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Research Article

Cytogenetic effects of bendiocarb, a carbamate insectide on *Calotes versicolor* assessed by chromosomal aberration assay

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Abstract: The present work deals with the cytogenetic effects of a carbamate insecticide, bendiocarb on the males of garden lizard *Calotes versicolor*. Metaphase plates were prepared from the animals injected intraperitonealy (IP) with a daily dose of 8 mg/kg body weight (1/2 LD_{50}) of bendiocarb after 7th, 14th, 21st and 28th days of treatment. The metaphase plates prepared from bone marrow cells of the lizard were observed for scoring the frequencies of chromosomal aberrations (CA). Our findings revealed structural aberrations in the form of gap, break, addition and deletion in treated lizards. Statistical analysis showed no significant change in CA frequency after 7 days. Significant increase in induction of CA frequencies in the form of gap was observed after 14th, day and for break, addition and deletion after 21st day of treatment. This study indicates that chronic exposure to bendiocarb for an extended period induces structural aberrations in the metaphase chromosomes in a time dependent manner at the tested dose in the reptilian model *Calotes versicolor*. Based on this work, it can be asserted that bendiocarb induces genotoxicity in male *Calotes versicolor*.

Keywords: Carbamate, Bendiocarb, Genotoxicity, *Calotes*, Chromosomal aberration.

Abbreviations: Chromosomal Aberration (CA), Intraperitoneal (IP), Lethal Dose 50% (LD_{50}) Standard Error of Mean (SEM).

INTRODUCTION

In order to protect agricultural, household and industrial products from various plant and animal pests several synthetic pesticides are being used in India as well as all other parts of the world. Most of the synthetic pesticides are complex mixture of chemicals and majority of these acts as environmental contaminants or pollutants. Due to lack of proper guidelines and regulations by the manufacturing companies and unaware public, these pesticides are being indiscriminately used which is a cause of great concern all over the world. The degradation product of such complex chemicals and their accumulation in water bodies and soil leads to further deterioration of the natural habitat of several organisms.

Pesticide pollution has thus, become a serious threat for the modern society, and are potentially toxic for several non target organisms.

There are three major groups of synthetic pesticides namely, organophosphates, organochlorines and carbamates. Carbamates are used as insecticides, fungicides, nematocides, acaracides, molluscicides, sprout inhibitors or herbicides (Paíga *et. al*., 2009). Bendiocarb (Chemical Name- 2, 3-isopropylidenedioxyphenyl methylcarbamate, Trade names: Ficam, Dycarb, Garvox, Turcam, Niomil, Seedox and Tattoo) is an insecticide belonging to the N-methyl carbamate group (Holovska *et. al*., 2014). It is a broad spectrum insecticide, used against mosquitoes, flies, wasps, ants, fleas, cockroaches, silverfish, and ticks. Many investigations regarding toxicity of carbamate pesticides on various non target organisms revealed that some of the carbamate pesticides are cytotoxic, mutagenic, clastogenic and carcinogenic (Pandey & Guo, 2015; Priya *et. al*., 2014; Srivastava & Singh, 2013; Shrivastava *et. al*., 2014; 2015). Carbamate insecticides, namely, propoxur, methomyl and aldicarb, induce significant increase in structural and numerical chromosomal aberrations in mammalian cells as reported by Wei *et. al*., (1997).

Lizards inhabiting the agricultural farms, gardens and buildings are an important component of food chain, and these are constantly being exposed to various pesticides as a non target species either directly or through the food chain in a biomagnified manner. In reptiles, these are bioaccumulated and biomagnified to the levels equal to or greater than those described in birds and mammals (Hall and Henry, 1992). Reptiles have previously been shown to be excellent indicators of the potential association between contaminants and genetic damage (Hall & Clark, 1982; Talent *et. al*., 2002; Matson *et. al*., 2005; 2009; Capriglione *et. al*., 2011).

However, there is paucity of reports in reptiles regarding cytogenetic effects of bendiocarb. Hence, interest aroused and the present work is designed to investigate cytogenetic effects of bendiocarb, by evaluating its capability to induce chromosomal aberrations as revealed in the metaphase plates prepared from bone marrow cells in the male garden lizard, *Calotes versicolor.*

MATERIALS AND METHODS

Location and duration of study

This study was conducted in the Department of Zoology, Udai Pratap College Varanasi, India. The preliminary studies standardization of experimental procedures, animal acclimatization, actual animal experiment and evaluation of results lasted for a period of four months. However, the actual administration of bendiocarb to the test animals lasted for 28 days. Adult male garden lizards, *Calotes versicolor* (average snout - vent length 10±2 cm and body weight 30±2g) were housed in vivarium (wire net cages of size 18 x12x 10 inch). These were provided with food (crickets, maggots, flies) and water *ad libitum.* These were acclimatized for one week prior to experimentation. The guidelines of the Committee for the Purpose of Control and Supervision of Experiment on Animals, Ministry of Statistics & Programme Implementation, Government of India, were followed in maintenance and sacrifice of animals.

LD_{50}

According to the methods described by Miller and Tainter (1944) and modified by Randhawa (2009) the LD_{50} value for (IP) dose of bendiocarb was determined to be 16

mg/kg body weight. Wettable powder of bendiocarb purchased from SIGMA-ALDRICH, India was used for this study.

Experimental Design

A total of 32 lizards were taken for experimentation and the animals were divided into two equal groups. The first group was kept as control and received vehicle and the lizards of second group were injected daily with 8 mg/kg body weight (1/2 LD_{50}) of bendiocarb intraperitoneally. Four lizards from each group were selected to prepare metaphase plates after 7^{th}, 14^{th}, 21^{st} and 28^{th} days, post treatment.

Chromosomal Aberration (CA) Assay

Control and treated groups were colchicinised (Colchicine purchased from SRL) at the rate of 0.005 mg/g body weight after 24 hours of the last dose of bendiocarb administered. Lizards were sacrificed after 2 hours of colchicine treatment and bone-marrow from femur bone was flushed in hypotonic solution (0.56 % KCl) and then incubated at 30^{o}C for 10 min. The cell suspension was centrifuged at 1800 rpm for 30 min, fixed in acetomethanol (acetic acid: methanol, 1:3, v/v). Centrifugation and fixation was repeated three times at 10-min intervals. The material was re-suspended in a small volume of fixative, dropped onto chilled slides, flame-dried, and stained in 5% buffered Giemsa (pH 6.8). One hundred good-quality metaphases were examined under oil immersion for control and treated groups separately to observe CA (Brusick, 1980).

Statistical Analysis

Data was expressed as arithmetic Mean ± SEM. Significance of the data was analyzed using the test criterion, Student's t- test.

RESULT AND DISCUSSION

The evaluation of CA is a fully accepted method to reveal genotoxicity as it is indicative of real genetic effects (Tompa *et. al.*, 1992). The production of CA is a complex cellular process with mechanisms of chromosome breakage and re-joining that are not yet completely understood. According to the prevailing theories, structural CA results from : 1. direct DNA breakage, 2. replication on a damage DNA template and 3. inhibition of DNA synthesis (Assayed *et. al.*, 2010).

Metaphase plates prepared from the bone marrow of *Calotes* injected intraperitoneally with a daily dose of ½ LD_{50} of bendiocarb showed CA. Most of these aberrations were structural in the form of gaps, breaks, additions and deletions. Concerning genotoxic effects of bendiocarb, our results indicate that bendiocarb may have the potential to interact with the genetic system and cause alterations in the cellular DNA which may lead to structural aberration in the chromosome of our animal model *Calotes*. Our observation showed the following result when statistically analyzed by Student's t- test. No significant change was observed for frequencies of gaps, breaks, additions and deletions after 7^{th} day of treatment. Frequencies of CA in the form of gaps increased in the treated group significantly after 14^{th} day ($p \leq 0.005$) with a trend showing increased value of p (0.0005) after 21^{st} and 28^{th} days of treatment. CA in the form of break, addition and deletion increased significantly after 21^{st} day of treatment. The results illustrated in Figure are indicating a time dependent increase in the level of induction of various types of structural aberrations following chronic exposure for an extended period. Our finding on time dependent increase in cytogenetic damage induced by carbamate insecticide in *Calotes* bone marrow cells is similar to the findings of Shrivastava *et. al.*, (2014; 2015).

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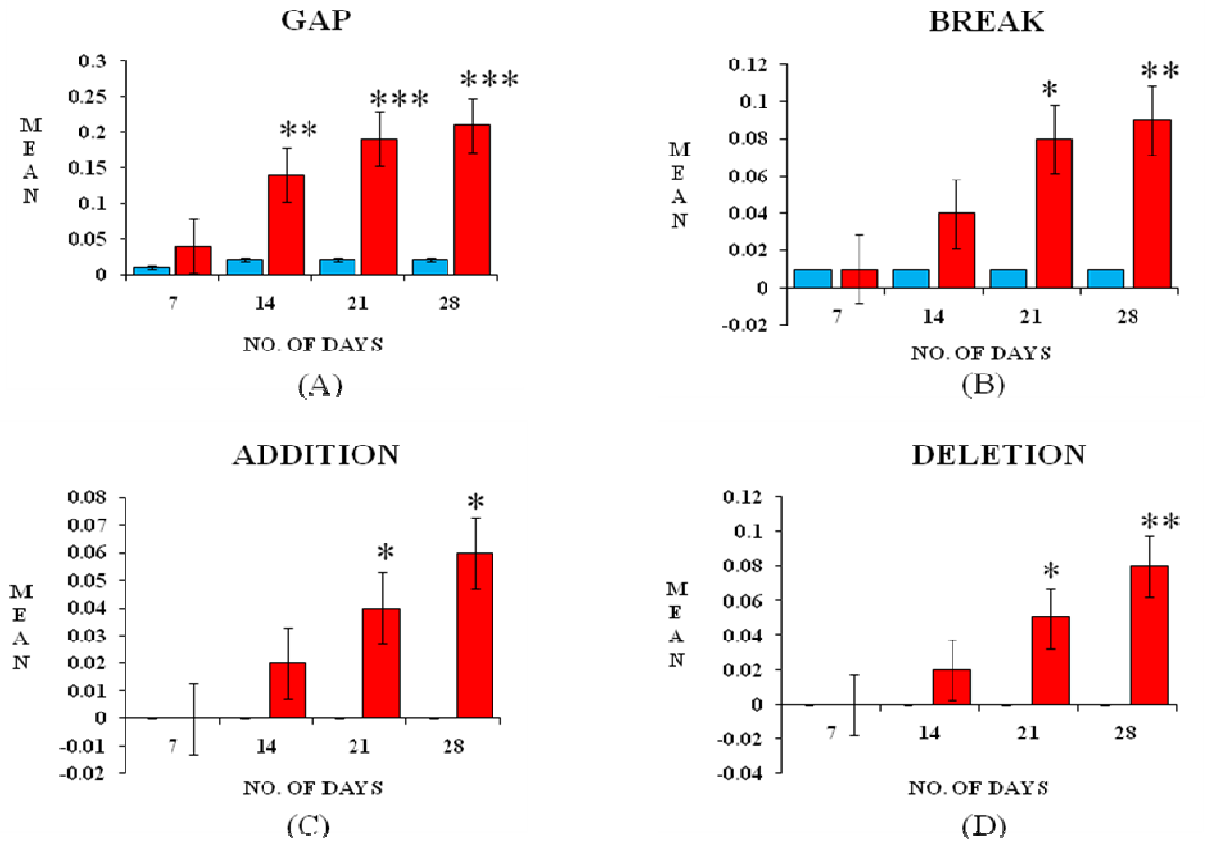
Figure: Time (in days) dependent induction in different types of chromosomal aberrations (A-Gap, B- Break, C-Addition and D- Deletion) in the bone marrow cells of *Calotes versicolor*, following intraperitoneal treatment with ½ LD_{50} of Bendiocarb.



In the present work, significant increase in CA, seen after 14th, 21st and 28th days of treatment suggests that bendiocarb has a tendency to accumulate and concentrate in the animal body or it may be degraded into harmful xenobiotic agents which may be clastogenic or mutagenic. Time dependent induction in CA also suggests that long term exposure of bendiocarb may lead to generation of active electrophiles capable of interacting with the biological macromolecules DNA, RNA and proteins in reptilian cells *in vivo*. This may lead to altered enzyme activity causing abnormal DNA repair response. On the other hand, there is increasing evidence of pesticide induced oxidative stress through the generation of reactive oxygen radicals, leading to lipid peroxidation and DNA damage (Leomanni *et. al*., 2015). Several different pathways by which oxidative DNA damage occurs have been proposed, including chemical modification of nucleotides leading to mutagenic effects. Bendiocarb may have the potential to induce oxidative stress leading to generation of free radicals and alterations in antioxidant status or the oxygen free-radical (OFR) scavenging system.

Our observations on structural aberrations in the metaphase chromosomes of bone marrow cells of *Calotes* treated intraperitonally with bendiocarb are similar to the findings on bone marrow cells of *Calotes* exposed to thiodicarb as reported by Shrivastava *et. al*., (2014). These findings are also in accordance with those of Chauhan *et. al*., (2000) and Giri *et. al*., (2002) on mouse bone marrow cells exposed to carbofuran and carbosulfan, respectively. Similar results have also been reported on cultured human lymphocytes exposed to mancozeb by Srivastava *et. al*., (2012). Present observation concurrent with earlier reports on several carbamates discussed above substantiates the genotoxic potential of carbamate pesticides. From the present work it may also be concluded that bendiocarb a synthetic carbamate pesticide leads to genotoxicity in the reptilian model *Calotes*. However, the exact mechanism of genotoxicity induced by bendiocarb in *Calotes* needs further investigation.

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