IJPSR (2014), Volume 5, Issue 10 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 29 March 2014; received in revised form, 16 May 2014; accepted, 13 July 2014; published 01 October 2014

IN-VITRO ANTI-INFLAMMATORY SCREENING OF A POLY HERBAL SIDDHA MEDICINE, "*ASHWATHI CHOORANAM*"

A. Shifana Raheema, C. Aswini, M. Sathya, S. Merish and Thomas M. Walter *

Department of Pharmacology, Government Siddha Medical College, Palayamkottai - 600106, Tamil Nadu, India.

Keywords:

Siddha Medicine, Poly herbal formula, *In-vitro* studies, THP-1 cells

Correspondence to Author:
Dr. Thomas M Walter

MD (s)
Department of Pharmacology, Government Siddha Medical College, Palayamkottai - 600106, Tamil Nadu, India.

E-mail: dr.thomaswalter@gmail.com

ABSTRACT: Siddha is one among the ancient systems of Indian medicine. Its reliability lies in the fact that it is time-tested, devoid of any drastic adverse effects and deep-rooted in the culture and heritage of India. The role of this system in treating chronic diseases is being widely felt nowadays. The challenges in treating such conditions with the currently available drugs (Non-Steroidal Anti-inflammatory Drugs – NSAID) often results in gastric irritation, renal damage, dependence, etc. Traditional Siddha medicines offer a wide range of Anti-inflammatory drugs which are polyherbal in nature and devoid of the above-said side-effects [1]. This paper deals with Anti-inflammatory screening of such a medicine '*Ashwathi chooranam'* documented in Classic Siddha text *"Agathiar Vaithiya Kaaviyam"* indicated for arthritis [2]. *Withania somnifera* (Ashwagandha) is widely used in Siddha medicine. It is the main ingredient in many formulations prescribed for a variety of musculoskeletal conditions (*e.g.*, arthritis, rheumatism), and as a general tonic to increase vitality, improve overall health and facilitates longevity [1]. In our study, we reproduced an inflammatory state by treating THP-1 cells (human myelomonocytic leukemia) with pro-inflammatory stimuli, such as LPS obtaining an up-regulation in the expression and the activity of nitrate level. Our results show a significant increase in the expression and activity of Tissue nitrite level when cells were treated with the Test drug in different concentrations.

INTRODUCTION: The usage of NSAID in the treatment of painful musculoskeletal conditions often results in adverse effects such as gastric irritation, renal damage, *etc*. On the other hand, polyherbal medicines which are safe, effective, time-tested and devoid of drastic side-effects are the need of the hour.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(10).4395-99

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(10).4395-99

Traditional Siddha Indian Medicine has many such herbal medicines indicated for the treatment of Vatha (arthritis). This research paper deals with the *in-vitro* anti-inflammatory screening of such a medicine documented in Classic Siddha text, *"Agathiar Vaithiya Kaaviyam"* indicated for arthritis.

MATERIALS AND METHODS: The test drug (Ashwathi Chooranam) was prepared as per the Standard Operative Procedure (SOP) based on the Siddha literature, '*Agathiyar Vaithiya Kaaviyam.'* The ingredients of the test drug along with descriptions regarding their botanical names, phyto-chemistry, actions, and uses in Siddha

Medicine are given in **Table 1**. The prepared medicine was subjected to *in-vitro* Cox inhibitor studies using THP-1 Cell line.

Ingredients of the Test Drug [3, 4]:

1. Dry ginger (*Zingiber officinale*)
2. Pepper (*Piper nigrum*)
3. Long Pepper (*Piper longum*)
4. Nutmeg (*Myristica fragrans*)
5. Arillus of the nut (*Myristica fragrans*)
6. Indian liqorice (*Glycyrrhiza glabra*)
7. Cloves (*Syzygium aromaticum*)
8. Picrorrhiza (*Picrorhiza scrophulariiflora*)
9. Bishops weed (*Trachyspermum roxburghianum*)
10. Henbane seeds (*Hyocyamus niger*)
11. Winter cherry (*Withania somnifera*)

TABLE 1: INFORMATION ABOUT THE INGREDIENTS OF ASHWATHI CHOORANAM [1, 5, 6, 10]

S. no.	Common name Tamil/English	Botanical name/ Family	Phytochemistry	Actions	Uses in Siddha
1	Chukku/Dry ginger	*Zingiber officinale* / Zingiberaceae	β- sitosterol, palmitate, hexa cosanoic acid, gingerol, omega glycerol	Stomachic Carminative Digestive Rubefacient	Indigestion, cold, Anti- vatha, Headache.
2.	Milagu / Pepper	*Piper nigrum* / Piperaceae	Thyone, pinene, camphene, sabene, linalol, carypophyllene	Carminative Antiperoidic Rubifacient Antivata Antidote	Stabilizes Tridosa, effective in treating Cold, Anaemia, Jaundice, zndigestion.
3	Thippili/Long pepper	*Piper longum* / Piperaceae	Coumaperine, Piperolactum, Demethoxy curcumin, Prolidine	Stimulant Carminative	Cough, Anaemia, Sinusitis.
4	Sathikkai/ Nutmeg[9]	*Myristica fragrans* / Myristicaceae	Terpinine, α-terpineol, Dimethoxy phenol	Stimulant Narcotic Carminative Aphrodisiac	Cough &cold, Headache, Body heat, Increases Sperm count.
5	Chathi paththri/ Arillus of Nutmeg	*Myristica fragrans* / Myristicaceae	Terpinine, α-terpineol, Dimethoxy phenol.	Stimulant Carminative Aphrodisiac Hypnotic	Dysentery, Increases body weight.
6	Ati maduram/ Indian liquorice	*Glycyrrhiza glabra* / Papilionaceae	Glyccrhizin, Glabramin, Glycrrheroel, Chlycrrhizinc acid.	Emollient Demulcent Expectorant Laxative	Eye diseases, Lecoderma, Hiccup, urinary problems.
7	Kirambu/ Cloves [11]	*Syzygium aromaticum* / Myrtaceae	Thymol, Eugenol, Cinnamoldehyde, Carvacrol.	Anti-spasmodic Carminative Stomachic	Relieves Pain, Vomiting, Diarrhea, Dysentery, Dental Problems.
8	Katukurohani/ Picrorhiza	*Picrorhiza scrophulariiflora*/ Scrophulariaceae	Saponins; many oleanane-derived phytolaccosides, 3-acetyl myricadiol.	Anti-periodic Stomachic Cathartic	Fever, Vatha diseases, Stomach pain, Psoriasis
9	Omam / Bishops weed[13]	*Trachyspermum roxburghianum* /Apiaceae	Limonene, Sabinene, Trpinen 4-ol, Dipentene, D-linalool.	Stomachic Carminative	Stomach pain, Peptic ulcer, kidney stone.
10	Kurosani omam/ Henbane seeds	*Hyoscyamus niger*/ Solanaceae	Hyoscyamine, atropine, hyoscine, Scopolamine, Hyoscrytricin.	Hypnotic Sedative Anodyne Anti -spasmodic, Mild diuretic	Uterine disorders, Kapha diseases, useful in Insomnia.
11	Amukara / Winter cherry	*Withania somnifera* / Solanaceae	Withanolides, Withaniferrin, Steroidal, Lactones, Tropine.	Febrifuge Diuretic Alterative Aphrodisiac Hypnotic Sedative	Increases body weight, useful in Psoriasis, Swelling.

***In-vitro* Anti-inflammatory Effect on Cultured THP-1 Cell Lines:**

Cell Culture: THP1 (Human monocytic cell lines) was cultured in RPMI 1640 [HIMEDIA] media, supplemented with 10% heat-inactivated FBS, antibiotics (Penicillin and Streptomycin) and 1.5% sodium bicarbonate. The media was filtered using 0.2µm pore sized cellulose acetate filter (Sartorius) in completely aseptic conditions. The cells were then grown to 60% confluency, followed by activation with 1µl LPS (1µ/ml).

LPS stimulated THP 1 cells were exposed with different concentrations of samples such as 10µg/ml, 50µg/ml and100µg/ml from a stock of 100mg/ml dissolved in 1% DMSO and incubated for 24 hours. The anti-inflammatory effects of samples were determined by assessing the inhibition of COX, spectrophotometrically. The isolation was done by spinning at 6000 rpm for 10 min. The supernatant was discarded, and 200µl of cell lysis buffer (1MTris HCl, 0.25M EDTA, 2M NaCl, 0.5% Triton) was added. The incubation was done for 30 min at 4°C, and enzymes assay was done in pellet suspended in a small amount of supernatant.

Cox Inhibitory Assay: Cyclooxygenase (COX) is an enzyme that is responsible for the formation of prostanoids. The three main groups of prostanoids -- prostaglandins, prostacyclins, and thromboxanes are each involved in the inflammatory response. Lipoxygenases are non-heme iron-containing enzymes that catalyze the stereospecific incorporation of molecular oxygen into polyunsaturated fatty acids with a 1, 4-*cis*, *cis*-pentadiene motif leading to the production of leukotrienes leading to inflammation [7].

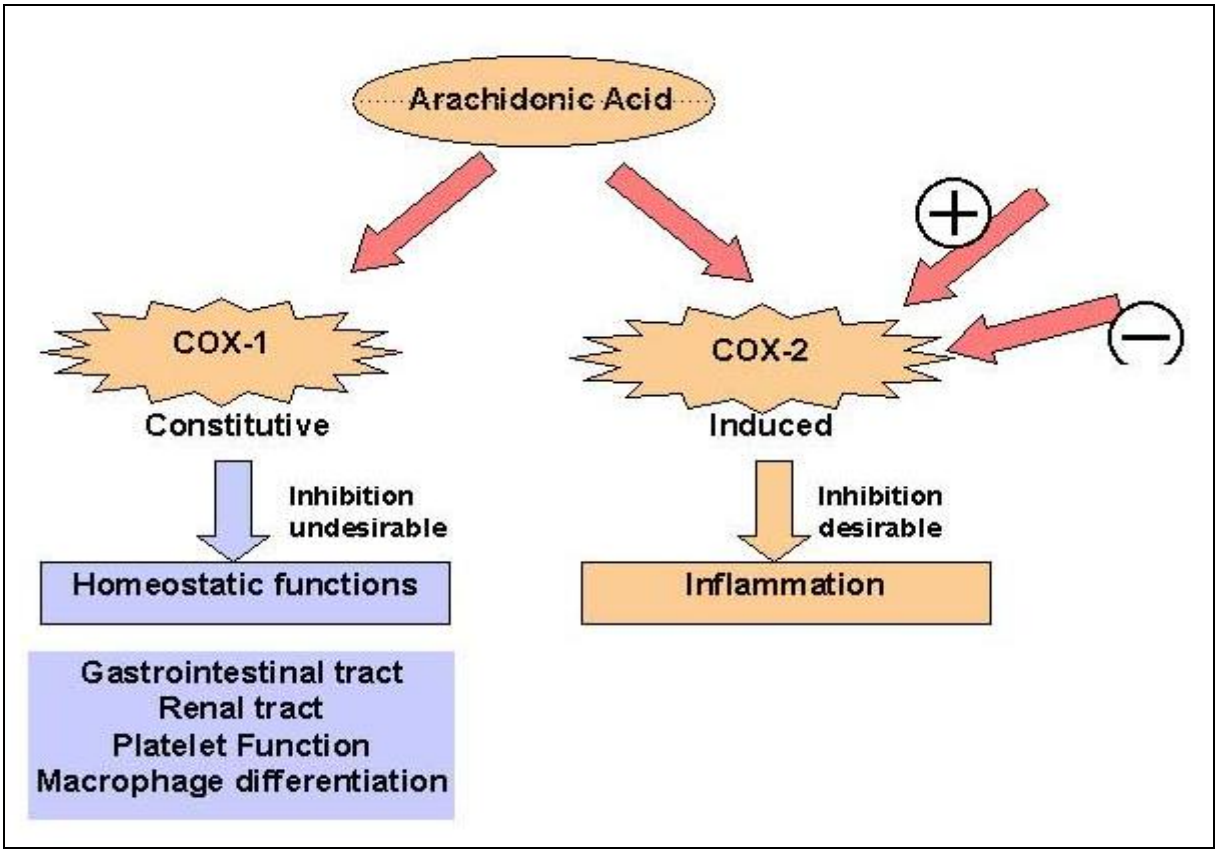

FIG. 1: *IN-VITRO* ANTI-INFLAMMATORY MECHANISM

Assay of Cycloxygenase (Co-X):

Reagents used*:*

- **a)** 100Mm Tris HCl (pH8)
- **b)** 5mM GSH
- **c)** 5µM Hemoglobin
- **d)** 200µm arachidonic acid
- **e)** 10% TCA in HCl
- **f)** 1% Thiobarbituric acid

Procedure: The assay mixture contained Tris- HCl buffer, glutathione, hemoglobin & enzyme. The reaction was started by the addition of arachidonic

acid and terminated after 20 min incubation at 37 ºC by addition of 0.2ml of 10% TCA in 1N HCl, mixed and 0.2ml of TBA was added, and contents heated in a boiling water bath for 20 min, cooled and centrifuged at 1000rpm for 3 min. The supernatant was measured at 632nm for COX activity and the results are noted.

Estimation of Tissue Nitrite Levels: The level of nitrite level was estimated by the method of Lepoivre *et al.*, 1990). To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 min. The samples were then centrifuged at 5,000 rpm for 15 min. The protein-free supernatant was used for the estimation of nitrite levels. To 200 µL of the supernatant, 30 µL of 10% NaOH was added, followed by 300 µL of Tris-HCl buffer and mixed well. To this, 530 µL of Griess reagent was added and incubated in the dark for 10–15 minutes, and the absorbance was read at 540 nm against a Griess reagent as blank. Sodium nitrite solution was used as the standard. The amount of nitrite present in the samples was estimated from the standard curves obtained.

RESULTS:

TABLE 2: ASSAY OF CYCLOXYGENASE

Sample concentration (µg/ml)	OD at 632nm	% inhibition
Control	0.559	
10 µg/ml	0.449	19.67
50 µg/ml	0.389	30.41
100 µg/ml	0.287	48.65

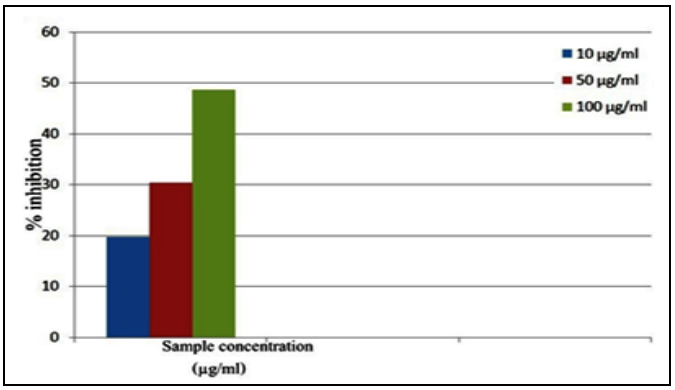

FIG. 2: INHIBITION OF TEST DRUG

TABLE 3: TISSUE NITRITE LEVEL

Sample concentration(µg/ml)	OD at 540nm	Concentration (µg)
Control	0.1415	721.65
10 µg/ml	0.1245	634.95
50 µg/ml	0.1062	541.62
100 µg/ml	0.1005	512.55

DISCUSSION: The role of Siddha polyherbal medicines in the treatment of painful Musculoskeletal conditions have been well documented since centuries. In this current scientific world where every claim should be evidence-based, the Authors' of this research paper tried to prove the efficacy of such a Siddha Medicine through *in-vitro* cell line studies for its Anti-inflammatory properties and shown in **Table 2**. When a tissue undergoes inflammation, it regularly releases iNOS which can simultaneously generate NO (nitric oxide) and superoxide. These react rapidly to yield nitrite to peroxynitrite (ONOO). Thus measuring nitrite in a sample can tell you something about ONOO production (and indirectly about NO production) [12, 14].

When tissue gets damaged, Nitric oxide is released constantly along with Nitrite. This released Nitric oxide combines with oxygenated Hemoglobin to form Methemoglobin. Nitrite again combines with oxy hemoglobin and free hydrogen ion to form nitrate, hemoglobin, oxygen, and water. Normally meth hemoglobin level is less than 2.5 of the body's total Hemoglobin. Nitrite act to increase the meth hemoglobin, which results in oxygen Starvation leading to cyanosis [15].

From the available **Tables 2** and **3** results, it is inferred that the test drug has potent anti-inflammatory property. **Fig. 2** shows the percentage of inhibition, increases from 20, 30, and 49 in 10, 50 & 100 µgm per ml concentration, respectively. The ability of the test drug to lower the tissue nitrite levels are also evident through the concentration ranging from 635, 542, 513 µgm in 10, 50 & 100 µgm/ml concentrations. These concentrations are much lower than the concentration of the control which 722 µgm.

CONCLUSION: It is concluded that the polyherbal Siddha medicine *Ashwathi Chooranam* which is being widely used by Traditional Healers and Practitioners of Siddha Medicine to treat arthritis since decades exhibit 48.65% of inhibition at 100 µgm/ml concentration level. It is also evident that the test drug has lowered tissue Nitrite levels of 513 µgm at100 µgm/ml concentration when compared to the control concentration of 722 µgm. This result clearly shows the Anti-inflammatory properties of the test drug along with

its efficacy in lowering tissue nitrite levels, thereby increasing Oxy-hemoglobin in the blood circulation.

ACKNOWLEDGEMENT: Sincere thanks are due to all those who helped in preparing this paper.

CONFLICT OF INTEREST: Nil

REFERENCES:

1. Muthaliar M: Siddha Materia Medica (Vegetable section), Publisher; Tamilnadu Siddha Medical Council, Chennai. Fourth edition, 1988; 1: 132, 232, 448.
2. Anonyms, Siddha classical Text, Agathiyar Vaithya kaviyam, First Edition, Vol 1; 1983: 763-68.
3. Sambasivampillai TV: Dictionary Based on Indian Medical science, published by Directorate of Indian Medicine and Homeopathy, Chennai, India, Vol. 2, Second edition; 1991.
4. "The Wealth of India", Publication and Information Directorate, CSIR, New Delhi 1985; 10: 281.
5. Thomas M. Walter, Catalogue_of_Siddha_medicinal_ plants. [ONLINE] Availableat:https://www.researchgate. net/publication/36447396_Catalogue_of_Siddha_medicina l_plants?ev=prf_pub. [Last Accessed December 20, 2013].
6. Nadkarni KM: Indian Materia Medica. Popular Prakashan, Mumbai, 3rd Edition, Vol. I, 2000.
7. Mohan H: Textbook of Pathology, Jaypee Brothers Medical Publishers (P) Ltd, 6th Edition, 2010: 32, 130-135.
8. Prajapati ND: A Handbook of Medicinal Plant – A complete Source book, Agrobios, India 2003; 396.
9. Maya KM, John Zachariah T and Krishnamoorthy B: Chemical composition of essential oil of nutmeg (*Myristica fragrans* Houtt.) accessions. Journal of Species and aromatic Crops 2004; 13(2): 135-39.
10. Sofia N and Walter TM: Review of *Glycyrrhiza glabra*, Linn., Siddha Papers 2009; 2(01).
11. Bankar R, Kumar A and Puri S: Phytochemical constituent of Syzygium *aromaticum* L., International Journal of Current Research 2011; 3(7): 215-17.
12. Murugan V, Walter TM, Nihar SS and Kumar BS: Pharmacological evaluation of the anti-inflammatory activity of Sangu Parpam (Conch) – A Siddha Medicine. Siddha Papers, 2004, World Siddha Day Special 2010; 1-5.
13. Paul BK, Saleh-e-In MM, Hassan SMM, Rahman MZ, Saha GC and Roy SK: Chemical composition and biological activities of Carum roxburghianum Benth. (Radhuni) seeds of three Bangladeshi ecotypes. Journal of Essential Oil Bearing Plants 2013; 16(2): 201-11.
14. Walter TM, Priya TS, Paargavi AS, Devi NSP and Thanalakshmi S: A review of herbs to treat skin disorders in traditional siddha medicine. Res and Rev Journal Pharm Toxicology Studies 2014; 2(1): 7-14.
15. *In-vitro* and *in-vivo* kinetic handling of nitrite in blood: effects of varying hemoglobin oxygen saturation, Heart and Circulatory Physiology. [ONLINE] Available at http://ajpheart.physiology.org/content/293/3/H1508. [Accessed 22 March, 2014].

How to cite this article:
Raheema AS, Aswini C, Sathya M, Merish S and Walter TM: *In-vitro* anti-inflammatory screening of a poly herbal siddha medicine, "ashwathi chooranam". Int J Pharm Sci & Res 2014; 5(10): 4395-99. doi: 10.13040/IJPSR.0975-8232.5(10).4395-99.