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Assessment of Genotoxicity of BHT as Food Preservatives Using *Allium Cepa L.* as a Test Plant

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ABSTRACT

Preservatives play a crucial role in modern food preservation, ensuring the safety, quality, and longevity of food products. Preservatives are substances added to food to inhibit the growth of microorganisms, delay spoilage, and extend shelf life. A synthetic substance called butylated hydroxyl toluene (BHT) is frequently added to processed foods as a preservative. BHT is an antioxidant that is soluble in oils and animal fats, much like the artificial preservative butylated hydroxyl anisole (BHA), with which it is frequently employed (in fact, it is more soluble than BHA). BHA and BHT are both used to prevent oxidation and rancidity, therefore extending the shelf life of processed foods BHT appears to have the ability to decouple phosphorylation, a crucial process that produces cellular energy. This would reduce the amount of cellular energy available to power the body's cells. Assessment of genotoxicity of BHT was done by using *Allium cepa L.* as a test plant. There has been increasing cases of cancers, tumors and complicated ailment from the population across the world. There is therefore, a need to control the use of these preservatives to the minimum safe levels in chemically preserved food products and ingredients.

Keywords: Preservatives, butylated hydroxytoluene (BHT), rancidity, phosphorylation, genotoxicity .

Introduction :

Preservatives are substances added to food to inhibit the growth of microorganisms, delay spoilage, and extend shelf life. They can be classified into two main categories: natural and synthetic. Natural preservatives include substances such as salt, sugar, vinegar, and spices, which have been used for centuries to preserve food. Synthetic preservatives, on the other hand, are chemical compounds specifically designed to prevent microbial growth and oxidation in food products. Examples of synthetic preservatives include benzoates, sulfites, nitrites, and sorbates.

Food Preservation is basically done for three reasons.

In order to maintain the inherent qualities of the food.

To ensuring that the look of food is maintained.

To boost the value of food that can be stored for longer periods of time

Benefits of Preservatives: The use of preservatives offers several benefits to both food producers and consumers:

Food Safety:

Preservatives help prevent the growth of harmful bacteria and pathogens, reducing the risk of foodborne illnesses and ensuring the safety of food products

Reduced Food Waste:

By extending the shelf life of perishable foods, preservatives help reduce food waste by minimizing spoilage and allowing for longer storage and distribution.

Concerns and Considerations: Despite their benefits, the use of preservatives has raised concerns among some consumers and health experts.

Convenience: Preserved foods are convenient for consumers, as they can be stored for longer periods without the need for frequent restocking or refrigeration.

Health Risks: Certain synthetic preservatives, such as sulfites and nitrites, have been linked to adverse health effects in sensitive individuals, including allergic reactions and increased cancer risk.

Regulatory Oversight: Government agencies regulate the use of preservatives in food products to ensure safety and compliance with established guidelines and maximum allowable levels.

Category of food preservatives.

The three main types of preservatives,

(i) **Antimicrobials:** Because they kill or slow the growth of germs, yeast, and moulds, nitrites and nitrates are in charge of avoiding botulism in meat products. The presence of sulphur dioxide helps prevent the further degradation of fruits, wine, and beer. Jams, salads, cheese, and pickles all use anti-fungal chemicals like benzoates and sorbates in their manufacturing.

(ii) **Anti-oxidants:** When exposed to air, fats and oils in food degrade, causing rancidity. These either delay or prevent this process totally. There are three main types of antioxidants: The redox potential of protective agents, such ascorbic acid, is lower than that of the medicine or excipients, whereas the redox potential of antioxidant synergists, like sodium edetate, is higher. Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are two examples of true antioxidants that may interact with free radicals to inhibit chain reactions.

(iii) **Anti-enzymatic preservatives**: Even after harvesting, these chemicals still prevent foodstuffs from undergoing certain enzymatic processes. To illustrate the point, erythorbic acid and citric acid inhibit the action of phenolase, the enzyme that gives sliced fruits and potatoes their characteristic brown hue.

Butylated hydroxytoluene (BHT) :A synthetic substance called butylated hydroxytoluene (BHT) is frequently added to processed foods as a preservative. BHT is an antioxidant that is soluble in oils and animal fats, much like the artificial preservative butylated hydroxyanisole (BHA), with which it is frequently employed (in fact, it is more soluble than BHA). BHA and BHT are both used to prevent oxidation and rancidity, therefore extending the shelf life of processed foods. BHT is often applied to the packing material, where it vaporizes into the food during storage, as opposed to being put directly to the meal itself.

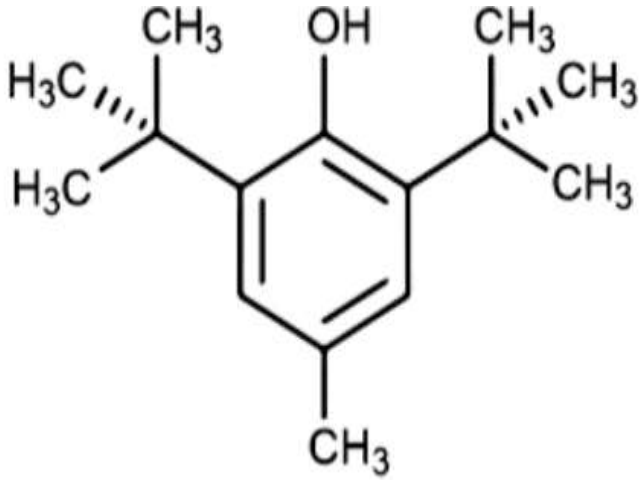

Figure 1: Butylated hydroxytoluene (BHT)

Harmful effects : It has been discovered that BHT causes reversible liver enlargement and inhibits normal development patterns in animals, among other negative consequences. BHT has significantly altered the brain and behavior of animals when it is present at high levels. It has been discovered that BHT inhibits the enzymes that phagocytes, or white blood cells, employ to kill germs, which interferes with the immune system's ability to operate normally. Furthermore, BHT appears to have the ability to decouple phosphorylation, a crucial process that produces cellular energy. This would reduce the amount of cellular energy available to power the body's cells.

Material and methods :

The food we eat now is processed and more diverse than in the past. As a result, one of the main issues for human health is food quality. Many nations have put in place rules that outline acceptable levels of specific chemical additives and harmful substances in food products and label those components according to their concentration in order to guarantee the nutritional value and safety of food. In this study we have selected food preservatives, popularly used in India that is butylated hydroxytoluene (BHT). BHT is referred to as an antioxidant. When it comes to viral infection, BHT may disrupt or harm a particular protective outer coating of a viral cell, which prevents the virus from proliferating or further harming its host. Additionally, BHT can be used to treat acquired immunodeficiency syndrome and genital herpes. There are a few pieces of evidence suggesting BHT causes allergic reactions.However, BHT is a food additive that is widely used in dehydrated mashed potatoes, salted peanuts, potato crisp, and packet cake mix. There is evidence to support the claim that certain food additives have genotoxic antimicrobial effects across a variety of test systems. Additionally, some of these preservatives are

highly noticeable substances that aid in reducing or decreasing equational division and promoting or increasing different kinds of deoxyribonucleic acid idiosyncrasy along with an elevated concentration while also lengthening the duration of therapies.

Assessment of genotoxicity

The toxicology of food preservatives can be evaluated using the *Allium cepa*, or onion, root tip test, which assesses their cytogenetic effects. This test examines the potential for preservatives to induce chromosomal abnormalities, such as breaks or changes in mitotic figures, in onion root cells. The results can help determine the genotoxicity of a preservative, indicating its potential to damage DNA and cause genetic mutations. At 1000 ppm, 1500 ppm, 2000 ppm, 2500 ppm concentrations for 4 h, 8 h and 16 h of exposure period, the effects of BHT, were examined and tested on Allium cepa root tips. Cytologic test results exhibit significant inhibition in the mitotic index as food preservative concentration increases compared to the control group Cytological aberrations such as C-mitosis, bridges, stickiness, multipolarity, and cell death were observed— with abnormality rate increasing as time duration and concentration of food preservatives' increase. The preservatives generally decreased mitotic index (MI) of Allium cepa treatment groups as compared with the controls.

The root-tip of *A. cepa (*2n = 16) were used as the test material and selected food preservative were butylated hydroxytoluene (BHT),as the test substances. Small bulbs (1.5–2.0 cm in diameter) of the common onion, *A. cepa* (2n = 16), were procured from local market. Prior to initiating the test, the outer scales of the bulbs and the dry bottom plate were removed without destroying the root primordia. Bulbs of *A. cepa* were placed in sand and allowed to germinate at room temperature (25 ± 2 ◦C). When the newly emerged roots were 1–2 cm in length, the bulb were removed from the sand washed and placed on a blotting paper to remove excess water and used for the treatment. Roots of *A. cepa* were treated with a series of concentrations, i.e. 1000, 1500, 2000, 2500 ppm (w/v, preservatives/distilled water), for 4, 8, 16 h. The control bulbs were grown in distilled water. After treatment, root tips were cut and washed and treated with 0.05% colchicines for 3 h. Root tips were washed and fixed in a mixture of ethanol and glacial acetic acid (3:1) for 24 h, washed thrice with distilled water, and then dyed with 2% aceto-carmine. Squashes were prepared as suggested by Sharma, 1980 to determine the mitotic index and the presence of chromosomal aberrations. Three replicates were performed for each treatment and scoring was done from the three roots of each replicate. A minimum of 500 well spread metaphase cells were scored for each concentration. The MI (mitotic index) was calculated for each treatment as number of dividing cells per approximately 500 cells. The percentage of aberrant cells was calculated on the number of aberrant cells per dividing cells scored at each concentration for each preservatives. The cytological abnormalities were scored in the mitotic cells and the results are shown in the tables and figures. Necrotic cells were identified by light microscopy using morphological characteristics of the nucleus which exhibits a pale cytoplasm or loss of cytoplasm, and a damaged/irregular nuclear membrane with a partially intact nuclear structure. The most frequent abnormalities are shown in photomicrographs.

Mitotic index (MI) = $Total\ mitosis/Total\ cell \times 100$

Total abnormalities = Add all abnormalities (B+CB+BN+Lob+C.mitosis+Stickiness)

% of abnormalities = $Total\ abnormalities\ /Total\ mitosis \times 100$

Result and discussion :

Cytogenetic testing is the examination of chromosomes to determine chromosome abnormalities such as aneuploidy and structural abnormalities. Cytogenetic testing can be performed in a variety of situations, including solid organ malignancies, hematologic malignancies, congenital diseases. It can be performed prenatally after biochemical screening or ultrasound with abnormal findings. It is also used for parents with multiple miscarriages or significant findings in their pedigree analysis. Post natally, cytogenetic testing plays a role in distinguishing patients with mosaicism, intellectual disability, autism, or developmental delays. Cytogenetic analysis can also be utilized to diagnose malignancies, determine appropriate therapy for prognostic stratification. Table 1 shows cytogenetic analysis of *A. cepa* root exposed to different concentration of

butylated hydro toluene.

Table 1: Cytogenetic analysis of *A. cepa* root tips exposed to different concentration of butylated hydrotoluene for different periods

Total mitosis	MI (mean± S.E.[a])	B	Stick iness	CBand laggard	BN	Lobu lated nucle i	c- Mito sis	% of abno rmali ties	Total abnormalities
320.67±1.85	61.74±2.53b	0.83	–	–	–	–	–	0.26	0.83±0.33a
308.20±2.61	60.51±1.71ab	3	2	–	–	–	1	1.94	6.00±0.58b
324.33±1.73	63.67±.62ab	7.33	1	–	–	–	1.69	3.09	10.02±0.58c
299.67±2.45	59.10±2.29ab	1.33	3	–	–	–	2.67	2.33	7.67±0.33b
290.00±3.08	55.70±1.50a	4	4	–	–	–	1.33	3.22	9.33±0.88c
330.67±2.45	64.66±1.66b	–	0.52	–	–	–	–	0.16	0.33±0.33a
319.67±1.33	63.25±2.34b	2	–	–	–	–	4	1.98	6.00±3.21b
286.33±1.55	54.89±1.70a	3.67	2	–	–	–	4	3.37	9.67±2.40b
277.00±2.15	52.39±1.51a	6.45	4.67	–	–	–	2.67	4.98	15.67±0.33c
256.33±3.45	50.27±1.60a	8.24	2	1.45	2.00	1.00	2	6.51	16.69±1.15c
314.00±7.02	60.89±2.54d	2	–	–			–	0.64	2.00±0.00a
272.00±2.83	52.78±2.22c	2	3	1.67	0.67	2.00	1.87	4.12	11.21±0.00b
268.33±2.71	51.97±1.54bc	10	1.33	4	2.00	3.12	2.24	8.12	21.33±0.88c
248.67±5.36	46.34±1.80ab	6	4	2.57	5.33	2.33	4.33	9.12	22.69±1.00c
228.67±6.01	43.94±1.81a	12.33	5.67	1.33	1.67	5.00	5.33	13.7	31.33± .88d

MI – mitotic index

B – Bridges

MP – multi polarity

CB – chromosomal break

BN – binucleated.

Table 1 shows cytogenetic analysis of *A. cepa*root exposed to different concentration of butylated hydrotoluene for different periods During 4 h of treatment total mitosis, mitotic index and c-mitosis were 290, 55.70 and 1.33, respectively at 2500 ppm of A.cepa root. However, the percentage abnormalities and total abnormalities were 3.22 and 9.33, respectively. On the other hand, 8 h treatment showed 256.33, 50.27 and 2 of total mitosis, mitotic index and c-mitosis, respectively at 2500 ppm concentration of A. cepa root. The percentage abnormalities and total abnormalities at 8 h were 6.51 and 16.69, respectively. Finally, at 16 h the total mitosis, mitotic index and c-mitosis were 228.67, 43.94 and 5.33, respectively at 2500 ppm. The percentage abnormalities and total abnormalities at 16 h were 13.7 and 31.33, respectively. Interestingly, chromosomal break, binucleated and labulated nuclei were mostly seen during 16 h of treatment. Decrease in mitotic index might be due to inhibition of DNA synthesis (Schneiderman et al, 1971) or a blocking in the G2-phase of the cell cycle, preventing the cell from entering mitosis (Sharma, 1980). According to Jain et al., 1988 inhibition of DNA synthesis due to decrease in ATP level and pressurefrom the functioning of energy producing centre. Earlier reports revealed that food preservatives and several other chemicals have been reported as inhibitor of MI.

Conclusion :

In spite of the fact that food preservatives play a significant role in the food industry , the multiple negative consequences that are associated with them continue to be a problem that we need to strive as hard as possible to fix. This is because preservatives are able to alter the chemical composition of the food. From the present investigation it appears that BHT which is frequently used in packaged food have genotoxic effects on the chromosomes in a reliable plant assay, then it might be harmful to the other organisms especially to human being. In view of above, it is necessary to be aware about the level of chemicals at the time of using. if we consume an excessive amount of these products on a regular basis, it will have a detrimental effect on our health. In spite of the fact that food preservatives serve a crucial role in preserving the safety of food and extending its shelf life, there is a possibility that

excessive usage of these compounds or prolonged intake of them might have adverse effects on human health. Among the many various forms of food preservatives, some of the possible impacts that are associated with them include allergic reactions, an increased risk of certain cancers, cardiovascular difficulties, and metabolic alterations.

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