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A REVIEW ON THE TOXICOLOGICAL EFFECTS OF FOOD PRESERVATIVES USING ALLIUM CEPA L. AS TEST PLANT

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ABSTRACT

Food preservatives are predominant in preserving foods to steer clear of food deterioration, spoilage and poisoning. The effects of food preservatives have minor and major concerns in terms of health. Many researchers have reported that some of these preservatives can even cause death. With that, this review article focuses on summarizing the main toxicological findings of five certain preservatives of food to *Allium Cepa* L. as the test plant. The review article is the outturn of different literature reviews of all relevant papers from peer review and high impact journals. In an effort to pique the reader's interest in the selected food preservatives that were being tested in *Allium cep*a. Cytologic tests result in a Cytological abnormality that the mitotic index of the tested Allium cepa treatment group decreases as it was compared to the controlled groups. As indicated, there are Cytological abnormalities in which the deoxyribonucleic acid synthesis inhibited its activity that resulted in inhibition of plant growth and a drastic reduction of its protein content. Furthermore, food preservatives play a crucial role in preserving food shelf life and its physical appearance through time. Some studies have found that preservatives have high effects in genetics which are considered lethal and dangerous to any living species. As time progresses, further studies and research may be able to obtain a drastic change and a compelling result in the preservation of foods that affect every organism.

Keywords *- Food preservative; Allium cepa; Genotoxic*

1. INTRODUCTION

One of the earliest methods that humans have utilized is food preservation. Due to the massive world's growing population, and to prevent foods from oxidizing, flavor enhancers (preservatives) have been habitually added to provide longer expiration and freshness to a variety of foods. Preservatives like sugar and salt are regularly utilized. Chemical preservatives are also widely employed in the food industry and are essentially guaranteed in terms of preservation. Certain antimicrobial food additive compounds are genotoxic (known to damage DNA and cause cancer) in several test systems. In this review article we have selected five (5) commonly used preservatives for food namely, BHA or Butylated hydroxyanisole, BHT or Butylated hydroxytoluene, PG or Propyl gallate, SN or Sodium nitrate, and SA or Sorbic acid.

BHA or Butylated hydroxyanisole, BHT or butylated hydroxytoluene, and PG or propyl gallate are considered as synthetic antioxidants and are frequently employed as preservatives in the food products. In the food industry, synthetic antioxidants can be introduced as direct additives or indirect additives through the diffusion from packaging materials [1]. A Japanese house musk shrew was tested using the carcinogenicity test utilizing butylated hydroxyanisole, all animals succumbed from gastrointestinal hemorrhage when given higher doses; however, the groups of animals with lower dose, and mild-dose revealed a 50% – 60% of occurrence of adenomatous hyperplasia (in lungs) [2]. It was suggested the greater doses of BHA may cause cancer in humans. BHT is known for its chemical name 3,5-di-tert-butyl-4-hydroxytoluene. In vitro investigations have shown that BHA has a modest estrogenic impact as well as anti-androgenic effects. BHT was less estrogenic than BHA and was included in the non-estrogenic chemicals list based on cell proliferation studies (Pop et. al., 2013) [3]. In vitro testing revealed that PG is one of the strongest ligands among the xenochemicals. According to studies estimating BHA and BHT daily consumption, the population can receive close to the ADI (acceptable daily intake) through an ordinary diet, and a small percentage population may be susceptible to higher dose compared to ADI [3]. The additives BHT and BHA are said to be cytotoxic, which also causes damage to cardiac (cultured cells) and cell lysis following prolonged exposure at higher doses. PG is frequently combined with BHA and BHT. Chewing gums, various meat products, and base for chicken soups [4]. Moreover, PG occur naturally, and is considered as a propyl ester (gallic acid). It has been shown to be an antioxidant that can help retain freshness and preserve the nutritional and therapeutic products. SA or Sorbic acid is the version of the unsaturated fatty acid (trans–trans) hexa2,4-dienoic acid. It's a powerful antibacterial agent that works mostly on molds and yeast, but is also effective against bacteria [4]. At greater concentrations, sorbic acid is not harmful, however potassium salt and SA have been shown to cause sister chromatid exchange (SCE) and chromosomal aberration [5]. Sodium Nitrate is the preservative, coloring, and flavoring ingredient typically found in, luncheon meat products, hot dogs, corned beef and any red meats. It is a naturally occurring substances and white crystalline powder which can be found in the environment in various forms. Adults have developed cancer as a result of higher nitrate levels, as well as an increased risk of brain tumors, leukemia, and nasopharyngeal cancer [5].

According to experiments, food preservatives produce toxicity in organisms when fed in excess amounts. Despite the preservatives are present in our meals or foods, we do not have adequate data and information regarding its negative impacts. Utilizing Allium cepa to estimate toxicity has certain advantages. It usually has a substantial number of chromosomes and low number of chromosomes, a elevated meristematic cells proliferative rate, a simple assay, and does not necessitate extensive plant laboratory which are a valuable resource for evaluating genetic changes caused by environmental toxins (Khan et. al., 2020) [6]. Allium cepa L., has been proven to be a effective organism model in studies (genetic) for chromosome aberration assays. Variety of food preservatives' toxicity were assessed using Allium cepa as a marker. Bioassays utlizing plant samples are more straightforward and conscious than animal bioassays for determining a chemical compound's genotoxicity and cytotoxicity [6]. Previous studies have considered allium cepa as one of the leading-established test systems. Due to the good correlation with the test system (mammalian), it was commonly used to assess and evaluate the potential genotoxicity of chemicals in the environment. Considering the fact that the root meristem cells (A. cepa) were discovered to produce similar or near effects in the lymphocytes of human as a variety of insecticides and pesticides have shown to induce and influence the Allium cepa's genotoxicity [5]. Hence, in this article review, we looked at the subsequent journals and articles on the food preservatives focusing on Butylated hydroxyanisole, Butylated hydroxytoluene, Propyl gallate, Sodium nitrate, and Sorbic acid, as well as their toxicity in human, plant and animal models, and their toxicity in the test plant Allium cepa L. Allium cepa has been proven to be the finest model plant for environmental monitoring and cytological investigation.

2. METHODS

This article review utilized reliable evidence and published literature from journal databases in ScienceDirect, Elsevier, Research Gate, and PubMed. The review included up-to-date journals and articles that focused on the food preservatives butylated hydroxyanisole, butylated hydroxytoluene, propyl gallate, sorbic acid, and sodium nitrate, their toxic effects on human, animal, and plant models, and their corresponding toxicity on *Allium cepa L* as a test plant. After gathering relevant and substantial literature, the data were evaluated based on their validity, relevance, significance, and appropriateness to the research topic.

Butylated hydroxytoluene::

Butylated hydroxytoluene, a cresol derivative, is one of the important laboratory made chemicals that is added as a food preservative, and a widely used medicine for humans. BHT is known as an antioxidant. In terms of viral infection, BHT may interrupt or damage the specific protected outer layer of a viral cell that results in incapability of the virus to multiply or cause more damage to its host. BHT is also useful in treating genital herpes and acquired immunodeficiency syndrome or AIDS [7]. A few existing pieces of evidence were found that BHT leads to allergic reaction [8]. However, BHT is an additive of food that are extensively used in packet cake mix, potato crisp, salted peanuts and dehydrated mashed potatoes [9]. In some supporting evidence that some food additives have been proven to have a antimicrobial effects that are genotoxic in different arrays of tests or test systems [10][11]. And some of these preservatives are very prominent agents that help decrease or lessen the equational division and increase or promt a various types of deoxyribonucleic acid idiosyncrasy together with an escalated concentration at the same time increased the period of treatments [12].

Butylated hydroxyanisole:

Butylated hydroxyanisole is among the many antioxidants that are added in cosmetics, foods, packaging, and pharmaceuticals. BHA is available as white waxy flakes and is very soluble in fats and oil, but is insoluble in water. It is capable of preventing lipid oxidation— particularly with its chain-breaking function in lipid peroxidation— making it one of the mainly used food preservatives. It also has superior antioxidant effectiveness in the prevention of flavor and color deterioration of essential oils. Since 1947, it has been integrated as an antioxidant in the production of fatty foods and edible fats. It is also added to confectionery products and cereals. In addition, it is essential in the preservation of food by inhibiting fats and oils from going rancid due to oxidation. It also prevents oil-soluble vitamins from going inert [13-16].

It is also incorporated into packaging materials to preserve foods inside packages by antioxidant volatilization [13]. During the late 1950s, the food industry authorized its usage in delaying the deterioration, discoloration, and rancidity of fats, oils, and lipid-containing foods during the processing, packaging and storage stages to extend the products' shelf-lives [14][16]. Presently, it is used in various food products within the permissible range of 2ppm to 5000ppm in various food product categories across countries. [17]. BHA is also utilized as an antioxidant in dental sealants along with BHT and is suggested by present-day studies to be used clinically in treating several diseases. Its antioxidant property is capable of suppressing autoimmune diabetes development in rats and is reported to protect rats from multiple xenobiotics and acute radiation exposure [14][18].

However, multiple studies and literature have reported in vivo and in vitro toxicities of BHA. Several scientific works of literature reveal that BHA shares inexplicable properties with other antioxidants, referring to BHA as both antioxidant and pro-oxidant, anticarcinogenic and carcinogenic, and a promoter of tumors [15]. An in vivo study [19] using a rat model inferred that BHA is a mild inhibitor of energy metabolism and is both an antioxidant and a pro-oxidant. Results of the study revealed that BHA decreases respiration and gluconeogenesis while increasing glycogenolysis and glycolysis. In addition, a robust database on BHA's mechanism of action indicates its rodent carcinogenic activity from chronic high-dose exposure and is toxic to rat liver. BHA however does not pose safety concerns at recommended levels in our diet; in vitro mammalian test systems indicate that BHA does not show mutagenicity hazard [13].

In the same manner, the genotoxic properties of BHA could affect developmental and reproductive systems where BHA may exert estrogenic and anti-estrogenic actions in vivo [16][20-21]. Also, Ham et al. [22] elucidated BHA toxicity on the cell lines of mouse testis where BHA induced dysfunction in the testis cells of rats as manifested by the dysregulation of regulation and stimulated reticulum stress [22]. Rodents and humans have different responses to endocrine-disrupting chemical exposure but it was speculated that their toxicological metabolism and steroidogenesis are similar, making this literature significant in the effects of BHA on humans [23][24]. Furthermore, BHA increases anemic conditions, production of cytokine, and

pancytopenia; and poses hematoimmunotoxic activity in adult male albino rat models. This indicates that BHA evokes immunosuppressive effects in rats and may reduce the immune system's response to pathogens and tumors [25].

Moreover, BHA exerts neurotoxic effects by promoting accumulation of systolic calcium and stress in the endoplasmic reticulum of astrocytes. Astrocytes are glial cells that provide nutritional support, perform synaptic functions and regulate inflammation in the human brain [26]. In vitro and in vivo results from the study of Park et al. [27] of BHA's neurotoxic effects provided evidence of cytotoxicity on human astrocytes. BHA was found to have neurotoxic effects on human astrocyte NHA-SV40LT cells and zebrafish whole brain development, suggesting that BHA causes cycle arrest and downregulates regulatory protein expression, causing brain and nerve development to be disrupted. This indicates that BHA toxicity is possible in doses higher than the authorized one.

Sorbic acid:

Sorbic acid, with a chemical formula of $C_6H_8O_2$, is an unsaturated fatty acid, straight chain. It is hexa 2,4-dienoic acid in its trans–trans form. It is a compound occurring by nature that has become the world's most frequently used food preservative, and it is in charge of the global food industries. Sorbic Acid is a powerful antimicrobial drug that operates mostly against molds and yeast, although it is also a bacterial growth inhibitor. It is remarkably good in preventing mold development, which may degrade food and spread deadly illnesses. The most common uses include desserts, dairy, sour soup container, fruit, liquid, sausage, and ground beef [12][29]. In comparison to nitrate that can also generate cancerous effects, sorbic acid is a favored preservative. It prevents the development of C. botulinum as well as further decreases nitrosamine production. Its usage helps people by avoiding microbial activity while transporting and storing meat securely. Sorbic acid is also found in canned foods such as pickle, prune, maraschino cherry, fig, and ready-to-eat salad. It is administered to meals by spraying or immersing it in a sorbic acid plus water solution [29][30].

Sorbic has not been linked to any health issues, and it is deemed to be non-toxic. Sorbic Acid, in accordance with JECFA, is not harmful at higher concentrations, and respective ADIs are specified at 25 mg/kg body weight [1]. Sorbic acid and its salts have undergone a series of testing, which includes rapid, brief, and long term toxicity/carcinogenicity tests, as well as multiple propagation and teratogenic effects investigations. Notable investigations reveal sorbic acid and sorbates having extremely little toxicity in mammals, including in long-term tests at as much as 10% of the intake, and are cancer-causing. In vitro and in vivo, they are not mutagenic or clastogenic. The decreased toxicity is explained because of the case that sorbic acid is rapidly absorbed by mechanisms similar to those used by additional fatty acids. There are a few incidences of idiosyncratic intolerances in humans that were cited. It may combine with nitrite to create mutant-causing compounds under severe conditions such as in high concentrations and temperature, although these mutagens are unnoticeable under regular settings of usage, even in the brines used for curing [31].

Propyl gallate:

With the chemical name propyl 3,4,5-trihydroxybenzoate, propyl gallate is a phenolic antioxidant widely utilized in foods, pharmaceuticals, and cosmetics companies, particularly in emulsions, oils, waxes, and fats. [31]. The crystallized powder form of propyl gallate is white to cream-colored. It is the n-propyl ester of gallic acid. It is dissolvable in ethyl ether, oils, lards, ethanol, and polyethylene glycol (PEG) ethers of cetyl alcohol, but water solubility is minimal [32]. It is used as a stabilizer for synthetic vitamin A and transformer oils' components [33].

Since 1948, propyl gallate has acted as a food antioxidant to combat rancidity caused by the formation of peroxides in oils, fats, and foods rich in fat. It is used to suppress the oxidation of oxidation-sensitive ketones, aldehydes, and monoterpenes in some essential oils. Moreover, concentrations of propyl gallate in alcoholic and nonalcoholic drinks, fats and oils, snacks, baked goods, icings, gums, candies, meat, nuts, grain, dairies, and gelatinous foods range from 0.01484 to 0.00001 percent. Furthermore, The FDA has capped the overall antioxidant content of food to 0.02 percent of the product's fat or oil composition [34].

Although propyl gallate is regarded as an antioxidant in food and cosmetics, studies have shown that it can affect multiple cell types. Hamishehkar et al. documented that propyl gallate decreases intracellular glutathione levels and alters the redox characteristics of cells by elevating the number of reactive oxygen species by inhibiting superoxide dismutase (SOD) and catalase (CAT) [35]. Chinese hamster fibroblast cells were tested with propyl gallate at concentrations of 0.04 mg/ml in saline. At 0.023 mg/ml, 20% of the cells exhibited chromosomal gaps, breaks, exchanges, and fragmentation. The mutagenic potential of propyl gallate was assessed using L5178Y tk+/ mouse lymphoma cells. At all tested doses, significant mutagenic responses were observed [37]. A relatively high dose of PG as a food additive induces toxicity and carcinogenicity [34].

Sodium Nitrate:

Sodium nitrate with a chemical formula of $NaNO_3$ is a type of sodium salt of nitrate. This is considered a frequent type of nitrate used as additives or preservatives (food) in meat or meat products, and fish in particular, due to the natural or appealing distinct color and flavor retention and their antimicrobial properties. The inclusion or incorporation of sodium nitrate into processed meat products has an equivalent compound of roughly around 500 mg/kg varies by country [38]. Nitrates can also be found in plants like celery and spinach and in tap water as contaminants. Food-based nitrate consumption has been linked to several potential health risks. Around 60%–70% of these chemicals are quickly absorbed and eliminated in urine when they are ingested. In individuals, roughly (3%) of nitrate is excreted as ammonia or urea in the urine [39]. Nitrates can also pass into the circulatory system through stomach. Under a acidic stomach circumstances or in blood and tissues, a range of highly bioactive nitrogen oxide molecules is produced. When amines (secondary) are seen in the stomach, these may be involved in producing hazardous nitrosamines [39]. According to the World Cancer Research Fund, sodium nitrate has been linked to a variety of diseases, including reproductive toxicity, anemia, and thyroid hypertrophy. The inclusion of nitrates in processed meat, which can be further converted to nitrites, has been related to an elevated risk of cancer in the colon [40]. To provide the body with healthy compounds, it is better to intake nitrates from vegetables rather than salt from processed meats. Processed or red meats are low in nutritional value, but veggies are high in beneficial carbohydrates and fiber. These natural alternatives also help to reduce nitrosamine conversion. They prevent carcinogens from forming from the nitrates consumed [41].

According to several research, nitrates may play an etiologic role in poor chilbearing complications and other problems in health. A classification of compounds in genotoxic that compromise both nitrosamines and nitrosamides is calee N-nitroso compounds, it form sodium nitrates or nitrates as precursors. These compounds (N-Nitroso) have shown to cause congenital abnormalities in animal models, and their significance in pregnancy outcomes needs more exploration [42]. Humans or individuals are largely exposed and suceptible to nitrates through diet (food intake) and water supplies, moreover vegetables are supplying the most dietary intake (nitrates) every portion or serving. The nitrates are naturally found in all plant substance (field crops, and vegetables) as the plant develop they build up in a nitrate-rich environment [43]. Vegetables and fruits are the most significant sources of dietary intake of nitrates, as they contribute for 50% - 75% of the overall nutrient intake. Nitrate salts are given in little amounts to meats, poultry, and fish to preserve them; this has been a regular practice for generations. Exogenous sources and endogenous production both expose humans to N-nitroso compounds. Cured meats, beer, and smoked salmon are dietary sources of nitrosamines; these products may contain synthesized nitrosamines due to cooking and/or preservation processes [42].

Although several studies show the meat production is completely focused on lowering sodium nitrate levels, and the comparison between the vegetables and water contribute more to the nitrate levels in the diet than cured meat, [44]. One of the challenging problems in the meat market is reducing food additives, particularly nitrates, to consumer expectations. Because of the health dangers associated with nitroso compounds, consumers/public prefer additives which are natural over chemicals in meat compositions. As a result, research into reducing or eliminating the use of nitrates and the utilization of substances that are natural as substitutes has risen in recent years. Some manufacturers of processed meat have started utilizing natural nitrate sources such beetroot, celery juice, or spinach extract. Nevertheless, bacterial cultures degrade the nitrates in to nitrites in vegetables, which contribute to nitrosamine production. Bacteria with reductase activity in nitrates, such as micrococci and staphylococci , present naturally in any added meat processing, are primarily responsible for converting nitrates to nitrites. Cultures with reductase activity of nitrate to create nitrite, such as Staphylococcus carnosus, are also utilized in natural curing with natural sources such as concentrates of dried, natural fruits, and vegetable [38][45].

Sodium nitrates have become one of the disputable compound or substances in food preservatives. However, because nitrate is linked to health problems, its conversion (nitrite) and subsequent nitrgen metabolism of molecules to nitrosamines are linked to consumers' harmful adverse effects. On the other hand, many studies have linked the benefits of nitric oxide produced by nitrate conversion to blood pressure regulation and improved cardiovascular health. Because nitrates and nitrites are considered multifunctional food additives in meat curing, they are mainly connected with the meat industries [38]. In the meat market, nitrates and nitrites should be controlled due to their probable carcinogenic effect. However, determining the right substitute for the processing of meat is quite challenging because of nitrates multifunctional nature of nitrates.

3. RESULTS AND DISCUSSIONS

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, BHA, sorbic acid , propyl gallate, and sodium nitrate 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 (Figure 1) [5]. Cytological aberrations such asC-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 [5].

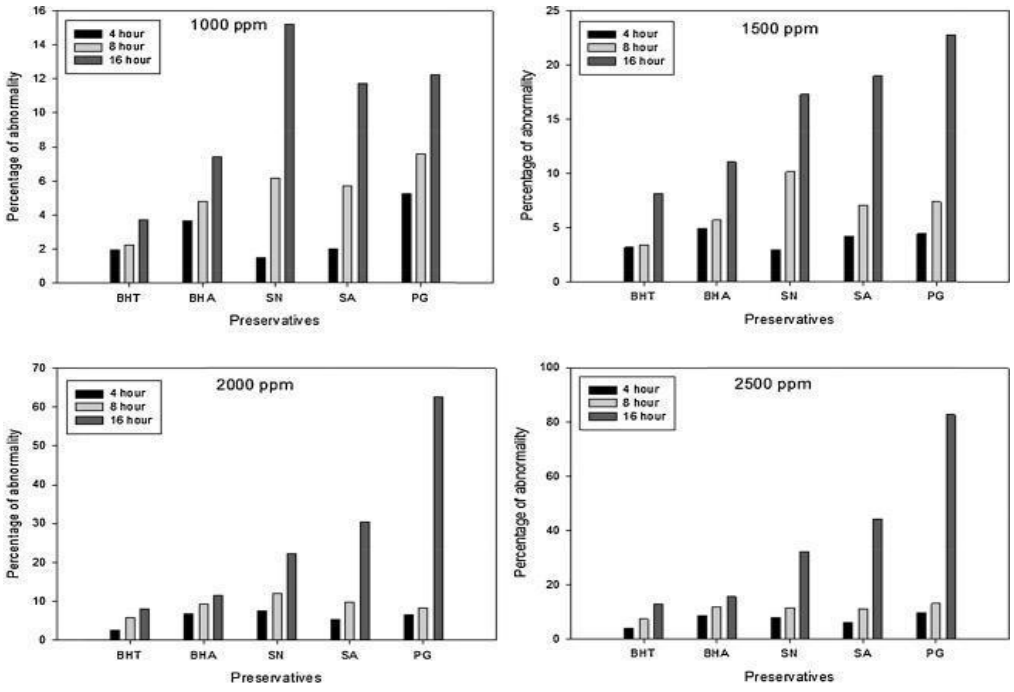

Figure 1. Graphical presentation of abnormality percentage of the five food preservatives at different concentrations and different time periods [5]

Specifically, roots treated with BHT decreased MI after 4 hours from exposure and significantly reduced its MI as exposure increases, compared to the control. However, it was insignificant at 1500, 2000 and 2500 ppm concentration at 8-16 hours of exposure. On the other hand, BHA showed significant mitotic index reduction at 1000 to 1500 ppm concentration after 4-16 hours of exposure. The reduced MI activity and intensified chromosomal aberrations substantiates the DNA synthesis inhibition activity of BHT and BHA [48][49]. Both impede the root development in Allium cepa, and decrease its protein content, indicating cytotoxic action [50]. In addition, BHT and BHA similarly affect the onion bulbs' genomic template stability (GTS) as reflected in the band intensity variation, and appearance or disappearance of DNA bands— indicating that both preservatives induce genotoxicity and cytotoxicity in Allium cepa root tips. Genotoxicity of BHT and BHA may possibly affect the lowering of mitotic index scores [49].

Sodium nitrate displayed a maintained reduction in MI after 4 hours of exposure at 2500 ppm dose concentration. It then exhibited an insignificant decrease at 8 hour of exposure and significant decrease in MI at 1500, 2000 and 2500 ppm concentration at 16 hours of exposure. The reduction in MI indicates increased frequency of abnormal mitosis and mitotic inhibition which showed similar results observed in the study of Antofie and Doroftei [51] that assessed the cytotoxic and genotoxic effects of varying sodium nitrate concentrations to the Allium cepa root tips. The same results were also observed in a study of Aledwany et al [52] on the root tips of onion bulbs treated with potassium nitrate. Abnormalities such as sticky chromosomes, chromosomal bridges and laggards, and increased chromatin expression during mitosis were similarly observed in the studies [5][51-52]. Moreover, sorbic acid significantly elucidated a decrease in MI at 2500 ppm dose and subsequently showed a maximum MI reduction at 16 hours of exposure. Concentrations of 1000, 1500, and 2000 ppm were ineffective in reducing MI. According to Samoylov et al [53], sorbic acid causes toxicity in Allium cepa roots which results in chromosomal aberrations that correspond with mitotic spindle disorder. Besides these, propyl gallate displays high effectivity and toxicity at 2500 ppm compared to the other preservatives at 16 hours exposure but was insignificant with the rest of the concentrations and exposure time. Nevertheless, PG causing stickiness of cells suggests its significant effect on the proteins of Allium cepa roots [54]. A statistically significant association of concentration and chromosomal aberrations was also most observed in propyl gallate.

Based on the gathered data, it is evident that all the chosen preservatives caused abnormalities and toxicities in Allium cepa roots. All preservatives exhibited significant reduction of MI in correlation to dose and longer treatment durations as compared to the control. The preservatives also showed a wide scope of abnormalities in the mitosis of *Allium cepa* root tips. Most significantly, PG showed the highest percentage of abnormalities observed.

4. CONCLUSION

Food preservation is a method that has long been used to prevent food from oxidizing and that increases shelf life as there is a growing population in the world. According to the current research, the five common preservatives that are often used in packaged foods which are BHA, BHT, sorbic acid, propyl gallate, and sodium nitrate, have chromosomal impacts that are genotoxic in a credible plant test (Allium cepa L.), which may be detrimental to other species, including humans. Through the data presented, it should be critical to be mindful of the levels of chemical compounds present while it is being used. Given the potential risks, it is with extreme caution that higher than the authorized concentrations of these compounds should not be incorporated as food preservatives. Therefore, additional cytogenetic study on the clastogenicity and genotoxicity among these preservatives is necessary to bring substantial results.

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