ORIGINAL ARTICLE

Acute and sub-chronic toxicity study of *Brahmi ghrita* in rodents

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Context: *Brahmi Ghrita* (BG) contains *Brahmi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*), *Kushtha* (*Saussurea lappa*), *Shankhapushpi* (*Convolvulus pluricaulis*) and *Puran Ghrita*, mentioned for treatment of various diseases. **Aim:** To assess acute and sub-chronic toxicity of *Brahmi Ghrita* in mice and rats, respectively. **Materials and Methods:** In acute toxicity study, Swiss strain albino mice were administered orally *Brahmi Ghrita* doses of 1, 2.5 and 5 g/kg and observed for behavioural changes and mortality, if any. In sub chronic toxicity study, Charles Foster albino rats were administered two doses of *Brahmi Ghrita* i.e., 400 and 800 mg/kg, p.o. for 30 consecutive days. During 30 days of treatment, rats were observed for any changes in body weight and daily food and water intake. After 30 days, rats were sacrificed for haematological, biochemical and histopathology study. **Result:** There was no mortality or abnormal behaviour, observed in acute toxicity study in mice at all the three dose levels. In sub-chronic toxicity study, *Brahmi Ghrita* did not produce any significant changes in body weight and daily food and water intake of rats when compared to control group rats. Further, haematological and biochemical parameters were also found normal. Histopathological study revealed normal architecture of kidney and liver of *Brahmi ghrita* treated rats. **Conclusion:** *Brahmi Ghrita* is safe in rodent and mice.

Key words: Acute toxicity, *Brahmi ghrita*, sub chronic toxicity

INTRODUCTION

Safety is a fundamental principle in the provision of herbal medicines and herbal products for health care, and a critical component of quality control. Among consumers, there is a widespread misconception that "natural" always means "safe", and a common belief that remedies from natural origin are harmless and carry no risk. However, some medicinal plants are inherently toxic.[1] The growing number of herbal drug users around the globe and scarcity of scientific reports regarding safety aspects of herbal products make it imperative to conduct toxicity study of herbal drugs.[2] The concern for herbal toxicity has alarmed many national and international administrative bodies to formulate and implement various guidelines for assessing, monitoring and preventing toxicity arising due to herbal products. For example Uppsala Monitoring Centre of WHO compiles and disseminates information regarding herbal drug adverse reactions whereas Organization for Economic Cooperation and Development (OECD) sets guidelines regarding toxicity study. Very often marketed herbal products are identified with significant toxic effects. One research study reported severe hepatotoxicity with use of herbal plants.[3] In this context proper screening of herbal products for various toxic effects before they become consumable, is quite necessary.

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Brahmi ghrita (BG) contains *Brahmi* (*Bacopa monneri*), *Vacha* (*Acorus calamus*), *Kushtha* (*Saussurea lappa*), *Shankhapushpi* (*Convolvulus pluricaulis*) and *Puran Ghrita*. Among them *Brahmi*, enhancing cognitive effect especially memory, learning and concentration[4,5] but it causes marked degenerative changes in the somniferous tubules and alterations in the male reproductive organs.[6] Brahmine, present in *Brahmi* is highly toxic even in therapeutic doses, it toxicity resembles strychnine poisoning and saponins like Bacoside A & B possess haemolytic activity.[7] Aqueous extract of *Acorus calamus* potentiated phenobarbitone-induced seizures.[8] Beta-asarone of *Vacha* also showed hallucinogenic[7] effect. *Ghrita* has desirable and undesirable effect on body, desirable effect in non-observation of hyperlipidaemia in spite of giving good amount of fat and undesirable effects on cardiac and renal disorders.[9] In *Brahmi ghrita* formulation, *Brahmi* and *Ghrita* present in maximum percentage and both have some toxic properties besides this *Vacha* also incorporated its untoward effect in it. Rule 170 of Drug and Cosmetic act 1940, safety study is required for those classical

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formulations which contain any ingredient specified in Scheduled E (1) and it is also required if intended human use is more than 3 month or any report suggesting toxicity. *Brahmi ghrita* is specified for the treatment of *Apasmara*; in this disorder it is used for more than 3 months, according to rule 170 of Drug and Cosmetic act 1940 safety study of *Brahmi ghrita* is required.

MATERIALS AND METHODS

Preparation of *Brahmi Ghrita*

Brahmi ghrita was prepared with *Murchhita ghrita*. First of all *Murchhita ghrita* was heated on mild heat, when *Ghrita* was slightly warm then *Brahmi Swarasa* (Juice) was added into it and mixed thoroughly, during mixing of *Swarasa* heating process was continued. Then *Kalka dravya* (Paste) was added. After adding the *kalka dravya* continuous stirring of whole material was done. In first day whole material was heated up to boiling for one hour, after that heating process was stopped on first day. In second day heating process was started again and heated for 5 hours after that, heating process was again stopped. In third day heating process again started and continued up to obtaining *Sneha siddhi lakashana* (completion test) like *varti-vat Sneha kalka* (wick-like shape), *sabdhinoagni nikshipto* (does not produce crackling sound on fire), etc., When *Sneha siddhi lakashana*[10] was obtained, then *Ghrita* was filtered with the help of cotton cloth. This filtered *Ghrita* was known as *Brahmi Ghrita*. Thus *Brahmi Ghrita* was prepared in 3 days of discontinuous heating.[10]

Analytical Study

Brahmi ghrita has higher acid, saponification, iodine and refractive index in comparison to *Ghrita* and phytochemical like bacosides are present in it.[11]

Animals

Charles Foster rats weighing between 160 and 180 g and Swiss albino mice weighing between 20 and 30 g were used for experimental study. The animals were obtained from the Central Animal House. The animals were housed in polypropylene cages at an ambient temperature of 25°C ± 1°C and 45-55% relative humidity, with a 12:12 h light/dark cycle. Animals were provided with commercial food pellets and water *ad libitum* unless stated otherwise. They were acclimatized to laboratory conditions for at least 1 week before using them for the experiments. Principles of laboratory animal care (NIH publication number # 85-23, revised in 1985) guidelines were always followed and prior approval of Institutional Animal Ethics Committee (No. Dean/10-11/150) was obtained before commencing experiments.

Drug Treatment

In sub-chronic toxicity, rats were administered *Brahmi ghrita*, once daily for 30 consecutive days in the dose of 400 and 800 mg/kg body weight through oral route. In acute toxicity study *Brahmi ghrita* was administered orally at 1, 2.5, and 5 g/kg doses to mice.

Toxicity Study

Acute Toxicity: Acute toxicity of *Brahmi ghrita* was evaluated in Swiss strain albino mice (20–25 gm), as per protocol,[12] forty Swiss strain albino mice were equally divided in to four groups (A, B, C, D) including control i.e., 10 animals in each group. *Brahmi ghrita* was administered orally at the single dose of 1, 2.5, and 5 g/kg body weight in groups B, C, D, respectively. No food or water was given up to 4 h after drug treatment. Mice were closely observed for the initial 4 h after the administration of *Brahmi ghrita* and then once daily during the following days. The behavioural changes closely observed for were: hyperactivity, ataxia, tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. Total observation period for eventual mortality was 14 days.[13]

Sub-chronic Toxicity Study: Thirty-six adult Charles Foster albino rats (150 ± 10 g) were distributed into 3 groups (A, B and C) of 12 animals each.[12] *Brahmi ghrita* was administered orally for 30 consecutive days in the doses of 400 and 800 mg/kg. Body weights of animals, as well as their food and water consumptions were monitored at 10-day intervals throughout the study period. They were fasted overnight prior to blood collection by retro-orbital technique on the 31st day of the study. Blood samples were analysed for haematological [haemoglobin, total leukocyte count (TLC), differential leukocyte count (DLC)] and biochemical (glucose, cholesterol, alkaline phosphatase, aspartate transaminase (AST), alanine aminotransferase (ALT), blood urea nitrogen, creatinine, total protein and albumin) parameters. After blood collection, animals were scarified for isolation of kidney and liver to observe histopathological changes, if any. The kidney and liver were dissected out and were fixed in 10% formalin solution. Paraffin sections were made and stained with haematoxylin and eosine for detailed histopathology study.[14]

Blood Analysis

Haematological analysis was performed by Sysmex XE-2100 haematology auto analyser (Sysmex-Corporation, Kobe, Japan) using fluorescent dye and hydrodynamic focusing method. For serum biochemical analysis ERBA CHEM-7 auto analyser (ERBA diagnostics, Manheim, GmbH, Germany) was used. AST, ALT, alkaline phosphatase and creatinine were analysed using kinetic method whereas glucose, cholesterol, albumin, total protein and blood urea nitrogen were analysed by end-point method.

Statistical Analysis

The data, expressed as Mean ± SD, were subjected to Kruskal–Wallis one-way analysis of variance (ANOVA).

Inter group comparisons were made by Mann–Whitney U test (two tailed) for only those responses which yielded significant treatment effects in the ANOVA test. $P < 0.05$ was considered statistically significant.

RESULTS

Acute Toxicity Study

Mice administered with *Brahmi Ghrita* did not show abnormal behaviour for initial 4 h after drug administration. No mortality was observed during 14 days after treatment with *Brahmi ghrita*.

Sub-chronic Toxicity Study

The body weight of rats treated with *Brahmi ghrita* was normal in comparison to control group rats [Table 1]. As compared to the control group, daily food and water intake was also not significantly different in *Brahmi ghrita*-treated groups. As summarised in Tables 2 and 3. *Brahmi ghrita* treatments did not significantly change the level of haemoglobin, TLC, and DLC. It is apparent from Table 4 that the serum levels of glucose, cholesterol, alkaline phosphatase, AST, ALT, blood urea nitrogen, creatinine, total protein and albumin were also not altered significantly in *Brahmi ghrita*-treated groups. Histopathological examination of control and *Brahmi ghrita*-treated rats revealed the absence of any gross pathological lesion in kidney and liver (as summarised in Table 5).

Table 1: Effect of BG on body weight of rats in sub-chronic toxicity study

Treatment	Dose (mg/kg)	Body weight (g)		
		0 day	15th day	30th day
Control	-	140±3.67	163±1.89	185±3.12
A	400	147±2.45	171±5.74	189±4.23
B	800	153±4.71	176±1.23	193±6.15

BG – *Brahmi ghrita; n*=12 animals in each groups; values are mean±SD

Table 2: Effect of BG on food intake of rats in sub-chronic toxicity study

Treatment	Dose (mg/kg)	Food intake (g)			
		0 day	10 day	20 day	30 day
Control	-	220±6.04	231±5.33	239±7.21	241±3.76
A	400	215±5.19	228±2.11	234±5.31	238±4.06
B	800	211±4.12	223±3.67	229±7.81	236±1.98

BG – *Brahmi ghrita*; *n*=12 animals in each groups; values are mean±SD

Table 3: Effect of BG on water intake of rats in sub-chronic toxicity study

Treatment	Dose (mg/kg)	Water intake (ml)			
		0 day	10 day	20 day	30 day
Control	-	380±2.81	410±4.12	425±3.94	440±5.51
A	400	400±1.68	420±3.66	435±2.81	450±4.33
B	800	415±2.41	430±5.86	445±1.07	460±2.18

BG – *Brahmi ghrita*; *n*=12 animals in each groups; values are mean±SD

DISCUSSION

Bacopa adversely affect gastrointestinal tract (GIT) like increased stool frequency, abdominal cramps, etc.[15] Saussure amines A, B, C, costunolide and dehydrocosts lactone, present in *Saussurea lappa* showed gastroprotective effect[16] along with this *Saussurea lappa* and *Shankhapushpi* showed antiulcer[17,18] and anti-inflammatory activity.[19] It may be possible that gastrointestinal adverse effect of *Brahmi* is counterbalanced by beneficiary effect of Kushtha and *Shankhapushpi*. Anti-fertility effect of *Brahmi* might be counterbalanced by properties of *Shankhapushpi* for e.g., uterine affections promoting fertility[7] and aphrodisiac property of *Kustha*.[20] *Brahmi ghrita* have high saponification and iodine values that plain *Ghrita*. It indicates that *Brahmi ghrita* have more short chain fatty acid and unsaturated fats, which might be due to pharmaceutical processing of *Brahmi ghrita*. Short chain fatty acids (SCFAs) are readily absorbed; a greater increase in SCFAs production and potentially a greater delivery of SCFAs, specifically butyrate, to the distal colon may result in a protective effect[21] and recognized as an essential fuel source for colonocytes, particularly in the distal colon.[22] Unsaturated fat supplementation increases total dietary energy intake to recommended levels, has no adverse impact on blood lipids. It also improves nutritional status, reduces systemic inflammation[23] and possesses health benefits, such as regulating blood cholesterol levels, etc.[24] *Ghrita* beyond treating serious mental disorders can be used to pacify anxiety[25] which may pacify the untoward effect of *Vacha*.

In our study, the haematological parameters of *Brahmi ghrita*-treated rats showed absence of any significant increase or decrease in count of any such component as mentioned above when compared to control rats. Low level of haemoglobin, abnormal count of total leucocytes and changed ratio of different constituents of leucocytes such as neutrophils, lymphocytes, eosinophils, monocytes and basophils shows possible haemotopoietic toxicity.[26] But all the above-mentioned haematological parameters were found normal and no significant changes were observed. Thus, *Brahmi ghrita* seems to devoid of any adverse effect on haematopoietic system of rats.

In *Brahmi ghrita*-treated rats level of AST and ALT was found normal when compared to control rats. Increased level of AST and ALT are due to damage of hepatic cells used as marker test for hepatic toxicity.[27] *Brahmi*, which is present in maximum percentage in *Brahmi ghrita* is reported for hepatoprotective activity[28] besides this *Acorus calomus* also confer the hepatoprotective[29] and *Saussurea lappa*, displayed anti-HBsAg activity by suppressing HBsAg

Table 4: Effect of BG on various haematological parameters of rats in sub-chronic toxicity study

Treatment	Dose (mg/kg)	Haemoglobin (mg/dl)	TLC (X 10^3/µl)	Neutrophil (%)	Lymphocyte (%)	Eosinophil (%)	Monocyte (%)	Basophil (%)
Control	-	15.23±0.94	7.02±0.61	34.83±4.31	61.5±5.24	1.5±0.55	1.0±0.00	0.33±0.52
A	400	13.95±1.13	7.12±0.52	31.83±6.21	65.67±6.47	0.83±0.41	1.0±0.00	0.0±0.00
B	800	14.02±1.47	7.07±0.55	30.17±4.26	68.50±4.04	1.33±0.52	1.17±0.41	0.0±0.00

BG: *Brahmi ghrita*; *n*=12 animals in each group; Values are mean±SD; TLC – Total leukocyte count; % – Percentage

Table 5: Effect of BG on biochemical parameters of rats in sub-chronic toxicity study

Treatment	Dose (mg/kg)	S. Urea mg/dl	Creatinine mg/dl	Total Bil. mg/dl	Bil. Direct mg/dl	Bil. Ind. mg/dl	SGOT U/l	SGPT U/l	Total protein mg/dl	Alk. Phosph. U/l	Globulin mg/dl	Albumin mg/dl
Control	-	73.25±5.34	1.20±0.19	0.47±0.08	0.27±0.12	0.20±0.09	25.67±3.33	28.00±1.79	8.82±0.21	63.72±7.81	3.98±0.51	4.02±1.55
A	400	71.08±5.29	0.90±0.40	0.50±0.18	0.20±0.09	0.30±0.09	23.33±3.61	32.0±7.77	8.77±0.74	58.52±2.52	4.08±0.67	3.98±1.21
B	800	75.82±4.66	0.7±0.19	0.57±0.16	0.40±0.17	0.20±0.09	20.17±2.32	24.5±2.51	8.77±0.44	59.27±4.03	3.88±0.47	4.67±0.97

BG – *Brahmi ghrita*; *n* – Six animals in each group; SGOT – Serum glutamic-oxaloacetic transaminase; SGPT – Serum glutamic pyruvate transaminase; A. Phosphate – Alkaline phosphate values are mean±SD; mg/dl – Milligram/decilitre; u/l – Unit/litre

gene expression in human hepatoma cell.[30] *Brahmi* have significant protective effect against morphine-induced kidney damage in term of Serum urea and serum creatinine[31] and effect of *Shankhapushpi* on kidney may be counterbalanced by effect of *Vacha* i.e., *Shankhapushpi* increase Serum creatinine and decreases Serum Urea[32] and *Vacha* decreases creatinine and increases urea[33] to achieve normal function of kidney. Normal serum total protein, alkaline phosphatase and albumin signify healthy liver and normal condition of metabolic stage while normal level of blood urea nitrogen and creatinine indicate normal excretory function of kidney.[34]

These normal biochemical parameters indicate non-toxic aspects of *Brahmi ghrita* towards kidney and liver which was further confirmed by findings of histopathological examinations. Examination of section of kidney in *Brahmi ghrita*-treated rats show normal renal architecture. Renal glomerulii, collecting tubules, interstitial tissue and blood vascular channels were found in normal condition. Interstitium of kidney was devoid of any kind of necrosis or degeneration. In photomicrographs of *Brahmi ghrita*-treated rats liver, no focal or diffuse foci of necrosis of hepatocytes, and infiltration of chronic inflammatory cells were observed. *Brahmi ghrita*-treated rats showed normal lobular architecture of liver with normal central portal vein, radiating plates of hepatocytes and peripheral portal tracts composed of hepatic artery, bile ductule and distal portal vein.

CONCLUSION

In acute toxicity study mice do not showed abnormal behaviour for initial 4 hours after drug administration. In sub-chronic toxicity study, normal body weight increase of rats along with proportionate food and water intake signifies that *Brahmi ghrita* did not affect any physiological process adversely. In the same way all haematological, biochemical parameters as well as histopathological finding do not any abnormal finding. *Brahmi ghrita* (containing *Brahmi Swarasa*, *Vacha*, *Kushtha*, *Shankhapuspi* and *Puran Ghrita*) have a broad safety margin in experimental animals may be due interaction of ingredients.

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