ISSN (Online): 2091-2609
DOI Prefix: 10.3126/ijasbt
International Journal of Applied Sciences and Biotechnology (IJASBT)

Research Article

Effect of Carbafuran Pesticide on Mitotic Chromosomes of *Mus musculus*

Manisha Kumari[1*], Rohit Kumar Verma[1], Prabhat Kumar Roy[2]

[1]Post Graduate Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur-812007, India
[2]University Department of Zoology, T. M. Bhagalpur University, Bhagalpur-812007, India

Abstract

The Genotoxic effect of the carbafuran pesticide was evaluated in the metaphasic chromosomes of bone marrow cells of female Swiss albino mice, *Mus musculus* . Six week old mice were put into different groups(six mice per group) and treatment were done according to experimental plan (CLD_{15} i.e. lower dose =30ppm or 0.03mg/ml and CHD_{30} i.e. higher dose =300ppm or 0.3mg/ml).The test material was administered orally for 15 days in CLD_{15} and for 30 days in CHD_{30} test groups of female mice. The result revealed that the frequency of chromosomal abnormalities i.e. structural and numerical changes were11.5% in control, 74.0%in CLD_{15} and 99.0%in CHD_{30}, respectively in bone marrow cells of female mice.

Keywords: Carbafuran; genotoxic; bone marrow cells; Swiss albino mice.

Introduction

Pesticide is a massive poisoning substance; spread a major global health problem and killing 250-350 000 people each year (Jeyarathnam 1990; Gunnell *et al*., 2007). Carbafuran is one of the most known toxic carbamate pesticides. It is marketed under the trade names Furadan by =FMC corporation and cutrated among several others. Carbafuran (2,3-dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, molecular weight of 221.25 and a melting point of 150-152°C). It is a broad spectrum systematic insecticide, acaricide and nematicide, currently registered for use on agriculture crops for example alfalfa (*Medicago sativa*), peanuts (*Arachis hypogaea*), rice (*Oryza sativa*), sugar cane (*Saccharum officinarum*), and especially corn (*Zea mays*) (Gupta 1994; Osten *et al*., 2005). The use of Pesticide is not specific otherwise, it lead to a number of number of toxicological consequences in the environment. (Milatovic *et al*., 2006).

About 5.6 billion pounds of pesticides are used world wide. The 11% of total pesticides used world wide corresponds to carbamates which shows higher amount of its use world wide in agricultural crops as (Annonymons). About 2.5 million agricultural workers worldwide experienced in initial pesticide poisoning (Jeyaratnam, 1990) Out of which about 16% have at least one pesticide poisoning encountered during their lifetime.(Alavanja *et al*., 2001). The lower toxicity and lesser environmental persistence of organocarbamates have led their application more frequent

Cite this article as:

M. Kumari et al. (2018) Int. J. Appl. Sci. Biotechnol. Vol 6(2): 169-173. DOI: 10.3126/ijasbt.v6i2.20434

***Corresponding author**
Manisha Kumari,
Post Graduate Department of Biotechnology, T.M. Bhagalpur University, Bhagalpur-812007. India
Email: kumarimanisha677@gmail.com

Peer reviewed under authority of IJASBT

than any other pesticide i.e. organophosphate (OP),organochlorines(OCI). Reversible inhibition of AChE is caused by carbamate pesticides by carbamylation of serine residue AChE. Therefore, the inhibition is short lived. Thus, its effect relatively low toxicity as compared to Organochlorine pesticide and Organophosphates (Kaur and Sandhir, 2006). The presence of carbafuran has been reported in the non target mammalian systems such as umbilical cord, maternal plasma and blood of American and African women and new born babies respectively (Whyatt *et al*., 2003). The effect of different vital organs such as brain, liver, skeletel muscles and heart (Kaur & Sandhir 2006; Gupta 1994; Rai *et al*., 2011) has shown to generally accumulated in the fat depots and to exert adverse effects and also induces the carbafuran caused the neuronal injury of mammalian system and also induces the oxidative stresses. Rai *et al*. (2011) reported that the neural injury is caused by carbafuran through dose administration of aqueous extract of *Cynodon dactylon* and vitamin C.

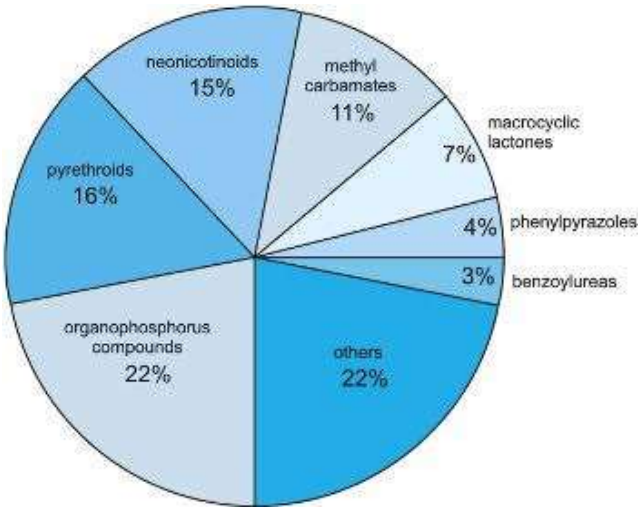


Fig. 1: Pie chart showing percentage use of different pesticides world wide.

Material and Methods

Experimental Animals

Healthy adult swiss albino female mice weighting 25 to 30g body weight were taken from University Department of Zoology, T.M.B. University, Bhagalpur. All experimental mice were maintained under hygienic condition in well ventilated room in polypropylene cages, with12 hrs light/dark cycle at 22±2˚C temperature. The animals were separated in to 3 major groups each consisting six mice i.e. control and treated ones. The treatment groups were further subdivided in to two groups according to concentration of chemicals for studying different parameters manifesting cytogenetic toxicity. All the experimental as well as control groups were fed twice with bread, dalia, green vegetables, milk, and supplemented with germinated grown seeds along with tap water *ad libitum*.

Chemicals

Commercialy available Carbafuran [2,2-Dimethyl-2,3-dihydro-1-benzofuran-7-yl Methylcarbamate] (50% E.C, trade name : 'Furadan, Curater, Furacarb'was purchased from local market.

Treatment Protocol

The animal were grouped in to three groups in which one was control and another was carbafuran treated at higher dose and lower dose. The LD_{50} for carbafuran was calculated through standard method and the LD_{50} for carbafuran was estimated as 3mg/kg b.w for swiss albino mice. Therefore, dose for carbafuran were taken as higher dose (0.03 mg/ml) and lower dose (0.3mg/ml) kg b.w .The dosing were done once a day for 30days.The dosing were done with 1000 microliter of aqueous medium of higher and lower dose in their respective test groups. The mice scarified after 15 days for lower dose test group similarly the higher dose test group mice were scarified after 30 days of dosing. To analyze the chromosomal damages, the bone marrow cell preparation was done (Adler, 1984). Colchicine was injected in the animals1.5 hr. prior to sacrifice. Bone marrow cells were aspirated with warmed (37°C) Potassium chloride (0.59%) from the femurs and treated hypotonically for 20 min. at 37°C in incubator. The sample was then centrifuged at 1000 rpm for 10 mins, and supernatant was discarded. The cell pellet was fixed in chilled methanol:acetic acid (3:1 v/v). The centrifugation and fixation steps were repeated thrice with an interval of 30 mins. The slides were prepared by dropping method, dried completely and then stained with 4% giemsa. The cytological or genotoxic assessment of carbafuran were done with the help of Olympus 41x microscopic examination of chromosomes for the studies of chromosomal abnormalities both in structure and numbers. The photographs of cytological slides were taken at 400 magnification from the Olympus 41x microscope with illumination. Structural chromosomal aberrations (SCAs) like chromatid breaks, chromosomal association (Ctb), stickness(st), pulverisation (Pv), stickness (st) translocation were scored. Two hundred (n=200) metaphase cells per animal were analyzed to determine the total chromosomal aberrations.

Results and Discussion

Comparision between Carbafuran Lower Dose (CLD_{15}) with Higher Dose (CHD_{30})

It was observed that exposer of carbafuran on mammalian model Swiss albino mice (balb/c) strain produces chromosomal anomalies. The oral doses for 15days provided for the test group CLD_{15} and 30days of test group CHD_{30} days. The cytological slides were prepared from the bone marrow cells of sacrificed mice treated with 4% giemsa stain (Prestone *et al*., 1987). The results of cytological slides revealed that different chromosomal abnormalities, such as Chromosomal association, chromatid

break, clumped, pulverised and stickness in both test groups with different degree of alteration, it was observed that in CHD_{30} the test group chromosomal stickness were more prominent than CLD_{15} test group similarly, chromosomal association were shown to be of similar occurrence in CLD_{15} test group and CHD_{30} test groups, clumping in CDH shown to be more prominent than CLD, chromatid break found in CDH_{30} separately (Fig. 1 and 2). Pulverisation is as similar as CLD_{15} and CHD_{30}. The alteration in mitotic chromosomes after the carbafuran treatment is similar with the work of (Ila *et al*., 2008; Tyrkiel *et al*., 2001).

The statistical analysis revealed that the p›0.05 indicates result come in the region of rejection. Therefore, interestingly the null hypothesis is not applicable and it gets rejected.

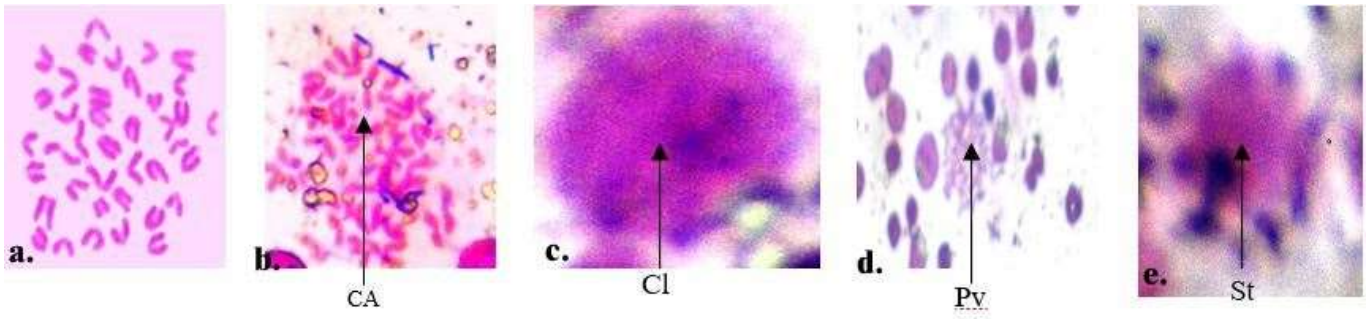


Fig. 2: Microphotographs showing structural chromosomal aberrations at CLD_{15} [a. Normal metaphase; b. Chromosomal association (CA); c. Clumped (Cl); d. Pulverised (P); e. Stickness (St)]

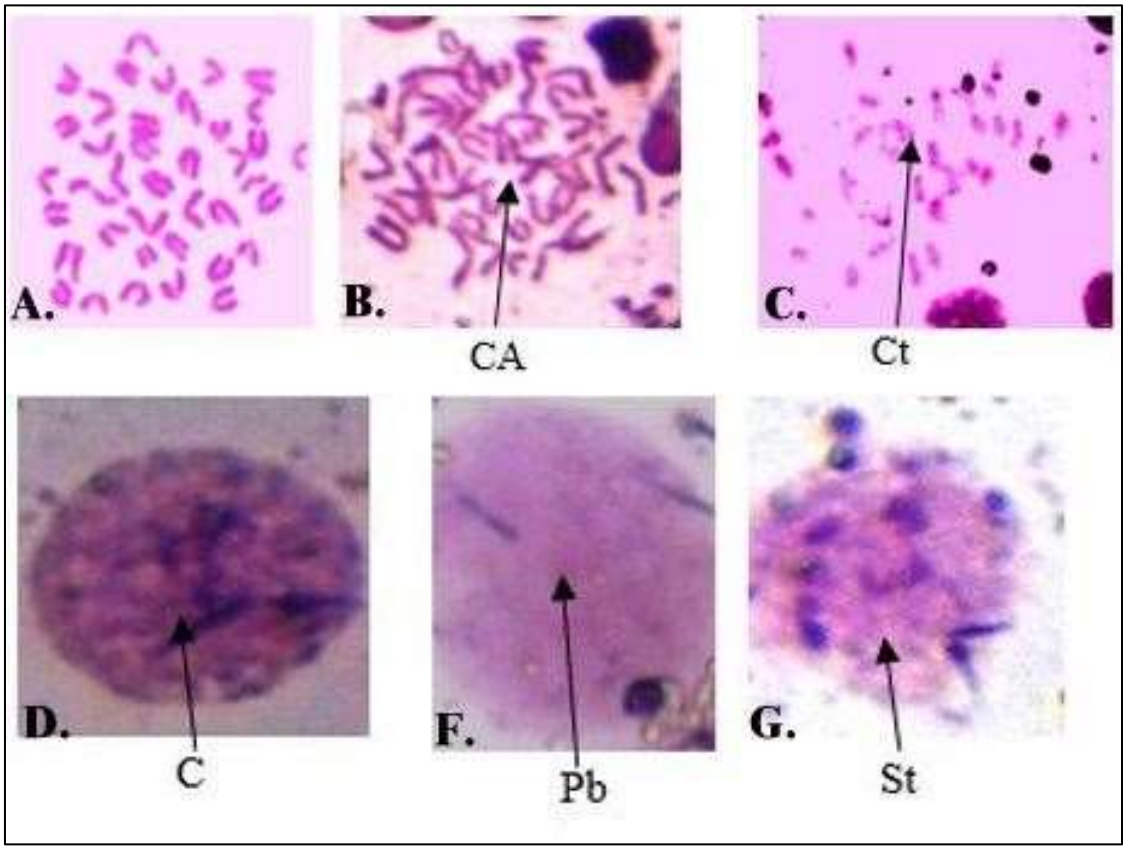
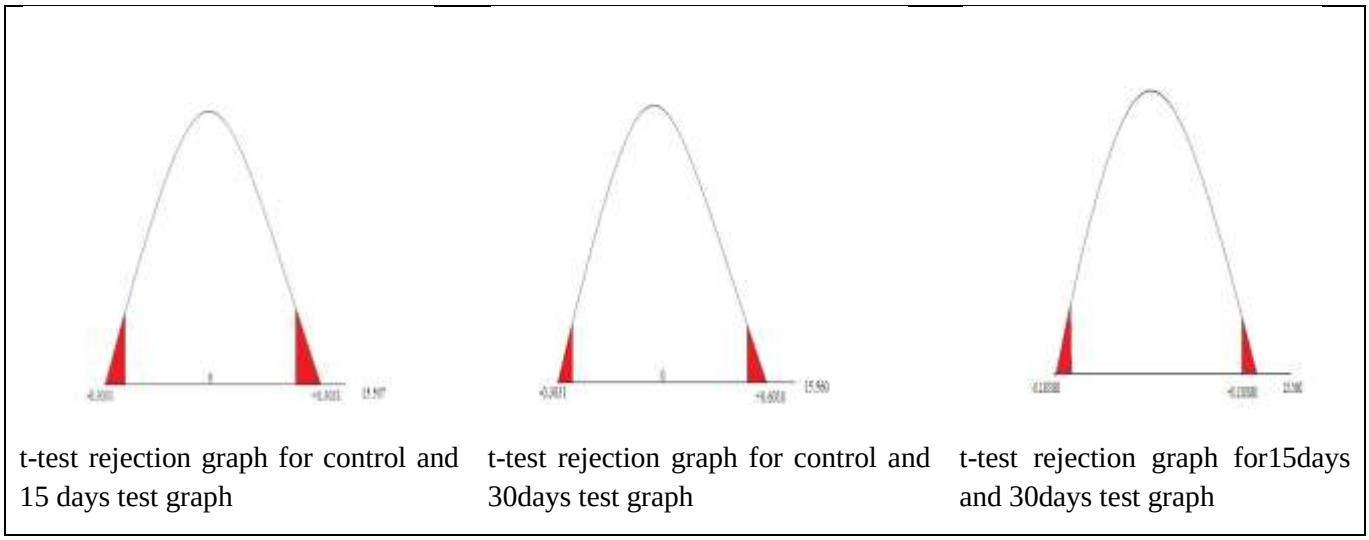


Fig. 3: Microphotographs showing structural chromosomal aberrations at $CLD_{30;}$ [A. Normal metaphase; B. Chromosomal association(CA); C. Chromatid break(Ctb); D. .Pulverisd(Pb); F. Pulverisd(Pb); G. Stickness (St)].

Table 1: Showing different chromosomal abnormalities at CLD_{15} and CLD_{30}.

Effect of carbafuran of different chromosomal changes (N=200)								
S.N.	*Groups*	*Mean ± SE*	Ctb	St	Cl	Ca	Pul	Unaltered
1.0	Control	33.33±4.73	03	02	07	09	02	177
2.0	CLD_{15}	33.33±7.09**	31	49	11	36	21	52
3.0	CHD_{30}	33.33±5.30*	61	58	08	37	34	2

df=8, 95%significance=15.507



t-test rejection graph for control and 15 days test graph

t-test rejection graph for control and 30days test graph

t-test rejection graph for15days and 30days test graph

Fig. 4: statistical analysis of data detracting the t-rejection graphs for different groups of test animals.

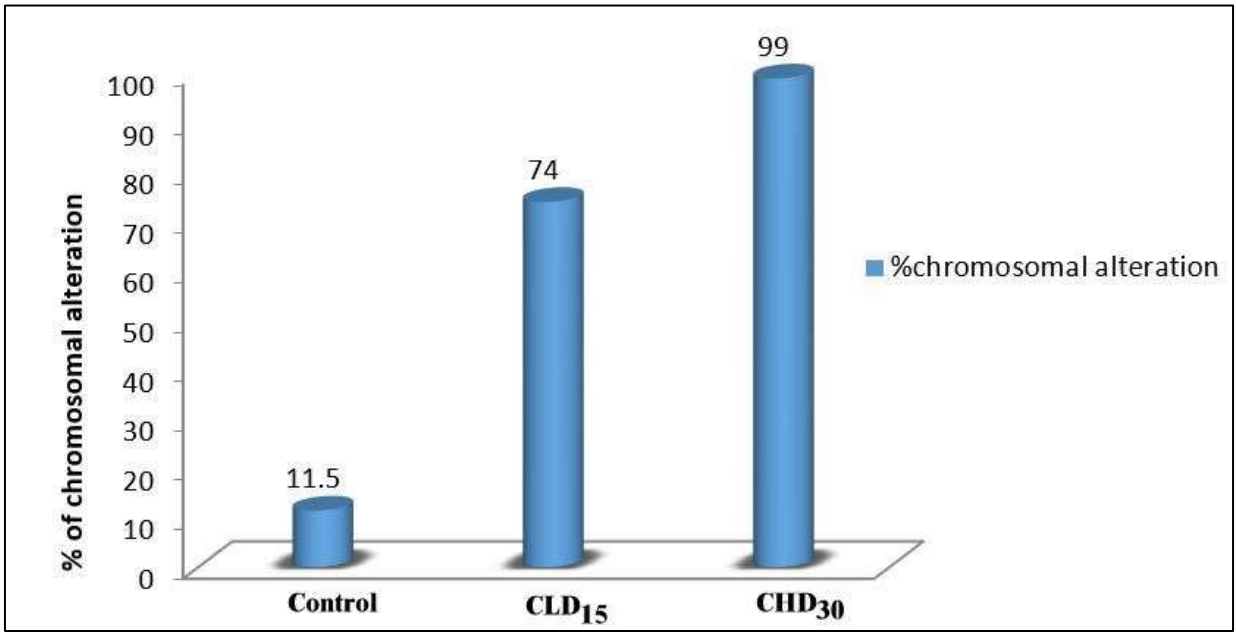


Fig 5: Histogram showing the percentage of aberrations at CLD_{15} and CHD_{30}

The tabular data 1.0indicates that for total number of 200 slides studied for each group having a mean value of 33.3 along with ± S.E as 4.73, 7.09 and 5.30 for control, CLD_{15} and CHD_{30} respectively.

The CLD_{15} group shows more variation amount different abnormality groups in comparison to CHD_{30}, it was reveal that the CLD_{15} group had lesser impact on total chromosomal aberration with respect to CHD_{30} groups.

The Table 2 indicates that the test group CLD_{15} shows about 74% of chromosomal alteration (Fig: 5).

The chromosomal alterations such as Ctb, St, Pul, Cbr produced in bone marrow cells have comparable results with the work of (Shudhanshu &Singh 2012).

The data was validated through the t-test along with standard error within each group. It was compared for p>0.05.The 95% confidence level shows that the null hypothesis comes in the region of rejection which suggests that the data of each group statically escapable.

Conclusion

It was observed that carbafuran is a potent pesticide causing structural changes in mammalian model. The higher exposure produced higher % of chromosomal alterations with some exception. Exposure for 30days has 74% more impact on chromosomal alterations.

Acknowledgement

The authors are grateful to the Course Co-ordinator of P.G. Department of Biotechnology, Tilkamanjhi University Bhagalpur for providing laboratory facilities for the present work.

References

Adler ID (1984) Cytogenetic tests in mammals. In: Venitt S and Parry J M (Eds). *Mutagenicity testing, a practical approach* (IRL Press, Oxford, England, UK. 275.

Alavanja MCR (2001) Nested case-control analysis of high pesticide exposure events from the Agricultural Health Study. *Am J Ind Med* **39**: 557–563. DOI: 10.1002/ajim.1054

Gunnell D, Eddleston M, Phillips MR, Konradsen F (2007): The global distribution of fatal pesticide self-poisoning: systematic review. *BMC Public Health* **7**: 357. DOI: 10.1186/1471-2458-7-357

Gupta RC (1994) Carbofuran toxicity. *Journal of Toxicology and Environmental Health* **43**(4): 383–418. DOI: 10.1080/15287399409531931

Ila HB, Topaktas M, Rencuzogullari E, Kayraldiz A, Donbak L, Daglioglu YK (2008) Genotoxic potential of cyfluthrin. Mutation Research/Genetic Toxicology and Environmental Mutagenesis **656**(1): 49-54. DOI: 10.1016/j.mrgentox.2008.07.005

Jeyaratnam J (1990) Acute pesticide poisoning, a major global health Problem. *World Health Stat* **43**: 139–144.

Kaur M and Sandhir R (2006) Comparative effects of acute and chronic carbofuran exposure on oxidative stress and drugmetabolizing enzymes in liver. *Drug and Chemical Toxicology* **29**(4): 415–421. DOI: 10.1080/01480540600837969

Milatovic D, Gupta RC and Aschner M (2006) Anticholinesterase toxicity and oxidative stress, *The Scientific World Journal* **6**: 295–310. DOI: 10.1100/tsw.2006.38

Osten JR, Soares AM, Guilhermino L (2005) Black-bellied whistling duck (*Dendrocygna autumnalis*) brain cholinesterase characterization and diagnosis of anticholinesterase pesticide exposure in wild populations from Mexico. *Environmental toxicology and chemistry* **24**(2): 313-317. DOI: 10.1897/03-646.1

Prestone RJ, Dean BJ, Galloway AF and Mcfree S (1987) Mammalian *in vivo* cytogenic assay-analysis of chromosomal aberration in bone marrow cells mutation. *Mutant Research* **189:** 157-165. DOI: 10.1016/0165-1218(87)90021-8

Rai DK, Rai PK, Rizvi SI, Watal G and Sharma B (2009) Carbofuran-induced toxicity in rats: protective role of vitamin C, *Experimental and Toxicologic Pathology* **61**(6): 531–535. DOI: 10.1016/j.etp.2008.11.003

Rai DK, Sharma RK, Rai PK, Watal G and Sharma B (2011) Role of aqueous extract of *Cynodon dactylon* in prevention of carbofuran-induced oxidative stress and acetylcholinesterase inhibition in rat brain" *Cellular and Molecular Biology*, **57**(1): 135–142.

Tyrkiel EB, Wiadrowska JK. and Ludwicki JK (2001) Comparative study of the effect of synthetic pyrethroids on the induction on genetic changes in mice somatic and sex cells depending on exposure route. *RoczPanstwZaklHig* **52**(2): 97-109.

Whyatt RM, Barr DB, Camann DE (2003) Contemporary use pesticide in personal air samples during pregnancy and blood samples at delivery among urban minority mothers and new borns. *Environmental Health Perspectives* **111**(5): 749–756. DOI: 10.1289/ehp.5768