ABHRAK BHASMA AND SiO$_2$, INFLUENCED FREE RADICAL STATUS IN LIVER AND KIDNEY OF CCl$_4$ -INDUCED ACUTELY INTOXICATED MALE ALBINO RAT

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INTRODUCTION

As the traditional and ethnic being tested for their efficacy, new formulations of hepatoprotective drugs have also been tested in rats [1]. Our laboratory is also engaged in testing bhasmas for their efficacies and probable mode of action against induced hepatotoxicity [2,3]. In our earlier study, abhrak bhasma and SiO$_2$, protective efficiency were tested against single dose of CCl$_4$ (3.0 ml/kg body wt given once) induced hepatotoxicity in male albino rat [4]. In the present study, the protective potency of abhrak bhasma and SiO$_2$, graded doses was tested against CCl$_4$-induced acute hepatotoxicity model [5]. The hepatotoxic effects of CCl$_4$ are largely due to its active metabolite/s, including the free radicals CCl$_3^-$ and CCl$_3$OO [6], causing lipid peroxidative degradation of biomembranes leading to centrilobular hepatotoxicity [7], which is referred as fatty degeneration. Metabolically produced aldehydes can act as second toxic messengers of free radicals [8]. Malondialdehyde (MDA), the cytotoxic aldehydes, is one of the final products of polyunsaturated fatty acids peroxidation in the cells [9]. MDA is a major aldehyde resulting from the peroxidation of biological tissue and it is an indicator of tissue damage [10-12].

The control of lipid peroxidation (LPO) in vivo is important for several reasons, in particular because it contributes to the development of atherosclerosis [13]. Thus to prevent free radicals associated damage to tissues/organ or to control/management of free radicals, drug/s are helpful. Thus, abhrak bhasma and SiO$_2$, are used to control oxidative damage that leads to atherosclerosis and further development of associated cardiac complications.

The experimental design evaluates the potency of hepato and nephroprotection of abhrak bhasma and distinguishes role of SiO$_2$, also, since abhrak bhasma is derived from ores of silica.

METHODS

Male albino rats (130–140 g each) were used for experiment. They were obtained from the departmental animal house (Reg. No. 233/ CPCSEA). They were basically derived from Rattus norvegicus breeding pairs obtained from National Institute of Virology, Pune (India). During breeding, maintenance, and experimentation, the animals were provided with standard pellet diet (by Amrit Feeds, Sangli, MS, India) and water ad libitum (during 8 am–9 am).

Preparation of abhrak bhasma and SiO$_2$

Abhrak bhasma was prepared as per Rasa Ratna Samucchaya [14]. SiO$_2$ was obtained from local chemical store.

Experimental schedule

A 3 ml of CCl$_4$ /kg body wt of rat/day was injected (SC) for 7 consecutive days to induce acute hepatotoxicity in animals. Graded doses (10, 20, 30, and 40 mg/kg body wt of rat) of abhrak bhasma and SiO$_2$, were administered (PO) simultaneously with CCl$_4$.

Doses of abhrak bhasma and SiO$_2$, were administered with honey (PO). Honey control rats (six animals) were also maintained. Since their results were similar to normal, they are not included in the present data. The male albino rats were assigned into the following groups, each containing six 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 dose of 3.0 ml CCl$_4$ /kg body wt/day for 7 days
- **Group 3** – 10 mg abhrak bhasma/kg body wt/day for 7 days was given po
- **Group 4** – 20 mg abhrak bhasma/kg body wt/day for 7 days was given po

RESULTS

Lipid peroxidation (LPO) in liver and kidney was studied by malondialdehyde (MDA) estimations as parameter of toxicity and also to study protection. Abhrak bhasma protected CCl$_4$-induced hepatotoxicity and also associated renal toxicity. Silicon from both SiO$_2$, and abhrak bhasma is hepatoprotective in 10 ml doses (10 and 20 mg) but silicon processed in abhrak bhasma by traditional Ayurvedic processes increased its potency and hepatoprotection and added the potency of renal protection.

CONCLUSION

Abhrak bhasma mediated liver and kidney protection in CCl$_4$-induced acute hepatotoxicity in male albino rats. Action of abhrak bhasma is compared with the action of SiO$_2$, in similar experimental conditions to differentiate the role of silicon.

REFERENCES

[1] [2] [3] [4] [5] [6] [7] [8] [9] [10] [11] [12] [13] [14]

ABSTRACT

Objective: The objective of the study was to study the mechanism of action of abhrak bhasma-mediated liver and kidney protection in CCl$_4$-induced acute hepatotoxicity-induced male albino rats. Action of abhrak bhasma is compared with the action of SiO$_2$, in similar experimental conditions to differentiate the role of silicon.

Methods: Male albino rats (Rattus norvegicus) were used for experiments. The acute hepatotoxicity was induced by daily dose of CCl$_4$ (3.0 ml/kg body wt for 7 days consecutive). Concurrent treatment of abhrak bhasma in graded doses (10, 20, 30, and 40 mg) was given for 7 days (PO). SiO$_2$, (10, 20, 30, and 40 mg) in graded doses was also given in independent groups of rats as silica control. Lipid peroxidation (LPO) in liver and kidney was studied by malondialdehyde (MDA) estimations as parameter of toxicity and also to study protection.

Results: CCl$_4$-induced hepatotoxicity (MDA levels) is partially managed by low doses of SiO$_2$, but not by high doses. Abhrak bhasma hepatoprotective activities were dose dependent. A 40 mg dose maintained normal levels of LPO. Abhrak bhasma also protected associated renal toxicity.

Keywords: Abhrak Bhasma, Acute Hepatotoxicity, Lipid Peroxidation, CCl$_4$, SiO$_2$.
- Group 5 – 30 mg abhrak bhasma/kg body wt/day for 7 days was given po
- Group 6 – 40 mg abhrak bhasma/kg body wt/day for 7 days was given po
- Group 7 – 10 mg SiO₂/kg body wt/day for 7 days was given po
- Group 8 – 20 mg SiO₂/kg body wt/day for 7 days was given po
- Group 9 – 30 mg SiO₂/kg body wt/day for 7 days was given po
- Group 10 – 40 mg SiO₂/kg body wt/day for 7 days was given po
- Group 11 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 10 mg AB/kg body wt po/day for 7 days
- Group 12 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 20 mg AB/kg body wt po/day for 7 days
- Group 13 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 30 mg AB/kg body wt po/day for 7 days
- Group 14 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 40 mg AB/kg body wt po/day for 7 days
- Group 15 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 10 mg SiO₂/kg body wt po/day for 7 days
- Group 16 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 20 mg SiO₂/kg body wt po/day for 7 days
- Group 17 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 30 mg SiO₂/kg body wt po/day for 7 days
- Group 18 – CCl₃ (3 mL/kg body wt) sc/day for 7 days+ 40 mg SiO₂/kg body wt po/day for 7 days

The rats were killed after 7 days by giving deep ether anesthesia, and liver and kidney tissues were separated from animals and were taken for LPO.

Estimation and evaluation of LPO
Free radical assessment was performed by MDA estimations per gram wt of tissue and per gram tissue protein by method described [15]. The results were statistically analyzed by ANOVA followed by the Student’s “t-test.” The values of p<0.05, p<0.01, and p<0.001 were considered statistically significant.

The results are presented in Tables 1 and 2.

RESULTS AND DISCUSSION
Four graded doses of abhrak bhasma used as seven consecutive doses in normal rat (daily once PO) have shown to maintain the normal levels of MDA both in liver and kidney and are not toxic to liver or kidney as it is true for single-dose studied [16].

In the present experimental schedule, SiO₂ control drug study showed no influence of MDA levels in liver by 10, 20, and 30 mg while 40 mg elevated the levels (moderately significant) (p<0.01), which differed as compared to abhrak bhasma. Same is true in case of single dose of SiO₂ [13]. In kidney, MDA levels are elevated by 30 mg and 40 mg seven doses (low significance, p<0.05). Thus, abhrak bhasma given alone (as graded doses) is not hepatotoxic or nephrotoxic. However, in similar conditions, high doses (30 and 40 mg) of SiO₂ are toxic with moderate significance.

An increase (of 2.55-fold p<0.001) was observed in the formation of MDA in the rats which are exposed to 7 consecutive daily doses of CCl₃ causing acute toxicity to liver. Hepatotoxicity is consequence of cytochrome P-450-mediated CCl₃ metabolism that generates free radicals CCl₃ and CCl₃O⁻ resulting in centrolobular fatty degeneration/ necrosis [17-19]. The reactive oxygen species subsequently induce LPO measured as MDA. In kidney, liver toxicity associated injury caused increase in MDA level (2.55-fold p<0.001) in male albino rats.

The potency to induce LPO in liver and kidney by CCl₃-induced acute hepatotoxicity seems to be equal as increase in MDA levels in both liver and kidney.

Table 1: Effects of seven doses of abhrak bhasma and SiO₂ influenced alterations in LPO in liver and kidney

| 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 | 102.16±3.01 | 0.85±0.02 | 74.16±3.90 | 0.85±0.02 |
| AB [10 mg/kg body wt] po | 10.02±5.18 | 0.87±0.007 | 73.89±3.27 | 0.87±0.007 |
| AB [20 mg/kg body wt] po | 96.19±4.62 | 0.79±0.06 | 70.16±4.01 | 0.79±0.06 |
| AB [30 mg/kg body wt] po | 90.04±3.75 | 0.71±0.06 | 67.98±3.16 | 0.71±0.06 |
| AB [40 mg/kg body wt] po | 92.16±3.34 | 0.73±0.05 | 63.64±4.94 | 0.73±0.05 |
| SiO₂ [10 mg/kg body wt] po | 98.26±3.03 | 0.76±0.06 | 74.88±3.15 | 0.76±0.06 |
| SiO₂ [20 mg/kg body wt] po | 109.01±4.13 | 0.80±0.09 | 83.14±4.19 | 0.80±0.09 |
| SiO₂ [30 mg/kg body wt] po | 111.06±3.19 | 0.99±0.03 | 88.93±3.09 | 0.99±0.03 |
| SiO₂ [40 mg/kg body wt] po | 120.69±4.54 | 1.14±0.03 | 90.03±4.01 | 1.14±0.03 |

Values are mean±SE of 6 animals p values: a<0.05; b<0.01; c<0.001 vs Normal

Table 2: Abhrak bhasma and SiO₂ influenced alterations in LPO in liver and kidney against acute CCl₃ toxicity (7 days)

| 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 | 10.13±3.24 | 0.86±0.03 | 72.91±4.34 | 0.86±0.03 |
| CCl₃ [3.0 mL/kg body wt] sc | 258.0±1.21c | 1.78±0.09 | 186.0±7.33 | 1.78±0.09 |
| CCl₃+AB [10 mg/kg body wt] | 184.09±1.23c | 1.39±0.04 | 153.34±1.03c | 1.39±0.04 |
| CCl₃+AB [20 mg/kg body wt] | 159.06±1.04c | 1.30±0.08 | 115.02±6.45c | 1.30±0.08 |
| CCl₃+AB [30 mg/kg body wt] | 122.69±8.14 | 1.03±1.10 | 79.98±3.16c | 0.89±0.09 |
| CCl₃+AB [40 mg/kg body wt] | 114.16±6.96c | 0.88±0.08 | 69.43±7.83c | 0.74±0.07c |
| CCl₃+SiO₂ [10 mg/kg body wt] | 22.11±9.10.12c | 1.54±0.04 | 149.10±10.16c | 1.62±0.09c |
| CCl₃+SiO₂ [20 mg/kg body wt] | 19.37±6.49c | 1.44±0.09c | 131.09±9.90c | 1.31±0.09c |
| CCl₃+SiO₂ [30 mg/kg body wt] | 17.12±4.13c | 1.30±0.11c | 156.64±10.14c | 1.88±0.08c |
| CCl₃+SiO₂ [40 mg/kg body wt] | 18.95±1.11c | 1.72±0.09 | 166.53±12.94c | 2.10±0.04c |

Values are mean±SEM of 6 animals p values: a<0.05; b<0.01; c<0.001 vs Normal x0.05; y<0.01; z<0.001 vs CCl₃ Treated
the organs is 2.55 folds. Abhrak bhasma graded doses showed dose dependent decrease in MDA levels in both liver and kidney. Highest dose (40 mg) maintained the normal MDA levels.

SiO$_2$ also showed decrease in levels of MDA. It was dose dependent in liver up to 30 mg dose but 40 mg dose elevated the levels. None of the doses fully managed the MDA levels to normal. In kidney also, same trend was observed, except that 40 mg dose also lowered the levels in kidney. However, none of the doses normalized the MDA levels. Thus, SiO$_2$ failed to manage CCl$_4$-induced MDA levels in acute toxicity status.

CCl$_4$-induced levels of MDA in acutely intoxicated rats were further elevated by 30 mg and 40 mg SiO$_2$ doses indicating either failure to manage increased levels directly or failure to influence the natural metabolism/s that scavenge the MDA levels in vivo.

Both these results indicate that silica seems to have potency to scavenge free radicals but is not capable to handle it fully when used as SiO$_2$. Thus, silicate from abhrak bhasma seems to play active role in free radical management as other components associated with silica or changed form of silicate in abhrak bhasma (on Shodhan and Maran of silica ore) is responsible for MDA levels management as revealed in the present observations.

This is in well verse with our earlier liver and kidney function observations against CCl$_4$-induced acute toxicity [20]. The lowest dose of abhrak bhasma that protected MDA levels is 30 mg in CCl$_4$ acute toxicity showing rats; while liver functions were fully protected with lowest dose of 20 mg, that is, in 20 mg abhrak bhasma treated rat even in the presence of high levels (1.5-fold) of MDA over normal range. Same is true for kidney functions also [16].

The high doses of abhrak bhasma required for MDA management seem to protect CCl$_4$-induced acute fatty degeneration as observed by histological studies of liver and kidney [21,22], thus protecting the membrane cytosolic hepatic and kidney functions or increasing the functional potency of normal cells, as lipolytic and lysosomal enzyme activities are involved in protection of liver toxicity (acute) as noted in rats by other Ayurvedic drugs [3,5,21]. Numerous studies have shown that antioxidants could protect CCl$_4$-induced hepatotoxicity [23-25].

In Indian traditional system of medicine, several Ayurvedic preparations have been reported to protect the liver against CCl$_4$-induced hepatic injury by mediating through intrinsic antioxidant and free radical scavenging activities [26-28]. In the present results, the metabolites/metabolism may be interacting to scavenge free radicals and/or subside the free radical generation from CCl$_4$ metabolism.

CONCLUSION

Pure silica partially protects LP0 production in the presence of CCl$_4$. However, processed form of silica o.m, abhrak bhasma in graded doses protected LP0 products MDH in liver and kidney (30 mg lowest effective dose). The results indicate that silica in SiO$_2$ form shows increased free radical levels. However, silica from Ayurvedic preparation abhrak bhasma is effective in all the doses indicating the processing of silica ore to form abhrak bhasma seems to modify the action of silica to fully protect liver and kidney from CCl$_4$-induced free radicals or other components added/modified during Shodhan and Maran may be enhancing protective action of silica in abhrak bhasma. Abhrak bhasma can be used in treatment in integral medicine as it protects both liver and kidney.

Honey is shown to have immunomodulatory effect against CCl$_4$ toxicity [29]. In present work, honey has not influenced the normal LP0 levels; but its catalytic activity may have added to abhrak bhasma influenced modulation of LP0 in CCl$_4$ induced injured rats, as honey has shown to have immunomodulatory effects. Its additive role in strengthening the protection of liver and kidney in present work cannot be neglected since low doses of SiO$_2$ also showed protective trend in modulation of LP0 levels, since SiO$_2$ is known to produce free radicals [20].

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AUTHORS CONTRIBUTIONS

Both the authors contributed equally in experimental work and manuscript preparation.

CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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REFERENCES

1. Manickam D, Ramamoorthy KP, Kumar MU, Kumar BS, Subramanium S, Subramaniam S. Antioxidant activity of traditional siddha formulation on CCl$_4$ induced liver fibrosis in rats. Int J Pharm Sci 2017;9:81-5.
2. Devarshi P, Kanase A, Kanase R, Mane S, Patil S, Varute AT. Effect of mandur bhasma on lipolytic activities of liver, kidney and adipose tissue of albino rat during CCl$_4$-induced hepatic injury. J Biosci 1986;10:227-34.
3. Kanase RN. Effects of Ayurvedic Drugs on the Lysosomal Enzymes of Liver and Kidney after CCl$_4$ Induced Injury in Albino Rats. A Ph. D. Thesis Submitted to Shivaji University, Kolhapur; 1998.
4. Teli P, Chougule P, Jadhav J, Kanase A. Abhrak bhasma mediated alterations in liver and kidney functions in male albino rats during CCl$_4$ induced toxicity. Int J Res Ayurveda Pharm 2013;4:696-700.
5. Patil S, Kanase A, Kulkarni PH. Effect of hepatoprotective Ayurvedic drugs on lipolytic activities during CCl$_4$ induced acute hepatic injury in albino rats. Indian J Exp Biol 1993;31:265-9.
6. Burk RF, Lane JM, Patel K. Relationship of oxygen and glutathione in protection against carbon tetrachloride-induced hepatic microsomal lipid peroxidation and covalent binding in the rat. Rationale for the use of hyperbaric oxygen to treat carbon tetrachloride ingestion. J Clin Invest 1984;74:1996-2001.
7. Kaplowitz N, Aw TY, Simon FR, Stolz A. Drug induced hepatotoxicity. Ann Intern Med 1986;104:826-39.
8. Esterbauer H. Lipid peroxidation products: Formation, chemical properties and biological activities. In: Poli G, Cheeseman KH, Dianzani MV, Slater TF, editors. Free Radicals in Liver Injury. Oxford: IRL Press Oxford; 1985. p. 29-47.
9. Gawel S, Wardas M, Niedworok E, Wardas P. Malondialdehyde (MDA) as a lipid peroxidation marker. Wiad Lek 2004;57:453-55.
10. Vaca CE, Wilhelm J, Hamms-Ringdahl M. Interaction of lipid peroxidation products with DNA. A review. Mutat Res 1988;195:137-49.
11. Esterbauer H, Schaur RJ, Zoller H. Chemistry and biochemistry of 4-hydroxynonenal, malonaldehyde and related aldehydes. Free Radical Biol Med 1991;11:81-128.
12. Isabella DD, Ranieri R, Roberto C, Daniela G, Aldo M. Biomarkers of oxidative damage in human diseases. Clin Chem 2006;52:601-23.
13. Romero FJ, Bosch-Morell F, Romero MJ, Jareno EJ, Romero B, Marin N, et al. Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect 1998;106:1229-34.
14. Sharma S, Rasa Ratna Samuchhaya. New Delhi: Motilal Banarasidas; 1977. p. 72-108.
15. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol 1978;52:304-10.
16. Teli P, Jadhav J, Kanase A. Comparison of abhrak bhasma and silicon dioxide efficacy against single dose of carbon tetrachloride induced hepatotoxicity in rat by evaluation of lipid peroxidation. Ann J Pharm Health Res 2014b;2:186-96.
17. Renold ER, Ree HJ. Liver parenchymal cell injury. VII. Membrane denaturation following CCl$_4$. J Cell Biol 1971;25:269.
18. Recknagel RO, Gleave EA. Lipid peroxidation a specific form of cellular injury. In: Lee DH, Falk HL, Murphy SD, editors. Hand Book of Physiology: Section 9: Reactions to Environmental Agents. Washington, DC: American Physiological Society; 1978. p. 591-602.
19. Recknagel RO. A new direction in the study of carbon tetrachloride hepatotoxicity. Life Sci 1983;33:401.
20. Teli PB, Chougule PB, Jadhav JT, Kanase AA. Curative effect of...
abhrak bhasma on liver and kidney functions in carbon tetrachloride intoxicated albino rats. Int J Bioassays 2014a:3:1624-9.

21. Buwa SK. Hepatoprotective and Curative Effects of Abhrak Bhasma on Liver, Kidney and Adipose Tissue of Male Albino Rats. A Ph. D. Thesis Submitted to Shivaji University, Kolhapur; 2000.

22. Priti C. Abhrak Bhasma Mediated Alterations in Lysosomal Enzymes Activities of Liver and Kidney in Male Albino Rats against CCI, Induced Hepatic Injury. A Ph. D. Thesis Submitted to Shivaji University, Kolhapur; 2007.

23. Yoshikawa T, Furukawa Y, Murakami M, Takemura S, Kondo M. Effect of Vitamin E on D-Galactosamine-induced or carbon tetrachloride-induced hepatotoxicity. Digestion 1982;25:222-9.

24. Halim AB, El-Ahmady O, Hassab-Allah S, Abdel-Galil F, Hafez Y, Darwish A. Biochemical effect of antioxidants on lipids and liver function in experimentally induced liver damage. Ann Clin Biochem 1997;34:656-63.

25. Farghali H, Kamenikova L, Hynie S, Kmonickova E. Silymarin effects on intracellular calcium and cytotoxicity: A study in perfused rat hepatocytes after oxidative stress injury. Pharmacol Res 2000;41:231-7.

26. Tripathi YB, Singh VP. Role of tamra bhasma, an Ayurvedic preparation, in the management of lipid peroxidation in liver of albino rats. Indian J Exp Biol 1996;34:66-70.

27. Suja V, Sharmila SL, Shyamala DC. Protective effect of Liv. 52 and Liv. 100, ayurvedic formulations on lipid peroxidation in rat liver homogenates an in vitro study. Indian J Exp Biol 1997;35:50-2.

28. Agte V, Mengale SS, Akkalkotkar M, Paknikar KM, Chiplonkar SA. Antioxidant and trace element potential of chyavanprash and some Ayurvedic preparation. Indian J Tradit Knowl 2003;2:215-23.

29. Elbakry KA, Malak CA, Howas MM. Immunomodulatory role of honey and propolis on carbon tetrachloride (CCl ) injected rats. Int J Pharm Pharm Sci 2015;7:259-62.