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RESEARCH ARTICLE

CHROMOSOMAL ABERRATIONS IN MICE AFTER TREATMENT WITH AROMATIC AMINE SULPHANILIC ACID AND ITS AEROBICALLY DEGRADED PRODUCT

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ABSTRACT

The present study was undertaken to evaluate the *in vivo* genotoxicity of sulphanilic acid and its biodegradation product using mice as a model. 0.5 mg of sulphanilic acid at 24 hrs, 48 hrs and 72 hrs time interval and its aerobic treated product at 48 hrs time interval were injected to different mice. The experiments were carried out in control group after treatment with DMSO. The air drying preparations were made by dissecting out the limbs of adult mice and processing it. A highly significant increase in mean aberration values were found among all treated groups when compared with the control. Chromosomal aberrations observed were stickiness, clumping, breaks, dicentric, rings and gaps. Chromosomal aberrations were found both in control as well as treated groups of mice but the number of aberrations was significantly high in all treated groups as compared to control mice. Among treated groups, the number of chromosomal aberrations was high in mice treated with 0.5 mg of sulphanilic acid at 72 hrs. It has been proposed that toxins released by aromatic amines might have caused chromosomal anomalies by coming in direct/indirect contact with the chromosomes after the disintegration of nuclear membrane during cell division. Such chromosomal anomalies may lead to the genetic instability of mice population. From these findings we conclude that sulphanilic acid is more genotoxic in nature as compared to its aerobic degradation product. These degraded samples also induced significantly higher genotoxicity as compared to control but found to be less genotoxic as compared to parent aromatic amine.

INTRODUCTION

Over the past century, humans have introduced a large number of chemical substances into the environment from various sources such as industrial wastes, agricultural run off and chemical spills. Water sulphonated dyes such as azo dyes generally enter into the environment through wastewater discharges. These dyes and their biodegraded products induce toxicity, mutagenecity and carcinogenicity. The parent azo dyes undergo reduction to liberate various aromatic amines. Sulphanilic acid is the most important aromatic amine that is found to be genotoxic and can induce chromosomal aberrations viz gaps, rings and dicentric. Sulphanilic acid (4- aminobenzene sulfonic acid) is a colourless crystalline solid produced from sulphonation of aniline. Biodegradation of aromatic amines can occur in both aerobic and anaerobic environment. The most commonly used aerobic bacterium for the biodegradation of sulphanilic acid is *Pseudomonas paucimobilis*. The present study was undertaken to evaluate the in vivo genotoxicity of sulphanilic acid and its biodegradation product using mice as model. The extent of chromosomal aberrations at different time intervals (24 hrs, 48 hrs and 72 hrs) was taken as an indicator of genotoxicity. *Chhabra et al.* (1991) observed that sarcomas of the spleen occurred in male rats where as fibrosis of the spleen was significantly increased in all p- chloro-aniline (PCA) treated groups of male and female rats. *Rajaguru et al.* (1999) found that increasing concentration of the azo dye Red 2 induced significant toxicity to erythroid compartment of mice bone marrow. *Andreasi et al.* (2001) reported that chronic long term nitrate therapy increased genotoxicity in mice. *Meng and Zeng* (2002) concluded that sulphur dioxide in low concentration caused both chromatid type and chromosome type chromosomal aberration in mice bone marrow cells. *Das and Mukherjee* (2004) observed that chromatid breaks were the main aberrations in case of dyes tartrazine and amaranth. *Krishnatrey and Mathur* (2005) found that the rats exposed to concentrated textile waste water were affected by haemolytic anaemia, thus the number of RBC's begin to decline. *Sharma et al.* (2008) observed the increase in body weight and liver weight but decrease in weight of kidney and testes of male albino mice when administered with Tomato Red dye. *Stern* (2011) reported that sulphonated azo dyes

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caused a temporary inhibition of the development of sarcoma and mammary carcinoma in mice. *Kesari, Kumar and Khan* (2012) found significant increases in the frequencies of chromosome abnormalities in the bone marrow cells of mice over the control level upon exposure to all the doses of arsenic including its reference dose. Same authors studied the genotoxic potential of arsenic at the exposure level of its human reference dose through micronucleus assay in mice in 2013 and reported significant increases in the frequency of micronucleated erythrocytes in mice. In 2014, they studied the arsenic toxicity through the analysis of induced sperm impairments in sperm head morphology and sperm count in mice and found a marked male reprotoxic effect of arsenic.

MATERIALS AND METHODS

The present study was undertaken to assess the induction of chromosomal aberrations in mice after treatment with aromatic amine- Sulphanilic acid and its treated product.0.5 mg of sulphanilic acid and its aerobically treated product at 6h interval were weighed. These were dissolved in 0.5 ml of DMSO (Dimethyl sulphoxide) separately in test tubes or cavity blocks. The solution was mixed properly. Then the solution was injected intraperitoneally into mice with the help of syringe and time was noted. A control mouse was injected with 0.5 ml of DMSO. Colchicine was injected intraperitoneally into the animal 1 hr before sacrifice, in order to block dividing cells at metaphase. After 1 hr of colchicine treatment, the mice was dissected. The limbs of mice were cut and bone marrow was removed from bone marrow cavity with the syringe filled with KCl into glass cavity.

The bone marrow material was incubated in KCl solution for about 45 minutes at 37 °C and centrifuged at 1000 rpm for 10 minutes. A pellet was formed at the bottom of the centrifuge tube after centrifugation. The supernatant was then discarded and fixative was added dropwise into the tube and mixed properly with the help of dropper.

The solution was kept for 5 minutes and again centrifuged for 10 minutes. After fourth centrifugation, few drops of supernatant were discarded and remaining was mixed properly with the pellet that remained at the bottom of the tube. New glass slides were dipped in absolute alcohol for about 10-15 minutes before their use. The slides were air dried and cleaned with tissue paper. The cell suspensions were dropped on glass slides from the height of 2 ft and were blazed on flame for 5 seconds. Then they were air dried and were left for 30 minutes in Giemsa staining solution. The slides were rinsed with water and allowed to dry at room temperature. The experiments were repeated thrice for each time interval. At last the slides were examined under microscope CX21 at 100X and good metaphases were photographed.

RESULTS AND DISCUSSION

During the present investigation, effect of aromatic amines which are being produced after the degradation of azo dyes were studied in inducing chromosomal abnormalities in bone marrow cells of mice. The investigation was carried out by injecting 0.5 mg of aromatic amine sulphanilic acid at 24 hrs, 48 hrs and 72 hrs time interval and its aerobically treated product at 48 hrs time interval (Table 2-3). DMSO was taken as control. Some aberrations were found in mice treated with control (Table 1).

Table 1. Total Chromosomal Aberrations in rat treated with DMSO (Control)

S.No	No. of Cells	Clump	Stickiness	Ring	Acentric	Dicentric	Chrd. Gap	Chr. Gap	Chrd. Break	Chr. Break	Total Abr.
1.	100	4	2	2	0	4	0	0	2	0	14
2.	100	3	2	1	1	3	0	0	1	1	12
3.	100	4	1	1	1	2	1	0	1	0	11
Total	300	11	5	4	2	9	1	0	4	1	37

Table 2. Total Chromosomal Aberrations in mice treated with 0.5 mg Sulphanilic acid at 24 hrs, 48 hrs and 72 hrs

S.No.	No. of cells	Clump	Stickiness	Ring	Acentric	Dicentric	Chrd. Gap	Chr. Gap	Chrd. Break	Chr. break	Total Abr.
24 hrs											
1	100	20	10	14	16	28	10	6	6	2	112
2	100	18	8	12	18	26	10	8	8	2	110
3	100	16	8	12	16	24	12	8	8	4	108
Total	300	54	26	38	50	78	32	22	22	8	330
48 hrs											
1	100	20	12	14	22	28	12	8	14	4	134
2	100	22	10	16	22	26	12	8	10	6	132
3	100	20	8	16	20	28	12	8	12	4	128
Total	300	62	30	46	64	82	36	24	36	14	394
72 hrs											
1	100	24	12	20	22	30	12	6	10	6	142
2	100	22	8	18	21	27	14	8	10	10	138
3	100	20	8	20	20	28	12	8	12	8	136
Total	300	66	28	58	63	85	38	22	32	24	416

Each experiment was repeated thrice and 100 cells were observed in each category and various kinds of chromosomal abnormalities were observed.

Table 3. Total Chromosomal Aberrations found in mice treated with 0.5 mg of aerobically treated product at 48 hrs

S.No.	No. of cells	Clump	Stickiness	Ring	Acentric	Dicentric	Chrd. Gap	Chr. Gap	Chrd. Break	Chr. Break	Total Abr.
1	100	10	6	12	10	12	6	4	4	2	66
2	100	8	8	11	9	10	4	4	4	4	62
3	100	8	8	12	8	8	4	4	4	4	60
Total	300	26	22	35	27	30	14	12	12	10	188

Table 4. Mean value of chromosomal aberrations among control, 0.5 mg of sulphanilic acid at 24 hrs, 48 hrs and 72 hrs time interval and 0.5 mg of aerobic treated dye product at 48 hrs

		Control	24 hrs	48 hrs	72 hrs
Sulphanilic Acid	Mean	12.33±0.88	110.00±1.15*	131.33±1.76*	138.67±1.76*
	t-value		67.21	60.34	64.06
Aerobic treated	Mean	12.33±0.88	.....	62.67±1.76*	.....
	t-value		.....	25.52	.....

*Very highly significant when compared to control group ($p<0.001$)

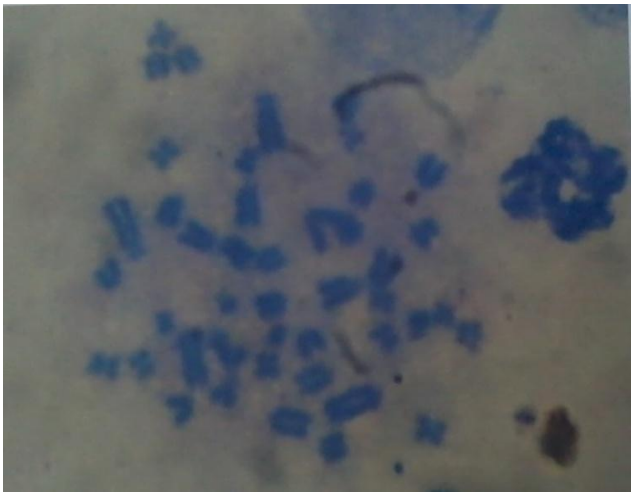

Figure 1. Chromosomes in Control Group

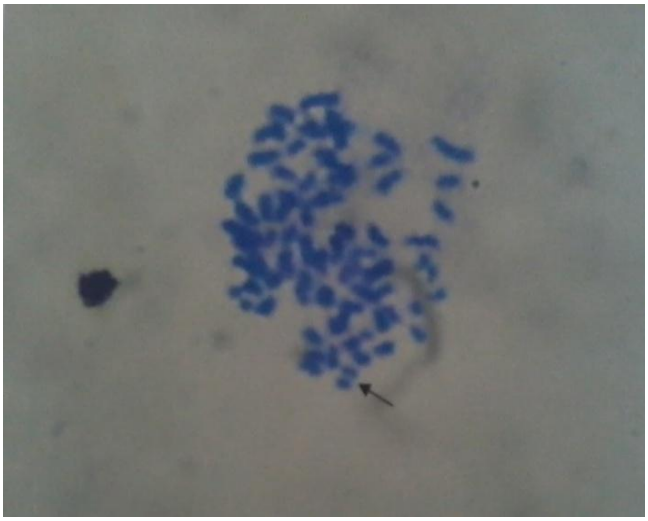

Figure 2. Acentric Chromosomes (Treated Product of Sulfanilic Acid 0.5 mg,48 Hrs)

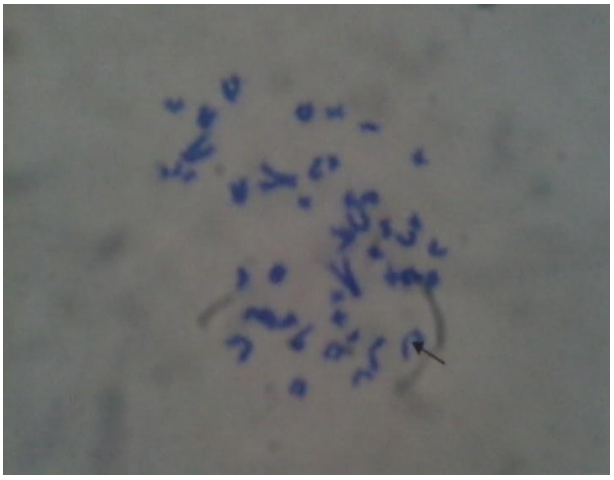

Figure 3. Chromatid Gap at Metaphase (Sulphanilic Acid, 0.5 mg, 72 hrs)

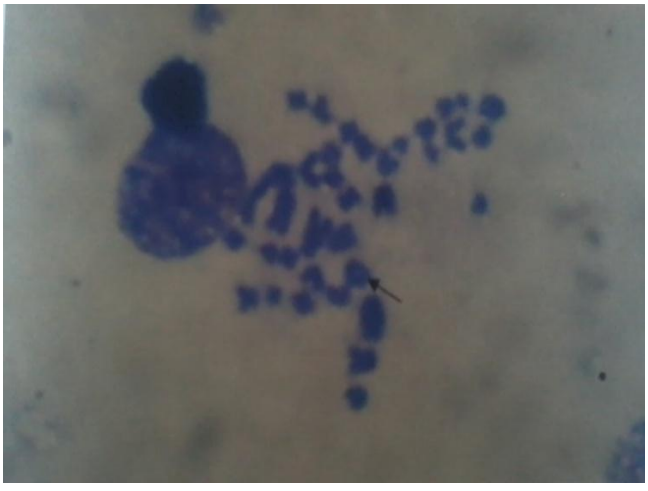

Figure 4. Chromosomes showing Ring (Sulphanilic Acid, 0.5 mg,72 hrs)

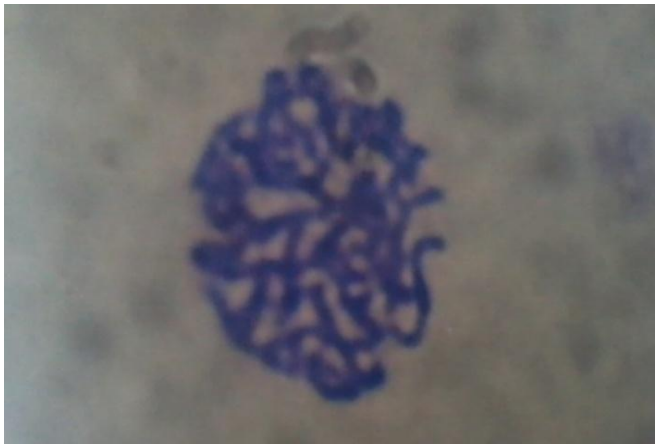

Figure 5. Chromosomes showing Stickiness (Sulphanilic Acid, 0.5 mg, 48 hrs)

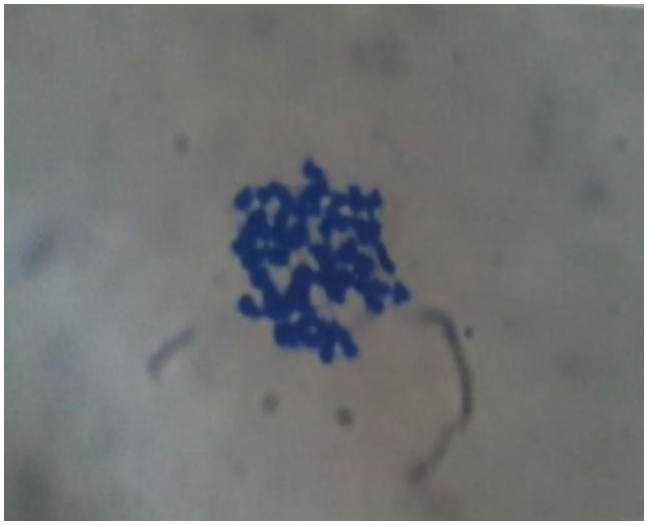

Figure 6. Chromosomes showing Clumping (Sulphanilic Acid, 0.5 mg, 24 hrs)

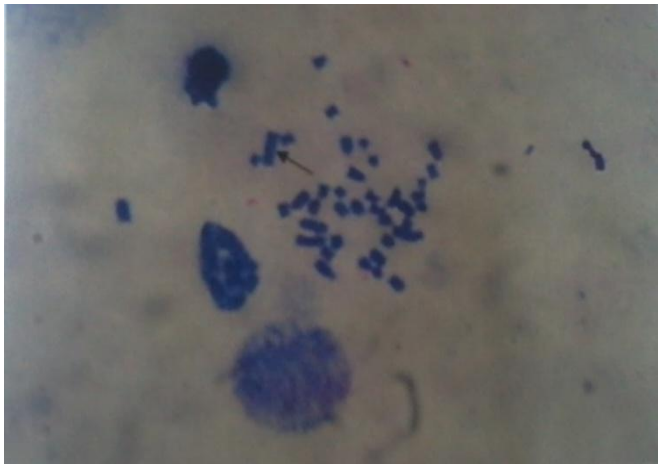

Figure 7. Dicentric Chromosomes (Sulphanilic Acid, 0.5 mg, 72 hrs)

Major chromosomal anomalies were clumping, stickiness, rings, dicentric, chromatid gaps, chromosome gaps, chromatid breaks and chromosome breaks (Figures 1-7). The mean value of aberrations in control was found to be 12.33±0.88 as shown in table 4. The mean value of aberrations found in chromosomes of mice treated with 0.5 mg of sulphanilic acid at 24 hrs, 48 hrs and 72 hrs were 110.00±1.15(t being 67.21), 131.33±1.76 (t being 60.34), 138.67±1.76 (t being 64.06) respectively in Table 4.

The mean value of aberrations found in chromosomes of mice treated with 0.5 mg of aerobically treated product at 48 hrs was 62.67±1.76 (t being 25.52) respectively as shown in table 4. The values were significantly higher than those of control ($p<0.001$). After applying one way ANOVA test, the value of 'f' was found to be 88.06 for sulphanilic acid. The value is found to be significant. Proportion of cells showing clumping and stickiness was highest in all groups of mice. The mean values of total aberrations were found to be significantly higher than in control mice.

Das and Mukherjee (2004) also reported chromosomal aberrations like gaps and breaks in bone marrow cells of mice treated with food colors Amaranth and Tartrazine. A dye blend, tomato red was found to induce degenerative changes in liver, kidney and testes of male swiss albino mice *(Sharma et al.,* 2008). Azirak and Rencuzogullari (2007) observed decrease in mitotic index in mouse bone marrow cells treated with carvacrol and thymol. They were found to induce total structural chromosomal abnormalities. Cytogenetic characterization and classification of different types of chromosomal aberrations have an important role because many types of cancers are associated with specific types of aberrations. Chromosomal aberrations are measurements of genomic instability and bone marrow cells are considered to be indicator cells of their high sensitivity to clastogenic agents. Clumping, stickiness and dicentric were the most common abnormalities reported during present investigation. According to Mishra and Banerjee (1986), chromosome stickiness is a result of profound changes occurring on surface of chromosomes due to loss of viscosity in their matrix because of treatment with the chemicals. Due to stickiness, most of the bivalents at first metaphase tend to remain together resulting in clumped bivalents (Saha and Khudabaksh, 1974). The results of present study clearly suggests that the aromatic amine, Sulphanilic acid is quite effective in inducing chromosomal abnormalities. Sulphanilic acid is more genotoxic in nature as compared to its aerobic degradation product. These degraded samples also induced significantly higher genotoxicity as compared to control but found to be less genotoxic as compared to parent aromatic amine.

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