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# Comparison of Abhrak Bhasma and Silicon Dioxide efficacy against Single dose of Carbon Tetrachloride Induced Hepatotoxicity in rat by evaluation of Lipid Peroxidation

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## ABSTRACT

Carbon tetrachloride ($CCl_4$) induces lipid peroxidation in liver and other tissues. Abhrak bhasma, an Ayurvedic drug known for its hepatoprotective potency and is derived from silicate/silicon ore. Its preparation involves heating and it is assumed by many that it is $SiO_2$. Thus to compare their hepatoprotective potency, both the drugs were tested against the single dose of $CCl_4$ induced hepatotoxicity in male albino rat. Graded single doses viz. 10mg, 20mg, 30mg and 40mg of abhrak bhasma and $SiO_2$/ kg body wt were used simultaneously. The hepatoprotective potency was evaluated by studying malondialdehyde (MDA) contents of liver and kidney in experimental rats. The results show that abhrak bhasma has counteracted $CCl_4$ induced MDA levels to bring them to normal levels. Though $SiO_2$ has influenced free radical scavenging positively. None of the doses used did not counteracted fully to normalize the MDA levels in $CCl_4$ induced lipid peroxidation in male albino rats. In kidney, $CCl_4$ induced levels of MDA were low which were protected by all the doses of abhrak bhasma and high doses of $SiO_2$ (30 and 40 mg/kg body wt). Thus, present results help to use abhrak bhasma as a potential drug which is capable of protecting liver and it can potentially be used for possible prevention of diseases associated with oxidative stress. However, further study need to be assessed for doses optimization in human.

**Keywords:** Abhrak bhasma, $SiO_2$, hepatotoxicity, lipid peroxidation, malondialdehyde, hepatoprotective potency.

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# INTRODUCTION

The liver and kidneys are extremely sensitive to many chemical and environmental agents. They are involved in detoxification of xenobiotics by biotransformation[1-3]. Carbon tetrachloride ($CCl_4$) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. The hepatotoxic effects of $CCl_4$ are largely due to its active metabolite, including the free radicals CCl3. and CCl(3)00.[4] which bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. This lipid peroxidative degradation of biomembranes is one of the principle causes of hepatotoxicity of $CCl_4$[5].

Lipid peroxidation (LPO) is probably the most extensively studied free radical induced process [6-10]. It is a crucial step in the pathophysiology of several disease states and in the process of aging[11-13], during which oxidative damage of lipids occur which can lead to the liberation of highly reactive aldehydes such as malondialdehyde (MDA) and 4-Hydroxynonenal (HNA). MDA is the aldehydic end product, which has frequently been used as an indicator of LPO and occur under conditions associated with the free radical damage[14].

Endogenous antioxidant that plays a major role in reducing reactive oxygen species formed during cellular metabolism. Many researchers have focused on natural antioxidants for the treatment of oxidative stress induced complications. Abhrak bhasma is an Ayurvedic drug used in various disorders including hepatitis. It is derived from mica, an ore of silica[15]. Since abhrak bhasma has a high penetrative ability, it spreads in the body at a faster rate and impacts micro tissues quickly. It supremely effective in cell turnover. It has been reported for a strong immune system. It is useful in anti-aging treatment, rejuvenation treatment etc. Hepatoprotective efficacy of abhrak bhasma was also studied earlier[16,17].

The present study was aimed to evaluate the effect of abhrak bhasma on LPO in $CCl_4$ in single dose induced hepatotoxicity in rat liver and kidney in experimental schedule of 24 hrs in male albino rats. Since abhrak bhasma is derived from crude ore of silica on processing by herbal treatments followed by heat cycles (Shodhan and Maran-Sharma[15]). It is always criticized that it is an oxide of silica. To differentiate role of silica and also to evaluate abhrak bhasma, pure $SiO_2$ was used as silicate control for drug to judge the efficiency.

# MATERIALS AND METHODS

### Animal:

Male albino rats, *Rattus norvegicus* originally derived from National Institute of Virology Pune,

were bred and maintained in the animal house (Reg. No. 233/CPCSEA). All rats were fed with standard pellet diet (prepared by Amrit feeds, Sangli, MS, India). Food and water were provided *ad libitum.* The rats, weighing about 130-140g each were used for experiment.

**Preparation of abhrak bhasma and Silicon dioxide:**

Abhrak bhasma was prepared in the laboratory as described in Rasa Ratna Sammucchaya[15]. $SiO_2$ treatment was given as positive control. To study the detailed dose dependent effects of abhrak bhasma and $SiO_2$ on lipid peroxidation in liver and kidney, different doses viz, 10, 20, 30 and 40 mg/kg body wt were administered orally with honey. Honey control rats were used which showed normal histology of liver and kidney at all doses. Therefore, honey control data is not presented.

**Experimental schedule:**

The male albino rats were assigned into following groups, each containing 6 animals and the various treatments were given as follows.

Group 1 - The rats were maintained as normal without any treatment.

Group 2 - Hepatotoxicity induced by single dose of 3.0 ml $CCl_4$/kg body wt for 24 hrs sc.

Group 3 - 10 mg abhrak bhasma/kg body wt was given po.

Group 4 - 20 mg abhrak bhasma/kg body wt was given po.

Group 5 - 30 mg abhrak bhasma/kg body wt was given po.

Group 6 - 40 mg abhrak bhasma/kg body wt was given po.

Group 7 - 10 mg $SiO_2$/kg body wt was given po.

Group 8 - 20 mg $SiO_2$/kg body wt was given po.

Group 9 - 30 mg $SiO_2$/kg body wt was given po.

Group 10 - 40 mg $SiO_2$/kg body wt was given po.

Group 11- $CCl_4$ (3.0 ml/kg body wt) *sc* + 10mg abhrak bhasma/kg body wt *po* for 24 hrs.

Group 12- $CCl_4$ (3.0 ml/kg body wt) *sc* + 20mg abhrak bhasma/kg body wt *po* for 24 hrs.

Group 13- $CCl_4$ (3.0 ml/kg body wt) *sc* + 30mg abhrak bhasma/kg body wt *po* for 24 hrs.

Group 14- $CCl_4$ (3.0 ml/kg body wt) *sc* + 40mg abhrak bhasma/kg body wt *po* for 24 hrs.

Group 15- $CCl_4$ (3.0 ml/kg body wt) *sc* + 10mg $SiO_2$/ kg body wt *po* for 24 hrs.

Group 16- $CCl_4$ (3.0 ml/kg body wt) *sc* + 20mg $SiO_2$/kg body wt *po* for 24 hrs.

Group 17- $CCl_4$ (3.0 ml/kg body wt) *sc* + 30mg $SiO_2$/kg body wt *po* for 24 hrs.

Group 18- $CCl_4$ (3.0 ml/kg body wt) *sc* + 40mg $SiO_2$/kg body wt *po* for 24 hrs.

The rats were killed after 24 hrs by giving deep ether anesthesia and liver and kidney tissues were separated from animals and taken for lipid peroxidation.

**Determination of Lipid Peroxidation***:*

For the assessment of lipid peroxidation malondialdehyde level per gm tissue and per gm protein of both liver and kidney tissues were determined by method described by Buege and Aust [18].

**Statistical analysis***:*

The obtained data were expressed as Mean ± SEM. Statistical analysis was performed by one-way ANOVA followed by the students 't' test. The values of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered statistically significant.

## RESULTS AND DISCUSSION :

Numerous studies have shown that oxidative stress in cells and tissues result from the increased generation of free radicals or reactive oxygen species (ROS). Free radicals are produced in cells by cellular metabolism and by exogenous agents that induce numerous pathological complications including aging[19-22]. Since $CCl_4$ metabolism in liver produces free radicals, lipid peroxidation was studied[24,25]. MDA used as the indicator of lipid peroxidation[26-30]. It is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids[31] and it is a secondary product of LPO which is used as an indicator of tissue damage by a series of chain reactions[32]. Here the extent of lipid peroxidation was estimated by measurement of MDA formation in the liver and kidney.

The normal range of MDA in liver was 102.10 µmoles/gm wt of tissue. Single dose of 10gm abhrak bhasma given for 24 hrs (one day) did not alter the rat MDA production. But single dose of 20, 30 and 40 mg abhrak bhasma marginally decreased (approximate 5%) the production of MDA (Table No. 1). The results indicate that 10mg and 40mg abhrak bhasma are not able to scavenge free radicals produced with normal metabolism. Similarly 20mg and 30mg abhrak bhasma mediated metabolisms in liver insignificantly decreased lipid peroxidation indicating influence of abhrak bhasma through different metabolisms on liver MDA production. With 10, 20 and 30mg $SiO_2$ lowered the lipid peroxidation indicating $SiO_2$ mediated liver metabolism resulting in scavenging of the radicals existing though normal metabolism but with 40mg dose it was unaffected (Table 1). Thus at least in lipid peroxidation resulting metabolism abhrak bhasma and $SiO_2$ both are similar except in 10mg dose where abhrak bhasma differs from $SiO_2$ in lowering the normally existing free radicals. As abhrak bhasma contains silica so also $SiO_2$ the results may be influence of silica and difference may be due to preparation of abhrak bhasma from basic silica ores. Same trend was exhibited by per mg protein expressions of MDA. The decrease being significant with all the doses except 30mg dose; which showed marginal increase

with abhrak bhasma. Thus, as per the above results the metabolisms influenced by abhrak bhasma significantly lowered the normal free radicals. $SiO_2$ at 20, 30 and 40mg doses showed increase trend; significant being at 40mg $SiO_2$ dose (p<0.01).

**Table 1. Abhrak bhasma (AB) & $SiO_2$ influenced alterations in Lipid peroxidation in liver and kidney of rats by single dose of treatment**

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | µMoles of MDA contents/ gm Tissue | µMoles of MDA contents/ mg Protein | µMoles of MDA contents/ gm Tissue | µMoles of MDA contents/ mg Protein |
| Normal | 102.10±2.54 | 0.80±0.02 | 71.11±3.89 | 0.77±0.02 |
| AB [10 mg/kg body wt]po | 102.09±3.41 | 0.92±0.09 | 68.19±1.89 | 0.67±0.02[b] |
| AB [20 mg/kg body wt]po | 97.41±2.98 | 0.87±0.06 | 64.04±3.21 | 0.65±0.04[a] |
| AB [30 mg/kg body wt]po | 97.44±3.85 | 0.81±0.04 | 63.15±3.22 | 0.67±0.089 |
| AB [40 mg/kg body wt]po | 103.15±4.01 | 0.85±0.07 | 59.27±1.48[a] | 0.62±0.05[b] |
| $SiO_2$ [10 mg/kg body wt]po | 97.05±2.04 | 0.70±0.04[a] | 64.04±3.45 | 0.75±0.06 |
| $SiO_2$ [20 mg/kg body wt]po | 94.14±3.47 | 0.72±0.089 | 68.14±4.35 | 0.79±0.02 |
| $SiO_2$ [30 mg/kg body wt]po | 95.09±1.89 | 0.84±0.01 | 73.36±2.07 | 0.77±0.03 |
| $SiO_2$ [40 mg/kg body wt]po | 101.98±4.02 | 0.95±0.03[b] | 75.14±2.31 | 0.92±0.03[b] |

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

Thus metabolisms in kidney mediated by abhrak bhasma and $SiO_2$ are involved in scavenging the free radicals existing as a result of normal metabolisms. 40mg dose of abhrak bhasma had lowered the free radicals but same dose of $SiO_2$ had increased the free radicals in normal kidney metabolism. This high dose of $SiO_2$ increases free radicals in kidney of normal rats. And thus differs in its metabolic behaviour from abhrak bhasma. The reason may be the preparation process of abhrak bhasma; which seems to influence the silica related metabolism in normal rat.

$CCl_4$ in known to induce free radicals in liver and kidney[33-38]. In present studies it is noted that abhrak bhasma, an ayurvedic drug influences free radical metabolism; similarly $SiO_2$ which is used as silica control for drug also influences free radical metabolisms. Therefore both of them was tested for their efficacy to scavenge the free radicals. The oral administration of single dose of varying concentrations of abhrak bhasma simultaneous with $CCl_4$ in albino rats for 24 hrs showed decline in the lipid peroxidation content in liver and kidney tissues. They tried to bring the normal levels of MDA in liver and kidney; more effective being in kidney.

In agreement with other studies[39-42], we observed a significant increase in MDA levels in liver of $CCl_4$ induced intoxicated rats when compared to normal rat (P<0.01). Treatment of abhrak bhasma at all studied doses caused a progressive decrease in MDA level per gm tissue. The values indicated through MDA level were not normalized but remained marginally high over MDA contents of normal rat liver. Similar trend was exhibited by $SiO_2$ dose treatments but not

efficiently as abhrak bhasma. With 40mg dose levels of MDA remained significantly high over normal ($p<0.01$) per mg protein expression(1.26 folds). The levels were expressed of changes in proteins and it seems 40mg abhrak bhasma dose has tried to bring MDA levels to MDA levels of normal rat. In per mg protein expression $SiO_2$ not seems to affect MDA levels by any of the doses studied.

In kidney, MDA levels per gm tissue are not significantly increased. With all the doses of abhrak bhasma studied progressively lowered even below the MDA levels in of normal rat (20, 30 and 40mg abhrak bhasma doses). $SiO_2$ treatment also showed maintenance of MDA levels in kidney. In per mg protein expression $CCl_4$ significantly ($P<0.05$) induced lipid peroxidation. 10mg and 40mg abhrak bhasma doses maintain MDA levels as in normal rats. While 20mg and 30mg doses of abhrak bhasma non significantly maintained them at low levels than MDA levels in normal rat (Table No. 2).

By $SiO_2$ treatments, progressive decrease was noted in MDA levels. 40mg dose brought the MDA levels to the levels as observed in normal rat. The alterations indicate that abhrak bhasma influenced changes in MDA levels in per gm tissue expression lowered in per mg protein expression; they showed significantly high at low doses (10mg and 20mg), but brought MDA levels near to MDA levels of normal rat by high doses (30mg and 40mg), thus it seems 30mg and 40mg doses of abhrak bhasma influenced metabolisms scavenge $CCl_4$ induced free radicals and thus protect the liver metabolism. With $SiO_2$ doses 10, 20, 30 and 40 mg doses showed progressive lowering in MDA levels in liver as expressed per gm tissue. But with per mg protein expression except 10mg and 20mg doses seem to not affect $CCl_4$ induced MDA levels in liver. 30mg and 40mg doses elevated the MDA significantly over $CCl_4$ induction levels (Table No. 2).

In kidney per gm tissue expression abhrak bhasma doses progressively lowered $CCl_4$ induced MDA levels. $SiO_2$ seems to maintain MDA levels to normal in per gm tissue expression, but in per mg protein expression $CCl_4$ induced MDA levels seems to be normalized by 10mg dose and they were further decreased by 20, 30 and 40mg doses non significantly. $SiO_2$ has normalized MDA levels in $CCl_4$ induced rats (Table 2).

The deflection in MDA levels per gm tissue expression and per mg tissue expression are due to protein content. Thus $CCl_4$ induced elevated MDA levels were progressively decreased by increasing doses of abhrak bhasma (which is deflected for 10mg and 20mg doses in per mg protein expression). It seems that in both the expressions scavenging of free radicals are effective at 30mg and 40mg doses significantly. But $SiO_2$, the silicon drug control used has not influenced free radical scavenging as effectively as abhrak bhasma. The MDA levels remained significantly

high over MDA levels of normal rat. In kidney as far as MDA levels are concerned all the four doses are free radical scavenging; while only 10 and 20 mg doses of $SiO_2$ seem to scavenge free radicals.

**Table 2: Abhrak bhasma (AB) & $SiO_2$ influenced alterations in Lipid peroxidation in liver & kidney of rats during induction of toxicity by single dose of $CCl_4$. Treatment.**

| Groups | Liver | | Kidney | |
|---|---|---|---|---|
| | µMoles of MDA contents/ gm Tissue | µMoles of MDA contents/ mg Protein | µMoles of MDA contents/ gm Tissue | µMoles of MDA contents/ mg Protein |
| Normal | 100.98±3.04 | 0.79±0.04 | 69.40±5.69 | 0.75±0.02 |
| $CCl_4$ [3.0 ml/kg body wt] sc | 146.77±11.23$^{b}$ | 0.99±0.08$^{a}$ | 74.61±5.34 | 0.94±0.07$^{a}$ |
| $CCl_4$ + AB [10 mg/kg body wt]po | 137.23±11.83$^{a}$ | 1.12±0.08$^{b}$ | 71.11±4.12 | 0.75±0.08 |
| $CCl_4$ + AB [20 mg/kg body wt]po | 123.26±10.30 | 1.03±0.04$^{b}$ | 67.07±6.71 | 0.68±0.08 |
| $CCl_4$ + AB [30 mg/kg body wt]po | 118.32±7.32$^{x}$ | 0.97±0.08 | 66.17±5.10 | 0.69±0.02 |
| $CCl_4$ + AB [40 mg/kg body wt]po | 109.96±7.82$^{x}$ | 0.89±0.03 | 66.27±4.11 | 0.71±0.01 |
| $CCl_4$ + $SiO_2$ [10 mg/kg body wt]po | 140.41±12.01$^{b}$ | 0.99±0.05$^{a}$ | 67.53±5.47 | 0.80±0.03 |
| $CCl_4$ + $SiO_2$ [20 mg/kg body wt]po | 134.84±7.14$^{b}$ | 0.98±0.04$^{b}$ | 68.13±3.40 | 0.83±0.04 |
| $CCl_4$ + $SiO_2$ [30 mg/kg body wt]po | 127.19±5.98$^{b}$ | 1.00±0.04$^{b}$ | 71.12±2.89 | 0.82±0.03 |
| $CCl_4$ + $SiO_2$ [40 mg/kg body wt]po | 117.19±4.41$^{ax}$ | 1.00±0.02$^{b}$ | 73.01±6.54 | 0.77±0.01 |

(Values are mean ± SEM of 6 animals. P values: a < 0.05; b <0.01; c <0.001 vs normal; x<0.05; y<0.01; z<0.001 vs $CCl_4$).

Since abhrak bhasma is processed, silica ore and $SiO_2$ is pure oxide of silica both of them have metabolisms which are having similar alterations while some of them differ from each other. Similarities seems to be due to silica since both of them have silica. But differences cannot be attributed to specified factor in case of abhrak bhasma since the silica ore is processed using natural substances. In our earlier work[17]; it was noted that kidney functions are not affected by abhrak bhasma and $SiO_2$. Liver functions were normalized by all doses in $CCl_4$ induced MDA levels in rats. But with $SiO_2$ same results were obtained only by 30mg and 40mg doses. Single dose of $CCl_4$ used induces centrolobular necrosis when not treated it is recovered intrinsically by 72 hrs. It maintains normal liver after 24 hrs when treated with abhrak bhasma showing hepatoprotective potency of abhrk bhasma [43].

Therefore, in present work free radical scavenging is dose dependent in abhrak bhasma. 20, 30 and 40mg has counteracted $CCl_4$ induced MDA levels to bring them to normal levels. Though $SiO_2$ has influenced free radical scavenging positively. None of the doses used did not counteracted fully to normalize the MDA levels in $CCl_4$ induced lipid peroxidation in male albino rats. In kidney, $CCl_4$ induced levels of MDA were low which were protected by all the doses of abhrak bhasma and high doses of $SiO_2$ (30 and 40 mg/kg body wt).

The results show that the metabolisms mediated by abhrak bhasma protected liver and kidney from $CCl_4$ induced hepatic stress expressed in kidney (even if in low level). But the silica control used ($SiO_2$) fail to manage the oxidative stress in liver and kidney fully. Thus abhrak bhasma which is considered by many as simple oxide is not metabolically behaving as oxide and hence differs from $SiO_2$ in action. In addition the silica ore from which it is derived contain many other trace elements or bulk elements which may have also been derived partially from the herbal treatments used in Shodhan and Maran processes (or eliminated/modified). This may be reason abhrak bhasma is more potent than $SiO_2$.

Thus, present results help to use abhrak bhasma as a potential drug which is capable of protecting liver and it can potentially be used for possible prevention of diseases associated with oxidative stress. However, further study need to be assessed for doses optimization in human.

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