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ABHRAK BHASMA AND SiO_2 MEDIATED PROTECTION OF PHOSPHOLIPID TURNOVER IN CARBON TETRACHLORIDE INDUCED ACUTE HEPATO-STEATOSIS AND ALLIED NEPHROTOXICITY SHOWING MALE ALBINO RAT

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ABSTRACT

CCl_4 (3.00ml/kg body wt/day) given for 7 days causes acute hepatic steatosis with associated kidney necrosis. This hepato-steatosis model was used to study Abhrak Bhasma (AB) mediated (10, 20, 30 & 40mg) and drug control SiO_2 (10, 20, 30 & 40mg) mediated turnover of phospholipids in liver and kidney and tracking its transfer through serum phospholipids content in male albino rat. Results indicated AB alone showed no change in normal rat phospholipids in liver, kidney and serum. In case of SiO_2 dose 10, 20mg given alone non-significantly influenced the phospholipids content in liver and kidney. But 30 and 40mg doses increased transitory phospholipids level with low significance.

Phospholipids content of liver were decreased significantly with CCl_4 induced acute hepato-steatosis coupled with deplete in renal phospholipids and reflective transitory rise in serum. Minimum effective dose of AB to normalize the phospholipids levels for liver was 20mg, kidney 10mg and for serum it was 30mg. In SiO_2 treated rats 10mg and 20mg doses showed trend of increased phospholipids in liver and kidney but high doses did not show this trend. Serum levels remained in mirror with the organ patterns and remained high over normal values.

Results are compared and interpreted with AB and SiO_2 protective potencies as appeared through phospholipid metabolism in liver and kidney. Serum phospholipids content indicate the transfer route in protective processes. The results also help to understand structural damage and protection of target organ liver, associated toxicated organ kidney in early phases of induced non-alcoholic fatty liver injury.

Key Words: Abhrak Bhasma, Steatosis, Phospholipids, NAFLD, SiO_2, CCl_4

INTRODUCTION:

In recent years Non-alcoholic fatty liver changes are revealing their roots in numerous important diseases and disorders that are expanding to cause serious health threat. Reviews and works in field shows that non-alcoholic fatty liver disease (NAFLD) are leading to cirrhosis Friedman (2008), hepatic carcinoma Yin *et al*., (2013). The concern increases Estes *et al*., (2018); Arab *et al*., (2018) as it is known that they lead to the hepatic and systemic insulin resistance (Arab *et al*., (2018); Amir and Czaja (2011); Amir *et al*., (2020). At this stage it becomes more important to know the development of NAFLD so that our expansion of its understanding possibly open more options for its management.

Considerable groups of the drugs being explored for management and cure of non-alcoholic fatty liver disease (NAFLD) using animal models. In non-alcoholic fatty liver disease (NAFLD) hepatic steatosis is the main part in its developments Amir *et al*., (2020). As being so the turnover of lipids in liver and its liaison of metabolic load on kidney becomes more important in mechanism of drug action analysis; since the mobility of lipids plays a central part in hepatic physiology and pathology. CCl_4 induced acute hepatotoxicity model offers induced early phase of lipid accumulation/steatosis to test the drug/s so that early metabolisms in the process can be unwrapped for further analysis.

In our early works on CCl_4 induced acute hepatotoxicity model and hepatic protection by abhrak bhasma (AB) and its control SiO_2; attempts have been made to reveal mode of action of abhrak bhasma studying structural changes Buwa (2000), lipid peroxidation Teli and Kanase (2020 b), free radical manager glutathione status Teli and Kanase (2020 a) and alterations in total lipids and its turnover Teli and Kanase (2021).

We have further expanded the lipid metabolism studies by exploring the phospholipids contents that involve the structural integrity of cells and hence organs.

In present studies acute CCl_4 toxicity model in albino rats was used to study the details of phospholipids contents and its changes in liver, kidney and serum, as the metabolism of phospholipids is known to involve in dynamics of membrane Albert *et al*., (1994); Xie *et al*., (2020); Amir and Czaja (2011).

MATERIALS AND METHODS:

The male albino rats were used for the present work. The detailed experimental work was conducted at Departmental animal house (Regi. No.233/CPCSEA) for breeding and maintenance of rats and mice, Department of Zoology, Shivaji University, Kolhapur, MS, India.

Male albino rats, *Rattus norvegicus* (Wistar strain) originally derived from National Institute of Virology, Pune, MS, India and were bred and maintained in animal house and further used to conduct the experimental work. The room temperature, humidity and a 12 hrs. light/dark cycle were maintained in the animal house. All rats were fed standard pellet diet (rat feed) prepared by Amrit Feeds, Sangli, MS, India. Drinking water was supplied in glass bottles, which were cleaned and refilled daily. Food and water were provided *ad libitum*. At the

beginning of the study, the rats were of 90-100 days old and weighed about 130-140gm. Feeding and treatment schedule/s were conducted during 8 to 9.00 am.

Abhrak Bhasma: Abhrak Bhasma (AB) preparation and quality testing was carried out as per the methods described in Ayurvedic text Sharma (1977). Krushna Abhrak (Vajrabhrak) was used for drug preparation. The ore was processed to eliminate any associated toxicity if present in crude element as mentioned in Ayurvedic text. Bhasma preparation was conducted through shodhan and maran procedures to get abhrak bhasma. It is insoluble in water.

Doses of Abhrak Bhasma and Control drug SiO_2: Graded doses of Abhrak Bhasma and SiO_2 10, 20, 30 and 40 mg/Kg body wt were used. This helped to select minimum effective dose/s and side effects if any of higher doses independently or along with CCl_4.

Dose of CCl_4: CCl_4 3.0 ml/kg body weight /day given for 7 days consecutively (SC) given during (8.00 – 9.00am) induced acute hepatotoxicity Patil *et al*., (1989, 1993); Buwa (2000); Teli and Kanase (2020a, 2020b, 2021).

Drug Treatment: The abhrak bhasma and SiO_2 (10, 20, 30 and 40 mg/kg body wt/day administered orally (PO) with known amount of honey. The doses were given orally between 8 to 9.00 am.

Experimental Schedule:

The experimental conditions used in present experimental model of acute toxicity are as given. Toxicity was induced by seven days treatment of CCl_4 (3.0ml/kg body wt/day). For protective studies daily dose of 10/20/30/40 mg abhrak bhasma (AB) and SiO_2 (drug control)/day was provided to the rats that were being treated with CCl_4 to analyze the influence of abhrak bhasma and SiO_2. The experimental rats were rested over night (24 hrs.) before sacrifice.

The drug vehicle control; honey is/as used vehicle and hence only honey treated rats were maintained independently but data does not differ from normal rat and hence it is not included here.

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 phospholipids estimation.

Estimation of Phospholipids:

Total phospholipid contents in liver, kidney and serum were estimated as per Zilversmit and Davis (1950).

STATISTICAL ANALYSIS:

The results of various experiments were analysed statistically and expressed as Mean ± SEM for six rats in each group. The statistical calculations were carried out with the help of XLSTAT 7.5 computer programme. Statistical significance between groups was analyzed by using One-Way ANNOVA.

Groups	Liver	Kidney	Serum
	mg/100gm Tissue	mg/100gm Tissue	mg/dl
Normal	1674.43 ± 123.70	1422.22 ± 66.13	117.03 ± 5.14
AB [10 mg/kg body wt]	1698.26 ± 92.48	1434.26 ± 83.17	129.45 ± 7.22
AB [20 mg/kg body wt]	1706.61 ± 109.34	1448.33 ± 59.76	129.36 ± 5.93
AB [30 mg/kg body wt]	1689.49 ± 111.92	1449.93 ± 51.36	122.38 ± 9.16
AB [40 mg/kg body wt]	1718.08 ± 128.14	1479.35 ± 38.47	124.42 ± 7.64
SiO_2 [10 mg/kg body wt]	1696.12 ± 102.26	1426.86 ± 56.19	109.18 ± 10.68
SiO_2 [20 mg/kg body wt]	1518.68 ± 128.13	1401.89 ± 101.51	119.76 ± 9.39
SiO_2 [30 mg/kg body wt]	1484.91 ± 141.39	1368.17 ± 72.06	136.92 ± 11.14^a
SiO_2 [40 mg/kg body wt]	1263.78 ± 136.26^a	1207.64 ± 43.16^a	142.68 ± 8.58^a

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 phospholipid contents in liver, kidney and serum.

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

Groups	Liver	Kidney	Serum
	mg/100gm Tissue	mg/100gm Tissue	mg/dl
Normal	1648.39 ± 102.10	1410.83 ± 78.16	118.14 ± 11.39
CCl_4 [3.0 ml/kg body wt]	1022.85 ± 109.60^{b}	1103.07 ± 88.14^{a}	289.34 ± 12.84^{c}
CCl_4 +AB [10mg/kg body wt]	1306.61 ± 110.22^{a}	1278.11 ± 92.48	238.18 ± 9.26^{cx}
CCl_4 +AB [20mg/kg body wt]	1479.26 ± 114.63^{x}	1352.91 ± 69.36	192.79 ±12.69^{bz}
CCl_4 +AB [30mg/kg body wt]	1592.29 ± 98.48^{y}	1399.24 ± 96.24^{x}	132.38 ± 11.78^{z}
CCl_4 +AB [40mg/kg body wt]	1603.94 ± 122.02^{y}	1392.26 ± 78.38^{x}	126.39 ± 9.89^{z}
CCl_4 +SiO_2 [10mg/kg body wt]	1268.14 ± 106.14^{a}	1208.91 ± 94.19	268.16 ± 14.49^{c}
CCl_4 +SiO_2 [20mg/kg body wt]	1294.37 ± 110.22^{a}	1216.19 ± 68.54	214.26 ± 8.87^{cz}
CCl_4 +SiO_2 [30mg/kg body wt]	1210.78 ± 94.35^{a}	1163.74 ± 72.42^{a}	182.88 ±12.36^{bz}
CCl_4 +SiO_2 [40mg/kg body wt]	1134.19 ± 106.48^{b}	1099.06 ±103.36^{a}	198.38 ±10.43^{cz}

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:

Phospholipids contents in liver of normal rat was 1674.43 ± 123.70 mg/100 gm tissue; which was not altered by the administration of 10, 20, 30 and 40mg Abhrak Bhasma (AB) and remained in normal range; indicating hardly any change in normal phospholipid content. Same was true in case of AB mediated phospholipids content in kidney. All these alterations can be related to the unaltered structure of liver and kidney Buwa (2000) in same experimental schedule where AB had hardly affected liver and kidney functions Teli *et al.*, (2013) even after seven consecutive doses. Thus AB is not toxic to liver/kidney. Similar doses of SiO_2 when given to the normal rat in same experimental condition, it showed non significant decrease in phospholipid contents at 10mg through 30mg doses of SiO_2 as compared to the normal liver. Dose 40mg of SiO_2 showed significant decrease ($P<0.05$) in hepatic phospholipids. Similar mode to alterations that was observed in liver, was also noted in phospholipids of kidney by SiO_2 treatment. Results indicate that 40mg dose influenced phospholipid content with low significance.

In same experimental schedule liver functions and kidney functions were altered adversely with significance Teli *et al.*, (2013) by 30 and 40mg doses of SiO_2. The results indicated that decreased phospholipids can be related to the

functions of liver and kidney directly especially with 40mg dose. Alterations in functions by 30mg dose seems to be related to changes occurring in organs before the appearance of the biochemically measurable alterations in phospholipids.

In serum of normal rats phospholipid content was 117.03±5.14 mg/dl; which was maintained at normal level when treated with 10mg through 40mg AB dose administration. These results are well agreed with unaffected liver functions and kidney functions studied earlier in same experimental schedule Teli *et al.*, (2013). These results indicate that treatment of AB alone with all studied doses are not affecting liver phospholipid content and maintain normal phospholipid turnover; while SiO_2 treatment influenced the phospholipid contents by graded doses used. 10mg and 20mg of SiO_2 had not influenced the phospholipid content and hence the turnover. But 30mg and 40mg doses increased the phospholipid content ($P<0.05$). These results agree with the altered liver and kidney functions by SiO_2 in same experimental schedule Teli *et al.*, (2013), which are directly reflected in transitory levels of phospholipids in serum.

As given in Table No. 2, there was a significant decline ($P<0.01$) in the concentration of phospholipids in liver tissues of CCl_4 induced acutely intoxicated rats as compared to normal rat. It seems due to the interference of CCl_4 induced free radicals in phospholipid synthesis Recknagel (1967) and damage caused to membranes. Simultaneous treatments of 10, 20, 30 and 40mg doses of AB to CCl_4 treated rats show dose dependent progressive increase in phospholipid contents to bring it towards normal level. It attained normal level with 20, 30 and 40mg doses. Thus 20mg is minimum effective dose and causes significant rise in the phospholipid content in liver, while in kidney all the doses maintained normal levels and that of serum of CCl_4 induced acutely intoxicated group of rats, 30 and 40 mg doses retrieved the levels to normal by AB. Though all the AB doses show potency to normalize phospholipid levels in liver, kidney and serum the minimum effective dose varies. In liver 20mg is minimum effective dose, while in kidney 10 mg is minimum effective dose and for serum levels 30 mg is minimum effective dose. Liver being target organ its effective dose is high than kidney, since kidney is influenced by associated toxicity it needed lower dose than liver. Even though organ toxicity is being protected the transport of metabolic products of intoxication and drug metabolism seems to be cleared slowly as the levels in serum phospholipids are normalized by 30mg abhrak bhasma dose. Which is low than the minimum dose required (40mg) to attain the normalization of total lipids content in liver, kidney and serum Teli and Kanase (2021). In similar experimental status full protection of free radicals (LPO) status of liver and kidney Teli and Kanase (2020b) also required 40mg dose of abhrak bhasma. But the corresponding glutathione contents normalization in liver and kidney required 30mg minimum dose of AB Teli and Kanase (2020a).

These results are well depicting the normalization of membranes as revealed by structure Buwa (2000), protection of phospholipids in liver in present condition and also the protection of free radical

scavenger glutathione Teli and Kanase (2020a) and required minimum dose of 30mg abhrak bhasma. All these results indicate that membrane protection is provided by AB which seems to initiate even at lowest of the dose and with the same dose it had moved 82.856% of phospholipid from liver and 73.36% of phospholipids from kidney. Thus AB seems to protect the membranes which is revealed through its protection of acid, alkaline and lipoprotein lipase activities of liver and kidney Buwa (2000) in earlier studies from our laboratory. Acid lipase protection indicates protection of membrane system of in lysosomal complexes that are involved in membrane turnover Alberts (1994); Xie *et al*., (2020); Amir and Czaja (2011).

The results also indicate that calcium homeostasis induced by CCl_4 in hepatocytes Casini and Farber (1981) is also recovered as indicated by membrane recovery by phospholipids content and other earlier studies.

In protective schedule of acute toxicity model experiments fats are moved to protect fatty degeneration of liver and necrotic hepatocytes being recovered, repaired/regenerated where increased phospholipids in liver are being utilized in liver as the present studies and earlier studies Buwa (2000); Teli and Kanase (2020a, 2020b, 2021) indicate the hepatoprotective potential of AB, which interferes with fatty degeneration hepatic steatosis to maintain cellular membrane system and the phospholipid content is reflection of it.

10mg and 20mg SiO_2 doses are inefficient to normalize phospholipid content. 30mg and 40mg also fail to normalize phospholipid content in CCl_4 treated rat.

Thus SiO_2 seems inefficient to influence membrane injury and failed in liver and associated kidney changes. The transitory levels in serum are reflections of alterations in liver and kidney since levels being significantly high. But high doses of SiO_2 may have contributed to CCl_4 induced injury in liver/hepatocytes necrosis and thus increased serum levels of phospholipids. These results support the earlier results of SiO_2 in same experimental status in case of liver and kidney lipid contents Teli and Kanase (2021), free radical status of liver Teli and Kanase (2020b). So also free radical scavenger glutathione status of liver and kidney Teli and Kanase (2020a).

From our earlier studies, total lipid content of liver remained between 95.659% - 100% with all SiO_2 doses and that of kidney remained in 98.930% - 89.118% by all the doses studied Teli and Kanase (2021) in protection. This is clear indication that hepato-steatotic status is not changed so also the lipid loading in kidney is affected. Similarly the production of free radicals remained in linear. 85.658%-66.279% range and in kidney they were in range of 89.247%-80.107% as compared to CCl_4 treated LPO in liver and kidney respectively which indicated that with any of the doses of SiO_2 it is not lowered than 20% Teli and Kanase (2020b), free radical scavenger glutathione Teli and Kanase (2020a) in same status was elevated in the range that shows failure to phospholipids content may be due to failure/delayed rapid clearance of free radicals by production of GSH in liver and kidney. Thus SiO_2 though stimulates the

phospholipid metabolism in the direction of liver steatotic removal, it is unable to proceed further as revealed by the present studies on phospholipids also protection of membrane system.

The serum levels of phospholipids are indicators of circulating phospholipids and reflect partially their delivery in required zones liver and kidney. The results also indicate that early changes in liver steatosis are sensitive to silica pure and silica abhrak bhasma (AB) form. Thus AB which is fully effective and also shows dose dependency seem to be influencing the other accessory metabolic factors and/or neurotrophic and similar other factors which are involved in pathogenesis of NAFLD Amir *et al*., (2020) and may be functional through them.

Thus AB is stimulating mobility of hepatic cell accumulated lipids that are being loaded or inhibiting the hepatic steatosis. It also seems that AB leads the protection of normal cells and or repair, regeneration, recovery of partially injured cells through interference at pathogenesis of steatosis, as it is observed through phospholipids turnover and other earlier studies stated earlier in same experimental status.

Silica toxicity is supposed to be due to surface silanol groups formed by hydrolysis McCoy (1987), it is also due to positive charges Bagachi (1992). Low doses with initiation of protection and high doses with toxicity may be due to bioavailability of SiO_2 in hepatocytes and other liver cells (kupffer cells & sinusoidal cells) may be differential with different doses. This may be tissue/organ specific or physiological status dependent. SiO_2 nephrotoxicity in chronic inhalation is known El-Safty *et al*., (2003).

Though SiO_2 low doses are showing relief to liver toxicity and showing adverse effects of higher doses, this data supports the antisteatotic effects of silica but its high dose toxicity is also indicating the necessity of processing of silica as indicated by data on AB. Protective effects of AB can be attributed to the drug preparation process. It is the processed silica ore derived drug used in Ayurveda. The processes like shodhan and maran may influence the silica ore content improving the effects of silica or may be through other trace elements or possibly modifying its properties with other modifications since the process involves heating with herbal treatment Sharma (1977).

The comparison of data on phospholipids compared with other earlier studies in same experimental status Teli and Kanase (2020a, 2020b, 2021) indicates that AB modulates the existing protective functions at cells and organs level leading to protection of membranes.

Ayurvedic drugs if properly studied will provide the options for integrated medicinal therapy and will also open up with the possibility of other physiological/cell biological conditions where modern drugs can be efficiently used.

CONCLUSIONS:

In conclusion AB efficiently modulates CCl_4 induced hepato-steatosis influencing phospholipid turnover in target organ so also in associated toxicity in kidney. Abhrak bhasma thus influences the early stages of lipid accumulation in liver especially the situation that can be

compared with NAFLD (non-alcoholic fatty liver diseases). This model may be further useful for pathogenesis analysis of NAFLD.

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REFERENCES

1. Friedman S. L. 2008. Mechanisms of hepatic fibrogenesis. Gastroenterology. 134:1655-69.
2. Yin C., Evason, K. J., Asahina, K. and Stainier D. Y. 2013. Hepatic stellate cells in liver development, regeneration, and cancer. J. Clin. Invest. 123:1902-10.
3. Estes C., Razavi H., Loomba R., Younossi Z. and Sanyal A. J. 2018. Modeling the epidemic of nonalcoholic fatty liver disease demonstrates an experimental increase in burden of disease. Hepatology. 67:123-33.
4. Arab J. P., Arrese M. and Trauner M. 2018. Recent insights into the pathogenesis of nonalcoholic fatty liver disease. Annu Rev Pathol. 13:321-50.
5. Amir M. and Czaja M. J. 2011. Autophagy in nonalcoholic steatohepatitis. Expert Rev Gastroenterol Hepatol. 5:159-66.
6. Muhammad A., Michael Yu, Peijian H. and Shanthi S. 2020. Hepatic autonomous nervous system and neurotrophic factors regulate the pathogenesis and progression of Non-alcoholic Fatty Liver Disease, Front. Med. (Lausanne), 7:22
7. Buwa S. K. 2000. 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
8. Teli P. and Kanase A. 2020b. Abhrak bhasma and SiO_2 influenced free radical status in liver and kidney of CCl_4 induced acutely intoxicated male albino rat. Asian J. Pharm. Clin. Res. 13(9): 40-43.
9. Teli P. B. and Kanase A. A. 2020a. Deflections in glutathione content in SiO_2 and abhrak bhasma influenced protection in CCl_4 induced acutely intoxicated liver and kidney in male albino rat. Int. J. Recent Sci. Res. 11(06): 38941-38945.
10. Teli P. and Kanase A. 2021. Abhrak Bhasma and SiO_2 influenced mobility of lipids in liver and kidney of CCl_4 induced acutely intoxicated albino rat. Int. J. Adv. Res. 9(12): 429-434.
11. Alberts B., Brey D., Lewis J., Raff M., Roberts K. and Watson J. D. 1994. 'Vesicular traffic in the Secretory and Endocytic Pathways' in Molecular Biology of the Cells, (3rd ed), Pub. By Garlard Publishing inc New York.
12. Xie Y., Li J., Kang R. and Tang D. 2020. Interplay between lipid metabolism and autophagy. Front. Cell Dev. Biolo., 8:431.
13. Sharma S. 1977. Rasa Ratna Samuchhaya, Published by Motilal Banrasidas, New Delhi: 72-108.
14. Patil S., Kanase A. and Varute A. T. 1989. Effect of hepatoprotective ayurvedic drugs on lipases following CCl_4 induced hepatic injury in rats. Ind J Exp Biol. 27: 955-958.
15. Patil S., Kanase A. and Kulkarni P. H. 1993. Effect of hepatoprotective Ayurvedic drugs on lipolytic activities during CCl_4 induced acute hepatic injury in albino rats. Indian J. Exp. Biol. 31: 265-269.

16. Zilversmit D. B. and Davis A. K. 1950. Microdetermination of phospholipids by TCA precipitation. J. Lab. Clin. Med. 35: 155-161.
17. Teli P., Chougule P., Jadhav J., Kanase A. 2013. Abhrak bhasma mediated alterations in liver and kidney functions in male albino rats during CCl_4 induced toxicity. Int. J. of Res. Ayurveda pharm. 4(5): 696-700.
18. Recknagel R. O. 1967. Carbon tetrachloride hepatotoxicity. Pharmac Rev. 19:145-208
19. Casini A. F. and Farber J. L. 1981. Dependence of the carbon tetrachloride induced death of cultured hepatocytes on the extracellular calcium concentration. Am. J. Pathol. 105:138-148.
20. McCoy S. 1987. Factors Affecting the Integrity and Stability of Q7-2159A Mammary Gel and Intermediates. Dow-Corning Quality Assurance Report.
21. Bagchi N. 1992. What makes silica toxic? Br J Ind Med. 49(3): 163–166.
22. EL-Safty I. A., Gadallah M. and Shouman A. E. 2003. Effect of silica exposure on urinary excretion of copper and zinc. Am J Med Sci. 326(3):122-7