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Review Paper

BROMOBENZENE: A HEPATOTOXIN

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Abstract

Liver damage can be induced by drugs or chemicals resulting from overdose or workplace exposure. Bromobenzene is an efficient model to perform studies on hepatotoxicity. After biotransformation into liver,it causes production of reactive oxygen species (ROS). Organs that have been found to be affected due to bromobenzene induced toxicity are liver, kidney and lungs. In the following review, mechanisms and various genes involved in the bromobenzene induced hepatotoxicity have been discussed. Also, the toxicokinetics, involving absorption, metabolism and elimination of bromobenzene from the body have been reviewed in this article. Various herbal extracts and chemicals have been found to exhibit protective effect against bromobenzene induced liver injury proving their potential in use as hepatoprotective agents.

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INTRODUCTION

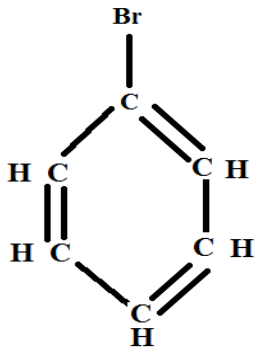
Liver is a vital organ as it performs the detoxification of xenobiotics and toxic substances and serves an important role in metabolism of drugs in the body. In the course of metabolism of xenobiotics and toxic substances large amounts of reactive oxygen species are produced which have been shown to be associated with liver diseases, such as hepatotoxicity,hepatic carcinoma and other liver pathological conditions [1, 2] .Antioxidant are present in the body in sufficient amounts and they scavenge the ROS [3] . However, when large acute dose or chronic exposure to toxic substances overpowers the hepatic antioxidant defence system, liver injury occurs [4]. ROS cause changes in gene expression system and alter the cellular membrane permeability

and bind to various macromolecules [3,4] thus resulting in DNA damage and ultimately cell death[5,6].

Modern medicines that are used for the prevention and cure of liver disorders have adverse side effects and are costlier. Therefore, phytocompounds which are relatively cheaper and easily available and have few or no side effects seem to be highly attractive. This creates an urge to evaluate the therapeutic potential of some natural products and for this chemically induced hepatotoxic models are being used. The chemicals used for inducing hepatotoxicity include Acetaminophen [7,8], Carbon tetrachloride[9,10], Galactosamine [11], Antituberculosis Drugs [12],Bromobenzene [13,14] ,etc. Recently it has been shown that bromobenzene induced hepatotoxicity represents a very good model for the study of lipid peroxidation, since in this experimental condition the level of detectable lipid peroxidation in the liver is by far greater than that of the others.

Bromobenzene(Fig. 1) is an aryl halide and is used for organic synthesis, as an additive to motor oils, as a flame retardant, in manufacture of various drugs and chemicals and as a crystallizing solvent. Release of bromobenzene to the environment may occur during its production or its use, resulting in its exposure to living beings. Bromobenzene being a hydrophobic molecule is subjected to biotransformation in the liver, where it elicits toxicity and causes necrosis. The purpose of this review is to understand the mechanism of Bromobenzene hepatotoxicity and its metabolism in liver.

Fig.1 Structure of Bromobenzene



Chemical and Physical Properties of Bromobenzene

Bromobenzene (BB) is a heavy, colourless liquid with a pungent odour [15]. Its Synonyms include monobromobenzene and phenyl bromide [16]. It has a molecular weight of 157.01 Da and chemical formula is C_6H_5Br. It has a high Boiling Point of 156.0°C and a very low melting point -30.6°C. It is very slightly soluble in water; its water solubility being 4.46 × 102 mg/L at 30°C and is miscible with chloroform, benzene, and petroleum hydrocarbons. The soil sorption constant or Koc of Bromobenzene is 150. Concentration of Bromobenzene more than or equal to 1 mg/m3 = 0.15 ppm, 1 ppm = 6.53 mg/m3 (17Verschueren, 2001) are considered to air pollution factors.

Models of bromobenzene(BB) induced liver injury

To study mechanisms of Bromobenzene toxicity, mouse or rats in vivo or their primary hepatocytes are most frequently used. The LD50 of BB was reported to be approximately 20 mmol/kg body weight for male Crl:Cd rats (Haskell Laboratory for Toxicology and Industrial Medicine, 1981, unreviewed). In most studies a dosage of 0.45 g/kg or 10mM or 460 mg/kg of body weight has been used. It can be administered alongwith 0.1m of corn oil or 0.1m of coconut oil. Route of administration for bromobenzene can be intraperitoneally [18], intragastric intubation[19,20,21] or oral gavage [23]. Usually animals are fasted for 24 h before and 19 h after Bromobenzene administration as fasting allows lower doses of Bromobenzene to be used and results in less variation of the injury but non-fasted animals can also be used [22]. Liver injury develops after 24 at a dosage of 2mmol/kg body weight, as pronounced centrilobular necrosis can be observed [22].

Metabolic Action of Bromobenzene

During the metabolism of bromobenzene(Fig.2) in the liver, it gets converted into either the 3, 4-oxide

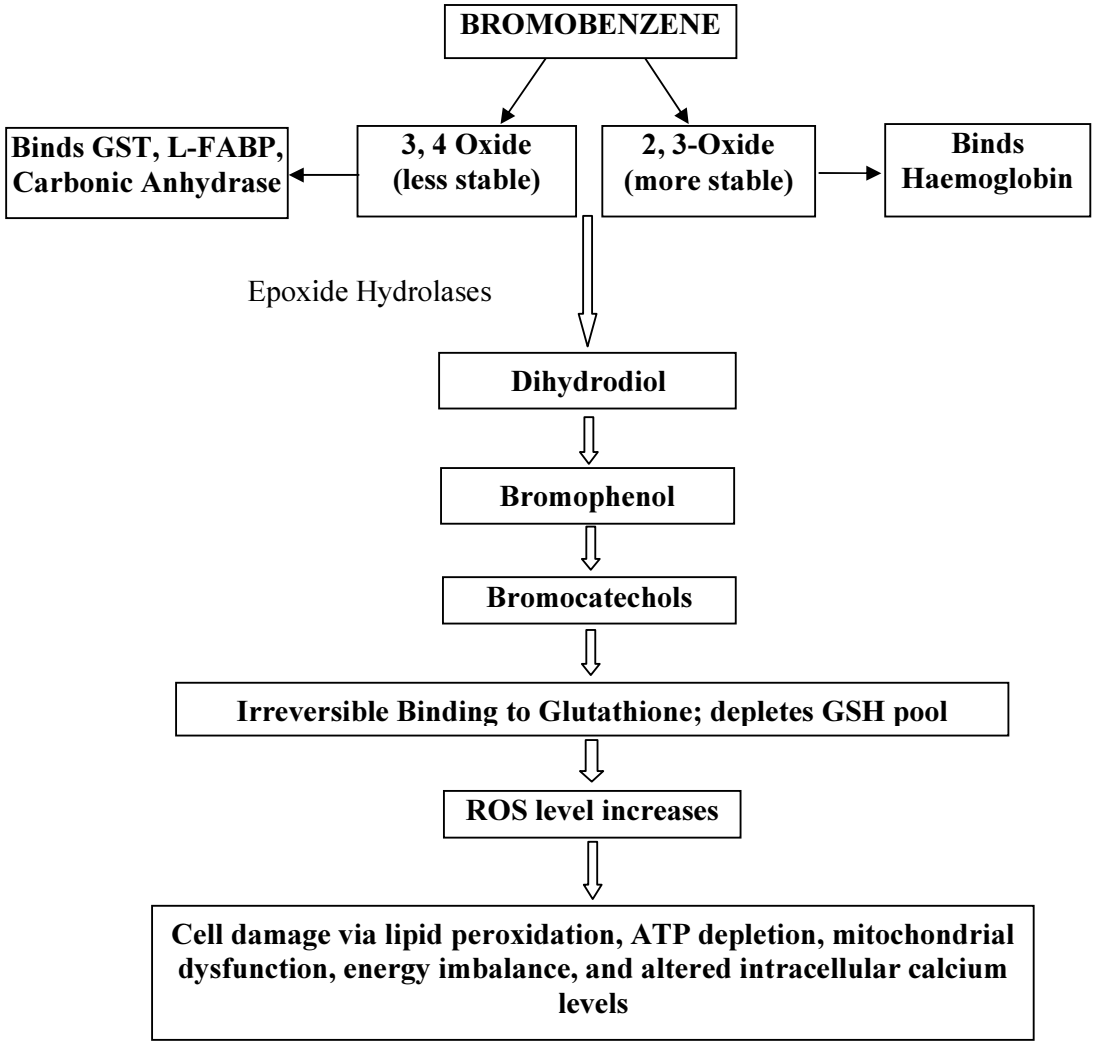
derivative or the 2, 3-oxide derivative catalysed primarily by cytochrome isozymes (CYP 450 1A2, 1B1, etc). 3,4-oxide derived from bromobenzene binds with glutathione-S-transferase (GST), liver fatty acid binding protein (L-FABP), and carbonic anhydrase. [24]. According to Lau and Zannoni [25] BB 2, 3-oxide is more stable than other alternative forms and it covalently binds to haemoglobin. These oxides are among the most reactive BB-metabolites and thus their hydrolysis is important in the hepatic detoxification. Also, both the 3, 4-oxide and 2, 3-oxide derivatives are converted to their corresponding dihydrodiols by epoxide hydrolase(EH) which is upregulated and increase in mEH messenger RNA (mRNA) levels by BB is observed thus proving its role; the hydrolysis of epoxide BB intermediates, enabling their excretion. The mEH is also induced by some other xenobiotics like phenobarbital, trans-stilbene oxide, and Aroclor 1254 [26]. Then bromophenols (2-, 3-, and 4-bromophenol) from the oxide derivatives are formed and many different pathways are proposed for this [27,14,28].According to Lertratanangkoon et al.[27] 2, 3-oxide derivative has a relatively short biological half-life so spontaneous rearrangement can be the primary pathway for conversion to 2-bromophenol. Also, it has been suggested that 2-bromophenol and 3-bromophenol may also be produced by non-enzymatic dehydration of the 2, 3-dihydrodiol [27,28].

The bromophenol metabolites can be subsequently converted to their respective bromocatechols (2-, 3-, or 4-bromocatechol) by Cytochrome isozymes and redox cycling of 2- and 4-bromocatechol and conjugation by glutathione S-transferase produces 2-bromo-3-(glutathione-S-yL) hydroquinone and 6-glutathione-S-yL-4-bromocatechol, respectively [14].

At high doses of bromobenzene, the levels of glutathione (GSH) are reduced as a result of conjugation to the metabolites and thus the intracellular protection against reactive oxygen species (ROS) and hazardous xenobiotic metabolites is lost. This may lead to a number of secondary events that damage the cell, like lipid peroxidation[29], ATP depletion [30,31] mitochondrial dysfunction, energy imbalance, and altered intracellular calcium levels. Gopi and Setty[20] found that in mitochondria there is a block in transfer of electrons through the electron transport chain at complex I. Complex I is a major site for the production of ROS and hence can be expected to have a greater damage under heavy oxidative stress. Level of ATP synthase beta subunit was found to decrease in study conducted by Heijne et al. [32].

Heijne et al. [32,22,34] observed increased production of more than 20 liver proteins (including γ-glutamylcysteine synthetase, rate limiting enzyme thus important in glutathione biosynthesis) Also, genes typically involved in oxidative stress such as Heme Oxygenase-1, Peroxiredoxin 1, Metallothionein, Ferritin were found to be induced and changes in the transcriptional expression of numerous genes involved in drug metabolism (epoxide hydrolase, aldehyde dehydrogenase, etc), cellular response to reduced glutathione levels, the acute phase response (Metallothionein, Vitronectin, etc) and intracellular signalling, following intraperitoneal administration of Bromobenzene to rats using transcriptomics and proteomics approach was noted. Some other studies such as those conducted by Waters et al. [34], Minami et al. [35]; Stierum et al. [36] have utilized toxicogenomics to characterize the relationship between Bromobenzene hepatotoxicity and hepatic gene expression profile.

Fig.2 Metabolism of Bromobenzene



Toxicokinetics of Bromobenzene

Findings of systemic effects due to oral ingestion by Casini et al. [13] or inhalation by Brondeau et al. [37] of Bromobenzene by animals serve as an indication that it is absorbed through the gastrointestinal tract and lungs. After absorption, Bromobenzene and its corresponding metabolites are widely distributed into the body and the highest levels are found in adipose tissue as observed in the experiments by Zampaglione et al. [38], Reid et al. [39]. At 4 hours after injection, the highest levels of Bromobenzene were found in fat (5,600 µg/g tissue), followed by the liver (282 µg/g), kidney (235 µg/g), brain (206 µg/g), heart (146 µg/g), lung (142 µg/g), stomach (132 µg/g), and blood plasma (34 µg/g). After 24 hours, measured concentrations were as follows: fat (400 µg/g), kidney (19 µg/g), stomach (17 µg/g), liver (11 µg/g), brain (7.0 µg/g), lung (6.2 µg/g), heart (5.0 µg/g), and blood plasma (2 µg/g) [39].

Monks et al. [40] determined the distribution of Bromobenzene inside the body by monitoring covalent binding to the protein fraction in various tissues following intraperitoneal injection of 3 mmol/kg (471 mg/kg-day) of [^{14}C]-Bromobenzene in male Sprague-Dawley rats and found that it was most pronounced in the liver, followed by the kidney, small intestine, lung, and muscle. The metabolism of Bromobenzene has been extensively studied in vivo and in vitro mammalian systems [41,27,14]. Results of animal studies indicate that urinary excretion of metabolites is the principal route of elimination of absorbed Bromobenzene [28,39], although biliary

excretion of the 3- and 4-glutathionyl conjugates formed from the 3, 4-oxide derivative has been demonstrated in bile-cannulated rats [42]. Rate of biliary excretion can be used as an index of in vivo activation of Bromobenzene [43].

In other rat studies, metabolites detected in the urine collected after 48 hours accounted for more than 90% of administered doses of 8 mg/kg-day (intravenous) or 1,570 mg/kg-day (intraperitoneal) [38]. Tanaka et al. [44] observed the changes in drug metabolizing reaction related to the metabolism and detoxification of Bromobenzene after repeated administration of Bromobenzene and found that the degree of the severity of the toxicity declined as the liver acquired resistance. It was noted from further studies that Multidrug resistance protein 3,mrp3, in Phase III reaction (drug elimination) contributed to the resistance of Bromobenzene hepatotoxicity in addition to the suppression of Phase I reaction(metabolic activation) and the induction of Phase II reaction(Detoxification) based on degree of the gene expression changes[45].

Therapeutics in treatment of bromobenzene hepatotoxicity

Few herbal drugs and chemicals (Table 1) have been found to be useful in protection from bromobenzene against liver injury. Some of them are as follows:

Bis (Maltolato) Zinc II Complex

Kawasa et al. [46] treated cultured rat hepatocytes with Bromobenzene and found that the number of viable cells decreased upto 40%.However, the viability was restores when Bis (Maltolato) Zinc II Complex was added to the culture.

Hispludin

Effects of the natural flavonoid hispidulin (6-methoxy-5, 7, 4′-trihydroxyflavone) on bromobenzene-induced hepatotoxicity in mice were evaluated and it was found to inhibit liver injury and lipid peroxidationThe hepatoprotective activity can be related to the antioxidant properties of hispidulin. [47]

Rosa rugosa

Hepatoprotective effects of a methanol extract of Rosa rugosa root and its triterpenoid glycoside, rosamultin, on hepatic lipid peroxidation and drug-metabolizing enzymes were investigated in rats treated with Bromobenzene.Both the methanol extract and rosamultin restored the activity of epoxide hydrolase, which had been reduced by bromobenzene. Hepatic glutathione concentrations were decreased and hepatic lipid peroxides were increased in rats intoxicated with bromobenzene which was prevented with the methanol extract and rosamultin. These results suggest that the extract of *R. rugosa* and its compound, rosamultin, may protect against bromobenzene-induced hepatotoxicity through its antioxidant properties [48].

Table 1: Treatment of Bromobenzene induced Hepatic Injury

S. No.	Drug/Chemical	Animal	Optimal Dose	Route	References
1	DimethylSulfoxide	Male Sprague Dawley Rats(300-325g)	2ml/kg	Oral Gavage	Lind and Gandolfi[49]
2	Aged Garlic Extract	Male Wistar Rats(200-300g)	10ml/kg	Orally	Wang et.al [50,31]
3	Aqueous extract of Phyllanthus fraternus	Male Wistar Rats(130-140g)	100mg/kg	Orally	Gopi and Setty [20]
4	Ginger Extract	Albino Male Rats(120-140g(	100mg/kg	Intragastric intubation	El Sharaky et al. [21]
5	Hemidesmus indicus Aqueous Extract	Male Albino Wistar Rats(130-140g)	100mg/kg	Orally	Gopi and Setty[51]

CONCLUSION

In the Liver the toxic effect of Bromobenzene are mainly seen in the centrilobular region, due to the high oxygen concentration and cytochrome P 450 activity. It is uniquely different as it causes centrilobular necrosis only after bioactivation. Many genes and proteins involved in drug metabolism, oxidative stress, reduced glutathione depletion involved in Bromobenzene toxicity have been found and some are yet to be resolved and therefore require more studies. Many Herbal drugs and Chemicals that have profound effect in decreasing the Bromobenzene induced hepatotoxicity have been identified and further exploration into the roles played by the potential mechanisms will produce new therapeutic interventions to help attenuate liver damage resulting from overdose of workplace exposure.

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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