IJPSR (2022), Volume 13, Issue 2 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 11 May 2021; received in revised form, 08 June 2021; accepted, 12 June 2021; published 01 February 2022

ACUTE AND SUB-ACUTE TOXICITY STUDIES OF THE SIDDHA PREPARATION MV KASHAYAMIN WISTAR RATS

S. P. Malarkannan [*1], P Dharumarajan [2] and Mkhariyohesh [3]

Department of Rural Development Science [1], Arul Anandar College, Karumathur, Madurai - 625514, Tamil Nadu, India.
Department of Indian Medicine and Homeopathy [2], Government Hospital, Aruppukottai, Virudhunagar Dist - 626101, Tamil Nadu, India.

Keywords:

Siddha preparation, MV kashayam, Acute and sub-acute toxicity, Biochemical, Histopathological study

Correspondence to Author:
Dr. S. P. Malarkannan

Department of Rural Development Science 1, Arul Anandar College, Karumathur, Madurai - 625514, Tamil Nadu, India.

E-mail:drspmalarkannan@gmail.com

ABSTRACT: The acute and sub-acute toxicity analysis of Siddha preparation MV kashayam was carried out with Wistar rats as per the guidelines 423 and 407of the Organization for Economic Co-operation and Development (OECD), respectively. In the acute toxicity study, a single dose of MV kashayam (1000 and 2000 mg/kg body weight) was administered orally to the rats and monitored for 14 days. In the sub-acute toxicity study, rats were orally administered with MV kashayam daily for 28 days at doses of 100 and 200 mg/kg body weight. In both the toxicity study, no rats have exhibited clinical signs of toxicity or mortality, and the doses were well tolerated by rats and no significant change in their mean body weight, food and water intake, haematological, biochemical parameters and his to pathological examinations as compared to that of control group rats. The findings suggest that MV kashayam has a wide margin of safety and a negligible amount of toxicity to ensure the safety and potency of the kashayam, which can be recommended to use as a novel prophylactic and therapeutic agent for COVID 19 based on the clinical study.

INTRODUCTION: Medicinal plants are used for the prevention and treatment of various diseases worldwide. The Indian subcontinent has an abundant variety of medicinal herbal plants and is evident from the Vedic era. Herbs are a rich source of diverse bioactive compounds that provide unlimited opportunities for the discovery of novel drugs and herbal remedies [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.13(2).912-20

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.13(2).912-20

The selection of medicinal plants for the discovery of new drugs and herbal remedies has mainly been facilitated by the wealth of knowledge stored in organized traditional medical systems around the world. Although a plethora of compounds from herbal medicinal plants have been isolated and their pharmacological properties have been reported, only a small percentage of them have gone through drug development processes providing therapeutic drugs for clinical applications.

According to a study carried out in 2001 by Fabricant and Farns worth, only a total of 122 distinct pure compounds from 94 plant species are reported to be in clinical use globally [2]. This scenario may be attributed to toxicity effects

exhibited by such compounds and safety challenges encountered during the drug development process. Therefore, it is important to ensure the safety and potency of medicinal plants, their preparations, and isolated compounds through well-controlled and validated scientific protocols for the development of novel drugs and herbal remedies [3, 4]. Moreover, toxicity studies play a significant role in the drug development process providing information on the limits of toxic dose and therapeutic indices of potential drugs. In this study, the acute and sub-acute toxicity studies of MV kashayam in Wistar albino rats has been investigated.

MATERIALS AND METHODS:

Chemicals and Reagents: All chemicals and solvents used for the study were of AR grade and purchased from Sigma-Aldrich. The biochemical parameters were analyzed with commercially available test kits (ALP, ALT, glucose, cholesterol, and urea) were purchased from Sigma-Aldrich. The preclinical study of acute and sub-acute toxicity of MV kashayam was conducted after obtaining due approval by the IAEC having approval No IAEC/ P. Dharmarajan /M.D(S)/KMCP/124/2020 from the Ethical committee of KM College of Pharmacy, Madurai.

Experimental Animals: Wistar albino rats (Species-Rattusnorvegicus), approximately 12 weeks old, weighing 160 g 180 g were purchased from the KM College of Pharmacy, Madurai, and utilized for toxicity studies. Animals were identified by colour markings on their body and housed in standard cages with sawdust as bedding material, and they were fed with a standard diet prepared according to a formula prescribed by Saboudry 5 and provided with water *ad libitum*. Rats were exposed to a 12 h light/dark cycle at room temperature. The rats were handled under the standard guidelines for the care and use of laboratory animals (CPCSEA guidelines). Rats were acclimatized to the above-mentioned conditions for one week before the toxicity studies.

Acute Oral Toxicity Study: Acute oral toxicity study of MV kashayam was performed using Wistar rats according to the Organization for Economic Co-operation and Development (OECD) guidelines 423 6. A total of 18 rats were segregated into three groups randomly, with six rats in each group used for this study, and their weight has been recorded [15]. In group I, normal control rats received vehicles only. In groups II and III, rats were administered with Siddha preparation MV kashayam orally under overnight fasting at a dose of 1000 and 2000 mg/kg body weight, respectively. The rats were maintained under the same conditions with normal food and water. They were observed individually for the first critical 4 h and thereafter twice daily (every day at 9.00 am and 3.00 pm) during the study period of 14 days for mortality, signs of toxicity (changes in the skin, fur, eyes, mucus membranes, respiratory depression) and behavioural changes (salivation, diarrhoea, sleep, coma, lethargy). Further, body weight changes, food, and water intake were also recorded during the study period. The percentage of body weight change was calculated according to the following equation 3.

Percentage body weight change = Body weight at the end of each week - initial body weight/ Initial body weight × 100

On the 14^{t} day, all the rats were kept fasting overnight. On the 15th day, they were weighed and sacrificed by overdose inhalation of the anesthetic ether. Blood samples were collected by cardiac puncture for haematological and biochemical analyses and followed by his pathological studies.

Biochemical Parameters and Haematology: Each blood sample was divided into two groups in two separate tubes, with and without the anticoagulant, Ethylenediamine-Tetraacetate (EDTA). The blood samples in the tubes without anticoagulant were kept for 2 h until complete clotting was observed. Afterward, the tubes were centrifuged at 4000 rpm at 4 °C for 10 min. Serum was then separated and subjected for analysis of biochemical parameters, alkaline phosphatase (ALP), alanine aminotransferase (ALT), urea, glucose, and cholesterol by standard methods using test kits with a Konelab auto analyzer. The blood samples in the tubes with the anticoagulant (EDTA) were immediately analyzed for haematological parameters (red blood count (RBC), haemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, white blood count (WBC), lymphocyte, monocyte, basophil, and neutrophil) using a haematological analyzer [7].

Sub-acute Toxicity Study: Sub-acute toxicity study of MV kashayam was carried out using Wistar rats according to the OECD guideline 4078. A total of 18 rats were used in the study. Their weights were recorded (periodically weekly once) and randomly divided into 3 groups, each containing 6 rats. The rats were kept fasting overnight and MV kashayam was orally administered to the rats at a dose of 100 mg and 200 mg/kg body weight. Respectively, separately daily for 28 consecutive days. Rats in group I was kept as the control, were administered with 5 ml/kg normal saline (vehicle) only.

The rats of all groups were maintained under the same conditions with normal fo**o**d and water. They were monitored for mortality, signs of toxicity, and behavioural changes twice daily (every day at 9.00 am and 3.00 pm) for 28 days. Bodyweight changes, food, and water intake, were also recorded from the onset of the study. On the 28th day, all the rats were kept fasting overnight. On the 29th day, they were weighed and sacrificed by an overdose inhalation of the anaesthetic ether. Blood samples were collected by cardiac puncture for haematological and biochemical analyses and followed by his pathological studies.

Statistical Analysis: All the qualitative data were expressed as mean ± Standard Error of Mean (SEM). Every statistical analysis was performed with a one-way analysis of variance (ANOVA) followed by the new Mann keels multiple range tests, using SPSS software.

RESULTS:

Acute Toxicity Study: In the acute toxicity study, there was no significant difference noticed between control and treatment groups by oral administration of a single dose of MV kashayam at the dose of 1000 and 2000 mg/ kg bodyweight and noticed no mortality in any of the treatment groups. Further, the experimental groups did not exhibit any visible signs of toxicity or behavioural changes **Table 1**. and were found to be normal throughout the study period of 14-day similar to the control. The mean body weights of the test and control group rats are given in **Table 2**. There was no significant difference in the mean body weight of the treated and control group.

TABLE 1: EFFECT OF ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BEHAVIOURAL CHANGES IN MICE

S. no.	Observation	GROUP-1	GROUP-2
		MV Kashayam (MVK) (1000 mg/kg)	MV Kashayam (MVK) (2000 Mg/kg)
1	Alertness	Normal	Normal
2	Grooming	Absent	Absent
3	Touch response	Absent	Absent
4	Torch response	Normal	Normal
5	Pain response	Absent	Absent
6	Tremors	Absent	Absent
7	Convulsion	Absent	Absent
8	Righting reflux	Present	Present
9	Gripping strength	Normal	Normal
10	Pinna reflux	Normal	Normal
11	Corneal reflux	Present	Present
12	Pupils	Normal	Normal
13	Urination	Normal	Normal
14	Salivation	Normal	Normal
15	Skin colour	Normal	Normal
16	Lacrimation	Normal	Normal
17	Hyperactivity	Absent	Absent

TABLE 2: EFFECTS OF ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BODY WEIGHT CHANGES IN RATS

Treatment	Day 1	Day 14
Control	180.60±4.10	186.20 ±4.75
Siddha formulation MV Kashayam 1000 mg.kg^{-1}	183.45 ±4.35	187.10 ±4.80
Siddha formulation MV Kashayam 2000 mg.kg^{-1}	184.80 ±4.55	188.80 ±4.95

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

Haematological and biochemical parameters were determined at the end of the 14-day study periods are presented in **Table 3** and **5** and b, respectively. The results of haematological parameters such as total red blood cell count (RBC), total white blood cell count (WBC), platelet count, haemoglobin, mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), and biochemical parameters such as serum urea, alkaline phosphatase (ALP), alanine amino-transferase (ALT), glucose and cholesterol levels of both treatment groups were similar to that of the control group.

TABLE 3: EFFECT OF ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON HAEMATOLOGICAL PARAMETERS SUCH AS HB, CALCIUM, RBC AND WBC IN RATS.

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	12.20±1.15	7.30± 0.85	9.40± 1.40	9.10 ±0.80
Siddha formulation MV Kashayam 1000 $mg.kg^{-1}$	12.70±1.30	7.85±0.92	9.65± 1.50	9.28 ±0.87
siddha formulation MV Kashayam 2000 $mg.kg^{-1}$	13.10±1.45	8.08±0.98	9.80± 1.64	9.42 ±0.94

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

TABLE 4: EFFECT OF ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BIOCHEMICAL PARAMETERS SUCH AS GLUCOSE, CHOLESTEROL, TRIGLYCERIDE, HDL AND LDLIN RATS

Treatment	Glucose ($mg.dl^{-1}$)	Cholesterol ($mg.dl^{-1}$)	Triglyceride ($mg.dl^{-1}$)	HDL ($mg.dl^{-1}$)	LDL ($mg.dl^{-1}$)
Control	86.45±3.15	30.42± 1.53	27.30±1.32	36.60±1.60	87.20±2.60
Siddha formulation MV Kashayam 1000 $mg.kg^{-1}$	83.15±2.85	32.30±1.65	20.60± 0.80	40.75±1.84	77.80±2.05
Siddha formulation MV Kashayam2000 $mg.kg^{-1}$	88.45±3.24	27.45± 1.33	23.80± 1.08	42.40±1.90	80.40±2.20

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

TABLE 5: EFFECTS OF ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BIOCHEMICAL PARAMETERS SUCH AS AST, ALT, ALP, TP AND ALBUMIN IN RATS

Treatment	AST ($IU.l^{-1}$)	ALT ($IU.l^{-1}$)	ALP ($IU.l^{-1}$)	TP ($g.l^{-1}$)	ALBUMIN ($g.l^{-1}$)
Control	295.3±5.10	73.7± 1.34	245.40± 5.20	70.40±2.60	37.30±1.50
Siddha formulation MV Kashayam 1000 $mg.kg^{-1}$	308.4±5.25	70.2± 1.22	252.10±5.35	73.10±2.87	40.35±1.65
Siddha formulation MV Kashayam 2000 $mg.kg^{-1}$	312.8±5.40	71.6±1.08	249.10±5.28	71.70±2.68	42.55±1.80

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

Sub-acute Toxicity Study: Mortality, General Signs of Toxicity and Food and Water Consumption of Rats. In the sub-acute toxicity study, oral administration of MV kashayam daily for 28 days at doses of 100, and 200 mg/kg body weight to rats did not result in any mortality. Similarly, the rats of any of the test groups did not show any visible signs of toxicity, such as changes in the skin, eyes, fur, and mucous membranes. Further, these rats did not show any behavioural changes, including salivation, sleep, coma, lethargy, and diarrhoea, and were found to be normal throughout the study period compared to the control group. The effect of MV kashayam on the mean body weight of rats in all test groups of sub-acute toxicity study is given in **Table 5**. According to the results, agradual increase in the mean body weight was observed in rats in experimental and control groups during the 28^{th} day. Weekly food consumption of experimental rats was similar to that of control group rats during the study period.

TABLE: 6 EFFECT OF SUB-ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BODY WEIGHT CHANGES IN RATS

Treatment	Day 1	Day 10	Day 20	Day 28
Group I- Control	188.85± 5.35	194.30 ± 5.48	197.35± 5.65	202.20 ± 5.70
GroupII- Siddha formulation MV kashayam100 $mg.kg^{-1}$	185.10 ±5.15	188.60 ± 5.40	192.30± 5.55	194.30 ± 5.75
GroupIII- Siddha formulation MV kashayam 200 $mg.kg^{-1}$	187.70± 5.30	190.50 ± 5.55	194.30± 5.65	198.40 ± 5.80

Haematology and Biochemical Parameters of Subacute Toxicity Study of MV Kashayam: The effect of oral administration of MV kashayam fed daily for 28 days in rats resultant effect on

haematological and biochemical parameters which were determined at the end of the study period in treated and control group rats are summarized in **Table 6** and **7** and **8** respectively. The haematological parameters of rats of all experimental groups were similar to those of control group rats. When considering biochemical parameters (serum urea, alkaline phosphatase (ALP), alanine aminotransferase (ALT), glucose, and cholesterol), there was no significant difference between the treated and control group rats.

TABLE 7: EFFECT OF SUB-ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON HAEMATOLOGICAL PARAMETERS IN RATS

Treatment	Haemoglobin ($mg.dl^{-1}$)	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium ($mg.dl^{-1}$)
Control	13.50±1.40	9.20± 0.75	10.60± 1.65	9.60 ±0.45
Siddha formulation MV kashayam 100 $mg.kg^{-1}$	13.80±1.65	8.78±0.68	9.40± 1.30	9.25 ±0.36
Siddha formulation MV kashayam 200 $mg.kg^{-1}$	13.95±1.80	9.05±0.72	9.60± 1.38	9.39 ±0.40

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

TABLE 8: EFFECT OF SUB-ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BIOCHEMICAL PARAMETERS- I (GLUCOSE, CHOLESTEROL, TRIGLYCERIDE, HDL AND LDL) IN RATS

Treatment	BIOCHEMICAL PARAMETERS - 1				
	Glucose ($mg.dl^{-1}$)	Cholesterol ($mg.dl^{-1}$)	Triglyceride ($mg.dl^{-1}$)	HDL ($mg.dl^{-1}$)	LDL ($mg.dl^{-1}$)
Control	97.38±3.70	35.25± 1.68	30.20±1.40	34.55±1.80	90.40±2.75
Siddha formulation MV kashayam100 $mg.kg^{-1}$	95.30±3.55	31.15±1.35	21.20± 0.84	45.35±1.87*	75.75±2.25
Siddha formulation MV kashayam200 $mg.kg^{-1}$	98.70±3.85	28.60± 1.20	19.20± 1.05	49.30±1.94*	72.60±2.18*

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III were *P<0.05

TABLE 9: EFFECTS OF SUB-ACUTE TOXICITY STUDY OF SIDDHA FORMULATION MV KASHAYAM ON BIOCHEMICAL PARAMETERS- II (AST, ALT, ALP, TP AND ALBUMIN) IN RATS

Treatments	BIOCHEMICAL PARAMETERS - II				
	AST ($IU.l^{-1}$)	ALT ($IU.l^{-1}$)	ALP ($IU.l^{-1}$)	TP ($g.l^{-1}$)	ALBUMIN ($g.l^{-1}$)
Control	328.2±9.40	76.4± 1.40	260.50± 5.25	73.50± 3.20	45.80±1.70
Siddha formulation MV kashayam100 $mg.kg^{-1}$	322.3±9.18	72.4± 1.18	267.30±5.55	75.40±3.38	38.20±1.30
Siddha formulation MV kashayam200 $mg.kg^{-1}$	316.5±9.00	70.8±1.05	265.50±5.45	78.20±3.55	41.30±1.45

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II and III.

His To Pathological Studies: The his to pathological sections of vital organs (liver and kidney) under light microscopic examinations of test and control group rats are shown in **Fig 1.** and **2**. On his to pathological analysis of tissue sections of vital organs in rats of all test groups showed a normal morphological architecture without any treatment-related pathological changes and were similar to that of control group rats.

Histopathology Study of Liver:

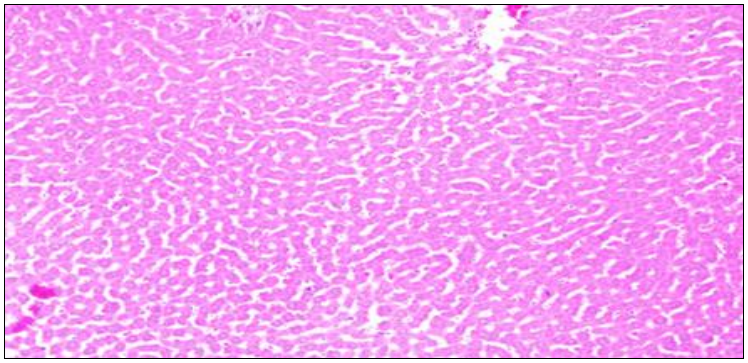

FIG. 1: SECTION OF NORMAL CONTROL ANIMALS (GROUP I) SHOWED NORMAL LIVER ARCHITECTURE WHICH WAS BROUGHT OUT THE CENTRAL VEIN, WERE PRESERVED CYTOPLASM AND PROMINENT NUCLEUS AND NUCLEOLUS

Histopathology Study of Kidney

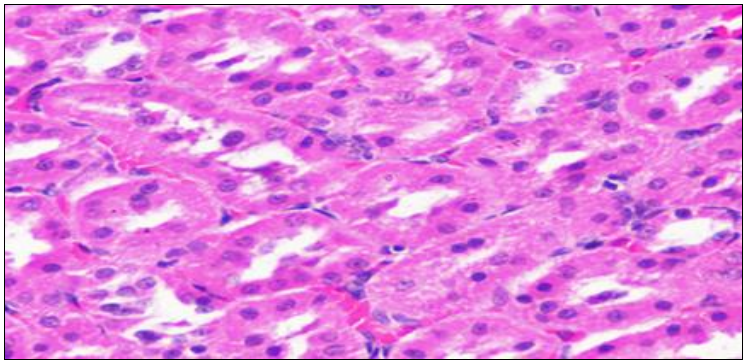

FIG. 2: SECTION OF NORMAL CONTROL ANIMALS (GROUP I) SHOWED THE STRUCTURE OF THE KIDNEY WITH GLOMERULI AND TUBULES, WHICH APPEAR NORMAL

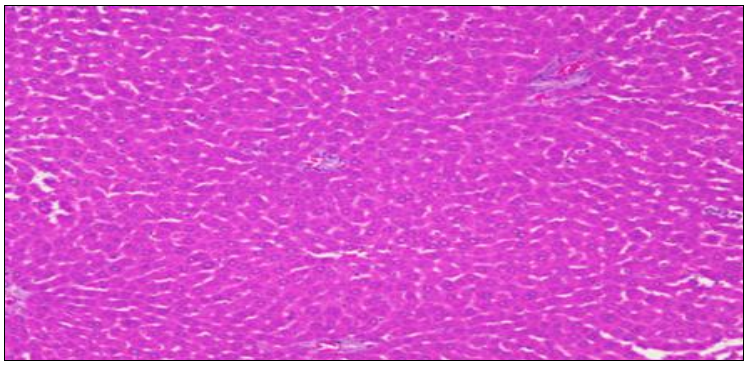

FIG. 3: TREATMENT CONTROL ANIMALS (GROUP 2, MV KASHAYAM 100MG/KG) SHOWED NORMAL LIVER ARCHITECTURE WITH WERE BROUGHT OUT THE CENTRAL VEIN, WERE PRESERVED CYTOPLASM AND PROMINENT NUCLEUS AND NUCLEOLUS

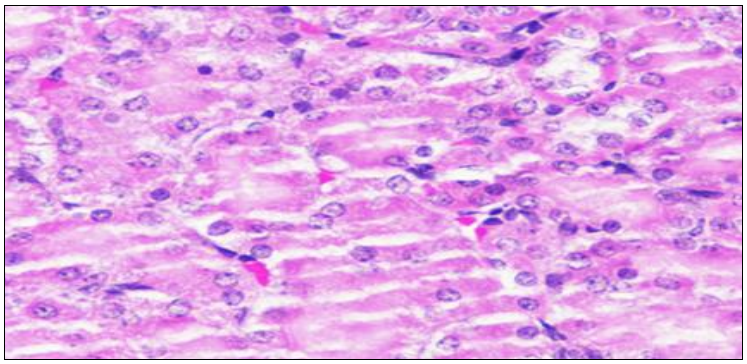

FIG. 4: TREATMENT CONTROL ANIMALS (GROUP 2, MV KASHAYAM 100MG/KG) SHOWED THE NORMAL STRUCTURE OF THE KIDNEY WITH GLOMERULI AND TUBULES, WHICH APPEAR NORMAL

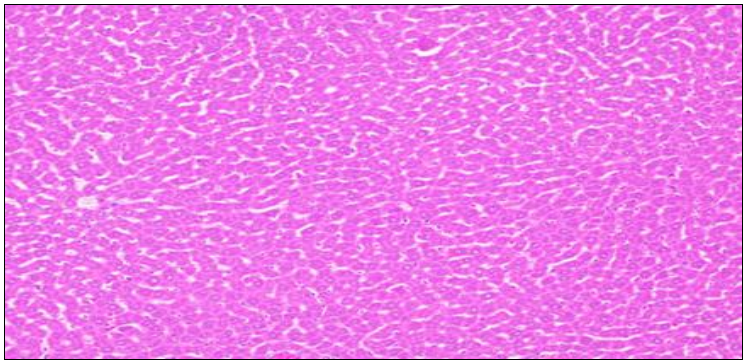

FIG. 5: TREATMENT ANIMALS (GROUP 3, MV KASHAYAM 200MG/KG) SHOWED NORMAL LIVER ARCHITECTURE WITH WERE BROUGHT OUT THE CENTRAL VEIN, WERE PRESERVED CYTOPLASM AND PROMINENT NUCLEUS AND NUCLEOLUS

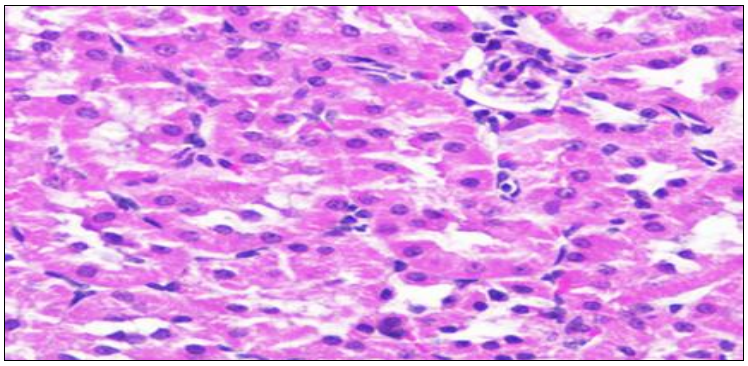

FIG. 6: TREATMENT ANIMALS (GROUP 3, MV KASHAYAM 200MG/KG) SHOWED THE STRUCTURE OF THE KIDNEY WITH GLOMERULI AND TUBULES, WHICH APPEAR NORMAL
FIG. 6 & 6: HIS TO PATHOLOGY OF LIVER AND KIDNEY BEFORE AND AFTER ADMINISTRATION OF MV KASHAYAM

DISCUSSION: Unusually, a higher dose of the test material is selected for the acute toxicity study. In this study, a limited dose of 2000 mg/kg body weight was selected as described in the OECD guidelines 423 6. A sub-acute toxicity study was performed according to the OECD guidelines 407. In this study, the highest dose, 200 mg/kg body weight which is 4x therapeutic dose, was selected to induce toxic effects, but no mortality or visible clinical signs of illness were found. Thereafter, two-fold intervals of the higher doses were selected for the setting of other descending dose levels [8].

In the acute toxicity study, rats treated at the dose level of 2000 mg/kg body weight did not reveal any mortality, visible signs of toxicity or behavioural changes similar to that of control group rats during 14 days of the study period. Similar observations were obtained for rats of all four treated groups in the sub-acute toxicity study of 28 days. Mortality and clinical signs of toxicity are not only important observations in toxicity studies and also include the indicators of toxicity effects induced by the test material [9].

The observation of bodyweight change is a sensitive indicator of the general health status of the animals in the toxicity study [10]. The bodyweight loss of animals more than 20% is considered critical according to CACC and OECD guidelines; this incident is defined as one of the human endpoints [11, 12]. In the acute toxicity study, there was no significant difference in the mean body weight of rats treated at the dose level 2000 mg/kg body weight compared to the control group rats during the study period. The percentage of body weight gain and the food intake of treatment group rats at the dose level of 2000 mg/kg body weight were similar to those of the control group rats at the end of the study period. In the sub-acute toxicity study, the mean body weight and percentage body weight gain of rats in all treatment groups were similar to those of the control group rats at the end of 7, 14, 21, and 28 days. Therefore, it can be suggested that the oral administration of MV kashayamto rats daily for 28 days at the above-mentioned dose levels did not produce any treatment-related body weight changes. Generally, there was no significant difference in the weekly food intake of these rats during the study period.

In toxicity studies, serum biochemistry analyses play a major role in evaluating the possible toxic effects induced by the oral treatment of the test material [13, 14]. There was no significant difference in biochemical parameters in rats of all treatment groups as compared to that of the control group in both acute and sub-acute toxicity studies. The serum biochemical parameters are important in the analysis of liver and kidney functions in the toxic evaluation of test materials as these organs are necessary for the survival of an organism, and they metabolzie and detoxify the organism from xenobiotic and endogenous compounds [15].

The liver is the major site of drug metabolism. It is considered the site of cholesterol synthesis, degradation, and disposal. The liver plays a key role in the synthesis of glucose, and it generates free glucose from hepatic glycogen stores [16]. As per the analysis, MV kashayam does not affect the lipid and carbohydrate metabolism since no significant changes were observed in glucose and cholesterol levels in rats of all treatment groups in both the

toxicity studies. Liver function tests such as serum alanine aminotransferase (ALT) and serum alkaline phosphatase (ALP) can be used to predict liver malfunction in toxicity studies. Elevated levels of ALP and ALT are indicative of liver disease or hepatotoxicity [17]. The levels of ALP and ALT in rats of both acute and sub-acute toxicity studies of treatment groups were similar to those of the control group rats. From these results, it is concluded that MV kashayam does not cause any acute toxicity effects in rats at the dose level of 2000 mg/kg body weight and sub-acute toxicity effects in rats at dose levels of 100 and 200 mg/kg bodyweight on the function of hepatocytes. Serum urea levels of rats can be used to assess renal dysfunction in toxicity studies. Non-significant changes were observed in serum urea levels in rats of all test groups in both toxicity studies may suggest that MV kashayam does not alter the normal kidney function.

In toxicity studies, haematology analyses also play a major role in evaluating the possible toxic effects induced by the oral treatment of the test material [13, 14]. Bone marrow is considered one of the most sensitive targets of toxic compounds. The status of bone marrow activity and the intravascular system of treated rats can be monitored by their haematological parameters [18]. Further, changes in the haematological system of treated rats have a higher predictive value for toxicity in humans compared to animals when data is extrapolated from animal studies [19]. In the acute (2000 mg/kg body weight) and sub-acute toxicity (100 and 200 mg/kg bodyweight for 28 days) study, all haematological parameters in rats treated with MV kashayam were similar to those of the control group rats. Considering no significant changes in all haematological parameters of treated rats compared to the control group rats in both toxicity studies, it is possible to suggest that the oral treatment of MV kashayam is nontoxic to haematological parameters.

The liver and kidney are primary organs, which are affected by metabolic reactions caused by toxic compounds [20]. The colour, texture, and hypertrophy of these internal organs are some of the initial indications of organ toxicity, induced by toxic compounds. Macroscopic examination of internal organs in rats of all test groups in sub-acute toxicity studies did not show any changes in colour and texture compared to the control group rats during necropsy. Further, no hypertrophy was observed in the internal organs of these rats. His pathological examinations of internal organs such as liver and kidney, of rats in all test groups in sub-acute toxicity studies showed normal cellular architecture and were similar to those of the control group rats. Sections of the liver of these rats showed no evidence of cellular injury, cholestasis or cell necrosis, and the arrangement of hepatocytes and lobular architecture was normal. His to pathological sections of renal tissues of these rats showed normal architecture with no evidence of glomerulosclerosis, interstitial inflammation, or parenchymal scarring.

Considering the results of the acute toxicity study, it is possible to suggest that a single dose of oral administration of MV kashayam to rats was well tolerated up to the dose level of 2000 mg/kg body weight. Therefore, it is possible to suggest that the LD50 of MV kashayam is above 2000 mg/kg body weight via the oral route. According to the Globally Harmonized System of Classification and Labeling of Chemicals under OECD guideline, 423, MV kashayam can be classified into category 5 (LD 50 > 2000 mg/ kg), which was the lowest toxicity class in this classification. According to the results of the sub-acute toxicity study, the oral administration of MV kashayam to rats daily at the rate of 100 and 200 mg/kg body weight dose levels for 28 days is safe.

CONCLUSION: Acute and sub-acute toxicity studies of MV kashayam in Wistar rats had no mortality/morbidity or signs of toxicity in treated rats during the acute toxicity study and it may be considered @ 2000 mg/ kg body weight as therapeutic dose as its LD50 is greater than 2000 mg/ kg body weight when administered through oral route. Observations made during the sub-acute toxicity study indicate that the long-term intake (28-days) of MV kashayam at tested dose levels, including the therapeutic dose rate, do not induce any toxic effects in experimental rats as compared to the control group rats. The findings suggest that MV kashayam has a wide margin of safety and a negligible amount of toxicity to ensure the safety and potency of the kashayam, which can be recommended to use as a novel prophylactic and

therapeutic agent for COVID 19 based on the clinical study.

ACKNOWLEDGEMENTS: The investigators' thanks to the Principal, Arul Anandar College, Karumathur, Madurai, and Tamilnadu State Council for Science and Technology, Chennai, for giving necessary permission and provided financial support to support to carry out the research work in a reputed institution.

CONFLICTS OF INTEREST: The authors have no conflicts of interest.

REFERENCES:

1. Ping KY, Darah I, Chen Y, Sreeramanan S and Sasidharan S: Acute and subchronic toxicity study of *Euphorbia hirta* L. methanol extract in rats. Bio-Med Research International 2013; 2013: 1-14.
2. Fabricant DS and Farnsworth NR: E value of plants used in traditional medicine for drug discovery. Environmental Health Perspectives 2001; 109(1): 69-75.
3. Al-Afifi NA, Alabsi AM, Bakri MM and Ramanathan A: Acute and sub-acute oral toxicity of Dracaena cinnabariresin methanol extract in rats. BMC Complementary and Alternative Medi 2018; 18(50): 1-14.
4. Bhushan B, Sardana S and Bansal G: Acute and sub-acutetoxicity study of Clerodendruminerme, Jasminummesnyi Hance and Callistemon citrinus. Journal of Acute Disease 2014; 3(4): 324-27.
5. Saboudry MA: Breeding and care of laboratory animals. world health organization. Health Laboratory Technology Unit Geneva Switzerland 1988.
6. Organization for Economic Co-operation and Development (OECD): Test no. 423: acute oral toxicity-acute toxic class method. x001De. OECD Guideline for Testing of Chemicals OECD Rome 2001.
7. Nadia Asyura SN, Hamzah H, Shaari RM, Sithambaram S and Mustapha NM: Blood profiles and his to pathological changes of liver and kidney tissues from male Sprague Dawley rats treated with ethanol extracts of *Clinacanthusnutan*s leaf. Journal of Clinical Toxicology 2016; 6(6): 1-10.
8. Organization for Economic Co-Operation and Development (OECD): repeated dose 28-day oral toxicity study in rodents x001De. OECD Guidelines for Testing of Chemicals OECD Paris France 1995.
9. Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY and Sasidharan S: Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Journal of Molecules 2011; 16(6): 5268-82.
10. Chitra B, Ramaswamy R and Suba V: Toxicity evaluation of pũrnacantirotayacentũram, a Siddha medicine in Wistar rats. International Scholarly Research Notices 2015: 473296: 1-10.
11. Canadian Council on Animal Care CCAC: Guidelines on choosing an appropriate endpoint in experiments using animals for research testing. 1998.
12. Organization of Economic Co-operation and Development: guidance document on the recognition, assessment, and use of clinical signs as humane endpoints for experimental animals used in safety evaluation 2000.
13. Yakubu MT, Akanji MA and Oladiji AT: Haematological evaluation in male albino rats following chronic administration of aqueous extract of Fadogiaagrestis stem. Pharmacognosy Magazine 2007; 3(9): 34-38.
14. Rao JV: Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromismossambicus*. Pesticide Biochemistry and Physiology 2006; 86(2): 78-84.
15. Olorunnisola OS, Bradley G and Afolayan AJ: Acute and subchronic toxicity studies of methanolic extract of Tulbaghia violacea rhizomes in Wistar rats. African Journal of Biotechnology 2012; 11(83): 14934-40.
16. Anderson N and Borlak J: Molecular mechanismsand therapeutic targets insteatosis and steatohepatitis. Pharmacological Reviews 2008; 60(3): 311-57.
17. Hilary JE, Israeli ZH and Lyoussi B: Acute and chronictoxicological studies of Ajugaivain experimental animals. Journal of Ethno Pharmacology 2004; 91(1): 43-50.
18. Mukunda JT and Syce JA: Acute and chronic toxicity of the aqueous extract of Artemisia afra in rodents. Journal of Ethno Pharmacology 2007; 112(1): 138-44.
19. Olson H, Betton G, Robinson D, Thomas K, Monro A, Kolaja G, Lilly P, Sanders J, Sipes G, Bracken W, Dorato M, Van Deun K, Smith P, Berger B and Heller A: Concordance of the toxicity of pharmaceuticals in humans and animals. Regulatory Toxicology and Pharmacology 2000; 32(1): 56-67.
20. Feldman BF, Zink JG and Jain NC: Schalm's veterinary hematology. lippincott williams and wilkins, 2002; philadelphia, Baltimore. New York London Buenos Aires Hong Kong Sidney Tokyo.

How to cite this article:
Malarkannan SP, Dharumarajan P and Mkhariyohesh: Acute and sub-acute toxicity studies of the siddha preparation mv kashayamin wistar rats. Int J Pharm Sci & Res 2021; 13(2): 912-20. doi: 10.13040/IJPSR.0975-8232.13(2).912-20.

All © 2022 are reserved by International Journal of Pharmaceutical Sciences and Research. This Journal licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 3.0 Unported License.

This article can be downloaded to **Android OS** based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)