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Toxicological Evaluation of Herbal Siddha Preparation Vajjiravalli Chooranam in Rats

Meena. S[1], Kaniraja.S[2] Muthu Kumar. N.J[3], Banumathi. V[4]
[1]*Resident Medical Officer, National Institute of Siddha Tambaram Sanatorium, Chennai.*
[2]*Professor, Department of Sirappu Maruthuvam, Government Siddha Medical College, Palayamkottai.*
[3]*Professor,Department of Sirappu Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, Chennai.*
[4]*Director, National Institute of Siddha, Tambaram Sanatorium, Chennai.*
Corresponding Author: Meena. S

ABSTRACT: *The Vajjiravalli chooranam is traditionally used in Siddha system for various skin diseases and pain management and osteo problems. Acute oral toxicity test for the Vajjiravalli chooranam was carried out as per OECD Guidelines 425. Acute toxicity studies were done on Swiss mice for 28 days repeated oral toxicity studies on both sex of Swiss mice under OECD guidelines 425. Acute toxicity studies, Vajjiravalli chooranam was administered single oral dose and observed for 14 days. In Acute toxicity studies no toxic effects were observed up to the dose level of various mg/kg body weight. Sub-acute toxicity studies were carried in four different groups in which Vajjiravalli chooranam was administrated orally to rats once daily for 28 days in various doses ranging from 250mg/kg, 500mg/kg, 1000mg/kg for Swiss mice respectively. Detailed hematological, biochemical, necropsy and histopathological evaluation of organs was performed for all animals. No toxic effect was observed upto 1000mg/kg in sub-acute toxicity studies of Vajjiravalli chooranam.*

KEW WORD: *Vajjiravalli chooranam, Siddha medicine, Toxicity study.*

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I. INTRODUCTION:

Medicinal plants have been used for centuries and are appreciated for their multiple effects in a wide variety of ailments. In recent decades concentration on medicinal plants has increased dramatically not only in our country but also globally. For this reason, the World Health Organization (WHO) encourages and promotes drugs from natural resources. Today scientist discovering many Medical plants because they are the major sources for drug discovery and development, the phytochemicals which are the secondary metabolic substance present in it which pays way for new potential therapeutic effects. New drugs derived from pure sources have available throughout the last couple involving years. Many new drugs have obtained approval for curing many chronic disease and the management involving cancer, neurological diseases, infectious problems, cardiovascular and metabolic diseases, immunological, inflammatory related diseases, also which encompass many of a normal human diseases [1-5]. Siddha medicine in India has proven track record of 5000 years and forms part of the National Health Service, offered alongside conventional medicine. Herbal medicines yielding about 25% of currently used crude drugs with another 25% derived from chemically altered natural products. However, to develop a proper medication which will be ecofriendly and having very less side effects that can be used for prophylactic and therapeutic purpose to control many diseases is still a big challenge to a scientific community [6,7]. The purpose of the present study was, evaluation the toxicological of Vajjiravalli chooranam - an acute and sub-acute oral toxicity in rats. The Vajjiravalli chooranam was also studied for analgesic and anti-inflammatory effects.

II. MATERIALS AND METHODS:

Drug and Stock solution:

The siddha drug Vajjiravalli chooranam was uniformly suspended in 2% Carboxy Methyl Cellulose with water to obtain 50mg/ml concentration as stock solution and used for the pharmacological investigations.

Animals:

Swiss mice (25—35 g) were housed at 22±2°C under a 12-h light/12 h dark cycle and with access to food and water ad libitum, were acclimatized to the laboratory for at least 1 h prior to testing. The animals were acclimatized for one week under laboratory conditions. The experiments reported here were carried out in accordance with the current ethical and care guidelines for the care of laboratory animals. The experiments were approved by the Institutional Ethics Committee of Vels University.

(XIII/IAEC/CPCSEA/290/2000/14a/VELS/8.8.2013).

Acute toxicity study:

Acute oral toxicity test for the Vajjiravalli chooranam was carried out as per OECD Guidelines 425. Animals are 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, except where they need to be removed from the study and humanely killed for animal welfare reasons or are found dead. All observations are systematically recorded and Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. The principles and criteria summarized in the Humane Endpoints Guidance Document taken into consideration. Animals found in a moribund condition and animals showing severe pain or enduring signs of severe distress was humanely killed. When animals are killed for humane reasons or found dead, the time of death was recorded [8-10].

Sub-acute toxicity study:

Animals:

Male and female albino wistar rats of average body weight of 182g were kept separately in individual polypropylene cages with stainless steel hopper in air conditioned room (24 °C) of the animal house under uniform animal husbandary conditions. The animals were fed basal diet (Sai meera foods. Bangalore) and water ad libitum. The animals were acclimatized to temperature and lighting (12 h light/dark) conditions of the animal house and used in this study. Three groups of 6 rats received Vajjiravalli chooranam by intra-gastric gavages at the dose of 250mg/kg, 500mg/kg, 1000mg/kg body weight every for 28 days. During the period of administration the animals were weighed and food and water intake were monitored. After 28 days all surviving animals were fasted overnight. Animals were sacrificed by excess anesthesia and blood samples were collected from retro orbital vein into heparinized tube for hematological parameters and non-heparinized centrifuge tubes for other biochemical evaluations. The Brain, Lung, liver, Pancrea, Spleen, Stomach, Testes, Ovary, Heart and Kidney were collected and weighed [11]. After instantaneous washing all the isolated organs were subjected for histopathological studies.

Haematological assay:

Blood samples collected in the heparinized tubes were used to investigate TRBC, Hb, PCV, MCV, MCH, ESR, Platelets, TLC and DLC using the standard assay methods.

Biochemical estimations:

Freshly removed liver separated from extraneous material in chilled saline medium were homogenised on 0·25M icecold sucrose solution 10% w/v in a homogeniser. The homogenate was centrifuged at 2000rpm for 10 min to remove cell debris. Blood was collected into non-heparinized tubes were then centrifuged at 3000 rpm for 10 min. The serum separated was analysed to evaluate Total protein, Albumin, Globulin, Blood glucose, Total Cholesterol, HDL, LDL, VLDL, Total Bilirubin, Direct Bilirubin, Indirect Bilirubin, SGOT, SGPT, ALP, Creatinine, Urea, Uric Acid, Sodium, Potassium and Chloride levels.

Histopathological study:

Histopathological investigation of the vital organs was done. The organ pieces (3-5 µm thick) were fixed in 10% formalin for 24 h and washed in running water for 24 h. Samples were dehydrated in an auto technicon and then cleared in benzene to remove absolute alcohol. Embedding was done by passing the cleared samples through three cups containing molten paraffin at 50°C and then in a cubical block of paraffin made by the "L" moulds. It was followed by microtome and the slides were stained with Haematoxylin-eosin [12, 13].

Statistical analysis:

The data was expressed as Mean ± SEM (standard error of mean). Analysis of variance (ANOVA) followed by post hoc and dunnet-t-test was used to statistically analyzed data P value less than 0.05 ($P<0.05$) were considered as significant.

III. RESULTS AND DISCUSSION:

Mortality in the acute oral toxicity test was not seen in the limit test up to dose 5000mg/kg. One-tenth of the upper bound dose was considered as therapeutic dose and 1/20 and $1/5^{th}$ dose as lower and higher doses for the sub acute toxicity experiments. No other toxic symptoms were observed in any of the dose treated animals. The results of sub acute toxicity study revealed that the treatment of Vajjiravalli chooranam on rats

possess significant changes in general behavioral pattern and produced minor or negligible signs of toxicity at the dose level of 500mg/kg and above. In the sub acute toxicity study, after 28 days of alternate day treatment of Vajjiravalli chooranam in single oral dose showed significant body weight changes compared to day 1 (Table-2) during the experimental period. But an insignificant change in food intake was observed in all the groups after one week of drug treatment. There was a gradual increase in water consumption was observed in all the groups throughout the study period (Table-3&4).In the haematological parameters, there was a marked increase in TLC was observed and also increase in haematocrit value ($P<0.01$) was noted in the animals given 500mg/kg dose of Vajjiravalli chooranam (Table-5).Similarly, from the biochemical analysis, the ALP, SGOT, SGPT levels were decreased significantly ($P<0.01$) in all the dose treated groups but there was no major modifications in the other biochemical parameters. A fall of blood glucose level was observed in the groups treated with 250 and 500mg/kg dose of Vajjiravalli chooranam (Table-6).Table-7 shows that the urea, sodium concentrations are drastically decreased in all the dose treated groups with respect to control. There was no effect on total cholesterol. The results of urine analysis indicate that the urine volume is gradually decreased in the dose dependent manner after Vajjiravalli chooranam treatment. In the same manner there was a slight alterations was observed in PH. The color intensity of the urine is increased on the basis of drug dose range. In the urine collected from the 500 and 1000mg/kg Vajjiravalli chooranam treated group few RBC, Pus and epithelial cells were seen (Table-9). The isolated vital organ weights were changed after 28 days of Vajjiravalli chooranam treatment in experimental animals. Particularly, liver, lung, spleen and stomach weights were decreased but it is statistically not significant (Table-10). So, based on the results it can be concluded that the Vajjiravalli chooranam falls under the category of drug with no toxicity and it can be suggested that the use of Vajjiravalli chooranam clinically for long term therapy may not cause toxic symptoms like liver damage, respiratory ailments and impotence in females.

Table.No. 1: Dose finding experiment and its behavioral Signs of Toxicity

Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
500	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
1000	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
2000	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-
5000	+	-	-	+	+	+	-	-	-	-	-	-	-	+	-	-	-	-	+	-

1. Alertness 2. Aggressiveness 3. Pile erection 4. Grooming 5. Gripping 6. Touch Response 7. Decreased Motor Activity 8. Tremors 9. Convulsions 10. Muscle Spasm 11. Catatonia 12. Muscle relaxant 13. Hypnosis 14. Analgesia 15.Lacrimation 16. Exophthalmos 17. Diarrhoea 18. Writhing 19. Respiration 20. Mortality

Table.No. 2. Body wt (g) of albino rats exposed to Vajjiravalli chooranam for 4 weeks.

Dose (mg/kg/day)	Days				
	1	7	14	21	28
Control	183.32±10.10	189.12±10.12	196.26±9.21	201.61±10.29	212.11±10.12
250	166.66±8.16	178.96±9.17	185.10±10.02	192.05±10.48	205.34±10.40**
500	158.24±12.11	162.25±10.84	175.71±10.62	180.31±9.50	184.33±11.44**
1000	170.83±10.2	172.5±10.84	179.16±8.42	182.96±9.82	202.21±8.74**

**P<0.01; N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table. No. 3. Food (g/day) intake of albino rats exposed to Vajjiravalli chooranam for 4 weeks.

Dose (mg/kg/day)	Days (gms/rats)				
	1	7	14	21	28
Control	42.25±2.31	40.10±2.51	42.46±2.54	40.18±2.82	42.78±2.45
250	41.60±2.52	41.16±2.28	38.25±2.00	40±2.84	42.83±2.40
500	38.43±2.00	39.45±3.00	38±2.78	37.43±2.71	38.46±2.41
1000	34.43±2.80	41.22±3.81	43.93±2.40	39.32±2.45	40.15±3.80

^{NS}P>0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No.4.Water (ml/day) intake of male and female albino rats exposed to Vajjiravalli chooranam for 4 weeks.

Dose (mg/kg/day)	Days(ml/rat)				
	1	7	14	21	28
Control	40.32±2.54	40.43±2.40	40.28±2.47	43.12±2.40	42.38±4.18
250	43.64±2.28	45.24±4.47	40.5±2.47	44.48±2.64	45.15±3.10
500	40.22±2.04	48.96±2.43	49.66±3.16	44.12±2.43	44.18±2.10
1000	41.46±2.24	49.48±2.08	43.5±2.46	42.43±2.48	45.66±2.67

^{NS}P>0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No. 5. Hematological parameters after 4 weeks treatment with the Vajjiravalli chooranam

Parameter	Control	250mg/kg	500mg/kg	1000mg/kg
Red blood cell (mm^3)	7.24±0.52	7.48±0.34	7.78±0.36	8.02±0.34
HB (g)	11.26±1.40	15.52±1.15*	12.03±1.30	14.42±1.33*
Leukocyte ($x10^6$/mL)	10247±205.30	12248±238**	12506±390**	12810±224**
Platelets/µl	1129±48.22	1200±37.07	1198±44.00	1168±39.65
MCV (gl)	51.72±0.64	52.50±0.61	55.52±0.72	52.10±0.74
DLC (%)				
Neutrophil	65.24±2.5	70.16±2.4	69.5±1.04	74.5±1.02
Lymphocyte	29.33±1.32	29.62±1.47	28.83±1.44	29.13±1.60
Monocyte	2.5±0.54	0.5±0.54**	1.66±0.81	1.5±0.54
Eosinophil	1.160.75	1.16±0.98	1±0.63	1.66±1.03
Basophil	0±0.00	0.5±0.54	0±0.00	0±0.00
ESR (mm)	1.33±0.51	1.33±0.51	1±0.00	1±0.00
PCV	44.40±3.72	43.74±2.41	44.47±2.30	44.13±3.71
MCH pg	19.14±0.10	19.24±0.47	18.18±0.41	18.19±0.90

**P<0.01; *P<0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No. 6. Effect of treatment with Vajjiravalli chooranam biochemical parameters-LFT

Dose (mg/kg)	Control	250mg/kg	500mg/kg	1000mg/kg
Total Bilirubin (mg/dL)	0.24±0.04	0.25±0.05	0.26±0.05	0.28±0.07*
Bilirubin Direct (mg/dL)	0.1±0.02	0.1±0.02	0.10±0.03	0.09±0.04
Bilirubin Indirect (mg/dL)	0.18±0.04	0.19±0.05	0.18±0.04	0.18±0.07
ALP (U/L)	270.5±9.52	285.16±10.80	277±8.02	264.38±11.06
SGOT (U/L)	148.18±10.6	140.15±10.44	147.21±12.16	118.46±10.14*
SGPT(U/L)	29.68±4.80	22.35±6.00*	23.34±4.72*	20.68±3.12**
Total Protein (g/dl)	7.35±0.40	7.01±0.53	6.4±0.62	6.32±0.46*
Albumin (g/dl)	3.12±0.30	3.32±0.52	3.80±0.54	3.45±0.43
Globulin (g/dl)	4.25±0.40	4.15±0.40	3.22±0.32	3.28±0.30
GGT (U/L)	8.5±0.32	9.18±0.46	9.00±0.41	8.42±0.48
Blood glucose (mg/dl)	102.10±8.82	98.85±10.22	100.58±4.70	97.43±4.44

**P<0.01; *P<0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No.-7 Effect of treatment with Vajjiravalli chooranam biochemical parameters -RFT

Dose (mg/kg)	Control	250mg/kg	500mg/kg	1000mg/kg
Urea (mg/dL)	159.16±12.54	114.80±14.75	118.10±10.20	110.21±10.24
Creatinine (mg/dL)	0.62±0.21	2.52±0.28**	2.94±0.24**	2.13±0.28**
Uric acid (mg/dL)	1.44±0.27	1.28±0.37	1.40±0.32	1.38±0.38
Na m.mol	143.62±10.15	132.10±9.28	128.25±9.88	124.62±10.11
K m.mol	6.16±0.82	6.53±0.80	6.51±0.67	6.22±0.58
Cl m.mol	112.10±9.12	114±8.32	119.25±6.82	118.80±9.24

**P<0.01; N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No.-8 Effect of treatment with Vajjiravalli chooranam biochemical parameters -Lipid Profile

Dose (mg/kg)	Control	250mg/kg	500mg/kg	1000mg/kg
Total cholesterol (mg/dL)	62.62 ± 3.00	62.42 ± 3.38	66.00 ± 3.52	65.42 ± 3.26
HDL (mg/dL)	11.12±1.00	12.15±1.32	12.04±1.12	12.00±2.10
LDL (mg/dL)	33.12±2.12	32.20±2.12	35.10±2.10	34.14±2.14
VLDL (mg/dl)	16.14±2.41	16.28±2.49	15.30±2.50	16.00±2.14
Triglycerides (mg/dl)	81.44±2.82	82.72±2.79	83.18±3.24	82.42±2.90
TC/HDL ratio (g/dl)	3.35±0.28	3.12±0.22	3.24±0.26	3.26±0.25
Blood glucose (mg/dl)	90.24 ± 2.12	88.24 ± 2.00	91.10 ± 1.62	93.12 ± 1.10

^{NS}P>0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

Table.No.-9 Effect of treatment with Vajjiravalli chooranam - Urine Analysis

Parameters	Control	250mg/kg	500mg/kg	1000mg/kg
Volume	2.6ml/24hr	2.2ml/24hr	2.0ml/24hr	2.1ml/24hr
Colour	Straw Yellow	Orange Yellow	Orange	Reddish Orange
Transparency	Clear	Clear	Clear	Clear
Specific gravity	1.010	1.010	1.010	1.010
PH	7.2	>7.8	>8.0	>8.0
Protein	Nil	Nil	Nil	Nil
Glucose	Nil	Nil	Nil	Nil
Bilirubin	-ve	-ve	-ve	-ve
Ketones	-ve	-ve	-ve	-ve
Blood	Absent	Absent	Present	Present
Urobilinogen	Normal	Normal	Normal	Normal
Pus cells	Nil	3-4cells/HPF	5-6cells/HPF	5-6cells/HPF
RBCs	Nil	Nil	Present	Present
Epithelial cells	1-2cells/HPF	1-2cells/HPF	2-3cells/HPF	2-3cells/HPF
Crystals	Nil	Nil	Nil	Nil
Casts	Nil	Nil	Nil	Nil
Others	Bacteria seen	Bacteria seen	Bacteria seen	Bacteria seen

Table.No.10. Effect of oral administration of Vajjiravalli chooranam on organ weight

Dose (mg/kg)	Control	250mg/kg	500mg/kg	1000mg/kg
Liver (g)	5.24±0.25	5.21±0.24	4.88±0.26	4.97±0.22
Heart (g)	0.52±0.04	0.50±0.02	0.47±0.08	0.46±0.08
Lung (g)	1.20±0.06	1.14±0.08	1.17±0.09	1.30±0.08
Spleen (g)	0.47±0.04	0.42±0.02	0.45±0.03	0.44±0.04
Ovary (g)	2.47±0.15	2.44±0.12	2.49±0.11	2.52±0.10
Testes (g)	1.32±0.12	1.29±0.08	1.30±0.05	1.34±0.07
Brain (g)	1.50±0.09	1.48±0.10	1.50±0.08	1.45±0.10
Kidney (g)	0.78±0.07	0.80±0.07	0.79±0.05	0.78±0.06
Stomach (g)	1.08±0.09	1.09±0.08	1.07±0.08	1.10±0.09

^{NS}P>0.05. N=6 Values are mean ± S.E.M. (One way Anova followed by Dunnett's test).

IV. CONCLUSION:

This study presents strong evidence of the non-toxic effect of the Vajjiravalli chooranam. These results showed that the use of the Vajjiravalli chooranam is safe and suitable for the extensive utilization or for long term therapy at the dose level used in this study.

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