Hepatoprotective activity of Somanathi Tamra Bhasma in paracetamol induced liver toxicity in albino- rats

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Abstract

To Investigate the hepatoprotective activity of Somanathi Tamra Bhasma against paracetamol induced hepatotoxicity. The hepatoprotective activity of Somanathi Tamra Bhasma was tested against paracetamol induced hepatotoxicity in albino rats. The degree of protection was determined by measuring levels of serum marker enzymes like serum glutamate oxaloacetate transminase (SGOT), Serum glutamate pyruvate transminase (SGPT), alkaline phosphate (ALP) etc., and histopasthological studies. Silymarin was used as standard drug for comparison. Administration of Somanathi Tamra Bhasma (67.5 mg/1kg. Bd. Wt.) markedly prevented paracetamol induced elevation of levels of SGOT, SGPT and alkaline phosphate etc,. The results are comparable to that of silymarin. A comparative histopathological study of liver exhibited almost normal architecture as compared to control group. Treatment with Somanathi Tamra Bhasma significantly reduced the paracetamol induced hepatotoxicity. A comparative histological study of liver from different groups further confirmed the hepatoprotective activity of Somanathi Tamra Bhasma.

Keywords: Hepatoprotective; Somanathi Tamra Bhasma; Paracetamol

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Introduction

The liver is the largest glandular organ in the body and it is responsible for detoxifying the poisonous substances in the body by transforming and removing toxins and wastes[1], therefore maintenance of healthy liver is essential for the overall well being of an individual.

There are numerous plants and traditional formulations available for the treatment of liver diseases, however, only few of them were pharmacologically evaluated for their efficacy.

In Ayurvedic literature also many herbal, herbo-mineral and mineral formulations were advised for the treatment of liver related disorders, Tamra Bhasma is one amongst them.

Tamra by its ushna, teekshna and srotoshodhaka property acts on the liver and induces secretion and circulation of yakrit pitta (bile). It reduces any inflammation or edema with its lekhana property[2].

Somanathitamra bhasma[2] was a special method of tamra bhasma preparation, which was prepared by using shudhatamra, parada, gandhaka, haritala and manashila and considered to be more effective and non toxic. It was indicated in Yakrita and Pliha vikaras[2] (liver and spleen disorders), so to assess the rationality behind the statement, the study on the assessment of hepatoprotective activity of somanathitamra bhasma was under taken in this present work.

Materials and Methods

All the materials required for the preparation of Somanathi Tamra Bhasma were procured from SDM Ayurveda Pharmacy, Udupi after proper authentication[2], and Somanathi Tamra Bhasma was prepared as per the reference[2] of rasaratnasamuchchaya at rasashastra and bhaishajyakalpana practical hall of SDM College of Ayurveda Udupi. After the processing of Somanathi Tamra Bhasma, it was subjected for bhasma siddhi pareekshyas to confirm the quality and after getting positive results, the bhasma sample was utilized for experimental study.

Animals: In bread wistar strain albino rats of either sex of body weight ranging from 170- 200 g. were obtained from the central animal house of the SDM Centre for Research in Ayurveda & Allied Sciences, Udupi. They were maintained at standard housing conditions and fed with standard animal pellet and provided with tap water ad libitum during the experiment. The institutional animal ethical committee (IAEC-SDMCAU/ACA-49/EC 13/10-11) permitted the study.

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The human dose of Somanathi Tamra Bhasma was converted to animal dose by using the conversion formula as- human dose × 0.018/200 g. body weight[3]. 1g. of paracetamol/ kg body weight of rats was used intramuscularly to induce hepatotoxicity[4] and silymarin was used as reference standard drug against test drug Somanathi Tamra Bhasma.

Treatment groups: Animals were divided in 4 groups (n=6/group)

Group I (Test): The animals of this group were administered with test drug Somanathi Tamra Bhasma 13.5 mg + 0.5% CMC solution for first five days, then on the 5th day after 1 hour of drug administration 1g. Paracetamol /kg bd. wt. was given intramuscularly, and again on 6th and 7th day test drug was given as usual.

Group II (Positive control): The animals of this group were administered with only 0.5% CMC solution for first five days, then on the 5th day after 1 hour of CMC administration same as above 1g. Paracetamol /kg bd. wt. was given intramuscularly, and again on 6th and 7th day 0.5% CMC solution was administered.

Group III (Normal control): The animals of this group were administered with 0.5% CMC solution for seven days.

Group IV (Standard): The animals of this group were administered with Silymarin 50 mg/kg bd. Wt. + 0.5% CMC solution for first five days, then on the 5th day after 1 hour of drug administration 1g. Paracetamol /kg bd. wt. was given intramuscularly, and again on 6th and 7th day Silymarin and 0.5% CMC solution was given as usual.

Assessment of hepatoprotective acticity

All the animals were killed after 48 hours of paracetamol administration that is on the 7th day. The blood samples were collected separately by carotid bleeding into sterilized centrifuge tubes and allowed to coagulate for 30 min at 37^{0}C. The clear serum was separated at 2500 rpm for 10 min and bio-chemical investigations were carried out to assess liver function[5] viz, total bilirubin, total protein and serum alkaline phosphate etc.

Statistical Analysis

Data obtained was analyzed by using modified 't' test and analysis of variance (ANOVA) followed by Dunnett's 't' test for determining the level of significance of the observed effects. P value of less than 0.05 was considered statistically significant.

Histopathology[6]

After draining the blood, liver samples were excised, washed with normal saline and processed separately for histopathological observations. Initially the materials were fixed in 10% buffered neutral formalin for 48 hours and then with bovine solution for 6 hours, paraffin sections were taken at 5mm thickness, processed in alcohol- xylene series and were stained with alum hematoxylin and eosin. The sections were examined microscopically for histopathological changes.

Observations and Results

Table 1: Effect of Test drug Somanathi Tamra Bhasma on body weight changes in paracetamol induced hepatotoxicity.

Group	Before (grams) Mean ± SEM	After (grams) Mean ± SEM
Control	186.6±7.032	190.16±8.052
Positive control	189.8±3.00	193±4.85
Standard	194.16±9.34	183.4±8.35**
Test	182.6±4.61	177±5.14*

*p<0.05, ** p<0.01 in comparison of final body weight to initial body weight

Table 2: Effect of Test drug Somanathi Tamra Bhasma on changes in liver weight in paracetamol induced hepatotoxicity.

Group	Absolute Liver weight(grams) Mean±SEM	Relative Liver weight (grams) Mean ±SEM
Control 0.5% CMC solution (No hepato-toxicity induced)	6.75±0.49	3.567±0.26
Positive control 0.5% CMC solution	7.18± 0.21	3.72±0.262
Standard (50 mg/kg of silymarin +0.5% CMC (Carboxy methyl cellulose) solution)	7.20±0.24	3.949±0.16
Test (13.5 mg of Somanathi Tamra Bhasma + 0.5% CMC (Carboxy methyl cellulose) solution)	6.60±0.42	3.72±0.15

Table 3: Effect of Test drug Somanathi Tamra Bhasma on bio-chemical parameters in paracetamol induced hepatotoxicity.

Group	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Protein (gm/dl)	Sr. Urea (mg/dl)	Sr. Crtn.(mg/dl)	Sr. Bil. total (mg/dl)	Sr. Bil.Direct(mg/dl)	Sr. Glucose (mg/dl)
Control	155.66±20.02	80.16±18.79	425±73.097	6.4±0.20	42±8.96	0.8±0.03	0.15±0.20	0.09±0.03	140±4.10
Positive control	694±6.112*	468±58.97*	1211±197.63*	6.4±0.10*	149.16±68.04*	0.88±0.02*	0.75±0.022*	0.1±0.00*	139±6.27*
Standard	402±95.692**	98.4±3.076**	692.4±133.68**	6.02±0.17**	44.6±9.07**	0.74±0.09**	0.18±0.012**	0.1±0.00**	120.4±7.84**
Test drug	183±17.609**	119.6±32.41**	456.6±64.45**	7.06±0.17**	40.8±5.19**	0.75±0.022**	0.75±0.022**	0.1±0.00**	128.2±10.66**

* p< 0.01 compared with normal control, ** p < 0.01 compared with positive control

Paracetamol (1g. /Kg.) given intramuscularly showed hepatotoxicity after 48 hours, as evident from biochemical, pharmacological and histopathological parameters of the study.

Paracetamol treatment significantly increased the SGOT, SGPT, Alkaline phosphate, Serum urea and Bilurubin direct. The toxic effect of paracetamol was controlled in the animals treated with test drug Somanathi Tamra Bhasma by way of restoration of the levels of the liver function biochemistry similar to that of the standard drug silymarin (Table 3).

Histopathological profile of the control animal showed normal hepatocytes (Fig 1a, 1b), positive control animal showed extensive disturbance in the liver cytoarchitecture, as necrosis, leukocuyte infiltration, micro & macro fatty changes and sinusoidal dilation (Fig 2a, 2b). The section of liver taken from the animals treated with standard drug silymarin showed mild to moderate disturbance in two rats and almost normal cytoarchiteture in the remaining rats, indicating very good hepatoprotection (Fig 3a, 3b). The animals treated with test drug Somanathi Tamra Bhasma exhibited significant liver protection against the toxicant as evident by mild cell infiltration

and the presence of normal hepatic cords and lesser fatty changes (Fig 4a, 4b).

Discussion

Paracetamol a well known compound for producing chemical hepatic injury in the rat has been used as an experimental model to test the potential hepatoprotective activity of Somanathi Tamra Bhasma.

The effect of paracetamol on ponderal parameters i.e., body wt. and liver wt. showed significant decrease, suggestive of paracetamol metabolism affected in reducing the body wt (Table1&2).

Serum enzymes such as SGOT & SGPT gets elevated due to paracetamol hepatotoxicity, which was well noted in the positive control group, indicative of liver inflammation and injury due to toxic effect of paracetamol, the elevation was significantly reversed by both test and standard group indicative of hepatoprotective activity.

Alkaline phosphate was significantly increased in the positive control group, when compared to normal control group, suggestive of cholestasis in the biliary tract leading to liver injury and hence can be considered as one of the bio- marker for the assessment of paracetamol induced hepatotoxicity, this elevation was reversed significantly in test and standard group indication of hepatoprotection.

The bilirubin a break down product of hemoglobin is the predominant pigment produced in the liver, excess bilirubin causes yellowing of body tissues (Jaundice). There are two tests for bilirubin, direct-reacting (conjugated) and indirect- reacting (unconjugated). Differentiating between the two is important diagnostically, as elevated levels of indirect bilirubin are usually caused by liver cell dysfunction (eg. hepatitis), while the elevation of direct bilirubin typically result from obstruction either within the liver (intra- hepatic) or source outside the liver (eg. Gall stone or tumor) [4]. In the present study, serum bilirubin was significantly increased in positive control suggestive of liver cell dysfunction. Decrease in serum bilirubin level in the test and standard group was again suggestive of hepatoprotective activity of test and standard drugs.

The blood urea level is considered as a good indicator of a balance in nitrogrn metabolism in the body. High blood levels of ammonia are found in acute hepatitis, cirrhosis & hepatic encephalopathy. The rise in serum ammonia is due to the inability of severely damaged liver to convert it to urea. Thus urea synthesis is reduced in chronic liver diseases[4]. In the present study significant increase in urea level of the positive control group was noted and in test and standard groups urea level was significantly reduced. Reverse as index liver toxicity & its reversal changes observed in this parameter did not correlate well with the observation of histopathological findings and changes observed in other parameters.

Serum creatinine is formed in muscle through conversion of creatinine phosphate. The concentration in serum depends on the balance between production & excretion. The serum creatinine level is considered as a marker of kidney function. The toxicant paracetamol increased the creatinine level in serum[4]. This elevation may be due to increase in muscle massor impairment in kidney functions in liver disorders reveals the impact of impaired lipid metabolism on kidney function leading to elevation in serum creatinine levels. In present study non significant reversal observed in the test and standard groups may be considered as representing reversal of the toxic effect of paracetamol through direct effects on the kidney or indirect through correction of other factors. It is possible that both the mechanisms may also be involved.

Thus the analysis of serum bio- chemical parameters shows that administration of paracetamol leads to significant changes in majority of parameters. These altered bio- chemical parameters were found to be reversed in most of the instance, though there were some exceptions like non alteration or wrong alteration of serum cholesterol, HDL cholesterol and Blood urea. The overall activity profile indicated a reversal of almost all impairments. This, along with the Histo pathological examinations provide strong & un- equivocal evidence for the presence of hepatoprotective activity in the test formulation which compares quite well with that of the reference standard silymarin.

Conclusions

The test formulation Somanathi Tamra Bhasma was indicated for Yakrita&Pliha (Liver & spleen) disorders in classical rasa granthas. To provide a pharmacological basis for the clinical efficacy it was evaluated experimentaly by paracetamol induced hepatotoxicity. The effect of Somanathi Tamra Bhasma on the toxicant induced changes in ponderal, bio- chemical & histopathological parameters are assessed. The Somanathi Tamra Bhasma was found to afford significant protection against paracetamol induced hepatotoxicity. It restored most of the parameters altered by the toxicants. The effect was further substantiated by histopathological examinations.

The analysis of serum bio- chemical parameters showed that administration of paracetamol leads to significant changes in majority of the parameters. The altered bio- chemical parameters were found to be reversed in most of the instances, though there were some exceptions like serum cholestrol. HDL cholestrol & blood urea. The overall activity profile indicated a reversal of almost all important parameters. This, along with the histopathological examination provides strong & un- equivocal evidence for the presence of hepatoprotective activity of Somanathi Tamra Bhasma which compares quite with that of the reference standard silymarin.

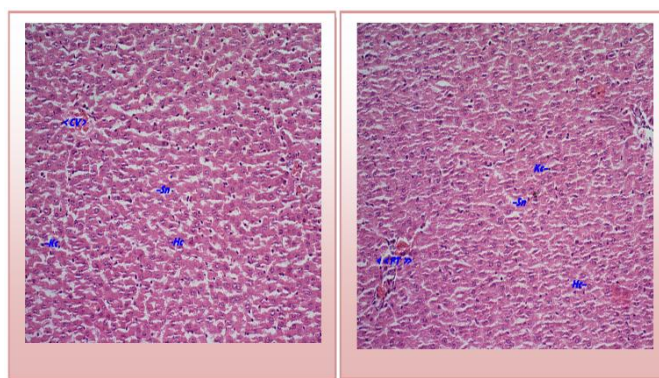The data generated can be considered as a strong basis for the clinical efficacy of the Somanathi Tamra Bhasma.

| Fig 1a: | Fig1b: |

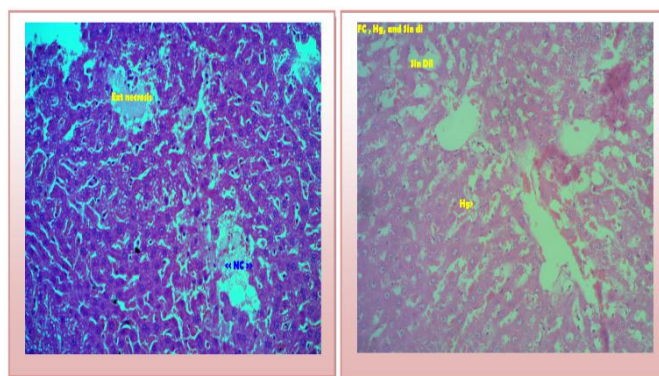**Fig 1a, 1b:** Sections of liver of control rats showing normal cytoarctitecture

| Fig 2a: | Fig 2b: |

Fig 2a, 2b: Sections of liver tissues of positive control rats treated with paracetamol showing necrosis hemorrhage and sinusoidal dilatation
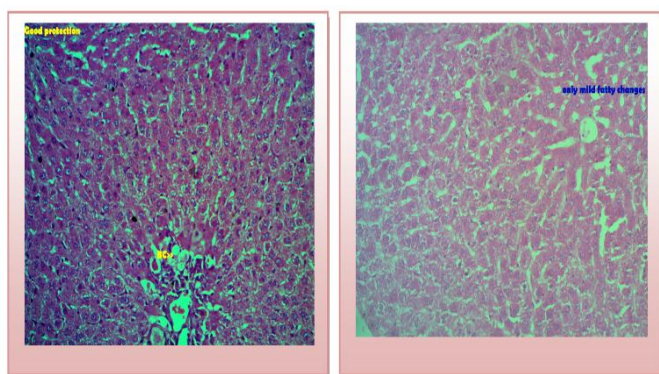
Fig 3a: Fig3b:

Fig 3a, 3b: Sections of liver tissues of silymarin treated rats showing good protection and mild fatty changes
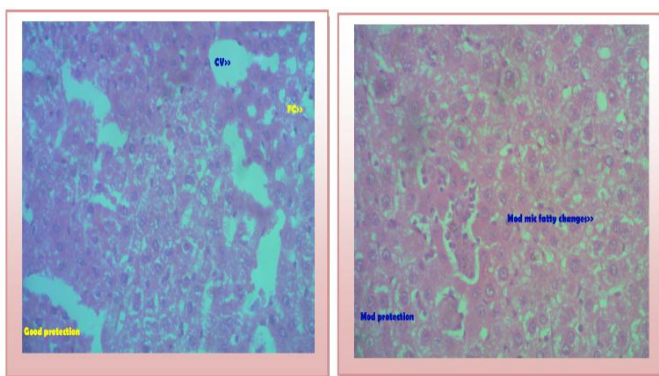

Fig 4a: Fig 4b:

Fig 4a, 4b: Sections of liver tissues of test drug S.T.B. treated rats showing good protection and mild fatty changes

Acknowledgment

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