Effect of abhrak bhasma and silicon dioxide on hepatic and renal glutathione status in rats: hepatoprotection testing against single dose carbon tetrachloride induced hepatotoxicity

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Abstract

Background: Glutathione (GSH) is an important intracellular antioxidant. Intrahepatic GSH levels are depleted in liver diseases.

Objectives: In present study, effect of abhrak bhasma (an Ayurvedic drug) and silicon dioxide (SiO\textsubscript{2}) on hepatic and renal GSH status against CCl\textsubscript{4} intoxicated male albino rats were investigated.

Methods: Single dose of CCl\textsubscript{4} (3.0ml/kg body wt, sc) was used to induce hepatotoxicity. Graded doses (10, 20, 30 and 40mg/ kg body wt) of abhrak bhasma and SiO\textsubscript{2} were concurrently given with CCl\textsubscript{4}. Hepatic and renal GSH content was studied after 24 hrs.

Results: Results showed that rats exposed to CCl\textsubscript{4} exhibited decreased GSH in liver. It was counteracted and maintained to normal levels by the treatment of abhrak bhasma (minimum protective dose-10mg). SiO\textsubscript{2} treatments did not affect GSH activity in liver significantly. Single dose of CCl\textsubscript{4} had not influenced GSH content in kidney alone or with any of the doses of abhrak bhasma or SiO\textsubscript{2}.

Conclusion: CCl\textsubscript{4} single dose depletes GSH content significantly in liver but not in kidney. These results suggest that single dose treatment of abhrak bhasma (10mg onwards) protects GSH content and thus manages CCl\textsubscript{4} induced free radical generation scavenging them.

Keywords: Abhrak Bhasma, Antioxidant, Glutathione, Hepatotoxicity, Silicon Dioxide.

1. Introduction

Liver and kidneys play a vital role in the metabolism, detoxification of xenobiotics by biotransformation and protect clearance (Edward & Celia, 1998; Majno & Joris; Nebbia, 2001). Carbon tetrachloride (CCl\textsubscript{4}) is one of the most commonly used hepatotoxin in the experimental study of liver diseases. CCl\textsubscript{4} induced oxidative stress is associated with increased free radical generation and lipid peroxidation (LPO) (Recknagel et al, 1992; Burk et al. 1984; Kaplowitz et al., 1986; Teli et al., 2014); which leads to fatty degeneration in liver. Oxidative damage induced by free radicals can be prevented by the use of antioxidants. LPO and cellular antioxidant defense have importance in the oxidative stability. Ongoing oxidative processes and decreased intrahepatic glutathione level induced oxidative stress in liver is known (Ljubuncic et al., 2000; Ljubuncic & Bonzom, 2006). Endogenous glutathione, a thiol compound synthesized mainly in liver plays an antioxidant role by reducing reactive oxygen species (ROS) formed during cellular metabolism and protects cells (Parke & Piotrowski, 1996; Deneke, 2000). It has negative correlation with LPO in liver and kidney.

Many researchers have focused on natural antioxidants for the treatment of oxidative stress induced complications. Abhrak bhasma is a commonly used Ayurvedic drug in various disorders including hepatitis (Sharma, 1977). In therapy it is useful in anti-aging treatment, rejuvenation treatment etc. It has been reported for a strong immune system, rapidly increasing the production of T-Cell phagocytes. In our earlier work abhrak bhasma has protected single dose of CCl\textsubscript{4} induced increased malondialdehyde content in liver (Teli et al., 2014). Thus, to study the possible scavenging activity in vivo; it was planned to study glutathione (GSH) content in single dose of CCl\textsubscript{4} induced hepatotoxicity and associated changes in kidney. SiO\textsubscript{2} was used as silicon control for abhrak bhasma.

2. Material and methods

2.1. Animal

Male albino rats, Rattus norvegicus weighing about 130-140g each were used for experiment. They were bred and maintained in the departmental animal house (Reg. No. 233/CPCSEA) under standard conditions and were given standard pellet diet (prepared by Amrit feeds, Sangli, MS, India). Food and water were provided ad libitum.

2.2. Preparation of abhrak bhasma and silicon dioxide

Abhrak bhasma was prepared in the laboratory as described in Rasa Ratna Sammucchaya (Sharma, 1977). SiO\textsubscript{2} treatment was given as silicon control. To study dose dependent effects of abhrak bhasma and SiO\textsubscript{2} on GSH content of liver and kidney, different doses viz. 10, 20, 30 and 40 mg/kg body wt were administered orally with honey. Honey control rats that were used; showed data as normal rat. Therefore, honey control data is not presented.

2.3. Experimental design
3. Results and discussion

Table 1: Abhrak bhasma (AB) & SiO₂ Influenced Alterations in GSH Contents in Liver and Kidney of Rats by Single Dose of Treatment.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td>LC₅₀</td>
<td>mg/gm Tissue</td>
<td>µg/mg Protein</td>
</tr>
<tr>
<td>Normal</td>
<td>27.97±1.28</td>
<td>0.22±0.002</td>
</tr>
<tr>
<td>CCl₄ (30 ml) sc</td>
<td>21.25±2.21</td>
<td>0.14±0.013</td>
</tr>
<tr>
<td>CCl₄ + 10mg AB</td>
<td>25.13±1.81</td>
<td>0.20±0.002</td>
</tr>
<tr>
<td>CCl₄ + 20mg AB</td>
<td>25.64±2.31</td>
<td>0.21±0.006</td>
</tr>
<tr>
<td>CCl₄ + 30mg AB</td>
<td>26.11±1.91</td>
<td>0.21±0.009</td>
</tr>
<tr>
<td>CCl₄ + 40mg AB</td>
<td>28.01±2.84</td>
<td>0.23±0.006</td>
</tr>
<tr>
<td>CCl₄ + 10mg SiO₂</td>
<td>39.21±3.11</td>
<td>0.28±0.012</td>
</tr>
<tr>
<td>CCl₄ + 20mg SiO₂</td>
<td>36.82±2.84</td>
<td>0.27±0.008</td>
</tr>
<tr>
<td>CCl₄ + 30mg SiO₂</td>
<td>35.14±1.33</td>
<td>0.28±0.009</td>
</tr>
<tr>
<td>CCl₄ + 40mg SiO₂</td>
<td>32.19±1.09</td>
<td>0.27±0.008</td>
</tr>
</tbody>
</table>

(Values are mean ± SEM of 6 animals. P values: a < 0.05; b <0.01; c <0.001 vs. normal).

Glutathione is a reducing agent that is in high concentrations in mammalian tissues. It is an antioxidant, rich in cytosol. GSH reacts efficiently with oxidizing substances such as active oxygen species and lipid peroxidation. Several studies have revealed that status of GSH contents is markedly decreased in human tissues, which was not altered by the administration of 10, 20 and 30 mg abhrak bhasma and remained in its normal range. When treatment of 40mg abhrak bhasma was given to the normal rat, GSH content was marginally increased (1.11 fold). Thus, abhrak bhasma alone with 10mg through 30mg doses are not affecting GSH contents while highest dose used marginally increased the GSH content. Similar doses of SiO₂ when given to the normal rat in same experimental condition, it showed significant elevation in GSH contents (P <0.001) which was noted at all SiO₂ doses studied. As contrast to abhrak bhasma, SiO₂ given alone showed highly significant increase in hepatic GSH, but not in renal GSH con-
tent. These results indicate that SiO$_2$ induces GSH content in liver. It is dose dependent in 10, 20 and 30mg, while 30mg and 40mg dose showed similar levels. Similar trend was also expressed in GSH status calculated per mg protein. Kidney GSH content remained unaffected either with abhrak bhasma doses or with SiO$_2$ doses used indicating single doses of any of the drugs is not influencing kidney GSH. These results are similar to malondialdehyde contents of kidney studied earlier (Teli et al, 2014); indicating free radical and free radical scavenger status is steady and normal. Single dose of CCl$_4$ reduced GSH significantly (P<0.05) in liver. Simultaneous treatment of abhrak bhasma (all the doses) normalized the GSH content. Minimum dose required was 10mg. But contrast to these results all the doses of SiO$_2$ induced GSH content in CCl$_4$ treated rats levels of which were significantly high over the levels reported in normal rat. These observations suggest that SiO$_2$ all the doses are potent inducers of GSH content in presence of CCl$_4$ so also in absence of CCl$_4$. But in kidney SiO$_2$ is not influencing GSH content either in presence of CCl$_4$ or in absence of CCl$_4$, same is true with abhrak bhasma doses in kidney. These results also show similarity with LPO product contents (Teli et al, 2014). Thus, indicating in kidney free radical (MDA) and free radical scavenger (GSH) status remains independent of single doses of CCl$_4$, abhrak bhasma and SiO$_2$.

Thus, primary dose response of CCl$_4$ toxicity in liver is protected by minimum dose of abhrak bhasma managing reduction in MDA (Teli et al, 2014) with increased GSH/ natural free radical scavenger. Single doses of SiO$_2$ are capable of inducing GSH content in normal and CCl$_4$ (single dose) treated rat liver. But GSH content of kidney is inert to CCl$_4$, single dose treatment, CCl$_4$+ single doses of abhrak bhasma (10/20/30/40mg), CCl$_4$+SiO$_2$ (10/20/30/40mg), abhrak bhasma alone single doses (10/20/30/40mg) and SiO$_2$ alone single doses (10/20/30/40mg). These studies require need of other parameter studies to resolve hepatoprotective mechanism of action/s of abhrak bhasma against CCl$_4$ induced toxicity.

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References


