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REVIEW ARTICLE

Health risks associated with benzene formation in health supplements – An appraisal

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ABSTRACT

Benzene, classified as class-1 human carcinogen by International Agency for Research on Cancer (IARC), is a well-studied chemical over the century and is directly associated with acute and chronic health effects. Evidences reflect that benzene exposure leads to Acute Myeloid Leukemia and Acute Non-Lymphocytic Leukemia. Benzene may enter food and formulations through various environmental factors and due to inferior manufacturing techniques. Moreover, the formulations containing sodium benzoate and ascorbic acid/citric acid as preservative/constituent of herbal ingredient are at greater risks of benzene contamination by oxidative decarboxylation reaction. Although FDA has set a limit for benzene content in products yet cases with high level have been reported. At the same time the long-term use of formulations, even with permissible limits of benzene, may increase risk of carcinogenicity. Harmful health effects due to environmental and occupational exposures to benzene have been sufficiently reported, however, no such reports for generation of benzene in food and pharmaceutical products exist. There is a need to make the scientific fraternity involved in food products, formulations, and food supplements and to be aware of the undesirable effects of multiple and indiscriminate use of preservatives leading to benzene generation. So, the present manuscript highlights the mechanism of benzene formation in food products/formulations, factors affecting benzene formation, metabolism, toxicity and other health effects.

KEY WORDS: sodium benzoate; ascorbic acid; decarboxylation; benzene; ayurvedic formulation

Introduction

Benzene, an aromatic organic compound, is extensively used solvent in various chemical industries as well as laboratories (Costa & Costa, 2002). It was classified as class-1 human carcinogen by International Agency for Research on Cancer (IARC) (Cogliano *et al.,* 2008; Santos *et al.,* 2015). The study report of IARC in 1987 gives the effective evidences that benzene exposure leads to Acute Myeloid Leukemia and Acute Non-Lymphocytic Leukemia (IARC, 2012; Yoon, 2018). Benzene is commonly employed in production process of various chemicals, plastics and paints and also its presence detected in environment due to various sources of air pollution like vehicular emission, burning of fossil fuels and wood and the human activities like cigarette smoking (Vinci *et al.,* 2012). Addition to this, benzene contamination is also reported in foods and beverages (Corriher, 2009; McNeal *et al.,* 1993; Heikes *et al.,* 1995, Fleming-Jones & Smith, 2003; Lachenmeier *et al.,* 2008; Nyman *et al.,* 2007; Poucke *et al.,* 2008). Apart from its existence in food and beverages, it is also expected to be present in different marketed Ayurvedic formulation available over the counter (Nishna *et al.,* 2012). Benzene contamination in formulations occurs through preservatives, water, packaging materials and inferior manufacturing techniques (Varner *et al.,* 1991). Formulations containing benzoate salts and ascorbic acid or citric acid as preservative or as the constituent of herbal ingredient are at greater risks of benzene contamination (Nishna *et al.,* 2012). In 1990, benzene formation in the beverages and foods with added preservatives such as salts of benzoates and ascorbic acid or its isomeric forms was observed (Nyman *et al.,* 2007; Poucke *et al.,* 2008; Nishna *et al.,* 2012; Varner *et al.,* 1991; Richardson, 2006).

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Later, the study in 1993 concluded that decarboxylation of benzoate in the presence of ascorbic acid and transition metal like Cu^{2+} and Fe^{3+} lead to the formation of benzene and the reaction is accelerated by heat and light (Gardner & Lawrence, 1993).

The study conducted on Dasamoolarishta, an Ayurvedic formulation, found that level of sodium benzoate was above the permitted level which could result in the formation benzene in the presence of ascorbic acid present as a constituent of herbal ingredient (Nishna *et al.,* 2012). The discovery regarding the production of benzene from the salts of benzoates in the various products demanded manufacturers to re-evaluate their formulations containing benzoate salts and ascorbic acid with the view to reduce benzene content (Azab *et al.,* 2019; Prnová *et al.,* 2019; Richardson, 2006). According to International Council for Harmonization (ICH) the residual solvent limit for benzene is kept as 2 parts per million (ppm) (ICH, 2018). Although maximum exposure to benzene occurs through inhalation from environment, the amount of benzene ingested plays a crucial role and interfere in biological processes (Maithani *et al.,* 2019; Dos *et al.,* 2015). So, the product with the benzene concentration above 2ppm is regarded as unsafe for human consumption. Looking at the risk, Margin of Exposure (MOE) value has been set, which ranges from 400,000 to 2,000,000. In general, a MOE ≥10,000 is considered to be protective. MOE value is regarded as the ratio between specific point on the dose- response graph that is responsible for tumor formation in various human and animal intake experiments (Benford *et al.,* 2010; Smith *et al.,* 2010).

Mechanism of benzene formation

Sodium benzoate or the other salts of benzoates react with ascorbic acid in the presence of transition metal like Fe^{2+} and Cu^{3+} form benzene as volatile organic compound (Gardner & Lawrence, 1993). Sodium benzoate gets converted to benzoic acid and finally, by the process of decarboxylation, benzene is produced. The chemical reaction of benzene formation is given in Figure 1 (Totally Wild, 2017). In this reaction, when the concentration of ascorbic acid is increased, the production of benzoic acid rises along with the formation of sodium L-ascorbate (Gardner & Lawrence, 1993). Further benzoic acid gets reduced to benzene by decarboxylation mechanism as illustrated in Figure 2 (Commons.wikimedia.org., 2015). In this mechanism benzoic acid reacts with hydroxyl group of sodium L-ascorbate forming water molecule and unstable benzoate ion as reaction intermediate. The unstable benzoate ion dissociates into carbon dioxide (CO_2) and positively charged benzene. From the positively charged benzene, H^+ ion reacts with another hydroxyl group and form water molecule. Hence, benzene molecule gets stabilised (Anon, 2018).

Factors affecting/influencing the benzene formation

Benzene formation in various food and beverages containing ascorbic acid and sodium benzoate depends upon various extrinsic and intrinsic factors. Concentration of sodium benzoate, ascorbic acid, pH, various raw materials, metal ions, chelating agent and artificial sweeteners/sugars are some of the intrinsic factors whereas temperature, UV radiation and storage time are extrinsic factors. Both intrinsic and extrinsic factors play an effective role in benzene formation in foods and health supplements.

Temperature

A study was conducted by Apera *et al.* (2008) on the influence of temperature on benzene formation using aqueous solution with similar concentration of sodium benzoate and ascorbic acid. They concluded that the benzene formation process enhances with the increase in storage temperature. The similar study was carried out by Morsi et al. (2012) on the soft drink samples. The samples were stored at 3 different temperatures (20 °C, 45 °C, and 90 °C)

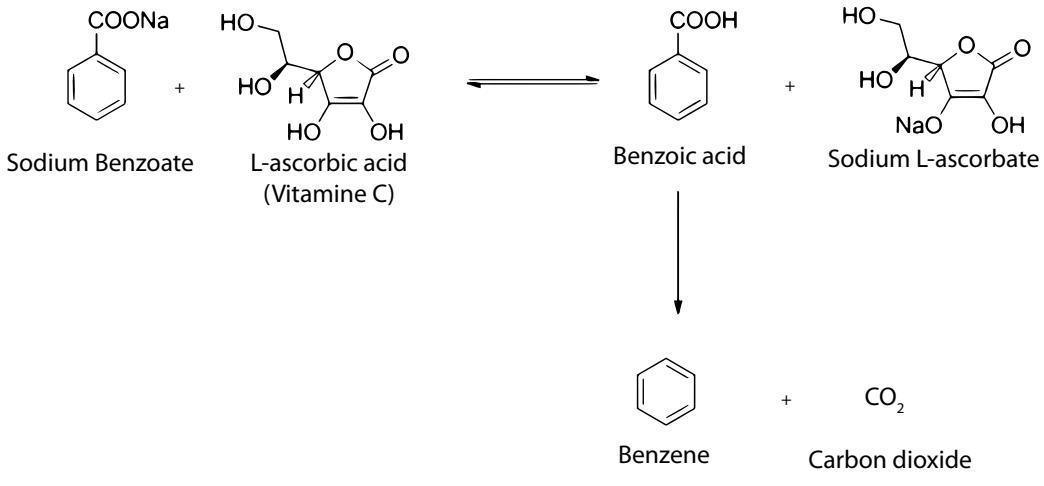

Figure 1. Chemical reaction for the formation of benzene.

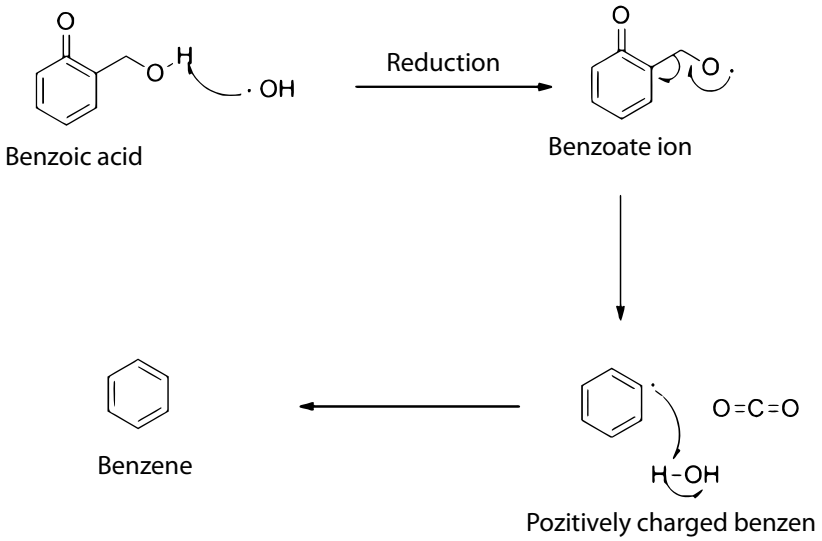

Figure 2. Decarboxylation mechanism of benzoic acid.

for 21 days. The samples stored at temperature 90 °C resulted in maximum benzene formation compared to samples stored at other two temperatures.

Sugar concentration

In the same study Aprea et al. (2008) discussed the effect of sugar on the formation of benzene at three different concentrations of sucrose, 0.1, 0.25 and 0.5. The study reflected that with the increase in sugar concentration the benzene formation was reduced.

Ultraviolet light

McNeal *et al.* (1993) studied the effect of temperature and UV on benzene formation. In order to conduct the study aqueous models containing sodium benzoate and ascorbic acid were stored at two different environmental conditions for 20 hours. One of them was storing at 45 °C or under intense UV light for 20 hours and the other condition was storing in dark and room temperature. Finally, it was discovered that benzene concentration in UV exposed sample was comparatively much higher than the samples stored in dark rooms.

Effect of copper and iron ions

Gardner & Lawrence (1993) studied the effect of transition metal catalyst in process of benzene production. According to the study Cu ion catalysis the electron reduction of oxygen molecule (O_2) by ascorbic acid or vitamin C and forms superoxide anion radical that further produce hydrogen peroxide by disproportionate reaction. Further, hydrogen peroxide (H_2O_2) get reduce to hydroxyl radical that attacks benzoic acid to produce benzene.

$Cu^{2+} + H_2Asc \rightarrow Cu^{+} + HAsc^{*}$

$Cu^{+} + O_2 \rightarrow Cu^{2+} + O_2^{-}$

$2O_2^{-} + 2H^{+} \rightarrow O_2 + H_2O_2$

$Cu^{+} + H_2O_2 \rightarrow Cu^{2+} + OH^{-} + OH^{*}$

Chang & Ku (1993) conducted similar study as McNeal *et al.* to determine the role of copper and iron ions in the benzene formation process. When the aqueous solution is stored in dark for eight days, the benzene concentration was found to increase more than McNeal *et al.* study. Hence, it was concluded that presence of these metal ions in water was enough to catalyze the reaction.

Effect of EDTA

Mahoney & Graf (1986) conducted the study on EDTA as oxidant in model system. In this study it was observed that EDTA complexation deactivates the catalytic activity of transition metal ions by utilizing their coordinating positions. Therefore, chelating agents like EDTA prevents the benzene formation when metallic ions are present in the solution (Codex Alimentarius commission, 2009).

Buffer system and antioxidants

Vinci *et al.* (2011) studied factors responsible for benzene formation. In this study, it was concluded that presence of buffer system and various sources of hydroxyl radical formation along with the presence of metal ions enhance the process of benzene formation. The factors like antioxidants, as comparative to EDTA, were more potent in inhibiting the benzene formation at concentration of 1M.

Incidents related to detection of benzene in Ayurvedic formulations

The presence of sodium benzoate is detected in various ayurvedic formulations like asava and arishta (Nishana *et al.,* 2012). These liquid formulations do not require any artificial preservative as in such formulations self-generated alcohol itself act as preservative. Nishana *et al.* study on the commercial samples of dasmoolarishta clearly indicated the presence of excessive amount of sodium benzoate as a preservative. This ayurvedic formulation contains vitamin C as a constituent of Amla. As per the reaction between ascorbic acid and sodium benzoate, benzene formation could occur in dasmoolarishta (Anon, 2017). Regular use of formulations containing high level of

benzene could lead to acute and long-term health effects. Apart from the dasmoolarishta, various other asava and arishta marketed formulations contain sodium benzoate (Nishana *et al.,* 2012). There is need of conducting more research studies on Ayurvedic formulations in order to quantify benzene formation in them.

Benzene metabolism

According to animal data, ingested benzene gets completely (100%) absorbed in the gastrointestinal tract and gets freely distributed throughout the body (ATSDR, 1993). Adipose tissues contain high levels of benzene metabolites on uptake. The metabolism of absorbed benzene follows the same pathway in both laboratory animals and humans. Benzene is converted mainly to phenol by the multi-function oxidase system, primarily in the liver followed by bone marrow. Low amount of phenol is metabolized to hydroquinone and catechol. Further, a very little amount gets converted to phenylmercapturic or *trans*-muconic acid. About 14% is excreted unchanged in expired air and a small unchanged part is excreted via urine (WHO, 2003; Cooper & Snyder, 2017).

Procedure of benzene metabolism

Biotransformation

Metabolic pathway for biotransformation begins with the formation of phenol. The metabolic process, carried out by Cytochrome P4502E1 and Cytochrome P450 in the liver, generates hydrogen peroxide. Hydrogen peroxide forms hydroxyl radical, which hydroxylate benzene to yield phenol (Snyder & Hedli, 1996). An alternative pathway for the formation of phenol involves the benzene oxide-oxepin system (Rappaport *et al.,* 2009). When metabolism of benzene forms benzene oxide, it rearranges itself nonenzymatically to form phenol (Rappaport *et al.,* 2010). Benzene oxide, so formed, yields 1,2-benzene dihydrodiol by hydration in the presence of epoxide hydrolase (Ross, 1996). Further, 1,2-benzene dihydrodiol gets oxidized in the presence of dihydrodiol dehydrogenase to form catechol (Smith, 1996). Phenol can also form hydroquinone by the process of hydroxylation (Bleasdale *et al.,* 1996).

Urinary metabolites

The metabolites of benzene found in urine are categorized as urinary metabolites. These includes phenolic metabolites–L-phenylmercapturic acid, 6-N-acetylcysteinyl-S-2,3-cyclohexadienol and 2,5-diOHphenylmercapturic acid (Nerland & Pierce, 1990); opened ring metabolites–trans-trans-muconic acid and 6-OH-t,t-2,4-hexadienoic acid (Kline *et al.,* 1993); and covalently bound DNA adduct – N^7-phenylguanine (Qu *et al.,* 2000).

Microsomal metabolism

In this process, hydroquinone is formed in much larger amount at lower benzene concentration. Addition of microsomal epoxide hydrolase also yields hydroquinone instead phenol (Snyder *et al.,* 1993).

Benzene metabolism with metabolites is summarized in Figure 3.

Benzene toxicity and health effects

Benzene toxicity causes damage to multiple classes of hematopoietic cells and a variety of hematopoietic cell functions resulting in both bone marrow depression and

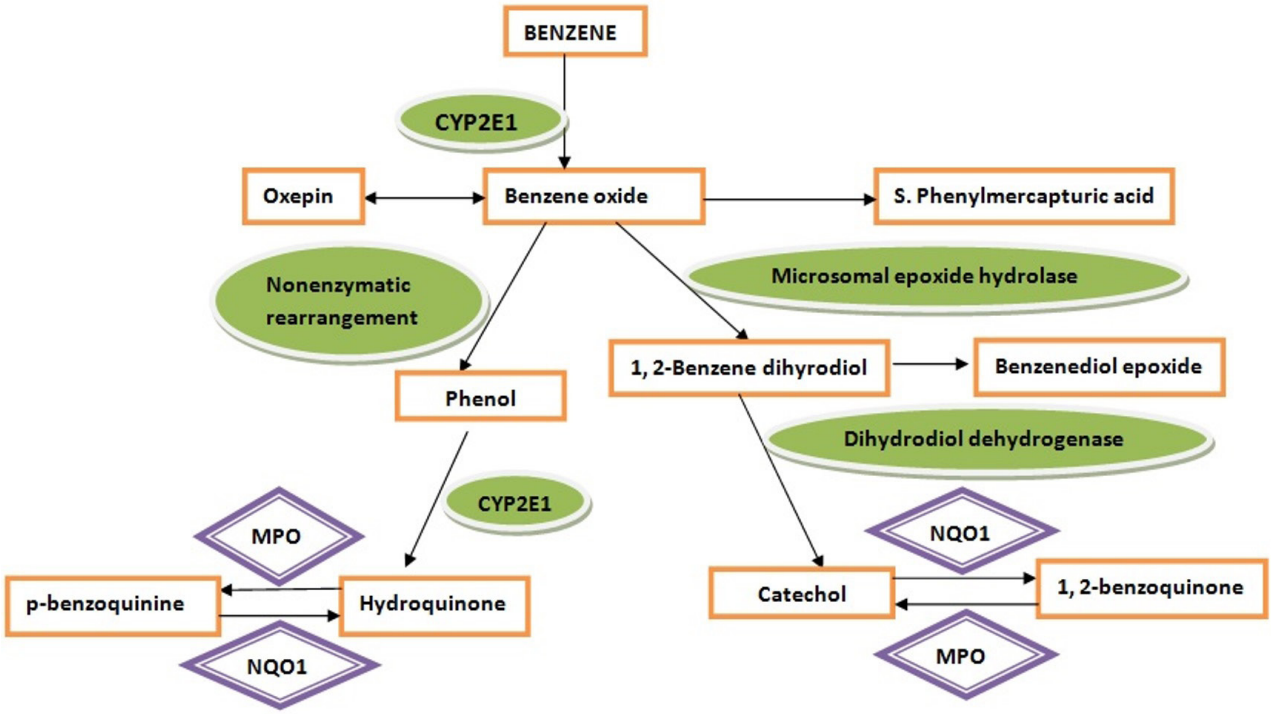

Figure 3. Schematic diagram of benzene metabolism.

leukemogenesis. Benzene metabolites damage cellular macromolecules from two ways, either by covalent binding of reactive metabolites of benzene or by inducing oxidative damage (Chen *et al.,* 2017). Relative contributions of these mechanisms to toxicity remains unrecognized hence it is clear that different mechanisms contribute to the toxicities associated with different metabolites (Snyder & Hedli, 1996). The general scheme of benzene metabolism, its metabolites and various adverse health effects is illustrated in given Figure 4 (D'Andrea & Reddy, 2018).

Benzene induced leukemia and hematotoxicity

Various national and international agencies conducted studies to determine the environmental substances that can cause cancer (a substance that causes cancer or multiplies its growth is called a carcinogen). The American Cancer Society collects data from these agencies to evaluate the risk of cancer based on evidence from animal and human research studies. From such animal and human evidence, various expert agencies have concluded that benzene has enough cancer-causing potential (Cancer.org, 2016).

The International Agency for Research on Cancer (IARC) is part of the World Health Organization (WHO) (Grosse *et al.,* 2009). Among its various functions, its major function is to detect causes of cancer. IARC classifies benzene as "carcinogenic to humans," based on enough evidence that benzene causes acute myeloid leukemia, acute lymphocytic leukemia, chronic lymphocytic leukemia, multiple myeloma, and non-Hodgkin lymphoma (Grosse *et al.,* 2009). Several different US government agencies, including the National Institutes of Health, the Centres for Disease Control and Prevention, and the United State Food and Drug Administration (USFDA) jointly established National Toxicology Program (NTP). NTP classified benzene as "known to be a human carcinogen" (Cancer.org, 2019). Apart from this, the US Environmental Protection Agency (EPA) also classified benzene as human carcinogen (Cancer.org, 2016). US EPA maintains the Integrated Risk Information System, an electronic database that contains information on human health effects from exposure to various substances in the environment (IPCS, 1999).

Quantitative risk extrapolation was used to estimate lifetime cancer risks due to clear evidences of carcinogenicity and chromosomal effects of benzene on laboratory animals and humans (WHO, 1993). It was calculated from different concentrations in drinking water using data on leukemia from epidemiological studies involving inhalation exposure. Increased lifetime cancer risk with various concentration of benzene in water is given in Table 1.

Due to lack of data on carcinogenic risk to humans following the ingestion of benzene, risk assessment was also carried out on the basis of a 2-year gavage study in rats and mice. The robust linear extrapolation model was used. The estimated range of concentrations in drinking-water corresponding to excess lifetime cancer risks of 10^{-4}, 10^{-5} and 10^{-6} are 100–800, 10–80, and 1–8 µg/l, respectively. This was calculated based on leukemia and lymphomas in female mice and oral cavity squamous cell carcinomas in male rats (WHO, 2003). The results were similar to those derived from epidemiological data that formed the basis for the previous guideline value of 10 µg/l associated with a 10^{-5} excess lifetime cancer risk.

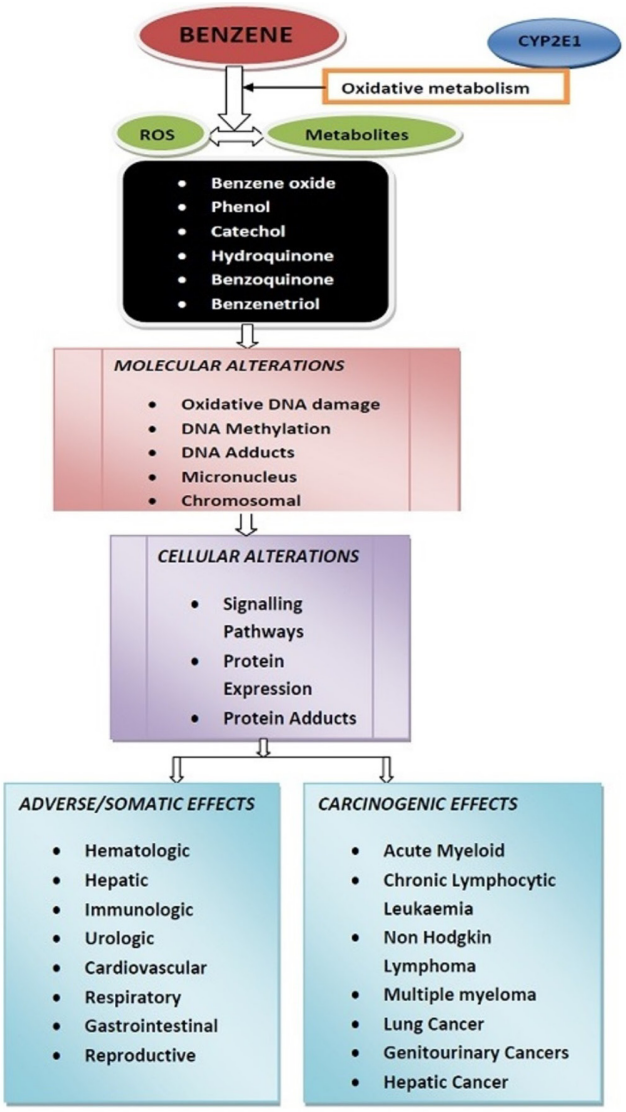

Figure 4. Schematic representation of benzene toxicity and health effects.

Table 1. Increased risk associated with benzene concentration.

Concentration (µg/l)	Increased risk
1	10^{-6}
10	10^{-5}
100	10^{-4}

Mechanism of benzene induced carcinogenicity

The metabolites produced in liver undergo secondary metabolism in bone marrow to produce benzoquinone (Rappaport *et al.,* 2009; Linhart *et al.,* 2011). Benzene induced leukemia begins when these metabolites targets genes that are unfavorable to hematopoiesis in

hematopoietic stem cells (HSCs) (Schoch *et al.,* 2005). Benzoquinone forms adduct with protein and DNA, which results in myelotoxicity (Linhart *et al.,* 2011; Loomis *et al.,* 2017). This adducts damages hematopoietic cells along with chromosomal aberrations, oxidative stress, alteration in gene expression, error prone DNA repair, apoptosis, and epigenetic regulations.

Damage to DNA causes bone marrow depression resulting in aplastic anemia, which could further leads to dysplasia and finally to acute myeloid leukemia (Snyder & Kali, 1994).

Along with adducts of protein and DNA free radicals are also generated. These free radicals cause oxidative stress and dysregulation of immune system (Kolachana *et al.,* 1993; Wiemels & Smith, 1993).

The transcription factor aryl hydrocarbon receptor (AhR) works as a negative regulator of HSCs. The AhR plays an effective role in benzene-induced hematotoxicity as it induces HSCs cycling from quiescence (Hirabayashi & Inoue, 2009; Singh *et al.,* 2009; Westphal *et al.,* 2009). The damage caused by benzene in HSCs results in apoptosis that is observed as hematotoxicity (McHale *et al.* 2011).

HSCs in bone marrow interact with mature lymphocytes in the stem cell niche. Hematotoxic damage to stem cell microenvironment results in abnormal hematopoiesis and finally leads to clonal expansion of leukemic stem cells (LSCs) as illustrated in Figure 5 (LSCs) (McHale *et al.,* 2011).

Benzene metabolites leading to reproductive failures

Benzene is colorless, volatile, and highly flammable liquid (ATSDR, 2007). Several epidemiological studies showed that the long-term exposure to benzene results in adverse impact on both male and female reproductive system (Mihaic & Borgert, 2019). It has been proven to cause high rates of functional disturbances in the system. Benzene is believed to cause negative impact on gonadotrophin hormones. Disturbances in such hormones, directly or indirectly, lead to reproductive failures (Reutman *et al.,* 2002).

Female reproductive system

Brown *et al.* (1998) disclosed in his study that women metabolize 23%–26% more benzene than men under similar exposure conditions. It was stated in research study conducted by Reutman *et al.* (2002) that regular benzene exposures for longer period results in abnormal menstruation and reduction in the size of ovaries. Continuous exposure to the benzene causes hormonal disturbances, which in turn are responsible for irregular menstruation among women and also effects size of ovaries at an adolescence age (Reutman *et al.* 2002). The study conducted by Alviggi *et al.* (2014) concluded that with increase in the concentration of benzene in the ovaries disturbs the gonadal functions and ovaries respond negatively to gonadotrophin hormones (FSH, LH). As a result, the effected ovarian sensitivity to FSH reduces the oocyte quality, which in turn effects pregnancy and increases the chances of frequent abortion. Apart from

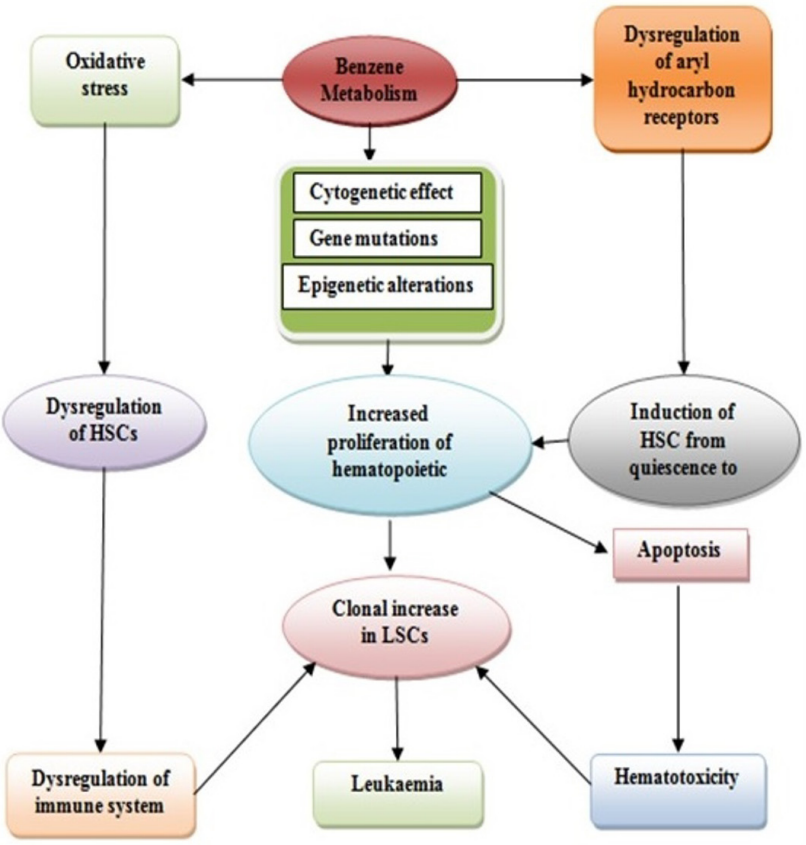

Figure 5. Illustration of benzene induce leukaemia and hematotoxicity.

this, various benzene metabolites cause damage to ova, decreases fertility, and increase the risk of tumor formation among young women (Alviggi *et al.,* 2014). In NTP bioassay various reproductive tract tumors (uterus, ovary) were reported (Cal/EPA, 1997).

Benzene also passes into umbilical cord of pregnant mother. From umbilical cord it reaches to fetus and causes abnormal development (Cal/EPA, 1997). The various adverse effects on fetal growth includes decreased birth weight, prematurity, growth retardation, spontaneous abortion, congenital diseases, and even childhood leukemia. Benzene exposure at particular stage of pregnancy affects the immune system of fetus, which becomes a major cause of childhood leukemia. Various other factors like maternal anemia lead to abnormal organogenesis in fetal development. Benzene also causes reduction in platelets (thrombocytopenia), as a result, there occur excessive bleeding (hemorrhage) during delivery. This condition could be life threatening to both baby and mother (Cal/EPA, 1997).

Male reproductive system

Recent experimental verifications reveal that males might be more susceptible than females to benzene toxicity. Long term benzene exposure among men is responsible for abnormal chromosomal number in sperm, which in turn affects fertility and fetal development (Cal/EPA, 1997). Benzene metabolites, phenol-hydroquinone and catechol, affects sperm motility, sperm viability, sperm nuclear DNA integrity and effective sperm count (Mandani *et al.,* 2013).

Sperm motility

In study conducted by Mandani *et al.* (2013), when the semen sample is exposed to phenol- hydroquinone significant decrease in sperm motility was observed than in normal sample. Another semen sample was exposed to catechol, again decrease in sperm motility was observed but less than by phenol-hydroquinone.

Sperm viability

Similarly, sperm viability was tested in two phases. In phase 1 spermatozoa were exposed to phenol-hydroquinone and in phase 2 spermatozoa were exposed to catechol (Mandani *et al.,* 2013). At lower concentration catechol reduces sperm viability greater than phenol hydroquinone.

Sperm nuclear DNA integrity

In the same research study, it was found that the number of spermatozoa with intact double stranded DNA was significantly decreased when exposed to phenol-hydroquinone and catechol (Mandani *et al.,* 2013). Hence, benzene exposure significantly reduces sperm DNA integrity.

Effective sperm count

On benzene exposure, it was also found that there was decrease in effective sperm count as compared to sperm density (Mandani *et al.,* 2013).

In all the above ways, benzene decreases fertility and also causes developmental effects. Reduce in quality of sperm lead to abnormal fetal development and various birth defects.

Cardiovascular disease risk associated to benzene exposure

Urinary metabolites like trans, trans-muconic acid and acrolein increase with increase of benzene level in blood. These metabolites were found to be responsible for causing cardiovascular effects. With the increase in these metabolite level circulating angiogenic cells (CACs) decrease and as result cardiovascular disease risk increases (DeJarnett *et al.,* 2014; O'toole *et al.,* 2010).

Research study conducted by Abplanalp *et al.* (2017) reported that every 20 (parts per billion) ppb increase in benzene increases cardiovascular mortality by 33%. Increase in benzene exposure marks the onset of acute myocardial infarction due to the suppression of CACs (Abplanalp *et al.,* 2017).

Hepatotoxicity due to benzene metabolism

Liver is primary organ for metabolism of benzene and toxic effect of these metabolites on liver is reported in initial pilot study. Higher levels of liver functioning enzymes alkaline phosphatase, aspartate aminotransferase, and alanine aminotransferase were observed in this study. Increased level of these enzymes in serum indicate the impairment of liver functions (D'Andrea & Reddy, 2014; 2016). The toxicity profile of benzene over the various parts of body is summarized in the Table 2 given (ATSDR, 2014).

Current legislative of benzene

Several government agencies regulate benzene levels and exposures. The Occupational Safety & Health Administration (OSHA) is the federal agency responsible for health and safety regulations in most workplaces. OSHA limits exposure to benzene in the air in most

Table 2. Benzene exposure and Health effects.

S.N.	Health effects	Description	Concentration of exposure	Time
1	Leukemia & hematoxicity	Aplastic anemia, pancytopenia, dysplasia, AML	2 ml	Years
2	Reproductivity failures	Irregular menstruation, damage to ova, reproductive tract cancer, decreased sperm count, viability and motility	–	–
3	Cardiovascular	Ventricular fibrillation, artherosclerosis	1000 ppm	Hours
4	Hepatotoxicity	Impairement of liver functions	–	–
5	Central nervous system	Vomiting, dizziness, sleepiness, convulsions, rapid heart rate and coma	300 ppm	60 minutes
6	Death	–	10 ml (8.8 g)	Minutes

workplaces to 1 ppm during an average workday and a maximum of 5 ppm over any 15-minute period. When working at potentially higher exposure levels, OSHA requires employers to provide personal protective equipment such as respirators. The Environmental Protection Agency (EPA) limits the percentage of benzene allowed in gasoline to an average of 0.62% by volume (with a maximum of 1.3%). The EPA limits concentrations of benzene in drinking water to 5 ppb. Some states may have lower limits. Likewise, the USFDA sets a limit of 5 ppb in bottled water. The Consumer Product Safety Commission (CPSC) considers any product containing 5% or more by weight of benzene to be hazardous, requiring special labeling.

Future perspective

Looking through the doors of present research studies on the various hazardous affects caused by benzene, it becomes an essential need to carry out extensive research in the field of Ayurvedic sciences to detect its presence. Research in these areas provides a better understanding about benzene formation and its level in various Ayurvedic formulations. Although number of studies has been carried in food sciences, where the preserved foods consist of sodium benzoate along with ascorbic acid, similar studies are also required in Ayurvedic sciences. Most of the nonalcoholic syrups are added with salts of benzoate to enhance its shelf life ignoring the drastic step that take place when such syrups already consist of ascorbic acid.

An oral reference dose (RfD) for benzene set by EPA is 0.004 mg/kg/d based on hematological effects in humans. The RfD is an estimate of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without appreciable risk of deleterious noncancer effects during a lifetime. At exposures above RfD, the potential for adverse health effects increases. Hence, the formulations that are at risk of benzene contamination should be necessarily investigated.

Various research studies are conducted on the factors that play crucial role in benzene formation by the process of decarboxylation. As even the presence of sodium benzoate and ascorbic acid do not necessarily lead to the formation until the various intrinsic and extrinsic factors favor the formation. In 2006, American beverage association recommended certain guidelines for benzene reduction. It includes removing, reducing, or replacing benzoates with other microbial growth inhibitors like potassium sorbate and checking the product storage conditions since strong light and high temperatures speed up the formation of free radicals. In case of formulations containing transition metals, the addition of chelators such as EDTA or sodium polyphosphates was recommended to reduce benzene formation.

Owing to the various serious health issues related to benzene exposure and increasing degree of relying on herbal drugs or formulations in the people for both, the purpose of health and personal needs, it becomes necessary to carry out preclinical trials and, whenever necessary, clinical trials should be carried out before the various formulations are made available over the counter.

Conclusion

Present manuscript highlights the mechanism of benzene formation in food products/formulations, factors affecting benzene formation, metabolism, toxicity and other health effects. In light of oral exposure to benzene, study reports are scarce but to clarify the risk of oral exposure and for establishing more appropriate limit for benzene in foods and drugs more experimental studies are required to be conducted and also the cumulative effect of oral and environmental exposure should also be studied for finding out the health risk increment. Presence of higher level of benzene in food can further impact their acceptability at international level and cause loss to export revenues of our country. There is a need to assess the level of benzene formation in marketed formulations so that alternative preservatives can be sorted out and regulatory authorities may issue new directions for use of alternative and safe preservatives. It is high time that regulatory authorities recognize the problem of noncompliance of GMP for ASU drugs and take steps to enforce the provisions of Drug and Cosmetics Act in letter and spirit of the law.

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