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Anti-Oxidant effects of *Swarnamakshika Bhasma* : A Experimental Study

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ABSTRACT

Shodhana of *Swarnamakshika* carried out by *Bharjana* in *Eranda Tila*. *Marana* of *Swarnamakshika* by finely powdered *Shudda Swarnamakshika* was taken in a *Khalvayantra*. Then equal quantity of *Shudda Gandhaka* was added and triturated together till they become homogenous. To this mixture 100ml of *Jambhira Rasa* was added triturated well till it becomes semisolid consistency. The paste were made into shape of *Chakrikas* weighing 25gm and 8cm uniformly and kept for drying. Subjecting into 5 required number of *Varahaputa*s. The present day lifestyle and food habits have increased the production of free radicals. These cytotoxic free radicals not only raise the oxidative stress but also play an important role in the immune-system dysfunction due to which the mankind is prone to various major ailments and it is now proved that diseases like *Prameha*, *Pandu*, *Vatavyadhi* etc. are free radical mediated ones. To tackle these free radicals our body needs antioxidants. An antioxidant is a molecule which is capable of inhibiting the oxidation of other molecules. Oxidation reactions can produce free radicals which in turn start chain reactions that damage cells. Antioxidants terminate these chain reactions by removing free radical intermediates and inhibit other oxidation reactions. Many herbals drugs and compound herbal preparations have been screened for their antioxidant and immuno-modulatory properties but still there is a need for effective antioxidants. This dearth and also the fact that *Swarnamakshika* is being used in treating many of the free radical mediated diseases prompted us to take the present study which aims to validate the Antioxident effect of *Swarnamakshika Bhasma* scientifically and explain its probable mode of action at the cellular level.

Key words: *Makshika, Shodhana, Marana, Amritikarana, Immunomodulatory.*

INTRODUCTION

Free radicals and other reactive oxygen species are derived either from normal essential metabolic processes in the human body (endogenous) or from external sources (exogenous). Free radical formation occurs continuously in the cells as a consequence of both enzymatic and non-enzymatic reactions. Enzymatic reactions which serve as sources of free radicals include those involved in the respiratory chain, in phagocytosis, in prostaglandin synthesis and in the cytochrome P450 system. Free radicals also arise in non-enzymatic reactions of oxygen with organic compounds as well as those initiated by ionizing radiations. An immuno-modulator is a substance which has an effect on the immune system.

Rasayana, listed as a class in the texts of traditional Ayurvedic medicine literature, consists of a number of drugs reputed to promote physical and mental health, improve the ojus and defense mechanisms of the body and enhance longevity.

In Ayurveda particularly *Rasayana* is recommended for Immune system. Many herbs, minerals, metals, gem stones and some poisonous drugs also having property of *Rasayana*. In my studies proved that *Swarnamakshika* is having anti-oxidant property effect, Keeping this in mind this a experimental work is started.

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Materials and Methods

Pharmaceutical study of *Swarna Makshika Bhasma* is designed as below

- Collection of raw drugs.
- *Swarna Makshika Shodhana*.
- Preparation of *Swarna Makshika Bhasmas* by using *Varaha Puta*.

Collection of raw drugs

The Raw *Swarna Makshika* was collected from (Hinustan Copper Ltd.) Khetri Copper Complex Khetrinagar, Rajastan. It was having all *Grahya Lakshanas*[1] told in the classics. The Raw drug was confirmed by Quantitative analysis for Copper, Iron and Sulphur using Atomic Absorption Spectrophotometer study.

Swarna Makshika Shodhana[2]

Powder of *Swarna Makshika* was taken in an iron vessel. 1000ml of *Eranda Taila* was added to it and mixed properly. Then this iron vessel was kept on a gas stove for heating *Agni* is in *Tivragni*. The mixture was then continuously stirred with the help of a small *Darvi* throughout the procedure. Heating was stopped when the bottom of an iron vessel attained red color and all sulphur fumes stoped. Then the mixture was washed thoroughly with the hot water for two times and kept spread for drying on a clean cloth.

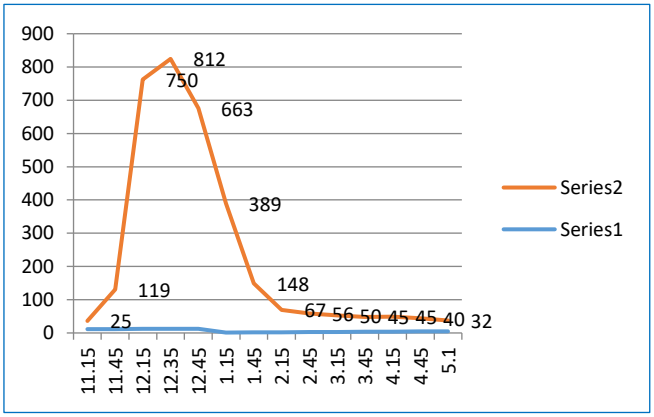
Table 1: Showing the colour change of *Swarna Makshika Churna* during *Bharjana*.

Before *Shodhana*	Warm gray
After addition of *Eranda Taila*	Light gray
After 5 min addition of *ErandaTaila*	Greenish gray
After 15 min of heating	Blackish gray
After 40 min of heating	Blackish gray red color

Preparation of *Swarna Makshika Bhasmas* by using *Varaha Puta*[3]

- Finely powdered *Shudda Swarna Makshika* was taken in a *Khalva Yantra.*
- Then equal quantity of *Shudda Gandhaka* was added and triturated together till they become homogenous.
- To this mixture 100ml of *Nimbu Swarasa* was added triturated well till it becomes semisolid consistency.
- The paste were made into shape of *Chakrikas* weighing 25gm and 8cm uniformly and kept for drying.
- In first *Sharava* the *Swarna Makshika Chakrikas* were arranged separately leaving little space to avoid overlapping.
- Over this *Sharava*, another *Sharava* was placed invertedly. Facing mouth of two *Sharavas* in contact, without leaving any space at the juncture.
- *Sandhi Bhandana* was done with the help of a cloth smeared with *Multani Mitti.*
- Seven Layers of *Multani Mitti* were applied, allowed to dry.
- Completely dried *Samputa* was subjected to *Varaha Puta*.
- Measurement of *Varaha Puta*: 16" x 16" x16" *Angulas* (lxbxd)
- *Vanopalas* used: 12½ kgs

Graph 1: Showing the time & temp. during *Varaha Puta*.



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Amritikarana of *Swarnamakshika*[4]

Swarna Makshika Bhasma with equal quantity of *Panchamrita* is taken in a *Lohapatra* and this mixture is covered with *Sharava* and subjected to *Paka*, till it becomes *Nirdhuma*. After *Swangasheeta* this mixture is collected and subjected to *Bhavana* with *Triphala Kwatha, Chakrikas* are prepared and subjected to five *Varahaputa*s.

Experimental Study

Albino Wister rats weighing 150-200g of either sex were 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 tolaboratory conditions for 48 hours prior to experimental protocol to minimize if any of the non-specific stress.

Screening of Free Radical Scavenging Activity

Materials Required

- Drugs: *Swarna Makshika Bhasma* and Vitamin C.
- Rat liver homogenate
- Glasswares: Beakers, Test tubes, Pipettes (1 ml, 5 ml and 10 ml) and Stirrers.
- Equipment: Micropipettes, Incubator, Centrifuge, Vortex mixture, Shimadzu UV spectrophotometer 1700 and Water bath.

Method in General

- Healthy rat liver was taken.
- Liver homogenate was prepared.
- Oxidative stress was induced in the homogenate by CCl_4.
- Drug was added to homogenate and the parameters were assessed.
- One day, two days and four days study was done.

A. Lipid Peroxidation[5]

End products of Lipid peroxidation have a property that they react with Thiobarbituric acid and produce colour. Malondialdehyde (MDA) is one of the end products of lipid peroxidation. By measuring the colour produced by photometric method we can assess the concentration of Malondialdehyde (MDA) that gives the extent of Lipid peroxidation.

The color produced by the reaction of thiobarbituric acid with Malondialdehyde (MDA) was measured at 540 nm with the help of spectrophotometer. The results were expressed as nmol/ml.

Preparation of Chemical Reagents

1. 20% Trichloroacetic acid: 20 gm of TCA was dissolved in 100 ml of distilled water and kept in a bottle.
2. 0.05 N Sulphuric acids : 1.23ml of conc. H_2SO_4 was diluted with 250 ml of Distilled water and kept in bottle.
3. 2 molar Sodium Sulphate : 28.4gm of unhydrous sodium sulphate was dissolved in 90ml of distilled water by heating and stirring. Then volume was made up to 100ml with distilled water.
4. Thiobarbituric acid reagent : 0.67gm (670mg) of TBA was dissolved in 2 molar sodium sulphate solution by heating and the volume was made upto 100 ml, kept in brown colored bottle.

Preparation of 30% Rat liver homogenate in 0.15M KCl[6]

Animals

Healthy adult albino rats (150-200 gm) of either sex were used for the study. They were kept in polypropylene cages at 25 ± 2°C, with relative humidity 45-55% under 12 hr light and dark cycles. All the animals were acclimatized to the laboratory conditions for a week before use. They were fed with standard animal feed and water.

- Rats were kept for fasting for 16-18 hours before sacrificing.

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- Rats were sacrificed by the method of decapitation.
- Liver removed immediately and rinsed with cold water.
- Then it was perfused with ice cold normal saline to remove the excess blood as for as possible.
- Liver were chopped into small pieces and weighed.
- 15 gm of liver was mixed with 50 ml of 0.15M KCl and homogenized with *Remi*homogeniser.
- This homogenate was centrifuged at 6000 rpm for 10 minutes filtered and stored in conical flask.

1. Preparation of *Swarna Makshika Bhasma* suspension

- Compound powder of *Tragakanth* was used as suspending agent. Preparation of compound powder of Tragakanth[7]: 7.5gm of *Tragakanth*, 10 gm each of gum acasia and soluble starch and 22.5 gm of finely powdered sucrose were taken and mixed well.
- Method of preparation of *Swarna Makshika Bhasma* (SMB) suspension: 1%, 2% and 5% suspensions of *Swarna Makshika Bhasma* were prepared with Compound Powder of *Tragacanth* (CPT). 1% *Swarna Makshika Bhasma* suspension was prepared by adding 1gm of *Swarna Makshika Bhasma* with 2gm compound powder of tragacanth into 100ml of distilled water (D.W.). 2% *Swarna Makshika Bhasma* suspension was prepared by adding 2gm of *Swarna Makshika Bhasma* with 4gm of compound powder of tragacanth into 100 ml of distilled water. 5% *Swarna Makshika Bhasma* suspension was prepared by adding 5gm of *Swarna Makshika Bhasma* with 10gm of compound powder of Tragacanth into 100ml of distilled water. Tablet of Vitamin C weighing 550 mg dissolved in 100 ml of distilled water and 1ml was used. The same suspension was used in testing all the four parameters.

Table 2: *Swarna Makshika Bhasma* suspensions

Ingredients	1% Suspension of SMB	2% Suspension of SMB	5%Suspension of SMB
Swarna Makshika Bhasma	1 gm	2 gm	5 gm
Compound Powder of Tragacanth	2 gm	4 gm	10 gm
Distilled water	100 ml	100 ml	100

Estimation of Lipid Peroxidation

In 6 conical flasks of 25ml quantity, 4ml of liver homogenate was taken. All the test tubes were added with 6ml of Potassium sulphate buffer (pH 7.4) and 8ml of 0.15M Potassium chloride solutions. Test sample was excluded in first two flasks (control groups). In test group, three different conical flasks were added with 1ml of three different concentrations of drug like 1%, 2% and 5%. In standard group, one ml of Vitamin-C was added. Finally 60 µl of carbon tetra chloride (CCl_4) was added except second control. Totally six flasks were incubated at 37°C in incubator.

Table 3: Groups with induction of oxidative stress and administration of drug

Groups	Flask	Contents	
Control	1	Homogenate without CCl_4	Without drug
	2	Homogenate with CCl_4	Without drug
Standard	3	Homogenate with CCl_4	Vit C
Test	4	Homogenate with CCl_4	1% drug suspension

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	5	Homogenate with CCl_4	2% drug suspension
	6	Homogenate with CCl_4	5% drug suspension

Lipid peroxidation was assessed on 1st day (after 45min) as follows: The reaction mixture (0.5ml) which was kept for incubation was taken in a test tube and 4 ml of 10% Trichloroacetic acid (TCA) was added to it. Contents were then centrifuged at 4000 rpm for 10 minutes. Then 2ml of clear supernatant fluid was taken in a graduated tube. 2 ml of 0.67% Thiobarbituric acid (TBA) was then added and heated on water bath for 15 minutes. Then the tube was allowed to cool and pH was adjusted to 12-12.5 and the colour which developed was stabilized. Absorbance was measured at 540 nm in an UV spectrophotometer.

In 2 days and 4 days study addition of 1 ml of the different test drug suspensions and the standard drug were done to the homogenate mixture daily and the estimation of LPO was done as above at the end of 2 days and 4 days.

B. Superoxide Dismutase (Sod)[8]

This method is based on the ability of SOD to inhibit autoxidation of pyrogallol under specific conditions. Superoxide dismutase is an enzyme which inhibits oxidation. Pyrogallol is autooxidative substance which gets oxidized when exposed to atmosphere. When pyrogallol is kept with homogenate it starts getting oxidized. But the SOD present in homogenate inhibits oxidation of pyrogallol. So by calculating the extent of inhibition of auto-oxidation of pyrogallol we can assess the concentration of SOD. Reading was taken at 420 nm and expressed as units/ml.

i. Preparation of Chemical Reagents

1) 0.25M Sucrose solution: 4.278 gm Sucrose dissolved in 50 ml distilled water gives 0.25M sucrose solution.
2) 50 mMTris buffer: 0.605gm of TrisHcland 0.037 gmTris base dissolved in 100 ml distilled water.
3) 20 mMpyrogallol: 25.2mg pyrogalloldissolved in 10ml distilled water gives 20mM solution of pyrogallol. This was used freshly prepared.

ii. Preparation of 10 % Rat liver homogenate in 0.25M sucrose solution

Rat liver homogenate was prepared in the same way as previously prepared in lipid peroxidation test. But this time only 2 gm of liver was mixed with 20 ml of 0.25M sucrose solution and homogenized with Remihomogeniser.

iii. Estimation of SOD

As done in previous test, in 6 conical flasks, 2ml of Rat liver homogenate prepared in 0.25M sucrose solution was added with 60 µl of CCl_4 except control I. Control II was added with only CCl_4 without any test drugs or standard drug suspension. In test group, 1ml suspensions of different drug concentration (1%, 2% and 5%) were added. In standard group vitamin-C was added. The flasks were then kept for incubation at 37°C.

SOD was estimated on 1st day as follows: 50µl of reaction mixture from the homogenate which is kept for incubation was taken in a test tube. To it, 2.8 ml of Tris buffer solution was added. Just before checking its optical density, reaction mixture was added with freshly prepared 0.1 ml of 20mMpyrogallol. Immediately after addition of pyrogallol O.D. was seen using U.V. spectrophotometer at 420nm at an interval of 1.5 min and 3 min. ΔA obtained for control (C) and Test (T).

Table 4: Reaction mixture content in SOD test

SN	Contents	Test (T)	Control (C)
1.	Tris buffer	2.8 ml	2.8 ml
2.	Homogenate	50µl	-
3.	Pyrogallol	0.1 ml	0.1 ml

SOD was calculated by formula (C-T/Cx50) × 2000

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In 2 days and 4 days study addition of 1 ml of the different test drug suspensions and the standard drug were done to the homogenate mixture daily and the estimation of SOD was done as above at the end of 2 days and 4 days.

C. Reduced Glutathione (Gsh)[9]

Glutathione protects cells from the free radicals produced through oxidation. It can only do this by remaining in its naturally reduced state so that it is readily available to neutralize free radicals by bonding with them. As GSH bonds, it converts to its oxidized form, called glutathione disulfide. Then an enzyme-glutathione reductase-reverse it back to its reduced state. The ratio of reduced GSH to oxidized GSH within the cells can be used to measure cellular toxicity.

i. Preparation of Chemical Reagents

1. EDTA solution: readily available.
2. 0.3 M disodium hydrogen phosphate: 10.68 gm of disodium hydrogen phosphate dissolved in 200 ml distilled water gives 0.3 M solution.
3. 0.001% DTNB solution: 5mg of DTNB dissolved in 50 ml distilled water.

   (DTNB = 5, 5'-dithiobis (2-nitrobenzoate))
4. Precipitating buffer: 1.67gm of metaphosphoric acid and 0.2 gm of EDTA disodium salt dissolved in 1litre of 30%NaCl solution.
5. 0.15M trisHCl solution: 908 mg of TrisHCl dissolved in 50 ml distilled water gives 0.15 M TrisHCl.

ii. Preparation of 10 % Rat liver homogenate in 0.15M trisHCl solution:

Rat liver homogenate was prepared in the same way as previously done for lipid peroxidation test. But this time only 2gm of liver was mixed with 20ml of 0.15M trisHClsolution and homogenized with Remihomogeniser.

iii. Estimation of Glutathione

- As done in previous test, in 6 conical flasks, 2ml of Rat liver homogenate prepared in 0.15M trisHCl solution was added with 60µl of CCl_4 except control I. Control II was added with only CCl_4 without any test drugs or standard drug. In test group, 1 ml suspension of different drug concentration (1%, 2% and 5%) was added. In standard group, vitamin-C was added. Flasks were then kept under incubation at 37˚C.
- On 1st day 0.2ml of homogenate which was incubated was taken and mixed with 1.8ml of EDTA. To this, 3ml of precipitating reagent was added and mixed thoroughly with Vortex mixer and centrifuged for 15 min at 2800 rpm. 2ml of Supernatant was taken and added with 4ml of 0.3M disodium hydrogen phosphate solution and 1ml DTNB reagent. Then absorbance was read at 412 nm in UV spectrophotometer.
- In 2 days and 4 days study addition of 1 ml of the different test drug suspensions and the standard drug were done to the homogenate mixture daily and the estimation of GSH was done as above at the end of 2 days and 4 days.

D. Catalase (Cat)[10]

The enzyme catalase reacts with Hydrogen peroxide and converts it in to H_2O and O_2. In our study, liver homogenate was added with fresh solution of Hydrogen peroxide and the amount of Hydrogen peroxide utilized by the homogenate was calculated. By this we calculated the concentration of CAT present in the homogenate.

i. Preparation of Chemical Reagents

1. Phosphate buffer:60mM and pH 7.4

   Soln A: 2.04 gm of KH_2PO_4 dissolved in 250 ml distilled water.

   SolnB: 5.34 gm of Na_2HPO_4 dissolved in 500 ml distilled water.

   Soln A and Soln B were mixed in the ratio of 1:18 and pH adjusted to 7.4.
2. This forms the Phosphate buffer solution.
3. Hydrogen peroxide: 0.3 ml of 30% H_2O_2 dissolved in 100 ml of distilled water.

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4. 0.15M KCl: 3.354 gm of KCl dissolved in 300 ml of distilled water.

ii. Preparation of 30% Rat liver homogenate in 0.15M KCl

Rat liver homogenate was prepared in the same way as previously done for lipid peroxidation test.

iii. Estimation of Catalase

- As done in previous test, in 6 conical flasks, 2ml of Rat liver homogenate prepared in 0.15M KCl solution was added with 60µl of CCl_4 except control I. Control II was added with only CCl_4 without any test drug or standard drug. In test group, 1 ml suspension of different drug concentration (1%, 2% and 5%) was added. In standard group, vitamin C was added. Then they were kept under incubation at 37˚C.
- On 1st day estimation, 10µl of reaction mixture from the homogenate which was kept for incubation was taken in a test tube and 5ml of Phosphate buffer solution was added. Just before checking its optical density, reaction mixture was added with freshly prepared Hydrogen peroxide solution. Immediately after addition of H_2O_2, O.D. was seen at 240 nm with the help of digital U.V. spectrophotometer from 0 to 60 min.
- Concentration of CAT was calculated with the formula: $\Delta A \times 4225.35$
- In 2 days and 4 days study addition of 1 ml of the different test drug suspensions and the standard drug were done to the homogenate mixture daily and the estimation of CAT was done as above at the end of 2 days and 4 days.

Total Protein Content of Homogenates

Total protein content of homogenates was estimated by Biurete method.

- Homogenate for LPO: 6.0 gm
- Homogenate for SOD: 5.8 gm
- Homogenate for GSH: 5.3 gm
- Homogenate for CAT: 7.5 gm

Antioxidant Activity Results

Estimation of Lipid Peroxidation

Table 5: Results of LPO

SN	Groups	Concentration of MDA in nmol/ml		
		1 day	2 days	4 days
1.	Control	20.69	28.62	31.25
2.	Homogenate with CCl_4	35.26	37.25	39.52
3.	Standard with CCl_4	17.56	11.29	10.74
4.	1% SMB with CCl_4	30.24	28.35	27.35
5.	2%SMB with CCl_4	21.25	11.58	10.07
6.	5%SMB with CCl_4	23.87	24.56	18.02

Maximum decrease in the concentration of MDA was observed with the treated with 2% in 2 days and 4 days study, where as 5% was also found to be significant in 4 days study as compared to normal control group. Noted that comparatively 2% Suspension was effective than 5% Suspension, this indicates 2% was sufficient to prevent lipid peroxidation.

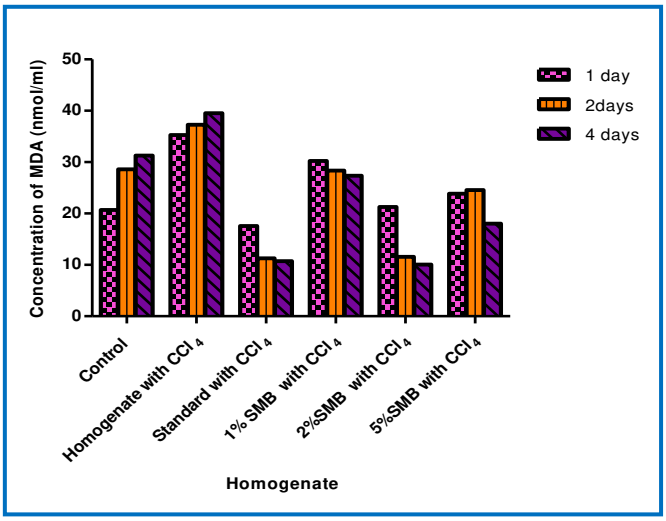

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Table 6: Estimation of Superoxide Dismutase (Sod)

SN	Groups	Concentration of SOD (units/ml)		
		1 day	2 days	4 days
1.	Control	24.17	25.36	25.47
2.	Homogenate with CCl_4	12.45	11.26	9.35
3.	Standard with CCl_4	22.35	23.25	22.12
4.	1% SMB with CCl_4	11.24	16.25	21.24
5.	2% SMB with CCl_4	15.25	23.45	22.14
6.	5%SMB with CCl_4	14.25	23.89	22.13

SOD concentration was significantly increased with 2% and 5% suspension treated group in 2 days and 4 days as compared to normal control group. No activity was observed in 1 day study at the concentrations studied.

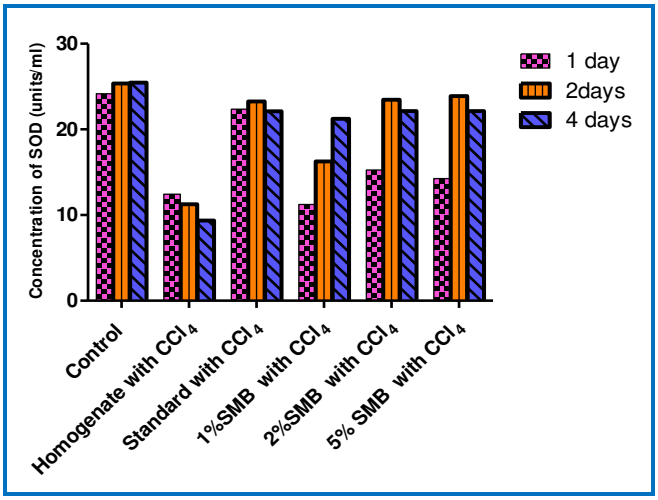

Table 7: Estimation of Reduced Glutathione (Gsh)

SN	Groups	Concentration of GSH (Units/mg protein)		
		1 day	2 days	4 days
1.	Control	5.21	5.14	4.28
2.	Homogenate with CCl_4	10.24	10.68	6.25
3.	Standard with CCl_4	4.23	3.25	3.24
4.	1% SMB with CCl_4	7.22	6.24	3.98
5.	2% SMB with CCl_4	5.24	3.45	3.02
6.	5% SMB with CCl_4	6.21	4.25	3.95

Significant decrease of elevated GSH levels was observed with all the concentrations studied in 1 day, 2 days and 4 days study.

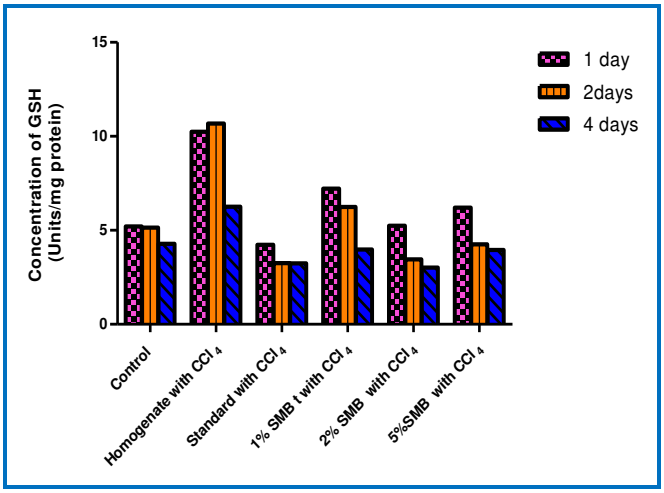

Table 8: Estimation of Catalase

SN	Groups	Catalase (Units/mg protein)		
		1 day	2 days	4 days
1.	Control	109.859	301.056	258.80
2.	Homogenate with CCl_4	88.732	67.60	115.14
3.	Standard with CCl_4	135.436	132.25	154.24
4.	1% SMB with CCl_4	69.718	135.31	219.71
5.	2% SMB with	140.23	131.28	142.36

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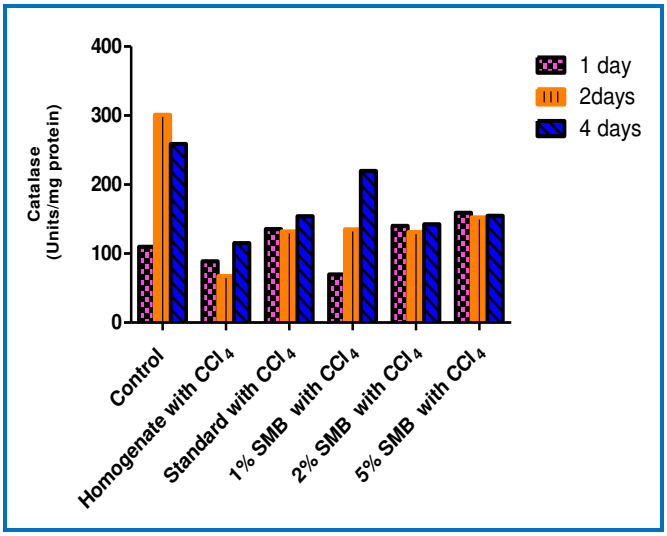
	CCl_4			
6.	5% SMB with CCl_4	159.01	152.36	154.89

Maximum decomposition of H_2O_2 was observed with the groups treated with 1% and 5% suspension in 4 days study as compared to normal control group.



Discussion

It was tested in three different doses against CCl_4 induced liver toxicity for free radical scavenging activity. In favor of antioxidant study, parameters like LPO, SOD, CAT, & GSH were estimated. MDA is the major oxidant result of polly unsaturated fatty acid & its increase is an important indicator of lipid peroxidation induced tissue damage due to failure of antioxidant defense mechanism.[11] Treatment with *Swarna Makshika Bhasma* significantly reversed the lipid peroxidation indicating antioxidant potential of *Swarna Makshika Bhasma*. Super oxide dismutase is one of the enzymatic antioxidant, high activity found in liver. SOD concentration was significantly increased with 2% and 5% suspension treated group in 2 days and 4 days as compared to normal control group. No activity was observed in 1 day study at the concentrations studied. Superoxide radical Swarna Makshika 200mg increased the level of SOD significantly which was near to the Vit-C value. Catalase is enzymatic antioxidant high activity found in liver. It decay hydrogen peroxide & protects the tissues from highly reactive hydroxyl radicals. Hence, reduction in the activity of CAT may result in harmful effects due to absorption of superoxide radical & hydrogen peroxide. *Swarna Makshika Bhasma* 200mg increased the level of CAT significantly which was near to the Vit-C value. Glutathione is a non-enzymatic activity, 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. 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 *Swarna Makshika Bhasma* 200mg/kg showed increased GSH level significantly which was near to Vit-C value.

Conclusion

Shodhana by *Bharjana* process in *Eranda Taila*, *Bhavana* with *Jambhira Swarasa* & *Marana* by 12 *Varaha Puta* were sufficient to obtain *Swarna Makshika Bhasma* which passed all the *Bhasma Parikshas*. Reduction in the MDA level, increase in CAT and GSH represents good antioxidant activity of *Swarna Makshika Bhasma* at the dose of 200mg/kg. To inhibit lipid peroxidation, *Swarna Makshika Bhasma* 200mg/kg was found to be statistically significant when compared to CCl4 group. In LPO, CAT, SOD & GSH also, *Swarna Makshika Bhasma* 200mg/kg showed statistically significant results when compared to CCl4 group.

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