Int. J. Curr. Res. Med. Sci. (2019). 5(10): 40-52

International Journal of Current Research in Medical Sciences

ISSN: 2454-5716

P-ISJN: A4372-3064, E -ISJN: A4372-3061

www.ijcrims.com

Original Research Article

Volume 5. Issue 10 -2019

DOI: http://dx.doi.org/10.22192/ijcrms.2019.05.10.005

Safety Assessment of Siddha Formulation Manokara Chooranam by Acute and 28-Day Sub-acute repeated dose toxicity Studies in Wistar Rats

P. Ramachandran[*1], W. Zafiroon Nisa[2], N. Anbu[3], D. Sivaraman[4]

[*1&2] P.G Scholar, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.

[3] Head, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai - 600 106, Tamil Nadu, India.

[4] Scientist, Centre for Laboratory Animal Technology and Research, Col.Dr.Jeppiaar Research Park, Sathyabama Institute of Science and Technology , Jeppiaar Nagar, Rajiv Gandhi road, Chennai - 600 119,Tamil Nadu, India.

Corresponding Author: **Dr. P.Ramachandran,** P.G Scholar, Department of Maruthuvam, Government Siddha Medical College, Arumbakkam, Chennai 600 106, Tamil Nadu, India.

Abstract

Majority of the world population both in the developed and developing countries are utilizing herbal medicines in situation were modern medicines fails to provide adequate cure in treating specific diseases. Siddha system of healing is one of the premier ancient practice that emerged from southern part of India now exist globally. Siddha preparations offer unlimited opportunities for the discovery of new drugs. Most of the natural products used in siddha folk remedy have solid scientific evidence with regard to their biological activities. But there is a dire need of effective mechanism to regulate formulation and quality standards to explore the potential at global level. However, there is little information or evidence available concerning the safety level of most of the siddha formulations. The major objective of the present study is to evaluate the safety nature of the siddha drug manokara chooranam (MNC) by short term (acute) and long term (sub-acute) toxicity studies in rodents at preclinical level.In the acute study, a single dose of 5000 mg/kg was orally administered and experimental animals were monitored for 14 days. In the sub-acute study, repeated doses (250 and 500 mg/kg/day) of the test drug MNC were administered for the period of 28 days and biochemical, hematological and histopathological parameters were evaluated.Results of acute toxicity revealed that there was no mortality up to a maximum dose of 5000 mg/kg at single dose administration. No significant changes were observed with respect to bodyweight and other clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities and neuro muscular coordination. In sub-acute toxicity study treatment with MNC at 250 and 500 mg/kg reveals no significant change in food/water intake, serology and hematology parameters in treated rats. Microscopic observation of vital organ further justifies the safety level of the drug MNC by projecting normal histo morphological architecture in rats treated with250 and 500 mg/kg. It was concluded from the observation that the drug MNC has wide margin of safety and may render therapeutic benefits upon clinical application even on long term basis.

Keywords: Siddha preparation, Manokara chooranam, Acute, Sub-acute toxicity, Biochemical, Hematological, Safety margin

1. Introduction

Medicinal plants based traditional systems of medicines are playing important role in providing health care to large section of population, especially in developing countries. Interest in them and utilization of herbal products produced based on them is increasing in developed countries also. To obtain optimum benefit and to understand the way these systems function, it is necessary to have minimum basic level information on their different aspects. Indian Systems of Medicine are among the well-known global traditional systems of medicine. In this review, an attempt has been made to provide general information pertaining to different aspects of these systems [1].

Plants are always the key source of drug or treatment strategy in different traditional medicinal systems. In recent years, many people are choosing to plant based medicines or products to improve their health conditions or as curative substance either alone or in combination with others. Siddha system of medicine majorly relies on ancient traditional preparations for treating several infectious and non-communicable diseases. As per the vedic literature it has been provoked that this method of treatment has emerged from southern region of India and progressed though out the world [2,3]. According to the WHO, herbs or herbal products are used by the large number of populations for basic healthcare needs. Herbal medicine includes herbs, herbal materials (like plant parts) or preparations, processed and finished herbal products, active ingredients [4,5].

Manokara chooranam is a novel siddha formulation indicated for rejuvenation and reproductive cell proliferation as per the standard siddha literatures. It consist of several herbs such as *Vitis venifera,Hygrophila auriculata,Saraca asoca, Cycas circinalis,Cinnamomam veram, Syzygium aromaticum, Crocus sativus, Trigonella foenum, Trianthema decandra ,Mesua ferrea, Borneo camphor, Tribulus terrestris, Costus specisous, Mucuna pruriens, Withaniya somminifera, Ziziphus mauritiania Cinnamomam tamala* and *Borassus flabellifer*.

In recent years, a huge resurgence of the use of herbal product due to the side effects of modern drugs, failure of modern therapies for against chronic diseases, and microbial resistance. It is estimated that nearly 75% of the plant based therapeutic entities used worldwide were included from traditional/folk medicine. In India, approximately 70% of modern drug are discovered from natural resources and number of other synthetic analogues have been prepared from prototype compounds isolated from plants [6,7].

Toxicity studies on herbal extracts are commonly used to evaluate the possible health risk of the intrinsic chemical compounds in the plant which could result in adverse effects from the plant [8]. Specifically, acute toxicity and LD_{50} determination have been described as initial steps in the toxicological evaluations of herbal medicine [9], and data from such evaluations provide comprehensive information on the toxicological classification and labelling of such compounds [10].Substances with LD_{50} values of ≥5000 mg/kg b.w. are said to be safe and practically nontoxic[11]. The main aim of the present research work is to evaluate the toxicological profiling of the siddha formulation Manokara chooranam in rodents by acute and sub-acute (repeated oral) toxicity study in accordance with OECD guideline.

2. Materials and Methods

2.1. Animal

Healthy adult Wistar albino rats were used for the study. The animals were housed in poly propylene cages and were kept in well ventilated with 100% fresh air supported by air handling unit (AHU). A 12 light / dark cycle were maintained. Room temperature was maintained between 22 ± 2˚Cand relative humidity 50–65%. They were provided with standard pelleted feed and water *ad libitum*. All the animals were acclimatized to the laboratory for 7 days prior to the start of the study. The experimental protocol was approved by The Institutional Animal Ethics Committee of Sathyabama Institute of Science and technology, Chennai, Tamil Nadu, India with the IAEC approval number: SU/CLATR/IAEC/X/090/2018

2.2. Acute toxicity Study

The animals were fasted overnight (08- 12 hrs) with free access to water. Study was conducted with single oral administration of study drug Manokara chooranam (MNC) at the dose of 5000mg/kg (p.o) to experimental rats. The animals were observed continuously for first 72 h and then 14 days for emerging signs of behavioral changes, body weight changes and for mortality.
Occurrence of toxicity in animals were observed continuously for the first 4 to 24 h and observed periodically for the next 14 days. Observation includes the change in skin, fur, eyes and mucus membrane. Appearance of C.N.S,C.V.S and A.N.S related toxicity such as tremors, convulsions, sedation, steric behavior, respiratory distress, cardiovascular collapse, response to sensory stimuli, salivation, diarrhea, lethargy, sleep, coma and mortality were observed with special attention [12].Body weight was recorded periodically. At the end of the experiment all animals were subjected to gross necropsy and observed for pathological changes.

2.3. Sub-Acute toxicity Study

Animals were allowed acclimatization period of 7 days to laboratory conditions prior to the start of treatment. The female rats used for the study were nulliparous and non-pregnant. The animals were randomly divided into control group and drug treated groups of 18 wistar albino rats (09 males and 09 females) were selected and divided into three groups. Each group consist of 06 animals (03 Males and 03 Females). First group served as a control and other two groups were treated with test drug MNC (250 and 500 mg/kg/day) for 28 days.

The rats were weighed periodically and observed for signs of toxicity pertains to C.N.S, C.V.S, A.N.S including behavioral changes, food - water intake and morphological changes. At the end of 28^{th} day, the animals were fasted for overnight with free access to water. On 29^{th} day the animals were sacrificed with excess dose of anesthesia as listed in the CPCSEA annexure. Blood samples were collected from aorta and stored in EDTA (ethylenediamine –tetra acetate) for Hematological analysis and for serum generation for biochemical analysis. The vital organs were harvested and carefully examined for gross lesions. The organs were preserved in 10% formalin for histopathological assessment and interpretation [13].

2.4. Hematological analysis

Blood samples were analyzed using established procedures using automated mindray hematology analyzer 2800. Parameters evaluated includes Packed Cell Volume (PCV), Red Blood Cells (RBC) count, White blood cell count (WBC), Platelet Count, Hemoglobin (Hb), Mean cell Haemoglobin Concentration (MCHC), Mean Red Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean platelet volume (MPV), Neutrophils, Eosinophil's, Basophils, Lymphocytes and Monocytes.

2.5. Biochemical analysis [14]

Serum samples were analyzed for High Density Lipoprotein (HDL), Low density Lipoprotein (LDL), Very low density Lipoprotein (VLDL), Triglycerides (TGL), Total Cholesterol, Blood urea nitrogen (BUN), Creatinine, Albumin, Total Protein, Glucose, Uric acid, Aspartate Transaminase (AST), Alanine amino Transaminase (ALT) and Alkaline phosphatase (ALP) using Mind ray auto analyzer model BS 120.

2.6. Histopathological evaluation [15]

Vital organs were harvested and the histological slides of organs were made and observed under the microscope. The pathological observations of cross section of these organs were performed on gross and microscopic analysis. Histological examinations were performed on the preserved tissues with particular emphasis on those which showed gross pathological changes.

2.7. Statistical analysis[16]

The statistical analysis will be carried by one-way ANOVA (GRAPH PAD PRISM 5 computer program). Results were expressed as mean ± standard error. A statistical comparison was

carried out using the Dunnet's test for the control and treatment group. P-values less than 0.05 were set as the level of significance.

3. Results

3.1. Assessment of clinical signs in rats treated with MNC on Acute toxicity study

The dose of MNC used for acute toxicity study is 5000mg/kg is higher than the normal therapeutic dose. No mortality observed at this dose level, further no significant change with respect to clinical signs on acute toxicity observed for (24-48 h) and a long period (14 days). The results were tabulated in Table 1.

Table 1: Clinical signs in rats on Acute toxicity study

Clinical Signs Parameters for the duration of 14 days	Test Drug 5000mg/ Kg
Lacrimation	Absence
Salivation	Absence
Animal appearance	Normal
Tonic Movement	Absence
Clonic Movement	Absence
Laxative action	Absence
Touch Response	Normal
Response to Sound	Normal Response
Response to Light	Normal Response
Mobility	Normal Response
Respiratory Distress	Nil
Skin Color	Normal
Stereotype behavior	Absence
Piloerection	Absence
Limb Paralysis	Absence
Posture	Normal
Open field behavior	Normal
Giat Balancing	Normal
Freezing Behaviour	Absent
Sings of Stress and Anxiety	None Observed
Muscular coordination	Normal
Muscle grip	Normal
Sedation	Absence
Social Behavior	Normal
Urine Analysis	No Abnormality
Urine Colour	Yellowish
Urine pH	6
Urine -Glucose	Absence
Urine -Ketones	Absence
Urine- Bilirubin	Absence
Urine-Blood Cells	Negative
Urine - Pus cells	Negative
Mortality	Nil

3.2. Quantitative data on the body weight of rats treated with MNC in Acute toxicity study

No significant change was observed in body weight of female rats treated with MNC at the dose of 5000mg/ kg. The results were tabulated in Table 2.

Table 2: Body weight of rats in Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	Final Body Weight (After Treatment)
MNC 5000 mg/kg	182.3 ± 2.251	185.8 ± 2.483

Values are mean ± S.D (n = 6 per group).

3.3. Fecal Pellet consistency analysis of rats treated with MNC in acute and sub-Acute toxicity study

Rats of control and treatment group were allowed to explore to open field on clean and sterile Stainless steel tray. The collected pellets were analyzed for consistency, color, Shape, Presence of blood cells etc. The results were tabulated in Table 3.

Table 3: Fecal Pellet consistency analysis of rats in acute and sub-Acute toxicity study

Acute Toxicity Study	
Analysis	**MNC**
Consistency	Soft
Shape	Round Headed
Colour	Brownish Green
Mucous Shedding	Absent
Blood Cells	Absent
Signs of Infection	None Observed

Sub-Acute Toxicity Study			
Analysis	**Control**	**Low Dose**	**High Dose**
Consistency	Rigid	Soft	Soft
Shape	Oblong	Round Headed	Round Headed
Colour	Greenish	Pale greenish	Pale greenish
Mucous Shedding	Absence	Absence	Absence
Blood Cells	Absent	Absent	Absent
Signs of Infection	None Observed	None Observed	None Observed

3.4. Assessment of clinical signs in rats treated with MNC on Sub-Acute toxicity study

The dose of MNC used for sub-acute toxicity study is 250 and 500 mg/kg. No mortality observed at this dose level, further no significant change with respect to clinical signs on sub-acute toxicity observed for the period of 28 days. The results were tabulated in Table 4.

Table 4: Clinical signs of rats in Sub-Acute toxicity study

Clinical Signs Parameters for the duration of 28 days	Control	MNC 250 mg/kg	MNC 500 mg/kg
Lacrimation	Absence	Absence	Absence
Salivation	Absence	Normal	Normal
Animal appearance	Normal	Absence	Absence
Tonic Movement	Absence	Absence	Absence
Clonic Movement	Absence	Absence	Absence
Laxative action	Absence	Normal	Normal
Touch Response	Normal	Normal Response	Normal Response
Response to Sound	Normal Response	Normal Response	Normal Response
Response to Light	Normal Response	Normal Response	Normal Response
Mobility	Normal Response	Normal Response	Normal Response
Respiratory Distress	Nil	Nil	Nil
Skin Color	Normal	Normal	Normal
Stereotype behavior	Absence	Absence	Absence
Piloerection	Absence	Absence	Absence
Limb Paralysis	Absence	Absence	Absence
Posture	Normal	Normal	Normal
Open field behavior	Normal	Normal	Normal
Giat Balancing	Normal	Normal	Normal
Freezing Behaviour	Absent	Absent	Absent
Sings of Stress and Anxiety	None Observed	None Observed	None Observed
Muscular coordination	Normal	Normal	Normal
Muscle grip	Normal	Normal	Normal
Sedation	Absence	Absence	Absence
Social Behavior	Normal	Normal	Normal
Urine Analysis	No Abnormality	No Abnormality	No Abnormality
Urine Colour	Yellowish	Yellowish	Yellowish
Urine pH	7	7	7
Urine - Glucose	Absence	Absence	Absence
Urine -Ketones	Absence	Absence	Absence
Urine- Bilirubin	Absence	Absence	Absence
Urine-Blood Cells	Negative	Negative	Negative
Urine - Pus cells	Negative	Negative	Negative
Mortality	Nil	Nil	Nil

3.5. Effect of MNC on Body weight of Rats in Sub-acute toxicity study

No significant change was observed in body weight of both male and female rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w.The results were tabulated in Table 5.

Table 5: Body weight of rats in Sub-Acute toxicity study

Dose	Body weight in gms	
	Initial Body Weight (Before Treatment)	Final Body Weight (After Treatment)
Control	184.5 ± 4.37	211.5 ± 42.02
MNC 250 mg/kg	184.7 ± 4.676	188.5 ± 6.091
MNC 500 mg/kg	183.2 ± 3.545	188.2 ± 4.708

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.6. Quantitative data on the food and water intake of rats treated with MNC for 28 days in Sub-acute toxicity study

No statistically significant differences were recorded in food and water intake observation of rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 6.

Table 6: Food and water intake of rats in Sub-acute toxicity study

Dose	Average Food and Water Intake	
	Food Intake in gms	Water intake in ml
Control	14.83 ± 1.722	25.33 ± 1.211
MNC 250 mg/kg	14.67 ± 3.502	27.67 ± 3.983
MNC 500 mg/kg	14.83 ± 3.189	26 ± 5.899

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.7.Effect of MNC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 7.

Table 7: Hematological parameters of rats in Sub-acute oral toxicity study

Group	RBC ($\times 10^6$ µl)	WBC ($\times 10^3$ µl)	PLT ($\times 10^3$ µl)	HGB (g/dl)	MCH (pg)	MCV (fl)
Control	6.517 ± 0.7468	10.37 ± 1.479	418.3 ± 84.88	10.73 ± 4.054	19.13 ± 1.655	63.63 ± 4.752
MNC 250 mg/kg	6.633 ± 0.8847	6.383 ± 1.214	740.7 ± 170.2	11.8 ± 1.176	20.27 ± 2.473	56.17 ± 5.867
MNC 500 mg/kg	7.467 ± 0.5854	8.35 ± 1.962	591.2 ± 168.5	11.77 ± 1.256	19.98 ± 2.066	55.67 ± 4.266

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.8.Effect of MNC on Hematological parameters of rats in Sub-acute oral toxicity study

No statistically significant differences were recorded in hematological parameters of rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 8.

Table 8: Hematological parameters of rats in Sub-acute oral toxicity study

Group	Neutrophils 10^3 /mm ^{3}	Eosinophils (%)	Basophils (%)	Lymph (%)	Mon (%)
Control	2.15 ± 0.6979	1.55 ± 0.251	0 ± 0	80.2 ± 8.415	2.25 ± 0.8313
MNC 250 mg/kg	2.45 ± 0.8826	1.517 ± 0.2787	0.1667 ± 0.4082	72.27 ± 9.07	3.383 ± 0.9621
MNC 500 mg/kg	2.617 ± 0.4167	1.133 ± 0.1033	0 ± 0	80.12 ± 5.91	3.15 ± 1.792

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.9.Effect of MNC on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 9.

Table 9: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	Neutrophils 10^3 /mm ^{3}	Eosinophils (%)	Basophils (%)	Lymph (%)	Mon (%)
Control	2.15 ± 0.6979	1.55 ± 0.251	0 ± 0	80.2 ± 8.415	2.25 ± 0.8313
MNC 250 mg/kg	2.45 ± 0.8826	1.517 ± 0.2787	0.1667 ± 0.4082	72.27 ± 9.07	3.383 ± 0.9621
MNC 500 mg/kg	2.617 ± 0.4167	1.133 ± 0.1033	0 ± 0	80.12 ± 5.91	3.15 ± 1.792

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one way ANOVA followed by Dunnett's test.

3.10.Effect of MNC on Serum Bio-chemistry profile of rats in sub-acute toxicity study

No statistically significant differences were recorded in serum biochemistry parameters of rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 10.

Table 10: Serum Bio-chemistry profile of rats in Sub-acute oral toxicity study

Group	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	TG (mg/dl)
Control	138.1 ± 7.627	59.83 ± 3.251	63.17 ± 6.242	15.08 ± 1.388	28.17 ± 4.262
MNC 250 mg/kg	134 ± 13.92	67 ± 7.457	52.33 ± 17.6	14.65 ± 3.182	38.17 ± 7.834
MNC 500 mg/kg	133.3 ± 20.77	61.83 ± 9.988	57.33 ± 20.58	14.13 ± 2.292	41.33 ± 7.659

Values are mean ± S.D (n = 6 per group of which 3 males and 3 females). Control and treatment groups were compared statistically using one-way ANOVA followed by Dunnett's test.

3.11. Quantitative data on absolute Organ weight of male rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of male rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 11.

Table 11: Quantitative data on absolute Organ weight of male rats in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Testes
Control - Male	1.425 ± 0.1886	0.762 ± 0.1525	1.7 ± 0.7264	1.379 ± 0.5352	6.824 ± 1.46	0.69 ± 0.4311	1.586 ± 0.2905	1.9 ± 0.5
MNC 250mg/kg - Male	1.527 ± 0.1026	0.38 ± 0.06245	1.17 ± 0.04583	1.163 ± 0.07024	4.827 ± 0.993	0.58 ± 0.2022	0.9567 ± 0.2579	1.76 ± 0.21
MNC 500mg/kg - Male	1.523 ± 0.07234	0.52 ± 0.04359	1.217 ± 0.2871	1.157 ± 0.2747	4.98 ± 0.737	0.62 ± 0.07	0.9367 ± 0.2325	1.723 ± 0.3995

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically

3.12. Quantitative data on absolute Organ weight of female rats belongs to control and drug treated group in sub-acute toxicity study

No statistically significant differences were recorded in organ weight of female rats treated with MNC at low and high dose of 250 and 500 mg/ kg b.w. The results were tabulated in Table 12

Table 12: Quantitative data on absolute Organ weight of female rats in sub-acute toxicity study

Group	Brain	Heart	Lung	Stomach	Liver	Spleen	Kidney	Uterus	Ovaries
Control - Female	1.75 ± 0.07835	0.9737 ± 0.09408	1.994 ± 0.4402	1.787 ± 0.09815	5.126 ± 0.6717	0.956 ± 0.1266	1.335 ± 0.08231	0.7957 ± 0.1555	0.4473 ± 0.04244
MNC 250mg/kg - Female	1.523 ± 0.07234	0.5233 ± 0.08083	1.323 ± 0.2065	1.2 ± 0.1908	4.347 ± 0.6901	0.45 ± 0.1825	0.8767 ± 0.2669	1.037 ± 0.04041	0.3033 ± 0.05508
MNC 500mg/kg - Female	1.58 ± 0.1386	0.4367 ± 0.1041	1.027 ± 0.2804	1.323 ± 0.1069	4.447 ± 0.6413	0.7267 ± 0.05859	0.7833 ± 0.1185	1.21 ± 0.1212	0.06333 ± 0.01528

Values are mean ± S.D (n = 3 per group). Control and treatment groups were compared statistically

3.13. Effect of MNC on Histopathological changes of Male rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to male rats presenting the following architecture as shown in figure 1.

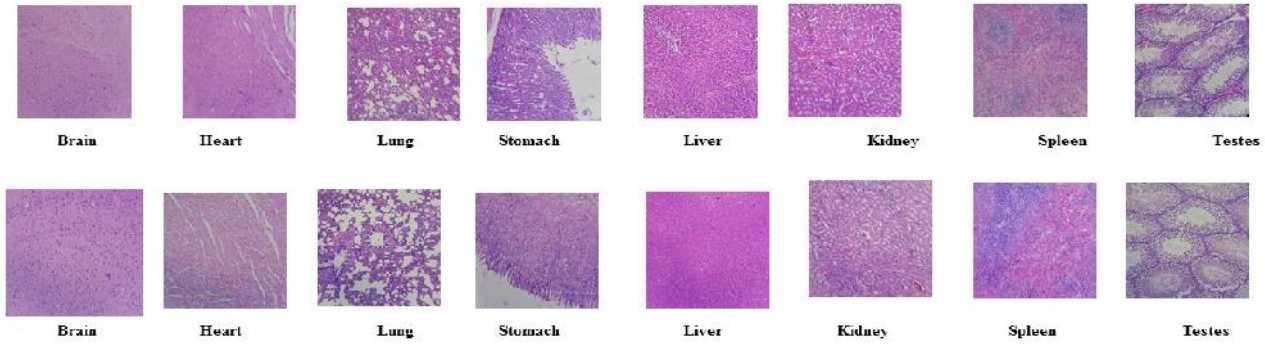

Figure 1: Histopathology of Male belongs to control and high dose treated group

3.14. Effect of MNC on Histopathological changes of Female rat in Sub-acute oral toxicity study

Microscopic observation of vital organs belongs to female rats presenting the following architecture as shown in figure2.

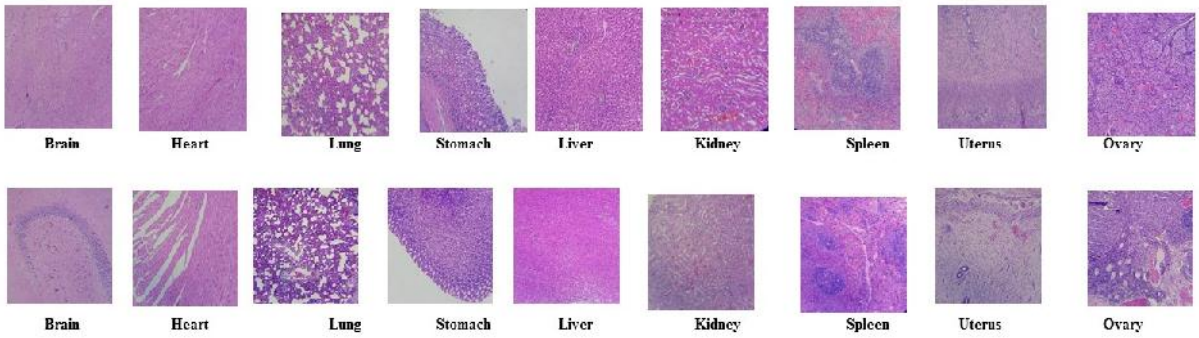

Figure 2: Histopathology of Female belongs to control and high dose treated group

4. Discussion

Subacute toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rodents. This test provides information on target organs and on the potential of the test chemical to accumulate in the organism and then is used as the basis for the determination of the no observed effect level (NOEL) [17].

In acute toxicity study, there was no mortality up to a maximum dose of 5000 mg/kg body weight of MNC after per oral administration. The changes in bodyweight and other Clinical signs like skin color change, fecal consistency, gait analysis, urine analysis, sensory responses, animal behavior abnormalities, neuro muscular coordination have been used as an indicator of adverse effect. Since no remarkable changes were observed in animal behavior, body weight and organ weight at dose in treated rats as compared to control group, it can be inferred that siddha formulation MNC is nontoxic at the administered dose of 5000mg/kg.

In sub-acute toxicity study treatment with MNC at 250 and 500 mg/kg reveals no significant change in the body weight of the treated rats. The low and high dose selected for toxicity covers the average therapeutic dosage of the test drug MNC in humans. Assessment of hematological parameters can be used to determine the extent of harmful effect of foreign compounds including plant materials on blood [18]. In the present subacute toxicity study, the hematological parameters (RBC, WBC, PLT, HGB, HCT, MCV, MCH, and MCHC) were within the reference range for rats.
SGOT and SGPT were found primarily in the liver and is the most sensitive marker for liver cell damage. The abnormal elevation of the liver enzymes is usually associated with liver damage or alteration in bile flow. When a cell is damaged, it leaks this enzyme into the blood. Similarly, BUN and creatinine are the index of kidney function. Serological analysis report of the sub-acute toxicity study revealed no significant elevation in any of the liver and kidney function markers. Further lipid profile results of both the treatment and control group rats are within the normal.

The gross pathological examination of the vital organs of the treated rats showed no change in color, shape, size, and texture compared to the control group. Remarkable change in relative organ weight between treated and untreated animals is an indicator of toxicity as organ weight is affected by the suppression of body weight [19] . In the present study there was no significant change in weight of any of the vital organs of both treated groups compared to control group.

Light microscopic observation of brain reveals normal morphology of neurons in CA1, CA2 and CA3 zones are normal, endocardium appears normal with no evidence of necrosis in heart, No signs of airway secretion and bronchial secretion in lung. Histology of stomach projects normal gastric mucosa containing intact gastric gland cells, parietal cells which are spherical cell with deeply stained dark nucleus. Normal, homogenous, intact hepatic parenchyma appeared in the liver. Appearance of Proximal Convoluted Tubule (PCT), Distal Convoluted Tubule (DCT) and Collecting Duct (CD) was normal in the kidney. Regular appearance of red pulp is composed of a three dimensional meshwork of splenic cords and venous sinuses were observed in spleen. Section of testes showing normal interstitial connective tissue. Observation of uterus exhibits normal histological aspect of endometrium and myometrium, appearance of antral follicle, primary oocyte and secondary follicles are normal in the ovary of female rats.

5. Conclusion

Siddha traditional medicine pioneering the health care needs of the humans since centuries. Further it also known for its efficacy and safety for long term administration. In recent times several siddha preparations have been validation at preclinical level to ascertain the safety level to popularize the Indian traditional medicine to the global standard. It was concluded from the observation of the present study that siddha drug

Manokara Chooranam has wide margin of safety and may render therapeutic benefits upon clinical application even on long term basis.

Acknowledgements

I wish to acknowledge my thanks to The Tamil Nadu Dr. M.G.R. Medical University, Chennai, Tamil Nadu, India and The Noble research solutions, Chennai, Tamil Nadu, India for their support.

6. References

1. Prasad LV. In: Indian System of Medicine and Homoeopathy Traditional Medicine in Asia. Chaudhury Ranjit Roy, Rafei Uton Muchatar., editors. New Delhi: WHO-Regional Office for South East Asia; 2002. pp. 283–286.
2. Sivaraman D, Anbu N, Kabilan N, Pitchiah Kumar M, Shanmugapriya P, Christian GJ. Exploration of Anti-Urolithiasis Potential of Traditional Siddha Formulations Amukkara Chooranam and Karisalai Karpam Chooranam by Struvite Crystal Growth Inhibition Assay. Pharmacog J. 2019; 11(4):683-688.
3. D.Sivaraman, N.Anbu, N.Kabilan, M.Pitchiah Kumar, P.Shanmugapriya, G.J.Christian.Review On Current Treatment Strategy In Alzheimer's Disease And Role Of Herbs In Treating Neurological Disorders. International journal of translational research in Indian medicine.2019;1(1):33-43.
4. Pan S., Litscher G., Gao S. Historical perspective of traditional indigenous medical practices: the current renaissance and conservation of herbal resources. Evid Based Complement Altern Med. 2014; 2014:1–20.
5. Sen S., Chakraborty R., De B., Ganesh T., Raghavendra H.G., Debnath S. Analgesic and inflammatory herbs: a potential source of modern medicine. Int J Pharma Sci Res. 2010; 1:32–44.
6. Sen S., Chakraborty R., De B., Mazumder J. Plants and phytochemicals for peptic ulcer: an overview. Phcog Rev. 2009; 3:270–279.
7. Sen S., Chakraborty R., De B. Challenges and opportunities in the advancement of herbal medicine: India's position and role in a global context. J Herb Med. 2011;1:7–75.
8. Iniaghe O. M., Egharevba O., Oyewo E. B. Effect of aqueous leaf extract of *Acalypha wilkesiana* on hematological parameters in male wistar albino rats. British Journal of Pharmaceutical Research. 2013;3(3):465–471.
9. Oladipipo A. E., Saheed S., Abraham B. F. Four weeks daily dose oral administration assessment of *Cyperus esculentus* L. aqueous extract on key metabolic markers of wistar rats. Pharmacologia. 2016;7(2-3):125–133.
10. Ukwuani A. N., Abubakar M. G., Hassan S. W., Agaie B. M. Toxicological studies of hydromethanolic leaves extract of Grewia crenata . International Journal of Pharmaceutical Science and Drug Research. 2012;4(4):245–249.
11. Lorke D. A new approach to practical acute toxicity testing. Archives of Toxicology. 1983;54(4):275–287.
12. OECD guideline for testing of chemicals. Guideline 423, 17th December 2001.
13. OECD Guide lines 407 for testing of chemicals. Repeated dose 28-Day Oral Toxicity Study in Rodents. 2008:2- 8.
14. Oloyede O. B., Sunmonu T. O. Potassium bromate content of selected bread samples in Ilorin, Central Nigeria and its effect on some enzymes of rat liver and kidney. Food and Chemical Toxicology. 2009;47(8):2067–2070.
15. Suvarna SK, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 7th edn, Churchill Livingstone, London.2013.
16. Visweswara Rao. Biostatistics., A manual of statistic methods for use in Health, Nutrition and Anthropology, Rajkamal Electrical press, Delhi, 2007.226-312.

17. Chambers F. L. A textbook of modern toxicology. In: Hodgson E., Levi P. E., editors. *Trends in Pharmacological Sciences*. Vol. 8. Elsevier; 1987. p. p. 408.
18. Yakubu A., Adua M. M., Adamude H. Welfare and hematological indices of weaner rabbits as affected by stocking density. Proceedings of the 9th World rabbit congress; 2008; Verona, Italy.
19. Lu F. *Basic Toxicology: Fundamentals, Target Organs and Risk Assessment*. 3rd. Washington, Wash, USA: Taylor and Francis; 1996.

Access this Article in Online	
	Website: www.ijcrims.com
Quick Response Code	Subject: Siddha Medicine

How to cite this article:
P. Ramachandran, W. Zafiroon Nisa, N. Anbu, D. Sivaraman. (2019). Safety Assessment of Siddha Formulation Manokara Chooranam by Acute and 28-Day Sub-acute repeated dose toxicity Studies in Wistar Rats. Int. J. Curr. Res. Med. Sci. 5(10): 40-52.
DOI: http://dx.doi.org/10.22192/ijcrms.2019.05.10.005