WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 12, Issue 9, 2006-2016. Research Article ISSN 2277– 7105

ACUTE TOXICITY STUDY AND ANALGESIC ACTIVITY OF SIDDHA POLY HERBAL DRUG *AVURI KARPAM*

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Article Received on
06 April 2023,

Revised on 26 April 2023,
Accepted on 16 May 2023,

DOI: 10.20959/wjpr20239-28283

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ABSTRACT

Avuri Karpam (AK) is a poly herbal formulation mentioned in Classical Siddha literature *Pathartha Guna Vilakkam* and it contains *Avuri ilai (Indigofera tincotria), Kaiyanthagarai (Eclipta alba), Kuppaimeni (Acalypha indica), Kottaikkaranthai (Spaeranthus indicus), Vallarai (Centella asiatica), Pottrilai kaiyanthagarai (Wedelia chinensis), Seruppadai (Coldenia procumbens)* indicated for treating *Keelvatham* (Arthritis), *Udhirakkattu, Sarpavisham.* Safety and efficacy of the drug AK has not been evaluated scientifically. The aim of the present study was carried out the acute toxicity study accordance with OECD guidelines and analgesic pharmacological activity in rats. Acute toxicity study had been carried out at the dose level of 5 mg/kg and 2000mg/kg. At these two doses level does not reveal any abnormalities. No mortality was documented. Whereas analgesic activity study, Group- I is normal control (0.5% CMC p.o.), and Group- II received Pentazocine (30mg/kg, i.p.), whereas Groups - III and IV animals received AK (400 and 800 mg/kg, p. o respectively). Latency period of AK low dose and high dose significantly given significant results when compared to standard drug at the time interval of 30 mins, 45 mins, and 60 mins. Since it was concluded that Siddha drug AK is non-toxic and it has potent analgesic activity.

KEYWORDS: *Siddha medicine, Herbal drug, Avuri Karpam, Acute toxicity, Analgesics.*

INTRODUCTION

Siddha system of medicine has been recently getting more attention and recognition among public. At the same time toxic effects and adverse drug reaction also have been reported for few medicines. So many adulterated drugs are daily coming up in the market. The World Health Organization estimated that 80 percentage of the world's population still uses traditional remedies, including plants, as the main tool for health care.[1]

To achieve clinical success, the traditional medicines need to attain high standards of quality, safety and efficacy. Diverse approaches have been tested to evaluate the toxicity potential of chemicals, drugs including traditional medicines.[2] Assessment of toxicological evaluation of the drug is an important one in clinical approach.

Acute toxicity study is carried out in two animal species (one rodent, one non – rodent). Single, graded doses are administered to small groups of animals using two routes – one that is to be used in human. It is done to determine the general behaviour and median lethal dose (LD_{50}) following exposure to the test drug.[3]

The Selected drug *Avuri Karpam* (AK) is a poly herbal formulation mentioned in Classical Siddha literature *Pathartha Guna Vilakkam* and it contains *Avuri ilai (Indigofera tincotria), Kaiyanthagarai (Eclipta alba), Kuppaimeni (Acalypha indica), Kottaikkaranthai (Spaeranthus indicus), Vallarai (Centella asiatica), Pottrilai kaiyanthagarai (Wedelia chinensis), Seruppadai (Coldenia procumbens)* indicated for treating *Keelvatham* (Arthritis), *Udhirakkattu, Sarpavisham*.[4] Here the most of the ingredients are having analgesic and anti-inflammatory properties.

Arthritis means inflammation of the joints. Pain, redness, swelling, warmness in the joints are common symptoms of arthritis. Pain is an unpleasant sensory and emotional experience associated with actual and potential tissue damage. Various types of pain are seen in humans, e.g. somatic pain (arising from the skin, muscles, joints, ligaments and bones), visceral pain, referred pain, neuropathic pain, cancer pain, etc. pain is produced by the excitation of particular receptors, the nociceptors or of their afferent fibres.

Drugs in clinical use as analgesics belong to two main groups in modern medicine – narcotic or morphine group and analgesic – antipyretic (nonsteroidal anti – inflammatory drugs)

group. Morphine like drugs produces analgesia by acting on the central nervous system, while analgesic – antipyretic drugs act by both central and peripheral mechanisms.[5] The aim of the study is to evaluate the acute toxicity study and analgesic activity in rats.

MATERIALS AND METHODS

Avuri Karpam – Ingredients

Table 1: Ingredients of the study drug AK.

S.No	Tamil name	Botanical name	Quantity
1.	*Avuri ilai*	*Indigofera tinctoria*	50 gms
2.	*Kaiyanthagarai*	*Eclipta alba*	50 gms
3.	*Kuppaimeni*	*Acalypha indica*	50 gms
4.	*Kottaikkaranthai*	*Spaeranthus indicus*	50 gms
5.	*Vallarai*	*Centella asiatica*	50 gms
6.	*Pottrilaikaiyanthagarai*	*Wedelia chinensis*	50 gms
7.	*Seruppadai*	*Coldenia procumbens*	50 gms

Collection of the Plant materials

Avuri ilai and *Seruppadai* were purchased from Rajendran Herbals, Thuckalay. *Kaiyanthagarai, Kuppaimeni, Vallarai* and *Pottrilai kaiyanthagarai* were collected from my native place (Tiruppattur) Herbal garden. *Kottaikkaranthai* was bought from the Ramasamy Mudhaliyar Store, Parry's corner, Chennai.

Identification and Authentication of the drug

All the plant materials were identified and authenticated by the experts of Siddha Central Research Institute (Central Council for Research in Siddha, Chennai, Ministry of AYUSH, Government of India), Govt. Anna Hospital Campus, Arumbakkam, Chennai -106.

Purification of the drugs

Purification process was done as per classical Siddha literature.[6]

Method of Preparation

All the above purified ingredients were powdered separately and it was sieved by a cotton cloth. Then these powders were mixed together and it had gone for steaming process (*Pittaviyal murai*) for final purification. After this, the powder was dried and sieved again and stored in a clean air tight glass container for study purpose. It was labelled as *Avuri Karpam* (AK).

Administration of the Drug

Nature of the medicine : *Karpam*

Route of Administration : Oral

Dose : 800-1000 mg (twice a day)

Vehicle : Honey

Studies were conducted after obtaining prior approval No. 07/321/PO/Re/S/01/CPCSEA for animal studies from CPCSEA, Government of India through the Institutional Animal Ethics Committee (IAEC) of C.L. Baid Metha College of Pharmacy, Chennai – 97, Tamil Nadu, India.

ACUTE ORAL TOXICITY STUDY

The acute oral toxicity test was performed following 423 guidelines of Organization for Economic Co-operation and Development (OECD) for testing of chemicals.[7]

Test Animals and Test Conditions

Sexually mature Female Wistar albino rats (150-200gm) were obtained from Mass Biotech, Chennai. All the animals were kept under standard environmental condition (22±3°C). The animals had free access to water and standard pellet diet (Sai meera foods, Bangalore).

Preparation for Acute Toxicity Studies

The animals are randomly selected, marked to permit individual identification, and kept in their cages for at least 7 days prior to dosing to allow for acclimatization to the laboratory conditions. Rats were deprived of food overnight (but not water 16-18 hours) prior to administration of the *Avuri Karpam* (AK). The principles of laboratory animal care were followed and the Institutional Animal Ethical Committee approved the use of the animals and the study design.

Test Substance : *Avuri Karpam* (AK)

Animal Source : Mass Biotech, Chennai

Animals : Wister Albino Rats (Female 3+3+3)

Age : 6-8 weeks

Body Weight on Day 0 :150-200gm.

Acclimatization : Seven days prior to dosing.

Veterinary examination : Prior and at the end of the acclimatization period.

Identification of animals : By cage number, animal number and individual marking by using Picric acid.

Number of animals : 3 Female/group,

Route of administration : Oral

Diet : Pellet feed supplied by Sai meera foods Pvt Ltd, Bangalore

Water : Aqua guard portable water in polypropylene bottles.

Housing & Environment : The animals were housed in Polypropylene cages provided with bedding of husk.

Housing temperature : between 22ºC ± 3ºC.

Relative humidity : between 30% and 70%,

Air changes : 10 to 15 per hour and

Dark and light cycle : 12:12 hours.

Duration of the study : 14 Days

Administration of Doses

AK was suspended in honey and administered to the groups of wistar albino rats in a single oral dose by gavage using a feeding needle. The control group received an equal volume of the vehicle. Animals were fasted 12 hours prior to dosing. Following the period of fasting, the animals were weighed and then the test substance was administered. Three Female animals are used for each group. The dose level of 5mgand 2000 mg/kg body weight was administered. After the substance has been administered, food was withheld for a further 3-4 hours. The principle of laboratory animal care was followed. Observations were made and recorded systematically and continuously as per the guideline after substance administration. The visual observations included skin changes, mobility, aggressively, sensitivity to sound and pain, as well as respiratory movements. Finally, the number of survivors was noted after 24 hours and these animals were then monitored for a further 14 days and observations made daily. The toxicological effect was assessed on the basis of mortality.

OBSERVATIONS

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. It should be determined by the toxic reactions, time of onset and length of recovery period, and may thus be extended when considered necessary. The times at which signs of toxicity appear and disappear are

important, especially if there is a tendency for toxic signs to be delayed. All observations are systematically recorded with individual records being maintained for each animal.

Observations include changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behaviour pattern. Attention was directed to observations of tremors, convulsions, salivation, diarrhoea, 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 humanly killed. When animals are killed for human reasons or found dead, the time of death was recorded.

Behaviour

The animals will be observed closely for behaviour in the first four hours which includes abnormal gait, aggressiveness, exophthalmos, ptosis, akinesia, catalepsy, convolusion, excitation, head twitches, lacrimation, loss of corneal reflex, loss of traction, piloerection reactivity of touch, salivation, scratching, sedation, chewing, head movements, sniffing, straub, tremor and writhes, diarrhea, leathery, sleep and coma.

Body Weight

Individual weight of animals was determined before the test substance was administered and weights will be recorded at day 1, 7, and 14 of the study. Weight changes were calculated and recorded. At the end of the test, surviving animals were weighed and humanly killed.

Food and water Consumption

Food and water consumed per animal was calculated for control and the treated dose groups.

Mortality

Animals were observed for mortality throughout the entire period.

ANALGESIC ACTIVITY OF *AVURI KARPAM* (AK) - EDDY'S HOT PLATE METHOD[8]

The hot plate assay method was employed for the purpose of preferential assessment of possible analgesic effects of *Avuri Karpam*. The analgesic drug, Pentazocine, was used for positive control group. In this experiment, four groups (n=6) of wistar rats (200–250 g) were placed on a hot plate maintained at room temperature for 15 min. Food was withdrawn on the

preceding night of the experiment. Group- I normal control (0.5% CMC p.o.), and Group- II Pentazocine (30mg/kg, i.p.), whereas Group III and IV animals received AK (400 and 800 mg/kg, p. o respectively). Each animal was then individually placed gently on Eddy's hot plate at 55℃. Latency to exhibit nociceptive responses such as licking paws or jumping off the hot plate, were determined 15, 30, 45 and 60 min after administration of the test drug or vehicle.

Table 2: Grouping of the animals.

Groups	No. of rats
Group- I Normal control (0.5% CMC p.o.)	6
Group- II Pentazocine (30mg/kg, i.p.)	6
Group- III *Avuri Karpam* (400 mg/kg)	6
Group- IV *Avuri Karpam* (800 mg/kg)	6

RESULTS AND DISCUSSION

The present research work was aimed to document the acute toxicity study and to validate the efficacy of analgesic activity in wistar albino rats.

Acute toxicity study

Effect of AK on clinical signs of rats in acute toxicity study

The test drug AK administered at the dose level of 5mg and 2000 mg/kg b.w, Observational results given below in Table 3.

Table 3: Effect of AK on clinical signs of rats in acute toxicity study.

S.No	Control group	Observation	S.No	Test group - I 5mg/kg	Test group – II 2000mg/kg	Observation Test group – I&II
1	Body weight	Normal	1	Body weight	Body weight	Normally increased
2	Assessments of posture	Normal	2	Assessments of posture	Assessments of posture	Normal
3	Signs of Convulsion Limb paralysis	Normal	3	Signs of Convulsion Limb paralysis	Signs of Convulsion Limb paralysis	Absence of sign (-)
4	Body tone	Normal	4	Body tone	Body tone	Normal
5	Lacrimation	Normal	5	Lacrimation	Lacrimation	Absence
6	Salivation	Normal	6	Salivation	Salivation	Absence
7	Change in skin color	No significant color change	7	Change in skin color	Change in skin color	No significant color change
8	Piloerection	Normal	8	Piloerection	Piloerection	Normal
9	Defecation	Normal	9	Defecation	Defecation	Normal

10	Sensitivity response	Normal	10	Sensitivity response	Normal	Normal
11	Locomotion	Normal	11	Locomotion	Normal	Normal
12	Muscle gripness	Normal	12	Muscle gripness	Normal	Normal
13	Rearing	Mild	13	Rearing	Mild	Mild
14	Urination	Normal	14	Urination	Normal	Normal

Behavioural signs of acute toxicity for study drug AK

No significant change was observed treated with AK at low dose and high dose. The results were tabulated in table 4.

Table 4: Behavioural signs of acute toxicity for study drug AK.

S.No.	Dose mg/kg	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
1.	**Control**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
2.	**5mg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
3.	**2000mg**	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-

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. Diarrhea 18. Writhing 19. Respiration 20. Mortality. (+ Present, - Absent).

Effect of AK on body weight of rats in acute toxicity study

The results of body weights exposed to low dose and high dose of AK exhibit the mild weight gain. Results were given in table 5.

Table 5: Effect of AK on body weight of rats in acute toxicity study.

DOSE	DAYS		
	1	7	14
CONTROL	320.2±42.30	322.4 ± 60.10	323.6 ±52.10
LOW DOSE	280.1±23.51	282.3±12.33	283.2±22.03
HIGH DOSE	302.4± 1.21	302 ± 2.04	304.2 ± 2.10
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Water intake (ml/day) and food intake (gm/day) of Wistar albino rats group exposed to AK

No significant changes on the average of water and food intake via oral route with AK at the low dose and high dose. Results are tabulated in table 6 and 7.

Table 6: Water intake (ml/day) of Wistar albino rats group exposed to AK.

DOSE	DAYS		
	1	7	14
CONTROL	58 ± 1.02	58±9.20	59.4±1.04
LOW DOSE	61±1.22	60±1.33	61±2.11
HIGH DOSE	59.4±2.20	59.8±3.40	59.9±6.24
P value (p)*	NS	NS	NS

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

Table 7: Results of food intake (gm/day) of Wistar albino rats group exposed to AK.

DOSE	DAYS		
	1	7	14
CONTROL	61.04±2.62	62.2±4.76	64.3±6.26
LOW DOSE	63.2±1.08	64.3±2.11	65.4±2.10
High DOSE	69.4±4.23	70.4±6.22	71.6±4.18

*N.S- Not Significant,**(p > 0.01), *(p >0.05), n = 10 values are mean ± S.D (One-way ANOVA followed by Dunnett's test)*

The test drug AK administered at the dose level of 5mg and 2000 mg/kg b.w. There are no abnormal clinical signs in any of the animals observed for 14 days. All the rats survived and no treatment related mortality occurred during the period of 14 days. No significant difference in body weight gain was observed between control and test group. Weight loss was not observed in any of the groups treated with AK. No significant changes in the feed intake, water intake was observed between control and test groups. Similarly, there is no significant change in C.N.S, A.N.S and C.V.S related behavioural activity in drug treated groups. In this present study, dose levels of 5mg/kg, 2000mg/kg did not produce any toxicity or adverse reactions to the animals. High dose level also can be taken if it is necessary.

Analgesic activity of *Avuri Karpam* (AK) - Eddy's hot plate method

The analgesic property of *Avuri Karpam* was measured through Eddy's hot plate method. Latency period of AK low dose and high dose significantly given good results when compared to standard drug at the time interval of 30 mins, 45 mins, and 60 mins. In this

method, when compared to control and standard drug group the latency period gradually increased which shows the potential of the drug. No mortality and morbidity were observed in all groups during the experiment.

Table 8: Analgesic effect on AK in rats.

Groups	Dose mg/kg	Reaction time			
		15 mins	30 mins	45 mins	60 mins
1.Control	10	2.3±.0.2	2.3±0.4	2.2±0.6	2.2±0.2
2. Pentazocine	30	4.4±0.2	7.1±0.4	8.1±0.6	9.6±0.6
3. *Avuri Karpam* (AK)	400	2.6±1.6	3.9±1.8	4.9±1.6	6.1±2.6
4. *Avuri Karpam* (AK)	800	4.5±0.8	6.9±0.4	7.2±0.2	7.9±0.6
n=6; Statistical analysis one way ANOVA followed by Dunnett t-test.					

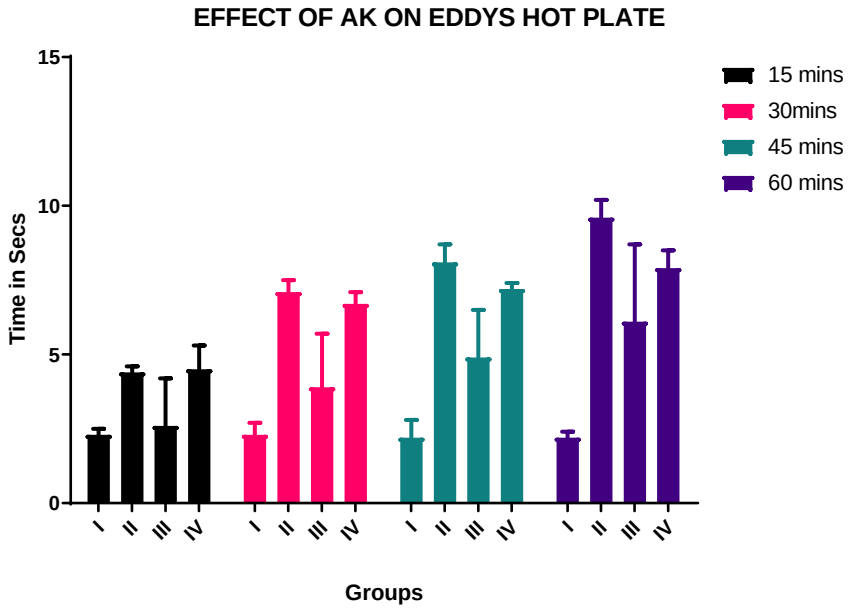

Fig. 1 Effect of AK on Eddy's hot plate method.

CONCLUSION

Acute toxicity study of *Avuri Karpam* (AK) on rats reveals that the drug AK is safe and there were no adverse effects during the study. The study drug *Avuri Karpam* shows significant analgesic activity when compared to the standard drug. However further clinical studies are needed to treat painful clinical condition in a successful way.

ACKNOWLEDGEMENT

We register our thanks to Department of Siddha, the TamilNadu Dr. M. G. R. Medical University, Chennai for their suggestions and support. Also we acknowledge our thanks to

Department of Pharmacology, C. L. Baid Metha College of Pharmacy, Thuraippakam, Chennai for helping in doing this research work.

Funding: This study was financially supported by The Ministry of AYUSH, CCRS, Chennai.

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