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Assessment of Hepatoprotective Activity of Rajata Bhasma in CCl_4 Induced Hepatotoxicity Rats

Anand Prakash Rai[1]*, Shalini Tripathi[2] and Om Prakash Tiwari[3]

[1]Institute of Pharmacy, H.C.P.G College,BawanBegha,Varanasi - 221102 (U.P.), India.
[2]Rameshwaram Institute of Technology & Management, Sitapur road, Lucknow - 227202 (U.P.), India.
[3]Varanasi college of Pharmacy, Near Babatpur Airport, Varanasi - 221105 (U.P.), India.
*Corresponding author E-mail: anandphd256@gmail.com

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Rajata Bhasma (RB)is an Ayurvedic formulation and used for the treatment of liver disorders.Till date scientific validationaccording to modern toolof RB has been not performed. Hence it was planned to evaluate the hepatoprotective activity of RB in CCl_4 induced liver cirrhosis rats. The different formulation RB1 (9 puta) and RB2 (17 puta) were prepared by following classical methods. The SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood significantly enhance in CCl_4 treated rats compared to normal group rats. After treatment with the different doses of RB1 (50 mg/kg and 100 mg/kg) and RB2 (50 mg/kg and 100 mg/kg) significantly decreased the CCl_4 induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin in blood. The RB1 and RB2 treated rats significantly increased the level of catalase, glutathione peroxidase, superoxide dismutase enzyme, whereas lipid peroxidation was decreased, when compared to CCl_4 treated group rats. Theses property confirmed the antioxidant properties of RB. The findings suggest that hepatoprotective activity of RB may be due to free radical scavenging property.

Keywords: Rajata Bhasma, hepatoprotective activity, antioxidant actiivit, CCl_4

Liver is the biggest organ in the vertebrate body and the site for exceptional digestion. Liver toxicity stay one of the genuine medical issues and are mostly brought about by toxic chemicals. In spite of the colossal advances made in allopathic prescription, no compelling hepatoprotective drug is accessible[1,2]. The use of natural remedies for the treatment of liver diseases has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of traditional medicines. The 21st century has seen a change in outlook towards remedial assessment of Ayurvedic products in liver disease models via cautiously synergizing the qualities of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization and randomized placebo controlled clinical trials to support clinical efficacy[3]. A large number of Ayurvedic formulations have been claimed to have hepatoprotective activity.

Ayurveda, a traditional Indian System of Medicine, is accepted to in presence from days of yore. There are confirmations for the utilization

of medications got from minerals, vegetable and animal products. It is accepted, that the teachings have been gotten from Lord Dhanvantari, which has taken its shape during the Vedic time and the substance referenced in Ayurvedic exemplary messages specifically CharakaSamhita and SusruthaSamhita. CharakaSamhita features the analysis of sickness, though SusruthaSamhita manages surgeries and careful devices. Aside from treating unpleasant maladies, Ayurvedic practices fit the prosperity of a person through Yoga practices and meditation[4].

The bhasmas derived using several metals are known for their numerous therapeutic uses against dreadful diseases. For instance, Swarnabhasma (Gold based bhasma) has been indicated for many degenerative diseases. Tamara bhasma (Copper based bhasma) has been used to treat leucoderma, cardiac problems, liver and stomach related disorders. RajataBhasma (Silver based bhasma) has known for effect against diabetes, fever, anaemia and psychological disorders. Vangabhasma (Tin based bhasma) is prescribed to patients suffering from diseases like diabetes mellitus, asthma, anaemia and gastric ulcers[5]. The present study was intended to evaluate hepatoprotective activity of two different samples acquired from Rajata Bhasma.

MATERIAL AND METHODS

Preparation of Rajata Bhasma

The Rajata Bhasma was prepared in two different form, RB1 prepared by 9 puta while RB2 prepared by 17 puta. The detailed procedure is given below[6]:

• Rajat foil cut into small pieces and amalgam was formed with parade in mortar
• Purified gandhaka was added to amalgam and triturated till formation of proper Kajjali
• Followed by impreganation with kumara swarasa to preparation of Chakrikas (Pellets)
• Dried chakrikas were placed in sharvana and laghuputa was given
• After first puta, Rajata was in completely powder form
• In subsequent two putas, half amount of kajjali was added, triturated with kumara swarasa and puta was given
• From 4-9 puta, half part of gandhaka was added in place of kajjali (Considered as a RB1)
• Remaining puta were followed without addition of Kajjali or Gandhaka
• 17 puta were given to obtain RajatBhasma that passing all classical parameter (Considered as a RB2)

Hepatoprotective activity of RB1 and RB2

Assessment of hepatoprotective activity was carried out on wistar albino rats. The animals were segregated into SIX groups of six animals each. Group I served as normal control receiving 5% CMC (10ml/kg). All other groups received CCl_4 (1ml /kg i.p.) with equal volume of olive oil (50% v/v) for two successive days. Group II animals were maintained as CCl_4 group, while Group III rats were administered RB1 (50 mg/kg body weight) orally, Group IV rats were administered RB1 (100 mg/kg body weight) orally, Group V rats were administered RB2 (50 mg/kg body weight) orally and Group VI rats were administered RB2 (100 mg/kg body weight) orally for seven days.After the drug treatment all the animals were sacrificed by cervical dislocation. Blood was collected from the carotid artery and was allowed to clot for 45 min at room temperature; serum was separated by centrifugation at 2500 rpm for 15 min, used for the estimation of various biochemical parameters.

Biochemical estimation

Biochemical parameters such as serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), alkaline phosphatase (ALP), acid phosphatase (ACP) and serum bilirubin were determined[7,8].

Analysis of antioxidant enzymes of liver tissue

The antioxidant activities in the rat liver homogenate were assayed for superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH) and activity lipid peroxidation (LPO)[9-12].

Statistical analysis

The results are expressed as mean ± SD of six independent experiments. Statistical significance between the groups was evaluated by one-way analysis of variance (ANOVA) followed by Dunet's test. A $P < 0.05$ value was considered as statistically significant.

RESULTS AND DISCUSSIONS

Bhasma is sole of Ayurveda metal-based preparations made by following complex

pharmaceutical processes incorporating herbs, converting them into a suitable form. They are preventive and complementary alternate medicine used in the Indian subcontinent since seventh century BC and widely recommended for treatment of a variety of chronic ailments. Conventional modern medicine is not always successful to control liver toxicity in all cases.RajataBhasmaare safe to detoxify the liver toxicity.

Hepatoprotective activity of RB

Hepatoprotective activity of RB was determined in $CC1_4$ induced hepatotoxicity rats. It was observed that the rats treated with CCl_4significantly altered the biochemical parametersin serum compared to normal group of rats (Table 1). This indicates the severe damage of liver of rats.

The rats treated with different doses of RB1 (50 mg/kg and 100 mg/kg) and RB2 (50 mg/kg and 100 mg/kg) leads to significantlyreduced the CCl_4 induced alteration in SGOT, SGPT, ALP, ACP, total bilirubin and direct bilirubin of blood. It was found that the RB1 and RB2 offer protection against toxin as evidenced by remarkable reduction in all biochemical parameters ($P<0.05$). The RB2 exhibited maximum hepatoprotective activity compared to RB1.

Antioxidant activity

The findings of antioxidant study are shown in table 2. The administration of the RB1 (50 mg/kg and 100 mg/kg) and RB2 (50 mg/kg and 100 mg/kg) counteracted the CCl_4-induced free radical activity. The SOD, GPx and CAT enzyme levels were statistically significant increased, whereas lipid peroxidation was decreased, when compared to CCl_4treated rats ($P < 0.05$). These results suggest that the RB1 and RB2 displays an antioxidant activity. The RB2 exhibited maximum antioxidant activity compared to RB1.

Liver participate in several metabolic activities, and in order to fulfill this role, release a wide variety of enzymes. Liver can be injured by many toxicants, as well as by chemicals or drugs. In our model, CCl_4 serves as a toxicant. CCl_4-related hepatotoxicity is associated with elevation in enzyme levels, which may be attributed to the generation of trichloromethyl free radical during metabolism by the hepatic microsomes, which in turn begin lipid peroxidation. Hepatocellular necrosis decreases SOD, CAT and GPx activities,

Table 1. Effect of RB1 and RB2 on liver function test for different parameters in animals treated with CCl_4

Treatment	SGOT (AST) (U/L)	SGPT (ALT) (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct (mg/dl)	Total (mg/dl)
Normal rats	80.17±2.56	72.35±5.13	115.43±3.54	125.64±5.24	0.21±1.53	0.47±1.23
Control rats [CCl_4 (0.5 ml/ kg i.p.)]	195.41±6.23*	223.18±4.18*	251.39±7.53*	242.69±6.24*	3.14±0.14*	5.47±1.19*
RB1 (50 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	91.36±6.32[a]	92.13±2.65[a]	162.53±4.23[a]	156.24±6.54[a]	0.51±1.63[a]	0.71±0.18[a]
RB1 (100 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	80.64±4.84[a]	76.53±2.56[a]	120.32±6.23[a]	130.59±5.64[a]	0.28±0.53[a]	0.42±0.43[a]
RB2 (50 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	96.72±4.51[a]	89.12±7.22[a]	141.75±5.74[a]	147.24±6.31[a]	0.45±0.62[a]	0.63±1.34[a]
RB2 (100 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	76.25±1.86[a]	69.49±3.32[a]	110.48±4.23[a]	121.57±3.63[a]	0.23±0.41[a]	0.41±1.37[a]

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal group, [a]P<0.05 when compared with CCl_4 treated group considered as statistically significant.

Table 2. Effect of RB1 and RB2 on oxidative stress induced by CCl_4 in the liver of experimental animals

Treatment	Enzymes involved in oxidative stress in liver			
	LPO (Mole/gm)	SOD (U/gm)	GSH (iMole/gm)	Catalase (U/mg)
Normal rats	58.17±2.34	63.58±2.54	4.61±1.73	9.14±0.43
Control rats [CCl_4 (0.5 ml/kg i.p.)]	189.42±4.32*	12.51±6.24*	0.24±1.53*	0.92±0.96*
RB1 (50 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	88.73±2.46[a]	40.58±1.32[a]	3.71±1.86[a]	7.15±1.35[a]
RB1 (100 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	62.24±4.35[a]	65.42±1.75[a]	5.29±1.63[a]	9.43±0.37[a]
RB2 (50 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	82.36±1.23[a]	42.79±3.74[a]	3.89±0.32[a]	8.11±0.75[a]
RB2 (100 mg/kg)+ CCl_4 (0.5 ml/kg i.p.)	61.47±3.46[a]	60.17±2.63[a]	5.09±0.57[a]	10.73±1.34[a]

Values are expressed as mean ± SEM, n = 6 in each group. *P<0.05 when compared with normal group, [a]P<0.05 when compared with CCl_4 treated group considered as statistically significant

and the increase of such activities into basal values, is a clear indication of plasma membrane stabilization and tissue repair as well. Such an effect is in agreement with the view that enzyme activities are restored into normal conditions and healing of the hepatic parenchyma, as well as hepatocyte regeneration, are observed. SOD, CAT, and GPx constitute an enzyme defense mechanism against oxidative damage. Under CCl_4 conditions such enzyme activities are decreased, but under RB1 and RB2 treated conditions, a significant increase in their activities is observed, which may serve as a biochemical strategy to reduce lipid peroxidation[1,2,8]. The study revealed that the RB1 and RB2 under evaluation, at both studies doses, showed a hepatoprotective activity against CCl_4-induced liver damage. The present findings demonstrated that the RB1 and RB2 have free radical scavenging activity and were prominent in inhibiting the lipid peroxidation. The free radical scavenging properties may be one of the mechanism for protective effects of liver.

CONCLUSION

The RB1 and RB2have resilient antioxidant activity and may confer a favorable effect against oxidative stress. The RB1 and RB2 exhibited significant hepatoprotective activity in rats intoxicated with CCl_4. Further the metal-based nanoparticles size of bhasma enhances the bioavailability of therapeutic efficacy. This properties assist the development of newer drugs in modern medicine of Ayurveda.

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