



ISSN: 0975-833X

Available online at <http://www.journalcra.com>

INTERNATIONAL JOURNAL  
OF CURRENT RESEARCH

International Journal of Current Research  
Vol. 15, Issue, 08, pp.25638-25640, August, 2023  
DOI: <https://doi.org/10.24941/ijcr.45860.08.2023>

## RESEARCH ARTICLE

### EVALUATION OF GENOTOXIC EFFECT OF SODIUM BENZOATE USING MICRONUCLEUS TEST IN BONE MARROW CELL OF MICE (*Mus musculus*) AND ITS AMELIORATION BY FENUGREEK (*Trigonella foenum greacum*) SEED EXTRACT.

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#### ARTICLE INFO

##### Article History:

Received 19<sup>th</sup> May, 2023  
Received in revised form  
15<sup>th</sup> June, 2023  
Accepted 17<sup>th</sup> July, 2023  
Published online 30<sup>th</sup> August, 2023

##### Key words:

Micronucleus, Sodium benzoate,  
Normochromatic erythrocytes,  
Polychromatic erythrocytes,  
preservative, food additives,

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#### ABSTRACT

Now a days it is difficult to imagine the food products without any food additives. Sodium benzoate (SB) is one of the most commonly used food preservative in food processing industries. The aim of present study, is to ensure the ameliorating effect of fenugreek seeds were evaluated against genotoxic effect induced by Sodium benzoate(SB) on polychromatic erythrocytes(PCEs) and normochromatic erythrocytes(NCEs) in bone marrow cells in mice using micronucleus test, twenty four adult swiss albino mice of average weight 25 gm was selected and divided into four groups. First group included control(C) with normal diet, the second group was treated with sodium benzoate at concentration (2.25 mg/ml) and the third group was treated with fenugreek seed powder(ameliorating agent) at a concentration( 400 mg/Kg) body weight and fourth concurrent group was treated with sodium benzoate and fenugreek same as 2nd and 3rd group concentration for 35 days, after that animals were sacrificed and samples were taken for study of micronucleus test, the result shows that the micronucleus formation in Sodium benzoate (SB) treated groups were significantly (2.80%) higher than the control (0.16%) and the treatment of concurrent (sodium benzoate and fenugreek) group shows the value of (0.27%), this value was significantly lower than Sodium benzoate treated group and almost equivalent to the control group. The results thus suggested that fenugreek seed showed ameliorating effect against Sodium benzoate (E211) induced genotoxicity in bone marrow cells of mice.

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Citation: Gopal Roy, Dharmshila Kumari and Abha Rani, 2023. "Evaluation of genotoxic effect of sodium benzoate using micronucleus test in bone marrow cell of mice (*mus musculus*) and its amelioration by fenugreek (*trigonella foenum greacum*) seed extract.". *International Journal of Current Research*, 15, (08), 25638-25640.

## INTRODUCTION

With the advancement of processed foods, the food additives play an important role in food technology (Saad *et al.*, 2005). The food additives are used for various purposes, including, coloring, sweetening, and preservation. Preservatives are added to fruits, foods, vegetables, cosmetics and pharmaceuticals in order to increase their shelf life and keep them fresh and maintain the quality by inhibiting or arresting their fermentation, microbial contamination and decomposition. Now-a-days it is difficult to imagine the food product without any food additives. The preservatives are commonly used as antimicrobial substances in various kind of foods, such as soft drinks, salad cream. Fruits based fillings, jam, marinated fish, and beer. They have been found to arouse urticaria, angioedema, and asthma (Michaelsson and Juhlin, 1973; Food intolerance and Food Aversion, 1984; Miller and Millstone, 1987; Tuormaa, 1994; Dogruyol, 2006). And they have also been linked with hyperactivity in children (Egger *et al.*, 1985).

## MATERIAL AND METHODS

The aim of present study the ameliorating effect of fenugreek (*Trigonella foenum greacum*) seed were evaluated against genotoxic effect induced by sodium benzoate(SB) on polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NECs) in mice bone marrow cells using micronucleus test, twenty four adult swiss albino mice(*Mus musculus*) of average weight 25 gm were collected from animal house colony, University Department of Zoology, Tilka Manjhi Bhagalpur University, and divided into four groups, six (6) mice in each group. First group included control(C) with normal diet, the Second group was treated with sodium benzoate at concentration (2.25 mg/ml) and the third group was treated with fenugreek seed powder (ameliorating agent) at a concentration( 400 mg/Kg) body weight and fourth concurrent group sodium benzoate and fenugreek with same as 2<sup>nd</sup> and 3<sup>rd</sup> group concentration for 35 days as shown as Table – 1

Table 1.

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	F	400 mg/kg (Roaa <i>et al.</i> , 2016)
iv	Sodium benzoate + Fenugreek	SB + B	As i and ii

After 35 days animals were sacrificed without colchicine injection, collected the bone marrow from femur of treated mice, kept in 1 % sodium citrate at 20°C, centrifuged the sample immediately at 1000 rpm for 5 minutes, slides were prepared by smears of bone marrow (Schmid, 1976) with little modification (Das and Kar, 1986; Salamone and Heddle, 1983). Slides were screened, the normochromatic erythrocytes (NCEs) were stained pink and polychromatic erythrocytes (PCEs) were stained blue. Approximate 1000-2000 PCEs and corresponding number of NCEs were counted in each group. statistical analysis were done using T-test

## RESULTS

Evaluation of micronucleus test the polychromatic erythrocytes (PCEs) and normochromatic erythrocyte (NCEs) done for all four experimental groups.

Table 2. Frequency of micronucleated polychromatic erythrocytes (PCEs) and normochromatic erythrocytes (NCEs) in the bone marrow cells of sodium Benzoate (SB), fenugreek (*Trigonella foenum greacum*) seed extract treated group of mice (*Mus musculus*) for 35 days

Treatment	PCEs			NCEs			PCEs+NCEs		
	Score	Mn	%±S.E	Score	Mn	%±S.E	Score	Mn	%±S.E
Control	2187	4	0.18±0.08	1957	3	0.15±0.08	4144	7	0.16±0.06
SB	2253	83	3.68±0.39	2173	41	1.88±0.28	4426	124	2.80±0.24
F	2150	3	0.13±0.07	1925	2	0.10±0.07	4075	5	0.12±0.04
SB+F	2285	7	0.30±0.11	2125	5	0.23±0.10	4410	12	0.27±0.07

Total 7 (0.16±0.161) micronucleus formed in control group among which PCEs were 4(0.18±0.089) and NCEs were 3(0.15±0.083). This showed low frequency of micronucleus in control group. The second group, the sodium benzoate (SB) treated group had total micronucleus 124(2.80±0.246) comprising PCEs 83(3.68±0.396) and NCEs 41(1.88±0.289). The result showed that the treatment of sodium benzoate group was significant value comparison to control group thus sodium benzoate causes cells damages and lead to the formation of micronucleus. When fenugreek seed extract was given to the third experimental group it noticed a total of 5 (0.12±0.044) micronucleus, in which PCEs were 3 (0.13±0.077) and NCEs were 2 (0.10±0.070). These value were less than the control group, so fenugreek seeds showed a potential to decrease in the formation of micronucleus than control group and was insignificant. When fenugreek seed powder was given concurrently with sodium benzoate a total of 12 (0.27±0.077) micronucleus were formed among which PCEs were 7 (0.30±0.114) and NCEs were 5 (0.23±0.103). Thus it is concluded that fenugreek seeds was effective in decreasing the formation of micronucleus induced by Sodium benzoate. The occurrence of micronucleus was comparatively higher in PCEs in all four groups.

Table 3. The relative ratio of PCEs% and NCEs %

PCEs/NCEs Ratio	C	SB	F	SB + F
	1.2	2.0	1.3	1.3

The relative ratio of PCE% and NCE% (P/N) (Tab-3) in control and fenugreek seeds treated group was almost same, so it suggest that the frequency of formation of two types of cell is equal. The relative ratio of PCE% and NCE % of Sodium benzoate group and concurrent treated group (sodium benzoate and fenugreek) was 2.0 and 1.5 respectively which suggest that the erythrocyte formation process was affected by sodium benzoate and fenugreek seeds. This result suggest the fenugreek seeds is potent ameliorating agent against sodium benzoate induced genotoxicity in bone marrow cell of mice.

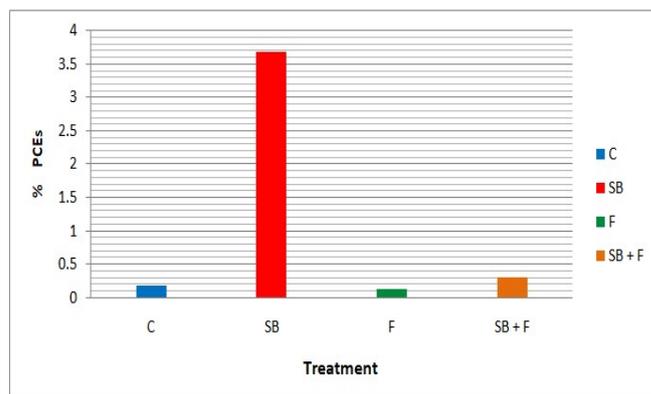


Figure 1. Graph Showing the incident of micronucleus in PCEs in four different groups of treatment.

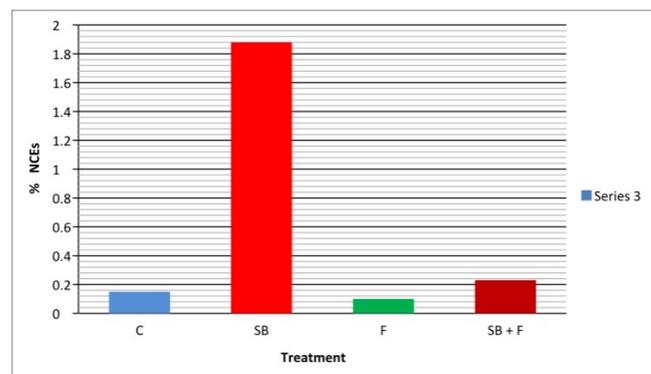


Figure – 2. Graph Showing the incident of micronucleus in NCEs in four different groups of treatment

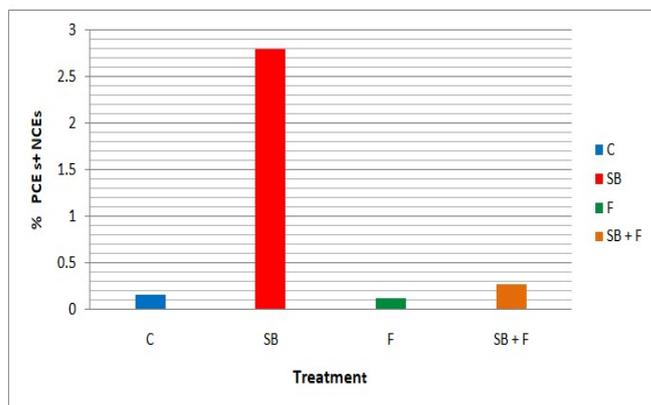


Figure –3. Graph Showing the incident of micronucleus in PCEs + NCEs in four different groupsof treatment

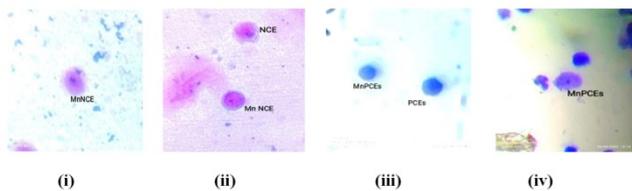


Figure – 4. Micronucleus in bone marrow cells of mice (*Mus musculus*) treated with sodium benzoate. i,ii) NCEs iii,iv) PCEs

## DISCUSSION

From the above result it can be assumed that sodium benzoate is a potent genotoxic agent which causes increase in the formation of micronucleus in both PCE and NCE. The toxicity of sodium benzoate may be implemented because the formation of toxic substance.

## CONCLUSION

From the above discussion it is concluded that sodium benzoate is a genotoxic agent which induces micronuclei formation and fenugreek seeds can reduce its toxicity due to presence of antioxidant, flavinoids (Blumenthal *et al.* 2000, Sauvare *et al.* 2000, Meghwal and Goswami 2012), Polyphenols (Anuradha *et al.* 2001, Kaviarasan *et al.* 2007, Preet *et al.* 2005), Trigonelline (Anuradha *et al.* 2001), Saponins (Gupta *et al.* 2001), richness. Therefore we must supplement our diet with antioxidants rich species. So that toxicity of various chemical preservatives can be minimized.

**Acknowledgments :** I thankfully acknowledge my Ph.D. supervisor Prof. & Head Dharmashila Kumari, and special thank to Prof. Ashok Kumar Thakur & Abha Rani, Department of Zoology, T.M. Bhagalpur University for their affectionate guidance and providing necessary laboratory facilities for the completion of this research work.

**Conflicts of Interest:** The authors declare no conflicts of interest.

**Author Contributions:** Experimental work and analysis has been perform by GR, Manuscript preparation and Discussion done by DK, and technical support given by AR.

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