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RESEARCH ARTICLE

ABHRAK BHASMA AND SiO_2 INFLUENCED MOBILITY OF LIPIDS IN LIVER AND KIDNEY OF CCl_4 INDUCED ACUTELY INTOXICATED ALBINO RAT

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Abstract

In our earlier studies on CCl_4 induced acute toxicity model (CCl_4 3ml/kg body wt). Abhrak bhasma protects fatty degeneration of liver and associated nephrotoxicity in male albino rats. It had shown to function through production and management of free radicals (Teli and Kanase, 2020a, b). To study further the paths of Abhrak Bhasma mediated protection of acute hepatotoxicity and associated nephrotoxicity, liver, kidney and serum lipid contents were studied in present work. It shows no lipid accumulation in liver or kidney of normal rats by Abhrak Bhasma (10, 20, 30 and 40mg doses). But a same dose of silica in pure form (SiO2) is hepato and nephrotoxic in high doses in normal male albino rat. In acutely intoxicated rat also all the doses of Abhrak Bhasma influenced lipid contents of liver, kidney and serum show the protection of liver from fatty degeneration and also associated nephrotoxicity. Doses 30 and 40mg normalized the contents from liver, kidney and serum. The results are discussed to reveals the probable mode of action of Abhrak Bhasma.

Introduction:-

Earlier work using CCl_4 induced acute toxicity model [3.00ml CCl_4/kg body wt/day for 7 days consecutively (SC)] in albino rat had shown that Abhrak Bhasma, a silica ore derived Ayurvedic drug (Abhrak Bhasma) and its drug control SiO_2 (Silica not processed) both protect hepatotoxicity (Abhrak Bhasma all doses and SiO_2 10 and 20mg doses with partial protection) as studied by monitoring free radicals (Lipid peroxidation studies) and GSH/GSSH metabolism (Teli and Kanase, 2020a, b). The studies also revealed that CCl_4 associated Kidney toxicity was protected by only Abhrak Bhasma (40mg dose for full protection of liver and 20, 30, 40mg for full protection of Kidney) but not by SiO_2.

Since in CCl_4 induced toxicity, fatty degeneration of centrolobular hepatocytes occur, the protection of liver cells involve mobility of lipids and hence the same experimental schedule was used to study serum lipids and lipoproteins.

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Material and Methods:-

Experimental Animal:

Rattus norvegicus (Male albino rats) were bred and maintained in the Departmental Animal House (Reg. No. 233/CPCSEA). They were originally derived from National Institute of Virology, Pune. Males weighing about 130-140gm were selected for experiments. During breeding, maintenance and experiments, they were provided with standard rat pellet feed (Prepared by Amrit Feeds, Sangli, MS, India). Food and water were provided *ad libitum*. Feeding and drug treatments schedule was between 8.00am to 9.00am.

Preparation of Abhrak Bhasma (AB) and SiO_2:

The method adapted for Abhrak Bhasma preparation in laboratory was as per Rasa Ratna Sammucchaya (Sharma, 1977). SiO_2 was used as drug control for silica. Since Abhrak Bhasma is derived from mica-ore (Sharma, 1977). SiO_2 sample was obtained from the market. Graded doses 10, 20, 30, 40mg/kg body wt. of both drugs were used for experimental schedule.

Drug Treatment:

The doses mixed in honey were fed to individual animals (PO).

Experimental Schedule:

The experimental conditions used in present experimental model of acute CCl_4 toxicity work were acute hepatotoxicity induced by seven days treatment of CCl_4 (3.0ml/kg body wt/day) with daily dose of 10/20/30/40 mg Abhrak Bhasma/SiO_2 (drug control) /day po; to analyze the protective influence of Abhrak Bhasma and SiO_2. The experimental rats were rested over night (24 hrs.) before sacrifice. The drug vehicle control honey treated rats were maintained but since data does not differ from normal rat and hence not included.

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 dose of 3.0ml CCl_4/kg body wt/day for 7 days.
Group 3 - 10mg abhrak bhasma/kg body wt/day for 7 days was given po.
Group 4 - 20mg abhrak bhasma/kg body wt/day for 7 days was given po.
Group 5 - 30mg abhrak bhasma/kg body wt/day for 7 days was given po.
Group 6 - 40mg abhrak bhasma/kg body wt/day for 7 days was given po.
Group 7 - 10mg SiO_2/kg body wt/day for 7 days was given po.
Group 8 - 20mg SiO_2/kg body wt/day for 7 days was given po.
Group 9 - 30mg SiO_2/kg body wt/day for 7 days was given po.
Group 10 - 40mg SiO_2/kg body wt/day for 7 days was given po.
Group 11- CCl_4 (3ml/kg body wt) sc/day for 7 days+10mg AB/kg body wt/day for 7 days.
Group 12- CCl_4 (3ml/kg body wt) sc/day for7 days+20mg AB/kg body wt/day for 7 days.
Group 13- CCl_4 (3ml/kg body wt) sc/day for 7days+30mg AB/kg body wt/day for 7 days.
Group 14- CCl_4 (3ml/kg body wt) sc/day for 7days+40mg AB/kg body wt/day for 7 days.
Group 15- CCl_4 (3ml/kg body wt) sc/day for 7days+10mg SiO_2/kg body wt/day for 7 days.
Group 16- CCl_4 (3ml/kg body wt) sc/day for 7days+20mg SiO_2/kg body wt/day for 7 days.
Group 17- CCl_4 (3ml/kg body wt) sc/day for 7days+30mg SiO_2/kg body wt/day for 7 days.
Group 18- CCl_4 (3ml/kg body wt) sc/day for 7days+40mg SiO_2/kg body wt/day for 7 days.

The rats were killed after 7 days by giving deep ether anesthesia and liver and kidney tissues were removed from animals and were further processed for total lipid estimation.

Preparation of Tissue Homogenates:

The liver and kidney were perfused with chilled phosphate buffer saline (PBS). After dissection of rat, liver and kidneys were removed, minced and wash with PBS. The minces were suspended in 10mM Tris-HCl buffer (pH-7.0). The minces were homogenized with Potter-Elvehjam homogenizer with Teflon piston at 1500rpm with 8 up and down strokes. The liver and kidney homogenates were centrifuged in refrigerated centrifuge at 4^oC for 10 minutes at 3000×g, the supernatants were collected and used for biochemical estimation.

Collection of Serum:
On termination of experimental schedule animals were killed by deep ether anesthesia. The blood samples were obtained immediately with sterilized syringes through left ventricle and were kept at room temperature until they are clot. On clotting, the colourless serum samples were obtained by centrifuging the clots by table top centrifuge. The colourless samples were stored at 10^oC until use.

Biochemical Assays:
Total lipid content in liver, kidney and serum was estimated as per Frings et al., 1972.

Statistical Analysis:
The expressions of results in tables are mean±SEM of different groups. The significance in differences between the groups was evaluated. One way analysis of Variance (ANOVA) was used for the evaluation.

Results are given in Table 1 and Table 2.

Table 1:- Effects of seven doses of Abhrak Bhasma and SiO_2 influenced alterations in total lipid contents in liver, kidney and serum.

Groups	Liver	Kidney	Serum
	mg/100gm Tissue	mg/100gm Tissue	mg/dl
Normal	6294.33 ± 129.26	1912.22 ± 49.92	154.84 ± 10.03
AB [10 mg/kg body wt]	6214.26 ± 116.13	1899.69 ± 92.16	169.09 ± 9.74
AB [20 mg/kg body wt]	6292.63 ± 133.41	1863.31 ± 88.32	154.92 ± 10.16
AB [30 mg/kg body wt]	6322.93 ± 153.16	1960.38 ± 72.09	168.16 ± 11.05
AB [40 mg/kg body wt]	6338.41 ± 163.33	1948.93 ± 101.10	159.15 ± 8.26
SiO_2 [10 mg/kg body wt]	6331.22 ± 168.31	1964.36 ± 84.61	158.13 ± 14.28
SiO_2 [20 mg/kg body wt]	6360.91 ± 149.16	1999.82 ± 91.22	169.07 ± 12.74
SiO_2 [30 mg/kg body wt]	7213.84 ± 153.64^b	2014.80 ± 101.09	192.96 ± 19.14
SiO_2 [40 mg/kg body wt]	7978.49 ± 176.31^c	2334.22 ± 94.14^b	212.44 ± 15.16^a

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 total lipid contents in liver, kidney and serum against acute CCl_4 toxicity.

Groups	Liver	Kidney	Serum
	mg/100gm Tissue	mg/100gm Tissue	mg/dl
Normal	6310.18 ± 149.70	1934.61 ± 34.18	162.16 ± 9.26
CCl_4 [3.0 ml/kg body wt]	7834.51 ± 119.76^c	2711.24 ± 54.28^c	346.34 ± 12.18^c
CCl_4 +AB [10mg/kg body wt]	7478.47 ± 148.19^c	2628.36 ± 57.72^c	289.36 ±10.30^{cy}
CCl_4 +AB [20mg/kg body wt]	6959.63 ± 167.47^y	2184.96 ± 72.52^{az}	243.69 ± 9.28^{cz}
CCl_4 +AB [30mg/kg body wt]	6491.26 ± 149.23^z	1989.17 ± 89.16^z	194.48 ±10.88^{az}
CCl_4 +AB [40mg/kg body wt]	6418.92 ± 161.12^z	1971.86 ± 64.24^z	183.86 ± 16.28^z
CCl_4 +SiO_2 [10mg/kg body wt]	7889.74 ± 178.26^c	2682.18 ± 70.19^c	311.18 ± 12.18^c
CCl_4 +SiO_2 [20mg/kg body wt]	7534.16 ± 116.78^c	2306.70 ± 95.67^{by}	289.34 ±10.19^{cy}
CCl_4 +SiO_2 [30mg/kg body wt]	7494.19 ± 146.33^{cx}	2493.69 ± 96.36^{cy}	289.48 ±13.77^{cx}
CCl_4 +SiO_2 [40mg/kg body wt]	7768.89 ± 191.27^c	2416.78 ± 92.32^{cx}	300.73 ±12.48^{cx}

Values are mean ± SE 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

Results and Discussion:-

The total lipid contents in liver of normal group of albino rats was 6294.33 ± 129.26 mg/100gm tissue; which was maintained at its normal level by treatments of all studied doses of Abhrak Bhasma. Similarly administration of 10mg and 20mg doses of SiO_2 also maintained normal lipid levels in liver. But treatments of 30mg and 40mg doses of SiO_2 exhibited significant (P<0.001) increase in the total lipid contents. Thus liver lipid contents were not affected significantly by Abhrak Bhasma in normal rat. But 30mg and 40mg of SiO_2 doses seem to accumulate

lipids in normal rat indicating adverse effect. In kidney, treatments of all the studied doses of abhrak bhasma showed normal values of total lipid contents. Treatments of 10, 20 and 30mg SiO_2 showed practically no change in total lipid contents of kidney; thus showing no influence on renal lipids in normal rat. But 40mg SiO_2 dose exhibited significant increase ($P<0.01$) in lipid contents as compared to the normal rat kidney and thus indicates accumulation of total lipids in kidney which is adverse effect. In serum of normal rats total lipid contents were 154.84 ± 10.03 mg/dl. Treatment of 10mg, 20mg, 30mg and 40mg doses of Abhrak Bhasma did not affect lipid contents. Administration of 10mg and 20mg dose of SiO_2 showed non-significant alterations in serum total lipids, but 30mg and 40mg doses of SiO_2 showed significant increase by 1.24 and 1.37 folds as compared to the lipid contents of normal rat. Results indicated increased transport of lipids under SiO_2 influence (high doses). These results indicate that Abhrak Bhasma administration to albino rats maintain normal state of lipid metabolism. But in SiO_2 treated control groups with 30mg and 40mg doses (seven doses) increased the lipid content. Thus, the results indicate that Abhrak Bhasma doses not affect normal liver, kidney and serum total lipid contents. This indicates hardly any influence on kinetic of normal lipid metabolism. In case of SiO_2 doses, liver total lipids are increased by 30mg and 40mg doses, this is also reflected in serum. In kidney, additionally 20mg dose is not influencing kidney lipids. The alterations indicate serum lipids levels are the reflection of their traffic towards liver. The results also support the earlier finding that lipids mobility is hardly influenced by Abhrak Bhasma in normal rat so also lipid peroxidation and glutathione content (Teli and Kanase, 2020a, b) but SiO_2 shows (with higher doses) adverse influences.

Administration of seven doses of 3.0ml of CCl_4 per kg body wt to the normal rats caused a significant increase ($P<0.001$) in liver total lipid contents (by 1.24 fold). Treatments of 10, 20, 30 and 40mg Abhrak Bhasma simultaneous with CCl_4 to the rats counteracted and showed progressive reduction in total lipid contents towards normal level; more significant change was noted with 30 and 40mg doses ($P<0.001$). Minimum dose required to lower increased contents is 30mg. Doses of SiO_2 showed no significant influence in presence of CCl_4 on lipid contents in liver.

In kidney, increase ($P<0.001$) in total lipid contents was noted with CCl_4 administration (by 1.40 fold); which was progressively counteracted by Abhrak Bhasma treatments and brought it to normal level with 30 and 40mg doses. Treatments of 10, 20, 30 and 40mg SiO_2 doses showed significant reduction in total lipid contents in kidney but failed to bring it to normal level, indicating less protective potency. The doses of Abhrak Bhasma that protected the liver also protected the kidney. Minimum effective dose being 30mg, but SiO_2 failed to do so.

Administration of 3.0ml CCl_4/kg body wt/day for seven days exhibited significant increase ($P<0.001$) in total lipids levels in serum (2.13 fold). Treatments of all studied doses of Abhrak Bhasma against CCl_4 induced toxicity; showed progressive reduction in the total lipid contents and brought the levels to normal. The significant protective response was noted at 30mg and 40mg doses of Abhrak Bhasma. Similar trend was noted with the administration of various doses of SiO_2 to the CCl_4 intoxicated rats; which have shown progressive reductions in total lipid contents, but failed to bring it to normal levels. Serum lipid levels indicate the movements of lipids to organs. Abhrak Bhasma mediated changes in serum relate with the reduction of levels with liver and kidney. Same is true reversely in case of SiO_2. Abhrak Bhasma mediated protection of CCl_4 induced acute centrolobular necrosis in liver and protection of associated toxicological effects histologically reflected in kidney (Buwa, 2000) in same experimental schedule. This indicates that lipids are utilized for the protection of tissue necrosis or fatty degeneration in liver and concurrently associated kidney toxicity protection. SiO_2 mediated results can be related with the liver and kidney functions which are also not protected by SiO_2 (Teli et al., 2013) in similar experimental schedule.

It is revealed from results that pure SiO_2 is not potent in protecting liver and kidney against CCl_4 toxicity; silica component of Abhrak Bhasma parent substance mica from ore is not independently acting as effective drug. The processes involved in Abhrak Bhasma preparation seems to play major role as both the drugs mediated metabolic alterations differ. In protective experimental schedule against acute toxicity induced by CCl_4 in liver and kidney having 20 and 30mg effective doses for Abhrak Bhasma. But in serum 40mg dose is needed for normalization.

The doses of SiO_2 against CCl_4 show reduction in lipids in liver (10, 20, and 30mg) and kidney (10, 20mg). But 30mg and 40mg doses in kidney elevated the levels. In serum the levels show equal amount of contents in 10 and 40mg group and 20 and 30mg group. In protective schedule, where induction of CCl_4 toxicity and Abhrak Bhasma mediated protection are occurring simultaneously high levels of metabolic turnover seem to occur. The minimum effective doses for liver and kidney are 30 and 40mg. But in serum turnover being high the highest dose seems to control the levels.

In toxicity studies the total lipid contents and liver and kidney are not influenced by Abhrak Bhasma (10, 20, 30, 40mg doses). Even serum levels are also not altered. This indicates Abhrak Bhasma is hardly influencing lipid metabolism by any of the doses used or possibly it may be maintaining equilibrium in transport from organs to adipose tissue (Buwa, 2000) as indicated by changes in different lipases activities in liver, kidney, adipose tissue and serum. As results reveal SiO_2 low doses (10 and 20mg) are not influencing the liver and kidney lipid contain but 30 and 40mg are influencing significantly by increase in lipid content which is also indicated by high levels in serum. This shows that high doses of SiO_2 are mobilizing the fats possibly from adipose tissue and being deposited in liver and kidney and probably for the same reason, the serum lipid levels are also high. Accumulation of lipids in liver and kidney is an adverse effect on their functions as it is known incase of fatty liver and incase of CCl_4 induced kidney toxicity in present results (Teli et al, 2013).

CCl_4 treatments for 7 days have also shown the accumulation of total lipids in liver and kidney. Serum high levels indicate lipids in movement from adipose tissue to liver and kidney (Buwa, 2000).

Abhrak Bhasma graded doses showed graded stimulation of liver fats drain. The minimum dose required for normal levels of lipids content is 30mg but 40mg also gave the similar results. These results are also reflected in serum lipid content but for serum the minimum effective dose is 40mg to normalize. Both indicate clearance of lipid load from liver and kidney. This is complemented by liver and kidney functions recovery in acute protective schedule (Buwa, 2000; Chougule, 2007) as revealed by histological and liver, kidney functions studies.

In drug control i.e. SiO_2 treated animals 10mg dose had not affected kidney contents significantly but serum levels were marginally lowered and lipid content in liver was marginally increased which indicates that by 10mg SiO_2 dose liver fat deposition is stimulated. But by 20mg dose and 30mg dose drop in stimulation of lipids in liver, kidney and serum is observed. But highest dose used 40mg SiO_2 reversed the trend and showed drop in lipid content in liver coupled with high serum lipid content. None of the doses of SiO_2 had shown lipid content of liver, kidney and serum to near the contents reported content in normal rat.

These alterations indicate that Abhrak Bhasma and SiO_2 both influence lipid metabolism in normal pathways. Lipid contents of liver and kidney are normalized by 30mg and 40mg doses of Abhrak Bhasma. The serum levels remained marginally/slightly high in case of both the doses. Probably it is due to high turnover of lipids under the influence of Abhrak Bhasma.

All these results have also indicated that abhrak in SiO_2 form or Abhrak Bhasma form influences liver, kidney and lipid metabolism in CCl_4 treated rat. But protective stimulation initiated by SiO_2 is not continued by high doses of 30 and 40mg and show adverse influences while Abhrak Bhasma which is processed form silica ore seems to have channelized its protective properties and showed dose dependent protection of primarily of liver and associated damage of kidney. It also confirmed that Abhrak Bhasma seems to use, modulate or strengthen the natural physiological pathway used by the animal in facing the adverse effects or natural protection. Thus the procedures adapted in bhasma preparation i.e. Shodhan and Maran (Sharma, 1977) which involve treatment by herbs like Tamarind fruits juice; *Vitex nigunda* leaves respectively. This may be changing contents/forms of silica and helping to modify the adverse effects of silica in pure form. It may also be true that use of ore in preparation may also be influencing purity of silica since the drug control SiO_2 is a pure form of silica.

Conclusion:-

As the results indicate Abhrak Bhasma is not hepatotoxic and nephrotoxic in male albino rat. It is hepatoprotective and nephroprotective against CCl_4 induced acute toxicity (7 days), most effective being 30mg and 40mg doses. It protects accumulation of lipids in liver also moves accumulated lipids from liver. CCl_4 induced acute hepatotoxicity associated accumulation of lipids in kidney leading to nephrotoxicity is also protected by the same drug. This is not true incase of use of pure silica in SiO_2 form. Thus processing of silica ore (Mica) to prepare Abhrak Bhasma seems to be key process in Abhrak Bhasma – an Ayurvedic drug preparation.

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