Research Article

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Acute and sub chronic toxicity study of *Saraswata Ghrita*

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Abstract:
Saraswata ghrita was commonly used in traditional medicine for memory enhancing and various other mental disorders from centuries and are known not to produce any toxic or adverse effect in humans but probably there is no scientific evidence available on toxicity till date. For present study *Saraswata ghrita* mentioned in *Bhaisajya Ratnawali Swarbheda Rogadhikar* (chapter 17) was taken. The acute toxicity study was carried out in Wister mice (20±5) and observed for any adverse effects and mortality for 72 hrs after the oral administration of single dose of drug at several levels higher than the therapeutically equivalent dose. The subchronic toxicity study was carried out in the Charles Foster strain albino rats of male sex weighing between 150±10 g on continued oral administration of SG for one month period.
Animals treated with *Saraswta Ghrita* (SG) did not show any signs of toxicity in the acute toxicity study up to the dose of up to 20 times of therapeutic dose. No mortality was observed during 72 hours after drug treatment. After oral administration of SG,(5.40gm / kg and 10.80gm / kg) for 30 consecutive days there were no treatment related toxic effects were observed in body weight, growth pattern, hematological and biochemical parameters of rats. Also there were no any histopathological changes found in various organs of rats. Depends on our observations it can be concluded that the preparation as a whole was found to be safe in all routine lab investigations both in short and long term even on higher than normal therapeutic doses.
Key Words: *Saraswata Ghrita,* Toxicity, *Ayurveda.*

Introduction:

According the W.H.O.(1993) guidelines for Research and evaluation of Traditional medicine for safety and efficacy, a pharmacological effect for observed in vitro or in animal models is not necessarily applicable to humans, but such data would verify the reported mechanism of action in animals or humans and well-documented reports in pharmacological activity may be viewed as having scientific rationale. Preclinical studies on herbal drugs provide a scientific justification for their traditional use and prove that they are safe and efficacious.[1]
Saraswata Ghrita is a unique combination of *Ayurvedic* herbal drugs, containing mainly *Brahmi (*Centiala asiatica *L.)*, which is well known drug for its Nootropic, antiaging and antidepressant properties and has been proven through various clinical and experimental study. *Saraswata Ghrita* also contain *Rasayana* drug like *Haritaki and Amlaki*, which have been described by Acharya Charak in a separate chapter of *Rasayan Adhyaya* and while describing the properties of Haritaki it has been said that it is best among the all *"Vayasthapana dravyas a*nd while describing the properties of Amalaki he said that it is endowed with the same attributes and the same action as that of Haritaki, except in potency.(Amalaki is cold while Haritaki is hot). Both drugs promote longevity, and give nourishment, prevent aging, and promote intellect, sense perception and vitality.[2] Though *Saraswata Ghrita* was commonly used in traditional medicine for memory enhancing and various other mental disorders from centuries and are known not to produce

any toxic or adverse effect in humans but probably there is no scientific evidence available on acute and subchronic toxicity effects till date. For present study ***Saraswata ghrita*** mentioned in *Bhaisajya Ratnawali Swarbheda Rogadhikar* was taken (table 1) and prepared on the basis of classical Ghrita Paka method.[3]

Table 1. *Saraswata Ghrita* (composition at a glance)

1.	Brahmi Swarasa	***(Centella asiatica (Linn.)***	256part
2.	Haridra	*Curcuma longa* (rhizome)	4 part
3.	Amalaki	*Embellica officinalis* (fruits)	4 part
4.	Kustha	*Saussurea lappa (roots)*	4 part
5.	Nisotha	*Operculina terpethrum* (roots)	4 part
6.	Haritaki	*Terminalia chebulaa (fruits)*	4 part
7.	Pippali	*Piper longum* (fruits)	1 part
8.	Vidanga	*Embelia ribes* (fruits)	1 part
9.	Vacha	*corus calamus* (roots)	1 part
10.	Saindhava	*Rock Salt*	1 part
11.	Sarkara	Sugar	1 part
12.	Go ghrita	*Clarified cow Butter*	64 part

Material and methods

Animal used

Adult Charles Foster rats (150 ± 10 g) of male sex and Wistar mice (20 ± 5 g) of male sex were used in the study. Animals were obtained from Central Animal House of Institute of Medical Sciences, Banaras Hindu University, Varanasi. The animals were housed in groups of 6 in polypropylene cages at an ambient temperature of 25 ± 1°C and 45-55 % relative humidity, with a 12:12 h light/dark cycle. Rats were provided with commercial Amrit brand food pellets and water ad libitum, unless stated otherwise. Rats were acclimatized to laboratory conditions for at least one week before using them for experiments. Body weight of rats was measured periodically. Principles of laboratory animal care guidelines (NIH publication number 85-23, revised 1985) were followed. This proposal was approved by the Central Animal Ethical Committee of the Banaras Hindu University, Varanasi (Ref. no. Dean/13-14/CAEC/348).

Drug administration and **Dose:**

The dose for the animal study was calculated by extrapolating the clinical human dose of *Saraswata Ghrita* (12 gm/day) to an animal dose based on body surface area ratio by using conversion factor 0.018 by referring to the standard table of Paget & Barns (1964).[4]

For rats:

Human dose x 0.018 = Xg / 200g Rat

X x 5 = Yg /kg of Rat

12gm x 0.018 = 0.216gm / 200g Rat

0.216 x 5 = 1.08gm / kg Rat

For mice:

Human dose x 0.0026 = Xg /20g Mice

X x 50 = Yg /kg of Mice = 32mg x50 = 1600mg/kg body wt of mice

12gm x 0.0026 = .0312gm / 20g of Mice= 32mg

The test drugs were administered orally with the help of a special small-bore blunt catheter.

Acute toxicity study:

It refers to recording of adverse effects over 72 hrs after the administration of single dose of drug at several levels higher than the therapeutically equivalent dose. **24 Wister mice (20±5g), of male sex** weighing between 20±5gm were taken from the animal house, I.M.S. BHU Varanasi, maintained on 'Amrit' Brand animal feed and tap water given under normal climatic conditions. Animals were divided into three groups of *Saraswaa ghrita.* Each group contained six animals, and one more group containing six animals of control was taken making total of 4 groups and 24 animals.The dose schedule was prepared as per OECD guidelines for acute toxicity Study with slight modification (Organization for Economic Cooperation and Development, Guideline-423, adopted on 17th December, 2001) (Three dose levels- TED × 4, 10 and 20 times)(table 2). No food or water was given up to 4 h after drug treatment.[5] The drug was given by oral route once and animals were observed for overt changes in gross behaviour and activity over next 72 hrs including mortality.[6]

Table 2: Showing study protocol of acute toxicity study

Group	Drug	Dose
Control	Distilled water	6400mg/kg body wt
A1	*Saraswata ghrita*	6400mg/kg body wt
A2	*Saraswata ghrita*	16000 mg/kg body wt
A3	*Saraswata ghrita*	32000mg/kg body weight

Sub-chronic toxicity study

It refers to effect of the drug on continued exposure for one month period. Charles Foster strain albino rats of male sex weighing between 150±10 g were taken from the animal house, I.M.S., B.H.U., Varanasi, maintained on 'Amrut' Brand animal feed and tap water given under normal climatic conditions. Animals were divided into three groups with 6 animals (male) in each group.(table3)

Table 3: Showing study protocol of Sub-chronic toxicity study

Group	No. of animal	Drug	Dose
Control	06	Distilled water	5.40gm / kg Rat
I	06	*SG*	5.40gm / kg Rat
II	06	*SG*	10.80gm / kg Rat

Five and ten times to the normal therapeutically equivalent dose was taken for sub acute toxicity study. The test drugs were administered for a period of 30 days orally and were sacrificed on 31st day by cervical dislocation. (OECD guideline (Organization for Economic Co-operation and Development, Guideline-407, adopted on 3rd October, 2008) was followed during the study with slight modifications.)

Investigations

Rats were fasted overnight prior to blood collection by retro-orbital technique on the day 31st of the study. Then the rats were dissected and organs were separated, weighed and transferred to a glass bottle containing 10% formalin. These were sent for histo-pathological studies.

Parameters studied - change in wt., water and food intake,

a) ***Biochemical parameters*:** Blood sugar, Sr. Cholesterol, blood urea, S. creatinine, S. bilirubin, SALP, SGOT, SGPT, total protein etc .

b) ***Haematological parameters*:** TLC, DLC, Hemoglobin and platelets.

c) ***Autopsy*:** examination of the viscera and general animal profile at the time of sacrifice.

d) ***Histopathological studies*:** Histopathology of important organs like brain, kidney and liver.

Observation and Results

Toxicity Study

Acute toxicity study: Animals treated with *Saraswta Ghrita* (SG) did not show any signs of toxicity in the acute toxicity study up to the dose of up to 20 times of therapeutic dose. There was no significant change in the behavior of animals. No mortality was observed during 72 hours after drug treatment.

Sub-chronic toxicity study:

Body weight: The mean change in body weight of rats is depicted in table 4. There was no significant difference in body Weight gain of *Saraswta Ghrita* (SG) treated rats compared to control rats during sub chronic toxicity study, showing normal growth pattern

Treatment (p.o.)	Body weight (gms)			
	Day 0	Day 10	Day 20	Day 30
DW	152.83±03.87	160.83±05.07	165.17±04.82	167.33±03.08
SG-5.40gm / kg Rat	149.83±05.49	161.50±05.39	164.33±06.01	170.17±06.36
SG-10.80gm / kg Rat	150.50±03.50	159.17±03.64	165.00±05.40	169.34±06.90

Table 4: Effect of saraswat ghrita(SG) on body weight of rats in sub chronic toxicity study

SG denotes saraswat ghrita. N= 6 in each group, values are mean ± SD. There was no significant change in body weight of rats during treatment in Comparison to control group.

Effect on hematological parameters: There were no treatment related effects in hematological parameters. After oral administration of SG ,5.40gm / kg and 10.80gm / kg for 30 consecutive days no significant alterations in the hemoglobin and blood cell values were observed. There was no major shift in white cell population in SG treated rats compared to vehicle. There was no leucocytosis or leucopenia. The observations are summarized in table 5.

Table 5: Effect of saraswat ghrita(SG) on hematological parameters of rats in sub chronic toxicity study

Treatment (p.o.)	Platelet count(x 10^3/mm^3)	Hemoglobin (gm/dl)	Total WBC (x 10^3/dl)	Differential Leukocytes Count (%)				
				Neurrophil %	Lymphocyte %	Eosinophil %	Monocyte %	Basophil %
DW	490.00±96.658	14.75±.87	8.30±0.22	25.53±3.626	69.17±3.656	.76±.216	4.46±.750	.067±.103
SG-5.40gm / kg Rat	525.67±126.44	14.63±.85	8.83±0.16	29.10±6.000	66.17±5.636	.55±.151	4.13±1.390	.05±.054
SG-10.80gm / kg Rat	485.50±68.421	15.08±.34	8.82±0.4 9	26.70±1.685	68.33±1.862	.78±.213	4.06±1.017	.11±.183

SG denotes *Saraswata Ghrita*, DW denotes distilled water N= 6 in each group, WBC= White blood corpuscles, values are mean ± SD, There was no significant change in hematological parameters of rats during treatment in comparison to DW.

Effect on biochemical parameters: *Saraswata Ghrita* (SG) did not cause any disturbance in hepatic functions as compared to vehicle. The excretory and other functions of liver of *Saraswata Ghrita* (SG) treated rats were not disturbed, because alkaline phosphatase, SGOT and SGPT values were not elevated in comparison to vehicle. *Saraswata Ghrita* (SG) treatment did not cause any adverse or toxic effect on renal functions. Biochemical parameters revealed that excretory function of kidney was well maintained, as there was no rise in the values of blood Urea, Serum creatinine levels in plasma of rats treated with *Saraswata Ghrita* (SG). There is no increasing effect on cholesterol and blood sugar levels also. (table 6)

Table 6: Effect of *Saraswat ghrita*(SG) on biochemical parameters of rats in sub-chronic toxicity study

Treatment (p.o.)	Glucose (mg/dl)	choles-terol (mg/dl)	AST (IU/L)	ALT (IU/L)	Alkaline Phosphata- se (IU/L)	Blood Urea (mg/dl)	Creatinine (mg/dl)	Total Protein (gm/dl)	Bilirubin (gm/dl)
DW	94.00± 17.029	81.50± 14.488	55.83± 11.531	25.67± 3.077	80.83± 5.529	24.700± 5.9501	00.52± 00.146	6.41± 00.231	00.36± 00.10
SG*-5.40gm / kg Rat	91.00± 20.179	75.83± 12.384	56.33± 08.066	26.00± 02.366	74.83± 13.977	27.15± 5..184	00.53± 00.146	06.55± 00.350	00.34± 00.12
SG*-10.80gm / kg Rat	94.00± 15.608	75.83± 11.125	58.83± 01.47	26.50± 04.764	83.50± 23.330	23.33± 06.04	00.61± 00.138	06.73± 00.423	00.42± 00.07
Normal range	50-135	40-130	45.7-80.8	17.5-30.2	56.8-128	15-35	0.2-0.8	5.6-7.6	0.2-0.55

*SG denotes Saraswat ghrita. N= 6 in each group, AST= Aspartate transaminase, ALT= Alanine transaminase, values in mean ± SD. There was no significant Change in biochemical parameters of rats during treatment in comparison to distilled water.

Histopathological study: Histological sections of rat's liver and kidney of control group show normal architecture. Liver of *Saraswata Ghrita* (SG) treated rats show normal lobular architecture of liver with normal central portal vein, radiating plates of hepatocytes and peripheral portal tracts composed of hepatic artery, bile ductile and distal portal vein. No focal or diffuse foci of necrosis of hepatocytes and infiltration of chronic inflammatory' cells were observed. Kidney of *Saraswata Ghrita* (SG) rats shows normal renal architecture composed of normal renal glomeruli, collecting tubules, interstitial tissue and blood vascular channels. There were no foci of necrosis, degeneration or fibrosis in interstitium. Histopathological study of brain also shows no any abnormality in the brain parenchyma. (The photomicrographs are shown in figures1-9)

Figures (1-9) Histological Examination of Subchronic Toxicity Study

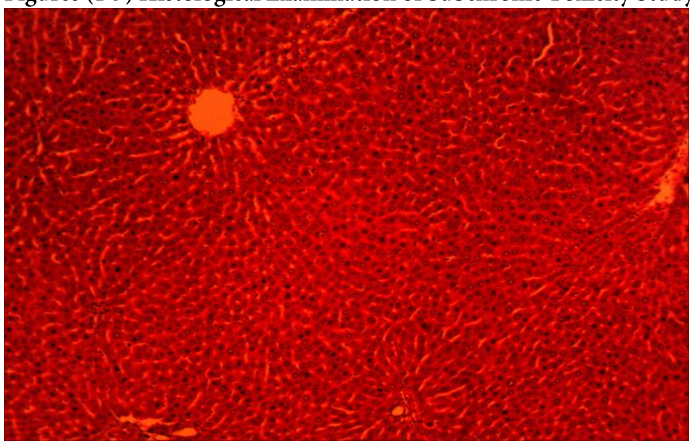

Plate 1 Liver tissue of control rat showing normal hepatocytes (H&E)

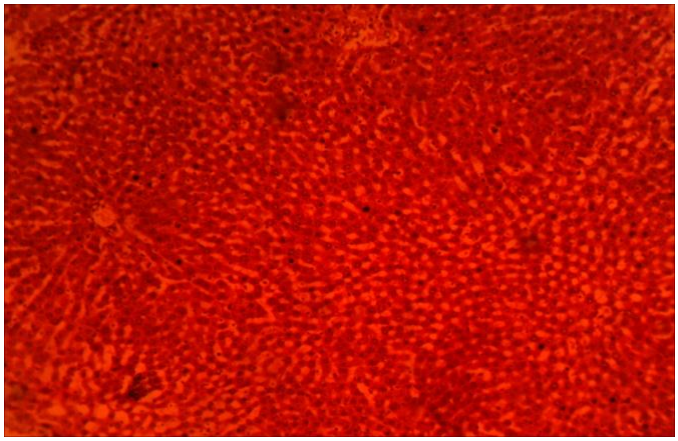

Plate 2 Plate 2-Liver tissue SG (5.40gm / kg Rat) showing normal hepatocytes (H&E)

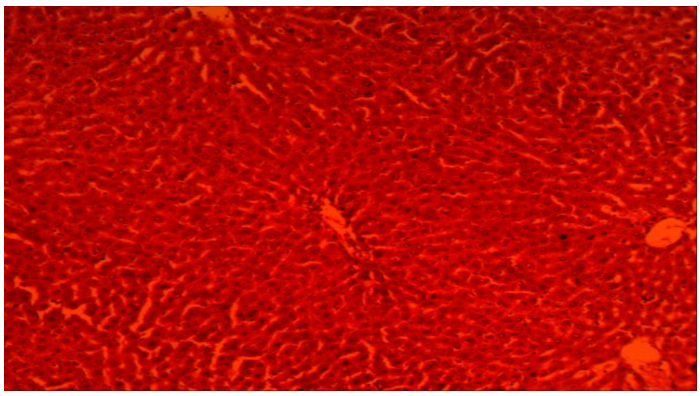

Plate 3 Plate 2-Liver tissue SG (10.80gm / kg Rat) showing normal hepatocytes (H&E)

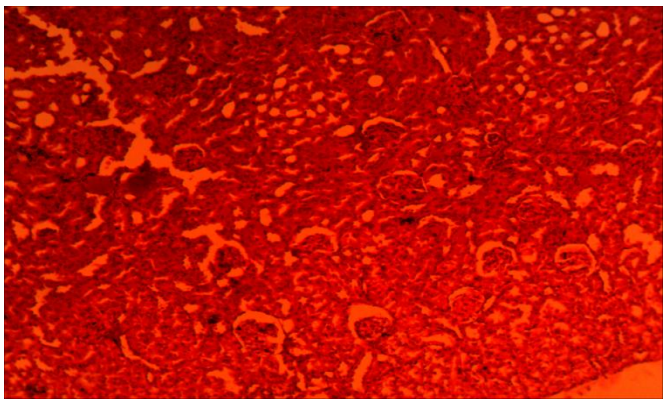

Plate 4 kidney tissue of control rat(DW) showing normal corticomedulary cells (H&E)

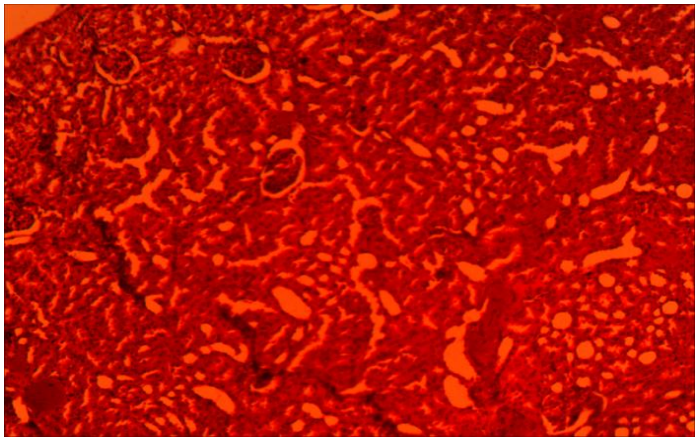

Plate 5 Kidney SG (5.40gm / kg Rat) showing normal corticomedulary cells (H&E)

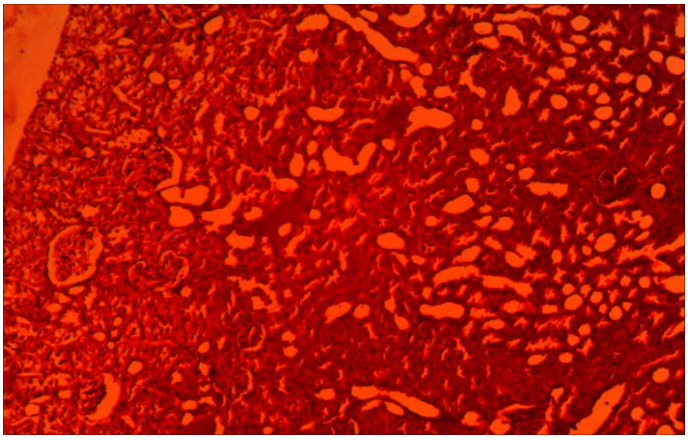

Plate 6 Kidney tissue SG (10.80gm / kg) showing normal corticomedulary cells (H&E)

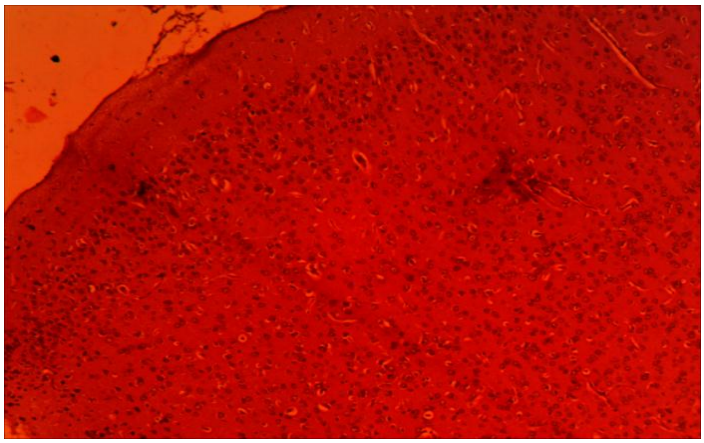

Plate 7. Brain (cerebrum) tissue of control rat showing normal cerebral parenchyma (H&E)

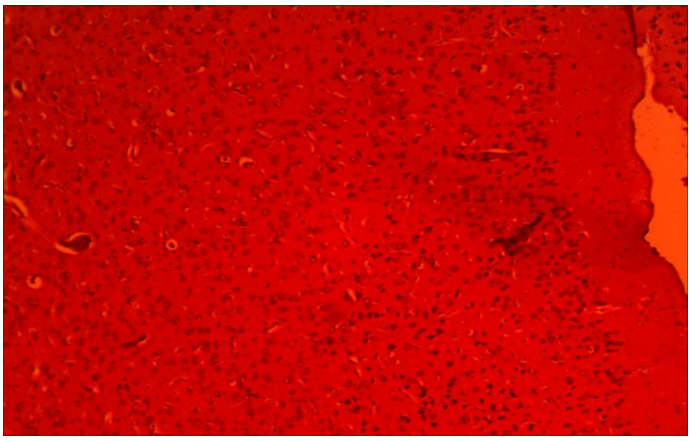

Plate 8. Brain (cerebrum) tissue of *Saraswata ghrita(5.40gm/kg)* treated rat showing normal cerebral parenchyma (H&E)

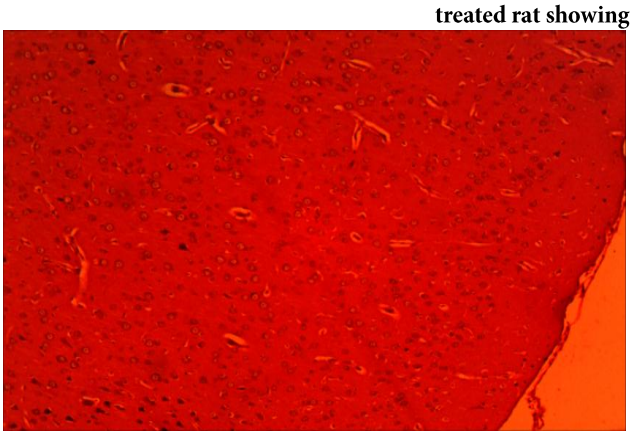

Plate 9. Brain (cerebrum) tissue of *Saraswata ghrita(10.80gm/kg)* treated rat showing normal cerebral parenchyma (H&E).

Results of acute and sub chronic toxicity study no toxic effects of *Saraswata Ghrita* (SG) on any of the parameters studied. Hence *Saraswata Ghrita* (SG) in daily doses up to ten times greater than therapeutic dose can be considered as toxicologically safe ones.

Discussion

For the determination of safety of drugs and plant products for the clinical use, toxicological evaluation is carried out in various experimental animals to predict toxicity and to provide guidelines for selecting "safe" dose in humans. Acute toxicity study with a range of doses have to be Conducted first to select proper doses for chronic and sub chronic studies, The doses selected for chronic and sub chronic studies should be higher than the Suggested human dose. The evaluation of adverse effects of sub chronic and chronic dosing in experimental animals may be more relevant in determining the overall toxicity of the herbal preparations.[7]

Acute toxicity: For acute toxicity study suitable animal dose of *Saraswata Ghrita* was calculated using Paget and Barnes table and animals were administered up to 20 times the normal therapeutic equivalent dose. No mortality was observed in any of the group that received the test drugs. The highest dose administered was 32000mg/kg body weight of mice; this suggests that the LD50 is higher than this dose. From this study, it can be

suggested that the test preparations are unlikely to produce any drastic acute toxic effects at the dose levels employed clinically. Further evaluation of effect on gross behavior did not reveal any significant change indicating lack of any adverse effect on CNS and ANS.

Sub chronic Toxicity: The sub chronic toxicity study on oral administration of *Saraswata Ghrita* (5.40gm / kg Rat and 10.80gm/kg of rat) clearly demonstrates that there was no abnormal change in weight gain in experimental animals. The average daily feed and water intake remained normal throughout the 30 days of sub chronic toxicity study. There were no adverse effects in haematological parameters like haemoglobin and blood cell counts. *Saraswata Ghrita* did not cause any disturbances in the functioning of liver and kidneys, which were evident by the normal ranges of biochemical parameters.[8] Though the preparation was in ghrita form which may be blamed to increase the serum cholesterol but surprisingly the preparation dose not alter the serum cholesterol level on very high doses too. This is probably due to hypolipidimic action of its many ingredients like *Embelica officinalis* and *Curcuma longa*.[9,10] Histopathological examinations of the liver and kidneys and brain also suggest the same. Hence it was concluded that *Saraswata Ghrita* did not show any toxic effects in the sub chronic toxicity study.

Summery and conclusion

Saraswata ghrita, a polyherbal *Medhya* compound drug used in traditional medicine for cognition and memory related problems is blended with the drugs which exert a variety of pharmacological actions including anti-inflammatory, anti-amyloidogenic, anti-cholinesterase, hypolipidemic, and antioxidant properties. In the animal experiments, however the *ghrita* preparation should largely serve absorption and other pharmacokinetic properties. The preparation as a whole was found to be safe in all routine lab investigations both in short and long term. Hence it can be concluded that the *Saraswata ghrita* is a non toxic compound *Ayurvedic* drug that can be used for long term at therapeutic and above the therapeutic doses.

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