WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.074
Volume 7, Issue 18, 1337-1344. Research Article ISSN 2277– 7105

ACUTE ORAL TOXICITY AND HISTOPATHOLOGICAL EVALUATION OF SIDDHA DRUG MAHA ANALURUVA CHOORANAM (MAC) IN WISTAR RATS – A STUDY.

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Article Received on
20 August 2018,

Revised on 07 Oct. 2018,
Accepted on 29 Oct. 2018

DOI: 10.20959/wjpr201818-13552

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ABSTRACT

In Siddha system of traditional medicine, *Maha analuruva Chooranam (MAC)* is an Herbal formulation. In the reference text of *"Agasthiyar vaithiya vallathi 600"*, it has been used for the treatment of *Kumbavatham (periarthritis of shoulder or Adhesive capsulitis or frozen shoulder). Kumbavatham* is a condition which deals with the involvement of painful and restricted movements of the shoulder joint, a kind of arthritis attended with boring pain in the shoulders and arms and restricted movements of the upper limb. Siddha medicine MAC is a good remedy for the periarthritis of shoulder. In this study the author has aimed to evaluate safety and efficacy of the trail drug MAC in female Wistar rats (Nulliparous, Non-Pregnant). Acute oral toxicity was performed as per organization for economic co-operation for the development (OECD) guideline 423 methods. Acute toxicity studies were carried out in 5 groups of 15 female Wistar rats, a dose of 100mg, 250mg, 500mg, 1000mg; 2000mg of MAC was administered and monitored for any toxicity effects. After 14 days the animals were sacrificed and the histopathological analysis of the liver, lung, kidney, spleen, of treated groups did not show any sign of toxicity. *Maha analuruva chooranam* was Non-toxic in acute toxicity study.

KEYWORDS: *Siddha System, Maha analuruva chooranam, Acute Oral Toxicity, Histopathology, Wistar rats.*

INTRODUCTION

Periarthritis is also known as adhesive capsulitis or frozen shoulder, an insidious painful condition of the shoulder persisting more than 3 months. This inflammatory condition that causes fibrosis of the glenohumoral joint capsule is accompanied by gradually progressive stiffness and significant restriction of range of motion (typically external rotation). However, the patients may develop symptoms suddenly and have a slow recovery phase. The recovery is satisfying in most of the cases, even though this may take up to 2-3 years.[1]

Siddha literatures have prescribed many medicines for the treatment of *kumbavatham.* In siddha system, *Maha analuruva chooranam* is a mixture of a single or combination of herbs. Herbs have been in use since long time to treat various diseases. However, many issues related to a lack of scientific evidence about the efficacy and safety of the drugs remains unresolved.[2]

The pre-clinical toxicity screening is essential for determining a safe dose for the human trails.[3] The siddha drug *Maha analuruva chooranam* quoted in siddha literature "*Agasthiyar vaithiya vallathi 600"* has been used for the treatment of Vatha diseases *(Kumbavatham).* Consequently an effort has been made by the author to evaluate acute toxicity of the siddha poly herbal formulation MAC in laboratory animals.

MATERIALS AND METHODS

The drugs used to prepare *Maha analuruva chooranam (MAC)* are Roots of *Plumbago indica (Kodiveli ver),* Roots of *Pongamia pinnata (Pungam ver),* Roots and bark of *Holoptelea integrefolia (Ayilium ver, Ayilium pattai), Terminalia chebula (Kadukkai), Zingeber officinale (Chukku), Piper longum (Thippili), Embelia ribes (Vaivilangam), Brassica junceae (Kadugu) and Nigella sativa (Kariya seeragam).*[4] The drugs were purchased from the local market in Thirunelveli town and authenticated by the Department of pharmacology, government siddha medical college, palayamkottai, Tamilnadu, India. The drugs were purified and the medicine was prepared as per the methodology in the Siddha text "Agasthiyar vaithiya vallathi 600".

Table: 1.

S.No	Botanical Name	Tamil Name	Family	Activities
1.	*Plumbago indica*	*Kodiveli ver*	*Plumbaginaceae*	Antimicrobial[5] Antimalarial[6] Antiinflammatory[7]
2.	*Pongamia pinnata*	*Pungam ver*	*Fabaceae*	Antiinflammatory Antioxidant[8]
3.	*Holoptelea integrefolia*	*Ayilium ver*	*Ulmaceae*	Antiinflammatory Antioxidant Antitumor activity[9]
4.	*Holoptelea integrefolia*	*Ayilium pattai*	*Ulmaceae*	Antiinflammatory Antioxidant Antitumor activity[9]
5.	*Terminalia chebula*	*Kadukkai*	*combretaceae*	Antidiabetic[10] Antioxidant[11] Antiulcer[12] Wound healing[13]
6.	*Zingeber officinale*	*Chukku*	*Zingeberaceae*	Hypolipidaemic[14] Antioxidant[15]
7.	*Piper longum*	*Thippili*	*Piperaceae*	Immunomodulatory[16] Antitumor activity[16] Antibacterial[17]
8.	*Embelia ribes*	*Vaivilangam*	*Myrsinaceae*	Anthelmintic Carminative Stimulant[18]
9.	*Brassica junceae*	*Kadugu*	*Brassicaceae*	Anticancer Antibacterial Antifungal[19]
10.	*Nigella sativa*	*Kariya seeragam*	*Ranunculaceae*	Antiinflammatory Analgesic[20]

Experimental protocol

Acute toxicity study of MAC was done adhering to the guidelines of OECD 423 method.[21] The study was done in KMCH College of pharmacy, Coimbatore, after obtaining the needed approval for the study from the Institutional Animal Ethical Committee (IAEC) *Ref.no: KMCRET/MD(S)/04/2016-17*. Fifteen healthy young adult female Wistar rats, nulliparous and non-pregnant weighing about 150-200gm were selected for the study. The rats were divided into 5 groups, with 3 animals in each group. 2000mg/kg dosage of the test drug in 200g body weight was given in a single dose of 1ml. Honey was used as the vehicle for the per oral administration of the drug through oral gavage.

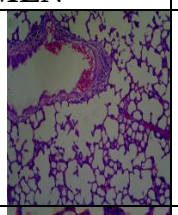
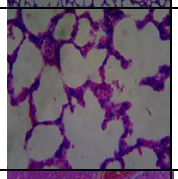
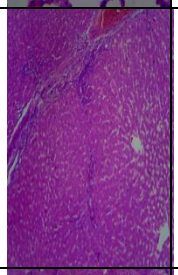
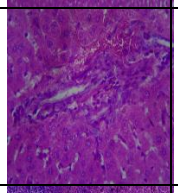
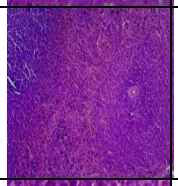
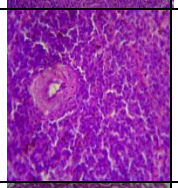
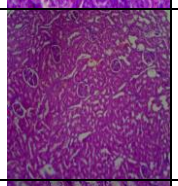
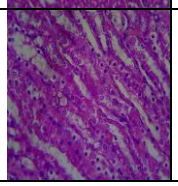
Study Procedure: To evaluate the acute oral toxicity of *Maha analuruva chooranam*, the test drug was administered in a single dose by a tuberculin syringe. Animals were fasted 3 hr prior to dosing (food was withheld for 3 hr but not water). Following the period of fasting

animals were weighed and the test substance was administered orally at a dose of 100mg, 250mg, 500mg, 1000mg and 2000mg/kg. After the *Maha analuruva chooranam* administration, food was withheld for 2 hr in mice. The animals are observed individually for at least once during the first 30 minutes, periodically during the first 24 hrs. With special attention given during the first 4 hrs and daily thereafter, for a total of 14days. The animals were sacrificed after 14 days and the histopathological examination was done.

Table 2: Effect of Maha analuruva chooranam on acute toxicity test in mice.

S.no	Response	Head		Body		Tail	
		Before	After	Before	After	Before	After
1	Alertness	Normal	Normal	Normal	Normal	Normal	Normal
2	Grooming	Absent	Absent	Absent	Absent	Absent	Absent
3	Touch response	Absent	Absent	Absent	Absent	Absent	Absent
4	Torch response	Normal	Normal	Normal	Normal	Normal	Normal
5	Pain response	Normal	Normal	Normal	Normal	Normal	Normal
6	Tremors	Absent	Absent	Absent	Absent	Absent	Absent
7	Convulsion	Absent	Absent	Absent	Absent	Absent	Absent
8	Righting reflux	Normal	Normal	Normal	Normal	Normal	Normal
9	Gripping strength	Normal	Normal	Normal	Normal	Normal	Normal
10	Pinna reflux	Present	Present	Present	Present	Present	Present
11	Corneal reflux	Present	Present	Present	Present	Present	Present
12	Writhing	Absent	Absent	Absent	Absent	Absent	Absent
13	Pupils	Normal	Normal	Normal	Normal	Normal	Normal
14	Urination	Normal	Normal	Normal	Normal	Normal	Normal
15	Salivation	Normal	Normal	Normal	Normal	Normal	Normal
16	Skin colour	Normal	Normal	Normal	Normal	Normal	Normal
17	Lacrimation	Normal	Normal	Normal	Normal	Normal	Normal

Table 3: Histopathological Examination.

S.NO	SPECIMEN		OBSERVATIONS
1.	LUNG		10x lung mild peribronchiolar inflammation
1a			40x shows normal alveoli
2.	LIVER		10x liver shows bile duct hyperplasia
2a.			40x shows bile duct hyperplasia
3.	SPLEEN		10x spleen shows normal
3a.			40x shows normal spleen, pencilar artery.
4.	KIDNEY		10x kidney shows normal
4a.			40x shows normal interstitium.

RESULTS AND DISCUSSION

The present study was conducted to know single dose toxicity of *Maha analuruva chooranam (MAC)* on female Wistar rats. MAC was administered single time at the doses of 100mg, 250mg, 500mg, 1000mg and 2000mg/kg to female Wistar rats and observed

consecutively for 14 days after administration. Doses were selected based on the pilot study and literature review. All animals were observed daily once for any abnormal clinical signs. Weekly body weight and food consumption were recorded. No mortality was observed during the entire period of the study. Data obtained in this study indicated no significance physical and behavioural signs of any toxicity due to administration of MAC at the doses of 100mg, 250mg, 500mg, 1000mg and 2000mg/kg to female Wistar rats.

At the 14th day, all animals were observed for functional and behavioural examination. In functional and behavioural examination, home cage activity, hand held activity were observed. Home cage activities like Body position, Respiration, Clonic involuntary movement, Tonic involuntary movement, Palpebral closure, Approach response, Touch response, Pinna reflex, Sound responses, Tail pinch response were observed. Handheld activities like Reactivity, Handling, Palpebral closure, Lacrimation, Salivation, Piloerection, Papillary reflex, abdominal tone, Limb tone were observed. Functional and behavioural examination was normal in all treated groups. Food consumption of all treated animals was found normal as compared to normal group. There were no physical and behavioural changes observed in female Wistar rats during the 14 days. Mortality was not observed in any treatment groups. The histopathological study was done after sacrificing the animals after 14 days and no toxic effects of the test drug was observed.

RESULT

From acute toxicity study it was observed that the administration of *Maha analuruva chooranam (MAC)* to female Wistar rats did not induce drug-related toxicity and mortality in the animals up to 2000mg/kg in 200g female Wistar rats. So No-Observed-Adverse-Effect-Level (NOAEL) of MAC is 2000mg/kg equal to human dose. The behavioural changes observed in the study are given in Table 2. In the histopathological evaluation, section from the lung shows normal alveoli with mild peribronchial inflammation, section from liver shows normal architecture with mild bile duct hyperplasia and the section from spleen and kidney shows no significant pathology which is given in Table 3. The histopathological study shows that the trial drug has no evidence of any toxic effects.

CONCLUSION

The study shows that the test drug *Maha analuruva Chooranam* did not produce any toxic effect at doses of 100mg, 250mg, 500mg, 1000mg and 2000mg/kg to rats. So No-Observed-Adverse-Effect-Level (NOAEL) of *Maha analuruva chooranam* is 2000 mg/kg. The

Histopathological observation of the given drug *Maha analuruva chooranam* has not induced any obvious abnormalities in liver, lung, spleen, and kidney. Hence it can be concluded that the given drug have no evidence of toxic effect.

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