International Journal of Pharmaceutical Research and Development (IJPRD)
Platform for Pharmaceutical Researches & Ideas
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NEUROTOXICITY STUDY OF TWO IRON CONTAINING BHASMAS, SWARNA MAKSHIKA BHASMA AND KASIS BHASMA

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ABSTRACT
The aim of this research work was oriented towards the evaluation of Neurotoxicity profile of two iron containing bhasmas swarna makshika and kasis bhasmas. The methodology is pertaining to this study, acute toxicity study of above-mentioned drugs was evaluated for the determination of LD50 on Swiss albino mice according to OECD 425 guidelines. while neurotoxicity study of above mentioned drugs was evaluated on wistar rats of either sex , divided equally in 7 groups for both bhasmas including one control group (n=10 female,10male) according to the OECD 424 guidelines .In neurotoxicity study Swarna makshika and kasis bhasma was administered orally in various dose ranging from 250mg/kg,500mg/kg and 1000mg/kg, for 28 consecutive days. Body weight of rats, was monitored once in a week, while feed and water consumption were monitored daily throughout the study. Neurotoxicity evaluation was done by detailed clinical observation for both the test drug using circular open field arena four times: prior to exposure, after one week, two weeks, and fourth weeks of the experiment, parameters observed are ambulation, grooming, rearing and defecation score. While Functional tests for neurotoxicity study was conducted using rota rod apparatus and actophotometer prior to exposure and during the fourth week of treatment, parameters measured are limb grip strength and motor activity respectively. On day 28th animals were sacrificed for the isolation of brain to observe histopathological changes, if any. The results of the detailed clinical observation showed that both kasis and swarna makshika bhasma does not produce any significant differences in the open field test statistically. However results of functional tests showed that animal from high dose treatment groups 500mg/kg and 1000mg/kg of kasis bhasma shows a significant decrease in % fall off time that is 64.24% and 68.35% respectively and % change of locomotor activity that is 62.5% and 64.2% respectively. Histopathological examination of control and experimental rat's revealed absence of any gross pathological lesion in brain. It can be concluded that there are chances of Neurotoxicity along with the clinical administration of higher doses of kasis bhasma. While low doses of both bhasmas was found to be safe. However, chronic toxicity studies are required to know the long term safety of these Bhasmas.

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Key words: Neurotoxicity, Oecd 424, Bhasmas etc.

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INTRODUCTION

For centuries detoxified digestible metals (bhasmas) are used for the treatment in ayurveda, this comes under rasasastra a branch of ayurveda. Rasasastra includes the extraction of metals from their mineral, their purification and conversion in to digestible metallic bhasmas. By this processes the metals are detoxified and converted in to wonder substances. [1-2]

Nowadays information's about ayurvedic medicines are widely available in internet and are exported largely to western countries. The prevalence of metals in ayurvedic medicines sold via the Internet and exported from India is under scanner. One-fifth of ayurvedic medicines made in India and sold in western countries through the Internet contain more than permissible levels of toxic metals includes mercury, zinc ,lead and arsenic. Although ayurvedic medicines are time tested for its efficacy proper toxicological studies are sorely missing so at fundamental level the bhasma as a medicine needs detailed scientific scrutiny about its toxicological profile and physicochemical characteristics.[3-4]

X-ray crystallography shows that Raw Swarna makshika bhasma contains $CuFeS_2$ also known as chelco pyrite. Final product of S.M.B contains Fe_2O_3, FeS_2, CuS and SiO_2 and raw kasis bhasma is $FeSO_4$ $7H_2O$. but final product consist of ferrous sulphate , ferric oxide and various metals like copper, nickel cadmium, lead etc. they are indicated for anemia, insomnia, convulsions, poor digestion and skin diseases .Excess iron causes iron overloading syndrome. Both the test drug contains iron. The present study of swarna makshika and kasis bhasmas has been undertaken to evaluate the Neurotoxicity profile of these bhasmas. The present study confirms to the guidelines laid by OECD.[5-6]

MATERIALS AND METHODS

Acute toxicological studies are carried out according to OECD Guidelines 425. Acute toxicity study of swarna makshika bhasma and kasis bhasma was evaluated for the determination of LD50.[7-8]

The neurotoxicity study was carried out according to OECD guidelines 424 using both male and female wistar rats. About 140 rats (70 male and 70 female) are purchased from Kerala Agricultural University, College of Veterinary and Animal Sciences Mannuthy, Thrissur, Kerala with the registration number 887/ac/05/CPCSEA distributed into 7 groups(20 animals in each group) I-VII for Control, Swarna makshika bhasmas; 250mg/kg, 500mg/kg, 1000mg/kg, Kasis bhasmas; 250mg/kg, 500mg/kg, 1000mg/kg. The temperature in the experimental animal room was 22°C (± 3°C). Although the relative humidity should be at least 30% and preferably not exceed 70% other than during room cleaning, the aim should be 50-60%. For feeding, food pellets are purchased from Kerala Agricultural University, College of Veterinary and Animal Sciences Mannuthy, Thrissur used with an unlimited supply of drinking water. All above mentioned drugs were administered orally for 28 consecutive days at required doses in the form of suspension in 3 % gum acacia Body weight of rats was monitored at least once in a week. Food consumption and water intake were monitored daily throughout the period of study.[8-9]

DETAILED CLINICAL OBSERVATIONS

Open field test

The open field arena was circular, 85 cm in diameter, with a white floor and a 50 cm high white wall. The floor had three concentric black circles, and the two outer circles were divided into segments by six radiating lines. Six additional short radiating lines subdivided all of the segments in the outermost circle into two, resulting in a total of 19 sections of equal area in the floor. The light level was 310 lux. For testing, each animal was placed on a starting point in the centre of one of the middle circle segments and then observed for 2 min. The number of the floor sections visited by the rat with all four feet, the number of rearing to the hind paws, and the number of faeces boluses were recorded.[10-11]

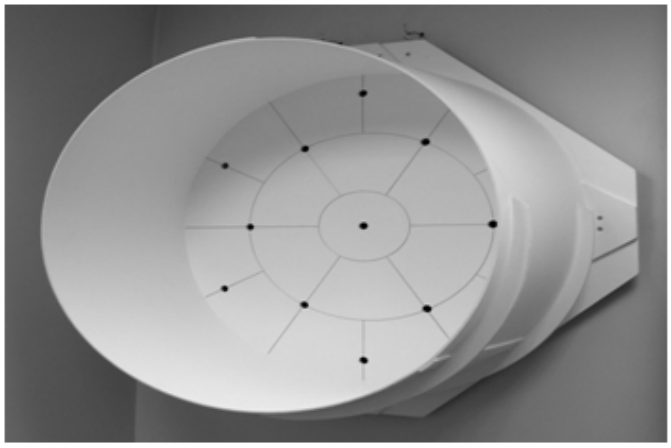
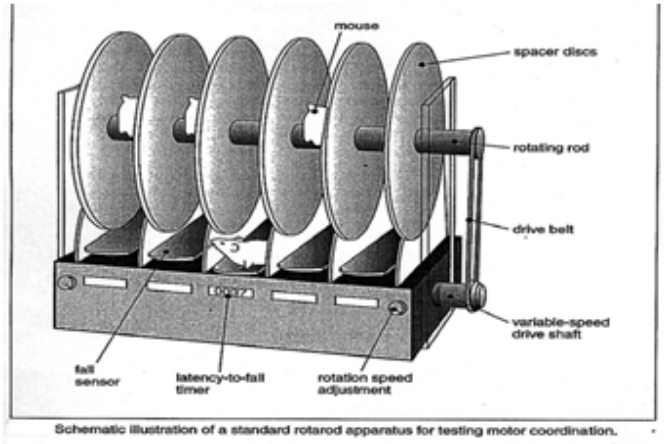


Fig: 1

FUNCTIONAL TESTS

Rota rod test

The apparatus consist of a Rotating rod diameter is ca. 5cm made of hard plastic material covered by grey rubber foam lanes width is ca. 5cm. The apparatus must allow an accelerating speed from 4rpm to 40rpm in 300 sec. The limb grip strength was assessed by means of rota rod testing, two times; prior to exposure and fourth week of treatment for both test drugs. parallely a group of un exposed animals also assessed for limb grip strength. On the day of testing, mice had been kept in their home cages and acclimate to the testing room for at least 15 min. Three trials separated by 15 min inter-trial intervals (ITI) were conducted for all groups. For testing rota rod was turned on at an appropriate speed (20-25) .animals was placed one by one on the rotating rod. 'Fall of time' was recorded when the rat falls from the rotating rod. Similarly fall of time was recorded at the fourth week of treatment also.[12]



Schematic illustration of a standard rotarod apparatus for testing motor coordination.

Fig; 2 Rotarod apparatus.

Fig: 3 Actophotometer.

Actophotometer

It consist of six built in photo electric cells which are connected in circuit with a counter .when the beam of light falling on the photocell was cut off by the animal , a count is recorded. The locomotor activity was assessed by means of actophotometer testing, two times; prior to exposure and fourth week of treatment for both test drugs. parallely a group of un exposed animals also assessed for motor activity. Actophotometer was turned on (check and make sure that all the photocells are working for accurate recording) and each rat was placed individually in the activity cage for 10min.Basal activity score of all the animals was recorded. Similarly basal activity score at fourth week of exposure was also recorded. Difference in the activity before and after treatment of both the test drugs was compared and % decrease in activity was calculated.[12]

HISTOPATHOLOGICAL STUDY

. On day 28th animals were sacrificed for the isolation of brain to observe histopathological changes,if any. The Brain was dissected out and was fixed in 10 % formalin. Paraffin sections of organs were stained with haematoxylin and eosin for detailed histopathological study.[9]

STATISTICAL ANALYSIS

Results were expressed as mean ± standard deviation. (S.D.) In acute and sub acute toxicity, statistical significance was determined by one-way analysis of variance (ANOVA) and Dunnett test. P values less than 0.001 were considered significant.[8-9]

RESULTS AND DISCUSSION

Acute toxicity study: At limit test 2000mg/kg, no death is observed. Hence the LD_{50} of swarna makshika and kasis bhasma was found to be greater than 2000mg/kg. But at limit test 5000mg/kg, all animals died during experiment. So the LD_{50} is below 5000mg/kg. Hence from the above observations we can conclude that the LD_{50} of swarna makshika and kasis bhasma was between 2000mg/kg and 5000mg/kg.

Neurotoxicity: The criteria for the assessment of effect of above mentioned drug administration in rats was based on the appearance of any kind of abnormal signs and symptoms, feed and water intake and growth pattern. Detailed clinical observation, Functional tests and biopsy were also taken into consideration for assessing the neurotoxicity of above-mentioned drugs.

Body Weight: Average body weight gain in swarna makshika and kasis bhasma treated groups of rats showed a slight decrease in high(1000mg/kg) doses when compared to control, but the decrease in weight is non significant. So body weight analysis of this study could suggest that there are no or less harmful effects of test drugs on body function as a whole.

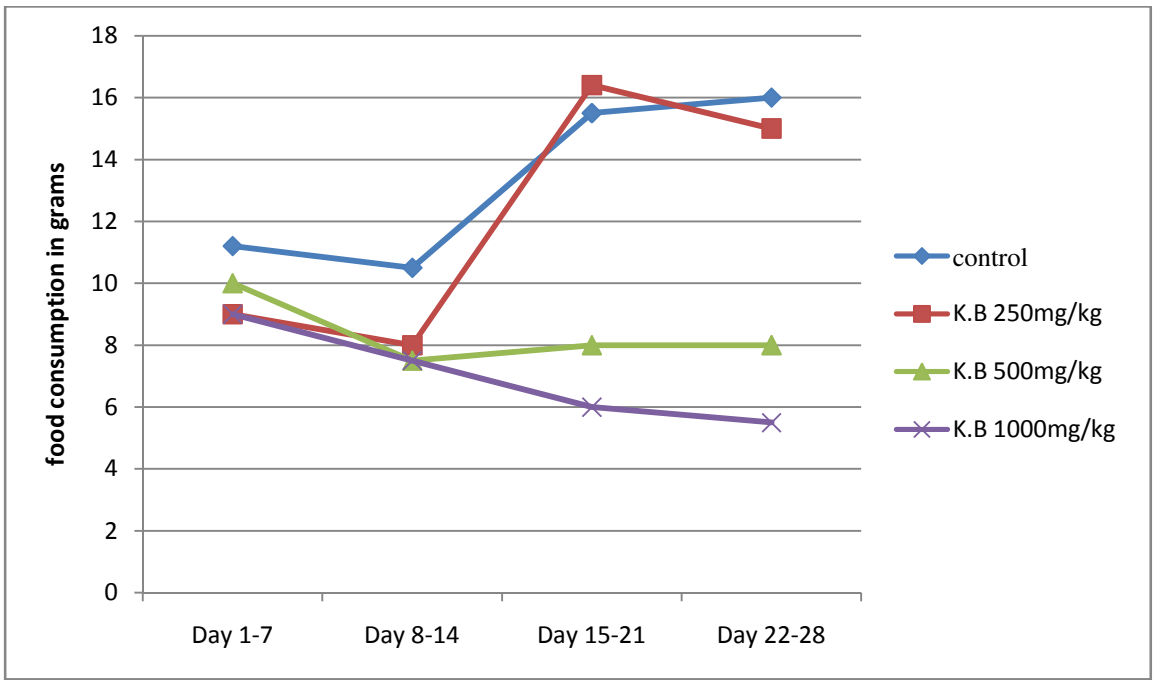
Food/Water Consumption: the food/water consumption of swarna makshika and kasis bhasma treated groups of rats showed a slight decrease but was not significantly different from those of the control group. Analysis of food and water consumption by treated rats doesn't show any signs of toxicity.

Table.1 body weight analysis

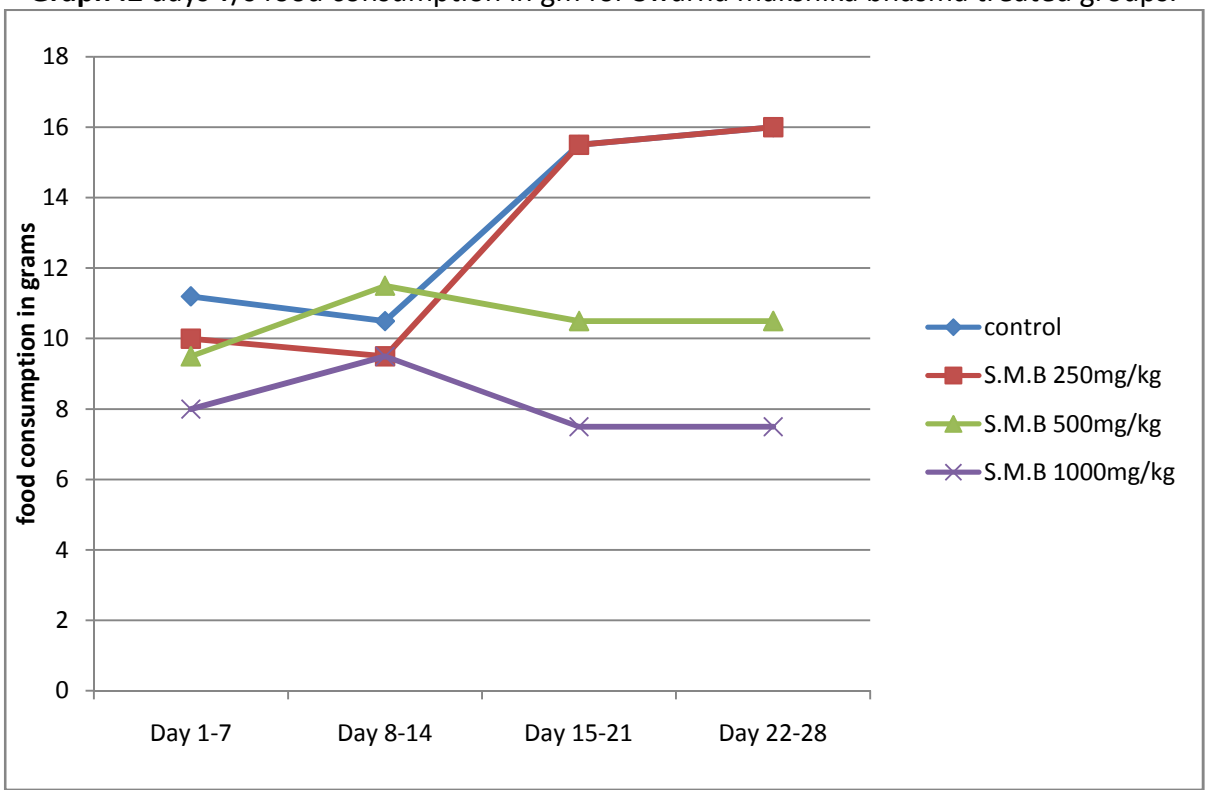
DRUG USED	GROUPS	DAY 0 Gm	DAY 7 gm	DAY 14 gm	DAY 28 gm	Total weight gain gm
	CONTROL	125±10.42	155±11.2	165±12.5	180±10	55±1.667
SWARNA MAKSHIKA BHASMA	250mg/kg	123.7±2.44	130.5±3.91	160±5.09	165.5±5.244	42±2.87
	500mg/kg	121.5±2.37	146.5±4.848	153.75±6.09	160.7±10.41	39±8.04
	1000mg/kg	148.7±7.31	160.2±5.543	166.2±2.5	170.2±5.00	22±2.31
KASIS BHAMA	250mg/kg	141.2±3.5	153.7±5.5	165±4.6	182±9.4	40.8±5.9
	500mg/kg	148.7±4.19	158.7±2.3	148.7±8.22	165.7±5.8	17±1.61
	1000mg/kg	158.7±5.2	160.7±11.5	170±4.7	170.5±2.4	11.8±2.8

Results are expressed in mean: n=20, * significant at $p < 0.05$, ** significant at $p < 0.01$, *** significant at $p < 0.001$.

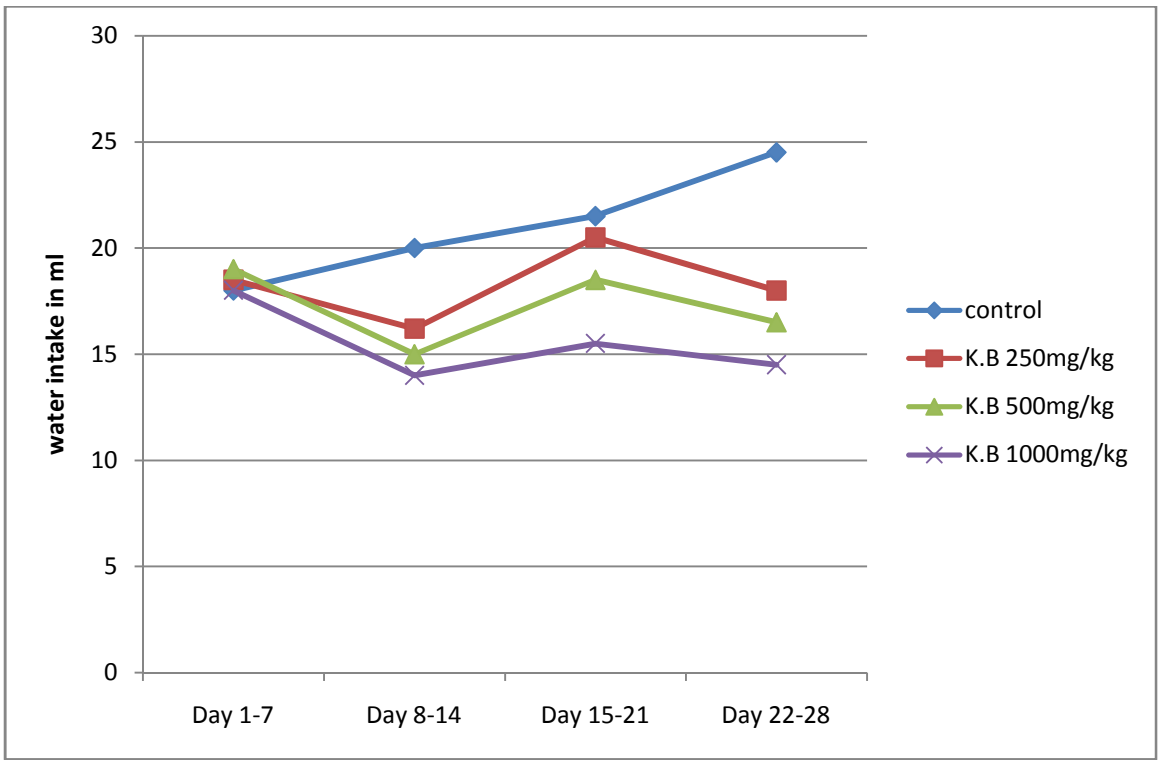
Graph .1 days v/s food consumption in gm for Kasis bhasma treated groups



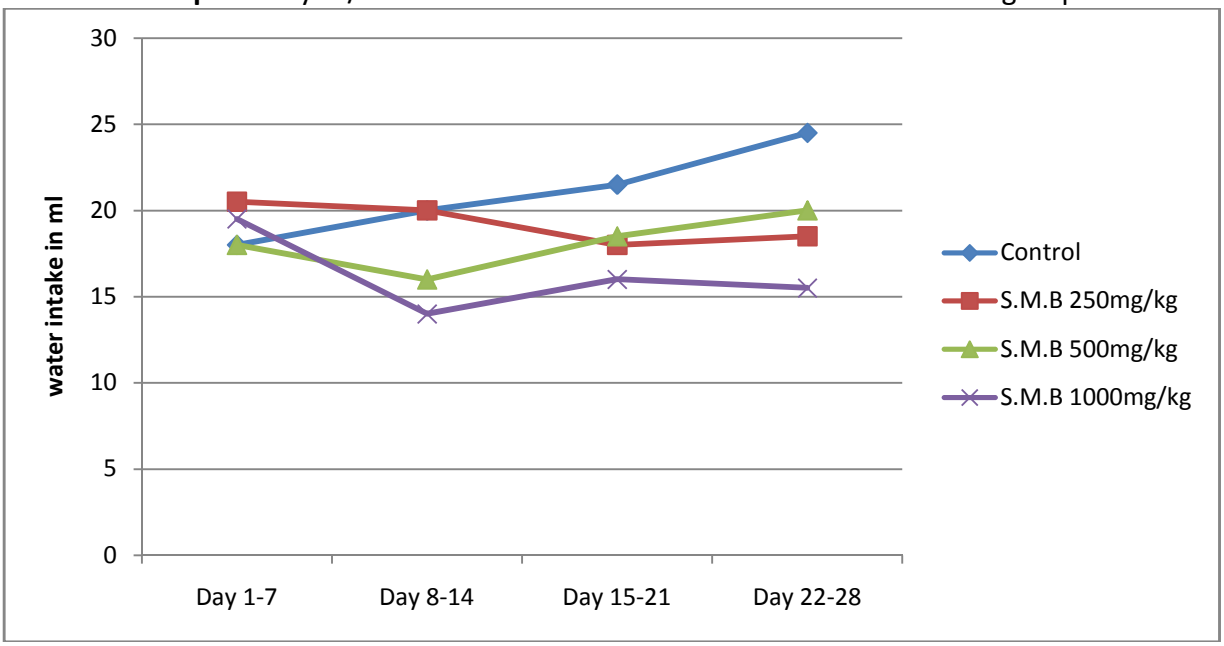
Graph .2 days v/s food consumption in gm for Swarna makshika bhasma treated groups.



Graph .3 days v/s water intake in ml for kasis bhasma treated groups



Graph .4 days v/s water intake in ml for swarna makshika treated group



DETAILED CLINICAL OBSEVATIONS

Open Field Test

The results of the present study (table 6.4) showed that both kasis and swarna makshika bhasma does not produce any significant differences in the open field test statistically. From this above observations it's clear that there were no differences in the measures of anxiety and emotionality statistically.

Table: 2 open field test

Examined Groups		CONTROL	KASIS BHASMA			SWARNA MAKSHIKA BHASMA		
			250 mg/kg	500 mg/kg	1000 mg/kg	250 mg/kg	500 mg/kg	1000 mg\kg
Prior to Exposure	A	38.3±11.0	36.2±8.0	40±6.82	51.4±12.42	44.4±8.43	48.2±7.25	47.8±10.349
	B	2.00±0.57	3.00±0.62	2.00±0.50	1.9±0.20	3.2±0.67	2.8±0.68	2.4±0.56
	C	18.20±3.1	16..4±2.40	16.2±2.2	13.2±2.2	19.4±2.1	19.8±3.4	18.2±2.8
	D	2.40±0.87	2.50±0.54	2.8±0.62	3.00±0.24	2.8±0.64	2.5±0.45	2.4±0.54
After 1 Week Exposure	A	38±8.71	30.2±6.4	21.4±9.42	24.4±14.2	38±11.4	31±12.04	26.4±5.02
	B	2.00±0.91	2.4±0.45	2.8±0.64	2.2±0.54	1.5±0.62	2.6±0.24	2.00±0.68
	C	6.00±2.54	10.4±4.62	11.2±6.31	8.62±3.2	15.2±2.84	13.2±4.2	14.2±3.4
	D	3.20±1.39	2.8±0.94	4.2±1.50	4.8±2.2	3.2±1.1	5.2±0.67	2.2±1.2
After 2 Week Exposure	A	40±8.05	25.2±9.4	17.2±6.23	24.4±14.45	37±8.4	31±6.04	23.8±11.2
	B	1.40±0.45	1.2±0.20	2.2±0.89	2.4±0.62	3.2±1.2	2.1±0.022	1.9±0.69
	C	9.60±1.80	8.2±1.4	4.2±0.43	5.4±1.72	12.2±1.8	14.2±0.56	15.8±2.8
	D	1.40±0.93	1.9±0.89	3.2±0.52	3.9±2.48	2.9±0.68	1.9±0.56	1.5±.97
After 4 Week Exposure	A	40±12.95	24.1±11.2	14.4±8.42	19.2±8.45	34±11.23	28±9.42	22±6.29
	B	1.20±0.29	1.2±0.52	1.8±0.89	2.1±0.67	1.9±1.2	1.6±0.41	1.9±0.24
	C	4.70±1.20	6.4±1.9	8.9±0.81	12.2±0.92	4.2±0.21	6.2±1.8	6.9±1.6
	D	1.90±0.86	1.2±0.20	1.5±0.68	2.9±0.92	1.8±0.54	2.00±1.7	1.9±0.65

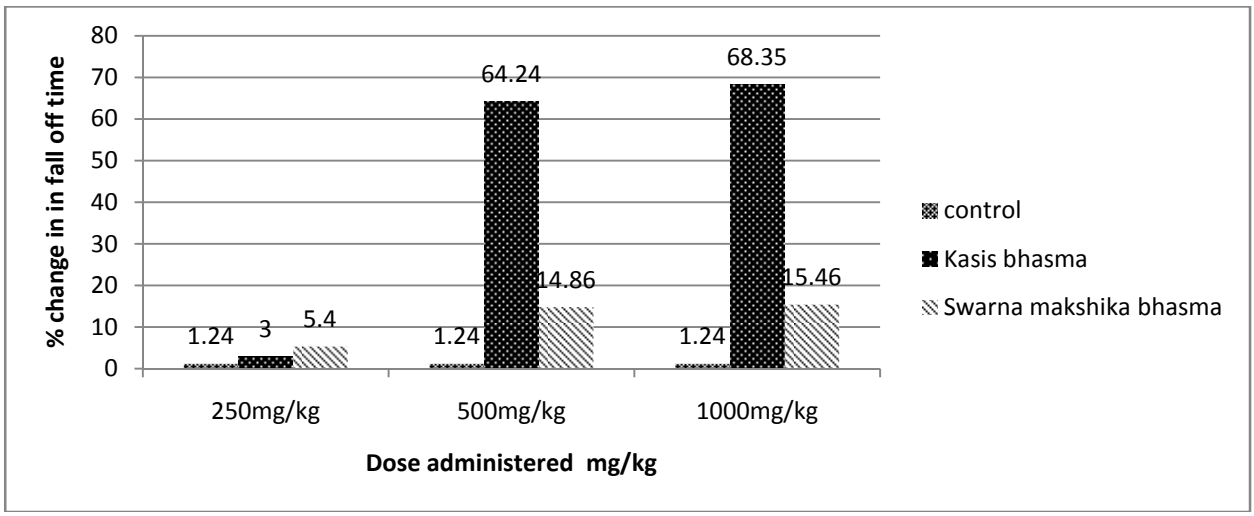
A -ambulation; B - grooming; C - rearing; D – defecation

Results are expressed in mean: n=20, * significant at $p < 0.05$, ** significant at $p < 0.01$.

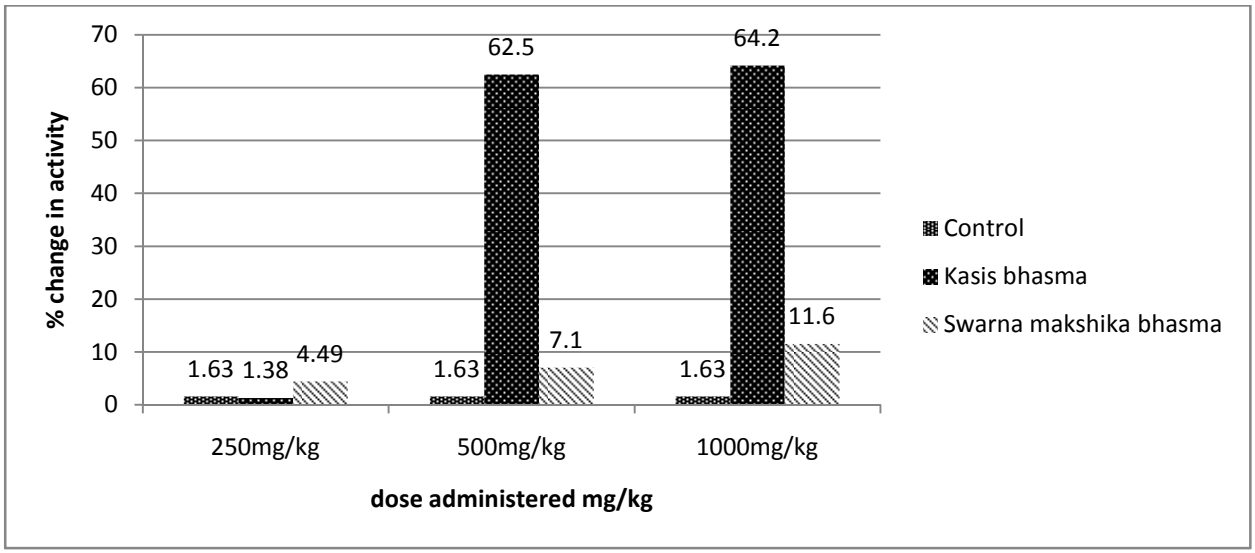
FUNCTIONAL TESTS OBSERVATION

Results of functional tests showed that animal from high dose treatment groups 500mg/kg and 1000mg/kg of kasis bhasma shows a significant decrease in % fall off time that is 64.24% and 68.35% respectively and % change of locomotor activity that is 62.5% and 64.2% respectively.

Graph: 5 % change in fall of time vs. dose administered by both bhasmas



Graph: 6 % change in locomotor activity vs. dose administered for both bhasmas



HISTOPATHOLOGICAL STUDIES

Effect on brain

Brain biopsy of all the dose range of kasis and swarna makshika bhasma treated rats when compared with untreated group shows normal architecture composed of normal cell bodies of neurons and the glial cells with normal fibrillary cytoplasm except there is a congestion in the choroid plexus of kasis bhasmas 1000mg/kg treated rats brain.

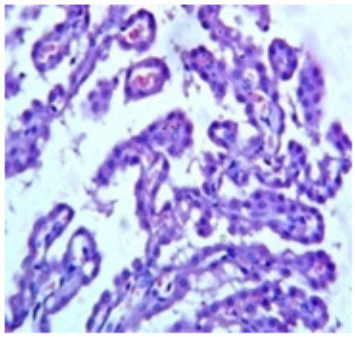


Fig: 1

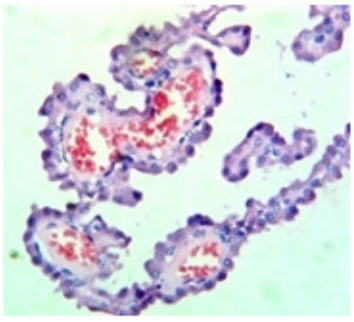


Fig: 2

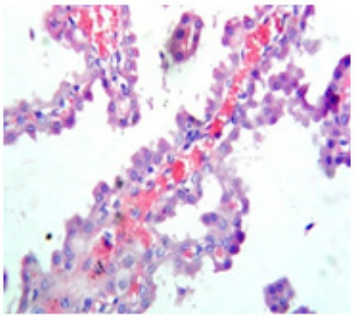


Fig: 3

Fig. 1: *Microphotograph of rat brain of control group.*
Fig.2: *Microphotograph of rat brain treated with swarna makshika bhasma 1000mg/kg.*
Fig.3: *Microphotograph of rat brain treated with kasis bhasma 1000mg/kg.*

The results of detailed clinical studies and functional tests (rotarod test and locomotor activity test) revealed that kasis bhasma given in high doses are susceptible to neurotoxicity or CNS depressant action, kasis bhasma high dose treatment groups (500mg/kg and 1000mg/kg) showed decrease in motor activity, muscle strength and exploratory behavior. This may be due to over dose and excessive absorption of iron in to mucosal cells of the duodenum and jejnum results in an increased free iron concentration in blood.[13] Excessive absorption of kasis bhasma may be due to the presence of organic acids in its preparation process. The organic acids and reducing agents (all acid in character) that promote iron absorption appear to do so by keeping the PH of the intestine down for a longer time.[13]

Increased free iron concentration in blood may lead to the dysregulation of brain iron homeostasis .dysregulation of iron homeostasis may results in the accumulation of iron in the brain cells. Excessive cellular iron is harmful to brain cells and promotes ROS formation through Fenton chemistry. Which may results in the development of neurodegenerative disorders like AD, PD and ALS. However, neither the mechanisms underlying iron accumulation nor its complete role in the pathogenesis of the diseases are clear.[14,15,16]

Whereas all the dose range tested for swarna makshika bhasma doesn't produce any significant difference in the detailed clinical and functional tests when compared with the control. No signs of neurotoxicity were observed for swarna makshika bhasma treated groups.

CONCLUSION

Thus in conclusion, present study indicates that there are a chance of neurotoxicity along with the clinical administration of higher doses of kasis bhasma .while low doses of both bhasmas was found to be safe. So both bhasmas are considered to be safe at clinically administered doses (9mg/kg for S.M.B and 4.5mg/kg for K.B). However, further detailed studies are required to know the long term chronic toxicity of swarna makshika and kasis bhasma.

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