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Research Article

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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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.

Conclusion: 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.

Keywords: Abhrak Bhasma, Acute Hepatotoxicity, Lipid Peroxidation, CCl_4, SiO_2.

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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 of CCl_4/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 centrolobular 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/organs 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

- 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 SiO_2/kg body wt/day for 7 days was given po
- Group 8 – 20 mg SiO_2/kg body wt/day for 7 days was given po
- Group 9 – 30 mg SiO_2/kg body wt/day for 7 days was given po
- Group 10 – 40 mg SiO_2/kg body wt/day for 7 days was given po
- Group 11– CCl_4 (3 ml/kg body wt) sc/day for 7 days+ 10 mg AB/kg body wt po/day for 7 days
- Group 12 – CCl_4 (3 ml/kg body wt) sc/day for7 days+ 20 mg AB/kg body wt po/day for 7 days
- Group 13 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+30 mg AB/kg body wt po/day for 7 days
- Group 14 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+ 40 mg AB/kg body wt po/day for 7 days
- Group 15 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+10 mg SiO_2/kg body wt po/day for 7 days
- Group 16 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+20 mg SiO_2/kg body wt po/day for 7 days
- Group 17 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+30 mg SiO_2/kg body wt po/day for 7 days
- Group 18 – CCl_4 (3 ml/kg body wt) sc/day for 7 days+40 mg SiO_2/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, SiO_2 control drug studied 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 SiO_2 [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 SiO_2 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 CCl_4 causing acute toxicity to liver. Hepatotoxicity is consequence of cytochrome P-450-mediated CCl_4 metabolism that generates free radicals CCl_3· and CCl_3OO· 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 CCl_4-induced acute hepatotoxicity seems to be equal as increase in MDA levels in both

Table 1: Effects of seven doses of abhrak bhasma and SiO_2 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±0.05
AB [10 mg/kg body wt]po	100.02±5.18	0.87±0.007	73.89±3.27	0.87±0.04
AB [20 mg/kg body wt] po	96.91±4.62	0.79±0.06	70.16±4.01	0.83±0.07
AB [30 mg/kg body wt] po	90.84±3.75	0.71±0.06	67.98±3.16	0.77±0.08
AB [40 mg/kg body wt] po	92.16±6.34	0.73±0.05	63.64±4.94	0.75±0.05
SiO_2 [10 mg/kg body wt] po	98.26±3.03	0.76±0.08	74.88±3.15	0.79±006
SiO_2 [20 mg/kg body wt] po	109.01±4.13	0.80±0.09	83.14±4.19	0.8±0.08
SiO_2 [30 mg/kg body wt] po	111.06±3.19	$0.99±0.03^b$	$88.93±3.09^a$	$1.01±0.04^a$
SiO_2 [40 mg/kg body wt] po	$120.69±4.54^b$	$1.14±0.03^c$	$90.03±4.01^a$	$1.17±0.06^b$

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 SiO_2 influenced alterations in LPO in liver and kidney against acute CCl_4 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	101.13±4.26	0.86±0.03	72.91±4.34	0.86±0.08
CCl_4[3.0 ml/kg body wt]sc	$258.00±12.11^c$	$1.78±0.09^c$	$186.08±7.33^c$	$1.88±0.12^c$
CCl_4+AB [10 mg/kg body wt]	$184.09±12.32^y$	$1.39±0.04^{cy}$	$133.34±11.03^{cy}$	$1.34±0.09^{bx}$
CCl_4+AB [20 mg/kg body wt]	$159.06±10.43^{cz}$	$1.30±0.08^a$	$115.00±6.45^{cz}$	$1.21±0.08^{by}$
CCl_4+AB [30 mg/kg body wt]	$122.69±8.14^{az}$	$1.03±1.10^z$	$79.98±3.16^z$	$0.89±0.09^z$
CCl_4+AB [40 mg/kg body wt]	$114.16±6.96^z$	$0.88±0.08^z$	$69.43±7.83^z$	$0.74±0.07^{cz}$
CCl_4+SiO_2 [10 mg/kg body wt]	$221.19±10.12^{cx}$	$1.54±0.04^{cx}$	$149.10±10.16^{cx}$	$1.62±0.09^c$
CCl_4+SiO_2 [20 mg/kg body wt]	$193.76±4.97^{cy}$	$1.44±0.08^{cx}$	$131.09±9.98^{cy}$	$1.31±0.08^{by}$
CCl_4+SiO_2 [30 mg/kg body wt]	$171.22±14.33^{bz}$	$1.30±0.11^{by}$	$156.64±10.14^{cx}$	$1.88±0.089^c$
CCl_4+SiO_2 [40 mg/kg body wt]	$189.51±11.16^{cy}$	$1.72±0.09^c$	$166.53±12.84^c$	$2.10±0.04^c$

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 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 S*hodhan* 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 (1.57-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/ metabolisms may be interacting to scavenge free radicals and/or subside the free radical generation from CCl_4 metabolism.

CONCLUSION

Pure silica partially protects LPO production in the presence of CCl_4. However, processed form of silica ore, abhrak bhasma in graded doses protected LPO 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 LPO levels; but its catalytic activity may have added to abhrak bhasma influenced modulation of LPO 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 LPO 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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