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Assessment of toxicity of formalin use as food preservative evidenced by cytotoxic and genotoxic effects in mice: An in vivo study

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Abstract

The present study was conducted to find out the deleterious effects of formalin by studying cytogenetical parameters, namely chromosome aberration (CA), mitotic indices (MI) and sperm head anomaly (SHA) in mice. Healthy mice weighing between 20-25 gms were fed with formalin at a dose 0.042mg/kg body weight to find out whether there is any toxicity induced by this chemical. Experiments were carried out at three different fixation intervals, namely 7 Days (7D), 14 Days (14D) and 21 Days (21D), maintaining suitable controls. The investigation reveals that feeding of formalin induced geno-toxicity and cyto-toxicity. This produced greater amount of CA and MI along with general increase in the SHA. Thus the increased level of geno-toxicity and cyto-toxicity could be attributed to chronic feeding of the chemical. Although our present investigation is an initial attempt to test the toxic effects of formalin, hopefully it will open up new avenues to solve critical issues yet to be discovered.

Keywords: Cytotoxicity, formalin, genotoxicity, mice.

Introduction

Formalin is cadaverous chemical (Kawamata and Kodera, 2004) of 37 to 50 percentage aqueous solution of dissolved formaldehyde (CH_2O) in water. It is flammable, highly reactive with many substances and readily polymerizing colourless gas at normal temperature and pressure (World Health Organization (WHO) IARC Monographs on the evaluation of carcinogenic risk to human, 2006; Agency for Toxic Substance and Disease Registry (ATSDR), 1999). In air, it is readily broken down by sunlight, with a half life of approximately 30 to 50 minutes (WHO, 1999). But in liquid form, it is stable over time (World Health Organization, 1999).

This formalin is very harmful to human health. Continuous addition of formaldehyde through fish and other food materials preserved in it, in human body can cause uncontrolled cell growth or cancer in any part

of body like stomach, lung, and respiratory system (Ross et al., 2002). Also, inhalation of formaldehyde cause respiratory system cancer such as sulphuric acid mists, mineral acid, metal dust and heat (Marsh et al., 2007). In our practical life, it is illegally used to preserve various kinds of food stuffs. It can prolong the shelf life of food by protecting against deterioration caused by microorganisms (Kusumawat and Trisharyanti, 2004; Kollu et al., 2009). Some dishonest traders use formalin in perishable foods to prevent from its decay. These are reported that currently formalin is being used widely and illegally in fishes to keep it fresh, vegetables (tomato and cucumber), fruits (apple and grapes), milk, drinks, sweet meat, ice cream and spices (The Daily Star, 2014).

Above fact is inspiring the fishermen for preferring formalin rather than ice block for preservation of fish. Fruits, vegetables and fishes are the most common food item that are the major target of the dishonest food vendors and merchants (Tang et al., 2009). Therefore our present work was undertaken to determine the degree of cytotoxic and genotoxic changes caused by consumption of formalin.

Materials and Methods

In the present study 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, served as materials. Mice were provided with food and water *ad libitum*. The food was generally made up of wheat, gram and powdered milk without any animal protein supplementation, unless mentioned otherwise. The experimental protocols were in accordance with the guidelines laid down by the Animal Welfare Committee, Maulana Azad College.

In the present study for the induction of toxicity, mice were fed with 0.042mg/kg body wt. formalin [(M) CG5F650563] following the method of several workers (Mamun et al., 2014). For the cytogenetical (bone marrow chromosome aberration, mitotic indices, sperm with abnormal head shapes) studies, which form the major part of the present investigation, experiments were carried out at three different fixation intervals, namely, 7D, 14D and 21D, maintaining suitable controls. Healthy mice weighing between 20 and 25 grams (about two months old) were chosen for the present investigation. Mice were fed with formalin (0.042mg/kg body wt. daily at 9.30 A.M. 5 mice each were used in each series for each fixation intervals. For cytogenetical preparations, bone marrow cells were processed for observation of aberrations in somatic metaphase chromosomes (7 Days interval only) and mitotic index [7 Days (7D), 14 Days (14D) and 21 Days (21D)] interval]. For sperm head anomaly studies, 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 intraperitoneally with 0.03% colchicine @ 1 ml per 100 gram body weight, at all fixation intervals 1 hour and 15 minutes before sacrifice. Marrow of the femur was flushed in 1% sodium citrate (Merck, India, MJ6M562587) (hypotonic) solution, and brought into suspension by repeated flushing in and out of a pipette with rubber tit and incubated for 7-10 minutes at 37°C. 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 : 3 parts methanol) 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 air-dried by keeping in a slanting position and 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. Slides were kept on glass rods in a horizontal position and flooded with the diluted Giemsa stain (Procured from 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-Giemsa stain as per the routine procedure by mixing 1 part of stock solution and 1 part of Milli-Q 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) (Schmid, 1976).

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 (Wyrobek et al., 1984).

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 (Fisher and Yates, 1953). 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 Parameters

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 formalin fed mice the total chromosome aberrations were 10% at 7D. The summarized data of CA in mice of different groups have been presented in Fig.1. The differences were also analyzed for their statistical significance and the levels of significance have been denoted in Fig.1.

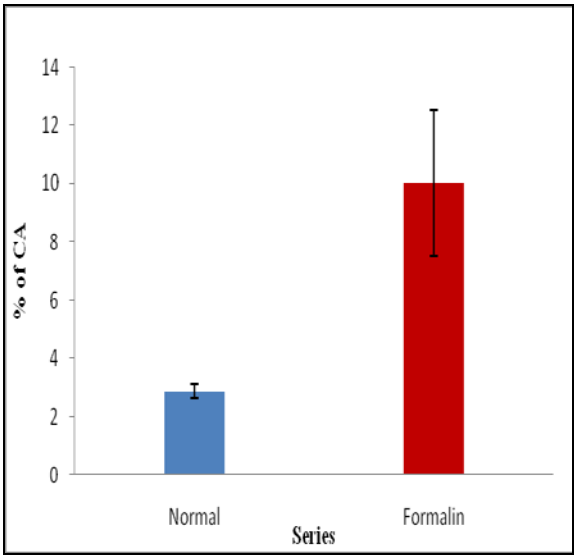

Fig.1. Frequency distribution of chromosome aberrations (CA) in different series of mice at 7D fixation interval.

Mitotic Index (MI)

The mitotic Index in normal mice was 2.33%, which was considerably increased in formalin fed series being 8.33% at 7D, 5% at 14D, and 6.66% at 21D. There was an increment in MI in the formalin fed series at all fixation intervals. The summarized data of MI in mice of different groups have been presented in Fig. 2. There was significant statistical change in 7D and 21D. The statistical significances have also been denoted in Fig. 2.

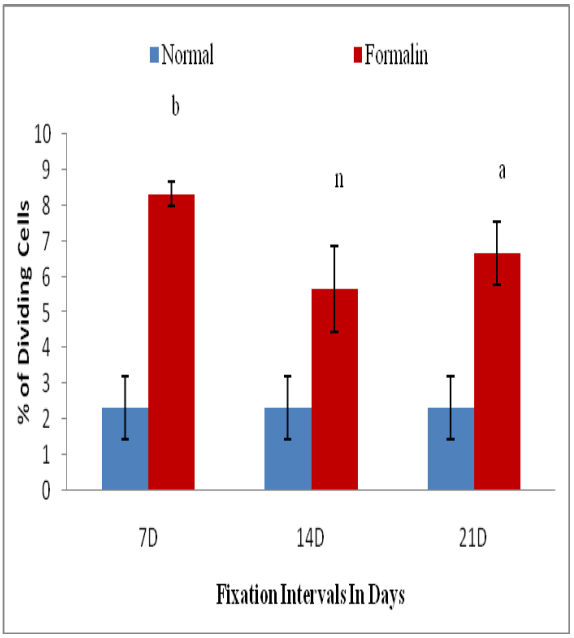

Fig. 2. Frequency distribution of mitotic index (MI) of bone marrow cells in different series of mice at different fixation intervals (7D, 14D and 21D).

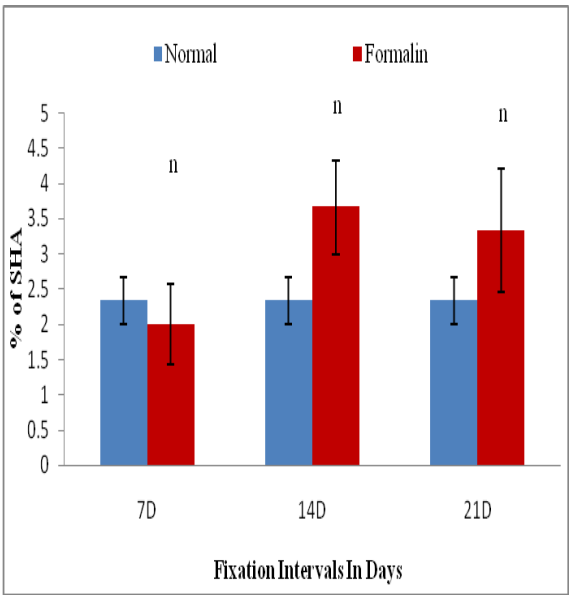

Fig. 3. Frequency distribution of abnormal sperm heads (SHA) in different series of mice at different fixation intervals (7D, 14D and 21D).

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	
Formalin	0.053 ± 0.005	0.003^{n}	0.003 ± 0.002	0.001^{n}	0.012 ± 0.002	0.002^{n}	0.007 ± 0.000	0.000^{n}
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	
Formalin	0.051 ± 0.002	0.005^{n}	0.005 ± 0.000	0.001^{n}	0.013 ± 0.003	0.001^{n}	0.006 ± 0.000	0.001^{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	
Formalin	0.055 ± 0.005	0.001^{n}	0.005 ± 0.002	0.001^{n}	0.019 ± 0.007	0.005^{n}	0.006 ± 0.000	0.001^{n}

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

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 formalin fed mice the frequency was elevated, being 2% at 7D, 3.66% at 14D, and 3.33% at 21D. In formalin fed series there was some decrease in the occurrence of abnormal sperm head at 7D, but at 14D and 21D the abnormal sperm data got increased. However, the statistical changes were denoted in Fig. 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. At normal level the ratio of LW/BW was 0.056 and after formalin treatment it became 0.053, 0.051, 0.055 at 7D, 14D and 21D respectively. At normal level SW/BW was 0.004 and after treatment it became 0.003, 0.005, 0.001 at 7D, 14D and 21D respectively. At normal level KW/BW was 0.014. After treatment this value became 0.012, 0.013, 0.014 respectively. At normal level TW/BW was 0.007 and after

treatment it became 0.007, 0.006, 0.006 at 7D, 14D and 21D respectively. Although no statistical significance was found in the body and tissue weight ratio.

Discussion

In the present study it was clearly revealed that the feeding of preservative induced cyto-toxicity and geno-toxicity. These produced greater amount of chromosomal aberration, a general increase in the mitotic index of bone marrow cells and abnormality in sperm head. Thus, enhanced mitotic index could be correlated with the abnormality of the spindle fibre formation. The frequency distribution of chromosomal aberration was increased after treatment. In this connection it has earlier been well established that in majority of toxicity induced neoplastic cells undergo chromosomal alteration, often of highly complex nature, usually exhibiting both structural and numerical aberrations (Dave et al., 1994). Further suggested that random breakage lesions on chromosomes were often linked to toxicity in the target tissue and also causes various chromosomal aberrations by their interaction with chromosomal DNA. Correspondingly the study of sperm head anomaly (SHA) would throw significant light on spermato-toxic activity of formalin as preservative.

Due to abundant use of formalin in different food items and consumption of foods contaminated with this dangerous chemical, a huge number of population particularly the kids and the elderly are exposing themselves everyday to severe health hazard in developing countries (Tang et al., 2009; Frankin et al., 2000). Thus the present study indicated a cytotoxic, genotoxic effects and change in liver and kidney function due to the effect of the chemical but further study needs to ensure more hazardous effects of the chemical. Hopefully the present findings will open up further avenues of research towards solving these critical issues.

Conflict of interest

Authors declare that there is no conflict of interest.

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