IJPSR (2018), Volume 9, Issue 8 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 28 November, 2017; received in revised form, 18 June, 2018; accepted, 29 June, 2018; published 01 August, 2018

FREE RADICAL SCAVANGING ACTIVITY OF RASASINDURA (RED SULPHIDE OF MERCURY)

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Keywords:

Rasasindura, Free radicals, Anti-oxidant parameters

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ABSTRACT: The main aim of the study was to scientifically validate the free radical scavenging activity of classically prepared Rasasindura (Red sulphide of mercury). With this intention Rasasindura (Red sulphide of mercury) was subjected to the screening of free radical scavenging activity in rat's liver homogenate with four parameters like lipid peroxidation (LPO), super oxide dismutase (SOD), catalase (CAT) and reduced glutathiosone (GSH). In lipid peroxidation assay maximum decrease in concentration of MDA was seen in 1% of Rasasindura suspension in 1st, 2nd and 4th day of study. There was no significant difference when control and test sample compared with standard drug, this shows that the action of standard and test samples were same in concentration of SOD and GSH. 1% and 5% of Rasasindura has shown significant decomposition of H_2O_2 in 4th and 2nd day respectively when compared to standard group. There was no significant difference when control and test compared with standard drug. This shows that the action of standard and test drugs were same in decrease of elevated GSH level with all the concentrations.

INTRODUCTION: The immune system will create free radicals in order to fight off disease. So not all free radicals are bad for mankind. It becomes a problem when the amount of free radicals is greater than the body can handle. As a result they can do some serious damage to our body. Other than the normal chemical reactions that occur in the body, there are a few environmental factors like smoking, air pollutants, radiation, industrial chemicals and processed foods which increase free radical production.

QUICK RESPONSE CODE

DOI:
10.13040/IJPSR.0975-8232.9(8).3360-66

Article can be accessed online on:
www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.9(8).3360-66

Due to which the mankind is prone to various major ailments like Prameha (diabetes), Pandu (anemia), Vata vyadhi (neurological disorders), Arbuda (tumour) *etc*. Following defects that are common to every degenerative disease. These are pH imbalance-primarily acidemia, anaerobic tendency, free calcium excess, chronic inflammation, connective tissue break down and oxidative stress.

To tackle these free radicals, our body needs antioxidants. Antioxidants terminate the chain reactions of oxidation by removing free radical intermediates and inhibit other oxidation reactions. Immuno modulation is a process which alters the immune system of organism by interfering with its function either by immune stimulation or immune suppression. Modulation of immune responses to alleviate the diseases has been of interest for many years and the concept of 'Rasayana' is based on

related principles. Rasayana, listed as a class in the texts of traditional Indian medicine literature, consists of a number of drugs reputed to promote physical and mental health, improve defence mechanisms of the body and enhance longevity. In Ayurveda particularly Rasayana is recommended for increase life span (indirectly keeping immune system in good condition) and cure disease.

Ayurveda Pharmacopeia prescribes Rasasindura as an effective medicine for various diseases like Prameha (diabetes), Pandu (anemia), Vata vyadhi (neurological disorders), Arbuda (tumour), Bhagandara (fistula in ano), Rajayakshma (tuberculosis), Kushtha (lephrosy), Mahajwara (fever), Gulma *etc*. Rasasindura is Deha-Bala-Virya-vardhaka and Rasayana. It alleviates Vatadi Dosha, increases longevity and vitality. The Rasasindura is being used diseases like Prameha (diabetis), Rajayakshma (tuberculosis), Jwara (fever), Kushtha (leprosy) *etc.* provoked us to take the present study which aims to validate the free radical scavenging activity of Rasasindura (Red sulphide of mercury) scientifically and explain its probable mode of action at the cellular levels.

MATERIALS AND METHODS:

Preparation of Rasasindura: [1] Authenticated raw drugs were procured from K. L. E. Ayurveda Pharmacy Khasbag, Belgaum, and Karnataka. Parada (mercury) and Gandhaka (sulphur) were subjected to Shodhana (purification) procedure according to Rasatarangini. Then equal quantity of Shodhita Parada (processed mercury) and Gandhaka was triturated for 79 h to get Kajjali, to this 200 ml of Vatankuraswarasa (juice of banyan leaf bud) was added and Mardana (trituration) was done till the evaporation of liquid *i.e* about 5 and 1/2 h. This Samyak Bhavita Kajjali (properly processed black sulphide of mercury) was dried in shade and was filled in kachakupi (glass bottle) which was rapped with 7 layers of kapadmitti (rags and mud) and kept in valukayantra (sand contained iron vessel), heat was given in mridu (100-250 ºC), madhyam (250 - 450 ºC) and teevragni (450-600ºC). The whole procedure was completed in 21 hours. After swangasheetala (self cooling) kupi (glass bottle) was taken out from the Valukayantra (sand contained iron vessel). The mud smeared cloth layers of the kupi (glass bottle) were scrapped out with a knife. A jute thread dipped in kerosene was tied to the kupi (glass bottle) below the level of sublimated product and ignited, broken. Rasasindura was collected.

Materials required for the assessment of free radical scavenging activity:

- Drugs: Rasasindura (Red sulphide of mercury) and ascorbic acid.
- Rat liver homogenate
- Glass wares: Beakers, test tubes, pipettes (1 ml, 5 ml and 10 ml) and stirrers.
- Equipment: Homogenizer, Micropipettes, Incubator, Centrifuge, Vortex mixture, Shimadzu UV spectrophotometer 1700 and Water bath.

Lipid Peroxidation: [2]

Principle: End products of lipid peroxidation have a property that they react with thiobarbituric acid and produce colour. By measuring the colour produced by photometric method we can assess the concentration of MDA that gives the extent of lipid peroxidation. The colour produced by the reaction of thiobarbituric acid with MDA was measured at 540 nm with the help of spectrophotometer. The results were expressed as nmol/ml.

Procedure:

Preparation of 30% Rat Liver Homogenate in 0.15M KCl: [3]

Animals: IAEC Reg.No1017/C/06CPCSEA dated 19/12/2006. Healthy adult Albino rats (150 - 200 gm) of female sex were used for the study. They were kept in polypropylene cages at 25 ± 2 °C, with relative humidity 45-55% under 12 h 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 h before sacrificing.
- Rats were sacrificed by the method of decapitation.
- Liver removed immediately and rinsed with cold water.
- Then it was perfuse 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 homogeiser.
- This homogenate was filtered and stored in conical flask.

Preparation of Rasasindura (Red Sulphide of Mercury) Suspension: Compound powder of Tragakanth was used as suspending agent.

Preparation of Compound Powder of Tragakanth: [4] Tragakanth (7.5gm), gum acacia (10gm), soluble starch (10gm) and finely powdered sucrose (22.5gm) were taken and mixed well.

Method of Preparation of Rasasindura (Red Sulphide of Mercury) Suspension: 1%, 2% and 5% suspensions were prepared with compound powder of tragacanth (CPT). Rasasindura (Red sulphide of mercury) suspension of 1%, 2% and 5% was prepared by adding 1, 2 and 5 gm of Rasasindura (Red sulphide of mercury) with 2, 4 and 10 gm compound powder of tragacanth into 100 ml of distilled water (D.W.) respectively. 550 mg of ascorbic acid was dissolved in 100 ml of distilled water and 1 ml was used. The same suspension was used in testing all the four parameters.

TABLE 1: RASASINDURA (RED SULPHIDE OF MERCURY) SUSPENSIONS

Ingredients	1% suspension of R.S	2% suspension of R.S	5% suspension of R.S
R.S	1 gm	2 gm	5 gm
CPT	2 gm	4 gm	10 gm
D.W.	100 ml	100 ml	100 ml

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

Lipid peroxidation was assessed on 1st day (after 45 min) as follows: The reaction mixture (0.5 ml) 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 min. Then 2 ml of clear supernatant fluid was taken in a graduated tube and 2 ml of 0.67% thiobarbituric acid (TBA) was then added and heated on water bath for 15 min. 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.

TABLE 2: 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	Ascorbic acid
Test	4	Homogenate with CCl_4	1% drug suspension
	5	Homogenate with CCl_4	2% drug suspension
	6	Homogenate with CCl_4	5% drug suspension

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.

Superoxide Dismutase (SOD): [5]

Principle: This method is based on the ability of SOD to inhibit auto oxidation of pyrogallol under specific conditions. Superoxide dismutase is an enzyme which inhibits oxidation. Pyrogallol is auto oxidative 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.

Procedure:

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 Remi homogeniser.

Estimation of SOD: As done in previous test, in 6 conical flasks, 2 ml 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, 1 ml suspensions of different drug concentration (1%, 2% and 5%) were added. In standard group Ascorbic acid was added. The flasks were then kept for incubation at 37 ºC. SOD was estimated on 1st day as follows: 50 µl 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 20mM pyrogallol. Immediately after addition of pyrogallol O.D. was seen using U.V. spectro-photometer at 420 nm at an interval of 2 min and 3 min. ΔA obtained for control (C) and test (T). SOD was calculated by formula (C-T/C × 50) × 2000.

TABLE 3: REACTION MIXTURE CONTENT IN SOD TEST

S. no.	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

In 2nd and 4th day 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.

Reduced Glutathione (GSH): [6]

Principle: 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 reverses it back to its reduced state. The ratio of reduced GSH to oxidized GSH within the cells can be used to measure cellular toxicity.

Preparation of 10% Rat Liver Homogenate in 0.15M Tris HCl Solution: Rat liver homogenate was prepared in the same way as previously done for lipid peroxidation test. But this time only 2 gm of liver was mixed with 20 ml of 0.15M tris HCl solution and homogenized with Remi homogenizer.

Estimation of Glutathione: As done in previous test, in 6 conical flasks, 2 ml of rat liver homogenate prepared in 0.15M tris HCl 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. In test group, 1 ml suspension of different drug concentration (1%, 2% and 5%) was added. In standard group, ascorbic acid was added. Flasks were then kept under incubation at 37 ºC.

On 1st day 0.2 ml of homogenate which was incubated was taken and mixed with 1.8 ml of EDTA. To this, 3 ml of precipitating reagent was added and mixed thoroughly with Vortex mixer and centrifuged for 15 min at 2800 rpm. 2 ml of supernatant was taken and added with 4 ml of 0.3M disodium hydrogen phosphate solution and 1 ml DTNB reagent. Then absorbance was read at 412 nm in UV spectrophotometer.

In 2nd and 4th day 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 2nd and 4th day.

Catalase (CAT): [7]

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

Procedure:

Preparation of 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.

Estimation of Catalase: As done in previous test, in 6 conical flasks, 2 ml 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, ascorbic acid was added. Then they were kept under incubation at 37 ºC.

On 1st day estimation, 10µl of reaction mixture from the homogenate which was kept for incubation was taken in a test tube and 5 ml 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 day.

OBSERVATION AND RESULTS:

Estimation of Lipid Peroxidation:

TABLE 4: ESTIMATION OF LIPID PEROXIDATION

S. no.	Sample	Concentration of MDA in nmol/ml		
		1st day	2nd day	4th day
1	Control	24.59	32.17	26.57
2	Homogenate with CCl_4	37.17	39.02	41.42
3	Standard with CCl_4	19.36	16.58	10.24
4	1% Test drug with CCl_4	32.14	30.24	26.35
5	2% Test drug with CCl_4	25.35	21.47	18.25
6	5% Test drug with CCl_4	26.87	22.07	19.08

TABLE 5: COMPARISON OF DOSES OF RASASINDURA WITH DIFFERENT SAMPLES

S. no.	Different parameters	1st day		2nd day		4th day	
		P value	Significant?	P value	Significant?	P value	Significant?
1	Control *vs.* Standard	0.2296	NS	0.0001	Yes***	0.0002	Yes***
2	Control *vs.* 1% R.S.	0.0452	Yes*	0.5540	NS	0.8817	NS
3	Control *vs.* 2% R.S.	0.8005	NS	0.0060	Yes**	0.0567	NS
4	Control *vs.* 5% R.S.	0.6089	NS	0.6089	NS	0.0911	NS
5	Standard *vs.* 1% R.S.	0.0017	Yes**	0.0010	Yes**	0.0004	Yes***
6	Standard *vs.* 2% R.S.	0.1469	NS	0.2512	NS	0.0625	NS
7	Standard *vs.* 5% R.S.	0.0884	NS	0.1687	NS	0.0376	Yes*
8	1% R.S. *vs.* 2% R.S.	0.0785	NS	0.0280	Yes*	0.0765	NS
9	1% R.S. *vs.* 5% R.S.	0.1316	NS	0.0484	Yes*	0.1203	NS
10	2% R.S. *vs.* 5% R.S.	0.7954	NS	0.8170	NS	0.8244	NS

p<0.01,*p<0.001. Data were analyzed by Chi - Square test. 1% of Rasasindura has shown significant (p<0.01) decrease in the concentration of MDA on 1st, 2nd day and highly significant (***p<0.001) result on 5th day compared to standard group.

Estimation of Superoxide Dismutase (SOD):

TABLE 6: ESTIMATION OF SUPEROXIDE DISMUTASE (SOD)

S. no.	Sample	Concentration of SOD (units/ml)		
		1st day	2nd day	4th day
1	Control	26.32	28.35	29.36
2	Homogenate with CCl_4	15.23	13.25	10.28
3	Standard with CCl_4	24.58	26.02	25.15
4	1% Test drug with CCl_4	19.36	21.25	23.02
5	2% Test drug with CCl_4	22.35	24.35	24.86
6	5% Test drug with CCl_4	18.26	19.01	19.35

TABLE 7: COMPARISON OF DOSES OF RASASINDURA WITH DIFFERENT SAMPLES

S. no.	Different parameters	1st day		2nd day		4th day	
		P value	Significant?	P value	Significant?	P value	Significant?
1	Control *vs* Standard	0.8203	NS	0.7529	NS	0.7246	NS
2	Control *vs* 1% R.S.	0.2832	NS	0.8937	NS	0.6222	NS
3	Control *vs* 2% R.S.	0.6127	NS	0.7475	NS	0.7952	NS
4	Control *vs* 5% R.S.	0.1865	NS	0.2282	NS	0.5166	NS
5	Standard *vs* 1% R.S.	0.3889	NS	0.3556	NS	0.7495	NS
6	Standard *vs* 2% R.S.	0.7784	NS	0.9890	NS	0.9313	NS
7	Standard *vs* 5% R.S.	0.2631	NS	0.7234	NS	0.8847	NS
8	1% R.S. *vs* 2% R.S.	0.5541	NS	0.7377	NS	0.8198	NS
9	1% R.S. *vs* 5% R.S.	0.7785	NS	0.5695	NS	0.8577	NS
10	2% R.S. *vs* 5% R.S.	0.3900	NS	0.3685	NS	0.6921	NS

Data were analyzed by Chi – Square test. There was no significant difference when control and test compared with standard group. This shows that the action of standard and test drugs were same in increasing level of SOD concentration with all the concentrations.

Estimation of Reduced Glutathione (GSH):

TABLE 8: ESTIMATION OF REDUCED GLUTATHIONE (GSH)

S. no.	Sample	Concentration of GSH (Units/mg protein)		
		1st day	2nd day	4th day
1	Control	9.21	9.14	8.28
2	Homogenate with CCl_4	12.45	12.86	12.74
3	Standard with CCl_4	5.45	4.53	3.26
4	1% Test drug with CCl_4	9.36	7.25	6.14
5	2% Test drug with CCl_4	6.35	5.24	2.02
6	5% Test drug with CCl_4	7.25	6.89	4.21

TABLE 9: COMPARISON OF DOSES OF RASASINDURA WITH DIFFERENT SAMPLES

S. no.	Different parameters	1st day		2nd day		4th day	
		P value	Significant?	P value	Significant?	P value	Significant?
1	Control *vs* Standard	0.0816	NS	0.0405	Yes*	0.0405	Yes*
2	Control *vs* 1% R.S.	0.7482	NS	0.3865	NS	0.4076	NS
3	Control Vs 2% R.S.	0.8990	NS	0.0977	NS	0.0130	Yes*
4	Control *vs* 5% R.S.	0.5706	NS	0.3036	NS	0.1025	NS
5	Standard *vs* 1% R.S.	0.0977	NS	0.2191	NS	0.2059	NS
6	Standard *vs* 2% R.S.	0.6820	NS	0.6733	NS	0.6152	NS
7	Standard *vs* 5% R.S.	0.4142	NS	0.3053	NS	0.6534	NS
8	1% R.S. *vs* 2% R.S.	0.2059	NS	0.4142	NS	0.0833	NS
9	1% R.S. *vs* 5% R.S.	0.3865	NS	0.8548	NS	0.4076	NS
10	2% R.S. *vs* 5% R.S.	0.6820	NS	0.5368	NS	0.3458	NS

Data were analyzed by Chi - Square test. There was no significant difference when control and test compared with standard drug. This shows that the action of standard and test drugs were same in decrease of elevated GSH level with all the concentrations.

Estimation of Catalase:

TABLE 10: ESTIMATION OF CATALASE

S. no.	Groups	Catalase (Units/mg protein)		
		1st day	2nd day	4th day
1	Control	129.85	258.06	305.80
2	Homogenate with CCl_4	108.73	98.56	98.61
3	Standard with CCl_4	165.436	172.25	144.24
4	1% Test drug with CCl_4	178.718	155.21	236.71
5	2% Test drug with CCl_4	160.23	151.28	162.36
6	5% Test drug with CCl_4	169.01	117.56	170.25

TABLE 11: COMPARISON OF DOSES OF RASASINDURA WITH DIFFERENT SAMPLES

S. no.	Different parameters	1st day		2nd day		4th day	
		P value	Significant?	P value	Significant?	P value	Significant?
1	Control *vs* Standard	0.0082	Yes**	0.0300	Yes*	< 0.0001	Yes****
2	Control *vs* 1% R.S.	0.0013	Yes**	0.0038	Yes**	0.2870	NS
3	Control *vs* 2% R.S.	0.0172	Yes*	0.0020	Yes**	0.0013	Yes**
4	Control *vs* 5% R.S.	0.0046	Yes**	< 0.0001	Yes****	0.0036	Yes**
5	Standard *vs* 1% R.S.	0.5263	NS	0.4415	NS	0.0017	Yes**
6	Standard *vs* 2% R.S.	0.7785	NS	0.3261	NS	0.3334	NS
7	Standard *vs* 5% R.S.	0.8345	NS	0.0005	Yes****	-45.63	NS
8	1% R.S. *vs* 2% R.S.	0.3611	NS	0.8304	NS	0.0255	Yes*
9	1% R.S. *vs* 5% R.S.	0.6706	NS	0.0055	Yes**	0.0573	NS
10	2% R.S. *vs* 5% R.S.	0.6242	NS	0.0098	Yes**	0.7278	NS

p<0.01,**p<0.0005. Data were analyzed by Chi – Square test. 1% and 5% of Rasasindura has shown significant (p<0.01), (****p<0.0005) decomposition of H_2O_2 in 4th and 2nd day respectively when compared to standard group.

DISCUSSION:

Carbon Tetra Chloride: CCl_4 was used to produce oxidative stress in homogenate. CCl_4 is hepatotoxic due to its ability to destruct liver cells through free radical mechanisms. These free radicals induce the oxidative decomposition of the lipids and organic peroxides after reacting with oxygen (lipid peroxidation), which causes damage to plasma membrane that increases permeability to Ca^{2+}, Na^{+} and water which causes cell swelling and ultimately leading to tissue injury.

Ascorbic Acid: Ascorbic acid is naturally occurring antioxidant agent. The ascorbate ion is the predominant species at typical biological pH values. It is a mild reducing agent also. It is oxidized with loss of one electron to form a radical cat ion and then with loss of a second electron to form dehydro ascorbic acid. It typically reacts with oxidants of the reactive oxygen species, such as the hydroxyl radical. Such radicals are damaging to animals and plants at the molecular level due to their possible interaction with nucleic acids, proteins, and lipids. Sometimes these radicals initiate chain reactions. Ascorbate can terminate these chain radical reactions by electron transfer.

Methodology and Results: Malondialdehyde (MDA) is the major oxidant product and its elevation is an important indicator of lipid peroxidation induced tissue damage due to failure of antioxidant defense mechanism. Maximum decrease in the concentration of MDA was observed with the homogenate treated with 1% of Rasasindura in 1^{st}, 2^{nd} and 4^{th} day study as compared to standard group.

Superoxide dismutase is an enzyme which inhibits oxidation. Increase in the concentration shows the reduced cell damage. There was no significant difference when Control and test compared with standard group. This shows that the action of standard and test drugs were same in increasing level of SOD concentration with all the concentrations. Glutathione is a non-enzymatic, highly conc. intracellular antioxidant present in liver. Reduction in GSH triggers the process of apoptosis. There was no significant difference when control and test compared with standard drug. This shows that the action of standard and test drugs were same in decrease of elevated GSH level with all the concentrations. Catalase is one of the enzymatic antioxidant, high activities found in liver. It decomposes hydrogen peroxide and protects the tissues from highly reactive hydroxyl radicals. 1% and 5% of Rasasindura has shown significant decomposition of H_2O_2 in 4^{th} and 2^{nd} day respectively when compared to standard group.

CONCLUSION: Reduction in the malondial-dehyde level (MDA), increase in catalase (CAT) and reduced glutathione (GSH), concentration of superoxide dismutase (SOD) represents a free radical scavenging activity of Rasasindura in different proportion *i.e*. 1, 2 and 5%.

ACKNOWLEDGEMENT: I am extremely thankful to Dr. B. S. Prasad, Principal, KAHER'S Shri B.M.K. Ayurved Mahavidyalaya, Shahapur, Belagavi who provided continuous encouragement and all the necessary facilities for this study. I would like to thank Dr. Prasanna Habbu and Smt. Smitha Department Pharmacognocy SET College of Pharmacy, Dharawad for their help and support in experimental study.

CONFLICT OF INTEREST: Nil

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How to cite this article:
Kupati VB and Jadar PG: Free radical scavanging activity of Rasasindura (Red sulphide of mercury). Int J Pharm Sci Res 2018; 9(8): 3360-66. doi: 10.13040/IJPSR.0975-8232.9(8).3360-66.