TOXICITY STUDY OF BUTYLATED HYDROXYL TOLUENE (BHT) IN RATS

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INTRODUCTION

Butylated hydroxyl toluene (BHT) is also termed as 2,6-di-tert-butyl-p-cresol or 2,6-di-tert-butyl-4-methyl phenol. BHT does not occur in nature and is prepared synthetically from p-cresol and isobutylene. BHT is one of the most widely used synthetic phenolic anti-oxidant in foods, mainly in foods containing fats and oils, food containers, packaging materials and in cosmetics\(^1\,^2\). Fats, oils, and fat-containing foods are naturally susceptible to rancidity and other oxidation reactions. Lipid oxidation is autocatalytic reaction which proceeding as a complex chain of reactions involving free radicals. Here BHT act as a free radical scavenger, prevents rancidification by terminating this chain reaction. BHT has a molecular weight of 220.34 with the chemical formula C\(_{15}\)H\(_{24}\)O and it is a white odourless crystalline solid, insoluble in water, but freely soluble in ethanol and fatty oils\(^3\).

BHT is metabolized primarily in the liver microsome. Some metabolism of BHT also occurs in lung. In rat liver BHT is metabolized to a variety of sulfate and glucuronic acid conjugates which eventually excreted in the urine and faeces and to some extend through bile\(^4\,^5\,^6\). Cytochrome P\(_{450}\) mediated metabolism in liver causes the formation of a reactive and electrophilic metabolite BHT quinone methide (BHT-QM) 2,6-di-tert- butyl-4methylene-cyclohexa-2,5-dienone) an intermediate metabolite of BHT which can covalently bind to various cellular nucleophiles, especially those containing sulfhydryl groups such as cysteine and glutathione which consequently leads to acute hepatotoxicity and pneumotoxicity\(^7\). BHT compounds has been subjected to extensive toxicological studies in various animals. LD\(_{50}\) value of BHT in rats when administered orally is > 2930 mg/kg bw. At high doses it is known
to cause hemorrhagic death because of the inhibition of hepatic prothrombin synthesis. BHT is generally recognized as a safe food additive (E 321). The Joint Expert Committee on Food Additives of the FAO/WHO approved an acceptable daily intake for man is 0.5mg BHT/kg bw. It is rapidly absorbed from the gastrointestinal tract and distributed to liver and body fat. Genotoxicity studies revealed that BHT is not capable of inducing point mutations, chromosomal aberrations, or to interact with or damage DNA.[8,9,10] BHT is used to treat herpes in human beings at a dose ranging from 2500 – 3000 mg and is found to be effective against a wide variety of lipid coated viruses like Newcastle Disease virus[11,12]. BHT can act both as promoter and antipromotor of carcinogenesis. Despite the antioxidant and antiviral effects many questions about the use of BHT in human medicine remain unanswered.

Present study was carried out to evaluate the toxic effect of BHT in rats.

MATERIALS AND METHODS

Chemicals - BHT was procured from Sisco Research Laboratory Ltd, Chennai. All other chemicals and reagents were procured from Merck India Pvt, Ltd, Mumbai. BHT was dissolved in olive oil and was prepared fresh every day. BHT was administered as gavage using orogastric tube.

Animals – Male wistar rats weighing 100 – 150 g were used for the study. Animals were housed in appropriate cages in a well ventilated experimental animal room under 12 : 12 hr L:D cycle at 22 to 28°C with free access to standard rat pellet diet and drinking water. Experiments were conducted with the approval of the Institutional Animal Ethics Committee. Animals were randomly divided into 4 groups, each comprising 10 animals and were administered with BHT for a period of 14 days as shown below.

Group I – Normal control
Group II - 250 mg/kg body weight
Group III – 500 mg/kg body weight
Group IV – 1 g/ kg body weight

All the animals were observed twice daily for mortality during the 14 – day period of study. Blood samples were collected from the retro orbital plexus under mild ether anaesthesia, using heparinised capillary tubes, into sterile microcentrifuge tubes on 0, 7 and 14th day of experiment and centrifuged at 1000 x g for 10 min. at 15°C to separate serum. On day 15 the
surviving rats were euthanized, samples of liver were collected and fixed in 10 % formalin for histopathological examination.

**Biochemical analysis** – Serum enzymes ALT, AST and GGT activity and total protein and serum albumin concentrations were estimated using commercially available Kits (M/s E. Merck India, Ltd, Mumbai).

**Statistical analysis** – Data were analyzed using repeated measures ANOVA and the interaction effect were found significant. Hence simple analysis of variance (ANOVA) was done for all the variables in different periods followed by Duncan multiple range test to determine the level of significance (Steel and Torrie 1980). The value of $P <0.05$ was considered statistically significant and $P <0.01$ is statistically highly significant.

**RESULTS AND DISCUSSION**

BHT- induced hepatotoxicity mirrored by an increased serum ALT, AST and GGT levels ($P <0.01$) and decreased serum total protein levels$^{[13]}$ ($P <0.01$). The present findings correlate with the reports of many other workers. All the animals in group IV died within 2-3 days.

_**Serum enzymes** – ALT, AST and GGT activities increased significantly on BHT administration. As indicated in Table 2 group III on 14th day showed a 7 times increase in ALT value. AST values showed a 10 times increase when compared to control group. Duncan multiple range test to determine the level of significance ALT and AST level on 14th day for both group II and III ($P < 0.01$) was significantly different from group I. The increase in levels of ALT and AST occurred in a dose dependent manner. Maximum value shown by group III animals. ALT found in the cytoplasm of the hepatocyte and to a lesser extent its also seen in liver mitochondria. So any hepatocytic injury can lead to an increase in serum ALT. Serum ALT activity can also be increased as a result of injury to myocytes as well. Similar to increase in ALT activity increased serum AST activity observed in both reversible and irreversible hepatocyte injury, cholestasis and myocyte injury. Hepatic injury is often associated with alterations in the serum and liver levels of some enzymes notably ALT and AST$^{[14,15]}$. Both these enzymes are present in high concentrations in the liver. Hepatocytes contain number of enzymes and it takes place in number of metabolic reaction. When there is any hepatocytic injury or hepatocellular reactions to toxic agents serum level of ALT and AST will increase. Liver injury can result in plasma membrane damage, thereby causing an increase in serum levels of ALT and AST.
Serum GGT level showed a 5 times increase in group III animals on 14\textsuperscript{th} day of the experiment. On Duncan multiple range test to determine the level of significance serum GGT level on 14\textsuperscript{th} day for both group II and III (\( P < 0.01 \)) was significantly different from group I. Serum GGT is used as a clinical indicator of cholestasis. In cholestasis increase in level of serum GGT in toxic groups clearly indicates its hepatotoxicity. No animal survived in group IV clearly indicates toxic effect of BHT. The most accurate indicator of liver damage is probably be the appearance of the cellular constituents in blood stream. Determination of the activity of the hepatic enzymes which is released into the blood stream from the damaged hepatocytes is one of the most useful tool in the study of hepatotoxicity.

*Serum total protein*- Group III animals showed a significant decrease in serum total protein by 14\textsuperscript{th} day when compared to group II and control animals. Decrease in concentration of serum total protein might indicate the inhibition of protein synthesis. Low protein level results when there is extensive liver damage\cite{16,17}. The decreased levels of total protein and augmented activities of some of the liver specific enzymes like ALT, AST, GGT may be related to hepatotoxicity of BHT.

**Table 1** – Level of serum markers in normal and experimental group (values are mean \( \pm \) SD for 10 animals in each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total protein (g/dl)</th>
<th>Albumin (g/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0\textsuperscript{th} day</td>
<td>7\textsuperscript{th} day</td>
</tr>
<tr>
<td>I</td>
<td>6.94±.22</td>
<td>6.89±.23</td>
</tr>
<tr>
<td>II</td>
<td>7.02±.16</td>
<td>6.61±.82</td>
</tr>
<tr>
<td>III</td>
<td>6.95±.18</td>
<td>6.19±.43</td>
</tr>
</tbody>
</table>

**Table 2** – Level of serum alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma glutamyl transferase in rats induced with BHT toxicity. (values are mean \( \pm \) SE for 10 animals in each group)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum ALT activity</th>
<th>Serum AST activity</th>
<th>Serum GGT activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0\textsuperscript{th} day</td>
<td>7\textsuperscript{th} day</td>
<td>14\textsuperscript{th} day</td>
</tr>
<tr>
<td>I</td>
<td>35.41±.59</td>
<td>35.5±.59</td>
<td>35.41±.60</td>
</tr>
<tr>
<td>II</td>
<td>35.45±.12</td>
<td>101.83±2.7</td>
<td>56.33±3.5</td>
</tr>
<tr>
<td>III</td>
<td>35.30±.05</td>
<td>108.19±2.8</td>
<td>201.11±9.5</td>
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CONCLUSION
From above results obtained it could be clearly inferred that BHT induces a potent hepatotoxic effect.

REFERENCES


