IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
November 2017 Vol.:10, Issue:4
© All rights are reserved by Kullappan Shanmugam Uma et al.

Subacute Toxicity Study of Siddha Formulation- Aavaarai Vidhai Chooranam in Wistar Albino Rats

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Kullappan Shanmugam Uma[1*], Mockkaiya Devar Logamanian[2], Sekkizhar Geethalakshmi[1], Palayyan Muralidharan[3], Gaddam Dayanand Reddy [4], Thiyagarajan Balasubramanian[1], Ramamurthi Sukumar[5]

[1]*The TN Dr. M. G. R. Medical University, Chennai-32, Tamil Nadu, India.*

[2] *Professor, Department of Noi Naadal, National Institute of Siddha, Chennai-47, Tamil Nadu, India.*

[3] *Professor, Department of Pharmacology, CL Baid Metha College of Pharmacy. Chennai - 97, Tamil Nadu, India.*

[4] *Research Officer, Department of Pharmacology, Siddha Central Research Institute, Chennai-106, Tamil Nadu, India.*

[5] *Professor, Department of Medicine, Sri Muthukumaran Medical College, Chennai-69, Tamil Nadu, India.*

Submission: 27 October 2017
Accepted: 5 November 2017
Published: 30 November 2017

HUMAN JOURNALS
www.ijppr.humanjournals.com

Keywords: Siddha Medicine; Aavaarai vidhai choornam; *Cassia auriculata*; Sub-acute toxicity, Herbal - Medicine.

ABSTRACT

The aim of present study was to investigate the Sub-acute toxicity of Aavaarai Vidhai choornam (AVC) in wistar albino rats. IAEC approval got from CL baidMetha College of Pharmacy, Chennai. Wistar albino rats of either sex, 6 to 7 weeks age weighing 220–250gm were used. A total of 18Wistar rats, 3 males and 3 females in each group were selected based on the body weight and randomly distributed to 3 groups (Group-I Vehicle control, Group-II Low dose and Group-III High dose). Sub-acute toxicity study was carried out by oral administration of ACV daily at doses of 200 mg/kg body weight (Low dose) and 400 mg/kg body weight (High dose) in respective group II and III for 28 days. Buttermilk was used as the vehicle and administered to Group I rats. During the study period, rats were observed weekly for toxicity symptoms and feed consumption. All surviving animals were euthanized on 29th day and hematological and biochemical analysis was done. Group II and III were statistically compared with Group I and no significant difference was observed with respect to body weight gain and feed consumption. No abnormal behavioral activity and pre-terminal deaths were recorded in the rats exposed to low dose and high dose of AVC. This study demonstrates that 28 days oral intake of AVC did not cause any toxic effects up to the dose of 400 mg/kg b.wt in wistar albino rats.

1. INTRODUCTION

Herbal formulations have attained wide recognition in comparison to crude plant materials and extracts, due to reduction in dose, convenience and ease of administration [1]. These formulations are popular worldwide as therapeutic agents, in various ailments that impact the quality of life. In developing countries, most of the population relies on traditional medicines, considering their affordability, traditional background knowledge on medicinal plants, and a belief that they are harmless. Many synthetic drugs are known to act on a single molecular target and provide symptomatic relief. The multi target responses of herbal drugs are proven to be beneficial in chronic conditions such as diabetes, cancer and so forth, and also in restoring the health status. Although many natural plant extracts used traditionally have passed the test of time, in terms of toxicity and adverse effects, the safety of the active phytochemical from these plants must precede their pharmaceutical use. There is a need to assure the safety of herbal formulations in order to acquire their maximum benefits even though these have been proven to be efficacious in pharmacological studies or by clinical evaluation. Toxicity studies are considered necessary, especially on drugs that are to be used in chronic conditions.

Aavaarai Vidhai Choornam a polyherbal formulation contains crude drugs like *Cassia auriculata*, *Curcumma longa*, *Coscinium fenestratum*, *Strychnus potatorum* and *Acacia nilotica*[2]. These plants have been used in traditional medicine for treating many ailments of humans [3,4]. *Cassia auriculata* have been proved for safety profile in animal model [5]. The safety studies of Aavaarai Vidhai Choornam have not been established so far. In the present study, the safety profile of the Aavaarai Vidhai Choornam has been investigated at the therapeutic dose level by a sub-acute toxicity study in wistar albino rats, in order to optimize its safe use. The experiment was conducted as recommended by the *Organization for Economic Cooperation and Development* (OECD) guideline 407.

In the present study, sub-acute toxicity of AavaaraiVidhaiChoornam, a poly-herbal formulation, was investigated to assess its safety and tolerability profile in long-term treatment.

2. MATERIALS AND METHODS

2.1 EXPERIMENTAL ANIMALS

A total of 18 Wistar albino rats of either sex aging 6 to 7 weeks, were received from Animal Breeding station, TANUWAS, Madhavaram, Chennai, Tamil Nadu. Only nulliparous and non-pregnant females were used in the experiment. Animals were housed 1 animal /cage in each polycarbonate cage with rice husk bedding and metal tops. Each cage was identified with cage card, which displayed study number, cage number, sex and animal identification numbers. Temperature and relative humidity were maintained at 18 to 25^{0}C and 30 to 65 % respectively and illumination was controlled to give approximately a sequence of 12 hours' light and 12 hours' dark. The animals were provided free access to autoclaved water purified with reverse osmosis and autoclaved standard pelleted laboratory animal diet *ad libitum* during the study period. Animals were acclimatized for 7 days before initiation of the study. Drugs were purchased from authorized dealers and were identified and authenticated by the Research Officer Sasikala from Siddha Central Research Institute Chennai and voucher specimen was submitted.

2.2 PREPARATION AND PROCUREMENT OF AAVAARAIVIDHAI CHOORNAM

AavaaraiVidhaiChoornam was prepared as per Good Manufacturing Practice (GMP) Guidelines of the Drugs and Cosmetics act 1947 at Gunapadam Laboratory of The TN Dr. M. G. R. Medical University, Chennai. The study drug was prepared according to the method mentioned in the Siddha text [6,7,8] under the direct supervision by following Good Manufacturing Practice (GMP) guidelines [9] the drugs were taken separately and made into fine powder. Powder was sifted and stored in airtight container.

2.3 SUB-ACUTE TOXICITY STUDY:

Sub-acute toxicity study was carried out as per OECD guideline-407 (Organization for Economic Co-operation and Development). All the procedures were followed as mentioned in Repeated Dose 28-Day Oral Toxicity Study in Rodents in stored OECD guidelines for the testing of chemicals [10].

2.4. TREATMENT SCHEDULE

Sub-acute toxicity test was performed as per the guidelines of OECD guideline - 407 for the evaluation of safety of herbal medicines. A total 18 animals (9 Males +9 Females) based on the body weight were randomly distributed to 3 groups (Group I, II and III). Each group consisted of 6 animals (3 males and 3 females). Animals were distributed such that mean body weight variation will not be ± 20%. Group, I received buttermilk daily and Groups II and III were administered with 200mg/kg b wt, p.o, 400 mg/kg b.wt p.o respectively of Aavaarai Vidhai Chooranam for a period of 28 days. On 29th day, animals fasted overnight and blood was collected from retro orbital plexus under ether anesthesia. Serum was separated by centrifugation at 2000 rpm. After separation of serum, all the biochemical parameters estimations were performed. After all, the animals were euthanized and weights of major organs were measured followed by histopathological studies.

2.5. PARAMETERS MONITORED

2.5.1. BODY WEIGHT:

Body weight of each animal in the four groups was measured using an electronic weighing balance at the time of acclimatization, once before starting the experiment, weekly once during the experimental period and on the day of sacrifice after fasting the animals.

2.5.2. FEED CONSUMPTION:

Feed consumption of animals was recorded weekly throughout the study. The food intake was quantified once daily by weighing the leftover feed on standard electronic balance.

2.5.3. HEMATOLOGY:

The hematology parameters viz., total white blood cell count (WBC), total red blood cell count (RBC), hemoglobin concentration (HGB), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), Platelet count (PLT) and differential leucocytes count (DLC) were analyzed.

2.5.4. BIOCHEMICAL PARAMETERS:

Creatinine, Glucose, BUN, serum albumin, SGPT, SGOT, lipid profile and Total Protein (TP) were estimated using RA-50 autoanalyzer (Bayer).

2.5.5. ORGAN WEIGHTS:

Heart, liver, lungs, brain, kidney, eyes, stomach, intestine, pancreas, spleen, ovary and testis were taken from all surviving animals at the scheduled necropsies, weighed and recorded.

2.5.6. HISTOPATHOLOGY:

The histopathological examination was performed for all major organs and tissues collected from all three groups.

3. RESULTS AND DISCUSSION

In Sub-acute toxicity study, there was no death in the treatment period either in the vehicle control group or in the treated group II & III. Food and water consumption also did not differ significantly. There was no change in the general behavior or other physiological activities of the animals. The results showed that a very high oral dose was tolerated by the animals without producing any toxicity symptoms.

The sub-acute toxicity study was carried out in normal Wiastar albino rats considering biochemical, hematological and physiological parameters. The repeated dose sub-acute toxicity study on AVC has shown that 2 different doses of the formulation were tolerated and there was no cumulative toxicity as evidence from biochemical data and pathology report. The result of all the investigations showed no difference between the control and the test groups subject to low doses and high doses of AVC. Thus the absence of sub-acute toxicity, the study drug, AVC, confirmed its significant safety in the experimental animal models. The results of Sub-acute toxicity study were tabulated in table 1 to table 7.

Table-1: Parameter checked in Sub-acute toxicity study of AVC

Group	Day
Body weight	Normal
Assessments of posture	Normal
Signs of Convulsion Limb paralysis	Absence of sign (-)
Body tone	Normal
Lacrimation	Absence
Salivation	Absence
Change in skin color	No significant color change
Piloerection	Not observed
Defecation	Normal
Sensitivity response	Normal
Locomotion	Normal
Muscle grip ness	Normal
Rearing	Mild
Urination	Normal

Table 2. Body weight (weekly) of rats in sub-acute toxicity study of AVC

S. No	Dose mg/kg	Initial. wt	Ist week	2nd week	3rd week	4th week
1.	Control	220.5 ± 11.42	222.5 ± 11.42	225.2 ± 12.46	229.8 ± 9.89	235.1 ± 16.26
2.	AVC 200mg/kg	228.31 ± 12.25	225.3.32 ± 9.70	229.33 ± 7.52	232.13 ± 12.05	235.16 ± 10.08
3.	AVC 400mg/kg	224.33 ± 14.71	226.83 ± 10.68	229.83 ± 8.01	229.16 ± 8.61	223.33 ± 6.83

n=6, Values are expressed as mean ± SD

Table 3. Food Consumption (Weekly) of rats in sub-acute toxicity study of AVC

Sr. No.	Dose mg/kg	1st week	2nd week	3rd week	4th week
1.	Control	12.41 ± 1.50	12.25± 1.49	13.83 ± 2.55	13.33 ± 2.27
2.	360mg/kg	14.02 ± 0.91	13.33 ± 1.89	14.66 ± 1.63	12.26± 1.10
3.	1800mg/kg	15.06± 0.53	11.3± 1.51	15.16 ± 1.83	11.89± 1.13

n=6, Values are expressed as mean ± SD

Table 4. Hematological values of rats in sub-acute toxicity studies of AVC

S.no	Group	WBC	L	N	M	RBC	HGB	MCV	MCH	MCHC	PLT	PCT
1	Control	8.11 ± 1.12	60.21 ± 5.25	4.01 ± 0.56	30.78 ± 6.01	6.18 ± 0.50	15.27± 0.76	47.52 ± 1.52	15.15 ± 0.57	32.43 ± 0.60	223 ± 25.09	0.22 ± 0.01
2	AVC 200mg/kg	6.9 ± 0.71	51.28 ± 10.66	5.51 ± 0.33	45.46 ± 11.71*	7.10 ± 0.68	15.5± 1.15	49.20 ± 1.33	16.5 ± 0.57	33.25 ± 1.17	250 ± 30.46*	0.19 ± 0.02
3	AVC 400mg/kg	8.16 ± 1.13	50.16 ± 11.64	3.71 ± 0.21	41.55 ± 10.57*	7.55 ± 0.18	14.33 ± 0.88	51.13 ± 1.15	17.28 ± 0.73	32.96 ± 0.66	276 ± 41.13*	0.17 ± 0.03

L= Lymphocytes, N = Neutrophils, M= Monocytes, RBC= Red blood cells, HGB= Hemoglobin, MCV= Mean corpuscular volume, MCH= Mean corpuscular hemoglobin, MCHC= Mean corpuscular hemoglobin concentration, PLT= Platelets, PCT= Procalcitonin. n=6, Values are expressed as mean ± SD. **P*<0.05, compared to control

Table 5. Biochemical parameters of rats in sub-acute toxicity study of AVC

S.No	Group	Blood glucose	Cholesterol	TG	HDL	LDL	VLDL	SGOT	SGPT	TP	Serum albumin
1	Control	74.16 ± 11.30	96.33 ± 0.71	45.66 ± 0.13	25.16 ± 0.94	43.21 ± 0.23	33.46 ± 0.46	238.8 ± 16.03	62.33 ± 15.58	5.66 ± 80.42	2.73 ± 0.07
2	AVC 200mg/kg	73.33 ± 10.09	105.5 ± 0.26*	43.16 ± 0.08	29.11 ± 0.24	44.24 ± 0.24	40.45± 0.65	213.16 ± 9.30*	67.17 ± 19.29	4.38 ±0.11	2.78 ± 0.18
3	AVC 400mg/kg	74.83 ± 18.84	103.52 ± 5.12*	47.21 ± 0.06	23.15 ± 0.29	40.53 ± 0.62	33.53 ± 0.62	214.5 ± 7.39*	73.53 ± 15.62	4.18 ± 0.24	2.55 ± 0.12

n=6, Values are expressed as mean ± SD;**P*<0.05, compared to control

Table 6. Organ Weights of rats in sub-acute toxicity study of AVC

Groups	Kidney	Liver	Heart	Lungs	Spleen	Pancreas	Brain	Ovaries	Testes
Control	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
AVC 200mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
AVC 400mg/kg	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal

Table 7: Sub-Acute Toxicity studies: Pathology report

Sample	Observation
Kidney	Nephrotic bundles appears normal in all the three groups
Heart	No signs of infarct and fiber appears normal Myocardial cells appear normal
Liver	Marginal hepatocytes at regular intervals. Lumen of hepatic veins appears normal.
Brain	No signs of edema or degeneration in all the groups Neurons appears normal with prominent nucleus

Considering the numerous therapeutic potentials of ingredients of Aavaarai Vidhai Choornamas an alternative medicine effective for a wide range of diseases and infections, as reported in a number of scientific papers [11,12,13], it is only pertinent that a safety profile of the plant be established as a guide for the management of its applications and usage in herbal preparations. Toxicity studies in appropriate animal models are commonly used to assess potential health risks in humans. Such toxicity studies assess the hazard and determine the risk level by addressing the probability of exposure to that particular hazard at certain doses or concentrations [14].The sub-acute toxicity study, which involved rats given Aavaarai Vidhai Choornam orally at doses of 200 and 400 mg/kg b.wt., demonstrated there was no significant changes in animal behavior, as well as reductions in body weight in both male and female rats.

Guidelines of toxicity studies lay emphasis on reporting changes in body weight. 10% decrease in body weight on chronic exposure has been fixed as an acceptable limit. In this study, all the animals showed normal body weight gain till the end of study which shows that treatment with Aavaarai Vidhai Choornam did not affect the normal health status of animals.

Hematopoietic system is one of the targets for toxic compounds and is an important index of physiological and pathological states. Treatment with Aavaarai Vidhai Choornam had no significant effect on hematological parameters. The changes in enzymes like SGOT & SGPT levels showed liver impairment, due to toxicity. Serum cholesterol mainly regulated via synthesis in the liver and increase or decreases in serum concentrations of constituents suggest liver toxicity. Rats treated with Aavaarai Vidhai Choornam did not alter the hepatic and renal function as identified from the hepatic enzymes SGO &SGPT and renal markers such as serum creatinine and blood urea which shows that the developed Aavaarai Vidhai Choornam maintains the integrity of liver and kidney.

The results of the lipid profile of rats treated with Aavaarai Vidhai Choornam for subacute toxicity was found that no significant increase in lipid profile vise total cholesterol, TG, LDL, VLDL and HDL levels, suggests that the Aavaarai Vidhai Choornam does not impair lipid metabolism. Another advantage of sub-acute toxicity testing is the information which could be gathered on specific organ toxicity on repeated administration. In this study, any of the treated animals with pathology finings did not reveal any gross morphological changes in any of the organs. Weight of the isolated organs of treated animals was also found to be comparable to control group.

The results of this study demonstrated that treatment with Aavaarai Vidhai Choornam may be considered relatively safe without any toxicity. Due to its non-toxic effects on the various organ systems, there is a clear potential for the utilization of Aavaarai Vidhai Chooranam for therapeutic use.

4. CONCLUSION:

In conclusion, the present study provides information on sub-acute toxicity profile of the Aavaarai Vidhai Chooranam in rats demonstrated that Aavaarai Vidhai Chooranamat 200 and 400mg/kg body weight did not cause any adverse effects or toxic effects and considered as non-toxic and safe.

ACKNOWLEDGEMENT:

Authors are thankful to Chairman, Principal and Head of Department of Pharmacology, ClBaid Metha College of Pharmacy, Thuraipakkam, Chennai for providing Laboratory facilities to carry out this study.

Conflicts of Interest: None

This research did not receive any specific grant from funding agencies in the public, commercial or not – for- profit section.

REFERENCES

[1] Tatke P A, Nidhiya I S, Deshpande SG. Safety profile of a polyherbal formulation (Gynocare capsules) in female rats by subchronic oral toxicity study.ToxicolInt2012; 19(2):106-11.
[2] Kanthasamy C. Pathinen Siddharkal Arulicheitha Athmaratchamirtham EnumVaithya Sarasangrigam.Chennai: Rathnanayakar B & Sons,
[3] Murugesanv Mudaliar K S.Gunapadam PorutpanbuNool- Part - 1, Moligai Vagupu. 7th ed. Chennai: Department of Indian Medicine and Homeopathy, Government of Tamil Nadu; 2003.
[4] Kannuswamy C.Pathartha Guna Vilakkam (Moola Vargam)Chennai: Rathina Nayakar B & Sons; 2006.
[5] Senthilselvi R.Acute and Sub Acute Toxicity study of Aqueous extract of *Cassia auriculata* seeds. DOAJ 2010.
[6] Sarakku Suththicheimuraikal. Chennai: Department of Indian Medicine and Homeopathy, Government of Tamil Nadu;2000.
[7] Kannusamy Pillai C. Chikitcha Rathna Deepam Enum Vaidhya Nool. Chennai: Rathna Nayakar B & sons; 2007.
[8] Hakkim P M Abdhulla Sahib**.** Mega Nivarana Pothini Ennum Nerizhivu Maruthuvam.Chennai: Thamarai Noolagam;1998
[9] Health Products and Food Branch Inspectorate, Good Manufacturing Practices (GMP) Guidelines for Active Pharmaceutical Ingredients. (API), GUI-0104;2013.
[10] Repeated dose 28 - Days Oral In-toxicity study in rodents. Organization for Economic Co-operation and Development Guidelines for the Testing of chemicals. [Accessed on 12 -12-2012]
[11] Ravi Shankar V, Raj, Rajesh P S, Hyung - Min Kim. Medicinal use of *Cosciniumfenestratum*coleb: An short reviewOriental pharmacy and experimental medicine 2013; 13(1):1- 9.
[12] Pratibha Mehta Luthra, Rambir Singh, Ramesh Chandra. Therapeutic uses of *Curcuma longa* (turmeric). Indian Journal of Clinical Biochemist 2001;16(2):153-60.
[13] PrashithKekuda T R, Mukunda S, Sudharshan S J, Syed Murthuza, Rakesh G M.Studies on phytochemical and antimicrobial activity of ethanol extract of curcuma aromatica and *Cosciniumfenestratum.* Natural products An Indian Journal 2008;4(1):77-80.
[14] Anaigu, S.O., Nwinyi. Toxicity studies in rats fed nature curebitters. Afr. J. Biotechnol2005; 4: 72-8.