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Genotoxic effect of two commonly used food dyes metanil yellow and carmoisine using *Allium cepa* L. as indicator

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A B S T R A C T

Food dyes are important component of food in this fast life. Metanil yellow and carmoisine are two azo dyes which are being used at an alarming rate for increasing visual appearance and consumer validity of food. There is a lot of controversy regarding the genotoxicity of these two dyes. In the present study genotoxicity of two food dyes metanil yellow and carmoisine was evaluated using *Allium cepa* as indicator. The effect of these two azo dyes was determined at concentration of 0.25 %, 0.50 %, 0.75 % and 1.0 % for 24 h and 48 h of exposure period using root meristematic cells of *Allium cepa*. Some genotoxicity parameters like mitotic indices and chromosomal aberrations were studied. It was found that both metanil yellow and carmoisine caused a significant reduction in mitotic index and also produce different kinds of chromosomal aberrations mostly at higher concentration and longer exposure period. The different kinds of aberrations that were observed in meristematic cells after treatment with both metanil yellow and carmoisine are disorientation at metaphase, metaphase stickiness, anaphase stickiness, anaphase bridge, c-mitosis and chromosome breaks. The genotoxicity of carmoisine was found very high as compared to metanil yellow at all concentrations and exposure periods. Thus it was concluded from the present study that carmoisine and metanil yellow have genotoxic activities and should be taken in very control and limited doses.

1. Introduction

The use of food additives have increased from last few decades due to high population growth, industrialization and new trends in food technology. Food additives may be coloring agents, preservatives, emulsifiers and taste modulators. Coloring dyes are widely used among all food additives. Despite the beneficial use of food additives, many food chemicals have been found to possesses genotoxic nature in many test systems [1]. The overall worldwide turnover of food coloring agents is nearly 8000 tons per year and India accounts for only 2% of this output [2]. Food dyes are mostly hydrophilic in nature and are widely used in different kinds of foodstuffs like carbonated drinks, salads, juices, ice creams and sweets [3]. Among food additives maximum range are used as preservatives and coloring agents. Coloring dyes are mostly azo compounds which are added to different items to impart new color and making them more attractive and palatable [4]. Nearly a total of 3000 azo dyes are found which are used in every sector like textile firms, paper production industries, food industries, cosmetics and pharmaceutical companies [5]. Azo dyes are synthetic aromatic compounds with functional azo group (-N = N-) and coloring property of these dyes is mainly due to azo group [6]. Azo dyes constitute about more than 70 % of all dyes used in food and textile coloring.

Food additives are known to induce chromosomal abnormalities with increase in dosage concentration and time duration of treatment [7]. It has been found that many kinds of azo dyes produce carcinogenicity and induce genetic disorders in human beings [8]. It was experimentally found that many kinds of metabolic disorders were induced in rats after administration of some coloring dyes [9,10]. In some short-term genotoxicity tests many azo chemicals were found carcinogenic in animals [11]. Some of the coloring dyes are also capable of causing different types of chromosomal aberrations [12]. In this research work we select two food dyes metanil yellow and carmoisine which are mostly used in India and made efforts to evaluate their genotoxicity using *Allium cepa* plant. Genotoxicity estimation by using *A. cepa* has some advantages over mammalian system. It usually has large and low chromosome number, high proliferative rate of meristematic cells, inexpensive assay and do not require elaborate laboratory

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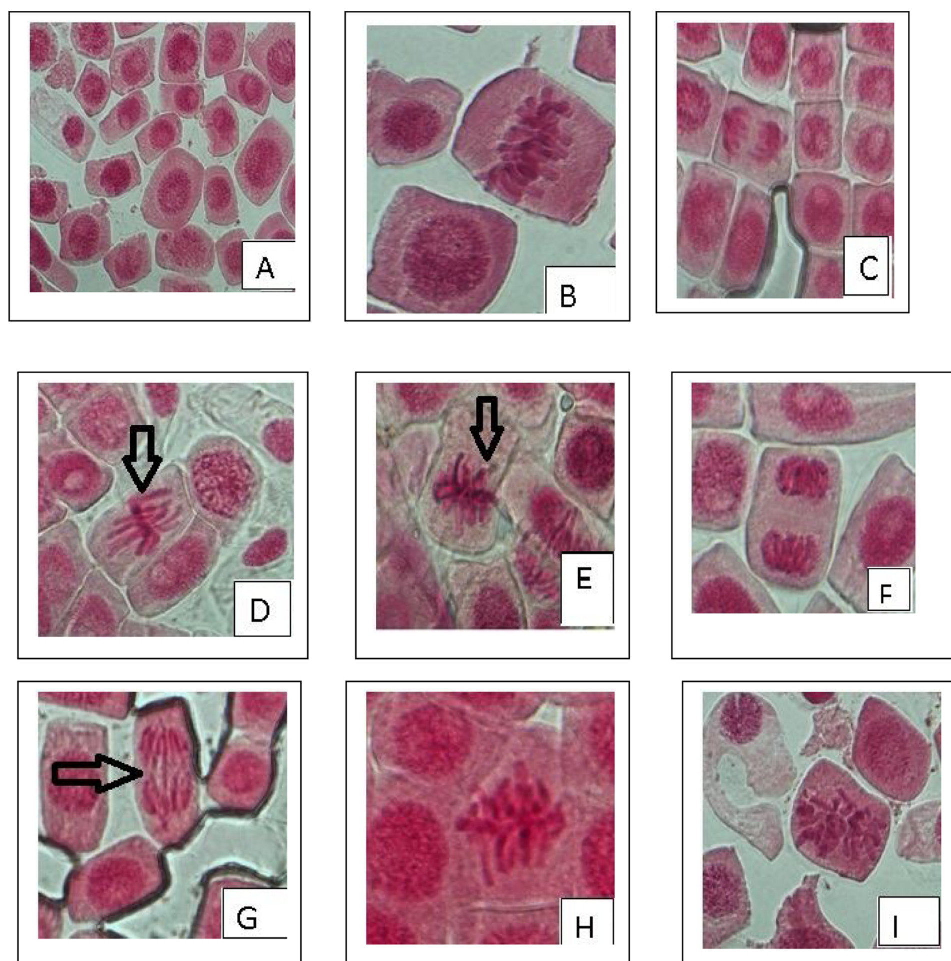

Fig. 1. Some pictures of chromosomal aberrations observed in root tips cells of *Allium Cepa* during exposure with metanil yellow and carmoisine at x1000.
A- Normal prophase; B- Normal metaphase; C- Normal anaphase: D- Disorientation at metaphase ; E- Stickiness at metaphase; F- Anaphase stickiness; G- Anaphase bridges; H- C-mitosis; I- Chromosomal break.

facilities. Plants constitute an important material for testing genetic alterations brought about by environmental chemicals [13]. The Allium cepa has been used as an efficient model organism in genetic tests for chromosome aberration assays. Genotoxicity of many food dyes have been evaluated using *A. cepa* as indicator [14]. Plant bioassays are quite sensitive and simple in comparison to animal bioassays to assess the genotoxicity and cytotoxicity of a chemical compound [15].

Metanil yellow commonly known as acid yellow 36, is a water-soluble yellow azo dye. It is basically 3-[[4-(Phenylamino) phenyl] azo] benzene sulfonic acid, with $C_{18}H_{14}N_3NaO_3S$ as chemical formula and 375.38 g/mol as molecular weight. It is commonly used as a yellow coloring factor in food stuffs, textiles, cosmetics and turmeric due to its cheap price and availability. Excessive presence of metanil yellow dye in food products and tissue samples was determined by simple spectrophotometry [16]. Metanil Yellow produce activation of many detoxification enzymes and cytochrome P-450 when orally administered in rats at dose concentration of 430 mg/kg body weight [17]. The acceptable daily intake (ADI) of metanil yellow was maintained by the Food and Agriculture Organization (FAO) at 0-0.3 mg/ 1 kg body weight. Metanil Yellow is known to be an illegal food dye worldwide. In India, it was deemed by the Government of India as illegal coloring according to the Food Adulteration Act 1954. Metanil yellow being an unauthorized food colorant is still used in many kinds of food items and has been found to cause damage in different key internal tissues in albino wistar rats after administration of metanil yellow [18].

Carmoisine or azorubine (E122) is an azo dye of red colour. Chemically carmoisine is Di-sodium salt of 2-(4 sulpho -1-naphthylazo)-1-naphthol-4-sulphonic acid, with chemical formula $C_{20}H_{12}N_2Na_2O_7S_2$ and molecular weight 458.459 g/mol. Although it is not listed as permitted food color in United States, Japan and Norway but is permitted in India and United Kingdom (UK). Eight food dyes are permitted by Food Safety and Standards Authority of India (FSSAI) and carmoisine dye is one of them. An acceptable daily intake (ADI) of 4 mg/kg bw/day was maintained by Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1983. It was reported that excess dose of carmoisine damages liver and also decreases the function of some main metabolic enzymes [19]. Carmoisine was reported to produce carcinogenicity and biochemical toxicity in mice by increasing the concentration of some serum marker enzymes and downregulate the expression of some important defensive genes [20].

2. Materials and methods

The *Allium cepa* (2n = 16) was used as the test indicator and the food dyes that were used in this experiment metanil yellow and carmoisine were procured from Sigma Aldrich company. Metanil yellow with chemical formula $C_{18}H_{14}N_3NaO_3S$, molecular weight 375.38 g/mol, carmoisine with chemical formula $C_{20}H_{12}N_2Na_2O_7S_2$, molecular

weight 502.43 g/mol. The onion bulbs used in this experiment were taken from an agricultural field where use of herbicides, fungicides or chemical fertilizers was avoided and for growth of onion bulbs only manure was used. The onion bulbs were placed in sand trays for about 5 days for the emergence of roots at temperature (25 ± 3 °C). When the emerged rootlets reach 2–2.5 cm in length the bulbs were removed from trays and used for experimental treatment. We prepare two set ups, in both cases *A. cepa* roots were treated with different concentrations 0.25 %, 0.50 %, 0.75 % and 1.0 % of metanil yellow and carmoisine for 24 h and 48 h of exposure period. In both cases some onion bulbs were kept in double distilled water as control.

After treatment the apical part of roots were broken, washed and then fixed in carnoys fixative (acetic acid and ethanol in 1:3 ratio) for 24 h. Some root tips were withdrawn from fixing solution and transfered to 70 % ethanol and stored at 4 °C for further experimental work. The root tips were withdrawn from fixative, washed two times with double distilled water and then hydrolyzed with HCl (1 N) at 50 °C for 20 min. The root tips were then washed after hydrolysis to remove excess HCl and then dried. After that staining of root tips was done with acetocarmine (2%) and the darkly stained apical tips were used for prepration of slides. The slides were examined for estimation of mitotic index and chromosomal aberrations in both treated and control groups. Analysis of slides was done under 1000x oil immersion light microscope and suitable pictures were captured as shown in Fig. 1 (A–I). In case of both metanil yellow and carmoisine five slides were designed from each concentration and 450 cells were considered per slide. The active mitotic index (AMI) and total abnormality percentage (TAP) was calculated as given below.

$$\text{Active mitotic index(AMI)\%} = \frac{\text{Number of dividing cells}}{\text{Total cells scored}} \times 100$$

$$\text{Total abnormality percentage(TAP)\%} = \frac{\text{Number of abnormal cells}}{\text{Total cells observe}} \times 100$$

2.1. Statistical analysis

One way ANOVA was carried out for statistical analysis of data with the help of SPSS software programme followed by student's t-test. Difference between control and exposure treatment groups was considered statistically significant at $p < 0.05$. The data was expressed as mean value with ± standard deviation (SD) for each concentration.

3. Results

The data of mitotic index (MI) and chromosomal abnormalities shown by metanil yellow and carmoisine is listed in Table 1–4. The active MI determined by overall number of dividing cells is commonly used to assess the genotoxicity of toxic chemicals. In the present work, *A. cepa* root meristematic cells were subjected to expose with different concentrations of metanil yellow and carmoisine at exposure period of 24 h and 48 h. It was found that MI was reduced at a greater extent at 48 h of exposure period than 24 h in both metanil yellow and carmoisine treatment. Table 1 and 2 shows the MI values obtained from the root meristematic cells of *Allium cepa* treated with varying concentrations of metanil yellow and carmoisine under exposure period of 24 and 48 h. It was found that in case of both metanil yellow and carmoisine treatment, the meristematic cells produce a dose dependent decrease in MI at both 24 h and 48 h of exposure when compared with control and decline in MI with extended exposure time was highly significant. However at exposure period of 24 h, dose concentration 0.25 % and 0.50 % of metanil yellow produce only significant decrease ($P < 0.05$) in MI in comparison to control treatment. From the data it was confirmed that carmoisine was producing more mitotic inhibition in comparison to metanil yellow at both exposure periods.

Both metanil yellow and carmoisine produces a dose dependent increase in chromosomal abnormalities as shown in Table 3 and 4. The cytotoxic effect of carmoisine on meristematic cells of *A. cepa* is shown in Table 3. The total abnormality produced by carmoisine was found to be concentration and exposure period dependent. The various types of chromosomal abnormalities produced by exposure with different concentrations of carmoisine in meristematic cells of *A. cepa* are shown in Fig.1 (A–I). These aberrations are disorientation at metaphase, stickiness at metaphase, anaphase stickiness, anaphase bridges, C- mitosis and chromosomal breaks. It was found that at exposure period of both 24 h and 48 h, the increase in total abnormality was highly significant at all concentrations (0.25, 0.50, 0.75 and 1.0 %) of carmoisine when compared with control. The increase in the number of aberrations was found very high at 48 h of exposure than 24 h.

The cytotoxic effect of metanil yellow on meristematic cells of *A. cepa* is shown in Table 4. Metanil yellow treatment produces the same kinds of chromosomal aberrations as observed when *A. cepa* was treated with carmoisine. In case of metanil yellow at 24 h of exposure period the total abnormality was found insignificant at concentration of 0.25 % when compared with control. At concentration of 0.50, 0.75 and 1.0 % the increase in abnormality was found highly significant in comparison to control. Similarly at 48 h of exposure period the increase in abnormality was found highly significant at all concentrations when compared with control.

4. Discussion

The main theme of this experiment was to determine the genotoxicity of two commonly used food azo dyes namely metanil yellow and carmoisine using meristematic cells of *Allium cepa*. Genotoxicity which is a major limitation of food additives is mostly evaluated by

Table 1
Mitotic index for Metanil Yellow at exposure period of 24 h and 48 h.

Duration of treatment	Concentration	Cells counted	Number of cells in division	% mitotic index (± SD)
24hrs	Control	450	320.80	71.11 ± 1.64
	0.25 %	450	314.40	69.86 ± 4.82*
	0.50 %	450	309.80	68.84 ± 5.35*
	0.75 %	450	289.60	64.35 ± 3.20**
	1.0 %	450	280.00	62.22 ± 6.67**
48 hrs	Control	450	319.00	70.88 ± 3.16
	0.25 %	450	301.00	66.88 ± 3.16**
	0.50 %	450	285.40	63.42 ± 5.12**
	0.75 %	450	273.40	60.75 ± 3.64**
	1.0 %	450	252.60	56.13 ± 4.27**

Data was expressed as mean ± SD.
Level of significance compared with control: * significant ($p < 0.05$), ** highly significant ($p < 0.001$).

Table 2
Mitotic index for carmoisine at exposure periods of 24 and 48 h.

Duration of treatment	Concentration	Cell observed	Number of cells in division	% Mitosis index (± SD)
24 hrs	Control	450	334.20	74.26 ± 4.49
	0.25 %	450	312.40	69.42 ± 2.50**
	0.50 %	450	299.20	66.48 ± 3.83**
	0.75 %	450	280.60	62.35 ± 2.07**
	1.0 %	450	272.00	60.44 ± 2.44**
48hrs	Control	450	318.20	70.71 ± 1.74
	0.25 %	450	294.20	65.37 ± 1.53**
	0.50 %	450	274.80	61.06 ± 2.20**
	0.75 %	450	260.40	57.86 ± 2.60**
	1.0 %	450	239.00	53.11 ± 2.54**

Data was expressed as mean ± SD.
Level of significance compared with control: * significant ($p < 0.05$), ** highly significant ($p < 0.001$).

ames test, micronucleus and chromosomal aberrations assay. Genotoxicity of food additives clearTaste and magnesium stearate was evaluated by using bacterial reverse mutation assay, micronucleus and chromosomal aberrations as genotoxic parameters [21,22]. According to the literature survey this is the first attempt to evaluate genotoxicity of food dyes metanil yellow and carmoisine using *Allium cepa* as indicator. *A. cepa* is considered an ideal model for determining genotoxicity due to high sensitivity of plant cells to environmental stress [23]. The structural and numeric changes in chromosomes have become an important trend for evaluating genotoxicity of food chemicals, pollutants, heavy metals, water samples and pesticides. Generally change in the structure of chromosome is considered good marker of genomic damage. It was reported that nearly all azo chemical colors and their oxidative end products have carcinogenic or mutagenic potential and can cause modification of DNA [24]. In the present experimental work it was found that both coloring dyes carmoisine and metanil yellow reduce the mitotic index and increase chromosomal abnormalities with increase in concentration treatment and exposure time. Our results are in positive agreement with a research finding in which genotoxicity of food azo dye sunset yellow was evaluated using root meristematic cells of *Brassica campestris*. A highly significant reduction in mitotic index and increase in chromosomal aberrations was observed [25]. Similar kind of result was also observed when genotoxicity of some common food preservatives (butylated hydroxytoluene, butylated hydroxyanisole, sorbic acid, propyl gallate and sodium nitrate) was evaluated using *A. cepa* as test model [26]. The reduction in the mitotic index may be due to errors in DNA synthesis or blocking of cell at some stage of cell cycle [27].

The most frequent type of chromosomal aberrations that were observed in our experiment are anaphase stickiness, metaphase stickiness, chromosome breaks, c-mitosis, anaphase bridges and disorientation at metaphase. The stickiness of chromosomes which was seen both in metanil yellow and carmoisine treatment may be due to inhibition of some specific proteins involved in the maintenance of chromosome condensation and segregation. This chromosome stickiness may arise also by some defective metabolic pathways in nucleic acids [28]. The presence of chromosome stickiness relates highly toxic effects associated with a particular chemical [7]. The anaphase bridge which was also seen as a frequent type of aberration in our experiment may be likely due to breakage of chromosomes. Some food additives like sodium sulphite and sodium benzoate were found to produce anaphase bridges in *Vicia faba* [29]. The disorientation of chromosomes at metaphase, which was seen during the treatment of both azo dyes may be due to the effect of these chemicals on the spindle fibers of microtubules and cause the misalignment of chromosomes at equatorial plate [30]. Another abnormality C-mitosis which was seen in meristematic cells indicates the inhibition of spindle formation during metaphase. Presence of C-mitosis is an indication of microtubule poison [31]. Chromosomal breaks were also frequently observed in our experiment during exposure of *A. cepa* roots to carmoisine and metanil yellow. This may be due to clastogenic action of these chemicals on DNA [22].

Comparing the result of Table 1–4, it was analyzed that the total abnormality and inhibition of mitosis was high at higher concentration and exposure period for both metanil yellow and carmoisine. This means that high dose of these dyes are genotoxic at cellular level. It was also confirmed that carmoisine shows more genotoxicity than metanil yellow because inhibition of cell division and frequency of chromosomal abnormality was found high in case of carmoisine. However, regarding their toxicity there is a lot of controversial report associated with these two dyes. In some research findings it has been proved that both of these studied dyes have genotoxic nature. However in some other findings these two dyes were found to be neutral regarding mutagenic changes.

In case of metanil yellow dose concentration 0.25 % was found to be safe threshold level. At this concentration the change in mitotic index and chromosomal aberrations were found nearly negligible when compared with control meristematic cells. In contrary to this carmoisine was found safe at threshold level of below 0.25 %. Above that concentration the mitotic index was highly reduced and chromosomal aberrations were very high. However, these results should be taken as preliminary reports and the detailed toxicity of metanil yellow and carmoisine must be evaluated by using animal models including human beings. The governmental authorities should make public awareness about the negative effects associated with the food additives and strict instructions should be given to food industries regarding use of these dyes at limited doses.

5. Conclusion

From the present study it was proved that both carmoisine and metanil yellow which are widely used as food coloring dyes have cytotoxic and genotoxic impact on meristematic cells of *Allium cepa*. Carmoisine was found to produce more toxic effects than metanil yellow. In case of both metanil yellow and carmoisine concentration above 0.25 % was found to produce negative effects in the *A. cepa* cells. Therefore it is necessary to use these dyes in limited doses.

Authors statement

We have added one more co author (Dr Showkat Ahmad Ganie) who works as co supervisor in our P.hd programme. We can not make confirmation mail from all authors regarding addition of one new author due to unavailability of internet connection in our state Kashmir India from August 4 2019.

Table 3

Chromosomal abnormalities induced in mitotic cells of *Allium cepa* by Carmoisine at different exposure periods.

Duration	Concentration	Disorientation at Metaphase (%)	Stickiness at metaphase (%)	Anaphase Stickiness (%)	Anaphase Bridge (%)	C-Mitosis (%)	Chromosomal break (%)	Total cells showing abnormality (%)	Total abnormality (%)
24 hrs	control	0.0 ± 0.00	0.2 ± 0.44	0.2 ± 0.44	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.44	0.6 ± 0.54	0.13 ± 0.54
	0.25 %	1.2 ± 0.44	1.2 ± 0.83	1.6 ± 0.54	1.4 ± 0.54	1.0 ± 0.0	1.0 ± 0.0	7.4 ± 1.14	1.64 ± 1.14**
	0.50 %	3.0 ± 0.70	3.6 ± 0.89	3.6 ± 0.89	3.6 ± 0.54	3.0 ± 1.0	3.0 ± 0.70	19.8 ± 1.64	4.40 ± 1.64**
	0.75 %	3.8 ± 1.30	3.6 ± 0.54	3.8 ± 0.83	4.0 ± 1.0	3.4 ± 0.54	2.6 ± 0.54	21.2 ± 1.78	4.71 ± 1.78**
	1.0 %	6.6 ± 0.54	5.6 ± 0.54	6.0 ± 0.70	6.0 ± 0.70	5.4 ± 0.54	5.4 ± 0.54	35.0 ± 2.00	7.77 ± 2.00**
48hrs	control	0.2 ± 0.44	0.6 ± 0.54	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.2 ± 0.44	1.0 ± 1.00	0.22 ± 1.0
	0.25 %	2.8 ± 1.30	4.0 ± 0.70	4.4 ± 0.54	4.6 ± 0.54	2.6 ± 0.54	2.4 ± 0.54	20.8 ± 1.64	4.62 ± 1.64**
	0.50 %	4.6 ± 0.54	5.6 ± 0.89	6.6 ± 0.89	6.2 ± 0.44	6.6 ± 0.54	5.4 ± 1.34	35.0 ± 1.87	7.77 ± 1.87**
	0.75 %	7.8 ± 1.09	10.2 ± 1.78	9.6 ± 1.51	9.0 ± 1.41	9.0 ± 0.0	9.0 ± 0.0	54.6 ± 2.30	12.13 ± 2.30**
	1.0 %	18.4 ± 2.30	19.4 ± 1.34	20.2 ± 0.44	18.4 ± 2.30	20.0 ± 0.0	20.0 ± 0.0	116.4 ± 1.34	25.86 ± 1.96**

Each value in the table was calculated as mean ± SD. Level of significance compared with control: * significant ($p < 0.05$), ** highly significant ($p < 0.001$).

Table 4

Chromosomal abnormalities induced in mitotic cells of *Allium cepa* by Metanil Yellow at different exposure periods.

Duration	Concentration	Disorientation at Metaphase (%)	Metaphase Stickiness (%)	Anaphase Stickiness (%)	Anaphase Bridge (%)	C-Mitosis (%)	Chromosomal break (%)	Total cells showing abnormality (%)	Total abnormality (%)
24 hrs	Control	0.0 ± 0.0	0.6 ± 0.54	1.0 ± 0.0	0.2 ± 0.44	0.0 ± 0.0	0.0 ± 0.0	1.8 ± 0.83	0.40 ± 0.30
	0.25 %	0.6 ± 0.54	1.6 ± 0.54	0.8 ± 0.83	0.2 ± 0.44	0.0 ± 0.0	0.0 ± 0.0	3.2 ± 1.30	0.71 ± 1.30
	0.50 %	0.8 ± 0.83	2.6 ± 0.54	2.4 ± 1.67	1.2 ± 0.44	2.0 ± 0.0	1.2 ± 0.83	9.6 ± 1.81	2.13 ± 1.81**
	0.75 %	2.6 ± 0.54	3.8 ± 0.83	4.2 ± 0.83	2.8 ± 0.83	2.6 ± 0.54	2.4 ± 0.54	19.4 ± 2.07	4.31 ± 2.07**
	1.0 %	3.4 ± 0.89	5.2 ± 0.83	5.0 ± 1.22	4.2 ± 0.44	3.0 ± 1.22	3.2 ± 0.83	24.0 ± 2.82	5.33 ± 2.82**
48hrs	Control	0.4 ± 0.54	0.8 ± 0.44	0.8 ± 0.44	0.6 ± 0.54	0.4 ± 0.54	0.4 ± 0.54	3.4 ± 1.14	0.75 ± 0.86
	0.25 %	1.4 ± 0.54	1.6 ± 0.54	1.4 ± 0.54	1.4 ± 0.54	1.6 ± 0.54	1.0 ± 0.70	8.4 ± 1.51	1.86 ± 1.53*
	0.50 %	4.4 ± 0.54	4.8 ± 1.92	5.2 ± 1.30	4.0 ± 0.70	3.6 ± 0.54	4.2 ± 0.83	26.2 ± 2.28	5.82 ± 2.28**
	0.75 %	4.0 ± 0.0	5.0 ± 1.00	10.4 ± 0.89	5.8 ± 1.09	5.2 ± 1.09	6.8 ± 0.44	37.2 ± 1.78	8.26 ± 1.78**
	1.0 %	6.8 ± 1.09	7.2 ± 1.09	19.8 ± 1.30	8.4 ± 1.51	7.2 ± 0.44	8.8 ± 1.64	58.2 ± 1.78	12.9 ± 1.78**

Each value in the table was calculated as mean ± SD.

Level of significance compared with control: * significant ($p < 0.05$), ** highly significant ($p < 0.001$).

Declaration of Competing Interest

None.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.02.009.

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