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Tamra Bhasma preparation by two methods and evaluating their effect on the Liver Function test pre-clinically

Research Article

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Abstract

Rasashastra is an important branch of Indian System of Medicine, which deals with the pharmaceutical processes, such as preparations of *Bhasmas, Pistis, Kharaliya Rasayanas, Druti, Kupipakva Rasayanas, Parpati, Pottali*. It is a well-known fact in the Ayurveda world that *Bhasmas* are highly efficacious and there are so many preparations of *Tamra* that are indicated for different ailments. Medicines (*kalpas*) mentioned in *Rasa shastra* are used freely in clinical practice by Ayurveda physicians after trial and testing. Now Ayurveda is accepted by world. Due to globalization, it is necessary to prove the Ayurveda drugs with modern parameters. So it can be utilized worldwide to get health. Hence the work taken here is preparation of *Tamra Bhasma* by two different methods and evaluating their effect on the Liver Function test pre-clinically. *Tamra Bhasma* preparation by two methods was chosen to compare the difference between direct heat and indirect heat method. Preparation with indirect heat by using sunlight method and with direct heat method resulted into *bhasma*. Biochemical and Histopathological changes in Liver were observed. Liver function assessment parameters namely serum Bilirubin, SGOT, SGPT, total protein were taken for biochemical evaluation. Also histopathology of Liver and Kidney was taken for study in support of biochemistry. The results were compared with standard drug Silymarine.

Key Words: *Shodhan of Tamra, Parad, Gandhak, Preparation of Kajjali, tamra Bhasma,* Liver Function Test.

Introduction

All branches of sciences are evolved to minimize pain and all *pravriti* of human being are for the happiness. (2) Ayurveda, the immortal science is practiced in the Asian Subcontinent since Vedic period, have given vital importance to the *Rasashastra*. This science is superior and It is useful to cure diseases as well as it helps to achieve *Moksha*. (3)

The main cause of choosing the *Tamra* and its efficacy on Liver Function Tests is that copper is quite well-known for action on liver. Different types of *puta's* are used to prepare *Bhasma* like *Suryaputa, Chandraputa, Agniputa*. These were the methods representing use of different energy sources for medicine preparation in ancient times. *Tamra Bhasma* preparation by two methods was chosen to compare the difference between direct heat and indirect heat method. Preparation with indirect heat by using sunlight method and with direct heat method resulted into *bhasma*.

The liver is one of the heaviest organs of the body (1.2 to 1.5 kg) and serves the principle function of maintaining the body's internal homeostasis. As almost all absorption of foreign material into the body takes place in the gut and the portal blood draining the gut flows to the liver, which subsequently controls the release of absorbed nutrients into the systemic circulation. The liver is able to store and release a variety of substrates, vitamins and minerals, and plays a crucial role in drug and bilirubin metabolism. The liver is also the largest reticulo-endothelial organ in the body and its situation is important on removing infecting bacteria and bacterial products which often enter the body from the gut. (4)

According to W.H.O. statistics regarding major diseases, prevalence of liver diseases in India is 56 per 1000 while that over the world is 42 per 1000.

- The mortality statistics of cirrhosis of liver show that, only 25 % of patients survive 5 years from diagnosis.
- Alcohol abuse causes 200,000 deaths annually, the fifth leading cause of death, approximately 40 % of deaths from cirrhosis are attributed to alcohol induced liver disease. (5)

Thus the above reasons are quite enough to prove the gravity of Liver diseases in India and the need for research in this field.

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Aims and objectives

- To prepare *Aputi Tamra Bhasma* as per the reference in *Ras ratnakar* without any use of direct heat. (6)
- To prepare *Puti Ttamra Bhasma* as per the reference in *Ras tarangini* by *lavana yantra* method. (7)
- To compare the physico-chemical properties of the same so that standard parameters for particular processes will be found and the difference in the values can be observed.
- Evaluating their effect on the liver function test to see weather both the drugs prepared have any improving action on damaged liver thereby restoring normal liver functions.

Materials and Methods

The entire study is divided into two sub titles as follows,

- Preparation of *Tamra Bhasma* by two different methods.
- Evaluating their effect on liver function test pre-clinically.

Preparation of Tamra Bhasma

Step 1 - *Shodhan of Tamra-*

A. *Samanya shodhana* as per the reference from *Rasaratnasamucchaya* 5 /13. (8)

Tamra patra taken for *shodhan*	460 gm	*Til Tel*	2 litres
Tamra patra after *shodhan* with *til tel*	445 gm	*Takra*	2 litres
Tamra patra after *shodhan* with *takra*	430 gm	*Gomutra*	2 litres
Tamra patra after *shodhan* with gomutra	410 gm	*Kanji*	2 litres
Tamra patra after *shodhan* with *kanji*	355 gm	*Kulattha kwatha*	2 litres
Obtained *Tamra* after *samanya shodhan*	345 gm		
Duration of experiment	10 days		
Method:	heating to red hot and quenching into said liquid		

B. *Vishesh shodhana* as per the reference from *Rasaratnasamucchaya* 5 /51 (9)

Weight of fresh *Nirgundi* leaves	6 kg
Water taken	4 lit
Duration of the experiment	02 days

Method: heating to red hot and quenching into said liquid

Step 2 - *Shodhan of Parada-* as per reference *Rasatarangini* 5/27-29. (10)

Amount of *Parad* initially taken	**500 gm**
Lime powder	500 gm
Amount of *Parad* obtained	**480 gm**
Rasona kalka	**480 gm**
Saindhav powder	240 gm
Obtained *Parad*	465 gm
Duration of experiment	04 days

Method: dry trituration with lime powder for 3 days and with *Rasona Kalka* and *Saindhav* for 1 day in *Khalwa Yantra.*

Step 3 - *Shodhan of Gandhak* – as per reference from *Ayurved Prakash* 2 / 21-24. (11)

***Gandhak* taken initially**	500 gm
Ghrita	**500 gm**
Milk	2 litres
Obtained amount	**465 gm**
Duration of experiment	01 days

Method: Melting *Gandhak* in *Ghrita* and pouring it into Milk.

Step 4 -Preparation of *Kajjali-* as per reference of *Rasratnasamucchaya* (12)

Amount of *Parad* taken	**465 gm**
Amount of *Gandhak* taken	**465 gm**
Time of trituration	8 Hrs
***Kajjali* obtained**	930 gms
Duration of experiment	01 day

Method: dry trituration of *Parad* and *Gandhak* in Khalwa Yantra till desired test comes.

Step 5 - Preparation of *Aputi Tamra Bhasma-* As per reference from Rasratnakar Grahani Chikitsa (13)

Weight of *Tamra Patra* taken initially	50 gm
***Kajjali* taken**	50 gm
***Nimbu swaras* needed**	1560 ml
***Tamra Bhasma* obtained**	55 gm
Duration of experiment	37 days

Method: *Tamra Patra* & *Kajjali* kept alternately in earthen pot, *nimbu swaras* added till conversion into *bhasma* form.

Step 6 - Preparation of *Puti Tamra Bhasma -* As per the reference from *Rastarangini* 17/32-33. (14)

In this preparation two steps are followed viz. *Lavan Yantra Pak* and *Putapak.*

Lavan Yantra Pak -

Weight of *Tamra Patra* taken initially	40 gm
***Kajjali* taken**	40 gm
***Kumari Swaras* taken**	80 ml
***Tamra Bhasma* obtained**	73 gm
Amount of *Lavana*	10 kg
Duration of experiment	12 hrs

Method: paste of *kajjali* and *kumari swaras* applied on *tamra patra* and subjected to *Lavan Yantra pak* for 12 hrs.

***Putpak* –**

Obtained *Tamra Bhasma*	50 gm
Total *Kajjali* needed	238 gm (5 *putas)*
Total *Kumari swaras* required	476 ml (5 *putas)*
***Tamra Bhasma* obtained**	43.5 gm
Duration of experiment	30 days (5 *putas)*

Method: trituration of obtained *Tamra Bhasma* with *kajjali* and *Kumari Swaras* and *putpak* 5 times.

Analytical tests

After the preparation of *Aputi Tamra Bhasma* and *Puti Tamra Bhasma*, both the drugs were sent for their physico-chemical analysis to Accurate Analytical Laboratory, Pune and following results were obtained.

Ash values were carried out by ignition and crucible method. Percentage of Mercury, Sulphur and Copper were carried out by Quantitative Volumetric titration method.

Test	*Aputi Tamra Bhasma*	*Puti Tamra Bhasma*
Total Ash (%)	46.3811	77.0242
Acid-soluble Ash (%)	1.4009	1.1908
Water-soluble Ash (%)	43.5005	69.8879
Mercury (%)	2.1049	0.003168
Sulphur (%)	7.4962	16.9293
Copper (%)	85.1347	51.5968
Solubility in 0.1 N HCl (%)	24.9415	59.9558
Solubility in Hexane (%)	2.0563	0.8220
Solubility in Ether (%)	Nil	2.6019

Animal Trial

The pre-clinical study for evaluating the effect of *Aputi Tamra Bhasma* and *Puti Tamra Bhasma* was carried out in the Animal laboratory of National Toxicology Centre, Pune. The rats were procured from the same laboratory.

- Species used – Wistar albino Rats
- Number of animals – 30
- Daily diet – 20 gm of rat chow
- Pattern – 12 hours' light and 12 hours' dark
- Weight – 120-140 gm 30 healthy lab animals (18 males and 12 female) were taken initially for the experiment.
- The rats were divided into 5 groups of 6 animals each as follows,

Group A – Control group of completely healthy animals.

Group B – Animals given carbon tetrachloride without any treatment.

Group C – Animals given carbon tetrachloride and treated with *Aputi Tamra Bhasma*.

Group D – Animals given carbon tetrachloride and treated with *Puti Tamra Bhasma*.

Group E – Animals given carbon tetrachloride and treated with standard drug Silymarine.

- Male and female rats were kept in different cages.
- During the experimental period all the animals were kept on their normal diet of daily rat chow.
- The IP injection of Carbon Tetrachloride in a dose of 0.7 ml/kg was given to the 24 animals for the induction of liver damage except control group of 6 animals.
- Practically the solution of carbon tetrachloride was prepared by adding equal amount of corn oil in 1:1 proportion.
- The mixture was mixed with the help of vortex mixture so as to obtain homogeneity in the solution. This solution was used for dosing.
- This dose of Carbon tetrachloride was given daily for first 6 days to induce toxicity in liver.
- After 6th day carbon tetrachloride was injected after every 72 hours until 21 days to maintain the toxicity in liver.
- Group C received *Aputi Tamra Bhasma* and group D received *Puti Tamra Bhasma* simultaneously along with carbon tetrachloride for a period of 21 days.
- Group E received Standard Hepatoprotective drug, Sylimarine in a dose of 100 mg/kg.
- The animals of group B, C, D, and E were bled and their Serum Glutamate Piruvate Transaminase(SGPT) values were recorded on day 7, 14 and 21.
- On day 22 all the animals were evaluated for biochemical liver function test parameters viz. serum bilirubin, total protein, SGOT and SGPT.
- On the same day animals were sacrificed and all organs preserved. After fixation of tissue Liver and Kidney of 2 animals from each group were send for the Histopathology and reports obtained were interpreted.

Calculation of drug dose

The dose to be administered in animals was calculated according to the reference of S.O.P. for drug trial of *Rasa bhasmas* and *kalpas* in rats. (15)

Accordingly, dose was calculated by the formula as,

For *Aputi Tamra Bhasma*:

Dose in Rat = Human Dose X Conversion Factor
= 240 mg X 2 X 0.018
= 8.64 mg/200 gm of rat
= 42.20 mg/kg (for 10 ml)

For *Puti Tamra Bhasma*: -

Dose in Rat = Human Dose X Conversion Factor
= 60 X 2 X 0.018
= 2.16 mg/200 gm of rat
= 10.80 mg/kg (for 10 ml)

Vehicle to be used – Honey and deionized water was used as a vehicle for the administration. It was prepared by adding 2 parts of Honey to 3 parts of water and vortex the mixture thoroughly.

Observation and Result

1. Observations during Preparation of *Tamra Bhasma*.
2. Observations during preclinical evaluation of their effect on the Liver function test.

Observations during Preparation of *Tamra Bhasma*: -

This part is divided into fractions as per procedure followed sequentially as under.

Purification of *Parada*

- *Parad* and *sudha churna* never mixed up with each other thoroughly.
- The color of *sudha churna* turned to dusky white or whitish gray after proper trituration.
- 4 % *Parada* was lost during separation procedure from *sudha churna*.
- When process was completed *rasona kalka* was converted into a dark black color.
- By washing with luke-warm water, *lasuna paste* and *saindhav* dissolved in water which made it easier to separate the *parad*.
- The shine of *parad* increased considerably after *shodhan*.
- Collection of *parad* from the *khalwa yantra* was done carefully to avoid any spillage (*Parada Gati*).

Purification of *Gandhak*

- Heat was maintained on a low flame.
- *Gandhak* was completely melted in *ghrita*.
- Milk was at room temperature.
- Cloth was tied on the pot, so it should not touch the milk.
- Repeated wash was done to remove *ghrita* and milk totally from *gandhak* after *shodhan*.

Preparation of *Kajjali*

- Color of *Gandhak churna* slowly started converting from yellow to ash color.
- Gradually *Parad* also lose its liquid nature and completely assimilated with *gandhak*.
- Mercurial luster was disappeared after completion of process.
- *Nischandra pariksha* was done by taking small amount of *kajjali* on palm and mixed it with few drops of water. It was checked in sunlight; no shining was found in it.

Purification of *Tamra*

- *Tamra patra* were converted to dark red hot color by heating them.
- Hissing noise was produced whenever the *patra* was merged into the liquids.
- Excess oil deposited on *tamra patra* can got fire on heating.
- *Tamra patra* turned from metallic red to dark black color.
- Deposited *Takra* (butter milk) converts *tamra patra* in shiny blue like the neck of peacock next day.
- *Tamra patra* become brittle after completion of the process of *shodhan*.

Preparation of *Aputi Tamra Bhasma*

- In early stage, the absorbing capacity of the content was more as they were in dry state which has got decreased on further addition of *Nimbu swaras*.
- When the *Nimbu swaras* dried totally on first day, hardness of *tamra patra* was reduced and on touching with pin it become easily breakable.
- Later on further addition of the *swaras, tamra patra* gradually converted into powder form.
- *Tamra patra* were totally converted into powder form after addition of 1200 ml of *Nimbu swaras*.
- To achieve *nishkalanka pariksha nimbu swaras* needed was 1560 ml.
- After the total conversion of *Tamra patra* into powder form the dried content subjected to dry trituration.
- On triturating the powder, moisture was released so it was again kept in sunlight for total drying.
- After obtaining total dry powder, it has achieved the *sukshmatva, varitaratwa* and *nishkalanka pariksha* also.
- *Tamra patra* were totally converted into powder form in 16 days. The powder was totally dried in 21 days. *Aputi tamra bhasma* was prepared totally in 37 days.

Preparation of *Puti Tamra Bhasma*

When *Puti Tamra Bhasma* was removed after each *puta*, three *bhasma parikshas* were done i.e. *Rekhapurnatva, Varitaratva and Nishkalank pariksha* which showed the following observations. (16)

***Puta* no.**	***Rekhapurnatva***	***Varitaratva***	***Nishkalankatva***
1	-	-	-
2	-	-	-
3	+	-	-
4	+	+	-
5	+	+	+

Observations during preclinical evaluation of their effect on the Liver function test

- Initially when the rats were dosed with Carbon tetrachloride, they have shown significant weight loss in comparison with normal healthy rats in first six days.
- But afterwards as the drugs were also been given along with Carbon tetrachloride, the loss of weight got decreased in drug dosed groups.
- The appetite of animals has also got decreased in the Carbon tetrachloride treated four groups as compared to normal healthy group.
- The value of Serum Glutamate Pyruvate Transaminase(SGPT) has been checked on day 7, 14, 22 to confirm the liver damage in only Carbon tetrachloride treated rats.
- It has also helped to compare the efficacy of both the drugs and that with the standard drug after every 7 days.

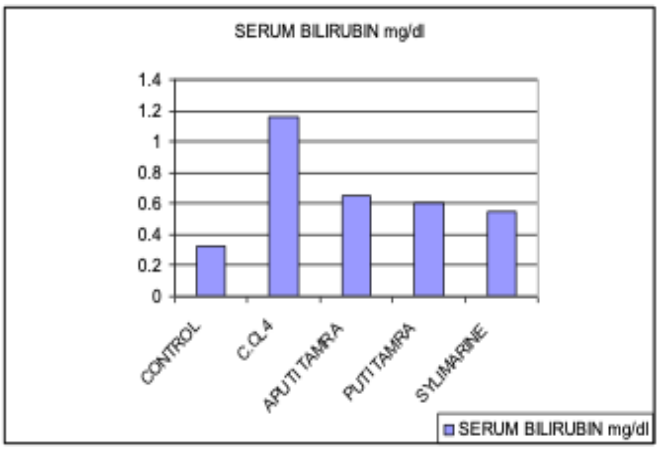
The total parameters of Liver function tests were checked on the 22nd day which gave the values depicted in the tables given below. The values in following table are Mean ± SE.

Group	Serum Bilirubin (mg/dl)	Total Protein (g/dl)	SGOT (U/L)	SGPT (U/L)
Control	0.32 ± 0.069	6.18 ± 0.208	36.6 ± 3.271	48.83 ± 3.68
$C.Cl_4$	1.16 ± 0.297	5.05 ± 0.388	79.8 ± 9.147	104.6 ± 18.15
Aputi Tamra	0.65 ± 0.111	3.98 ± 0.437	50.4 ± 4.874	144.2 ± 28.3
Puti Tamra	0.6 ± 0.228	3.86 ± 0.665	56.8 ± 5.3	117.8 ± 22.73
Silymarine	0.55 ± 0.104	4.57 ± 0.736	45.25 ± 3.567	88 ± 15.02

1. Bilirubin values:

Group A animals - 0.32 mg/dl.
Group B animals - 1.16 mg/dl which is raised due to Carbon tetrachloride.
Group C animals - 0.65 mg/dl
Group D animals - 0.60 mg/dl
Group E animals - 0.55 mg/dl.

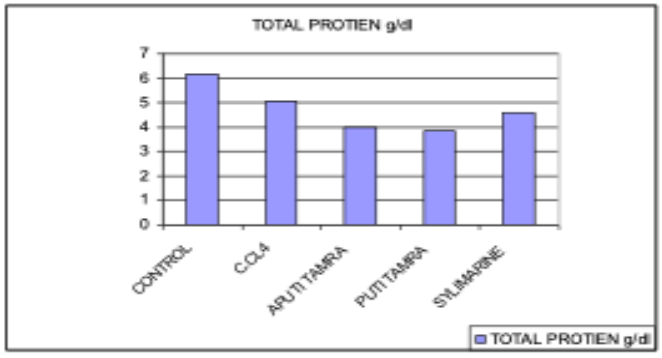
Hence both the drugs have significantly prevented the bilirubin rise as in Carbon tetrachloride treated group. For bilirubin values *Puti Tamra bhasma* is more effective than *Aputi Tamra Bhasma*. The effect is also comparable with standard drug (silymarine) which is as follows.



2. Total protein values:

Group A animals - 6.18 g/dl.
Group B animals - 5.05 g/dl which is decreased due to Carbon tetrachloride.
Group C animals - 3.98 g/dl
Group D animals - 3.86 g/dl
Group E animals - 4.57 g/dl

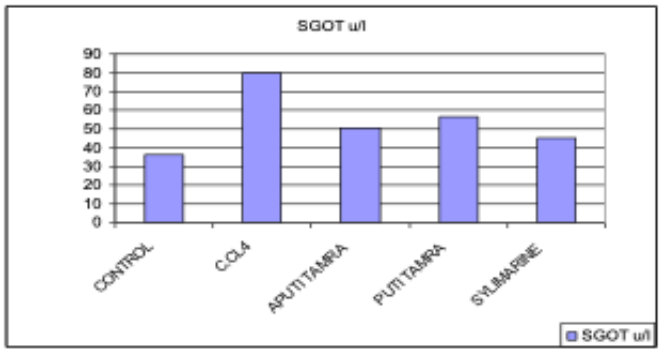
Thus only standard drug (silymarine) has prevented the decrease in the protein due to Carbon tetrachloride. Both the drugs have no much effect on protein depletion.



3. SGOT:

Group Wise mean SGOT values are as follows,
Group A animals - 36.60 U/L
Group B animals - 79.80 U/L which is raised due to Carbon tetrachloride.
Group C animals - 50.40 U/L
Group D animals - 56.80 U/L
Group E animals - 45.25 U/L

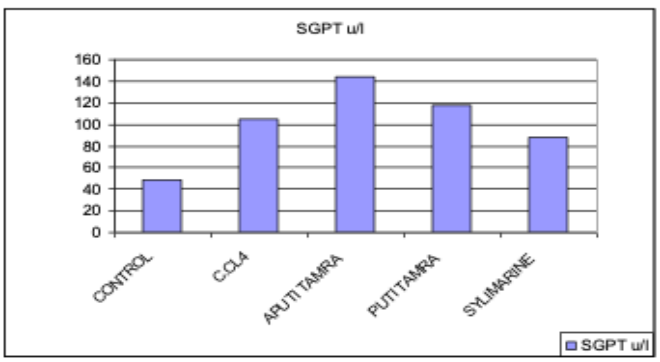
So in spite treated with Carbon tetrachloride, both the drugs have significantly prevented elevation in SGOT.



4. SGPT:

Group Wise mean SGPT values are as follows,
Group A animals - 48.83 U/L
Group B animals - 104.6 U/L which is raised.
Group C animals - 144.2 U/L
Group D animals - 117.8 U/L
Group E animals - 88 U/L

Thus only standard drug has prevented rise in SGPT value but both the drugs does not have any action in preventing SGPT rise.



Aputi Tamra Bhasma gives a better result as compared to *Puti Tamra Bhasma*. The results were also comparable with the standard drug (silymarine).

Histological changes in Liver and Kidney

Along with the biochemistry histopathology of liver and kidney was done. Organ samples of two animals from each group were send for the histopathology and following reports were obtained.

Animal NO.	Liver	Kidney
Control - 1	NAD	NAD
Control - 2	NAD	NAD
C.Cl4– 1	Micronodular cirrhosis with thin septae, large foamy histiocytes are seen scattered in the lobules.	NAD
C.Cl4– 2	Large foamy histiocytes are seen scattered in the lobules esp around portal tracts, No cirrhosis.	NAD
Aputi tamra – 1	Early micronodular cirrhosis with thin septae, large foamy histiocytes are seen scattered in the lobules with few apoptotic bodies.	NAD
Aputi tamra – 2	Large foamy histiocytes are seen scattered in the lobules esp. around portal tracts (less number) with lobular MNC infiltration and occasional apoptotic body.	NAD
Puti tamra – 1	Micronodular cirrhosis with thin septae, large foamy histiocytes are seen scattered in the lobules. Lobular parenchyma shows focal necrosis.	NAD
Puti tamra – 2	Lobular foamy cells, periportal and centrilobular areas show necrosis, lobular MNC infiltration, microvesicular steatosis and bridging fibrosis.	NAD
Silymarine - 1	Foamy cells are seen around portal tracts and around bridging fibrosis, periportal areas of necrosis.	NAD
Silymarine - 2	Foamy cells are seen around bridging fibrosis and portal tracts with evidence of early cirrhosis.	NAD

NAD: No abnormality detected.
MNC: Mononuclear cells.

Disscusion

Aputi Tamra Bhasma

The *Vaisheshika* mentioned Light rays are taken to be a stream of high velocity of *tejas (fire)* atoms. (17) Alhacen held light rays to be streams of minute energy particles. (18)

In the preparation of *Aputi Tamra Bhasma*, copper, the metal undergoes the endothermic reaction with *Kajjali* and *Nimbu swaras* in presence of sunlight to convert itself into *Bhasma* (an oxide form). This *Bhasma* shows all the positive signs as per Ayurveda text.

To find out the probable action of both types of *Tamra Bhasma*, it is necessary to know the biochemistry of copper.

Copper is present in 2 key enzymes of aerobic metabolism. (19) One is Cytochrome C Oxidase, which is responsible for the major part (90%) of the oxygen consumed by the life on this planet. And the other is Cytosolic Superoxide dismutase which catalytically scavenges the toxic free radical superoxide ion (O_2^-) generated during aerobic metabolism. The copper was found to be largely associated with the albumin fraction of the plasma immediately after its ingestion.

A decline in plasma radioactivity then occurs as copper attached to albumin is distributed to cuproproteins in the Liver and elsewhere. A secondary rise in plasma radioactivity then take place as copper incorporated into Liver ceruloplasmin is released into the blood.

Since copper in the plasma is largely bound to protein, it is not readily excreted in the urine. Most of it is excreted via the bile into the intestine & expelled with feces. (20)

Some Hepatoprotective mechanisms

To know the probable mechanism of action of *Tamra bhasma* as a hepatoprotective drug, some known recent hepatoprotective mechanisms are discussed below.

- Inhibition of Kupffer cell activation with gadolinium prevented hepatic lipid peroxidation and histopathology produced by carbon tetrachloride (Muriel et al. 2001). (21)
- Rats fed a diet mildly deficient in zinc showed elevated levels of hepatocyte injury, as assessed by serum sorbitol dehydrogenase activity (DiSilvestro and Carlson 1994). (22)
- Dietary zinc supplementation was associated with decreases in lipid peroxidation, collagen deposition, and proline hydroxylase activity, together with an increase in collagenase activity (Camps et al. 1992). (23)
- Decreased tissue levels of antioxidants such as glutathione influence the degree to which oxidative damage progresses following exposure to carbon tetrachloride. (24)
- Exogenous administration of SAM (S adenosyl methionine) or cysteine reduced carbon tetrachloride-induced liver injury by the increase in glutathione levels (Gasso et al. 1996). (25)
- Rats fed a low-copper diet were reported to be more sensitive to hepatic plasma membrane injury 24 hours following an intraperitoneal injection of carbon tetrachloride, possibly due to reduced Cu-Zn superoxide dismutase activities (DiSilvestro and Medeiros 1992). (26)

Thus both *Aputi Tamra Bhasma* and *Puti Tamra Bhasma* contains copper as major content which is an ingredient of enzyme Superoxide dismutase which removes the toxic free radicals from the body. As carbon tetrachloride induced Liver damage is a type of free radical injury, therefore both drugs are effective.

Antioxidant activity of copper is already proved and this action is due to the Glutathione. (27) Glutathione is one of the powerful antioxidant so it is useful in Carbon

tetrachloride induced liver damage. This is the second probable mode of action of both drugs due to Glutathione.

Sulfur is one of the content of amino acid cysteine and methionine. (28) It is also the content of tri-peptide Glutathione which plays a role as a chemical reduction potential in the cell. % of sulfur is more in *Puti Tamra Bhasma* than *Aputi Tamra Bhasma*, so *Puti Tamra Bhasma* is more effective than *Aputi Tamra bhasma*.

Conclusion

Animal Trial

- *Aputi* and *Puti Tamra Bhasma* have action on *Yakrit Vikar*.
- *Yakrit vikar* can be compared with Liver Damage related with altered Liver Function Tests.
- *Puti Tamra Bhasma* is drug for protecting the liver against chemical injuries. Effect of *Aputi Tamra Bhasma* is in early stage (effective in first week of treatment).
- Both drugs are used in elevated Bilirubin and elevated SGOT levels. As per results, *Puti Tamra Bhasma* is more effective in Bilirubin and *Aputi Tamra Bhasma* in SGOT.
- Histopathology of Liver for both the drugs is not much effective, but one of the animals from *Aputi Tamra Bhasma* group has shown minimal Liver damage.
- Both the drugs are safer as far as altered Liver Function is concerned because no physiological or adverse behavioral change was seen in the laboratory animals and histopathological reports of Kidney for both the drugs have shown normal echo texture.

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