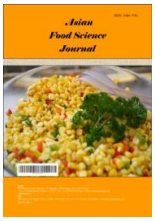

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Benzoic Acid Based Beverages: Health Implications

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MGA, DMS and MAM designed the review, wrote the protocol and supervised the work. Author SR carried out all the literature searches and wrote the first draft of the manuscript. Authors AD and JHB went through the manuscript and brought out the final draft. All authors read and approved the final manuscript.

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ABSTRACT

Soft drinks nowadays are becoming an important part of the modern diet consumed in many communities worldwide and are available in the market virtually in the same form almost anywhere around the globe. Additives such as antioxidants and preservatives are usually added to soft drinks to increase their shelf life. Several acids such as benzoic and ascorbic acid are used in beverages to prevent oxidation and degradation of matrix. Literature documented that combination of these preservatives in soft drinks results in benzene formation. Moreover, benzene has long been reported to inflict many public health problems. This review elucidated different health consequences such as hematological, neurological, reproductive and carcinogenic effects of exposure to benzene. Liver and kidney derangements were also reported from different epidemiological and experimental studies. Therefore, we suggest that combination of ascorbic acid and benzoic acid in beverages should be avoided by small scale and industrial manufactures. A closer monitoring of these preservatives in beverages by regulatory agencies is highly needed.

Keywords: Antioxidants; benzoic acids; beverages; health implications; preservatives.

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1. INTRODUCTION

Beverages nowadays are becoming an important part of the modern diet consumed in many communities worldwide. They are consumed probably due to their nutritional advantages, thirst-quenching abilities, stimulant or medicinal values. Fruit Juices are mostly fat-free, nutrient-dense beverages rich in vitamins, minerals and naturally occurring phytonutrients that contribute to good health [1,2].

Fruit juices, soft drinks and dairy drinks present are available in the market virtually in the same form almost anywhere around the globe [3,4]. Some are presented in bottles, cans, polyethylene terephthalate (PET) bottles, plastics bottles, laminated paper packs, pouches/sachets, cups, etc. Fruit juices are rich in Vitamin A, C, and minerals such as calcium, magnesium, zinc, iron, potassium, while soft drinks and dairy drinks could be fortified with more minerals and vitamins [5,6]. However, regular consumption of sugar-sweetened beverage is linked to an increased risk of developing type 2 diabetes mellitus and the most probable mechanism is via the weight gain. Beverages are densed in calorie but low in satiety, thus increase consumption of liquid sugars without decrease consumption of solid food [7]. Beverage glycemic index is also one of the factor that may explain the relationships betwee the diabetes mellitus and sotf drinks consumption. Beverages contain large amounts of rapidly resorbed carbohydrates, therefore, its consumption can leads to rapid increases in glucose and insulin concentrations. However, Sugar-sweetened beverages have a moderate glycemic index, but they contribute to a high glycemic load of the overall due to the consumption of large quantity [8].

Food additives are viewed as any substances added to food to preserve flavor, texture, enhance its taste, appearance, or delay the chemical degradation of food components [5]. Additives such as antioxidants and preservatives are usually added to increase the shelf life of food items. Ascorbic acid prevents oxidation by decreasing the available oxygen in the environment. Ascorbic acid is preferentially oxidized to the dehydroascorbic acid (DHA) form, thus preventing the oxidation of the matrix. Preservatives, such as benzoic acid prevent or inhibit the growth of microorganisms in food. Although, some of these antioxidants and preservatives occur naturally in some fruits [1,9].

Benzoic acid and its salts are well known preservatives (E210-E213), it works by the inhibition of an enzyme in the citric acid cycle of microorganisms and widely used in food, beverages, tooth pastes, mouth washes, cosmetics and pharmaceuticals at regulated dosage [4,10]. The preservatives are effective growth inhibitors of yeasts, mold, and fungi producing aflatoxins, but only partially against bacteria. Benzoic acid is not effective against oxidation or enzymatic rotting so manufacturers combine it with vitamin C or sulfur dioxide in treating fruits juices and soft drinks [1,11,12].Vitamin C (L-ascorbic acid) is probably the best known vitamin. It is present in many natural sources and is also a common synthetic product. Ascorbic acid is used preferentially in many food products to prevent oxidative degradation of food components of the matrix [6].

However, the use of these preservatives in food products, fruit juices, soft drinks and cosmetics has been known to be associated with many health implications [10,12]. Some people are allergic to benzoic acid; developing shortness of breath, urticarial, Metabolic acidosis, convulsion, hyperactive, immune-depression, irritate the eyes, skin, lungs, and digestive tract and hyperpnoea in experimental animals and humans. These implications may become worse after prolong exposure to the chemical. In recent years, the combination of these preservatives in food products, fruit juices, soft drinks and cosmetics raised a serious concern to academic communities [10,12]. Researchers observed that benzoates react with ascorbic acid (vitamin C) and form benzene, especially if they are stored for extended periods at high temperatures [13]. In the United States, the EPA has classified benzene as a known human carcinogen for all routes of exposure. Other medical implications such as reproductive failure, neurological, hematological as well as immunological effects have been identified in individuals exposed to bezoic acid.

In this respect, therefore, regulatory agencies especially in the developed world placed limitation on the use of preservatives in food products and beverages. Thus, it is imperative to note that consumers should in their own interest observe, examine and moderate what they eat, drink and rub.

1.1 Benzoic Acid and Its Salt

Benzoic acid [$C_7H_6O_2$; C_6H_5COOH]; benzenecarboxylic acid, phenyl carboxylicacid [E

210 [EU No. Regulation on Labelling of Foodstuffs]]; Sodium benzoate [$C_7H_5O_2Na$; benzoic acid, sodium salt E 211 (EU No. Regulation on Labelling of Foodstuffs) [2,6]. Benzoic acid is found naturally in small quantities in plums, tomatoes, cinnamon, cloves and apple and is formed in fermented dairy products by lactic acid bacteria [5]. It is one of the preservatives with the longest known histories, often effectively used by the manufacturers in canned fruit, pickle and soft drinks as preservative, yet its sodium salt is used mostly as it does not dissolve well in water [10,14]. Therefore, the salt was the first preservative cleared by the FDA for use in food products. The inhibitory effect of benzoic acid and its salt on microorganisms is related to pH and occurs best at low pH. The use of benzoic acid and its sodium salt is not bound to only products with high-acidity, because several mold and yeast thrive in acidic environments, yet benzoate acts as an effective deterrent force against mold and yeast [10,12].

1.2 Exposure

The general population is usually exposed to benzoic acid or sodium benzoate through the consumption of food products that contain the substances naturally or added as antimicrobial agents. The exposure can be orally via eating and drinking of food and beverages or through skin by the use of cosmetics, sanitary and pharmaceuticals products like in syrups, in containers used for liquid preparations, and in tablets formation, to make tablets transparent and smooth and to allow rapid decomposition of the tablets [5].

Analyses of some processed food products, fruit juices, and soft drinks from different countries revealed an inconsistent concentration of benzoic acid (Table 1) and benzene (Table 2), even far from regulatory limit set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In Philippine's products a range of 20 to 2000 mg/L benzoic acid concentrations were reported [15], 50 to 200 mg/L in Japanese products and the mean benzoic acid levels of 259.2 mg/L were also reported in Brazil [9] and 163.8 mg/L in Iran [16]. Out of the 34 soft drink samples analysed by Kusi & Acquaah (2014) in Ghanaian Markets, six samples had benzoic acid levels up to 564.0 mg/L with the rest within the range of zero to 150 mg/L. Similarly, Onwordi et al. [4] reported benzoic acid levels in soft drinks sold in Lagos, Nigeria with a range of 222 to 702 mg/L in carbonated drinks, 168 to 799 mg/L in fruit juice and 451 to 494 mg/L in soft drinks.

Table 1. Benzoic acid concentration in beverages

Country	Tested product	Concentration[mg/L]	Reference
Nigeria	Soft drink	451-494	[4]
	Fruit juices	168-799	
	Carbonated drinks	220-702	
Ghana	Soft drinks	150-564	[5]
Brazil	Soft drinks	200-259	[9]
Philippines	Soft drinks	20-2000	[15]
Iran	Soft drinks	50-163	[16]
Malaysia	Soft drinks	0-1260	[17]
Portugal	Soft drinks & Herbal Extract	0-2477	[11]
Romania	Soft drinks	0-110	[18]

Table 2. Benzene concentration in beverages

Country	Mean concentration [µg/L]	Reference
Egypt	20.0	[19]
Romania	8.8	[20]
Canada	23.0	[21]
Germany	41.8	[22]
Italy	3.9	[23]
Belgium	10.9	[24]
China	7.1	[25]
UK	8.0	[26]
USA	88.9	[2]

1.3 Pharmacokinetics and Toxicity of Benzoic Acid

The pharmacokinetic studies in human subjects and experimental animals reported by several workers indicated that after oral, benzoate is rapidly and virtually completely absorbed through the lining of the digestive tract. The percutaneous absorption in humans accounts for approximately 40%, dermal absorption up to 100%. Benzoic acid is eventually metabolized in the liver and kidney by conjugation with glycine, resulting in the formation of hippuric acid [up to 100%, e.g. in humans] followed by benzoyl-glucuronic acid (0 – 20%) [10,12].The metabolite is rapidly excreted to a high rate through urine after oral, intra-peritoneal and subcutaneous administration [80% - 99% within 24 hours]. Bioaccumulation of benzoic acid and its salt in organs and tissues are not to be expected due to the high rate and extent of elimination [27].

Although sodium benzoate is accepted as a safe substance, but short-term exposure can cause irritation of eyes, skin and respiratory tract, yet prolonged or repeated contact may cause high skin sensitization. Using high doses causes release of histamine and prostaglandin, ulcers and gastric mucus secretion changes. In a study conducted during the year 2007, sodium benzoate increased blood pressure, eventually tearing the vessels in the blood cells of the rat [10]. Damage to the hepatocyte cell membrane and crista losses in mitochondria, connection to outer shell of vacuole mitochondria in the cytoplasm and liver and kidney dysfunction are other adverse effects of consuming sodium benzoate [6]. Study showed that sodium benzoate at concentration of 200 mg/kg can decrease weight in mice and increase creatinine, urea and uric acid in the isolated serum from mice [28]. Fujitani [29] in a study on rats and mice showed that at a concentration of 2.4%, the average weight of the rats was reduced compared to the control group; weight gain in kidney and liver occurred at a concentration of 2.4% in rats and liver and kidney weight increased when compared with the control group at 3% concentration in the mice. In a study Sohrabi et al. [30] on mice, sodium benzoate at a concentration of 560 mg/kg reduced the weight of the ovaries and follicle-stimulating hormone (FSH) and luteinizing hormone (LH) hormones compared to the control group and decreased progesterone hormone at a concentration of 280 mg compared with the control group [30]. Amadikwa et al. [31] investigated the effect of benzoate sodium in rats and concluded that sodium benzoate could reduce white blood cells at concentrations of 60 and 120 mg/kg and the amount of hemoglobin at all concentrations compared to the control group and this decrease make their white blood cells more susceptible to infection. Yılmaz et al. [32] investigated the effect of benzoate sodium on human cell lymphocytes at concentrations of 200 and 500 µg/mL. Benzoic acid increased indices of sister chromatid exchanges (SCEs), chromosomal aberration (CA), and Micronucleus (MN) and at a concentration of 500 µg/mL decreased the mitotic division index (Mitotic index).

2. BENZENE FORMATION IN BEVERAGES

Benzoate and ascorbic acid are widely used as preservatives and antioxidants, respectively, in nonalcoholic beverages but they may also occur naturally in foods. Benzene can be formed when benzoate is decarboxylated in the presence of ascorbic acid and transition metals such as Cu (II) and Fe (III) and can be accelerated by light and heat [13]. Transition metals such as the ones mentioned above may catalyze the transfer of one electron from ascorbic acid to oxygen, producing the anion superoxide, which then undergoes spontaneous dismutation producing hydrogen peroxide. The further reduction of hydrogen peroxide by ascorbic acid is also catalyzed by those metals. This reduction may generate hydroxyl radicals, which can decarboxylate benzoic acid and form benzene (Fig. 1.) [13,33].

2.1 Factors that Enhance Benzene Formation in Beverages

Benzene formation in beverage and food samples has been reported to be influenced by both intrinsic and extrinsic factors. Raw materials, pH of the solution, concentration of preservatives (benzoic acid/its salt), antioxidants (ascorbic acid), and metal ions; sugars and hydroxyl precursors (example, riboflavin photooxidation and lipid peroxidation) are termed as intrinsic factors while, temperature, UV radiation and storage time are considered as extrinsic factors [13].

The influence of temperature an UV light on benzene formation was studied using aqueous model with ascorbic acid and benzoate concentration as those found in processed beverages. McNeal et al. [34] reported that about 300 ng of benzene per gram of solution were

formed at 45^0C under intense UV light for 20 hours. However, only 4ng of benzene per gram of solution formed in model stored in the dark and at 25^0C for 20 hours. Moreover, similar study was conducted using the same concentration of ascorbic acid and benzoate. The solution was kept at 45^0C and benzene formation was investigated at different time intervals. After 24 hours, 118 µg/L of benzene formed and increased to 125 µg/L after 48 hours [35,36].

Casado et al. [37] designed three experimental liquid models [1] without additives; [2] with sodium benzoate; [3] with sodium benzoate and ascorbic acid. The solutions were kept at room temperature for two weeks. Benzene only formed in experiment two [2] containing both benzoate and ascorbic acid. This indicated that benzene can only form in the presence of these two additives.

The influence of chelating agents likes EDTA and diethylenetriaminepentaacetic acid (DTPA) to effectively prevent benzene formation has also been evaluated. Complexation inactivates the ability of the metal ion by occupying its available coordination sites [38,39]. However, some essential minerals present in beverages may as well compete with copper or iron ions for EDTA thus, reducing the chelator's ability to curtail benzene formation [13].

Some antioxidants seem to occur naturally in fruits or intentionally be added to beverages to circumvent oxidation and hydroxyl radicals' formation. They work through many different mechanisms, like scavenging of metal ions or reactive species, and donation of a hydrogen atom to stabilize those species. In addition, the presence of other antioxidants and pH may also play an important role [40].

The effect of sugars on the formation of benzene in solution containing legitimate proportion of ascorbic acid and benzoate was investigated by Aprea et al. [35]. Three different concentrations of sucrose were added (0.1, 0.25 and 0.5 M). Benzene formation was successfully inhibited in concentration dependent fashion. Other sugars like glucose and fructose were found even more effective than sucrose in preventing benzene formation.

2.2 Adverse Effects

Animal and epidemiological studies show neurologic, immunologic, and hematologic effects from inhalation and oral exposure to benzene.

2.2.1 Neurologic effects

Pregnant rats intoxicated to acute benzene exposure have been reported to decrease motor activity and cognitive capacity [41]. Chronic oral exposure has been associated with distal neuropathy, difficulty in sleeping, and memory loss, cells of the intracerebeller nuclei had been reported to be altered in benzene treated rats [42]. Studies in animals suggest that inhalation exposure to benzene results in depressed electrical activity in the brain, loss of involuntary reflexes and narcosis, decrease in hind-limb grip strength and tremors, and narcosis, among other symptoms [43]. Neurobehavioural changes had been documented in human exposed to benzene [44].

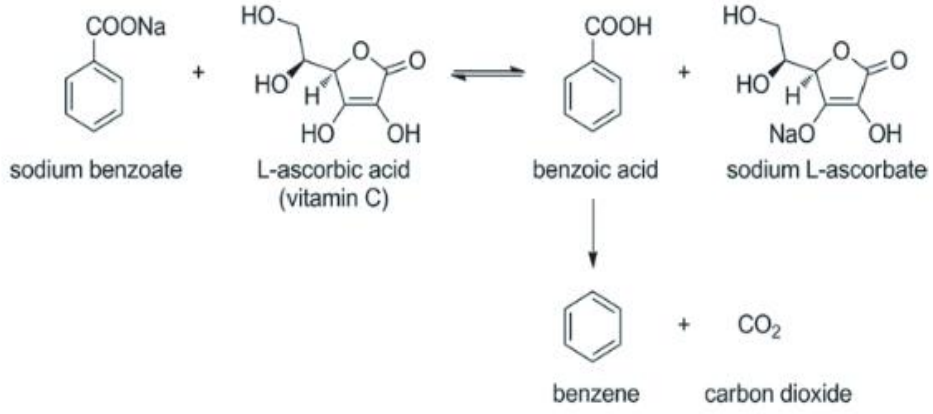

Fig. 1. Reaction of sodium benzoate and Ascorbic acid

2.2.2 Hematological effects

Both human and animal studies have shown that benzene exerts toxic effects on various parts of the hematological system. All of the major types of blood cells are susceptible (erythrocytes, leukocytes, and platelets). The most characteristic systemic effect resulting from intermediate and chronic benzene exposure is arrested development of blood cells [13,45]. Benzene also causes a life-threatening disorder called aplastic anemia in humans and animals. This disorder is characterized by reduction of all cellular elements in the peripheral blood and in bone marrow, leading to fibrosis, an irreversible replacement of bone marrow [1]. All major types of blood cells except WBC were significantly affected in human exposed to benzene [46].

2.2.3 Immunologic effects

Damage to both the humoral and cellular components of the immune system has been known to occur in humans following inhalation exposure. This is manifested by decreased levels of antibodies and decreased levels of leukocytes in workers [1]. Reduce both B-cell and T-cell proliferation and decrease the host resistance to infection were reported in animals exposed to benzene. Chromosomal aberrations in peripheral lymphocytes were reported in humans [47].

2.2.4 Effects on Liver and Kidney

Benzene is found to altered liver and kidney functional parameters. Direct bilirubin, alanine aminotransferase, aspartate aminotransferase, blood urea and plasma creatinine were significantly altered in individuals exposed to benzene [48]. These parameters had been used as biomarkers of liver and kidney functions. Increased serum ALT and AST had also been reported in benzene exposed individuals in pump station workers [49,50]. Similarly, Akinosun et al. [51] reported significant increase of ALT, AST, total proteins, total bilirubin and albumin in individuals exposed to gasoline.

2.2.5 Effects on reproduction

DNA fragmentation has been exploited as biomarker for evaluation of exposure to reproductive toxicants and a diagnostic tool for male infertility. Both occupational and environmental exposure to benzene had been reported to affect male infertility by altering DNA integrity in germ cells [52]. Studies had also suggested that damaged sperm DNA are implicated in anomalies of fertilization and pregnancy [53–55]. Decreased sperm count, sperm motility and increased abnormal sperm morphology and mean tail length were observed in occupational exposed workers [56].

2.2.6 Carcinogenic effects

Benzene and its metabolites (such as catechol, hydroquinone, and phenol] had been implicated in many types of cancers. Catecol has strong cancer promoting potentials and was reported to increase the occurrence of initiator- targeted tumors of the stomach, tongue and esophagus in mice [57,58]. Hydroquinone was reported to cause kidney tumors in male and female rats, leukemia in female rats, thyroid follicular cell hyperplasia in mice and liver tumors in male and female mice [5,59,60]. Phenol has been reported to promote skin cancer in mouse. Phenol in conjunction with benzo(a)-pyrene showed increased carcinogenicity of benzo[a)-pyrene by sixfold [57].

2.3 Metabolism of Benzene

The metabolism of benzene is required for expression of benzene toxicity, and the evidence has been summarized in several reviews. The current understanding of the benzene metabolic pathways involved the formation of the epoxide benzene oxide via a cytochrome P450-dependent mixed-function oxidase [47]. The specific oxidase identified to be responsible for this catalysis is cytochrome P450 2E1 (CYP2E1). The two metabolic pathways (Fig. 2), one involving ring hydroxylation and the second involving ring opening, leads to the formation of putative toxic benzene metabolites [61]. In the first pathway involving ring hydroxylation, acid-catalyzed opening of the epoxide ring is followed by aromatization resulting in the formation of phenol. Phenol is further converted into hydroquinone which is oxidized to benzoquinone. The conjugates formed from hydroquinone [hydroquinone glucuronide and hydroquinone sulfate] are markers for this toxification pathway leading to benzoquinone [62,63]. Phenol can also be metabolized to catechol and trihydroxy benzene. Metabolism of benzene oxide by epoxide hydrolase leads to the formation of benzene dihydrodiol. Catechol can also be formed from benzene dihydrodiol via metabolism by cytosolic dehydrogenases [62,63].

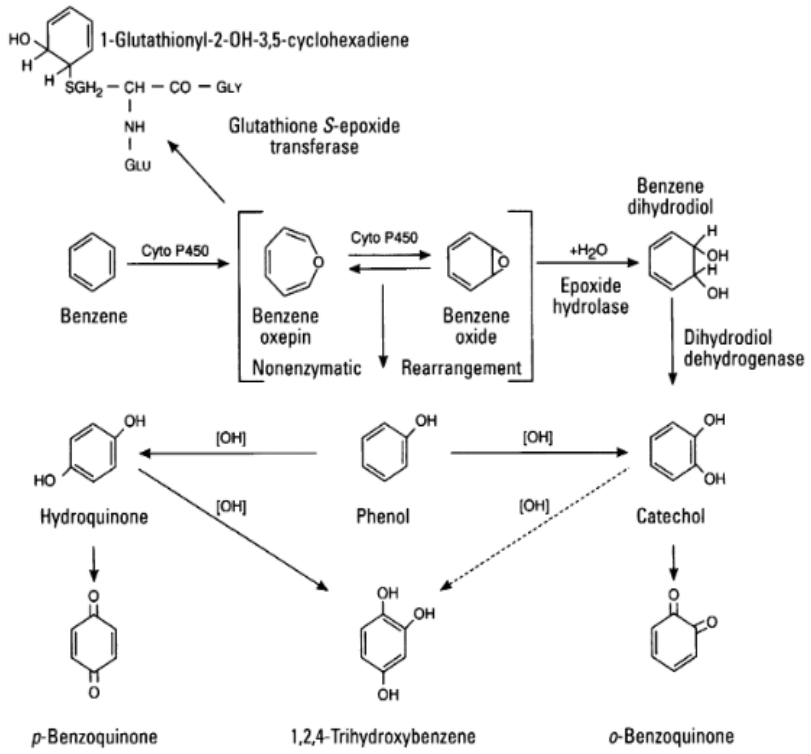

Fig. 2. The metabolic fate of benzene

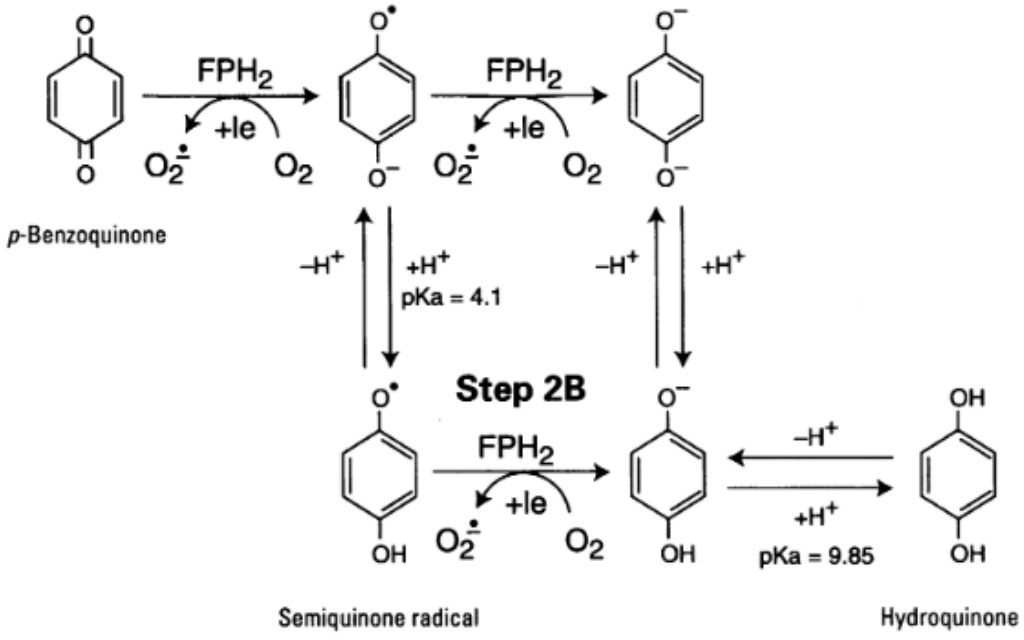

Fig. 3. Reduction of ρ-benzoquinone by reductase (FPH_2) to hydroquinone and sites (steps 1,2A and 2B) at which superoxide radicals are generated

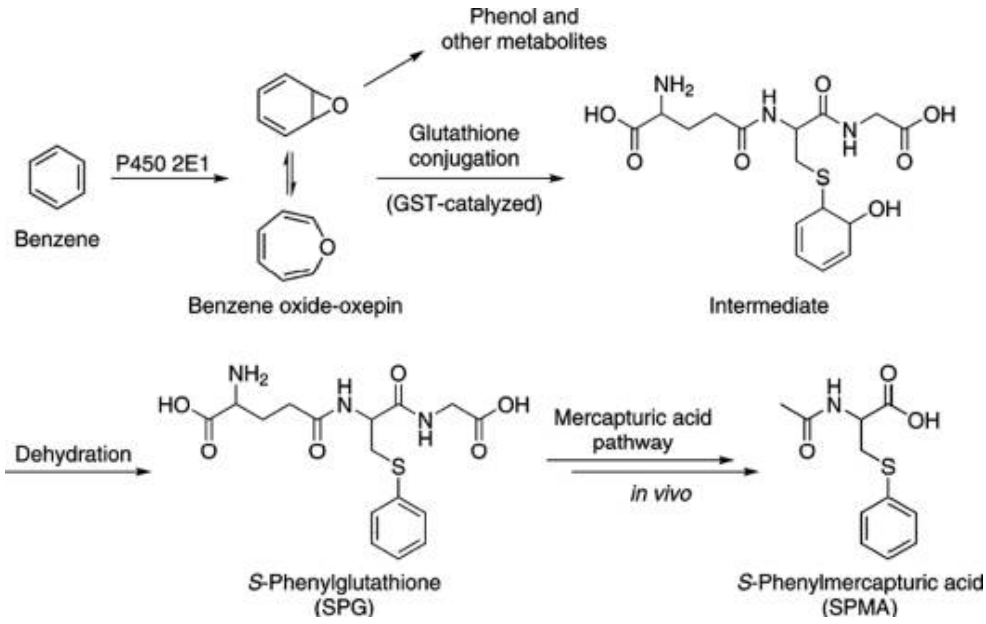

Fig. 4. Detoxification of benzene

Fig. 5. Potential interaction of ρ-benzoquinone with glutathione

The second pathway involving ring-opening leads to the formation of muconic acid viamuconaldehyde. Mouse liver microsomes and cytosol have been shown to catalyze ring-opening in the presence of nicotinamide adenine dinucleotide phosphate in vitro, producing trans, trans-muconaldehyde, a six-carbon diendialdehyde (muconic dialdehyde) and a well-known hematotoxin [44]. Metabolism of benzene and trans,trans-muconaldehyde in the isolated perfused rat liver indicated that benzene was metabolized to muconic acid, a ring-opened metabolite of benzene [64,65]. Trans,trans-muconaldehyde was metabolized to muconic acid and three other metabolites. These studies indicate that ring-opening of benzene occurs in the liver.

2.4 Mechanism of Benzene Toxicity

There are two potential mechanisms by which benzene metabolites may damage cellular macromolecules to induce toxicity include the covalent binding of reactive metabolites of benzene and the capacity of benzene metabolites to induce oxidative damage. The production of Benzene metabolites, largely in the liver, is followed by their transport to the bone marrow and other organs. The first pathway viewed the metabolic activation of benzene to metabolite species that covalently bind to DNA to produce mutagenic events that are expressed as leukemia. The second mechanism appreciates the production of those metabolites that cause oxidative stress [66].

2.4.1 Covalent Binding to Essential Macromolecules

Covalent binding to DNA to yield specific adduct formed by the reaction of hydroquinone with the bases guanine, adenine and cytosine. Because of two point attachment of the ring the result is the addition of a two ring system making the adduct bulkier than might be predicted from the addition of a single ring [67]. The covalent

binding of benzene metabolites to bone marrow DNA could result in bone marrow depression leading to aplastic anemia, which may ultimately cause myeloid leukemia [68].

2.4.2 Oxidative Stress

The second mechanism which can alter DNA structure is the reaction of active oxygen species causing hydroxylation of specific bases. The most common of these is the formation of 8-hydroxyguanine, but hydroxylation products of adenine; thymine and cytosine have been reported [69]. Benzene metabolites may engage in redox cycling, which involves autooxidation of a reduced form of the metabolite to yield an oxidized species plus reactive oxygen (Fig. 3). The bone marrow, which is a richly oxygenated organ, has the capability to generate reactive oxygen species. The four-electron reduction of oxygen [66] may then generate superoxide anion radical, hydrogen peroxide, and hydroxyl radical. The oxidized metabolite may undergo flavoprotein (FPH_2) reduction to yield the starting material that may reenter the redox cycle.

2.5 Detoxification

There are two detoxification pathways. One pathway leads to the formation of mercapturic acid via glutathione conjugates of benzene oxide, which are subsequently metabolized to prephenyl mercapturic acid and phenyl mercapturic acid and eliminated via biliary excretion (Fig. 4) [45,47,66]. The benzene oxide is detoxified by reaction with glutathione and excretion in the urine as S-phenylmercapturic acid. This process may be catalyzed by glutathione S-transferases (GSTs) (Fig. 5). The major portion of benzene oxide is nonenzymatically rearranged into phenol [13,70]. The second detoxification pathway involves the formation of water-soluble urinary metabolites, which are glucuronide or sulfate conjugates of phenol [66].

2.6 Guidelines to Suppress Benzene Formation

Different experimental and epidemiological studies have shown that nonalcoholic beverages appeared to be among the foods with highest benzene concentrations [67,71]. Therefore, the American Beverages Association draws the attention of industries concern to put more emphasis on some points in the manufacturing process as follows:

i. Raw water may be contaminated with benzene.
ii. Sugars may reduce benzene formation but do not inhibit it completely. Therefore, light/diet products are more vulnerable to benzene formation.
iii. Both ascorbic and benzoic acids may occur naturally in juices because they are present in many fruits.
iv. Raw carbon dioxide may be contaminated—the limit is20ppm of benzene (v/v).
v. When acidity is low, ascorbic acid together with sources of benzoic acid is very likely to produce benzene.
vi. Coloring agents and flavors may contain ascorbates.
vii. Consider removing, reducing, or replacing benzoates with other microbial growth inhibitors.
viii. Consider removing, reducing, or replacing ascorbates with other antioxidants.
ix. Check the product storage conditions since strong light and high temperatures speed up the formation of free radicals.
x. Transition metals may be present in raw water and sweeteners. Traces of copper and iron may catalyze reactions involving ascorbic and benzoic acids. In this case, the addition of chelators, such as EDTA or sodium polyphosphates, may help to minimize benzene formation.

3. CONCLUSION

It is noteworthy to mention that an appreciable amount of benzene was detected in many beverages sold around the globe. Data from the literature have implicated benzene as potential carcinogen with diverse health consequences. Application of good manufacturing processes by beverage producers; better control of environmental conditions during storage and retail and closer monitoring of preservatives levels by regulatory agencies are highly needed. Replacement of the antimicrobial agent (benzoate) or antioxidant (ascorbic acid) is always an alternative to be considered.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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