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Research Paper

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_2) on hepatic and renal GSH status against CCl_4 intoxicated male albino rats were investigated.
Methods: Single dose of CCl_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_2 were concurrently given with CCl_4. Hepatic and renal GSH content was studied after 24 hrs.
Results: Results showed that rats exposed to CCl_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_2 treatments did not affect GSH activity in liver significantly. Single dose of CCl_4 had not influenced GSH content in kidney alone or with any of the doses of abhrak bhasma or SiO_2.
Conclusion: CCl_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_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_4) is one of the most commonly used hepatotoxin in the experimental study of liver diseases. CCl_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 & Bomzon, 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_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_4 induced hepatotoxicity and associated changes in kidney. SiO_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_2 treatment was given as silicon control. To study dose dependent effects of abhrak bhasma and SiO_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

The experimental animals were divided into following groups, each comprising of six animals.
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 GSH estimation.

2.4. Preparation of tissue homogenate

The livers and kidneys were perfused with chilled phosphate buffer saline (PBS). They were dissected out, minced and washed with PBS. The minces were then suspended in 0.25M sucrose containing 10mM Tris-HCl buffer (pH 7.0) homogenizing buffer (HB) and washed 3 times with HB. The minces were homogenized with Potter-Elvehjam homogenizer with Teflon piston at 1500 RPM with 8 up and down strokes. The liver and kidney homogenates were centrifuged in refrigerated centrifuge at 4°C for 10 minutes at 3000 x g. The supernatants were collected and used for GSH estimation.

2.5. Estimation of GSH

Biochemical assay of GSH was performed as per method proposed by Grunert & Phillips (1951).
Bioassay was conducted in different tubes, one blank and sample tube as per experimental schedule of rats. To blank tube was added 6.0 ml 0.25 M sucrose in homogenate buffer and to sample tubes added 6.0 ml of supernatants. One ml sodium nitroprusside and 1.0 ml sodium cyanide solution in sodium carbonate were added to all tubes. Optical densities were measured at 520 nm against blank.

2.6. Statistical analysis

All the experimental data was statistically evaluated and expressed as mean ± standard error for six rats in each group. Hypothesis testing methods included one way analysis of variance (ANOVA) followed by students 't' test. Values P<0.05, P<0.01 and P<0.001 were considered to point out statistical significance.

3. Results and discussion

Table 1: Abhrak bhasma (AB) & SiO_2 Influenced Alterations in GSH Contents in Liver and Kidney of Rats by Single Dose of Treatment.

Groups	Liver		Kidney	
	mg/ gm Tissue	µg/ mg Protein	mg/ gm Tissue	µg/ mg Protein
Normal	27.97±1.28	0.22±0.002	13.10±0.62	0.14±0.009
CCl_4 [3.0 ml] sc	21.25±2.21^{a}	0.14±0.013^{c}	11.10±0.61	0.14±0.011
CCl_4 + 10mg AB	25.13±1.81	0.20±0.002^{y}	12.10±0.04	0.13±0.003
CCl_4 + 20mg AB	25.64±2.31	0.21±0.006^{z}	13.01±0.21	0.13±0.006
CCl_4 + 30mg AB	26.11±1.91	0.21±0.009^{y}	12.90±0.15	0.13±0.004
CCl_4 + 40mg AB	28.01±2.84	0.23±0.006^{z}	13.01±0.31	0.14±0.006
CCl_4 + 10mg SiO_2	39.21±3.11^{bz}	0.28±0.012^{cz}	11.34±0.97	0.13±0.004
CCl_4 + 20mg SiO_2	36.82±2.84^{ay}	0.27±0.008^{cz}	11.71±0.74	0.14±0.001
CCl_4 + 30mg SiO_2	35.14±1.33^{bz}	0.28±0.009^{cz}	9.24±0.96^{b}	0.11±0.007^{ax}
CCl_4 + 40mg SiO_2	32.19±1.09^{ay}	0.27±0.008^{cz}	10.10±0.98	0.11±0.009^{y}

(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 hepatic injury (Purucker et al., 1995). It is caused by reduced concentrations of liver GSH. In present work, we investigated abhrak bhasma and SiO_2 induced alterations in GSH status of liver and kidney of male albino rat, during hepatotoxicity induced by single dose of CCl_4 at 24 hrs. The results were shown in table No. 1 and 2.

Table 2: Abhrak Bhasma (AB) & Sio2 Influenced Alterations in GSH Contents in Liver & Kidney of Rats during Induction of Toxicity by Single Dose of Ccl4.

Groups	Liver		Kidney	
	mg/ gm Tissue	µg/ mg Protein	mg/ gm Tissue	µg/ mg Protein
Normal	28.96±0.65	0.22±0.003	14.57±0.71	0.15±0.004
10mg AB	28.64±0.11	0.25±0.006	13.34±0.54	0.13±0.008^{a}
20mg AB	29.39±0.14	0.26±0.009^{b}	15.22±0.31	0.15±0.001
30mg AB	29.35±0.14	0.24±0.004	15.36±0.65	0.16±0.004
40mg AB	32.19±0.61	0.26±0.005	15.54±0.94	0.16±0.008^{c}
10mg SiO_2	44.19±0.69^{c}	0.31±0.010^{c}	14.14±0.63	0.15±0.003^{b}
20mg SiO_2	55.24±0.34^{c}	0.42±0.014^{c}	15.24±1.03	0.17±0.004^{b}
30mg SiO_2	81.54±3.00^{c}	0.72±0.004^{c}	13.71±1.56	0.14±0.009^{c}
40mg SiO_2	81.23±2.85^{c}	0.75±0.007^{c}	12.29±0.96	0.15±0.008^{b}

(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).

GSH content in liver of normal rat exhibited 28.96±0.65 µg/ gm tissue, 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 SiO_2 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 SiO_2 doses studied. As contrast to abhrak bhasma, SiO_2 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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