ABHRAK BHASMA AND SiO₂ CONTEMPLATED LEVELS OF SERUM CHOLESTEROL, TRIGLYCERIDES AND FREE FATTY ACIDS IN SINGLE DOSE OF CCl₄ INTOXICATED RAT

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ABSTRACT

The serum levels of cholesterol (CHO), triglycerides (TG) and free fatty acids (FFA) were studied to analyse protective effects of abhrak bhasma in graded doses (10, 20, 30 and 40mg/kg body wt given once) against single dose of CCl₄ (3.00ml/kg body wt/sc given once) induced hepatotoxicity, administering (PO) it simultaneously. CCl₄ administration increased CHO, TG, FFA level significantly. Abhrak bhasma successfully protected the normal levels showing variations in minimum dose requirements viz. CHO-30mg, TG-10mg, FFA-30mg. All the above doses maintained the normal levels. SiO₂ treatment showed variance. 10mg and 20mg doses hardly influenced CCl₄ mediated elevated CHO and 30 and 40mg doses showed marginally high CHO levels than normal. While CCl₄ mediated increased FFA levels were hardly influenced by SiO₂. Same was true incase of TG where 30 and 40mg dose marginally lowered the TG. Results indicate that tendency of lowering cholesterol is shown by abhrak bhasma and SiO₂ but it is more potent incase of abhrak bhasma influence. Though the protective tendency is shared by SiO₂, it is not shown by SiO₂ high doses. TG and FFA levels were lowered by abhrak bhasma and not by SiO₂. Thus silica form in abhrak bhasma is more potent than pure amorphous silica form in SiO₂.

KEYWORDS: Abhrak bhasma, SiO₂, CHO, TG, FFA.

INTRODUCTION

Dyslipidemia is also one of the major risk factor for cardiovascular diseases (CVD), which is characterized by elevation of various lipid and lipoprotein fractions including cholesterol,
triglycerides and FFA. Which is also observed in CCl₄ induced toxicity[^1] The American heart association has identified the primary risk factor associated with atherosclerosis as elevated levels of cholesterol and TG in blood. Various Ayurvedic preparations have been reported to have hypolipidemic and hypocholesterolemic properties.[^2,3,4,5]

CCl₄ is known to induce Dyslipidemia[^6,7] and is used as centrolobular liver fatty degeneration inducing agent.[^8] Its single dose (3.00ml/kg body wt) is known to induce centrolobular necrosis in rat[^9,10], which is an appropriate model to test primary response of drug against single dose of CCl₄ induced hyperlipidemia.

Abhrak bhasma is mica-derived Ayurvedic drug and naturally should contain silica. For this reason we used SiO₂ as control drug.

In our effort to analyze mode of action of abhrak bhasma and to differentiate its effects from role of SiO₂ we have already studied the histological alterations against single dose of CCl₄ induced hepatotoxicity.[^8] Which showed abhrak bhasma and SiO₂ single dose mediated dose dependent (10, 20, 30 and 40mg/kg body wt) hepatoprotection with some hepatocytes showing hypertrophy in SiO₂ mediated changes. In abhrak bhasma and SiO₂ graded single doses (10, 20, 30 and 40mg/kg body wt) influenced toxicity studies in normal rat showed hypertrophy of hepatocytes[^8] and increased serum LDL levels[^11] with high doses of SiO₂ (30 and 40mg).

Present work was designed to study the serum cholesterol (CHO), triglycerides (TG) and Free fatty acids (FFA) during implementation of graded doses of abhrak bhasma (PO) and SiO₂ (PO) with single dose of CCl₄ (3.00ml/kg body wt once) administration in rats on 24 hrs of interval.

**MATERIAL AND METHODS**

*Animals*

Albino rats (male of *Rattus norvegicus*) were used for experimental work. They were derived from original stalk obtained from National Institute of Virology, Pune. The animals were bred and maintained in the Animal House (Reg. No. 233/CPCSEA). Required animals of about 130-140gm wt were segregated and were fed with standard pellet diet (Amrit Feeds, Sangli, MS, India). Food and water were given *ad libitum* during experimental work.
**CCl₄:** CCl₄ (EMerk, India) used was purchased from local trader and was preserved at 10⁰C. It was administered as 3.00ml/kg body wt/sc given once during 8.00 to 9.00 am.

**Abhrak bhasma:** To prepare abhrak bhasma the method described in Rasa Ratna Sammucchaya[12] was adapted. It was prepared under the guidance of traditional Ayurvedacharya.

**SiO₂:** As described in earlier work SiO₂ was used to distinguish role of silica from abhrak bhasma mediated changes since abhrak bhasma is derived from mica-silica ore. SiO₂ was obtained from local trader. Same sample was used throughout the experimental work.

Graded doses of both abhrak bhasma and SiO₂ (10, 20, 30 and 40mg/kg body wt given once) were administered orally (PO) with honey during 8.00 am to 9.00 am.

Honey control rats were maintained but they showed alterations similar to normal rat and hence data are not presented.

**Experimental Schedule**

The rats were maintained in the following groups. Each group contained six rats. The treatments/group were as follows,

- **Group 1** – No treatment. The group was maintained as normal.
- **Group 2** – CCl₄ (3.00ml/kg body wt/sc once)
- **Group 3** – CCl₄ (3.00ml/kg body wt/sc once) + 10mg abhrak bhasma/po once
- **Group 4** – CCl₄ (3.00ml/kg body wt/sc once) + 20mg abhrak bhasma/po once
- **Group 5** – CCl₄ (3.00ml/kg body wt/sc once) + 30mg abhrak bhasma/po once
- **Group 6** – CCl₄ (3.00ml/kg body wt/sc once) + 40mg abhrak bhasma/po once
- **Group 7** – CCl₄ (3.00ml/kg body wt/sc once) + 10mg SiO₂/po once
- **Group 8** – CCl₄ (3.00ml/kg body wt/sc once) + 20mg SiO₂/po once
- **Group 9** – CCl₄ (3.00ml/kg body wt/sc once) + 30mg SiO₂/po once
- **Group 10** – CCl₄ (3.00ml/kg body wt/sc once) + 40mg SiO₂/po once

**Collection of serum:** The rats were sacrificed after 24 hr by deep ether anesthesia. The blood samples were collected from the left ventricle using syringe and were allowed to clot at room temperature. On clotting the blood samples were centrifuged using table top centrifuge. The colourless serum from samples was stored at 10⁰C until use (within 6 hrs).
**Biochemical assays:** Estimations of cholesterol, triglycerides (TG) and free fatty acids (FFA) were made with the help of commercial kits (Purchased from Liquipath, marketed in India by Pathozyme Diagnostics, Kagal, Kolhapur, MS, India). Method used in kit for TG assay was based on Trinder (1969)[13] and Jacobs et al., (1960).[14] The bioassay described in kits for total cholesterol assay was based on Trinder (1969)[13] and Allain (1974).[15] Free fatty acids (FFA) were determined by the method of Itaya (1977).[16]

**STATISTICAL ANALYSIS**

The results were expressed as Mean ± SEM of different groups. The significant differences between groups were evaluated by one way analysis of variance (ANOVA) followed by student ‘t’ test. The statistical calculations were carried out with the help of XLSTAT 7.5 computer programme. Values P<0.05, P<0.01 and P<0.001 were considered to show statistical significance.

**RESULTS AND DISCUSSION**

Table: 1. Abhrak bhasma and SiO₂ influenced alterations in cholesterol, triglycerides and free fatty acids in serum against single dose of CCl₄ toxicity

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>Free Fatty Acids (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>116.50 ± 4.36</td>
<td>73.10 ± 1.93</td>
<td>62.98 ± 2.35</td>
</tr>
<tr>
<td>CCl₄ [3.0 ml/kg body wt] sc</td>
<td>144.05 ± 6.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.69 ± 2.32&lt;sup&gt;b&lt;/sup&gt;</td>
<td>76.39 ± 3.81&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + AB [10 mg/kg body wt] po</td>
<td>134.0 ± 4.52&lt;sup&gt;b&lt;/sup&gt;</td>
<td>77.00 ± 2.52</td>
<td>72.68 ± 3.25&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + AB [20 mg/kg body wt] po</td>
<td>137.30 ± 10.4</td>
<td>73.30 ± 5.98</td>
<td>73.47 ± 4.79</td>
</tr>
<tr>
<td>CCl₄ + AB [30 mg/kg body wt] po</td>
<td>122.84 ± 4.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.85 ± 3.54</td>
<td>64.01 ± 2.17&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + AB [40 mg/kg body wt] po</td>
<td>106.21 ± 6.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>72.34 ± 5.52</td>
<td>54.48 ± 4.71&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + SiO₂ [10 mg/kg body wt] po</td>
<td>143.68 ± 5.24&lt;sup&gt;b&lt;/sup&gt;</td>
<td>78.74 ± 3.23</td>
<td>77.39 ± 5.26&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + SiO₂ [20 mg/kg body wt] po</td>
<td>142.85 ± 6.32&lt;sup&gt;a&lt;/sup&gt;</td>
<td>79.16 ± 4.25</td>
<td>77.61 ± 4.67&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CCl₄ + SiO₂ [30 mg/kg body wt] po</td>
<td>127.10 ± 4.21</td>
<td>77.14 ± 2.22</td>
<td>70.39 ± 3.26</td>
</tr>
<tr>
<td>CCl₄ + SiO₂ [40 mg/kg body wt] po</td>
<td>127.46 ± 3.99</td>
<td>78.15 ± 2.14</td>
<td>71.25 ± 4.59</td>
</tr>
</tbody>
</table>

Values are mean ± SE of 6 animals. [Values expressed as mg/dl]

P values: a <0.05, b <0.01, c <0.001 vs normal

x <0.05, y <0.01, z <0.001 vs CCl₄ treated

**Cholesterol:** In normal rat serum total cholesterol was 116.50 ± 4.36 mg/dl. Administration of 3.0ml CCl₄ to the normal rat showed 1.23 fold increase (P<0.01) in total cholesterol level. Simultaneous treatment of single dose of 10, 20 and 30mg abhrak bhasma/kg body wt, to CCl₄ induced intoxicated male albino rats resulted in progressive decrease in serum cholesterol, 30mg and 40mg abhrak bhasma/kg body wt significantly reduced the cholesterol
contents. Except 10mg dose of abhrak bhasma remaining doses maintained the cholesterol levels in normal range i.e. 20, 30 and 40mg being equally potent and minimum effective dose being 20mg.

Simultaneous treatment of single dose of 10mg and 20mg SiO$_2$/kg body wt to the CCl$_4$ intoxicated albino rats showed no change and remained in the same range. Treatments of 30 and 40mg SiO$_2$/kg body wt to the CCl$_4$ intoxicated albino rats, showed marginal decrease in serum cholesterol contents. Only 30mg and 40mg SiO$_2$ doses protected cholesterol values and maintained the normal levels of cholesterol.

**Triglycerides:** Normal albino rats showed 73.10 ± 1.93 mg/dl of serum triglycerides. CCl$_4$ administration showed 1.14 fold increase ($P<0.01$) in triglycerides contents. Treatment of various doses abhrak bhasma showed progressive reduction in triglycerides contents towards normal level. 20mg, 30mg and 40mg doses of abhrak bhasma brought total triglycerides contents to the normal level. Thus showing 20, 30 and 40mg equally influenced triglycerides levels to maintain at normal range. Thus lowest minimum effective dose being 20mg.

Similar trend was observed with the simultaneous administration of various doses SiO$_2$ to the CCl$_4$ intoxicated rats. It showed progressive reduction in triglycerides contents towards normal levels. Thus all the doses are effective and 10mg dose being minimum effective.

**Free Fatty Acids (FFA):** In normal rat serum FFA level was 62.98 ± 2.35mg/dl. Administration of 3.0ml CCl$_4$ to the normal rat showed significant increase in FFA level (by 1.21 fold). Treatment of single dose of 10mg and 20mg abhrak bhasma doses/kg body wt to CCl$_4$ induced intoxicated male albino rats simultaneously showed non-significant depletion in serum FFA level while single dose treatments of 30mg and 40mg doses of abhrak bhasma/kg body wt significantly reduced FFA contents. 30mg dose of abhrak bhasma showed normalized FFA contents. But 40mg abhrak bhasma treatment showed further marginal decrease in FFA contents.

Treatment of single dose of 10mg and 20mg SiO$_2$/kg body wt to the CCl$_4$ intoxicated albino rats, showed no change in FFA contents. 30 and 40mg SiO$_2$/kg body wt to the CCl$_4$ intoxicated albino rats, showed non-significant decrease in serum FFA though it showed numerical further deplete.
Doses 10mg and 20mg abhrak bhasma did not affect CCl₄ influenced FFA levels. Thus, two doses of abhrak bhasma viz. 30 and 40mg doses protected CCl₄ induced FFA levels fully.

Thus 10mg and 20mg influences of abhrak bhasma on FFA levels are shared by same doses of SiO₂. But 30mg and 40mg abhrak bhasma fully protected FFA but same doses of SiO₂ failed to do so, maintaining high levels of FFA.

Levels of increased cholesterol in CCl₄ were induced by abhrak bhasma in dose dependent progressive influence and 30mg and 40mg normalized the levels.

SiO₂ 10 and 20mg doses did not influence. CCl₄ influenced high levels of cholesterol but 30 and 40 mg were equally potent to influence cholesterol level. Thus though abhrak bhasma and SiO₂ 30 and 40mg share the resultant influence. Tendency of lowering cholesterol show by abhrak bhasma by 10mg and 20mg doses is different from SiO₂ influenced behavior by same doses indicating the silica influence in sharing of similarity and differences being in the preparatory differences of drug.

Triglycerides levels in CCl₄ treated rat were normalized by 20mg and 40mg of abhrak bhasma but none of the SiO₂ doses shared this while FFA levels were normalized by 30mg of abhrak bhasma and was further lowered by 40mg dose. But SiO₂ did not affect FFA by any of the doses thus deflecting from abhrak bhasma. Thus FFA levels seem to inert to SiO₂ doses. Thus shared effects may be that of silica and difference can be due to basic processing of abhrak bhasma and SiO₂.

Abhrak bhasma is known to protect the single dose of CCl₄ induced centrolobular fatty degeneration⁹,¹⁷, minimum effective dose was 10mg⁹,¹⁷ and cholesterol, triglycerides and FFA may be influenced by mobility of accumulation fat by lipolytic enzymes under abhrak bhasma influence.⁹ Levels of cholesterol, triglycerides and FFA levels seem to be requiring higher doses abhrak bhasma to be normalized. While SiO₂ silica form is not influencing FFA.

In earlier studies of single doses of abhrak bhasma (10, 20, 30 and 40mg) given to normal rat¹⁷ is not toxic or adversely affecting either liver/kidney histologically. But SiO₂ single doses (10, 20, 30 and 40mg) given to normal rat showed hepatocytes hypertrophy at periarterial zone by 40mg dose only.
In light of above observations it seems that silica showed influences by SiO$_2$ are with stressed liver histologically indicating abhrak bhasma is not harmful even with higher doses.

CCl$_4$ is known to increase synthesis of cholesterol, triglyceride and FFA in liver.[18] Thus single dose induced all those levels apparently reflect liver metabolism of all these content since liver shows fatty degeneration.[8] It may be stimulation of other hepatocytes to damage necrotic area in centrolobular region. As with abhrak bhasma and SiO$_2$ centrolobular damage is protected the reduction in cholesterol, triglycerides and FFA is observed. But since composition of abhrak bhasma and SiO$_2$ differs the changes may be in minimum effective doses. The common effects of reduction can be considered as the influence of silica since both the drugs share this part. But variations in minimum effective doses can be attributed to differences in drug preparation and differences in synthetic processes and may be selective specific effects of these drugs’s induced biotransformed products in rat may be acting as influencing/modifying metabolites that give the net results.

CONCLUSIONS

Abhrak bhasma doses protect high levels of cholesterol, triglycerises, FFA levels in CCl$_4$ treated rat. Abhrak bhasma doses normalizes these levels. SiO$_2$ the silica drug control influenced cholesterol levels but not TG levels efficiently while it was inert towards FFA levels. Thus abhrak bhasma effectively lowers CCl$_4$ induced hypercholesterolemia, triglyceridemia and FFA in rat.

ACKNOWLEDGEMENTS

Authors are thankful to Late. Dr. S. S. Patil for his contribution to abhrak bhasma preparation and other constructive suggestions.

REFERENCES

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