

**TOXICOLOGICAL AND PHARMACOLOGICAL PROFILING OF
ATHIMATHURA CHOORANAM - A *SIDDHA* FORMULATION
INDICATED FOR RESPIRATORY AILMENTS AMONG CHILDREN**

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ABSTRACT

Respiratory tract infections seem to be the most common chronic disease in developing countries with bothersome symptoms affecting the quality of life in children causing emergency hospital visits, missed school days and mortality. *Athimathura Chooranam* is a *Siddha* classical Polyherbal formulation indicated in *Siddha* literature for *Kanam* (Respiratory ailments in Children). This study was aimed to evaluate the Anti-inflammatory, Antipyretic and Antihistaminic activity of the powdered extract of *Athimathura chooranam* supported by *in vivo* studies. The Acute oral toxicity and repeated oral toxicity study was performed for the extract of *Athimathura chooranam* in

Wistar albino rats to find the appropriate dosage to be administered. Anti-inflammatory activity of the extract was further assessed using Carrageenan induced hind paw edema and Cotton pellet granuloma and later Antipyretic and Antihistaminic activity was also studied using rat and Guinea pigs respectively. It was observed that the extract was found to be non-toxic at dosage of 2000mg/kg/po in rat and so it can be classified under category-5 of globally harmonized system of classification and labeling of chemicals. It poses anti-inflammatory activity at 450 mg/kg/po in both carrageenan induced hind paw edema and Cotton pellet granuloma. The extract had effective antipyretic activity even after 240 min of observation. *Athimathura chooranam* exhibited anti-histaminic activity at a concentration of

32mg/ml and all the results were supported by statistical significance. The results reveal that *Athimathura chooranam* had good Anti-inflammatory, Antipyretic and Antihistaminic activity which was supported by in vivo preclinical studies.

KEYWORDS: *Athimathura chooranam*, *Kanam*, Anti-inflammatory activity, Antipyretic activity, Antihistaminic activity.

INTRODUCTION

India is known for its traditional medicinal systems – *Ayurveda* and *Siddha* that are found mentioned even in the ancient Vedas and other scriptures. The Indian subcontinent is a vast repository of medicinal plants that are used in traditional medical treatments. Use of plants as a source of medicine has been an ancient practice and is an important component of the health care system in India. In India, around 20,000 medicinal plants have been recorded. However, traditional practitioners use only 7,000–7,500 plants for curing different diseases. The proportion of use of plants in the different Indian systems of medicine is *Ayurveda* 2000, *Siddha* 1300, *Unani* 1000, Homeopathy 800, Tibetan 500, Modern 200, and folk 4500.^[1]

The name *Siddha* medicine owes its origin to medicinal ideas and practices of class of Tamil sages called *Siddhars* – perfected or holy immortals who were, and are still believed to have superhuman power. *Siddha* medicine has immense faith in the miracles of herbal and mineral drugs and in the prolongation of life through rejuvenating treatment and intense yoga practices. The word *Siddha* means established truth.^[2] *Siddha* system of medicine is one such ancient traditional system of India and practiced mostly in its southern part for treating various diseases including even chronic conditions.^[3] *Siddha* propounds that the physical structure of the universe and man are basically made up of Five Elements. They are Nilam (Earth), Neer (Water), Thee (Fire), Kaatru (Air), and Vin (Sky).^[4]

Respiratory diseases are a major cause of morbidity and mortality in developing countries. Upper respiratory tract infections comprises 87.5% of total acute respiratory infections morbidity. Data suggest that children could suffer from 7 to 8 episodes of upper respiratory tract infections per year until they are 5 years of age, when their immune status reaches adult level. In *Siddha* system of medicine *Kanam* is referred to as group of respiratory disorders pertaining to both upper and lower respiratory tract infections.

Upper respiratory tract infections (URI) is highly prevalent incidence among young children and 61% results in Otitis media (OM) and 18% of children whom consumed antihistamines containing combination of preparation had contraindication^[5]. Colds occur all year round, but are more common in the winter months and the total burden of illness caused by them is greater than the burden caused by seasonal influenza.^[6] Respiratory viruses, especially rhinoviruses, are an important cause of exacerbation and may be isolated in 50% of cases.^[7 & 8]

Athimathura chooranam an herbal formulation mentioned in Sastric *Siddha* literature, *Balavagadam* and it is indicated for all types of *Kanam*. The major drug in the experimental formulation viz. *Athimathuram* (*Glycyrrhiza glabra*) had been reported to have anti-inflammatory activity, *Lavangapattai* (*Cinnamomum verum*) having anti allergic activity and *Korai* (*Cyperus rotundus*) having antipyretic activity and other drugs poses anti-inflammatory activity.

Though this formulation is used as a major drug in the treatment of respiratory infections and related symptoms no proper Scientific validation has been performed and hence this has led to an attempt to prove the efficacy of this *Siddha* formulation by appropriate clinical evidence.

MATERIALS AND METHODS

Ingredients of *Athimathura chooranam*

The required drugs for preparation of *Athimathura Choornam* were purchased from a well reputed country shop and raw drugs are authenticated with the help of an Herbal botanist and with Department of Gunapadam. The ingredients incorporated were *Glycyrrhiza glabra*, *Elettaria cardomomum*, *Cinnamomum verum*, *Michelia champaga*, *Costus speciosus*, *Zingiber officinale*, *Cuminum cyminum*, *Cyperus rotundus* and sugar. The medicine were prepared in Gunapadam lab of National institute of Siddha after proper purification. The prepared medicines were further authenticated.

Purification & Preparation

Elettaria cardomomum, *Cyperus rotundus*, *Cinnamomum verum*, *Michelia champaga* were dried in sun light, the outer layer of the root of *Athimathuram* were removed and cut in to small pieces and dried in sun light. The dust of *Cuminum cyminum* and *Costus speciosus* was removed and dried in sunlight, *Zingiber officinale* was soaked in limewater and filtered and

the outer layer was removed. All the eight raw drugs were weighed 35gm and fried until golden brown, then ground into fine powder and filtered in fine cloth finally equal quantity of sweet candy powder was added. The prepared choornam was stored in a clean, dry airtight container until use.

Phytochemical analysis

The powdered extract of the *Athimathura choornam* were tested for the presence or Absence of Calcium Sulphate, Chloride, Carbonate, Starch, Iron (Ferric), Iron (Ferrous), Phosphate, Tannic acid, Unsaturated compound, Saponins, Sugars, Steroids, Amino acids, Proteins, Flavanoids, Phenol, Tannins and Alkaloids.

Drugs and chemicals

Standard Drugs and fine chemicals used in these experiments were obtained from Sigma Chemicals Company, U. S. A. Other analytical grade chemicals were obtained from S. d. Fine Chemicals Ltd, Mumbai.

In vivo Pharmacology and Toxicity studies

The preclinical studies for acute toxicity were carried out in pharmacological laboratory in National institute of Siddha with the reference number of IAEC protocol No:1248/ac/09/CPCSEA and sub acute toxicity studies were performed in C. L. Baid Mehta College of Pharmacy. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC). (IAEC/XXXV/94/CLBMCP/2012)

Experimental animals

Colony inbred animals strains of Wistar rats of both male and female weighing 200 - 250gm were used for the pharmacological and toxicological studies. Male guinea pig ileum (0.5kg) was used for the assay of antihistaminic activity of the test drug. The animals were kept under standard conditions 12:12 (day/night cycles) at 22°C room temperature, in polypropylene cages. The animals were fed on standard pelleted diet (TANUVAS, Chennai) and tap water *ad libitum*. The animals were housed for one week in polypropylene cages prior to the experiments to acclimatize to laboratory conditions.

Acute oral toxicity study

Acute oral toxicity was conducted as per the OECD guidelines (Organization of Economic Cooperation and Development) 423 (Acute Toxic Class Method). The acute toxic class

method is a procedure in which three animals of a single sex were used in each step. Depending on the mortality and /or moribund status of the animals, on the average 2-4 steps may be necessary to allow judgment on the acute toxicity of the test substance. In this method the dosage used were 5, 50, 300, 2000 mg/kg body weight to rank the chemicals based on the acute toxicity. Wistar albino rats of both sex were fasted overnight, but allowed the intake of water. Since the formulation is relatively non toxic in clinical practice the highest dose of 2000 mg/kg/p.o (as per OECD guidelines "Unclassified") was used in the acute toxicity study. The animals were observed closely for behavioral toxicity, if any by using FOB (Functional observation battery).

Repeated oral toxicity study

Repeated oral toxicity studies were used to get additional information about the toxicity profile of a chemical. Usually, the dose levels are lower than for acute studies and allow chemicals to accumulate in the body before lethality occurs, if the chemical possess this ability.

Experimental procedure

To evaluate the repeated oral toxicity of the extract the animals were divided into two groups. Group I (Control animals) received 1% CMC, 2 ml/kg/p. o. for 14 days. Group II Received AMC at the dose of 450mg/kg/po in 1% CMC for 14 days. The dose for rats was calculated by multiplying the daily dose used in the clinical practice (i. e. 5000mg /day) divided by a factor 0. 018 corresponding to the body surface area of man weighing 70kg to rat weighing 200g. Body weight, food intake and water intake was recorded at two intervals with simultaneous observation for toxic manifestation and mortality, if any. At the end of 14 days treatment all the animals were sacrificed by over dosage of ether anaesthesia. Blood was collected and used for hematological studies. Section of liver, kidney, and heart were dissected out and kept in 10% formalin for histopathological studies.

Biochemical analysis

The powdered extract of *Athimathura chooranam* were evaluated for Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Cholesterol, Urea, Uric acid and Creatinine.

Haematological studies

Erythrocyte count, Total Leukocyte Count (WBC) and Haemoglobin were evaluated for the animals incorporated in the study.

***In vivo* Anti inflammatory activity**

Anti inflammatory activity of the powdered extract was evaluated in both acute model and chronic model of inflammation. Wistar rats either sex weighing 200-250g were divided into different groups with 6 animals in each group and were administered the test substance per oral. Group-1 (Control group) received CMC 10ml/kg. Group-2. Received Carrageenan (0.1% solution) and served as negative control. Group-3 Received test drug (AMC) at the dose of 450mg/kg. Group-4 received standard drug Diclofenac sodium (5mg/kg)

Acute model

Carrageenan induced hind paw edema

The carrageenan assay procedure was carried based on the method adopted by Winter *et al.*,^[9]. Edema was induced by injecting 0.1 ml of 1% solution of carrageenan in saline into the plantar aponeurosis of the left hind paw of the rats. The extracts, reference drug and the control vehicle (distilled water) were administered 60 min prior to the injection of the carrageenan. The volumes of edema of the injected and contra lateral paws were measured at +1, 3 and 5 hrs after induction of inflammation using a plethysmometer by adopting the method of and percentage of anti-inflammatory activity was calculated.

Chronic model

Cotton pellet granuloma

Sterile cotton pellets (weighing 10 ± 2 mg) were implanted subcutaneously along the flanks of axillae and groins of Wistar albino rats. The extracts, reference drug and the control vehicle (distilled water) were administered as per protocol to rats everyday for a period of 7 days. On day 8 the rats were sacrificed by cervical decapitation and cotton pellets were removed surgically, freed from extraneous tissue and weighed immediately for wet weight. One half of the pellets were dried in an incubator at 60°C until obtaining the constant weight.

***In vivo* Antipyretic activity**

Rats selected for the study were fasted overnight allowing water *ad libitum*. Initial rectal temperature was recorded using Hick's clinical thermometer. Pyrexia was induced by subcutaneous injection of TAB vaccine 1 ml/kg body weight, 6 hrs later pyrexia was assessed

and those animals that did not show a minimum rise of 1.5°C were rejected and rest of the animals was divided into 6 groups and drugs were administered. Pyrexia was recorded at hourly intervals for 3 hrs after drug administration.

***In vivo* Antihistaminic activity**

Guinea pigs weighing (300-500 g), starved over night with water *ad libitum*. The animals were killed by a blow on the head and the neck was exsanguinated. The abdomen was cut open and a suitable length of the ileum (approximately 2 cm long) was placed on a petridish containing Tyrode solution. The composition of the Tyrode solution in mM was NaCl 137 mM, NaHCO₃ 12 mM, NaH₂PO₄ 0.3 mM, KCl 2.7 mM, MgCl₂ 1.0 mM, CaCl₂ 1.0 mM and d- glucose 5.6 mM. Experiment was performed in a 30 ml organ bath containing Tyrode solution maintained at 37°C under a tension of 0.5 gm and gassed with air mixture (O₂+CO₂). Isometric contractions were recorded in a smoked kymograph paper with frontal writing lever. After an equilibration period of 30 min during which the Tyrode solution was changed intervals of 10 minutes, contractile responses were recorded for histamine (10 µg/ml). The contact time of 30 sec recorded at 5 min time cycle is kept for proper recording of the responses. The AMC - tissue contact time was 5 min before the addition of histamine. The effect of the extract on histamine induced contractions were recorded. The percentage inhibition of the AMC on contraction induced by histamine was recorded.

RESULTS

The drugs for the preparation of *Athimathura chooranam* were purchased and the raw drugs are authenticated with the help of an Herbal botanist. The extract was then prepared as per the formulation procedure. The prepared powder was further subjected to Phytochemical analysis and tested for the presence and absence of Calcium, Sulphate, Chloride, Carbonate, Starch, Iron (Ferric), Iron (Ferrous), Phosphate, Tannic acid, Unsaturated compound, Saponins, Sugars, Steroids, Amino acids, Proteins, Flavanoids, Phenol, Tannins and Alkaloids and the result were tabulated in (Table: 1). Biochemical analysis and Haematological pattern of *Athimathura chooranam* is given in (Table 2 &3)

Table 1: Phytochemical analysis of *Athimathura chooranam*.

Observation	Inference
No white precipitate	Calcium Absent
No white precipitate	Sulphate Absent
White precipitate obtained	Chloride Present
No effervescence	Carbonate Absent

No blue colour	Starch Absent
No blue colour	Ferric iron Absent
Blood red colour obtained	Ferrous iron Present
Yellow precipitate obtained	Phosphate Present
Black precipitate obtained	Tannic acid Present
No decolourisation	Absence of unsaturated compound
No Froth formation	Saponins Absent
No colour change	Sugar Absent
No Reddish violet zones	Carbohydrate Absent
No red colour	Steroids Absent
Violet colour formed	Amino acids Present
Deep blue colour formed	Proteins Present
Pink colour formed	Flavanoids Present
No green colour	Phenols Absent
White precipitate obtained	Tannins Present
Cream colour precipitate obtained	Alkaloids Present
Orange colour precipitate obtained	Alkaloids Present

Table 2: Haematological pattern of acute oral toxicity.

Groups	Hb (gm/100ml)	RBC (millions/cu. mm)	WBC (cells/cu. mm)	Differential leucocyte count (%)		
				Lymphocytes	Monocytes	Granulocytes
Normal	14. 0 ± 0. 354	5. 9 1 ± 0. 665	5785 ± 9. 434	75. 06 ± 3. 829	6. 30 ± 1. 904	17. 70 ± 5. 627
AMC(450mg/kg/p. o)	14. 88 ± 0. 710 ^{ns}	5. 89 ± 0. 737 ^{ns}	5886. 66 ± 3. 343 ^{ns}	76. 67 ± 3. 382 ^{ns}	6. 06 ± 1. 7 ^{ns}	17. 66 ± 1. 474 ^{ns}

n=6; Values are expressed as mean ± S. D followed by Students Paired 'T' Test ns – non significant when compared to control groups.

Table 3: Biochemical analysis of *Athimatura Chooranam*.

Groups	AST (IU/L)	ALT (IU/L)	Cholesterol (mg/dl)	Urea (mg/ 100ml)	Uric acid (mg/ 100ml)
Normal	69. 48±0. 273	30. 40 ± 0. 831	44209 ± 0. 797	24. 72 ± 0. 537	2. 01±0. 650
AMC (450mg/kg/p. o)	72. 65±5. 952 ^{ns}	33. 61 ± 6. 267 ^{ns}	41. 09 ± 0. 627 ^{ns}	23. 90 ± 1. 59 ^{ns}	2. 47 ± 0. 735 ^{ns}

n=6; Values are expressed as mean ± S. D followed by Students Paired 'T' Test ns – non significant when compared to control groups.

While performing acute oral toxicity study for the extract of *Athimathura chooranam* it was found that even at a dosage of 2000mg/kg/po did not exhibit any mortality in rats. As per OECD 423 guidelines the dose is said to be under category -5 of GHS toxicity scale. Since the dosage was comparatively high further higher doses analysis was not executed. Similarly

Repeated oral toxicity was performed for the extract at a dosage of 450mg/kg/po and the it was administered orally for 14 days and it did not exhibit ant toxicity rats in hematopoietic system, liver and kidney. The extract was administered at a dosage of 450 mg/kg/po daily for 14 days and there was no evidence of pathological lesions in the tissues. (Table: 4 and 5).

Anti-inflammatory activity

The extract of *Athimathura chooranam* found to possess significant anti-inflammatory activity at a dosage of 450 mg/kg/po in carrageenan induced hind paw edema (acute inflammation model) and Cotton pellet granuloma methods (Chronic model) of rats. The results were compared with the standard NSAID Diclofenac sodium (5 mg/kg/po) (Table: 4 and 5).

Table 4: Anti-inflammatory activity of extract in acute model

Treatment	0min	30min	60min	120min	240min
Control-Group-1	0. 871±0. 16	0. 890±0. 072	0. 852±0. 0. 117	0. 901±0. 083	0. 872±0. 764
Edematous control-Group-2	0. 873±. 0. 20	0. 967±0. 102 **a	1. 084±0. 021 ***a	1. 123±0. 310 ***a	1. 007±0. 764
Test(AMC)-Group-3	0. 871±0. 011	0. 931±0. 083	0. 910±0. 002 **b	0. 938±0. 09 ***b	0. 882±0. 078 **b
Standard (Dic. Sodium 5 mg/kg/po)Group-4	0. 893±0. 013 ns	0. 896±0. 067 *b	1. 12±0. 072 **b	0. 966±0. 041 ***b	0. 876±0. 028 **b

n=6; Values are expressed as mean ± S. E Values are compared with control and analyzed by Student's 't' test.

P<0. 01,*p<0. 001 as compared with respective control. a:group 1 vs group 2, b: group 2 vs groups 3 and 4

Table 5: Anti-inflammatory activity of extract in Chronic Model.

Groups	Cotton pellet Granuloma method
	Dry Weight (mg)
Control	107. 97 ± 9. 42
Test (AMC)	73. 08 ± 61. 30**
Standard (Dic. Sodium 5 mg/kg/po)	66. 31 ± 3. 12**

n=6; Values are expressed as mean ± S. E Significance was assessed by paired Student's 't' test . *P<0. 05 **p<. 01as compared with that of control. ns – non significant when compared to control groups.

Anti-Pyretic activity

The extract of *Athimathura Choornam* showed effective anti-pyretic activity even after 240 min compared to the standard drug Dic. Sodium 5 mg/kg/po and it was effective enough to control the pyretic condition similar to that of the standard for details refer (Table: 6).

Table 6: Anti-pyretic activity of the

Groups	Rectal temperature ($^{\circ}\text{C}$)				
	0 min	30 min	60 min	120 min	240 min
Control /Group-1	34.90 \pm 2.18	37.20 \pm 1.24	38.17 \pm 0.34	37.30 \pm 1.08	36.76 \pm 0.78
Pyretic control/Group-2	35.01 \pm 0.12	37.08 \pm 14*a	39.21 \pm 0.05**a	39.90 \pm 1.01***a	39.87 \pm 0.94***a
Test (AMC)/Group-3	35.13 \pm 0.20	36.80 \pm 0.50	35.94 \pm 0.41***b	36.02 \pm 0.05***b	36.31 \pm 0.30**b
Standard (Dic.Sodium 5mg/kg/po)Group-4	35.60 \pm 0.98ns	36.88 \pm 0.95 ns	35.99 \pm 0.61***b	35.75 \pm 0.20***b	36.51 \pm 0.72**b

extract

n=6; Values are expressed as mean \pm S. E Values are compared with control and analyzed by Student's 't' test.

P<0.01,*p<0.001 as compared with respective control. a:group 1 vs group 2, b: group 2 vs groups 3 and 4

Antihistaminic effect

The extract of *Athimathura Chooranam* exhibited antihistaminic effect in the contraction of g. pig ileum elicited with histamine. A dose dependent inhibition of contraction was observed and 100% inhibition was achieved with a concentration of 32mg/ml dose against 8 microgram of Histamine (Table: 7).

Table 7: Anti-histaminic activity of the extract

S. No	Treatment		Mean contraction (mm)	Percentage of inhibition of histamine
	Histamine ($\mu\text{g/ml}$)	AMC (mg/ml)		
1	8.0	Control	43 \pm 1.52	0
2	8.0	1	32 \pm 2.08	25.58*
3	8.0	2	24 \pm 1.15	44.18**
4	8.0	4	18 \pm 1.00	58.13***
5	8.0	8	11 \pm 1.00	74.41***
6	8.0	16	5 \pm 1.00	88.37***
7	8.0	32	0 \pm 0	100*

DISCUSSION

It is well known fact that medicinal plants play an important role in the development of potent therapeutic agents. An estimate that about 80% of people in developing countries still rely on traditional medicine for their primary health care. The multicomponent phytotherapy use has gained importance in the scientific years. Therefore the drugs used as single target may be less effective in combating multi inflammatory disorders. While herbal combinations show lower propensity of resistance and a good strategy to fight multifactorial diseases due to its action in synergy and holistically^[10], the *Athimathura Choornam* we have chosen for our study is also multicomponent phytotherapy and it is believed to possess multiple activity.

Glycyrrhizin and glycyrrhetic acid the main constituents of the hydrophilic fraction of licorice extracts and are known to be anti-inflammatory agents.^[11] Cinnamon has been reported to be beneficial for the amelioration of many inflammatory diseases including control of blood glucose levels in diabetes arthritic pain^[12,13] had reported on the significant anti-inflammatory activity of the essential oil of fruit of *Cuminum cyminum* in Carrageenan-induced paw edema in rats at the 2nd, 4th and 6th hours. Ginger has a long history of use as an antiinflammatory and many of its constituents have been identified as having antiinflammatory properties. Ginger has been found to inhibit prostaglandin biosynthesis.^[14]

Rhizomes of *Zingiber officinale* has antiinflammatory, cytoprotective, anti-ulcer action in NSIADs induced ulcer model, hepatoprotective and antioxidant property in paracetamol induced animal model.^[15, 16, 17] The anti-inflammatory activity of the alcohol extract of the rhizome of *Zingiber officinale* (100 mg/kg i.p.) was found to be superior to hydrocortisone in models of carrageenan induced paw edema and formaldehyde induced arthritis.^[18] Carrageenan induced hind paw edema is the standard experimental model of acute inflammation. Carrageenan is commonly used for testing anti-inflammatory drugs due to absence of apparent systemic effects and an antigenic nature.^[19] Carrageenan induced edema is the best method to evaluate the anti-inflammatory activity and the same has been used in our study for the powdered extract of athimathura chooranam.

The ethanolic extract of *Costus speciosus* rhizome possessed anti-inflammatory activities at doses of 400 and 800 mg/kg using carrageenan induced paw edema and cotton pellet induced granuloma formation methods.^[20] *Athimathura Chooranam* used in our study is the combination of *Glycyrrhiza glabra*, *Elettaria cardamomum*, *Cinnamomum verum*, *Michelia champaga*, *Costus speciosus*, *Zingiber officinale*, *Cuminum cyminum*, *Cyperus rotundus*

many studies have evaluated the anti-inflammatory activity of *Glycyrrhiza glabra*, *Costus speciosus*, *Zingiber officinale* individually but we have studied anti-inflammatory activity of Multicomponent formulation. Similarly we have studied the antipyretic activity and antihistaminic activity for the same and the results were encouraging.

CONCLUSION

While looking into the results obtained from the various studies conducted with *Athimathura Chooranam* a common drug of choice in *Siddha* medicine it is believed that the Chooranam have good anti-inflammatory, antipyretic and antihistaminic activity confirmed by preclinical studies and it can be concluded that *Athimathura Chooranam* can serve as drug of choice since it possess multiple therapeutic values. Further antimicrobial studies against common respiratory pathogens and clinical trials may be warranted to confirm its efficacy against respiratory ailments in Children.

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