Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 5, Issue, 02, pp. 129-131, February, 2013

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

A Study on Effect of Accumalate of Lufenuron in the Selected Organs (Resudval Effect of Lufenuron)

[1]*Deivanayagam, C. [1]Rajasekar, S. [2]Asokan, S. and [3]Suganthy, K.

[1]Advanced Zoology and Biotechnology, Dr. S. R. K. Government College, Yanam, Pondicherry- 533 464
[2] Department of Advanced Zoology and Biotechinology, AVC College (Autonomous), Mannampandal - 609 305, Mayiladuthurai, Tamil Nadu
[3]Vinayka missions Medical College and Hospital, Karikal 609 609

ARTICLE INFO

Article History:
Received 20th November, 2012
Received in revised form
22th December, 2012
Accepted 19th January, 2013
Published online 14th February, 2013

Key words:
Mice, Insecticide,
Protein

ABSTRACT

This work aims to disclosing the capability of the well known insecticide Match N-[2,5-dichloro-4-(1,1,2,3,3,3,-hexafluoro-propoxy)-(phenyl)amino]carbonyl]-2,6-difluorobenzamide (CA)that is widely used for controlling certain fruit and leaf caterpillars in inducing. The results also showed that Match has a capability to interfere with spindle fibers of mice spermatid cells, since a significant number of polyploid cells were obtained. Analysis of electrophoretic pattern of proteins indicated that Match was positive in causing changes in proteins specifically in high molecular protein patterns compared with the negative control group.

INTRODUCTION

The effect of environmental contaminations on human health is one of the most challenging problems that face the world today. The growing world economy and movement toward global market have driven competition in industrial and technological development at a high speed towards the betterment of mankind. However, in nearly all countries such developments have focused on increased production and economic gains before realizing their impact on the environment and human health (El-Seedy *et al*., 2006) High level demand and respiratory exposure to pesticides during on-farm and house use; and chronic exposure to low levels of pesticides residues in food and water represent a serious source for the induction of genetic lesions (Seehy, 2003 and Hafez *et al*., 2004) It is taken for granted that the degree of mutagenic potentiality of environmental pollutants evaluated in one test system may or may not be the same in another; therefore testing for the induction of DNA lesions and for mutagenicity using a variety of short-term assays, has become an essential part of the toxicological evaluation of contaminants (e.g. pesticides, cosmetics, drugs, food and feed additives etc). Evidences accumulated in the last two decades have indicated that a large number of pesticides are capable of inducing genetic damage to human as well as domestic animals and economical plant. In recent years, there has been increasing awareness of the genotoxic potential of a wide variety of chemicals to which the human population is exposed either environmentally or occupationally This awareness is paralleled by the recent development of appropriate ,sensitive and practical methods for detecting and assessing the possible genetic and biological effects of these substances.For many years, semen analysis has been routinely performed to diagnose testicular damage and infertility in human and domestic animals (Yousef *et al.,* 1996). The present investigation aims at disclosing the capability of the insecticide Match ininducing total proteins

**Corresponding author:* 15deivayagam69@gmail.com

MATERIALS AND METHODS

Chemicals Chemical

Lufenuron 5.4% (w/w) (Cigna) Chemical composition of Lufenuron 540% w/w Emulsifying agents caster of polyglcal, ether 36.40.6.00 w/w. Emulsifying agents linear alkylbenzone sulfonic acid. Calcium 4.00% w/w Solvent cycotoexanaon 20.00 solvent. (Solvent) 64.60% w/w. Manfactured by. Syngenta India limited. 14.1. Tata Road Mumbai.

Animals

Male albino mice, 7-8 weeks old, weighing 130-140g were used for the study. The animals were obtained from National Institute of Nutrition, Hyderabad and maintained in Central animal house, Rajah Muthiah Institute of Health Science, Annamalai University, Annamalainagar, India. The rats were housed in polypropylene cages at room temperatures (27 ± 2°C) with relative humidity 55 ± 5%, in an experimental room. In Annamalainagar, the LD (light: dark) cycle is almost 12:12h. The local institutional animal ethics committee (Registration Number 160/1999/CPCSEA), Annamalai University, Annamalainagar, India, approved the experimental design (Proposal No.527, dated 25.05.2007). The animals were maintained as per the principles and guidelines of the ethical committee for animal care of Annamalai University in accordance with the Indian National Law on animal care and use. The animals were provided with standard pellet diet (Amrut Laboratory Animal Feed, Mysore Feeds Limited, Bangalore, India) and water *ad libitum*.

Table 1. Composition, common name and manufacturer of the tested insecticide.

Composition	*Common*	*Manufacturers*
N-[[[2,5-dichloro-4-(1,1,2,3,3,3,-hexafluoro-propoxy)-(phenyl]amino]carbonyl]-2,6- difluoro-benzamide (CA) . Formula: $C_{17}H_8Cl_2F_8N_2O_3$ Activity	lufenuron	Syngenta India limited. 14.1. Tata Road Mumbai

Electrophoresis

Poly acrylamide gel electrophoresis

Preparation of Tissue homogenate and protein estimation

Tissue homogenates were prepared as described by Saad *et al.*((Saad *et al*., 1992). Tissues were homogenized in homogenization buffer (50 mM Tris-HCl pH 7.4, 1% Nonidet P-40, 0.25% sodium deoxycholate, 150 mM NaCl, 1mM sodium vandate, 1mM phenyl methyl sulfonyl fluoride, 1 mM aprotinin, 1mM leupeptin and 0.5 µg/ml okadaic acid) using a polytron homogenizer. The homogenates were centrifuged at 12,000g for 15 minutes at 4^0C and the protein content of the supernatant was estimated using Bradford reagent (Bradford., 1976). Cell lysate (50 µg of protein) was resolved by 12.0% sodium dodecyl sulfate – polyacrylamide gel electrophoresis (SDS-PAGE) as follows. The 30% acrylamide and bis-acrylamide mixture was prepared by dissolving 29 gms of acrylamide and 1gms of bis-acrylamide in milliQ water. The 12.0% separating gel was prepared by mixing 4.0 ml of 30% acrylamide mixture, 2.5 ml of lower Tris buffer (1.5M Tris-HCl, pH 8.8), 3.34 ml of milliQ water, 0.1 ml of 10 % SDS, 0.05 ml of 10% ammonium per sulfate (APS) and 10 µl of N,N,N',N'- tetramethyl ethylene diamine (TEMED). The 5.0% stacking gel was prepared by mixing 0.666 ml of 30% acrylamide mixture, 1.0 ml of upper Tris buffer (0.5M Tris-HCl, pH 6.8), 2.270 ml of milliQ water, 40 µl of 10% SDS, 20 µl of 10% APS and 4 µl of TEMED. This gel is mounted in the electrophoresis apparatus (Biotech, Yearcaud, MINI – vertical system) and the electrophoresis buffer (25mM Tris-HCl, pH 8.3, 250mM glycine and 0.1% SDS) was poured to the top and bottom of the reservoirs. The samples (50 µg of protein) were boiled with Laemmli (Laemmli, 1970) sample buffer (50mM Tris-HCl, pH 6.8, 0.1M β-mercaptoethanol, 2% SDS, 0.1% bromophenol blue and 10% glycerol) for 2minutes and proteins were separated at 100 voltage (approx 15V/cm of gel) until the bromophenol blue reaches the bottom of the separating gel. After the run, The gel was transferred to the coomassie brillant blue and aitated for 30 inutes.The gel was destained and photoraphed using a gel-doc system (Bio-rad, Gel Doc EQ, USA).

RESULTS AND DISCUSSION

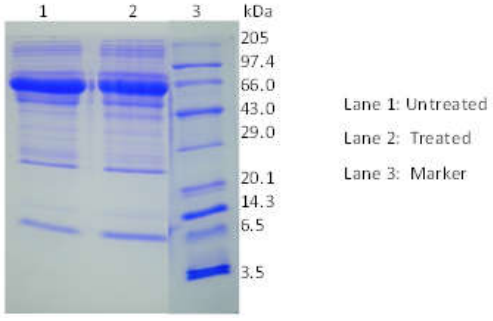

Fig. 1. Sds – Polyacrylamide gell electrophoresis – serum of albino mice

SERUM: Increases bonds level Lane 1: Untreated serum, Lane 2:Treated and Lane 3: Marker
KDa 205, 97.4, 66.0, 43.0, 29.0, 20.1, 14.3, 6.5, 3.5.,

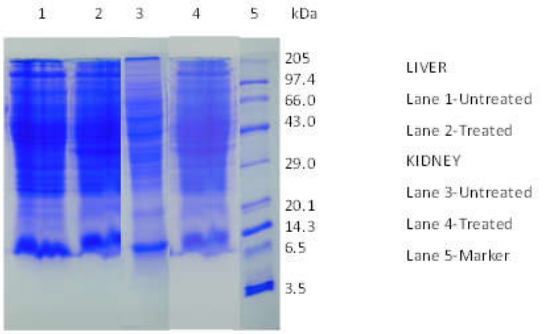

Fig. 2. Sds – Polyacrylamide gell electrophoresis –liver and kidey of albino mice

LIVER: Increases bonds Lane 1: Untreated, Lane 2: Treated.,
KIDNEY: Increases bonds Lane 3: Untreated, Lane 4: Treated, Lane 5: Marker.,
KDa 205, 97.4, 66.0, 43.0, 29.0, 20.1, 14.3, 6.5, 3.5.,

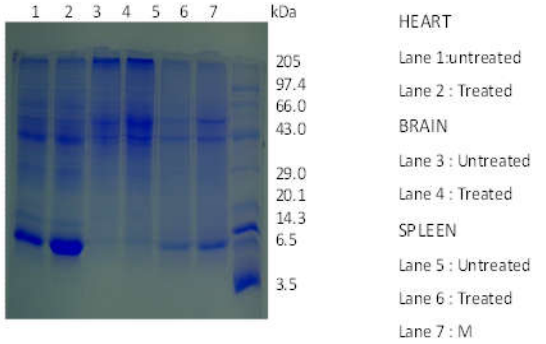

Fig. 3. Sds – Polyacrylamide gell electrophoresis – heart, brain, and spleen of albino mice

HEART: Increases bonds Lane 1: Untreated, Lane 2: Treated.,
BRAIN: Increases bonds Lane 1: Untreated, Lane 2: Treated.,
SPLEEN: Increases bonds Lane 3: Untreated, Lane 4: Treated, Lane 5: Marker.,
KDa 205, 97.4, 66.0, 43.0, 29.0, 20.1, 14.3, 6.5, 3.5.,

Blood Serum Protein

Electrophoretic patterns of proteins extracted from blood serum of mice treated with the tested insecticide Match are illustrated in Plate (4) and Table (5). The protein fractions were found to be distributed along a wide range of molecular weights. The total number of protein bands showed alteration value of βε. While males and females parents of the original population treated with Mach exhibited some changes in protein banding, average percentage of alteration were 17.71and 25.7٦ for chronic and acute treated parents, respectively. Testing variations in protein banding patterns in the F1's revealed a sizable variation, average percentages for alteration in litters produced from chronic treated parents were 63.19 versus 45.28 for litters produced from acute treated parents. As shown in Table (5), total number of scrorable bands was altered. They ranged from 15 bands in the negative control to 9 bands after the second week of chronic treatment, to 19 bands after the second week of acute treatment. Hussein and Salam (1985) reported that the protein banding pattern of an organism represents a biochemical genetic fingerprint of that organism and each band reflects a separate transcriptional event. Furthermore, electrophoretic analysis of the protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein. The changes in the banding pattern in certain progenies might be attributed to three alternatives, i.e. selection, genetic drift and mutational events. These alternatives are mutually exclusive. Elimination of certain alleles due to selection forces needs several generations. Meanwhile, genetic drift acts on several genes and causes a drastic changes in the concerned genotype considering that

the disappearance of some alleles which took place in one generation. Therefore, mutational event have to be considered as the reasonable interpretation for the observed changes. Subfractionation bands could be explained on the basis of the occurrence of gene duplication event, followed by point mutation in one or more of the duplicated genes that encode for particular band. Therefore, two bands will be formed, one of them with the original molecular weight, while the other with the changed one**,** this conclusion was in accordance with, Abedelsalam *et al.* (1997).

REFERENCES

Abdel-Galil A. M. (1996). Effects of the food antioxidant, butylated hydroxyanisole,on foresto-macch and lung tumorigenesis induced in balb/c mice with benzo (a) pyrene. J. Egypt. Ger. Soc. Zool. 21 (C): 87-97.

Abedelsalam, A. Z, Hassan, H.Z. Soliman, KH. A. and A. Bahieldin (1997).

Differential mutagenic activities of two aromatic compounds due to different side chains as revealed by cytological analysis and biochemical genetic indices Egypt J. Genet. Cytol., 26: 121-142.

Abedelsalam, A.Z, Hassan, H.Z. ELDomyati, F.M. Eweda, M.A. Bahieldin, A. and Ibrahim, S. A. (1993). Comparative mutagenic effects of some compounds using different eukaryotic systems.. Egypt . J. Genet. Cytol., 22: 129-153

Acharya, U.R., Rathore, R.M. and Mishra M. (2003). Role of vitamin C on lead acetate induced spermatogenesis in Swiss mice.Environmental Toxicology and Pharmacology,13: 9-14.

Alder, I.D. (1984). Cytogenetic Test in Mammals In: Mutagenicity Testing, a practical approach (Venitt, S.and J.Parry.Eds.), IRL press Oxford.

Anderson, D. (1984). The Dominant Lethal Test in Rodents. In :Mutagenicity Testing: a practical approach (Venitts &J. M. Parry eds). IRL Press. Oxford pp 307-335.

Brusick, D.(1986). Principls of Genetic Toxicology. Plenum Press, New York.

Chauhan, L. K., Pant, N. Gupta, S. K. andSrivastava S.P. (2000). Induction of chromo-some aberrations, micronucleus formation and sperm abnormalities in mouse following carbo-furan exposure. Mutat. Res.465(1-2): 123-9.

Chhabra, R. S., Herbert, R.A. Bucher, J. R.Travlos, G.S. Johanson J. D. and Hejmancik M.R. (2001) . Toxicology and carcinogenesis studies of p,p'- dichlorodiphenyl sulfone in rats and mice. Toxicol . Sci. 60 (1) : 28-37.

Choudhury, R.C., Mirsa, S. Jagdale, M. B.and A.K. Palo (2002). Induction and transmission of the cytogenetic toxic of 5-fluorouracil in male germline cells of Swiss mice. J. Exp. Clin. Cancer. Res. 21 (2) :277- 82

Christiansen, T., Korsgaard, B. And Jespersen ,A. (1998). Effects of nonylphenol and 17β-estradiol on vitellogenin synthesis, testicular structure and cytology in male eelpout *Zoarces viviparous*. Journal of Experimental Biology, 201: 179-192.

Cooper, G.H. (1997). The cell: a molecularapproach-ASM press. Washington, Dc.pp.248-9.

Ehling, .U. H., Cumming R. B. and Malling,H.V. (1968). Induction of dominant lethal mutations by alkylating agents in male mice .Mutat. Res., 5, 417- 428.

El –Seedy,A. S., Taha , T. A. Seehy, M. A.and Makhlof A. (2006). Ultrastructure sperm defects in male mice during carcinogenicity of urethane and indoxan. Arab j. Biotech. 9(1): 27-40

Fouad, W. (2003). Biological and biochemical seminal changes in rabbits treated with gossypol. M.Sc. Fac. Agric. Alexandria University.

Hafez, A. M., El Shehawi, A.M., Seedy, M.M. and Seehy M.A. (2004). DNA damage by Decis in human lymphocytes and experimental rodents. Proc. Int. Conf. Genet. Eng. & Appl. April, 8-11: 545-558.

Hart, J.W. and Engberg- Pederson H. (1983). Statistics of the mouse bone- marrow micronucleus test: Counting, distribution and evaluation of results. Mutat. Res. 111:195-207.

Hassan, H. Y. (1996). Evaluation of mutagenic effects of the two insecticides Basudin and Decis on *Vicia faba* plants. Egypt. J. Gent. Cytol., 25(1):27-38

Hussein, E. H. A. and El- Abdin Salam, A. Z. (1985). Evolutionary relationship among *Vicia* species as revealed by electrophoretic studies . Egypt. J. Genet. Cytol., 14:197-211.

Gollapudi, B. and Kamara O.P. (1979). Application of a simple Gimesa–staining method in the micronucleus test. Mutat. Res.64:45-6.

Miki K, W. Qu, Goulding, E.H. Willis, W.D. Bunch, D.O. Strader, L.F. *Genetic damage induced by the insecticide match in mice Arab J. Biotech. Vol. 9, No. (3) Oct. (2006): 437-452.*

Perreault, S.D. Eddy, E.M. O'Brien, D.A. (2004).Glycera-ldehyde 3-phosphate dehydrogenase-S, a sperm-specific glycollytic enzyme, is required for sperm motility and male fertility. Proc Natl Acad Sci U S A. 2004 Nov 23; 101(47):16501-6.

Murthy, R.C., Saxena, D.K. Guptaand, S.K. and Chandra S.V. (1991). Lead induced ultrastructural changes in the testis of rats. Exp. Pathol., 42(4): 95-100.

Reynolds, E.S.(1963). Staining tissue sections for electron microscopy with heavy metals. J. Cell Biol., 17: 203-212.

Odeigah, P. G. (1997). Sperm head abnormalities and dominant lethal effects of formaldehyde in albino rats. Mutat. Res. 17: 389 (2-3):141-8.

Saxena, A. K., Dhungel, S. Bhattacharya, S.Jha C. B. and Srivastava A. K. (2004).

Effect of chronic low dose of methotrexate on cellular proliferation during spermatogenesis in rats .Arch Androl .50(1):33-5.

Schmid,(1975). Seehy, M.M. A. (2003). Micro and Macro DNA lesions induced by Decis.M.Sc Thesis,Fac . Agri. Alex. Uni. Alexandria.

A.C., M. Kumar and Jha A. M. (2003). Genotoxicity of lomefloxacin an antibacterial drug in somatic and germ cells of Swiss albino mice in vivo. Mutat. Res. 535(1): 35-42.

Studier, F.W. (1973). Analysis of bacteriophage T7 early RNAs and proteins slab gels. J. Mol. Biol., 79: 237- 248.

Yousef, M.I., K.Bertheussen, M.H. Ibrahim, H.Z. Helmi, S. Seehy M.A. and Salem M.A. (1996).A sensitive sperm –mobility test for the assessment of cytotoxic effects of pesticides .J. Enviro. Science and health .3(1):99-115.

Padmalatha, R. S., Vijayalaxmi, K. And Tamoxifen K. (2001). Citrate induced sperm shape abnormalities in the *in-vivo* mouse. Mutat. Res. 492(1-2):1-6.

Parry, M.E. and Parry, J. M. (1984).The assay of genotoxicity of chemicals using the budding yeast Saccharomyces cerevisiae. In: Mutagenicity Testing; a practical approach Cs. Venitt & M. Parry.eds/ IRL Press,Oxford.

Perchec, G., Jeulin, C. Cosson, J. Andre, F.and Billard R. (1995). Relation between sperm ATP content and motility of carp spermatozoa. J. Cell Sci., 108: 747-753.

****