [70–72].