Int J Pharma Res Health Sci. 2019; 7 (2): 2948-51
DOI:10.21276/ijprhs.2019.02.05
Thavakeethan and Manoharan A. CODEN (USA)-IJPRUR, e-ISSN: 2348-6465

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International Journal of Pharma Research and Health Sciences

Available online at www.pharmahealthsciences.net

Original Article

Toxicity Study on Siddha Formulation *Appalakara chooranam* in Albino Rat

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ARTICLE INFO

Received: 19 Apr 2019
Accepted: 29 Apr 2019

ABSTRACT

Background: The Siddha medicine is an one among the AYUSH system, it is based on pancha bootha theory. Every human body is constructed by the Thathuvams. In Siddha Medicine widely used in herbals, mineral and metallic preparations. All metallic and minerals are purified before using the drugs. The mineral *Appalakara chooranam* (Sodium carbonate) is widely used in Siddha practice for curing Gastro-intestinal disorders and neuromuscular disease. **Objective:** To determine about the acute and sub-acute toxicity of *Appalakara chooranam* (AKC) in female Wister albino rat models. **Materials and Methods:** Acute toxicity was carried out female Wister albino rat, a single dose of 2000mg of *Appalakara chooranam*. **Results:** In acute toxicity studies showed no significant changes in normal growth pattern, body weight and safety profiles are within nomal limits. It was compared Standard and treatment groups. There was a slightly changed in plasma glucose level after administration of AKC. In Sub acute toxicity experiment showed no markedly changes in Liver enzymes and internal organs. **Conclusion:** The end of study, there was no an undesirable toxic effect of all internal organs, So AKC is a safety for consumption in long period.

Keywords: Siddha medicine, *Appalakara chooranam*, Rat models.Toxicity

1. INTRODUCTION

The World Health Organization (WHO) estimated that 80% of populations of developing countries really on traditional medicines, mostly plant drugs, for their primary health care needs [1]. In that way, Siddha medicine has profound role in disease prevention and prophylaxis through its herbal medicine and other form of medicine like chendooram, Parpam, waxy form etc [2, 3].

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Appalakaram is a potent mineral prepration in the Siddha system of medicine. Traditionally used in the treatment of vatha diseases, loss of appetite and in pain management. Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute and sub-acute toxicity [4]. Acute toxicity is involved in estimation of LD50 (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals. [13]

2. MATERIALS AND METHODS

The raw drug appalakaram was purchased from Traditional raw drug store around Madurai. The collected raw drug was authenticated by faculties of Department of Gunapadam, Government Siddha Medical College, Palayamkottai. After the purification process which is mentioned in Siddha classical text, it has ground into powder for dispensing.

Method: Acute oral toxicity of Appalakara chooranam is carried out as per the OECD-423 guidelines after the animal ethical clearance from Institutional Animal Ethics Committee (KMCP/22/2018)

The female Wister rat are fasted over night and provided only water, after which the Appalakara chooranam (AKC) was administered by gastric lavage to the relevant group of animals orally at the dose of 50 $mg.kg^{-1}$ body weight in Tween-80. The animals are then observed for 14 days and maintained with normal food. A mortality rate of 2 or 3 animals in 14 days is recorded and the dose is said to be toxic dose. But when mortality of one animal is observed, then the same dose is repeated again for confirmation. However, mortality is not observed, the procedure is repeated for further higher doses 300 and 2,000 respectively. Toxic symptoms are observed for 72 hrs including behavioral changes, locomotion, convulsions and mortality [5, 6]. Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of chemical compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute, sub-acute toxicity. Acute toxicity is estimated by LD_{50}. [13]

Cage Side Observations:

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. Special attention is directed for the observation of tremors, convulsions, salivation, diarrhea, lethargy, sleep and coma. Body weight, food and water intake are recorded at two-day intervals.

Sub acute test for Appalakara chooranam

Male and female Wister rats (180 ± 10 g) are divided into five groups of six animals, totally 30 animals is used for this experiment. The dose of the preparation is calculated based on the body weight of the animal. The animals in Group I are administered with a single daily dose of 0.5 ml of tween 80 orally for 20 days. The animals in Group II are administered with 50 $mg.kg^{-1}$ b.w. of the Appalakara chooranam orally once daily for 20 days. The animals in Group III are administered with 100 $mg.kg^{-1}$ b.w. of the APC orally once daily for 20 days. The animals in Group IV and V are administered once daily with 200 and 400 $mg.kg^{-1}$ b.w. of the APC for 20 days orally [7, 8]. The animals are then weighed every five days, from the start of the treatment, to record the weight variation. At the end of the treatment, blood samples are collected by puncturing retro orbital plexus after mild anesthesia for biochemical analysis. The collected blood sample is centrifuged within 5 min of collection at 4000 RPM for 10 min to obtain plasma, which is analyzed for Lipids,Renal profiles.

Appalakaram is a potent mineral prepration in the Siddha system of medicine. Traditionally used in the treatment of vatha diseases, , loss of appetite and in pain management. Determination of acute oral toxicity is usually the initial screening step in the assessment and evaluation of the toxic characteristics of all compounds. The types of toxicity tests which are routinely performed by pharmaceutical manufacturers in the investigation of a new drug involve acute and sub-acute and toxicity. Acute toxicity is involved in estimation of LD_{50} (the dose which has proved to be lethal (causing death) to 50% of the tested group of animals.[9-13]

3. RESULTS AND DISCUSSION

Acute toxicity study with Appalakara chooranam

The acute toxicity of Appalakara chooranam was found no mortality or morbidity in animals after 15-days periods followed by single oral administration of selected groups. The result was given in (Table-1). The morphological character is and physical appearance of all animals seems to normal, it was compared Group I , II and III and found no toxic effects up to 2gms.

Table 1: Result of Acute toxicity study of Appalakara chooranam

	Dose ($mg.kg^{-1}$)	Sign of Toxicity ($ST.NB^{-1}$)	Mortality ($D.S^{-1}$)
Group I	0	0/3	0/3
Group II	300	0/3	0/3
Group III	2000	0/3	0/3

ST- sign of toxicity; NB- normal behaviour; D- died; S- survive. Values are expressed as number of animals (n=3).

Subacute Toxicity of Appalakara chooranam(AKC)

Appalakara chooranam were evaluated for subacute toxicity.The effect of Appalakara chooranam was observed for their effect on the body weight changes from the study it was observed that, there was significant increase ($p<0.05$) in body weight in all the animals observed. The results are shown in Table.2. The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis

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was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Table 2: Results of Appalakara chooranam (AC) on body weight changes in rats

Treatment	Day 1	Day 5	Day 10	Day 20
Control	180.15±6.8	191.45 ±6.20	199.15 ±6.35	199.7±6.58
AC **50 mg.kg^{-1}**	187.30 ±6.4	196.30 ±6.30	201.25 ±6.70	201.30±6.72*
AC **100 mg.kg^{-1}**	179.35 ±5.7	192.30 ±6.40	199.55 ±7.10	200.36±6.30*
AC **200 mg.kg^{-1}**	188.30 ±7.2	200.15±6.50	201.90 ±7.20**	209.45±7.26**
AC **400 mg.kg^{-1}**	180.65 ±6.05	195.15 ±5.60	198.60 ±6.35**	300.66±7.38**

The effects of Appalakara chooranam on kidney, heart, liver and brain of the rats were observed. From the study it was clear that, significant (p<0.01) changes in the weights of various organs of the animals occurred with higher doses of the AKC (400 mg.kg^{-1} bwt), but macroscopic examinations did not show any changes in colour of the organs of the treated animals compared with the control. The results are shown in Table.3

Table 3: The effects of Appalakara chooranam on kidney, heart, liver and brain

Treatment	Heart (gms)	Kidney (gms)	Liver (gms)	Brain (gms)
Control	0.32 ± 0.05	0.66± 0.03	3.35± 0.05	0.70± 0.05
AC 50 mg.kg^{-1}	0.33± 0.02	0.82± 0.03	3.47± 0.03	0.73± 0.3
AC 100 mg.kg^{-1}	0.34± 0.06	0.80± 0.04	3.39±0.02	0.71± 0.2
AC 200 mg.kg^{-1}	0.33± 0.04	0.75± 0.02	3.37± 0.02	0.78± 0.05
AC 400 mg.kg^{-1}	0.32± 0.03	0.76± 0.03	3.40± 0.03	0.80± 0.05

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01.

Table no 4. Showed the effect of Appalakara chooranam was significant decrease (p<0.05) in the plasma glucose level in treated rats especially at higher dose (400 mg.kg^{-1}) compared with control groups. **P<0.01 *P<0.05. Significant decrease (p<0.05) in the plasma total cholesterol (TC), triglyceride (TG) and LDL-cholesterol levels. So, there is no evidence of severe toxicity associated with the administration of higher concentration of **AKC**.

Table 4: The result of Appalakara chooranam on biochemical parameters

Treatment	Glucose (mg.dl^{-1})	Cholesterol (mg.dl^{-1})	Triglyceride (mg.dl^{-1})	HDL (mg.dl^{-1})	LDL (mg.dl^{-1})
Control	90.65± 0.62	39.62± 0.56	28.25± 0.45	139.25± 0.55	83.15±1.72
AC 50 mg.kg^{-1}	88.50± 0.56	25.85± 0.25*	13.22± 0.23*	179.28± 0.65*	70.59±1.28
AC 100 mg.kg^{-1}	85.45± 0.47	26.74± 0.26*	15.42± 0.28*	169.18±0.78*	68.84±1.10
AC 200 mg.kg^{-1}	86.25± 0.55**	33.18± 0.30	17.84± 0.38*	188.30± 0.84*	47.60±1.30
AC 400 mg.kg^{-1}	82.25± 0.45**	32.78± 0.28	19.28± 0.34*	186.2± 0.85*	45.50±0.84

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The effect of Appalakara chooranamon biochemical parameters such as glucose, was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Table 5: The effects of Appalakara chooranamon biochemical parameters

Treatment	AST (IU.l^{-1})	ALT (IU.l^{-1})	ALP (IU.l^{-1})	TP (g.l^{-1})	ALBUMIN (g.l^{-1})
Control	321.5±12.40	74.5± 3.18	256.58± 8.80	72.85± 3.32	40.15±2.35
AC 50 mg.kg^{-1}	310.0±9.50**	72.5± 2.20**	269.10± 2.75**	73.30± 2.32	37.30±2.65
AC 100 mg.kg^{-1}	311.3±7.20**	70.1± 3.15**	263.18± 6.70**	83.15± 2.82	39.30±3.05
AC 200 mg.kg^{-1}	306.4±7.95	65.4± 2.90	268.00± 5.20	72.25± 3.32	41.20±2.75
AC 400 mg.kg^{-1}	316.2± 8.20	67.3± 3.52	272.40± 4.40	77.05± 2.58	40.48±2.70

The values are expressed as mean ± S.E.M. n=6. The results of group I were compared with other groups such as II, III, IV, and V. The statistical analysis was carried out using one way ANOVA method, where **P<0.01 *P<0.05.

Effect of Appalakara chooranamon haematological parameters in rats

The effects of Appalakara chooranam were observed for its effect on hematological parameters on the experimental rats. From the study it was evident that, a significant increase (p<0.01) were observed in the haemoglobin contents and RBC count,Serum Calcium level in the group treated with 200 mg.kg-1 body weight of Appalakara chooranam and a significant decrease of the parameters occurred in the group treated with 400 mg.kg-1/bwt. It was compared the control group. The statistical analysis was carried out using one way ANOVA method, where *P<0.05

Table 6: The effect of Appalakara chooranamon haematological parameters

Treatment	Haemoglobin (g.dl^{-1})	RBC (10^6 /mm^3)	WBC (10^6 /mm^3)	Calcium (mg.dl^{-1})
Control	14.3± 0.25	9.15± 0.02	11.9± 0.05	10.40 ±0.06
AC 50 mg.kg^{-1}	15.5± 0.26*	9.50± 0.04*	10.05± 0.01*	10.16 ±0.07
AC 100 mg.kg^{-1}	15.3± 0.15*	9.55± 0.02*	8.8± 0.32*	10.22 ±0.24
AC 200 mg.kg^{-1}	13.7± 0.20*	8.33± 0.12*	11.9± 0.03*	10.56 ±0.12
AC 400 mg.kg^{-1}	14.5± 0.35*	8.51± 0.45*	11.05± 0.13*	10.70 ±0.06

In acute toxicity, the more than the dose 2000 mg/kg can produced mortality, In sub acute toxicity study, the AKC was not affected the normal growth of the animals,which was compared the control and treated animal groups over the 20-days of treatment periods. There were no significant changes in liver enzymes (ALT,AST,ALP and IP) .The significant increased in the level of RBC,WBC and Hemoglobin was found, after using *Appalakara chooranam* (upto400 mg.kg$^{-1)}$.. The AKC was not produced undesirable effects,which was confirmed by necroplexy

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Conflict of Interest: None
Source of Funding: Nil

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