Toxicology International, *29(4) : 493-505; 2022. DOI: 10.18311/ti/2022/v29i4/30296*

Online ISSN : 0976-5131; Print ISSN : 0971-6580

Toxicology International

Contents available at: www.informaticsjournals.com/index.php/toxi



Safety and Anti-Fatigue Activity of Ayurvedic Formulation *Shramahara Mahaakashaaya* - A Pre-Clinical Study

Bidhan Mahajon[1], A. Ramamurthy[2], Rupashri Nath[3], Manish K. Gautam[4]*, Neeladri Das[5] and Partha Roy[6]

[1]*Central Council for Research in Ayurvedic Sciences (CCRAS), Ministry of AYUSH, New Delhi - 110058, India*
[2]*PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur - 302002, Rajasthan, India*
[3]*Department of Roganidana and Vikritivigyana, Uttrakhand Ayurved University, Dehradun - 248001, Uttrakhand, India*
[4]*Faculty of Pharmaceutical Science, Assam down town University, Panikhaiti, Guwahati - 781026, Assam, India; manishpharmacology@gmail.com*
[5]*Molecular Endocrinology Laboratory, Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee - 247667, Uttarakhand, India*
[6]*Department of Biotechnology, Indian Institute of Technology Roorkee, Roorkee - 247667, Uttarakhand, India*

ARTICLE INFO

KEYWORDS: Acute Toxicity, Anti-Anxiety, Anti-Fatigue, *Shramahara Mahaakashaaya*

ARTICLE HISTORY
Received: 18.05.2022
Revised: 22.06.2022
Accepted: 21.11.2022

Abstract

An experimental evaluation of the anti-fatigue activity of *Shramahara Mahaakashaaya* (SM) and its applicability in sports medicine has been systematically evaluated. Aqueous, ethanolic and petroleum ether extracts of SM granules (200mg/kg) were studied for a light-dark model test for assessment of anti-anxiety effect, rota-rod test for assessment of the effect on muscle tone and muscle balance, weight loaded forced swimming test for assessment of anti-fatigue activity. The acute toxicity study of SM granules has also been evaluated as per the OECD 423. SM granules showed significant anti-fatigue activity in different experimental models and were found safe up to 2000mg/kg body weight dose. The results provide an important basis for considering *Shramahara Mahaakashaaya* (SM) as an alternative medicine having anti-fatigue effects which can be further included in sports medicine.

1. Introduction

As Ayurveda has recuperated its eminence and popularity in the recent era, society is looking to this science for its contribution to the field of sports medicine. Sports medicine is an interdisciplinary subspecialty of medicine that deals with the overall care of an athlete at all levels, both amateur and professional. The sports medicine team includes speciality physicians and surgeons, athletic trainers, physical therapists, coaches and other personnel[1,2].

**Author for correspondence*

The aim of sports medicine is to maintain, sustain and at times regain peak physical fitness i.e., adaptability to physical and mental stress. The main objective of sports medicine is to prevent the damage to the human system caused mostly by inactivity i.e lack of movement or hypokinesia due to sedentary habits of the modern world with various push button facilities, automation and lack of physical exercise or the damage caused to the sportsmen because of excessive training and load in the competitive world. The main functions of sports medicine are promotive, educative, formative, recreative, competitive, therapeutic and rehabilitative in nature. Sports medicine services may also be extended to the prevention of obesity, cardiac illness, cardiac rehabilitation, and prevention and control of diabetes[3]. Ayurveda is not only a system of medicine, it emphasizes the culture, behaviour and huge heritage of India regarding individual lifestyle. It is a time-tested science. The time-honoured Ayurvedic science has proposed many concepts and principles for the overall well-being of mankind. Most of the information available in the *Samhitas* are in coded language and deciphering codes is key to understanding the concept. *Shrama* (fatigue) is one such concept that requires critical analysis and interpretation in comparison with contemporary science[4]. In *Charakasamhita*, it is mentioned that *Shramahara Mahaakashaaya* (SM) has 10 drugs to alleviate *Shrama* (fatigue) or to increase the physical endurance capacity. viz. *Draakshaa, Kharjura, Priyaala, Badara, Daadimba, Phalgu, Parushaka, Ikshu, Yava* and *Shastika*[5]. Out of 10 drugs, 7 drugs are fruits with more nutritive value and other active principles. So to explore the utility of Ayurveda in the field of sports medicine and to assess physical and mental strength enhancement present study has been undertaken. An experimental evaluation of the anti-fatigue activity of SM and its applicability in sports medicine has been systematically evaluated. According to modern science *Shrama* (fatigue) is a subjective feeling of tiredness, that can be alleviated by periods of rest. It is of 2 types viz. physical and mental. Physical fatigue is the transient inability of a muscle to maintain optimal physical performance and is made more severe by intense physical exercise. Mental fatigue is a transient decrease in maximal cognitive performance resulting from prolonged periods of cognitive activity. Prolonged fatigue is a self-reported, persistent (constant) fatigue lasting at least one month. Chronic fatigue is a self-reported fatigue lasting at least six consecutive months[6]. Chronic fatigue may be either persistent or relapsing. Chronic fatigue is a symptom of many diseases and conditions similar to *Shrama*[7,8]. Several theories explain the mechanism of fatigue, such as the exhaustion theory suggests that during exercise, many energy sources, such as glucose and liver glycogen, will be exhausted, thus leading to physical fatigue[9]. Several reports showed that post-exercise nutrition through the administration of proteins, peptides or amino acids can facilitate recovery from fatigue[10].

The clogging theory interprets that the over-accumulation of serum Lactic Acid (LA) and Blood Urea Nitrogen (BUN) will also result in metabolic disorders leading to fatigue. The free radical theory suggests that intense exercise can produce an imbalance between the body's oxidation system and its anti-oxidation system. The accumulation of reactive free radicals will put the body in a state of oxidative stress and bring injury to the body by attacking large molecules and cell organs[11]. Based on the literature review the *sharamahara* action can be explained through the theories of fatigue and also it can be evaluated experimentally by various well-established animal models.

2. Material and Methods

2.1 Collection and Authentications of Plant Materials

All ten drugs of SM were collected from the market of Jaipur, Rajasthan, in July 2017. All the ingredients were identified and authenticated by standard taxonomical method at the PG Department of Dravyaguna Vigyana, National Institute of Ayurveda, Jaipur and further authentication was done at CSIR-National Institute of Science Communication and Information Resources, Raw Material Herbarium, Delhi (RHMD). All the Voucher specimens (Ref. No. NISCARE /RHMD /Consult/ 2017/3096-45-1, 2, 3, 4, 5, 6, 7, 8, 9, 10), dated 10/08/2017 were deposited for additional future references. The collected samples were thoroughly washed with distilled water and dried in the shed.

2.2 Preparation of Test Drug i.e., *Shramahara Mahakashaaya* Granules

All the dried ingredients i.e., *Draaksha [Vitis vinifera* L.(Vitaceae)], *Kharjura* [*Phoenix sylvestris* (L.)Roxb. (Arecaceae)], *Priyaala* [*Buchanania lanzan* Spreng. (Anacardiaceae)], *Badara* [*Ziziphus zizyphus* (L.) H.Karst. (Rhamnaceae)], *Daadimba [Punica granatum*

L. (Lythraceae)], *Phalgu* [*Ficuscarica* L. (Moraceae)], *Parushaka* [*Grewia asiatica* L. (Tiliaceae)], *Yava* [*Hordeum vulgare* L.(Poaceae)] and *Shashtika* [*Oryza sativa* L.(Poaceae)], 1 kg each, were taken and grinded in mixer grinder, then 1 litter juice of Ikshu [*Saccharum officinarum* L.(Poaceae)] was added in the grinded materials. Then the whole materials were dried in a hot air oven at 70°C temperature for 5 hours. Finally, the dried materials were run through the sieve plate to make them into granular form.

2.3 Preliminary Pharmacognostical Study

2.3.1 Loss on Drying at 105°C

10 g of test sample i.e., granules of SM was placed in a tarred evaporating dish. It was dried at 105°C for 5 hours in a hot air oven and weighed. The drying was continued until the difference between two successive weights was not more than 0.01g after cooling in desiccators. The percentage of moisture was calculated with reference to the weight of the sample.

2.3.2 Total Ash Estimation

2 g of the test sample was incinerated in a tarred platinum crucible at a temperature not exceeding 450°C until carbon-free ash is obtained. The percentage of ash was calculated with reference to the weight of the sample.

2.3.3 Total Insoluble Ash Estimation

25 ml of dilute HCl was added to the crucible containing total ash. The insoluble matter was collected on ashless filter paper (Whatmann 41) and washed with hot water until the filtrate was neutral. Then the filter paper containing the insoluble matter was transferred to the original crucible, dried on a hot plate and ignited to constant weight. Subsequently, the residue was allowed to cool in suitable desiccators for 30 minutes and weighed without delay. The content of acid-insoluble ash with reference to the air-dried drug was calculated.

2.4 Preparation of Extracts

The test samples were prepared as per the standard method mentioned in Ayurvedic Pharmacopeia of India (API). Accurately weighed 500g SM granules were separately taken in three different round-bottomed flasks. 12.5 Liter of distilled water was added in the first flask, 12.5 litres of ethanol was added in the second flask and 12.5 litres of petroleum ether was added in the third flask. Each flask was occasionally shaken for 6 hours and then allowed to stand separately for 18 hours. Then the contents were filtered and three different extracts were concentrated by distillation and solvents were removed by evaporation on a water bath separately. Each extract was completely dried under vacuum and marked as

Test sample I: Aqueous extract of SM granules.
Test sample II: Ethanol extracts of SM *granules*.
Test sample III: Petroleum ether extracts of SM granules.

2.5 Heavy Metals and Minerals Estimation

Heavy metals like Hg, Pb, Cd, and As and minerals like K, Ca, Na, Mg, and Fe were estimated by Inductively coupled plasma mass spectrometry (ICP-MS). First, the standards of different concentrations were run and then the sample i.e., extract of SM granules in a concentration of 5 mg/ml was added to the tube with different dilutions. The samples were digested with nitric acid prior to running in ICP-MS. The readings were taken in Perkin Elmer, USA (Model-ELAN DRC-e).

2.6 *In-vivo* Animal Experimental Study

2.6.1 Animal Species, Acclimatization and Husbandry

Wistar strain albino rats (120-250 g) and Swiss albino mice of either sex were obtained from the Institute of Biomedical and Industrial Research, Jaipur (Rajasthan). Females were nulliparous, non-pregnant and caged separately. Animals were acclimatized (may use acclimatized instead) to the laboratory environment for seven days before the onset of the experiment. The housing provided was having controlled lighting of 12:12h light and dark cycle, a temperature of 25°C and relative humidity of approximately 50%. All the animal experiments were carried out in Pharmacology Laboratory, Institute of Biomedical and Industrial Research, Jaipur (Rajsthan) prior obtained the permission of the Institutional Animal Ethics Committee. The experimental protocol was approved by the institutional animal ethical committee under the IAEC approval number: IBIR/IAEC/2016/IV/10 dated 20/11/2016, after approval of the proposal from the Institutional Ethics Committee, National

Institute of Ayurveda, Jaipur, No. IEC/ACA/2016/6 dated 29/08/2016.

2.6.2 Acute Oral Toxicity Study

An acute oral toxicity study was conducted according to OECD guideline 423[12]. A total of 18 Wistar albino rats of either sex, weighing between 120-250 g were selected randomly and divided into different groups, each group having three animals. The starting dose was selected from one of two fixed levels 300 mg/kg and 2000 mg/kg body weight. The test samples were administered in a single dose by gavages using an oral feeding needle. Animals were in overnight fasted condition before dosing. Following the period of fasting, the animals were weighed and the test sample was administered. After the administration of the test sample, food was withheld for a further 3-4 hours in rats.

Three animals were selected for each group. The dose level was as follows:

Group 1: 3 rats and dose was 300 mg/kg test sample I

Group 2: 3 rats and dose was 2000 mg/kg test sample I

Group 3: 3 rats and dose was 300 mg/kg test sample II

Group 4: 3 rats and dose was 2000 mg/kg test sample II

Group 5: 3 rats and dose was 300 mg/kg test sample III

Group 6: 3 rats and dose was 2000 mg/kg test sample III

Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 hours, with special attention given during the first 4 hours and daily thereafter, for a total of 14 days. All observations were systematically recorded with individual records, maintained for each animal. Additional observations were not necessary as the animals do not display any signs of toxicity. Observations included changes in skin and fur, eyes and mucous membranes and also respiratory, circulatory, autonomic and central nervous systems and somatomotor activity in addition to behaviour patterns. Attention was given to observations of tremors, convulsions, salivation, diarrhoea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document had been taken into consideration. All test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal. Microscopic examination of organs showing evidence of gross pathology in animals surviving after 14 days had been considered because it may yield useful information[13,14].

2.6.3 Light-Dark Model Test for Assessment Anti-Anxiety Effect of Study Drug

Thirty healthy adult Swiss albino mice of either sex, weighing between 25-45g were randomly selected. Females were nulliparous and non-pregnant. They were divided into 5 groups having 6 animals in each group of either sex.

Group 1: Normal Control: Distilled Water (DW) was provided in this Group.

Group 2: Standard Control: Diazepam (Calmpose) Injection 2ml/10mg [RANBAXY Laboratory Limited Solan; HP; Batch. No-9021854] was administered through IP.

Group 3: Test sample I in therapeutic dose (200 mg/kg body weight) was provided.

Group 4: Test sample II in therapeutic dose (200 mg/kg body weight was provided.

Group 5: Test sample III in therapeutic dose (200 mg/kg body weight) was provided.

All the test drugs were administered through oral gavaging. The test and standard drugs were administered for 15 consecutive days before the onset of the experiment. On the fifteenth day one hour after drug administration the experimental procedure was carried out. The testing apparatus consists of a light and a dark chamber divided by a photocell-equipped zone. A polypropylene animal cage, 44 x 21 x 21 cm, is darkened with black spray over one-third of its surface. A partition containing a 13 cm long 5 cm high opening separates the dark one-third from the bright two-thirds of the cage. The cage rests on an animal activity monitor which counts total locomotor activity. An electronic system using four sets of photocells across the partition automatically counts movements through the partition and clocks the time spent in the light and dark compartments. Animals were placed into the cage and then observed for 10 min. Time spent in the light chamber was considered the key to observation.

2.6.4 Rota-Rod Test for Assessment of the Effect of Test Drug on Muscle Tone and Muscle Balance

Thirty healthy adult Wistar Albino rats weighing between 120-250 g were randomly selected. Females were

nulliparous and non-pregnant. They were divided into 5 groups. Each group was 6 rats of either sex.

Group 1: Normal Control: Distilled Water (DW) was provided in this Group.

Group 2: Standard Control: Diazepam (Calmpose) Injection 2ml/10mg [RANBAXY Laboratory Limited Solan; HP; Batch.No-9021854] was administered through IP.

Group 3: Test sample I in therapeutic dose (200 mg/kg body weight) was provided.

Group 4: Test sample II in therapeutic dose (200 mg/kg body weight) was provided.

Group 5: Test sample III in therapeutic dose (200 mg/kg body weight) was provided.

All the test drugs were administered orally with the help of a feeding needle. The test and standard drugs were administered for 15 consecutive days before the onset of the experiment. On the fifteenth day one hour after drug administration the experimental procedure was carried out. The apparatus consists of a horizontal wooden rod or metal rod coated with rubber with a 3 cm diameter attached to a motor with the speed adjusted to 2 rotations per minute. The rod is 75 cm in length and is divided into 6 sections by plastic discs, thereby allowing the simultaneous testing of 6 rats. The rod is at a height of about 50 cm above the table top to discourage the animals from jumping off the roller. Cages below the sections serve to restrict the movements of the animals when they fall from the roller. Only those animals that demonstrated their ability to remain on the revolving rod for at least 2 minutes were selected for the test. Time spent in the Rota-rod by each animal was considered the key for evaluation.

2.6.5 Weight Loaded Forced Swimming Test (WFST)

Thirty healthy adult Swiss albino mice of either sex, weighing between 25-45g were randomly selected. Females were nulliparous and non-pregnant. Then each animal was marked with saturated Picric acid solution in water for proper identification and weighing separately. They were divided into 5 groups. Each group was 6 mice of either sex.

Group 1: Normal Control: Distilled Water (DW) was provided in this Group.

Group 2: Standard Control: Imipramine 10mg/kg body weight was administered.

Group 3: Test sample I in therapeutic dose (200 mg/kg body weight) was provided.

Group 4: Test sample II in therapeutic dose (200 mg/kg body weight) was provided.

Group 5: Test sample III in therapeutic dose (200 mg/kg body weight) was provided.

All the test drugs were administered orally with the help of a feeding needle. The test and standard drugs were administered for 15 consecutive days before the onset of the experiment. On the fifteenth day one hour after drug administration the experimental procedure was carried out. 1 h after the last oral administration, mice were placed in the swimming pool (50 X 50 X 40 cm) filled with fresh water approximately 30 cm deep so that mice could not support themselves by touching the bottom with their feet. A tin wire (5% of body weight) was reported and this arrangement forced the mouse to maintain continuous rapid leg movement. The swimming period was regarded as the time spent by the mouse floating in the water, struggling, until exhausted. The mice were assessed to be exhausted when they failed to rise to the surface of the water to breathe within a 10s period. At the end of the session, the mice were removed from the water, dried with paper towels and placed back in their home cages. After the end of the process, the mice were taken out from each group and anaesthetized with an intra-peritoneal injection of chloral hydrate. After anesthetization, blood was collected in heparinized tubes and tubes without anticoagulant by retro-orbital puncture. Each animal was tested to determine the concentration of various biochemical parameters like-Glucose (mg/dl), Lactic Acid (mmol/L), Blood Urea Nitrogen (mg/dl) and Creatinine kinase (U/l), in addition to Anti-Oxidant Enzyme Assays- Supra Oxide Dismutase (SOD), Catalase (CAT) and Glutathione Peroxidase (GPx) were studied by spectrophotometric method (Jasco V530, Jasco, Tokyo, Japan).

3. Results

3.1 Pharmacognostical Study and Heavy Metal Estimation in SM

Table 1 shows the information on physiological parameters like- loss on drying, total ash, acid insoluble ash and pH value of the SM granules. Similarly, to detect the heavy metals as well as minerals in the test samples ICP-MS was performed. Percentage of heavy metals like- Hg, As, Pb and Cd were estimated (Table 2) and the percentage of K, Ca, Na, Mg and Fe (Table 3) were also detected. All the constituents were found in the normal range and there is

Table 1. Physicochemical study of SM

Parameters	Data (n=3, %, w/w)
Loss On Drying	0.98
Total ash	2.598
Acid Insoluble Ash	0.46
Alcohol Soluble Extractive Water Soluble Extractive pH value	35.67 49.83 5.43

Table 2. Heavy metal of SM

Analyte	Mass	Net Intens. Mean	Conc.Mean	Conc. SD	Conc.RSD	Sample Unit
Hg	202	5769.161	**12.867**	0.10	0.8	ppb
As	75	2341.859	**3.402**	0.05	1.4	ppb
Pb	208	33969.674	**2.812**	0.01	0.4	ppb
Cd	114	4269.047	**2.296**	0.62	27.1	ppb

Table 3. Mineral estimation of SM

Analyte	Mass	Net Intens.Mean	Conc.Mean	Conc.SD	Conc.RSD	Sample Unit
K	39	238646239.431	92977.982	1298.71	1.4	ppb
Ca	44	890893.760	6787.085	49.07	0.7	ppb
Na	23	23970367.672	12818.902	195.37	1.5	ppb
Mg	24	18325346.433	12012.670	181.27	1.6	ppb
Fe	57	12133.150	113.910	0.56	0.5	ppb

no possible heavy metal toxicity in the test drug i.e., SM granules.

3.2 Acute-Oral Toxicity of SM with Blood Biochemistry and Histopathological Evaluation

The effect of different extracts (aqueous, ethanol and petroleum ether) of the *Sramahara Mahaakashaaya* (SM) on the behavioural observation of the rats were tested in 300 and 2000 mg/kg doses. There were no significant abnormalities observed in the case of test samples I (aqueous extract), II (ethanol extract) and III (petroleum ether extract). In accordance with the above identification, haematological analysis was carried out in different groups. Different blood parameters like- haemoglobin, WBC, RBC, neutrophils, lymphocytes, eosinophils, monocytes and basophils were evaluated. Table 4 shows the mean value of all blood parameters in different groups along with the range of normal conditions. It was clearly observed that there was no significant variation in any of the studied parameters even if in the case of higher dosage groups. To support the above studies, histopathological evaluation of both the liver and kidney was carried out. Figure 1 & 2 shows the histopathological images of both the liver and kidney of different groups. Normal hepatic cells with central vein, sinusoidal dilation, liver lobules and portal areas were observed in all groups. Similarly, in the kidney, normal architecture and tubular arrangement were observed in all groups. No histopathological abnormalities

Table 4. Effect of SM in the hematological parameters of experimental animal

S. no	Parameters	Value (Mean ± S.E.M)						Normal Range
		G 1	G 2	G 3	G 4	G 5	G 6	
1	Haemoglobin	14.54	13.58	14.25	15.47	13.21	13.87	11.5-16.1 grams per deciliter
2.	WBC	8.54	9.54	7.83	9.65	8.48	10.24	6.6-12.6 x $10^3/mm^3$
3.	RBC	7.45	8.47	7.82	8.96	9.14	8.64	6.76-9.75 x $10^6/mm^3$
4.	Neutrophils	2.45	1.96	2.98	3.14	2.48	2.98	1.77-3.38 x $10^3/mm^3$
5.	Lymphocytes	7.54	5.68	6.57	5.98	7.14	6.47	4.78-9.12 x $10^3/mm^3$
6.	Eosinophils	0.054	0.048	0.059	0.049	0.054	0.067	0.03-0.08 x $10^3/mm^3$
7.	Monocytes	0.025	0.015	0.023	0.019	0.026	0.031	0.01-0.04 x $10^3/mm^3$
8.	Basophils	0		0	0	0	0	0.00-0.03 x $10^3/mm^3$

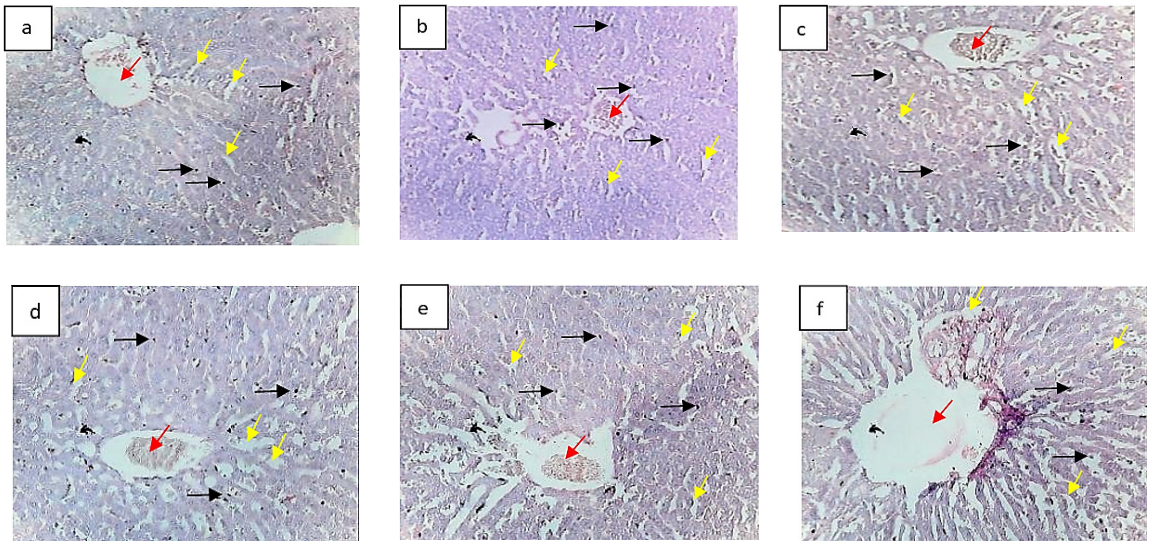


Figure 1. Histology of liver (H&E, 100x) of Group 1 **(a),** Group 2 **(b),** Group 3 **(c),** Group 4 **(d)**, Group 5 **(e)** and Group 6 **(f)**. Section of liver from all the groups showed normal hepatocytes (black arrow), hepatic sinusoid (yellow arrow), portal vein (red arrow) revealed, and normal architecture with granulated cytoplasm.

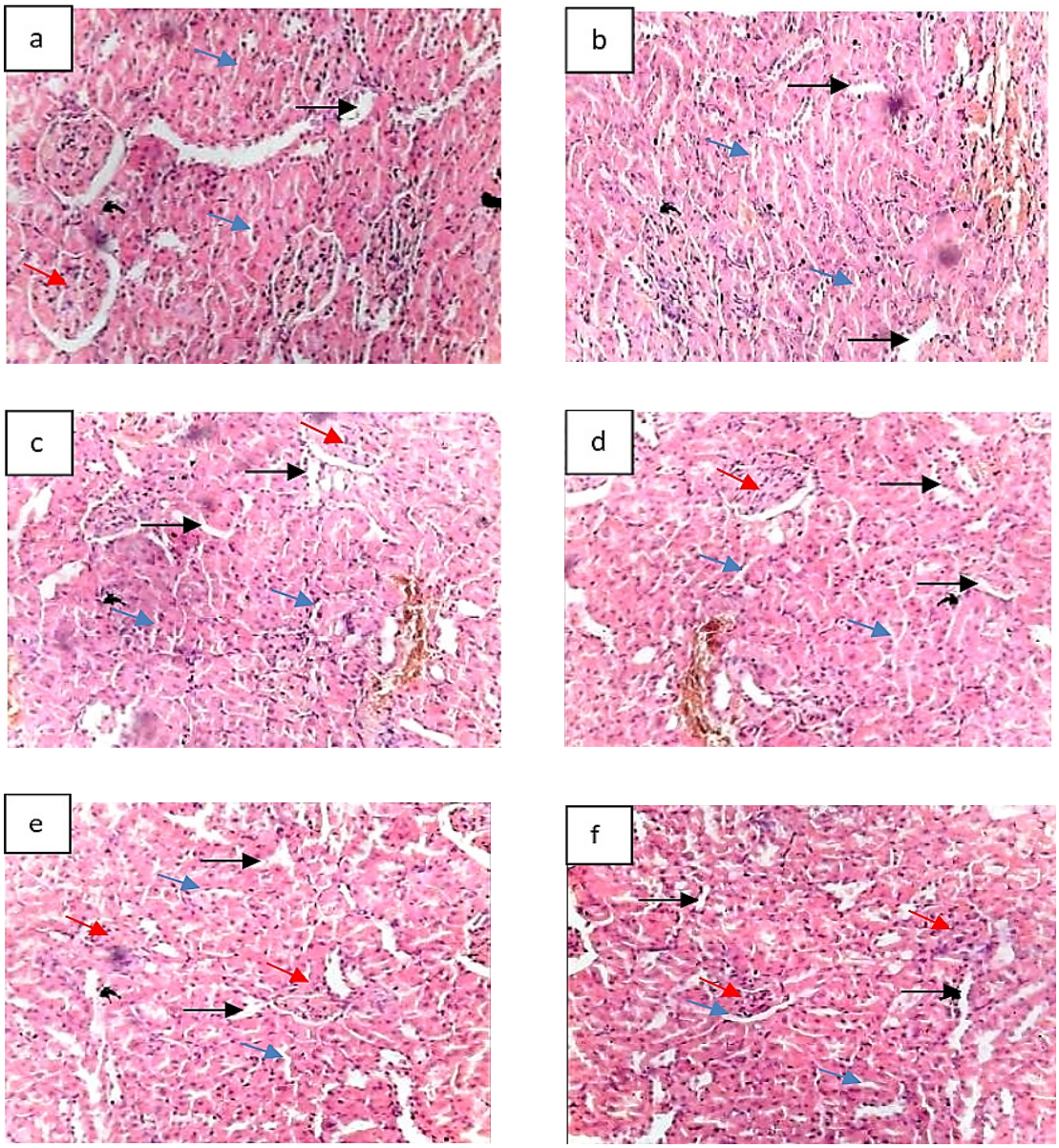


Figure 2. Histology of kidney (H&E, 100x) of Group 1 **(a),** Group 2 **(b),** Group 3 **(c),** Group 4 **(d),** Group 5 **(e)** and Group 6 **(f)**. Section of kidney from all the groups showed normal distal tubules (black arrow), proximal tubules (blue arrow), and size of glomeruli (red arrow) with normal tubules.

were observed in any groups which indicate that the drugs are safe even at a high dosage of 2000mg/kg.

3.3 Effect of SM on Light-Dark Model Test

In the Light-Dark Model experiment number of entries and time spent in the light chamber were evaluated. Table 5 shows the average number of entries in the light chamber as well as the average time spent in light. A significant improvement in time spent in the light chamber was observed in the test Group 4 ($p<0.05$) and Group 5 ($p<0.001$) compared to the control group (Group 1). However, Group 2 (Standard group) showed highly significant ($p<0.0001$) results on both the observed parameters (Table 5). To find out whether the result of the control group significantly differs from the test groups or not, one-way ANOVA was carried out followed by Dunnett's multiple comparisons 't'-test with post hoc test as $p<0.05$ on the parameters, - 'Number of entries in the light chamber'. Here the observed

Table 5. Effect of SM in Light and Dark Chamber Test

Groups	No. of entries in light chamber	Time spent in light chamber
Group 1	2.17±0.307	267.33±12.643
Group 2	5.67±0.422****	418.83±9.072****
Group 3	2.50±0.619^{NS}	338.00±20.118^{NS}
Group 4	2.67±0.333^{NS}	341.83±21.951*
Group 5	3.00±0.258^{NS}	374.67±19.692***

****P<0.0001; ***P<0.001; ** P<0.01; *P<0.05; NS= Not significant

p values were found 0.9407 for Test Group-3, 0.8004 for Test Group-4 and 0.4212 for Test Group-E compared to the control group (Group-1). All these observed data were found to be statistically not significant (p>0.05) except the standard group (Group-2) where the observed *p*-value was found to be statistically highly significant (*p* value-0.0001) compared to the control group.

A similar statistical analysis was done for the parameter-'Time spent in the light chamber'. The observed *p* values were found 0.0531 for Test Group-3, 0.0161 for Test Group-4 and 0.0006 for Test Group-E compared to the control group (Group-1). All these observed data were found to be statistically significant (p<0.05). In this case, also, the observed *p*-value of the standard group (Group-2) value was found to be statistically highly significant (*p* value-0.0001) compared to the control group.

3.4 Effect of SM on Rota-Rod Test

The rota-rod test was done to find out the endurance or the riding time of the test animals upon applying the test samples to evaluate the effect of the test drug on muscle tone and muscle balance. Table 6 shows the average time spent on the rota rod for all the groups. Here all the values of test groups were found to be statistically not significant (p>0.05) on the observed parameter-"Time spent on Rota rod (in a sec)", except the Group 2 (standard group) where observed data were found to be statistically highly significant (p<0.001). To prove the statistical significance as well as to check the performance among each of the treated groups compared to the control group, Dunnett's multiple comparisons tests were also done on the parameter- Time spent on Rota rod (sec).

3.5 Effect of SM on Weight Load Forced Swimming Test

Weight Loaded Forced Swimming Test (WFST) of the sample drug was conducted to verify the anti-fatigue effects of the different doses of the *Sramahara Mahaakashaaya* (SM). Table 6 shows the average swimming time of the animals in seconds. It was observed that test Group 3 (135.67±2.996) was found to be statistically significant (p<0.05) in comparison to the control group (Group 1).

Table 6. Effect of SM in Rota Rod Test and Weight Loaded Force Swimming Test

Groups	Time spent on Rota rod (sec) Mean ±SEM	Swimming Time in Sec (Mean ± SEM)
Group 1	51.83±3.081	118.50±4.808
Group 2	88.67±2.906****	158.67±5.708****
Group 3	57.33±4.137^{NS}	135.67±2.996*
Group 4	55.83±3.240^{NS}	120.33±3.602
Group 5	66.00±5.774^{NS}	108.00±3.347

****P<0.0001; *P<0.05; NS= Not significant

Data from other groups (Groups 4 and 5) were found to be statistically not significant (p>0.05). Whereas in Group 2 (standard group) statistical results showed highly significant (p<0.001) result compared to the control group. Dunnett's multiple comparisons test on 'Swimming Time in Sec' for the sample drug was performed and Data shows the result of one-way ANOVA followed by Dunnett's multiple comparisons 't'-test with post hoc test as p<0.05 on the parameter 'Swimming time in Sec'.

3.6 Effect of SM on histopathological study (biochemical parameters)

Analysis of various biochemical parameters like- Glucose (mg/dl), Lactic Acid (mmol/L), Blood Urea Nitrogen (mg/dl) and Creatinine kinase (U/l) was performed. Tables 7 show the changes in biochemical parameters in various groups. It was observed that the mean Glucose level was improved in Test Group-2, 3, 4 and 5 compared to the control group (Group 1). Another parameter Lactic Acid (mmol/L) was found to be decreased in all test groups (Test Groups 1, 2, 3, 4 and 5). Similarly, the observed parameters- Blood Urea Nitrogen (mg/dl) and serum creatinine kinase (U/l) were found to be decreased in all the test groups. Group 3 was found to have the most effective results in all the parameters, in which Blood Glucose (56.81 mg/dl) was the maximum, value for the Lactic Acid (9.58 mmol/L), Blood Urea Nitrogen (16.79 mg/dl) and serum creatinine kinase (386.50 U/l) was lowest among the other groups. Dunnett's multiple comparison 't'-test with post hoc tests on the various biochemical parameters (Glucose, Blood urea nitrogen, Creatinine kinase, Lactic acid) were conducted for the efficacy of the test drug show the statistical comparison among various groups for Glucose, Nitrogen, Creatinine and Lactic acid respectively. Anti-oxidant enzyme assays were also done to evaluate

Table 7. Effect of SM in Tissue biochemical parameter of experimental animals

Groups	Glucose (mg/dl)	Lactic Acid (mmol/L)	Blood Urea Nitrogen (mg/dl)	Creatinine kinase (U/l)
Group 1	30.22	11.99	21.00	499.50
Group 2	41.71	9.96	19.80	464.33
Group 3	56.81	9.58	16.79	386.50
Group 4	40.43	10.88	17.02	406.17
Group 5	31.72	11.45	17.02	480.00

Table 8. Effect of SM in Antioxidant enzyme assay of experimental animals

Parameter	Group 1 Mean ± SEM	Group 2 Mean± SEM	Group 3 Mean± SEM	Group 4 Mean± SEM	Group 5 Mean± SEM
Super Oxide Dismutase	3.50 ± 0.098	3.44 ± 0.063	3.29 ± 0.056	3.17 ± 0.077*	2.92 ± 0.132***
Catalase	53.29 ± 0.798	52.63 ± 0.503	52.34 ± 0.625	49.49 ± 0.807**	40.44 ± 0.742****
Glutathione Peroxidase	6.39 ± 0.317	5.74 ± 0.205	5.58 ± 0.252	5.56 ± 0.268	4.02 ± 0.137****

****P<0.0001; ***P<0.001; ** P<0.01; *P<0.05; NS= Not significant

the anti-oxidant effect of the test samples on the animal models. Table 8 shows the average values of enzymes-Super Oxide Dismutase (SOD), Catalase and Glutathione Peroxidase in all groups. The observed data on Super Oxide Dismutase of Test Group 4 (3.17 ± 0.077) and Group 5 (2.92±0.132) were found to be statistically significant (p<0.05) in comparison to the control group (Group-1). SOD values of other groups (Groups 3 and 2) were found to be statistically not significant (p>0.05). Similarly on the parameter Catalase, Group 4 (49.49±0.807**) and Group 5 (40.44±0.742****) were found to be statistically significant (p<0.05) in comparison to the control group (Group 1). Also, the observed data on the Glutathione Peroxidase test Group-5 showed a highly significant (P<0.0001) result in comparison to the control group.

4. Discussion

In the investigative research, the physiological manifestations of stress and fatigue are taken as the primary study condition for ensuring and asserting the anti-fatigue effect of the drug. To validate the anti-fatigue and antioxidant nature of the drug the animals were treated with test drugs and different anxiety and muscle fatigue-based studies were carried out. Initially, toxicity studies were done to determine the lethal dosage as well as the possible harmful effects of the drug on different physiological parameters. No anomalies in the drug-treated animals with respect to the control groups were observed. All the behavioural aspects were normal and the haematological parameters were within normal range.

To date anxiety in sports, a person hasn't been given that much attention but there is much evidence that understanding sports-related anxiety can help prevent injury and trauma, rehabilitation and return to sports[15]. It is a common problem faced by the sports person in addition to stress. This drug having the sportsmen as the primary target makes it important to analyze any anxiolytic effect it may have or not. For this purpose, the light-dark model animal test was performed to check the effect of the drug in reducing anxiety in mice wherein their aversions to brightly lit areas are exploited. Mice and rats tend to explore a novel environment, but to retreat from the aversive properties of a brightly-lit open field they tend to restrict themselves to a dark chamber[16]. The experiment points out that upon the drug administration the propensity of the mice to move into the brightly lit spaces and the quantity of time they were spending there vastly increased as opposed to the control group[17].

The rota-rod test performed on the animals is a quite prevalent test to judge the endurance of the rodents under episodes of intense exercise and physical activity. Skeletal muscle relaxation induced by a test compound could be evaluated by testing the ability of mice or rats to remain on a revolving rod, and in turn, the effect of the drug on motor coordination can be assessed[18]. The test drug in this case caused the mouse subjects to spend more time on the rotating rod, therefore indicating the anti-fatigue effect of the drug via increasing the endurance of the skeletal muscles and striving through physical stress. The forced swimming test is employed for evaluation of the potential anti-fatigue effect of the drug because it is very effective in evaluating the exercise endurance of animals and has a highly repeatable result[19]. The animals show a clear and conclusive increase in their physical ability all due to the increased stress tolerance upon administration of the drug because the swimming time remarkably increased in the test animals when compared with the control groups. As already mentioned, the fatigue in a physiological sense can be defined by three theories. The first one called "exhaustive theory" marked by the breakdown of liver glycogen into glucose increasing blood glucose levels[20]. The key to sustained physical strength in periods of intensive exercise is the maintenance of homeostasis in the blood glucose levels sustained hypoglycaemia can suppress the active brain functioning[21]. The analysis of the blood glucose of test drug-treated mice showed the ability of the drug to maintain basal glucose in circulation in drug-treated animal subjects actually validate the drug in alleviating fatigue in accordance with the exhaustive theory. Furthermore, the Creatinine Kinase (CK) enzyme is crucial for the rapid turnover of energy currency in times of exhaustive exercise. Previous research has confirmed the contribution of CK in fatigue development via heightened myoplasmic concentration of inorganic phosphate[22,23]. A marked reduced CK value in SM-administered subjects confirms the presence of the anti-fatigue effect of the test drug. Similarly, the second "clogging theory" is associated with the BLA and BUN levels. Continuous exercising causes the muscles to start respiring anaerobically, a process accompanied by the formation of lactic acid which leads to a condition of acidosis in muscles due to the lower than physiological pH of the by-product[24]. This imbalance can result in sub-optimal performance in turns of physical activity. The animals showed a significant

decrease in blood lactic acid levels. And hence the drug is somehow contributing to lowering the lactic acid in circulation, keeping the muscles in their normal healthy state.

The urea in the blood comes from the metabolism of protein and amino acids, which are basically the alternate sources of energy after carbohydrates and fats. Its elevated levels may indicate some renal dysfunction but similar spikes in BUN are seen in cases of stress and fatigue. However, this study shows a reduction in BUN levels as well under the effect of the drug. Hence proving that the integrity of the muscles is maintained even under stress and the metabolism of proteins directly contributes to the destruction and damage to the muscle tone. The drug thus inflicts a protective effect on the body under intensive exercise and protects against stress as defined by the clogging theory.

5. Conclusion

The above results provide an important basis for considering *SM* as an alternative medicine having anti-fatigue effects which can be further included in sports medicine. However, further research needs to be carried out to evaluate the effect of the drug on humans and the molecular mechanisms to be found.

6. Acknowledgment

The authors are thankful to the Department of Dravyaguna Vigyana, National Institute of Ayurveda (NIA), Jaipur, Rajasthan for assistance to carry out these studies.

7. References

1. Fu FH, Tjoumakaris FP, Buoncristiani A. Building a sports medicine team. Clin Sports Med. 2007, 26(2):173-179. https://doi.org/10.1016/j.csm.2006.12.003 https://doi.org/10.1016/j.csm.2006.12.003 PMid:17499620
2. Dave P, Vyas H, Vyas M. A pilot study on sports medicine in ayurveda with special reference to Sarira Bala Vriddhi. AYU. 2007; 28:3237. Available from: https://www.ayu-journal.org/article.asp?issn=0974-8520;year=2007;volume=28;issue=2;spage=32;epage=37;aulast=Dave;type=0
3. Mahajon B, Murthy AR. Relevance of *Shramahara mahakashaya* (An anti-fatigue formulation) in sports medicine. J Sci Innovative Res. 2018; 7(1):15-17. Available from: http://www.jsirjournal.com/Vol7_Issue1_04.pdf
4. Sir Williams M. Sanskrit-English dictionary. Varanasi: Mothilal Banarsidass Publications; 2005. p. 1096. Available from: https://www.mlbd.in/products/a-sanskrit-english-dictionary-etymologically-and-philologically-arranged-m-monier-williams-9788120831056-8120831055.
5. Acharya Trikamji Y. Agnivesha, Charakasamhita, Chaukhamba Sanskrit Sansthan, Varanasi; 2009. P. 34.
6. Jones JF, Nisenbaum R, Solomon L, Reyes M, Reeves WC. Chronic fatigue syndrome and other fatiguing illnesses in adolescents: A population-based study. J Adol Health. 2004; 35(1):34-40. https://doi.org/10.1016/j.jadohealth.2003.09.007 PMid:15193572
7. Mahajon B, Murthy AR. Applied knowledge of '*Shrama*' (Fatigue) in Sushruta Samhita. J Ayu Herbal Med. 2018; 4(2):76-78. Available from: http://www.ayurvedjournal.com/JAHM_201842_07.pdf
8. Mahajon B, Murthy AR. A comprehensive analysis on *shrama* (fatigue) in ancient transcript of charakasamhita. Global J Res Analysis. 2017; 6(6):346-347. https://doi.org/10.36106/gjra https://doi.org/10.36106/gjra
9. Jin HM, Wei P. Anti-fatigue properties of tartary buckwheat extracts in mice. Int J Mol Sci. 2011; 12(8):4770-4780. https://doi.org/10.3390/ijms12084770 PMid:21954324 PMCid:PMC3179131
10. Reilly T, Ekblom B. The use of recovery methods post-exercise. J Sports Sci. 2005; 23(6):619-627. https://doi.org/10.1080/02640410400021302 PMid:16195010
11. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clinical Int Aging. 2007; 2(2):219. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2684512/pdf/cia-2-219.pdf
12. OECD. Acute oral toxicity: Acute toxic class method. Test Guideline 423; 2001. Available from: https://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en
13. Gautam MK, Singh A, Rao CV, Goel RK. Toxicological evaluation of *Murraya paniculata* (L.) leaves extract on rodents. Am J Pharmaco Toxicol. 2012; 7(2):62-67. https://doi.org/10.3844/ajptsp.2012.62.67
14. Gautam MK, Goel RK. Toxicological study of *Ocimum sanctum* linn leaves: Hematological, biochemical, and histopathological studies. J Toxicol. 2014. https://doi.org/10.1155/2014/135654. PMid:24616736 PMCid:PMC3927567
15. Ford JL, Ildefonso K, Jones ML, Arvinen-Barrow M. Sport-related anxiety: current insights. Open Access J Sports Med. 2017; 8:205-212. https://doi.org/10.2147/OAJSM.S125845. PMid:29138604 PMCid:PMC5667788

16. Bourin M, Hascoët M. The mouse light/dark box test. Euro J Pharmacol. 2003; 463(1-3):55-65. https://doi.org/10.1016/s0014-2999(03)01274-3. PMid:12600702
17. Shimada T, Matsumoto K, Osanai M, Matsuda H, Terasawa K, Watanabe H. The modified light/dark transition test in mice: Evaluation of classic and putative anxiolytic and anxiogenic drugs. Gen Pharmacol. 1995; 26(1):205-210. https://doi.org/10.1016/0306-3623(94)00148-G PMid:7713361
18. Shiotsuki H, Yoshimi K, Shimo Y, Funayama M, Takamatsu Y, Ikeda K, Takahashi R, Kitazawa S, Hattori N. A rotarod test for evaluation of motor skill learning. J Neurosci Methods. 2010; 189(2):180-185. https://doi.org/10.1016/j.jneumeth.2010.03.026 PMid:20359499
19. Can A, Dao DT, Arad M, Terrillion CE, Piantadosi SC, Gould TD. The mouse forced swim test. J Visualized Exp. 2012; 59:e3638. https://doi.org/10.3791/3638 PMid:22314943 PMCid:PMC3353513
20. Coyle EF. Substrate utilization during exercise in active people. Am J Clinical Nutri. 1995; 61(4):968S-979S. https://doi.org/10.1093/ajcn/61.4.968S PMid:7900696
21. Wang B, Yan F, Cai L. Anti-fatigue properties of icariin from *Epimedium brevicornum*. Biomed Res. 2014; 25(3):297-302. Available from: https://www.alliedacademies.org/articles/antifatigue-properties-of-icariin-from-epimedium-brevicornum.html
22. Hody S, Rogister B, Leprince P, Wang F, Croisier JL. Muscle fatigue experienced during maximal eccentric exercise is predictive of the plasma Creatine Kinase (CK) response. Scand J Med Sci Sports. 2013; 23(4):501-507. https://doi.org/10.1111/j.1600-0838.2011.01413.x PMid:22107069
23. Dahlstedt AJ, Katz A, Wieringa BE, Westerblad H. Is creatine kinase responsible for fatigue? Studies of isolated skeletal muscle deficient in creatine kinase. The FASEB J. 2000; 14(7):982-990. https://doi.org/10.1096/fasebj.14.7.982 PMid:10783153
24. Wasserman K. Coupling of external to cellular respiration during exercise: The wisdom of the body revisited. Am J Physiol-Endocrinol Metabol. 1994; 266(4):E519-E539. https://doi.org/10.1152/ajpendo.1994.266.4.E519 PMid:8178973