Asian Journal of Biological and Life Sciences | Original Research

Dose-dependent genotoxic and cytotoxic activity of auramine in mice: A preliminary study

Sumitava Roy[1], Raktim Mukherjee[1], Surjyo Jyoti Biswas[2], Susanta Roy Karmakar[1]*

1 Post Graduate Department of Zoology, Maulana Azad College, Kolkata-700 013, West Bengal, India.
2 Department of Zoology, Sidho-Kanho-Birsha University, Purulia Sainik School, Ranchi Road, Purulia-723 104, West Bengal, India.

E-mail : subidar@rediffmail.com Contact No. : +91 - 9433564104.

Submitted : 02.08.2016 Accepted : 13.09.2016 Published : 30.12.2016

Abstract

The present study was conducted to find out the deleterious effects of Auramine by studying cytogenetical parameters, namely chromosome aberration (CA), mitotic indices (MI) and sperm head anomaly (SHA) in mice. Two different doses (10% and 15% of LD_{50}) of Auramine were fed to different groups of mice to find out whether there is any appreciable difference in toxicity. Experiments were carried out at three different fixation intervals, namely, 7D, 14D and 21D, maintaining suitable controls. The investigation reveals that feeding of dye induced geno-toxicity and cyto-toxicity. These produced greater amount of CA, SHA along with a general increase in the MI in both Auramine10% and Auramine15% fed series with some degree of variations. Thus, the increased level of genotoxicity and cytotoxicity could be attributed to chronic feeding of the chemical. Although our present experiment was an initial attempt to test the toxic effects of Auramine in two different doses, hopefully it will open up new vistas to solve the critical issues yet to be discovered.

Key words : Genotoxicity, Cytotoxicity, Auramine, Mice.

INTRODUCTION

For centuries various ingredients have served useful functions in a variety of foods such as to enhance the appearance and flavour of food and prolong its shelf life. In last 100 year the food industry has advanced rapidly and almost every food stores are stacked with packed, canned and processed foods which are laden with various artificial chemicals and dyes. More than 3,000 food chemicals are purposely added to prolong the shelf life[1].

Food colours which we encounter or consume without any prior concern are often synthetic dyes or petroleum-based and at times are not even purified chemicals[2]. These chemicals have a deleterious effect on our health and some of these chemicals are also carcinogenic in nature[3].

Auramine is a diarylmethane dye and a known food colouring agent[4] which is readily soluble in water and ethanol. Its solubility in water is 10 mg/ml H_2O and chemically known as bis [4-(dimethylamino) phenyl] methaniminium chloride, and commonly used as a fluorescent stain, it is also used as a dye for colouring leather, jute, tanned cotton and in paints. Further, it is also for colouring ribbon, ink of ball point pens, oils and waxes and in carbon paper. It is also used to colour smoke in fireworks and has wide application in military[5-6]. But the main concern about this dye is its application in several foods for a yellow colour[7]. It is widely used in India to colour fresh peas[8][9] and in China it has been reported that it is used in bean products[10].

Investigators reported that auramine is known to retard growth and damage kidneys and liver and gave positive result in differential toxicity assay, both in the absence and the presence of metabolic activation[11]. It also induced aneuploidy in wheat[12]. Commercial auramine induced DNA strand breaks in primary cultures of rat hepatocytes and also induced DNA single strand breaks in the liver and urinary bladder of intact rat has also been reported by several workers[13]. Scientific investigation by other workers revealed that it inhibits sporulation of *Bacillus subtilis*[14] and is also an inducing agent for toxicity in *E coli* DNA pol-1 deficient strain[15-16]. It induces recombinogenic activity in *Saccharomyces cerevisiae* D3 strain[17-18]. High frequency of bladder tumor in workers engaged in the manufacture of auramine has also been reported by other scientific investigators[19]. There are several case reports of incidence bladder cancer among Swiss auramine-production workers [20].

Therefore, the present investigation was carried out to find out the deleterious effects of auramine taking into consideration different genotoxic and cytotoxic endpoints in mice.

MATERIALS AND METHODS

Healthy inbred strain of swiss albino mice (*Mus musculus*), reared and maintained in the animal house of the Department of Zoology (under the supervision of The Animal Welfare Committee, F.No.25/250/2012-AWD), Maulana Azad College, Kolkata served as materials for the present investigation. Mice were provided with food and water *ad libitum*. The experimental protocols were in accordance with the guidelines laid down by the Animal Welfare Committee, Maulana Azad College.

For the induction of toxicity, the feeding method was followed which were earlier reported by several workers[21-23,24-25]. For the cytogenetical (bone marrow chromosome aberration, mitotic indices, sperm with abnormal head shapes) studies, experiments were carried out at three different fixation intervals, namely, 7D, 14D and 21D, maintaining suitable controls[26-27].

Healthy mice weighing between 20 and 25 grams (about two months old) were chosen for the present investigation. Mice were fed with Auramine (10% and 15% of the LD_{50} value respectively, daily at 9:30 A.M.) (LOBA Chemie), 5 mice each were used in each series for each fixation intervals.

For cytogenetical preparations, bone marrow cells were processed for analysis of aberrations in somatic metaphase chromosomes (7D interval only) and mitotic index (7D, 14D and 21D interval). For sperm head abnormality tests, testes from the males were processed (7D, 14D and 21D interval). Total body

weight of each animal and the weight of the tissues (namely liver, spleen kidney and testis) individually were also determined after they were sacrificed.

Study of Chromosomal Aberrations

Mice were injected intra-peritoneally with 0.03% colchicines (SRL, India) @ 1 ml per 100 gram body weight, 1 hour and 15 minutes before sacrifice. Marrow of the femur was flushed in 1% sodium citrate (hypotonic) solution (SRL, India), and brought into suspension by repeated flushing in and out of a pipette with rubber tit and incubated for 7-10 minutes at 37ºC. The materials were centrifuged for 6-8 minutes at 7000g and the supernatant was discarded. The materials collected at the bottom of the centrifuge tube were fixed in freshly prepared Carnoy's fixative (1 part acetic acid (SRL, India): 3 parts methanol (SRL, India)) and resuspended by flushing. By centrifugation and decantation the fixative was changed twice at an interval of 20 minutes. Materials were dropped with the aid of a pipette from a distance of 1-1½ feet above on clean grease-free slides pre-chilled in 50% alcohol (slides kept in 50% alcohol overnight in a freezing chamber of a domestic refrigerator). The slides were flame dried by touching on to a flame and allowing the alcohol and fixative to burn out. The slides were then air dried for overnight keeping in a slanting position. Slides were kept on glass rods in a horizontal position and flooded with the diluted Giemsa (HIMEDIA). After about 45 minutes, the slides were rinsed in tap water and air dried. Then they were scanned for suitable metaphase spreads.

Study of Mitotic indices

Bone marrow cells of control and treated series were smeared uniformly on clean grease free glass slides. Semidried slides were dipped in 90% ethyl alcohol briefly and allowed to air-dry. Air-dried slides were stained for 5 minutes in May-Grunwald (SRL, India)-Giemsa stain as per the routine procedure by mixing 1 part of stock solution and 1 part of Double Distilled water. The slides were rinsed in double distilled water and finally stained in diluted Giemsa (1 part stock Giemsa and 10 part double distilled water)[28].

Study of Sperm Head Anomaly

The epididymis of each side of the male mice was dissected out and taken separately into 5 ml of 0.87% normal saline. It was made free of fats, vas deferens and other tissues. The inner content of each side of the epididymis was taken out in normal saline and the material was thoroughly shaken to suspend the sperm in saline solution. The sperm suspension was filtered through silken cloth to remove the debris and the filtrate was collected in a graduated tube, more saline was added to make the volume 10 ml. The sperm suspension thus collected, was put in the center of a clean slide over which 0.02 ml methanol was added. The material was allowed to dry. A drop of diluted Giemsa stock solution (6:1) was put on the material. The material was covered with a cover glass and sealed temporarily for observation as per the routine procedure[29].

Calculation and Statistical analysis of cytogenetical studies

The differences in the frequencies of different types of chromosomal aberrations, mitotic indices and frequencies of occurrence of sperm with abnormal head morphology between different experimental series were critically analyzed by Student's t-test[30]. The observer was initially "blinded" as to the exact series he was studying and the coded slides were later deciphered. Uniformity in scoring of data of the different series was all along maintained.

Histo-Pathological Parameter

Tissue weight / Body weight Ratio (TW/BW)

The total body weight and the sexes of the mice were recorded before they were sacrificed. The total body weight of each mouse was recorded with the help of a pan balance After the mice were sacrificed the liver, spleen, testes and kidney were dissected out immediately and cleaned properly with the help of a clean forceps and tissue paper. Then each organ was weighed with the help of a digital Petit balance (BSA224S-CW). Then tissue weight and body weight ratio was calculated by the following formula.

TW/BW = Individual Organ (Tissue) Weight / Total Body Weight.

RESULTS

Chromosomal Aberrations (CA)

The frequency of total chromosome aberrations (CA) in normal mice was 2.89%, which would be considered as the baseline of spontaneous aberrations found in normal mice. In Auramine-10% fed mice, the total chromosome aberrations were 22.33% at 7D and that of Auramine-15% fed mice were 19.66%. The summarized data of CA in mice of different groups have been presented in histogram (H.1). The differences were also analyzed for their statistical significance and the levels of significance have been denoted in H.1.

Mitotic Index (MI)

The mitotic Index in normal mice was 2.33% which was considerably increased in Auramine-10% fed series being 9.33% at 7D, 4.33% at 14 D and 4.66% at 21D. In Auramine-15% fed series, it showed similar trend being 7.66% at 7D, 6.00% at 14D and 9.33% at 21D. The summarized data of MI in mice of different treatment and control groups have been presented in histogram (H.2). The statistical significances have also been denoted in H.2.

Sperm Head Anomaly (SHA)

In normal mice 2.33% sperm showed abnormal head morphology. Therefore this could be taken as the baseline data on the incidence of abnormal sperm head as a result of background effect. In the Auramine-10% fed mice, the frequency was elevated, being 4.33% at 7D, 4.66% at 14D and 10.66% at 21D. In Auramine-15% fed mice, the frequency was also gradually being 3.66% at 7D, 6.66% at 14D and 7.33% at 21D. The summarized data of SHA in mice of different treatment and control groups have been presented in histogram (H.3.). The statistical significances wherever obtained have been denoted in H. 3.

Tissue weight /Body weight Ratio

The comparative data of tissue weight-body weight ratio as obtained from the present investigation are summarized in the table.1. The statistical significances are also denoted in the same table (Table. 1). Some significant differences in Testis Weight/ Body Weight ratio were found both for Auramine 10% and Auramine 15% at 7D and only for Auramine 10% at 14D.

DISCUSSION

The present work is an attempt to correlate between the parameters under consideration to come in a probable conclusion in such a way that modulations of one could in some way be related either to the modulation of another parameter. As for

Table 1: Tissue weight/Body weight ratio of mice of different series at different fixation intervals (7D, 14D and 21D).

Series	7D							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ±0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
Auramine 10%	0.061 ±0.002	0.005^{n}	0.008 ±0.002	0.004^{n}	0.014 ±0.001	0.000^{n}	0.006 ±0.000	0.001^{a}
Auramine 15%	0.057 ±0.002	0.001^{n}	0.006 ±0.000	0.002^{a}	0.014 ±0.001	0.0126^{n}	0.006 ±0.000	0.001^{a}
Series	**14D**							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ±0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
Auramine 10%	0.055 ±0.003	0.001^{n}	0.005 ±0.001	0.001^{n}	0.013 ±0.001	0.001^{n}	0.006 ±0.000	0.001^{a}
Auramine 15%	0.060 ±0.006	0.024^{n}	0.007 ±0.002	0.003^{n}	0.014 ±0.001	0.0126^{n}	0.008 ±0.000	0.002^{n}
Series	**21D**							
	LW/BW ±SE	**Diff. in value**	**SW/BW** ±SE	**Diff. in value**	**KW/BW** ±SE	**Diff. in value**	**TW/BW** ±SE	**Diff. in value**
Normal	0.056 ±0.017		0.004 ±0.000		0.014 ±0.006		0.007 ±0.000	
Auramine 10%	0.050 ±0.001	0.006^{n}	0.005 ±0.000	0.001^{n}	0.013 ±0.002	0.001^{n}	0.006 ±0.000	0.001^{n}
Auramine 15%	0.0058 ±0.011	0.0502^{n}	0.006 ±0.001	0.002^{n}	0.017 ±0.007	0.0156^{n}	0.006 ±0.000	0.001^{n}

$^{a}p< 0.05$, $^{b}p< 0.01$, $^{c}p< 0.001$, n = non-significant.

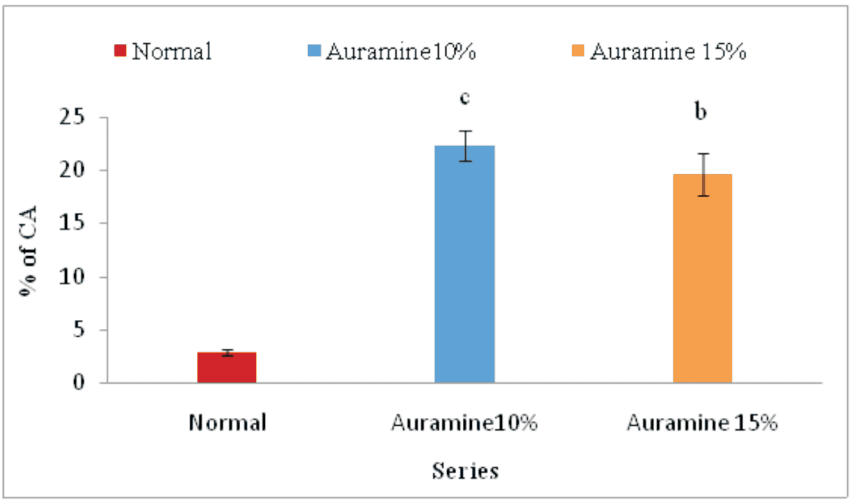

Figure 1 : Frequency distribution of chromosome aberrations (CA) in different series of mice at 7D fixation interval.

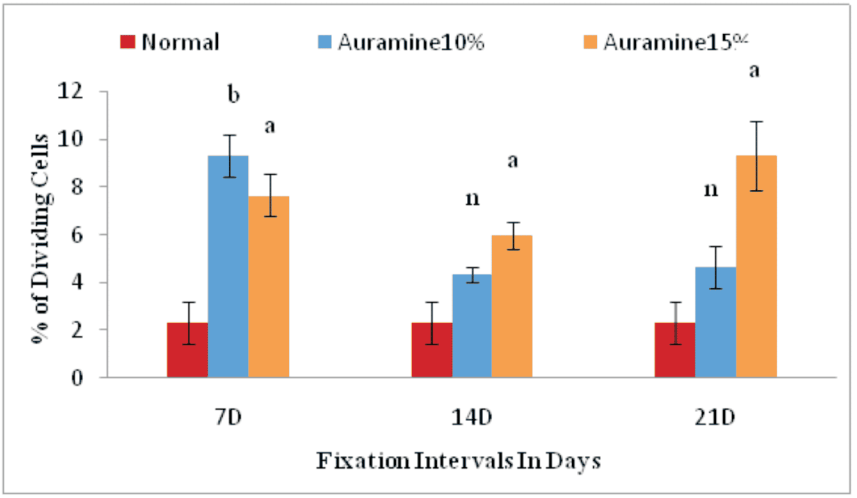

Figure 2 : Frequency distribution of mitotic index (MI) of bone marrow cells in different series of mice at different fixation intervals (7D, 14D and 21D).

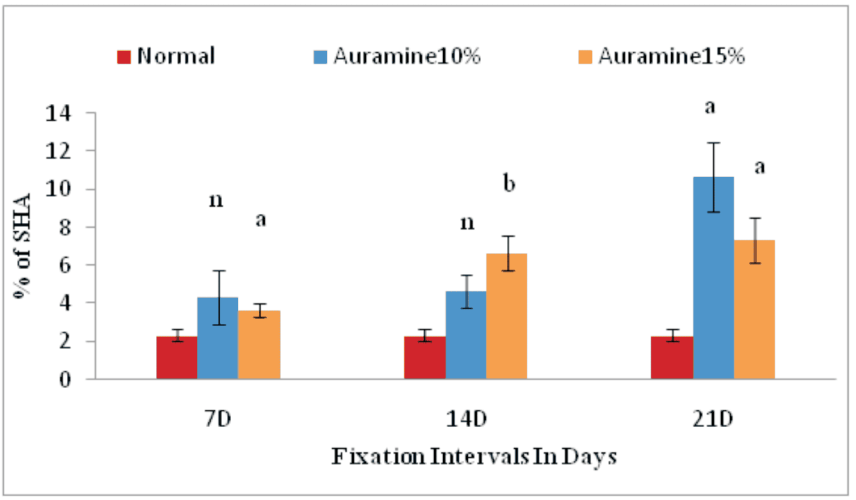

Figure 3 : Frequency distribution of abnormal sperm heads (SHA) in different series of mice at different fixation intervals (7D, 14D and 21D).

example, the mitotic indices (MI) can reflect the state of divisional activity and the study of sperm head anomaly (SHA) would throw significant light on spermatotoxic activity of the harmful colouring agent.

It was clearly revealed from the present study that the feeding of dye induced geno-toxicity and cytotoxicity. Administration of auramine produced greater amount of CA, SHA along with a general increase in the mitotic frequency of bone marrow cells when compared to normal control. Thus, the increased level of genotoxicity along with enhanced mitotic index could be correlated with the progress of toxicity induced as a result of chronic feeding of the chemical, auramine-10% and auramine-15% which corroborated well with earlier studies. Treatment with Auramine tended to elevate frequency of CA and MI of bone marrow cells[31]. Incidentally Auramine which was earlier concluded as a carcinogen of liver, bone marrow[25] supports the data of increasing CA in this study.

The more or less steady increase of MI percentage can be concluded as an increase in cellular proliferation which is widely recognized as a marker of carcinogenesis. The spermatotoxic effects of auramine in germ cells of mice were evident from the increasing frequency of sperm with abnormal head morphology. This indicates that the cytotoxic effect of auramine was also manifested in the germ cells. Further it has been suggested that many enzymatic functions are essential for the normal integrity and function of testis i.e. synthesis, development and maintenance of normal sperm. From the present investigation we can conclude that the two doses of auramine administration somehow hampered the normal enzymatic functions thereby showing an increase in abnormal sperm head shapes.

In all three intervals of this study it was obtained that there is no significant change in tissue weight/body weight in case of liver, spleen, kidney and testes and neither there is any abnormal growth in these organs during the study period.

CONCLUSION

From the preliminary study, we can conclude that auramine tend to show genotoxic and cytotoxic effect at 10% and 15% dose in mice. More in-depth and long term study are required to assess its proper effect and hopefully the present findings will open up further fields of study to solve the critical issues yet to be

discovered.

ACKNOWLEDGEMENT

Grateful acknowledgements are made to Prof. Subir Chandra Dasgupta, Head, Department of Zoology, Maulana Azad College, Kolkata, for providing infrastructural support to carry out the investigation and to Ms. Nabanita Ghosh, Assistant Professor, and Avijit Dey, Research Fellow, Department of Zoology for their help in some parts of the work.

REFERENCES

1. Food and Drug Administration. Overview of Food Ingredients, Additives & Colors, 2010.

2. Downham A, Collins P. Colouring our foods in the last and next millennium. International Journal of Food Science and Technology 2000: 35(1): 5-22.

3. Tomatis L, Agthe C, Bartsch H, Huff J, Montesano R, Saracci R, Walker E, Wilboun UD. Evaluation of the carcinogenicity of chemicals: a review of the Monograph Program of the International Agency for Research on Cancer (1971-1977). Cancer Res. 1978: 38: 877-885.

4. Chit K, Ohn HM, Aye KN, Zaw T, Hlaing TMM, Thant KS, Han M. Detection of Food Colorants in Packed Chilli Powder and Color Powder. Health Sciences Research Journal 2015:27(2).

5. Department of the Army. *Technical Manual: Military Explosives* (TM 91300214). Washington DC, 1990:1355.

6. Shimizu T. *Fireworks: the art, science, and technique:* Pyrotechnica Publications, Austin, Texas, 1981.

7. D'Aquino M, Santini P. Food additives and their possible genetic toxicity; microbiological determination. *Arch. Latinoam. Nutr.* 1977:27: 411424.

8. Tripathi M, Khanna SK, Das M. Surveillance on use of synthetic colours in eatables *vis as vis* Prevention of Food Adulteration Act of India. *Food Control* 2007:18:211219.

9. Rao P, Bhat RV. A comparative study on the synthetic food colours usage in foods procuredfrom urban and rural areas of Hyderabad. *Nutr. Food. Sci.* 2003:33:230234.

10. Lin Q. Simultaneous determination of chrysoidine and auramine O in bean products by HPLC. *Chinese Journal of Chromatography* 2007:5:776777.

11. Sharp DC, Parry JM. Induction of mitotic gene conversion by 41 coded compounds using the yeast culture JD1. Progmutat Res. 1981b:1:491-501.

12. Rédei GP, Sandhu SS. Aneuploidy detection with a short-term hexaploid wheat assay. Mutat. Res. 1988:201(2):337348.

13. Martelli A, Campart GB, Canonero R, Carrozzino R, Mattioli F, Robbiano L, Cavanna M. Evaluation of auraminegenotoxicity in primary rat and human hepatocytes and in the intact rat. Mutation Research 1998:414(1-3):37-47.

14. Macgregor JT, Sacks LE. The sporulation system of Bacillus subtilis as the basis of a multigenemutagen screening test. *Mutat. Res*. 1976:38:271286.

15. Rosenkranz HS, Gutter B, Speck WT. Mutagenicity and DNA-modifying activity: a comparison of two microbial assays. *Mutat. Res*. 1976:41 1 spel.no;6170.

16. Rosenkranz HS, Poirier LA. Evaluation of the mutagenicity and DNA-modifying activity of carcinogens and noncarcinogens in microbial systems. *J. Natl. Cancer Inst.* 1979:62:873892.

17. Simmon VF, Rosenkranz HS, Zeiger E, Poirier LA. Mutagenic activity of chemicalcarcinogens and related compounds in the intraperitoneal host-mediated assay. *J. Natl. Cancer Inst*. 1979:62:911918.

18. Simmon VF. In vitro assays for recombinogenic activity of chemical carcinogens and related compounds with Saccharomyces cerevisiae D3. *J. Natl. Cancer Inst*. 1979b:62:901909.

19. Case RA, Pearson JT. Tumors of the urinary bladder in workmen engaged in the manufacture and use of certain dyestuff intermediates in the British chemical industry. II. Further consideration of the role of aniline and the manufacture of auramine and magenta (fuchsin) as possible causative agents. Br. J. Ind. Med. 1954:11: 213216.

20. Müller V. Bladder alterations due to amines. Experiences from the industrial district of Basel. *Z. Urol. Chir. (Gynaekol)* 1933:36:202219.

21. IARC. Some inorganic substances, chlorinated hydrocarbons, aromatic amines, N-nitroso compounds and natural products. *IARC* Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Man 1972:1:1184. Lyon, France.

22. IARC. Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC* Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans 1987:7:1440. Lyon, France.

23. IARC. Some aromatic amines, organic dyes, and related exposures. *IARC* Monographs on the Evaluation of Carcinogenic Risk of Chemicals to Humans 2010:99:1678.

24. Bonser GM, Clayson DB, Jull JW. The induction of tumours of the subcutaneous tissues, liver and intestine in the mouse by certain dye-stuffs and their intermediates. *Br. J. Cancer* 1956:10:653667.

25. Williams MH, Bonser GM. Induction of hepatomas in rats and mice following the administration of auramine. *Br. J. Cancer* 1962:16:8791.

26. Biswas SJ, Pathak S and Khuda-Bukhsh AR. Assessment of the genotoxic and cytotoxic potential of an anti-epileptic drug, phenobarbital, in mice: a time course study. Mutat. Res. 2004:563:1-11.

27. Biswas SJ and Khuda-Bukhsh AR. Cytotoxic and genotoxic effects of the azo-dye p-dimethylaminoazobenzene in mice: A time-course study. Mutat. Res. 2005:587:1-8.

28. Schmid W. In Chemical Mutagens: Principles and Methods of Detection. (Eds: Hollaender AE): Plenum Press, New York, 1976.

29. Wyrobek AJ, Watchmaker G, Gordon L. In: Handbook of mutagenecity testing protocols. Eds: Kilbey BJ, Legator M, Nichols W and Ramel C, Elsevier Science, Netherland, 1984.p. 733-750.

30. Fisher RA and Yates F. Statistical tables for Biological, Agricultural and Medical Research. 4th Edition, Oliver and Boyd, Edinburgh, 1953.

31. Parodi S, Santi L , Russo P, Albini A, Vecchio D, Pala M,

Ottaggio L, Carbone A. DNA damage induced by auramine O in liver, kidney, and bone marrow of rats and mice, and in a human cell line (alkaline elution assay and SCE induction). Journal of Toxicology and Environmental Health 1982:9:941-952.