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Adverse effects of chemical preservatives: A review

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Abstract

Food preservatives are chemical or natural substances that are added to the food products which will help to increase the shelf life and also inhibits the growth of microorganisms that causes food spoilage. The objective of review is to know and understand the adverse effects of chemical preservative in food products. Even though the chemical preservative used is approved by the FDA, whereas the long-term usage of some preservatives can causedifferent health problems in humans including cancer. This review is also intended to understand how the preservative induce different health problem in the body if consumed for long term. It is not possible to review all the preservatives hence, mainly focused on sodium sulphite, sodium nitrite, sodium benzoate, Tertiary butylhydroquinoe (tBHQ) and Butylated hydroxylanisole (BHA) which are commonly used food preservatives in beverages, dried foodproducts, meat and other processed food. The long-term consumption of the food which is treated with the above-mentioned food preservatives showed signs of carcinogenicity, genotoxicity and other allergies. In future the usage of natural food preservative which is derived from plants and other natural sources can make a drastic change in growing health problems using chemical preservatives.

Keywords: *Chemical preservatives, Genotoxicity, Tertiary butylhydroquinone, Butylated hydroxyanisole, Sodium benzoate, Sodium sulphite, Sodium nitrite.*

Introduction

Preservatives are chemical or natural compounds that helps to inhibit the growth of microorganisms which results in food spoilage by inhibiting or stopping the fermentation, acidification or other decomposition of food. They also help to increase the shelf life of the food by preventing it from spoilage. Due to the increase in ready to eat and convenient foods has increased, the demand for chemical preservatives in market also came to a sudden rise (Mirza *et al*., 2017). Among the preservatives which are widely being used today, sodium chloride (salt) is perhaps the oldest preservative.

The use of food additives is directed by particular laws (specifically within the European Union - EU), considering the food where it can be used, most in usable amounts, chemical characterization and purity (Silva and Lidon, 2016). Long-term rat experiments revealed that large amounts of nordihydroguaiaretic acid (antioxidant) in diet were transformed to the corresponding orthoquinone, which is likely the source of the fecal bleeding and ulceration, as well as the mesenteric cysts. The alkyl gallates (antioxidants) in oils, margarine, sausage and meats were discovered to cause dermatitis in bakers and others who worked with alkyl gallates leads to cheek sensitization in a few customers; it was determined that a major cause of cheek sensitization was the consumption of large amounts of beer and other beverages treated with alkyl gallates, so the use of alkyl gallates as antioxidants in beer and other beverages was discontinued. (Parke and Lewis, 1992).

Several writers have examined the mutagenic and/or genotoxic effects of potassium sorbate, which is used as an antibacterial agent in a broad range of foods

such as cheeses, pickles, sauces, soft beverages, and other goods with the SCEs test, potassium sorbate seemed to have a modest genotoxic impact at two (4 and 8 mM) of the five (0.02, 0.2, 2, 4, and 8 mM) concentrations tested in human lymphocytes. Other results obtained in Chinese Hamster cells at 3-4 mg/ml concentration with Chromosomal aberration test and in Chinese Hamster V79 cells at 20 mg/ml concentration with CAs, SCEs, and gene mutation assays support a modest genotoxic impact. (Mamur *et al.*, 2010).

It is not possible to review all the preservatives that causes the adverse effect, hence the chemical preservatives that are discussed in this article are:

- Sodium sulphite
- Sodium nitrite
- Sodium benzoate
- Tertiary butylhydroqunione (tBHQ)
- Butylated hydroxyanisole (BHA)

Sodium sulphite

Sulphite is a popular food preservative that is widely used as a blanching agent and preservative in a variety of food products to enhance the appearance of the food product. They're also utilised to prevent food from oxidizing and can be employed to give a product a fresh scent and flavor. In general, they are regarded safe substances, but only when used in the smallest amount possible, Sodium sulphite, potassium bisulphite, and sodium bisulphite are only a few examples (El-Hefny *et al*., 2021).

Several allergic responses have been recorded in persons who have been exposed to sulphite-containing foods. Sulphite is a potentially deadly chemical that can be ingested, inhaled, or infused into the body. Rats administered with precise doses of sodium sulphite twice a week, the results revealed severe impacts on both liver and kidney cells. Sulphite compounds have been shown to have detrimental and hazardous effects on people. (Mahmoud *et al.*, 2015).

Mechanism of action

When sulphite is in contact with stomach acids SO_2 is formed this production on SO_2 will get enhanced when the temperature is high and the pH is low. The acidic and warm conditions in the stomach and mouth are efficient for the production of SO_2. Which causes asthma in some people. Sulphite also induces a health condition known as bronchospasm in individuals who are sensitive to sulphite, deaths are also reported due to sulphite sensitivity (Lester, 1995). Sulphite sensitivity is commonly seen in patients with asthmatic condition, but sulphite-induced health problems may be seen in patients without recorded asthma or other allergy-related disorders (Rangan and Barceloux, 2009).

Adverse effect Sulphite sensitivity

According to the Food and Drug Administration (FDA), one out of every hundred persons is sulphite-sensitive, and 5 % of that group has asthma. According to another source, 5% of asthmatics are sulphite sensitive, compared to only 1% of the general population. Sulphite intolerance symptoms might appear as soon as 5 minutes after parenteral exposure and 15-30 minutes after oral consumption. Individuals with sensitivity to sulphites have varying degrees of intolerance, with each having a different threshold for eliciting a response. In experimental animals, sulfiting agents are neither, teratogenic, mutagenic, or carcinogenic; nonetheless, a small percentage of the population is sulphite sensitive and vulnerable to a variety of side effects owing to acute allergic responses. Many allergic reactions have occurred in some people after consuming sulphite-containing food products, including urticaria, angioedema, hives and pruritus, flushing, tingling, and swelling; respiratory symptoms such as dyspnea, wheezing, and

bronchoconstriction; and gastrointestinal symptoms such as nausea and stomach cramps. (Grotheer, Marshall and Simonne, 2005).

Sodium nitrite

Sodium nitrite is a preservative mainly used to preserve meat products. They give bright red colour to meat products like beef and sausages. Nitrite is an active compound of nitric oxide which is formed when nitrite is dissolved. During the process of preservation of meat, the nitric oxide reacts with the meat pigment known as myoglobin to give its bright red colour this combination will produce a compound known as nitrosomioglobin which is considered as a carcinogenic compound (Ambarwati, 2012).

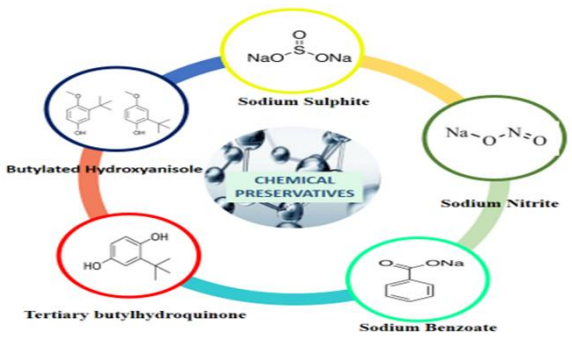

Fig 1: Structure of Chemical Preservatives

The long-term consumption of processed meat that

contain nitrites has shown the development of colorectal cancer (Crowe *et al.,* 2019). Long term exposure to N-nitroso-compounds can cause esophageal carcinoma. Nitrites are also involved in the synthesis of N-nitroso-compounds (Xie *et al.*, 2011). Sodium nitrites can induce colonic aberrant crypts. Colonic aberrant crypts are putative preneoplastic lesions for colon cancer. Experiments using rats were done to study the effects of sodium nitrite (Zhou *et al.*, 2016).

The results showed that the rats which was treated with sodium nitrite showed a small decrease in serum cholinesterase activity after 3-6 months of treatment (Helal *et al.*, 2008). When nitrates are consumed, they are converted to nitrites, which can combine with haemoglobin to form methemoglobin, which can cause loss of consciousness and death, especially in newborns (Anand and Sati, 2013). It's possible that sodium nitrite therapy caused foetal methemoglobinemia, which led to an increased requirement for new red blood cells (Globus and Samuel, 1978).

Mechanism of action

Among the various chemical carcinogens that have been recognized in human food and drink, N-nitroso compounds are exceptionally strong. N-nitroso compounds are generally formed by the combination of a secondary amino compound with a nitrite (Lijinsky, 1999). Nitrite is an active compound of nitric oxide which is formed when nitrite is dissolved. During the process of preservation of meat, the nitric oxide will get combined with the meat pigment known as myoglobin to give its bright red color this compound is known as nitrosomioglobin which is considered as a carcinogenic compound (Ambarwati, 2012).

Adverse effect

Formation of n-nitroso-compounds

Traces of NDMA were also found in cooked bacon. Nitrosomine and nitrosothiazolidine were discovered in bacon and other cured pork products and were generated by the interaction of cysteine, formaldehyde, and nitrite. There are reports showing the presence of nitrosodi-n-butaylamine in some meat products but at a very low concentration. This nitrosamine is a main compound for inducing tumours in the urinary bladder of rats (Kadhum *et al.,* 2015). In many Asian countries there were persistent reporting of nasopharyngeal cancer. On testing the samples of fish traces of NDMA was found. NDMA is an inducer for liver, kidney and lung tumours (Lijinsky, 1999). In fried bacon the presence of two volatile n-nitrosamines is always detected they are N-nitroso-sodimethlylamine and N- nitrosopyrrolidine (Joseph, 1988).

Santarelli *et al.*, 2010 conducted two studies on female rats, one for fourteen days and the other for one hundred days. In the fourteen days study the diet given to the female rats were processed pork of four different verities (a) was dark meat crossed with (b) light meat the cooking temperature for light meat was 70^{o} C and it was crossed with 50^{0}C raw meat, for (c) it was the meat treated with nitrite and (d) was oxidized meat.

The nitrite treated meat was cured with salt containing 0.6g of sodium nitrite per 100 gram of salt and the non- nitrite treated meat was cured with common salt. On the one hundred days experiment they used fifty rats and they were fed with experimental diets. This was done to show the dietary promotion of cancer. The body weight of the rats were monitored every week. The faecal water from the rats were collected and was analyzed the heme concentration of the faecal water was measured.

The processed meat that contained heme and nitrite which was cooked for 70^{0}c showed increased number of preneoplastic lesions in the rats which shows that nitrite processed meat can promote carcinogenesis (Gbemisola et al., 2020).

Sodium benzoate

Sodium benzoate is a sodium salt which is used as a preservative mainly in juices and beverages to inhibit the growth of harmful bacteria, yeast and molds. There are reports that states that, over intake of sodium benzoate treated food can cause utricaria, hyperactivity in children and also it is harmful to the DNA. Sodium benzoate is also considered to be genotoxic, neurotoxic and also it is responsible for the intercalation in DNA structure (Bruna *et al*., 2018).

It is also found that the long-term use of sodium benzoate treated food products might produce carcinogenic compounds when they get combined with vitamin C or ascorbic acid (Samal *et al*., 2017). Sodium benzoate treated food products was tested for genotoxic effects by using chromosomal aberrations, sister- chromatid exchanges and micronucleus analysis in cultured human peripheral lymphocytes and comet assay in lymphocytes (Zengin *et al.*, 2011).

Mechanism of action

The effect of sodium benzoate on human cell lymphocytes was examined at doses of 200 and 500 g/mL. At 500 g/mL, benzoic acid increased the

indices of sister chromosomal exchanges (SCEs), chromosomal aberration (CA), and Micronucleus (MN) while decreasing the mitotic division index (Mitotic index). The researchers looked at lymph node cells from mice that had been given different dosages of sodium benzoate to see if it may change the morphology of the lymphocytes and harm the cell membrane. When this chemical is exposed to higher concentrations and for longer periods of time, the negative effects become more pronounced (Shahmohammadi *et al*., 2016).

Adverse effect

Genotoxicity effects of sodium benzoate

To understand the genotoxicity effect of sodium benzoate preservative they used three assays chromosomal aberration, sister chromatid exchanges and micronucleus assay and it was carried out in human lymphocytes. Hungerford's standard technique was used to grow lymphocytes. Sodium benzoate showed an increased number in CA and CAs/cell in every concentration when it was compared with the control.

The preservative showed many structural aberrations in chromosomes like chromatid gaps, chromosome gaps, chromatid breaks, chromosomal breaks, acentric fragments and dicentric chromosomes. In the genotoxicity test they were able to detect substances that caused genetic damage and also carcinogenic compounds. The frequency of sister chromatid exchanges per cell is increased by sodium benzoate. In all concentrations of sodium benzoate, this rise is considerable. The increase in sister chromatid exchanges is proportional to the concentration. When the concentration of sodium benzoate is increased, the value of the cell cycle proliferation index decreases considerably (Mpountoukas *et al.*, 2008).

Table 1: Chemical preservative

Sl.No	Chemical preservative	LD_{50}/LC_{50} Dose mg/kg body weight	Duration	Mode of administration	Animal species	References
1	Sodium Sulphite	>400 mg/L	3-days	Oral-dose and Ocular exposure	Rat	Bindu Nair and Amy R. Elmore., 2003
			290 days	Oral-dose and Ocular exposure	Dogs	
2	Sodium Nitrite	218.7 mg/kg	3-days	Oral-dose	Rat	Kadhum *et al.*, 2015
3	Sodium Benzoate	400 mg/L	2 -days	Oral-dose	Zebrafish	Gaur *et al.,* 2018
4	Tertiary butylatedhydroqu in one	13367.79 mg/kg	1-day	Oral-dose	Rat	Saibu1 *et al.*, 2020
5	Butylated hydroxyanisole	2000 mg/kg	2 days	Oral-dose	Mice	Sasaki *et al*., 2002

Tertiary butylhydroquinone (TBHQ)

Tertiary butyl hydroquinone is food preservative used mainly in oils and some meat products. It helps to prevent rancidity and extends the shelf life of the food product, it also act as an antioxidant and prevents discoloration of the foods containing iron. Due to the high anti-lipid peroxidation property of tBHQ it is being widely used in fats and oils. In excessive consumption of foods containing tBHQ there is a possibility of stomach tumors and brain damage. Some studies have shown that tBHQ will cause in the formation of 8-hydroxydeoxy guanosine in calf thymus DNA due to the formation of reactive oxygen species like hydrogen peroxide and superoxide anion. It is reported that tBHQ showed cytotoxic effects on human umbilical vein endothelial cell (huvec) in a time dependent manner. Recent studies shows that over exposure to tBHQ can cause carcinogenicity (Eskandani *et al.,* 2014). A number of studies have shown that the excessive use of tBHQ can lead to stomach tumors and can damge the DNA (Dolatabadi and Kashanian, 2010).

Mechanism of action

The observed carcinogenic effects of tBHQ have been related to the metabolic synthesis of oxidized forms of tBHQ, their semiquinone anion radicals, and ROS formation (Gharavi *et al.*, 2006). tBH can also induce cytoxicity It depicts the development of DNA fragmentation and breaking (Eskandani, Hamishehkar and Ezzati Nazhad Dolatabadi, 2014).

Adverse effect carcinogenicity

Studies show that tertiary butyl hydroquinone can intensify the carcinogenic effects of other chemicals when they were tested in different animals like rats and hamsters. When rats were fed with 2% of tBHQ

for about 4-8 weeks, there was an increased hyperplasia of forestomach basal cell. When the rats were fed with 2% of tBHQ without the presence of $NaNO_2$ for about 4 weeks showed a small increase in the thickness of the forestomach mucosa. When 0.7% of tBHQ, 0.7% BHA, and 0.3% butylated hudroxytolune showed a combined effect of bladder tumours. In contrast to tBHQ alone, co-administration of 0.5–2 percent tBHQ with 0.2–0.3 percent NaNO2 for 4 or 36 weeks resulted in a more than 10-fold increase in fore stomach thickness mucosa (Saibu *et al*., 2020).

Thus, it is seen that tBHQ can cause an enhancing effect in bladder cancer which is induced by N-butyl-N-(4-hybroxybutyl) nitrosamine BBN. The metabolic production of oxidized forms of tBHQ, their semiquinone anion radicals, and ROS generation has been linked to the reported carcinogenic effects of tBHQ, at least in part. (Gharavi *et al.,* 2006). F344/N rat and B6C3F mice were fed with food cured with tBHQ for about 13 weeks. Oral consumption was selected because of the nature of human consumption. Genetic toxicology studies were conducted in Salmonella typhimurium and cultured rat ovary cells in vitro and in vivo bone marrow cells of mouse was tested. As a result, Introduction of tBHQ to rats in their feed resulted in diminished rates of mammary gland neoplasms in males and females (Naidenko *et al.*, 2021).

Cytotoxicity and DNA damage properties of TBHQ

A549 lung carcinoma cell line and HUVEC were used for the experiment. The cytoxicoty of tBHQ was determined by MTT assay. The DNA fragmentation assay was also done to identify the presence of fragmented DNA. The results for the cytotoxicity on tBHQ- treated A549 and HUVEC cells showed that tBHQ was able to induce cytotoxicity in both the cells depending upon the amount of dosage and the consumption rate (Mirza *et al*., 2017).

The study also showed that the excessive intake of food treated with tBHQ can also induce carcinogenicity. DNA fragmentation assay showed the formation of DNA-ladder which shows the formation of DNA breakage and fragmentation (Eskandani *et al.,* 2014). Rats were given various antioxidants including tBHQ in diet to identify if the food additive induced urinary bladder cancer. tBHQ showed weak promotion of urinary bladder cancer. They were given 0.5 % for 32 weeks as a result the rats that was fed by tBHQ diets showed increased DNA synthesis in the urinary bladder epithelium of the rats (Ito and Fukushima, 1989).

Butylated Hydroxyanisole (BHA)

Butylated hydroxylanisole is food preservative mainly used in oils and fats. It is a phenolic antioxidant. They prevent rancidity in oils and fats in food by preventing the lipid oxidation, BHA is also used in dry foods and cereals. They also maintain the freshness, colour, nutritive value of food. Many studies on BHA have shown that it promotes or induces cancer in many animals. The possibility of causing health problems is due to the excessive consumption of the products that contain the particular preservative. Studies have shown that BHA can cause cancer of the non-glandular stomach or the fore-stomach of male rats, in this particular study it was shown that the tumors were induced at 20000 ppm BHA in the given diet. Many other studies have shown that by 5000ppm of BHA in diet administrated to rats for about 51 week resulted in neoplasia and the increase in the squamous cell in rats (Whysner *et al.*, 1994).

The amount administrated by FDA for the usage of BHA in beverage and desserts made from dry mixtures is between 2 ppm and for active dry yeast it is between 1000 ppm. The daily consumption of the compound that contain BHA can get close to the ADI level. Pharmaceuticals also contain BHA, if one is to consume both the diet and the pharmaceutical product on a daily basis then can result in exceeding the ADI levels (Anca *et al.,* 2013). On A549 lung cancer cell the cytotoxic effect or the genotoxic effect of BHA and its mechanism still remains unidentified. By MTT assay, it helped to identify that BHA can induce ctytoxicity in A549 lung cells but it depended up on the time-duration and the dosage (Vandghanooni *et al.*, 2013).

Mechanism of action

The addition of the antioxidant BHA to the diet of F344 rats caused a significant incidence of papilloma and squamous cell carcinoma of the fore stomach in both sexes, male hamsters administered BHA developed papilloma, which grew downward into the fore stomach sub mucosa, according to carcinogenicity studies done by (Ito *et al*., 1985).

Adverse effect carcinogenicity

For this experiment five-four-week-old rats from both sexes were used as test animals. The addition of BHA to the diet of the rats were according to different levels that is from 0.5 to 2.0%. The rats of both sexes were divided into two different groups and was given high and low amount of BHA containing diets for about 104 weeks. After that they stopped giving them the test diet and started providing them with normal diet for about 112 weeks. The rats that survived the end of

112th week were sacrificed and examined. The rats that died during the experiment were also examined, and also that survived 41 weeks.

The first tumor appearance was in the pituitary of a female rat in the low dosage test group. In week 59, a male rat developed squamous cell carcinoma of the fore stomach; this rat had taken BHA at a higher dose. At week 82, a female rat with squamous cell carcinoma of the fore stomach was observed; this female rat had also got the higher dose of the BHA. Many tumors were found in the region of the fore stomach's constrained ridge on a gross level. Two or three tumors in the fore stomach were seen in around a third of the rats. In other rats, the tumors were greyish-white, wart-like, or villous nodules with ulceration. Cancer cells were also found to have invaded the lymphatic system. Squamous cell carcinoma has been linked to fibrosarcomatous malignant tissue growth in a few cases. There are two male rats and one female rat who received a higher dose of BHA had lymph node metastases (Ito *et al.,* 1985).

Table 2: Comparison of food chemical food preservatives.

Sl.No	Preservative name	Chemic al formula e	Adverse effect	Mechanism of action	Animal study	Reference
1.	Sodium sulphite	Na_2SO_4	Sulphite sensitivity	When sulphite is in contact with stomach acids SO_2 is formed which causes asthma in some people.	Abino Wister rats	(Rangan and Barceloux, 2009)
2.	Sodium nitrite	$NaNO_2$	Formation of n-nitroso-compounds	Generally formed by the combination of a secondary amino compound with a nitrite	Inducing tumours in the urinary bladder of rats.	(Lijinsky, 1999)
3.	Sodium benzoate	$C_7H_5Na\ O_2$	Genotoxicit y	Raised indices of sister chromosomal exchanges, chromosomal aberration, and Micronucleus (MN) and lowered the mitotic division index	Human lymphocyt es	(Mpounto ukas *et al.*, 2008).
4.	Tertiary butylatedhydr oqu in one	$C_{10}H_{14}\ O_2$	Carcinogeni city and Cytotoxicity	To the metabolic synthesis of oxidized forms of TBHQ, their semiquinone anion radicals, and ROS formation	rats and hamsters	(Naidenko *et al.*, 2021)
5.	Butylated hydroxylanisol e	$C_{11}H_{16}\ O_2$	Carcinogeni city	Significant incidence of papilloma and squamous cell carcinoma of the forestomach	F344 rats	(Ito, Fukushim a and Tsuda, 1985)

Conclusion

The current review revealed that the usage of chemical food preservatives in food can cause many severe health problems. The problems that it causes will depend up on the amount and the duration of the usage of the particular preservative. Some preservative may not act directly but still it may promote or induce many health problems like genotoxicity and carcinogenicity. The study also provided how the preservatives acts, when they induce different health problems and also gives information about health problems are caused by some specific synthetic food preservative. There are studies going on to find proper alternates for chemical preservatives. The usage of natural preservative in food will help to improve the shelf-life of the food product and the side effects from using it will be a minimum. So, a soft shift from chemical preservative to natural preservative can make a big difference in maintaining good health in the future.

References

1. Ambarwati, R. (2012). Effect of sodium nitrite (NaNO2) to erithrocyte and hemoglobin profile in white rat (rattus norvegicus). Folia Medica Indonesiana, 48(1), 1-5.
2. Anand, S. P., & Sati, N. (2013). Artificial preservatives and their harmful effects: looking toward nature for safer alternatives. Int. J. Pharm. Sci. Res, 4(7), 2496-2501.
3. ANTON, E. Practical 2: Risk Communication & Food Additives.
4. Nair, B., & Elmore, A. R. (2003). Final report on the safety assessment of sodium sulfite, potassium sulfite, ammonium sulfite, sodium bisulfite, ammonium bisulfite, sodium metabisulfite and potassium metabisulfite. International journal of

toxicology, 22, 63-88.

5. Crowe, W., Elliott, C. T., & Green, B. D. (2019). A review of the in vivo evidence investigating the role of nitrite exposure from processed meat consumption in the development of colorectal cancer. Nutrients, 11(11), 2673.
6. Dolatabadi, J. E. N., & Kashanian, S. (2010). A review on DNA interaction with synthetic phenolic food additives. Food Research International, 43(5), 1223-1230.
7. El-Hefny, I., Hozayen, W., AlSenosy, N., Basal, W., Ahmed, A. and Diab, A., 2021. Evaluation of genotoxicity of three food preservatives in drosophila melanogaster using smart and comet assays. Journal of Microbiology, Biotechnology and Food Sciences, 2021, pp.38-41.
8. Eskandani, M., Hamishehkar, H., & Dolatabadi, J. E. N. (2014). Cytotoxicity and DNA damage properties of tert-butylhydroquinone (TBHQ) food additive. Food chemistry, 153, 315-320.
9. Gaur, H., Purushothaman, S., Pullaguri, N., Bhargava, Y., & Bhargava, A. (2018). Sodium benzoate induced developmental defects, oxidative stress and anxiety-like behaviour in zebrafish larva. Biochemical and biophysical research communications, 502(3), 364-369.
10. Gharavi, N., Haggarty, S., & S El-Kadi, A. O. (2007). Chemoprotective and carcinogenic effects of tert-butylhydroquinone and its metabolites. Current drug metabolism, 8(1), 1-7.
11. Globus, M., & Samuel, D. (1978). Effect of maternally administered sodium nitrite on hepatic erythropoiesis in fetal CD-1 mice. Teratology, 18(3), 367-377.
12. Grotheer, P., Marshall, M., & Simonne, A. (2005). Sulfites: Separating Fact from Fiction: FCS8787/FY731, 4/2005. EDIS, 2005(5).
13. Helal, E., & Soliman, G. Z. (2008). Biochemical studies on the effect of sodium nitrite and/or glutathione treatment on male rats. The Egyptian Journal of Hospital Medicine, 30(1), 25-38.
14. Hotchkiss, J. H. (1988). A review of current literature on N-nitroso compounds in foods. Advances in food research, 31, 53-115.
15. Ito, N., & Fukushima, S. (1989). Promotion of urinary bladder carcinogenesis in experimental animals. Experimental pathology, 36(1), 1-15.
16. Ito, N., Fukushima, S., & Tsuda, H. (1985). Carcinogenicity and modification of the carcinogenic response by BHA, BHT, and other antioxidants. CRC Critical reviews in Toxicology, 15(2), 109-150.
17. Kadhum, H. J., & ASN, M. A. A. D. S. (2015). - HEPATOPROTECTIVE AND HYPOLIPIDEMIC EFFECTS OF BIS [4-(4'-HYDROXY-3'-METHOXYBEZYLIDINEAMINOPHENYL)] TELLURIDE (R2TE) AGAINST SODIUM NITRITE INTOXICATION IN MALE ALBINO RATS. Basrah Journal of Veterinary Research, 14(1).
18. Lester, M. R. (1995). Sulfite sensitivity: significance in human health. Journal of the American College of Nutrition, 14(3), 229-232.
19. Mahmoud, A. A. T., Hassan, G. M., Hassan, A. M. S., Latif, A. K. M. A., & Ramadan, M. F. (2015). Demonstrating adverse effects of a common food additive (sodium sulfite) on biochemical, cytological and histopathological parameters in tissues of albino Wister rats. European Journal of Integrative Medicine, 7(3), 234-242.
20. Mamur, S., Yüzbaşıoğlu, D., Ünal, F., & Yılmaz, S. (2010). Does potassium sorbate induce genotoxic or mutagenic effects in lymphocytes?. Toxicology in vitro, 24(3), 790-794.
21. Mirza, S. K., Asema, U. K., & Kasim, S. S. (2017). To study the harmful effects of food preservatives on human health. J. Med. Chem. Drug Discovery, 2, 610-616.
22. Mpountoukas, P., Vantarakis, A., Sivridis, E., & Lialiaris, T. (2008). Cytogenetic study in cultured human lymphocytes treated with three commonly used preservatives. Food and Chemical Toxicology, 46(7), 2390-2393.
23. Naidenko, O. V., Andrews, D. Q., Temkin, A. M., Stoiber, T., Uche, U. I., Evans, S., & Perrone-Gray, S. (2021). Investigating molecular mechanisms of immunotoxicity and the utility of ToxCast for immunotoxicity screening of chemicals added to food. International Journal of Environmental Research and Public Health, 18(7), 3332.
24. Parke, D. V., & Lewis, D. F. V. (1992). Safety aspects of food preservatives. Food Additives & Contaminants, 9(5), 561-577.
25. Pop, A., Kiss, B., & Loghin, F. (2013). Endocrine disrupting effects of butylated hydroxyanisole (BHA-E320). Clujul Medical, 86(1), 16.
26. Rangan, C., & Barceloux, D. G. (2009). Food additives and sensitivities. Disease-a-month, 55(5), 292-311.
27. Saibu, G., Adu, O. B., Faduyile, F., Iyapo, O.,

Adekunle, K., Abimbola, S., & Ogun, S. (2020). Investigation of the antioxidant potential and toxicity of the whole leaf of Solanum nigrum in albino rats. Journal of Research and Review in Science, 7(1), 9-16.

28. Samal, D., Gouda, S., & Patra, J. (2017). Food preservatives and their uses: a short report. Asian Journal of Biology, 4(1), 1-4.
29. Sasaki, Y. F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., ... & Tsuda, S. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutation Research/Genetic Toxicology and Environmental Mutagenesis, 519(1-2), 103-119.
30. Shahmohammadi, M., Javadi, M., & Nassiri-Asl, M. (2016). An overview on the effects of sodium benzoate as a preservative in food products. Biotechnology and Health Sciences, 3(3), 7-11.
31. Silva, M. M., & Lidon, F. (2016). Food preservatives–An overview on applications and side effects. Emirates Journal of Food and Agriculture, 366-373.
32. Vandghanooni, S., Forouharmehr, A., Eskandani, M., Barzegari, A., Kafil, V., Kashanian, S., & Ezzati Nazhad Dolatabadi, J. (2013). Cytotoxicity and DNA fragmentation properties of butylated hydroxyanisole. DNA and Cell Biology, 32(3), 98-103.
33. Whysner, J., Wang, C. X., Zang, E., Iatropoulos, M. J., & Williams, G. M. (1994). Dose response of promotion by butylated hydroxyanisole in chemically initiated tumours of the rat forestomach. Food and Chemical Toxicology, 32(3), 215-222.
34. Xie, T. P., Zhao, Y. F., Chen, L. Q., Zhu, Z. J., Hu, Y., & Yuan, Y. (2011). Long-term exposure to sodium nitrite and risk of esophageal carcinoma: a cohort study for 30 years. Diseases of the Esophagus, 24(1), 30-32.
35. Zengin, N., Yüzbaşıoğlu, D., Ünal, F. A. T. M., Yılmaz, S., & Aksoy, H. (2011). The evaluation of the genotoxicity of two food preservatives: sodium benzoate and potassium benzoate. Food and Chemical Toxicology, 49(4), 763-769.