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CYTOGENETIC STUDIES OF FOOD PRESERVATIVE IN *ALLIUM CEPA* ROOT MERISTEM CELLS

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Summary. *Cytogenetic effects of food preservative Potassium metabisulphite have been tested on root tip cells of Allium cepa L. The root tips have been treated with a series of concentrations, ranging from 25 to 150µg/ml for 1, 3, 5, 7 and 9 h. The results indicate that the food preservative has reduced mitotic division in A. cepa compared with the respective control. The percentage of Mitotic index has decreased with increasing dose and time. Chromosomal abnormalities increase with increasing concentrations of the test chemical and the longer period of treatment. We have found that chromatid break and multiple breaks increase with dosage.*

Key words: *Food preservative, potassium metabisulphite, Allium cepa, mitotic index, chromosomal abnormalities*

Introduction

In food preservation, sugar and salts are often used as preservatives. Chemical preservatives are being used and they seem to be the best and the most effective for a longer shelf-life. It has been reported that certain food additives, especially antimicrobial agents are genotoxic in different test systems (1, 2, 3). Sulfites are widely used in food processing to sanitize fermentation equipment and food containers, to prevent the microbial spoilage of foods to selectively inhibit the undesirable microorganisms in fermentation industries and to prevent oxidative discolouration and nonenzyme browning during preparation, distribution and storage of food (4, 5). There are still many food preservatives whose possible genotoxic effects are unknown.

Sulfites are used in restaurant foods to keep salad-bar vegetables and fruits looking fresh and to prevent the browning of avocado dips. They are also used in seafood, potatoes, beer, wine, fruit drinks, baked goods, dried fruits and in the processing of some food ingredients; including beet sugar, corn sweeteners, food starches and gelatin (6, 7, 8, 9). Many pharmaceuticals contain sulfites as antioxidants: they include some important chemicals such as alupent, injectable adrenalin; local anesthetics such as Novocain; injectable lidocaine; and solutions for total parental nutrition and dialysis (10, 11, 12). Sulfite update report (1984) (13) of Food and Drug Administration organization (FDA) has received more than 250 reports of suspected sulfite-related reactions; as of February 1984 the FDA had received 6 reports of deaths allegedly associated with restaurant food containing sulfites.

Nolan et al. (1983) (14) stated that people with asthma may be sensitive to metabisulphite. Twarong et al., (1983) (15) stated that approximately one million of the 9 million people with asthma in North America may be sulfite-sensitive. Although food preservatives are widely used in our foods, we do not have enough information about their genotoxic effects. Limited studies have been conducted with potassium metabisulphate in vitro and in vivo test systems. Therefore, it has been decided to test the genotoxic potential of the potassium metabisulphite in mitotic cells of *Allium cepa*.

Materials and Methods

In this study, the root-tip cells of *A. cepa* ($2n = 16$) are used to test the clastogenic effect of potassium metabisulphite (Fig 1). The potassium metabisulphite has been obtained from Himedia chemicals, Mumbai. The Chemical formula is $K_2S_2O_5$ and the molecular weight is 222.23. Onion Bulbs have been grown in distilled water at room temperature (25±2°C) in dark. When the newly emerged roots are 1–2 cm in length, they are used in the test. Roots of *Allium cepa* have been treated with a series of concentration 25, 50, 75,100,125,150µg/ml for 1, 3, 5, 7,9h. The controls have been treated with distilled water. After treatment root tips are treated with 0.05% colchicine for 3h and fixed in ethanol and acetic acid (3:1) mixture for 24h at 5°C. Slides have been prepared by using Feulgen squash technique to analyze mitotic

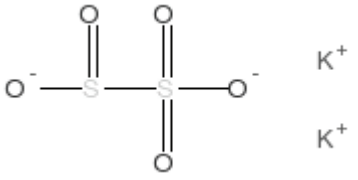


Fig. 1. Potassium metabisulphite

Table 1. Cytogenetic analysis of Root tips of *Allium cepa* exposed to different concentrations of Potassium metabisulphite for different periods

Time of Treatment (h)	Concentration (µg/ml)	Mitotic Index (Mean±SE)	NC	AC	Break	Gap	Multiple Breaks	Total±SE
	Control	19.5 ± 4.3	485.00	15.00	08.25	0.00	6.75	15.00 ± 0.5
	25	14.5 ± 4.3	486.00	16.00	10.50	0.00	5.50	16.00 ± 3.3*
	50	11.5 ± 4.3	460.50	39.50	27.80	5.60	6.10	39.50 ± 0.5*
1	75	11.0 ± 7.0	457.50	42.50	26.60	9.20	6.70	42.50 ± 2.0*
	100	11.5 ± 3.3	454.00	46.00	30.80	12.00	3.20	46.00 ± 4.3*
	125	11.5 ± 3.3	451.00	49.00	32.60	12.00	4.40	49.00 ± 4.2*
	150	8.0 ± 2.2	449.00	51.00	32.80	12.20	6.00	51.00 ± 5.3*
	Control	20.5 ± 4.3	478.00	17.25	09.50	0.00	07.75	17.25 ± 1.10
	25	12.5 ± 4.3	477.50	22.50	15.75	0.00	06.75	22.50 ± 1.70*
	50	10.5 ± 4.7	456.25	43.75	30.50	6.00	07.25	43.75 ± 0.9*
3	75	10.5 ± 5.3	452.25	47.75	27.00	10.25	10.50	47.75 ± 3.9*
	100	9.0 ± 3.5	444.75	55.25	34.50	10.00	10.75	55.25 ± 8.6*
	125	8.0 ± 2.6	437.50	62.25	36.50	15.00	11.00	62.25 ± 4.5*
	150	6.5 ± 1.7	432.75	67.50	36.50	17.25	13.75	67.50 ± 5.5*
	Control	21.5 ± 4.6	477.75	19.25	10.25	0.00	09.00	19.25 ± 0.9
	25	11.0 ± 4.6	471.00	29.00	16.25	0.00	12.75	29.00 ± 0.1*
	50	8.5 ± 7.5	452.50	47.50	31.00	3.00	13.50	47.50 ± 0.9*
5	75	9.0 ± 4.9	446.75	53.25	28.50	11.00	13.75	53.25 ± 1.2*
	100	8.0 ± 4.7	439.75	60.25	36.50	11.25	12.50	60.25 ± 0.9*
	125	6.5 ± 3.1	439.00	61.00	35.00	15.50	10.50	61.00 ± 2.5*a
	150	5.5 ± 2.9	432.50	67.50	36.00	18.50	13.00	67.50 ± 7.8*b
	Control	21.7 ± 5.5	475.25	19.75	13.50	0.00	06.25	19.75 ± 3.1
	25	8.5 ± 5.5	474.50	25.50	14.25	0.00	11.25	25.50 ± 3.1*
	50	7.2 ± 7.9	455.50	44.50	30.00	01.00	13.50	44.50 ± 6.4*
7	75	7.3 ± 9.6	446.50	53.50	26.50	12.00	15.00	53.50 ± 8.1*
	100	6.5 ± 1.7	440.50	51.50	35.00	11.25	13.25	51.50 ± 1.8*
	125	5.5 ± 5.9	432.25	67.75	37.75	15.00	15.00	67.75 ± 5.0*b
	150	5.1 ± 2.6	428.75	71.25	37.75	15.50	18.00	71.25 ± 4.5*b
	Control	21.9 ± 6.4	476.25	22.00	14.25	0.00	07.75	22.00 ± 0.5
	25	7.5 ± 6.4	474.25	25.75	20.50	0.00	05.25	25.75 ± 1.2*
	50	5.8 ± 6.8	454.25	45.75	35.00	0.00	10.75	45.75 ± 2.1*
9	75	5.1 ± 6.3	448.75	51.75	30.50	08.00	13.75	51.75 ± 5.4*
	100	4.9 ± 0.9	441.15	58.85	36.50	07.10	15.25	58.85 ± 4.9*
	125	4.7 ± 2.8	426.75	73.25	39.50	15.00	18.75	73.25 ± 2.5*[a]
	150	4.5 ± 2.9	424.75	75.25	40.25	12.50	22.50	75.25 ± 2.2*[b]

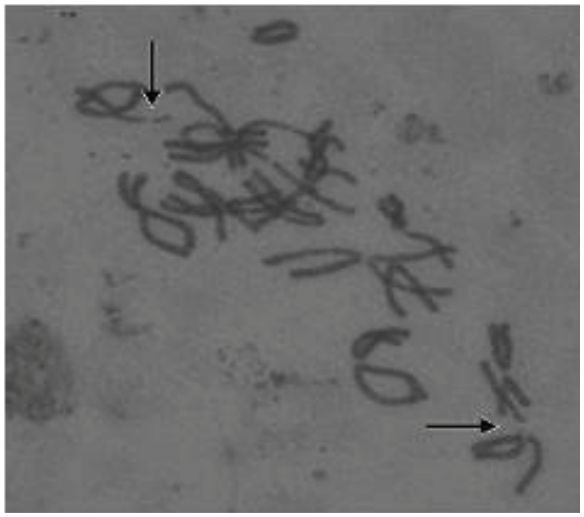
Abbreviations:* Statistically Significant when compared with untreated control, a = 0.005, b = 0.05 when compared with previous concentration NC – Normal cells, AC-Abnormal cells

index and chromosomal aberrations (16). Four replicates have been performed for each treatment and the slides were masked before scoring. A minimum of 500 well spread metaphase cells were scored for each concentration. The cytological abnormalities are scored in the mitotic cells and the results are shown in the Table 1 and Figure 2**.**

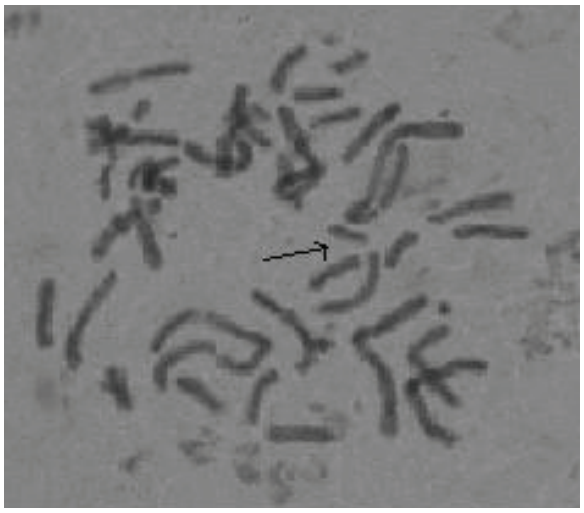
The clastogenic abnormalities such as break, gap, and multiple breaks were scored. The significance among the mean results of total number of aberrations, abnormal cells and mitotic index has been analyzed by ANOVA.

Result and Discussion

The data presented in the Table.1 show the clastogenic effect of potassium metabisulphite. The number of abnormal cells and aberrations are dose and time dependent. The frequencies of chromosomal aberrations increase with increasing concentrations. The differences among the concentrations have been significant, when compared with untreated control. The most frequent aberrations are break, gap, and multiple breaks (Fig. 2). The reduction in the mitotic activity increases when the concentration has been increased from 100µg/ml to 150µg/ml. There have been statistically significant differences between control and treated groups in chromosomal abnormalities.



a)



b)

Fig. 2. a) Gap, Break, b) Multiple Break

Plant systems have a variety of well defined genetic endpoints including alterations in ploidy, chromosomal aberrations and sister chromatid exchanges (17, 18). Potassium metabisulphite has decreased MI in the treatment groups compared with the control at all concentrations and treatment periods. Reduction in the mitotic activity could be due to inhibition of DNA synthesis (19, 20) or a blocking in the G_2-phase of the cell cycle, preventing the cell from entering mitosis (21). Beu et al. 1976 (22) have also showed that exposure of root tips of *V. faba* to high concentrations of the herbicide paraquat has led to inhibition of DNA synthesis. This suggests that potassium metabisulphite may cause inhibition of DNA synthesis.

Njagi and Gopalan (1982) (23) report that the food preservatives sodium benzoate and sodium sulphite has caused anaphase bridges in *V. faba*. But in the present study, the different concentrations of Potassium metabisulphite do not cause anaphase bridges. Sifa et al. (2005) (24) report that Chromosome bridges may be due to the chromosomal stickiness and subsequent failure of free anaphase separation or may be attributed to unequal translocation or inversion of chromosomal segments. Chemicals that induce chromosome breakage are known as clastogens and their action on chromosomes is generally regarded to involve an action on DNA (25, 26). Thus the induction of chromosome breaks by Potassium metabisulphite may be independent of its effect on the amount of DNA. Therefore, further studies that involve the effect of potassium metabisulphite on DNA and RNA are needed.

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CITOGENETSKA ISTRAŽIVANJA PREHRAMBENIH KONZERVANASA U MERISTEMSKIM ĆELIJAMA KORENA *ALLIUM CEPA*

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Kratak sadržaj: *Citogenetski efekti prehrambenog konzervansa kalijum metabisulfita testirani su na ćelijama korenskog vrha Allium cepa L.. Vrhovi korena tretirani su serijom koncentracija, počevši od 25 do 150µg/ml u toku 1, 3, 5, 7 i 9 h. Rezultati pokazuju da je prehrambeni konzervans redukovao mitotičku deobu u A. cepa u poređenju sa kontrolom. Procenat mitotičkog indeksa se smanjuje sa povećanjem doze i vremena. Sa povećanjem koncentracije konzervansa i dužine tretiranja povećava se broj hromozomskih abnormalnosti. Utvrdili smo da sa povećanjem doze dolazi do povećanog i višestrukog pucanja hromatida.*

Ključne reči: *Prehrambeni konzervans, kalijum metabisulfit, Allium cepa, hromozomske abnormalnosti*