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Research Article

Mukta Bhasma As Hepatoprotectant

Hepatoprotective and Anti-Oxidant Study of Mukta Bhasma (Calx of Cultured Pearls) Against CCl_4-Induced Hepatic Damage in Rats (In-Vivo).

Dr.Poornima B.Tukanatti[1*], Dr.Prashant Jadar[2], Dr. Wasedar Vishwanath[3]

[1] Assistant Professor, Department of Rasashastra and Bhaishajya Kalpana, KAHER's Shri B.M.Kankanwadi Ayurveda Mahavidyalaya, Shahpur, Belagavi-590003, Karnataka, India.*

[2]Professor, Department of Rasashastra and Bhaishajya Kalpana, KAHER's Shri B.M. Kankanwadi Ayurveda Mahavidyalaya, Shahpur, Belagavi-590003, Karnataka, India.

[3]Reader, Department of Panchakarma, KAHER's Shri B.M. Kankanwadi Ayurveda Mahavidyalaya, Shahpur, Belagavi-590003, Karnataka, India.

Abstract: *Mukta Bhasma* (MB) is a traditional Ayurvedic preparation for upper respiratory and lower respiratory conditions, eye disorders, powerful cardiac tonic, immune-regulator, mood elevator and known to promote strength, intellect and semen production. Mukta bhasma is prepared using direct heat as a media of transformation. Mukta Bhasma was evaluated for its hepatoprotective and antioxidant activities against Carbon tetrachloride (CCl_4) induced liver damage in wistar albino rats. Mukta Bhasma (100mg/kg, 200mg/kg and 300mg/kg) was administered to experimental rats for 10 days. Silymarin (25mg/kg) was given as the standard drug. The hepatoprotective activity was assessed using various serum biochemical parameters like (SGOT), (SGPT), Total and Direct bilirubin (TB and DB), Alkaline phosphatase (ALP), Total Triglycerides (TG) and Total Cholesterol (TC). Lipid peroxidation (LPO), Reduced Glutathione (GSH) and Catalase (CAT) were determined to explain the antioxidant activity of Mukta Bhasma. The substantially elevated levels of SGOT, SGPT, TB and DB, ALP, TG and TC due to CCl_4 treatment were restored towards near normal by Mukta bhasma (MB) of 200mg/kg dose. Administration of MB 200mg has shown significant reduction in the biochemical parameters like SGOT, SGPT, ALP, Bilirubin, Total cholesterol and Total triglycerides with significance of $p<0.001$ in all the parameters when compared to CCl_4 group. Also, they were near to the standard Silymarin group. Mukta bhasma 200mg has shown significant ($p<0.001$) reduction in the LPO level, increase in CAT and GSH representing significant antioxidant activity of Mukta bhasma. The histopathological study showed reduction in fatty degeneration of liver in MB 200mg/kg body weight. Thus, results revealed that Mukta bhasma afford significant hepatoprotective and antioxidant effects in CCl_4 induced hepatic damage.

Key words: Cultured Pearl, Mukta Bhasma, Hepatoprotection, Antioxidant, CCl_4.

***Corresponding Author**

Dr.Poornima B.Tukanatti , Assistant Professor, Department of Rasashastra and Bhaishajya Kalpana, KAHER's Shri B.M.Kankanwadi,Ayurveda Mahavidyalaya, Shahpur, Belagavi-590003, Karnataka, India.

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1. INTRODUCTION

Ayurveda exclusively describes about life and life care system. Along with herbs, metals & minerals, our ancient scholars were also well aware of the medicinal values of aquatic products like Mukta (Pearl) in different dosage forms i.e., Bhasma and Pishti. Bhasma is Agni samskarita (processed with fire) and Pishti is anagni samskara (processed without fire). The present-day lifestyle and food habits have increased the production of free radicals. Free radical mediated pathophysiology is the latest understanding of the diseases. To counteract such pathologies, antioxidants are essential. In recent years, many drugs have been screened for their antioxidant potential. However, deleterious effects of free radicals remain a problem. Thus, additional natural products need to be evaluated for their antioxidant potential. In the present study, standard study model was used i.e., CCl_4 was used to induce the hepatotoxicity in Wister strain albino rats which is mediated through free radical mechanism. The toxic effect of CCl_4 is due to its bioconversion by cytochrome P450 to the highly reactive free radical CCl_3^* (trichloromethyl). These free radicals further induce the tissue injury[1]. Oxidative damage plays an important role in carcinogenesis. ROS generated by mitochondria or from other intracellular or extracellular sites can cause cell damage and initiate various degradation processes. The role of ROS has been implicated in many human degenerative diseases including aging and Cancer[2,3]. Liver, an important organ actively involved in many metabolic functions is the frequent target for a number of toxicants. Hence, this compound has been used for the evaluation of free radical scavenging action *in vivo. Goa et al* opines that Pearl powder has the capacity of calming the mind, soothes the liver and protects liver, regulates endocrine, clears heat and detoxify and can supplement trace elements and promote metabolism. It improves sleep, the symptoms of constipation and disease resistance and enhance the immunity of the body. Pearl powder is a beneficial source of calcium supplement[4]. The beneficial effect of pearl powder may come from conchiolin, a protein made of 17 amino acids[5]. In Ayurvedic classics, Mukta (Pearl), being a marine originate, is indicated in the diseases like Rajayakshma (immuno-compressed conditions), Vata vyadhi (auto-immune disorders), Kasa-Shwasa (respiratory diseases)[6] etc. which are most of the free radical mediated ones. Keeping in view, all the above properties of Pearl powder and the potential health promoting benefits of Mukta, we have hypothesized that Mukta bhasma which is prepared according to our classics, may inhibit oxidative stress and toxicity induced by CCl_4 in rats. As such, no study has been carried out till date.

2. MATERIALS AND METHODS

2.1 Preparation of Mukta bhasma:[7]

Mukta bhasma was prepared according to the text *Rasa tarangini. Mukta shodhana* (purification) was done in *Jayanti swarasa* (Juice of *Sesbania aegyptica*) by *Swedana* (Mukta is tied in a cloth & suspended in an earthen vessel containing liquid media & heated for specific period) method for 3 hours. Then it was triturated with *Godugdha* (Cow's milk) and subjected to *laghu puta* (incineration with 200 cowdung cakes). Mukta bhasma passed all bhasma parikshas after four laghu puta.

2.2 Animals

Albino Wister rats weighing 150-200 g of either sex was used for the study. They were maintained in the animal house under controlled conditions of temperature (25 ± 2°C), humidity (50 ± 5%) and 12-h light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were randomized into experimental and control groups and housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad *libitum*. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of the non-specific stress. This study was approved by the Institutional Animal Ethics Committee (IAEC) of KAHER's Shri B. M. K. Ayurveda Mahavidyalaya, Belgaum (Reg.No:1017/C/06/CPCSEA) in accordance with the guidelines of Committee for the Purpose of Supervision and Control of Experiments on Animals, Govt. of India.

2.3 Acute Toxicity Studies

The acute toxicity test for *Mukta Bhasma* was performed on Adult Wistar Albino female rats weighing between 150-250g as per OECD Guidelines 425. Total of four rats were randomly allocated into two groups with 2 rats in each group. The Up and Down method (UDP) was adopted for acute toxicity studies. Prior to dose administration, rats were fasted overnight (12 hr) but had free access to water. Weight of each rat was recorded before test drug administration. Each group was administered with a single fixed dose of Mukta Bhasma in 3000mg/kg and 4000mg/kg. Any signs of clinical toxicity were closely monitored within 4 hour of treatment period upto 24 hr. After 14 days, they were humanly sacrificed. In rats of 4000mg/kg group, signs of sneezing, loose watery stools with mild weight loss were observed without any mortality. In rats of 3000mg/kg group, no signs of toxicity, no changes in general behavior, no mortality seen. The maximum non-lethal dose was found to be 3000 mg/kg. Hence 1/10th of the dose was taken as effective dose (300 mg/kg body weight) for the fractions to evaluate Hepatoprotective and Antioxidant activities

Table No 1: ***Experimental protocol (Grouping, Treatment & Observation)***

Sl.no	Group(N=6)	Treatment & dose/day	Observation
I	**Normal control**	Distilled water	Biochemical parameters and histopathological examination on 11th day in all groups
II	**Negative control**	CCl_4 1ml/kg i.p. in olive oil (1:1) on 3rd, 7th & 10th day.	
III	**MB 100 mg/kg**	MB 100mg/kg p.o daily + CCl_4 (i.p) on 3rd, 7th & 10th day.	
IV	**MB 200 mg/kg**	MB 200mg/kg p.o daily + CCl_4 (i.p) on 3rd, 7th & 10th day.	
V	**MB 300 mg/kg**	MB 300mg/kg p.o daily + CCl_4 (i.p) on 3rd, 7th & 10th day.	
VI	**Positive control**	Silymarin 25mg/kg p.o daily + CCl_4 (i.p) on 3rd, 7th & 10th day.	

2.4 Experimental design

Animals were grouped with six animals in each group and treated for a period of 10 days[8]. Group I received Distilled Water (5ml/kg body weight, p.o) and served as Normal control group. Group II (Negative control) received CCl_4 (1ml/kg) in olive oil (1:1; v/v)[9] and administered intraperitonially on 3rd, 7th and 10th day. Groups III, IV and V were treated with *MB* at doses of 100mg/kg, 200mg/kg and 300mg/kg orally respectively for ten days. Group VI (Positive control) was treated with standard Silymarin 25mg/kg[4] orally for ten days. Simultaneously group III-VI received CCl_4 (1ml/kg, i.p) after two hours interval of test drug on 3rd, 7th and 10th day. During this period of treatment, the rats were maintained under normal diet and water. All the animals were sacrificed by cervical decapitation under light anesthetic ether on the 11th day. Then retro-orbital blood was collected. Serum was separated by centrifuging blood (3000 rpm for 15 min.) and subjected to biochemical parameters like SGOT, SGPT, ALP, Total Triglycerides, Total and Direct Bilirubin and Total Cholesterol. Liver was dissected out, washed in the ice-cold saline. Further the liver was placed in 10% formalin solution for histopathological study. Then the liver homogenate was subjected for *in-vivo* antioxidant estimation like lipid peroxidation (LPO), Catalase (CAT) and Reduced Glutathione (GSH).

2.5 Morphological parameters

Processing of isolated liver for Histopathological study.

The animals were sacrificed and the liver of each animal was isolated. The isolated liver was cut into small pieces and preserved and fixed in 10% formalin for two days. Then they were taken for histopathological estimation.

2.6 Determination of Liver function tests[10]

SGOT, SGPT, ALP, TG, TB, DB and TC were measured in plasma samples obtained from all groups of rats. In all the parameters mentioned above, requisite quantity of serum was fed to the auto-analyzer which was automatically drawn in to the instrument for estimating different parameters.

2.7 Endogenous enzymatic and non-enzymatic antioxidant levels

2.7.1 Tissue preparation

1. Animals were sacrificed and were perfused transcardially with an ice-cold saline.
2. The whole Liver was perfused *in situ* with ice-cold saline, dissected out, blotted dry.
3. A 10% liver homogenate was prepared separately with ice-cold saline-EDTA using *Remi* homogenizer. The homogenate was used for the estimation of proteins and lipid peroxidation.
4. Liver homogenate was centrifuged at 10,000 rpm for 10 min and the pellet discarded. The supernatant was again centrifuged at 20,000 rpm for 1 hour at 4^0 C. The Liver supernatant obtained was used for the estimation of non-enzymatic antioxidants [LPO and GSH] and enzymatic antioxidants [Catalase].

2.7.2 Lipid Peroxidation (LPO)[11]

Thiobarbituric acid reactive substances (TBARS) in the liver homogenate were estimated by using standard protocol. Briefly, the homogenate was incubated with TCA-TBA-HCl (2 ml,15% TCA, 0.375% TBA and 0.25 N HCl) at 95°C for 15 min, the mixture was cooled, centrifuged and the absorbance of the supernatant measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using the formula $\epsilon = 1.56 \times 10^5 M^{-1}cm^{-1}$ and expressed as TBARS (μ moles) per g of tissue.

2.7.3 Reduced Glutathione (GSH)[11]

Following a reported method, the liver tissues GSH was measured. Briefly, the homogenates from the normal, positive control, drug-treated rats were made in 0.2 M Tris-HCl buffer, pH 8.2 containing 20 mM EDTA and centrifuged. An aliquot of the homogenate (1ml) was precipitated with ice-cold 20% TCA (1 ml) and centrifuged. The supernatant (1 ml) was added to 2 ml of 0.8 M Tris-HCl buffer, pH 9 containing 20 mM EDTA and mixed with 0.1 ml of 10 mM DTNB. The absorbance of the yellow chromogen at 412 nm ($\epsilon = 13.6 \times 10^4 M^{-1} cm^{-1}$) was read.

2.7.4 Catalase (CAT)[12]

Catalase activity was determined spectrophotometrically. Briefly, to 1.95 ml of 10 mM H_2O_2 in 60 mM phosphate buffer (pH=7.0), 0.05 ml of the Liver homogenate was added and rate of degradation of H_2O_2 was followed at 240 nm/ min. Catalase content in terms of U/mg of protein was estimated from the rate of decomposition of H_2O_2 using the formula $k=2.303/\Delta t \times \log (A1/A2)\ s^{-1}$ (A unit of Catalase is defined as the quantity which decomposes 1.0 μ mole of H_2O_2 per min at pH=7.0 at 25°C, while H_2O_2 concentration falls from 10.3 to 9.2 mM).

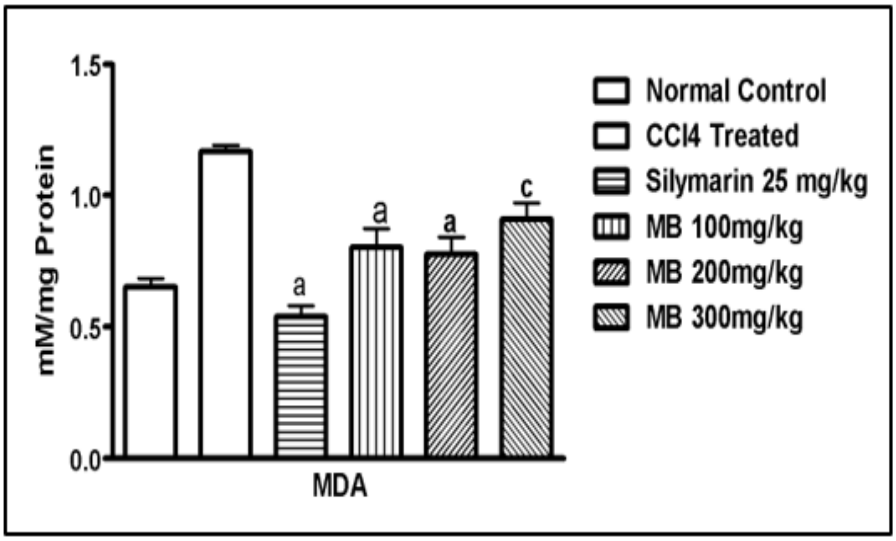
3 STATISTICAL ANALYSIS

Statistical analysis of all anti-oxidant parameters were performed by the use of Prism 5.0 software (graph-pad prism). The statistics were analyzed throughout one-way ANOVA and tukey's test. Data was presented as the Mean ±SD. P values<0.001 were considered significant.

4 RESULTS

4.1 *Lipid Peroxidation (LPO)*

LPO level was highly significantly increased ($p<0.001$) in CCl_4 treated group (Group II) as compared to control Group. *MB* 100mg/kg, *MB* 200mg/kg and Silymarin group showed highly significant ($p<0.001$) decrease in LPO when compared to CCl_4 Group. While *MB* 300mg/kg showed less significant ($p<0.05$) decline in LPO when compared to CCl_4 group. (Graph no.1)

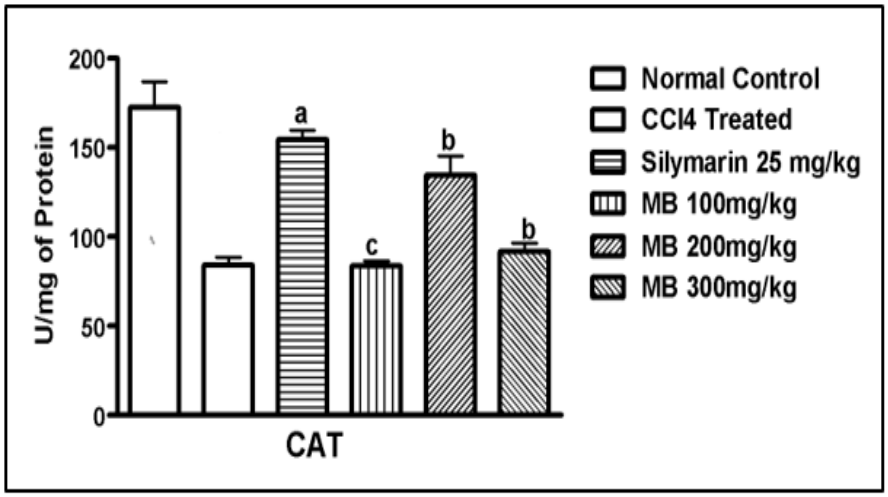


Graph No 1: LPO Levels in Various Groups

One-way ANOVA F=17.05, P<0.001, ** Tukey's test.
a==P<0.001 when compared with group II (CCl_4 group)***
b=**=P<0.01 when compared with group II (CCl_4 group)
c =* = P<0.05 when compared with group II (CCl_4 group)

4.2 Catalase (CAT)

CAT level was highly significantly decreased ($p<0.001$) in CCl_4 group as compared to Normal control group. The *MB* (200 mg/kg) showed moderately significant increase ($p<0.01$) of CAT in liver homogenate when compared to CCl_4 group. The *MB* (100 mg/kg and 300 mg/kg) showed no significant increase of CAT when compared with CCl_4 group. Silymarin treated group also showed highly significant ($p<0.001$) increase of CAT than CCl_4 treated group. (Graph.2)

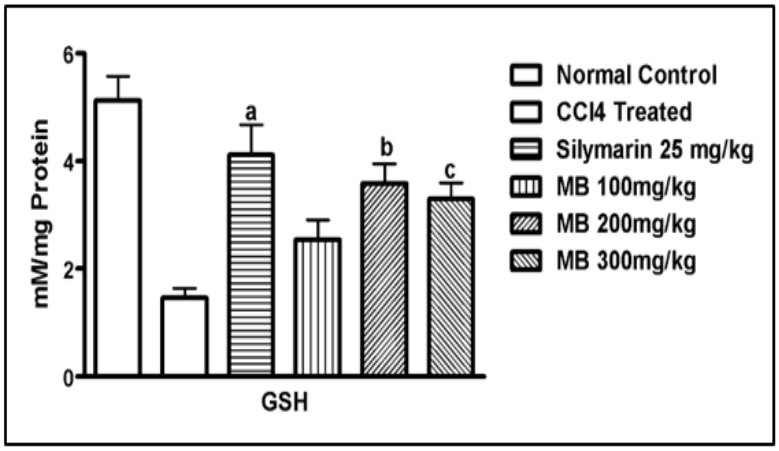


Graph No 2: Catalase levels in Various Groups

One way ANOVA F=22.49, P<0.001, ** Tukey's test.
a==P<0.001 when compared with group II (CCl_4 group)***
b=**=P<0.01 when compared with group II (CCl_4 group)
c =* = P<0.05 when compared with group II (CCl_4 group)

4.3 Reduced Glutathione (GSH)

GSH level was highly significantly decreased ($p<0.001$) in CCl_4 group as compared to Normal control group. The *MB* (200 mg/kg) showed moderately significant increase ($p<0.01$) of GSH in liver homogenate when compared to CCl_4 group. The MB (300 mg/kg) showed less significant ($p<0.05$) increase of GSH when compared with CCl_4 group. MB (100mg/kg) showed no significant increase of GSH when compared with CCl_4 group. Silymarin treated group also showed highly significant ($p<0.001$) increase of GSH as compared to CCl_4 treated group. (Graph 3)



Graph No 3: GSH levels in Various groups

*One way ANOVA F=10.49, P<0.001, ** Tukey's test.*
*a=***=P<0.001 when compared with group II (CCl_4 group)*
*b=**=P<0.01 when compared with group II (CCl_4 group)*
c = = P<0.05 when compared with group II (CCl_4 group)*

Table No 2: **Effect of MB* on Liver marker enzymes in Control and Experimental rats against CCl_4 induced toxicity**

Parameters	Normal Control	Negative control	*Mukta bhasma* + CCl_4			Positive control
			100 mg/kg	200 mg/kg	300 mg/kg	
SGOT	191.4±11.03	537± 183.81***	330.6±64.67@	246± 59.79#	335.6±97.10	271.8±89.57#
SGPT	84 ± 9.48	172.4± 32.34***	127±20.09@	112±24.27#	116±14.88@	94.6±14.02$
ALP	97.8±13.47	160.6± 21.37***	120.4±15.38@	116.2±18.83#	127.8±20.49@	103.6±14.11$
DB	0.116±0.02	0.264±0.07***	0.156±0.03@	0.136±0.02#	0.178±0.07	0.116±0.01$
TB	0.6±0.23	1.6± 0.46***	0.94±0.18@	0.76±0.27#	1.18±0.33	0.62±0.27$
TC	44.6±9.71	74±13.26**	50.6±7.95@	48.6±11.34#	63.2±8.95	47.6±8.98#
TG	51.4±12.77	81±12.10**	51.4±8.50#	46.0±12.56$	67.8±13.49	44.4 ±9.44 $

MB: Mukta bhasma. Values are expressed as Mean ± SEM of six rats in each group.
Doses are expressed as mg/kg
***P<0.01, ***P<0.001 compared to Normal control*
@ P<0.05, # P<0.01, $ P<0.001 compared to Positive (CCl_4 treated) group.

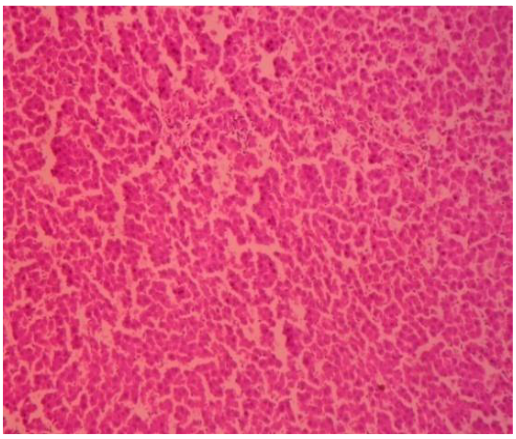
5. Histopathological Results (HPR)

The HPR of liver of all groups is shown in Table No:3

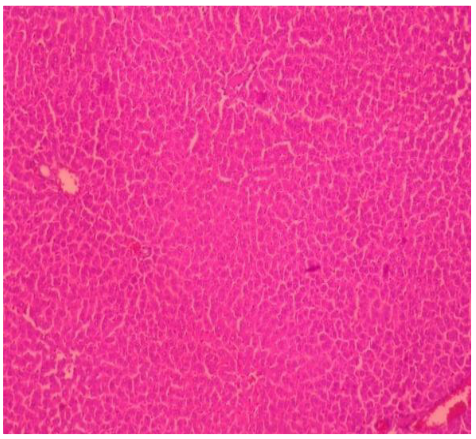
***Table 3*: Histopathological changes in liver in MB treated rats in normal and CCl_4 induced toxicity conditions**

Microscopic Appearance	Normal Control Group	Negative group	Positive group	MB 100mg/kg	MB 200mg/kg	MB 300mg/kg
Central vein Congestion	Mild	Marked	Mild	Moderate	Moderate	Moderate
Sinus congestion	Mild	Marked	Mild	Moderate	Moderate	Moderate
Focal hemorrhage	Nil	Present	Focal	Present	Focal	Focal
Portal triad	Nil	Moderate	Mild	Moderate	Mild	Mild
Inflammation	Nil	Focal	Mild	Moderate	Mild	Mild
Centrilobular degeneration	Nil	Marked	Nil	Present	Mild	Mild
Spotty necrosis	Nil	Nil	Focal	Present	Present	Present
Centrilobular necrosis	Nil	Marked	Nil	Mild	Mild	Mild
Fatty changes	Nil	Nil	Nil	Nil	Nil	Nil
Ballooning Hepatocytes	Nil	Present	Nil	Present	Present	Present
Overall grade of damage	Nil	Marked	Mild focal	Moderate	Mild	Mild
Overall protection	Normal	Poor	Good	Mild	Moderate	Moderate

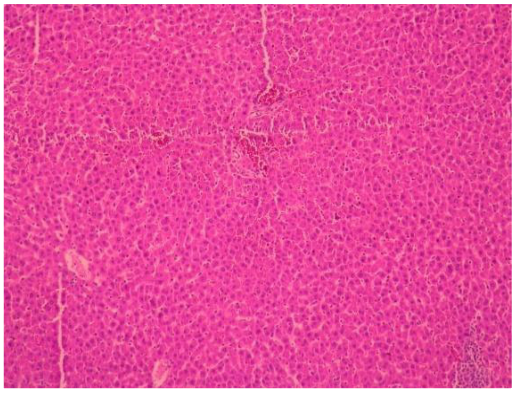
HPR images



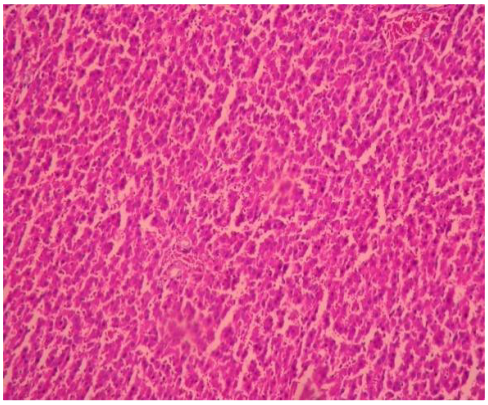
A) Normal group



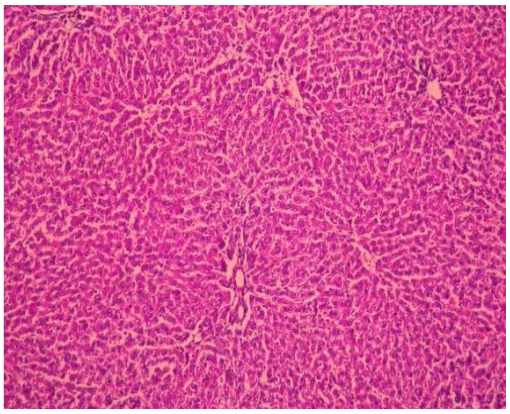
B) CCl_4 group



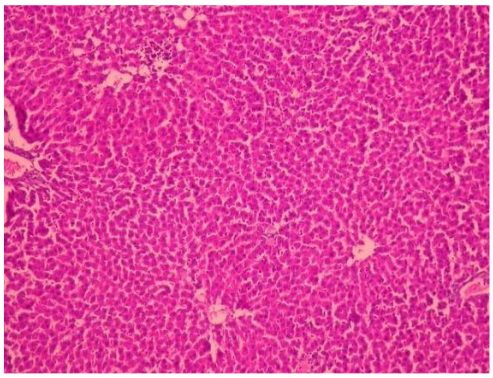
C) Silymarin group



D) MB 100mg



E) MB 200mg



F) MB 300mg

Histopathological images of Liver

A: Normal control group without any tissue damage. **B**: Negative control group showing centrilobular necrosis & degeneration with focal haemorrhage **C**: positive group showing mild congestion, focal hemorrhage **D**: MB 100mg showing moderate Overall grade of damage & mild protection **E**: MB 200mg showing mild Overall grade of damage & moderate protection **F**: MB 300mg showing mild Overall grade of damage & moderate protection.

6. DISCUSSION

This is the first of its kind study done to examine the anti-oxidant activity of Mukta Bhasma in experimental rats (in-vivo). The aim of the study is to explore the hepato-protective and anti-oxidant effect of Mukta Bhasma. In the present study, CCl_4 was used to induce the hepatotoxicity in rats which is mediated through free radical mechanism. The toxic effect of CCl_4 is due to its conversion by P450 to the highly reactive free radical CCl_3 (trichloromethyl). These free radicals induce the oxidative decomposition of the lipids & organic peroxides are formed after reacting with oxygen (lipid peroxidation) → damage to plasma membrane → increases permeability to Ca^{2+}, Na^{+} and water→ causes cell swelling→ ultimately leading to tissue injury. CCl_4 induced liver injury is both severe and extremely rapid in onset.[1] In toto, CCl_4 can injure hepatocytes directly through free radicals, through lipid peroxidation of the membrane[1]. Hepatotoxic drugs (CCl_4) will induce hepatotoxicity contributed to the hypothesis that this

category of drug reaction is immunologically mediated. In CCl_4 induced hepatotoxicity, there is non-progressive serum aminotransferase elevation which is dose dependent.[13] In this study, CCl_4-induced liver hepatotoxicity significantly reduced the levels of CAT and GSH and elevated the level of MDA, which is a marker of lipid peroxidation, compared to normal control. The *MB* pretreatment was found increased in the level of intrinsic antioxidants such as GSH and CAT, with reduction in the elevated level of MDA, caused by the CCl_4-induced liver hepatotoxicity. Silymarin acts against lipid peroxidation as a result of free radical scavenging activity and ability to increase cellular content of GSH. It regulates membrane permeability & increases membrane stability in presence of xenobiotic damage and inhibits the absorption of toxins, prevent them from binding to cell surface & inhibits membrane transport system.[14]. It is considered as hepatoprotective and it has been widely used in patients with cirrhosis, chronic hepatitis and liver diseases associated with alcohol consumption and exposure to environmental toxins[15,16]. Silymarin also caused significant favorable effects on the levels of GSH, CAT and MDA compared to the CCl_4-induced group. In the present study, results demonstrated that administration of MB 200mg has shown significant reduction in the biochemical parameters like SGOT, SGPT, ALP, Bilirubin, Total cholesterol and Total triglycerides with significance of $p<0.001$ in all the parameters when compared to CCl_4 group. Also, they were near to the standard Silymarin group. For antioxidant study, parameters like LPO, CAT & GSH were estimated. MDA is the major oxidant product of PUFA & its elevation is an important indicator of lipid peroxidation induced tissue damage due to failure of antioxidant defense mechanism[17].Treatment with Mukta Bhasma significantly reversed the lipid peroxidation indicating antioxidant potential of Mukta Bhasma. *MB* 200mg/kg showed ($p<0.001$) significant decrease in the LPO levels. L. Duan & his colleagues showed that intake of pearl powder could significantly reduce serum lipid peroxide products in a mouse model[18]. Catalase is one of the enzymatic antioxidants, high activity found in the liver. It decomposes hydrogen peroxide & protects the tissues from highly reactive hydroxyl radicals. Hence, reduction in the activity of CAT may result in deleterious effects due to assimilation of superoxide radical & hydrogen peroxide. MB 200mg increased the level of CAT($p<0.01$) significantly which was near to the Silymarin group value. Glutathione is a non-enzymatic, highly concentrated intracellular antioxidant present in liver. It appears to be a sensitive indicator of cell's overall health & its ability to resist toxic challenge[19]. Reduction in GSH triggers the process apoptosis. Higher GSH concentrations are associated with good health. Reduced level of GSH is associated with an enhanced lipid peroxidation in CCl_4 treated rats. Administration of MB 200mg/kg showed increased GSH level significantly ($p<0.01$) which was near to the Silymarin value. *Balcerczyk* and *Bartosz* demonstrated that thiols are the main determinants of the total antioxidant property of the cellular system[20]. The abundant presence of protein content (amino acids and minerals) in pearl powder demonstrated an increased total antioxidant capacity, antioxidant activity, total thiols (SH group) and glutathione content with suppressing lipid peroxides products (TBARS)[21]. Pearl powder significantly prolongs the lifespan of *Caenorhabditis elegans* owing to antioxidant activity[22]**.** Mother of pearl (nacre) of Pinctada maxima species was implanted into rat dermis and demonstrated that it fostered better skin tone while stimulating proper physiological functioning in skin fibroblast, having promising potential for skin regeneration[23]. Besides nacre, pearl extract was also demonstrated to be able to promote fibroblast migration in an in- vitro wound healing model by human fibroblast cells. *Li-Chen Li et al* demonstrated that Pearl extract (300 µg/mL) increased migrating fibroblast cell numbers by three times relative to a control without Pearl extract[24]. Physiologically, the integrity & permeability of cell membrane is regulated mainly by Calcium (Ca) which is abundantly present in the Mukta Bhasma. So, this fact may support in the prevention of cell damage when CCl_4 was administered. Additional to Ca, Iron (Fe) is also present in MB which serves as a cofactor for the enzyme Catalase which detoxifies Hydrogen peroxide by dismutation to water & oxygen. By the present study, Mukta Bhasma having antioxidant property mainly acts by inhibiting free radical mediated cell damage process. In the group treated with CCl_4, we noted massive centrilobular necrosis with hepatocyte degeneration and mild mononuclear cell infiltration. The livers of rats co-treated with Mukta bhasma showed minimal hepatocellular necrosis and maintained lobular architecture and hepatocyte structure with no evidence of inflammatory cell infiltration or fatty change. MB 200mg showed mild inflammation, mild Centrilobular degeneration & moderate overall protection when compared to CCl_4 group.

7. CONCLUSION

The present study demonstrates that Mukta bhasma 200mg has shown significant ($p<0.001$) attenuation in the LPO level, increase in CAT and GSH representing significant antioxidant activity of Mukta bhasma. The mode of action of Mukta bhasma in hepatoprotection and antioxidant activity against CCl_4 may be due to cell membrane stabilization, hepatic cell regeneration and activation of antioxidant enzymes such as CAT and GSH. Administration of MB 200mg has shown significant reduction in the biochemical parameters like SGOT, SGPT, ALP, Bilirubin, Total cholesterol and Total triglycerides with significance $p<0.001$ when compared to CCl_4 group. Histopathologically, MB 200mg showed mild inflammation, mild Centrilobular degeneration & moderate overall protection when compared to CCl_4 group. This study can be further taken up in network pharmacology and molecular docking, double blind study for the dose recommendation and clinical study to determine the efficacy of the drug. Also the same study can be conducted in comparison with Mukta pishti.

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9. AUTHOR CONTRIBUTION STATEMENT

Dr. Poornima carried out, analyzed and drafted the research work. Dr.Prashant Jadar designed the research. Dr. Vishwanath Wasedar helped in critical analysis, provided valuable inputs towards discussion. All authors read and approved the final version of manuscript.

10. CONFLICT OF INTEREST

Conflict of interest declared none.

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