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RESEARCH ARTICLE

SODIUM BENZOATE INDUCED GENOTOXIC EFFECT AND ITS AMELIORATION BY FENUGREEK (*TRIGONELLA FOENUM GRAECUM*) SEED EXTRACT IN MICE (*MUS MUSCULUS*) SPERM CELLS

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ABSTRACT

The food preservatives play an important role in the food industry which increases the life of food and protecting them from spoiling cause by micro-organism. Several investigation have been found that the food preservatives have various health problems leading to liver, kidney, etc. damages, it has genetic effect also if consume long time. The present investigation is carried out to study the effect of food preservative (sodium benzoate) and its amelioration by fenugreek (*Trigonella foenum graecum*) seed extract in mice (*Mus musculus*) sperm morphology. Twenty four adult male mice with average weight 25-35 gm have been used. They have been divided into four groups (6 mice to each group). First group included control with normal diet. The second group was treated with sodium benzoate at a concentration (1.125 mg/ml). The third group was treated with ameliorating agent fenugreek seed at a concentration (200 mg/kg) and the fourth concurrent (sodium benzoate + fenugreek) group with same concentration. After given the treatment of 35 days, the animals were sacrificed and samples were taken for the study of sperm head morphology. The result shows that the sperm abnormalities in sodium benzoate (E211) were significantly higher (11.08 %) than control (2.23%). The concurrent treatment of fenugreek seed extract with sodium benzoate (E211) has (2.28%) value. This value is highly significant then sodium benzoate treated group and almost equal to the control group. Different types of cytological abnormalities were observed in this investigation. The various abnormalities including pin-headed, funnel shaped, hook-less, club-headed, banana shaped, coiled tailed, double-headed, hammer shaped, spear shaped were observed.

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INTRODUCTION

With the increase of the production of processed food the food additive play an important role in the food industries. Preservatives are the substance added to food to prevent from decomposition or spoiling. It is also increases the life of the food and keep the food 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. Now a days many preservatives are used in food industries among them sodium benzoate is commonly used preservative. Fresh and dried food are always added preservatives to extend the life span of the food. The benzoate group such as sodium benzoate and potassium benzoate in diary industry. Food and drug administration (FDA) allows using sodium benzoate at 300 mg/kg. In several investigation it has been found that if it consume long time even though 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) . Sodium benzoate has short term effect also, it causes irritation of eyes, skin and respiratory tract, but if consume long term may cause large skin sensation (Lucio *et al.*, 2009) Research has also shown the formation of benzene in the reaction between benzoic acid and ascorbic acid (vit-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 (E211) significantly impaired memory and motor coordination and decreased the GSH and increased MDA level in brain (Muhammad jawad *et al.*, 2017). 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 plants for curative health care for long time (Jakhar et al., 2015). The very effective plant of Indian species "fenugreek" commonly called methi. 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 (Emtiazay et al., 2018). It has anticancer potential (Bain et al., 2017). A number of plants products such as neem leaf extract (Kumari and Chaurasia, 2007), Papaya fruit extract (Singh and Kumari, 2013), grape seed extract oil (Al-Attar, 2017) etc. reported to have beneficial effect against genotoxicity in mice.

MATERIALS AND METHOD

To evaluate the genotoxic effect of chemical preservatives and its amelioration by fenugreek seed, 4-6 week old albino mice (male) with an average weight ranging from 25 gm to 35 gm was used as test animal (experimental animal). Mice were collected from animal house colony, University Department of zoology, Tilka Manjhi Bhagalpur University, animals were grouped into the cage and they were provided normal Laboratory Condition with normal diet throughout the experimental period. Experimental mice were treated with preservative Sodium Benzoate (SB) in powdered form and fenugreek seed were dried and powdered for the purpose. Mice were divided into 4- groups, 6 mice in each group as shown in Table 1, for treatment duration of 35 days.

Table 1.

SL No.	Experimental group	Symbol	Dose
i	Control	C	Normal Diet
ii	Sodium benzoate (E211)	SB	1.125 mg/ml (Javed et al., 2017)
iii	Fenugreek	F	200 mg/kg (Roaa et al., 2016)
iv	Sodium benzoate + Fenugreek	SB + F	As i and ii

The slide were prepared by sperm suspension obtained by removing the cauda epididymis using methodology of (Wyrobek et al., 1983) approx 500 cells were screened for each group. The study of sperm head morphology under microscope (Das and Nayak, 1988).

STATISTICAL ANALYSIS

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

RESULT AND DISCUSSION

- Different types of abnormalities were observed including pinheaded, banana shaped, double headed, hammer shaped, coil shaped, tail less, funnel shaped, ring shaped after treated with sodium benzoate group.
- The sperm head morphological abnormalities in sodium benzoate group were significant (11.08%) which is higher than control (2.23%) group. After treated with fenugreek seed extract the abnormalities was (2.91%) which is almost equal to control group.

- However concurrent treatment of fenugreek seed extract with sodium benzoate shows the value 2.28 % 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.

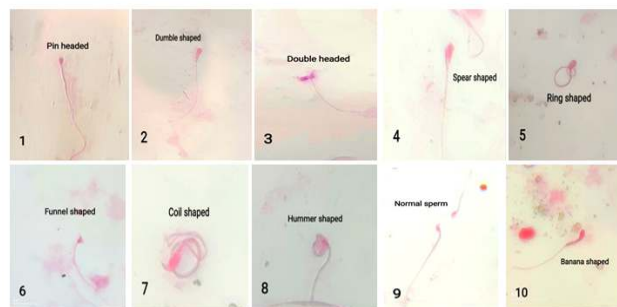


Figure 1. Abnormalities examined under microscope in sperms cells of chemical preservative sodium benzoate (E211) treated group

Table 2.

Treatment Variant	Symbol	No. of sperm studied	No. of abnormal sperms	% ± S.E
Control	C	492	11	2.23 ± 0.66
Sodium Benzoate	SB	478	53	11.08 ± 1.43 a
Fenugreek seed	F	412	12	2.91 ± 0.82 b
Sodium Benzoate + Fenugreek seed	SB + F	482	11	2.28 ± 0.67 b

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

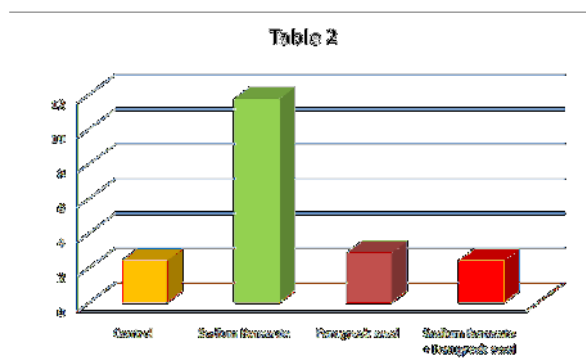


Figure 2. Graph showing the percentage of four experimental variant group

CONCLUSION

From the obtained results it is concluded that sodium benzoate (E211) produces genotoxic and mutagenic effect on sperm head morphology 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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