



RESEARCH ARTICLE

AMELIORATION OF SODIUM BENZOATE INDUCED MITOTIC CHROMOSOMAL ABNORMALITIES BY FENUGREEK (*TRIGONELLA FOENUM GRAECUM*) SEED EXTRACT IN BONE MARROW CELLS OF SWISS ALBINO MICE (*MUS MUSCULUS*)

Gopal Roy¹, Dharmshila Kumari² and Abha Rani³

¹Research Scholar, ¹University Department of Zoology, TMBU, Bhagalpur- 812007, Bihar, India; ²Professor, ²University Department of Zoology, TMBU, Bhagalpur- 812007, Bihar, India; ³Demonstrator, University Department of Zoology, TMBU, Bhagalpur- 812007, Bihar, India

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*Corresponding author: Gopal Roy

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ABSTRACT

Sodium benzoate is one of the commonly used chemical preservative in the food industry. It increases the life of food and protecting them from spoiling and keep them fresh. Several investigation have been found that the food preservatives have toxic effect on various organs including liver, kidney, brain, nervous system, etc. due to the formation of free radicals which in turn the damages the chromosome, it has genetic effect also if consume long time. In present study it is revealed that the toxicity of sodium benzoate was evaluated in mitotic metaphase chromosome of swiss albino mice (*Mus musculus*), isolated from the bone marrow cells. Structural and mitotic disruptive changes were reported and its amelioration was done by antioxidant rich fenugreek (*Trigonella foenum graecum*) seed extracts. Sodium benzoate (2.25 mg/ml) administrated orally to Swiss albino mice (*Mus musculus*) for 35 days increases the frequency of chromosome abnormality 48(16±2.11%) compare to 13 (4.3±1.17%) of control on mitotic chromosome which is significantly higher. The abnormalities were 11 (3.6±1.07%) in group fed only with fenugreek seed extract. However, when fenugreek seed extract and sodium benzoate fed concurrently the abnormalities were 15(5.0±1.25%) respectively. The result showed that sodium benzoate is potent enough to damage mitotic chromosome and present finding also showed that concurrent treatment of fenugreek seed extract and sodium benzoate decreases the frequency of mitotic chromosomal abnormalities. Therefore it is suggested that the fenugreek seed extract may reduce the risk of sodium benzoate induced genotoxicity.

INTRODUCTION

With the increasing the chemicalization of processed food in recent year, the food preservatives play an important role in the food industries. The consumers have declared their interest in such food. Now-a-days processed food make up 75% of diet of western society (Zengin *et al.*, 2011). Sodium benzoate is one of the commonly used Preservatives in food industry, pharmaceutical and cosmetic industries (Hong *et al.*, 2009; Williams *et al.*, 2005). Preservatives are the substance added to food to prevent from spoiling caused by microbes and increases the life of the food and keep them fresh for long time. Preservatives are used as bacteriostatic and fungistatic in acid food and drinks such as vinegar, carbonated drinks, jams, fruit juice and cosmetics. Fresh and dried food are always added preservatives to extend the life span of the food. In several investigation it has been found that if it consume long time even through it is small amount the preservatives may cause adverse effects.

The adverse effects of preservatives are nausea, vomiting, Diarrhea, rhinitis, migraine, hyper-reactivity in children (Feingold 1973, Smith 1991, Taurmaa 1994, Dogruyol 2006). It causes irritation of eyes, skin and respiratory tract, but if consume long term may cause skin sensation (Lucio *et al.*, 2009). Research has also shown the formation of benzene in the reaction between benzoic acid and ascorbic acid (vitamin - C) in presence of metal catalysts in drinks and juices (Gardner *et al.*, 1993) which is carcinogen that breaks down DNA-deficient RNA in the mitochondria (Chang and Ku, 1993). Sodium benzoate (SB) significantly effect on DNA breakage, micronucleus formation and mitotic index of rats (Saatci *et al.*, 2016). Hagmar *et al.*, (1994) investigated that sodium benzoate increased the levels of chromosomal aberrations (CA). It is reported that the effect of sodium benzoate as a preservative on the reproductive system of male mice (Manar M. Hadi *et al.*, 2018). and in another investigation it is suggested that excessive consumption of sodium benzoate induces impaired spermatogenesis and sperm quality (Dalal Redouane *et al.*,

2019). Human beings have been utilizing medicinal plants and their products for curative health care for long time (Jakhar *et al.*, 2015). It has been found that the fenugreek seed is effective against many disease such as cancer, hypercholesterolemia, diabetic, and inflammation (Abbas *et al.*, 2020) and are effective for mild asthma (Emtiazy *et al.*, 2018). Fenugreek has several medicinal properties (Roy *et al.*, 2023). It has properties to reduce the genotoxicity and increases the number of sperm cell (Roy *et al.*, 2023). It has anticancer potential (Alsemari *et al.*, 2014). A number of plants products such as neem leaf extract (Kumari and Chaurasia, 2007), Papaya fruit extract (Singh N. and Kumari, 2013), grape seed extract oil (Al-Attar, 2017) etc. reported to have beneficial effect against genotoxicity in mice.

MATERIALS AND METHODS

Both sexes of 4-6 week old Swiss albino mice (*Mus musculus*) with an average weight ranging from 25 gm to 35 gm was used as test animal (experimental animal).

Mice were obtained from the laboratory inbred stock and maintained in the animal house of University Department of zoology, Tilka Manjhi Bhagalpur University. They were separated into four groups (6 mice in each group) and were provided the standard Laboratory Condition ($25\pm 5^{\circ}\text{C}$) with normal diet throughout the experimental period for 35 days. All animal and protocols employed in this study received prior approval of the Institutional Head and Departmental Research Committee.

The summary of the experimental groups and treatment protocol are given in Table 1. After the completion of treatment animals were sacrificed and slides of mitotic metaphase chromosome of bone marrow cells were prepared by colchicines-hypotonic-aceto-alcohol-flame drying-Giemsa staining technique (Preston *et al.*, 1987). The chromosomal abnormalities were screened for the incidence of structural and mitosis disruptive changes in each experimental group. Total 300 well spread metaphase plates from each group of animals were screened by random selection and student's t-test was applied for the evaluation of the data.

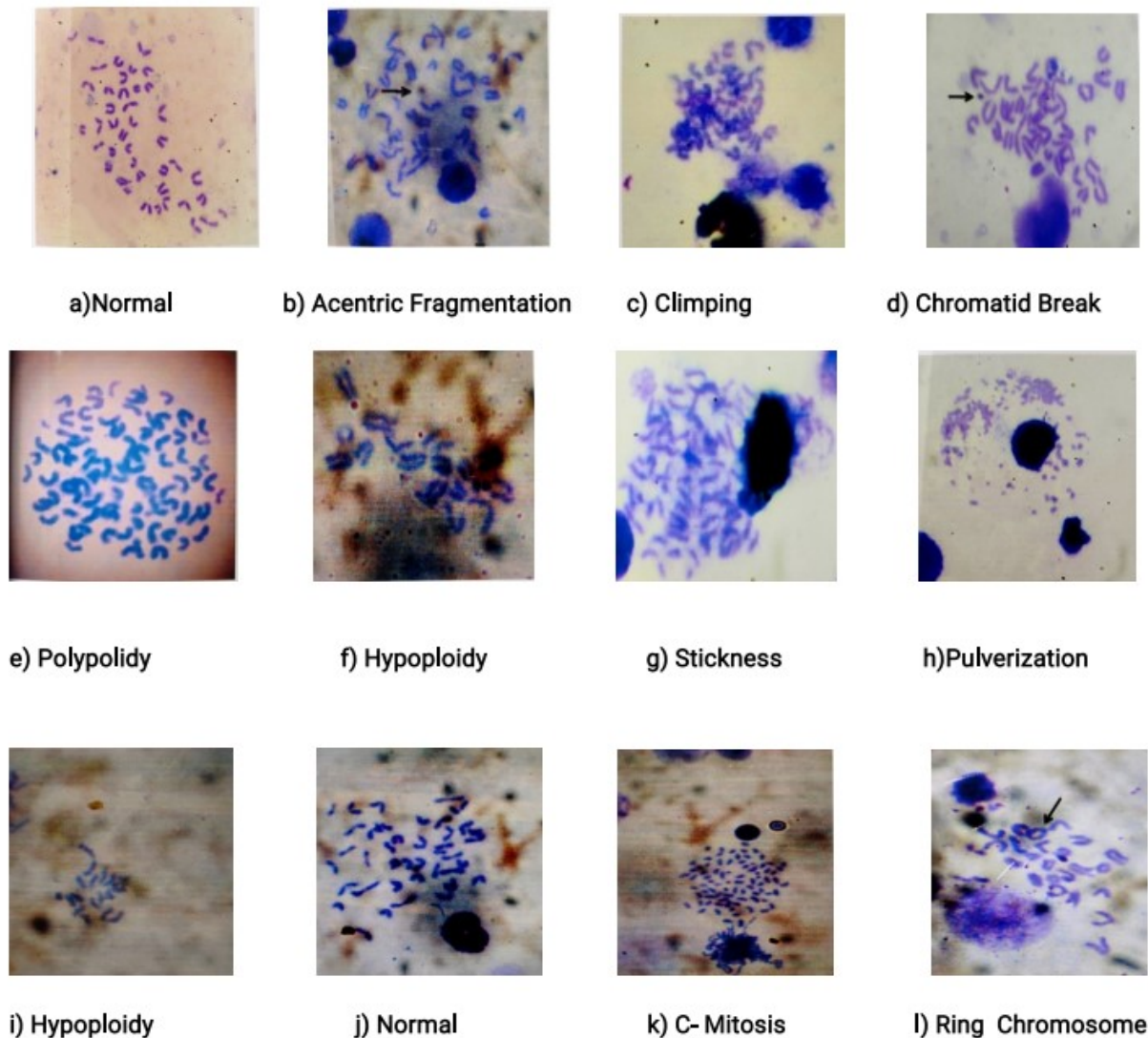


Figure 1. Abnormalities examined under microscope in bone marrow cell of mice (*Mus musculus*) of Chemical preservative. Sodium benzoate (E211) treated group

Table 1. Summary of the experimental group and treatment protocol

SL No.	Experimental group	Symbol	Dose
i	Control	C	Normal Diet
ii	Sodium benzoate (E211)	SB	2.25 mg/ml (Javed <i>et al.</i> , 2017)
iii	Fenugreek Seed extract	F	400 mg/kg (Roaa <i>et al.</i> , 2016)
iv	Sodium benzoate + Fenugreek seed extract	SB + F	As ii and iii

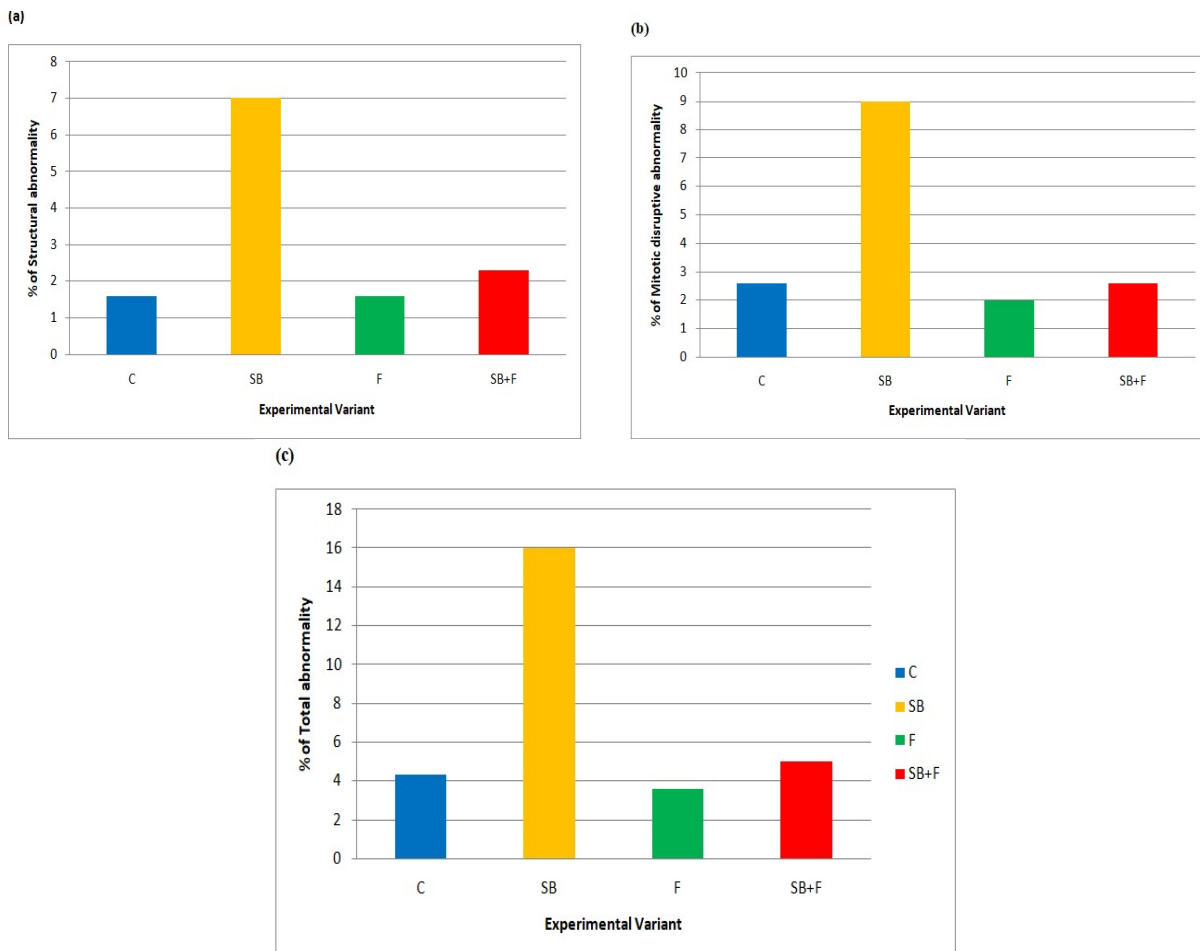
Table 2. Number of different types of abnormalities in bone marrow metaphase chromosome of mice treated with sodium benzoate and fenugreek

Exp. Variant	Structural abnormality								Mitosis- disruptive abnormality					
	Ctb	Ctg	Mf	Af	Ring	Iso-ctb	Iso-ctg	Met	Pol	Clum	Stic	Pulv	Hypo	C-mito
C	1	1	-	1	2	-	-	-	2	1	1	1	2	1
SB	7	1	-	2	7	-	1	3	12	2	-	1	10	2
F	1	1	-	1	-	1	-	1	2	1	-	-	2	1
SB+F	2	1	-	1	1	1	1	-	2	1	-	1	2	2

Table 3. Incidence of chromosomal abnormalities in mitotic cell of mice treated with treated with sodium benzoate and fenugreek

Experimental Variant	Symbol	Structural changes		Mitosis- disruptive changes		Total abnormality	
		Total	% ± S.E	Total	% ± S.E	Total	% ± S.E
Control	C	5	1.6± 0.72	8	2.6 ± 0.91	13	4.3 ± 1.17
Sodium Benzoate	SB	21	7.0± 1.47	27	9.0 ± 1.65	48	16.0 ± 2.11a
Fenugreek seed	F	5	1.6± 0.72	6	2.0 ± 0.80	11	3.6 ± 1.07b
Sodium Benzoate + Fenugreek seed	SB + F	7	2.3± 0.86	8	2.6 ± 0.91	15	5.0 ± 1.25b

•a indicates significant different with control and • b indicates significant different with sodium benzoate.

**Figure 2(a,b,c). Graphical representation of structural, mitosis disruptive changes and total abnormality in four different group of mice**

STATISTICAL ANALYSIS

The data were expressed as (mean± SE) and t- test (P<0.05) was used for evaluation of data.

RESULT AND DISCUSSIONS

- Different types of abnormalities were observed including Clumping, Ring chromosome, C-mitosis, Hypoploidy, Polyploidy, Stickness, Pulerization, Chomatid break Acentric fragmentation after treated with sodium benzoate group.
- The mitotic chromosomal abnormalities in sodium benzoate group were significant (16.0%) which is higher than control (4.3%) group. After treated with fenugreek seed extract the abnormalities was (3.6%) which is almost equal to control group.
- However concurrent treatment of fenugreek seed extract with sodium benzoate shows the value (5.0 %) which is highly significant and lower than sodium benzoate treated group and almost equal to the control group.
- The fenugreek seed extract with the concurrent treatment of sodium benzoate significantly minimized the genotoxic effect of sodium benzoate, hence it shows a useful amelioration effect against sodium benzoate induced genotoxicity.
- In our study we used fenugreek seed a common medicinal plant uses as common spice belong to family fabaceae is distributed worldwide and has been known by different names, including fenugreek (English), Methi (Hindi), Methika (Sanskrit). The fenugreek seed extract has numerous anti-oxidant and anti-inflammatory activity.

CONCLUSION

From the obtained results it is concluded that sodium benzoate (E211) produces genotoxic and mutagenic effect on bone marrow cell of mice and to minimize its toxic effect the fenugreek seed exhibited the good ameliorating agent. My objective behind this work is to make people alert against the harmful effect of chemical preservatives and aware them about the medicinal value of antioxidants rich herbal spices, Fenugreek (*Trigonella foenum graecum*).

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