ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Invitro Screening of a Poly Herbal Siddha Formula for Its Anti-Inflammatory Properties.

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

Department of Pharmacology, Government Siddha Medical College, Palayamkottai, Tirunelveli.

ABSTRACT

The awareness and use of Traditional Siddha Indian Medicine is on the increase in this globalized world. The specialty lies in its therapeutic potential in curing chronic diseases such as Arthritis, dermatological conditions, life style disorders etc. The usage of NSAID in the treatment of painful musculo-skeletal conditions often results in adverse effects such as gastric irritation, renal damage etc. On the other hand, poly herbal medicines are safe, effective, time-tested and devoid of drastic side-effects. Siddha 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, '*Yugi Muni Vaithiya Kaaviyam'* and specially indicated for '*Kudaithiri Vaatham'* – which can be correlated to a kind of Rheumatism attended with pain all over the body especially in the loins. 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 in 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.

Keywords: Siddha Medicine, Poly herbal formula, In-vitro studies, THP-1 cells.

*Corresponding author

ISSN: 0975-8585

INTRODUCTION

The usage of NSAID in the treatment of painful musculo-skeletal conditions often results in adverse effects such as gastric irritation, renal damage etc. On the other hand, poly herbal medicines which are safe, effective, time-tested and devoid of drastic side-effects are the need of the hour. 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, '*Yugi Muni Vaithiya Kaaviyam'* and specially indicated for '*Kudaithiri Vaatham'* – which can be correlated to a kind of Rheumatism attended with pain all over the body and boring pain especially in the loins [1-8].

MATERIALS & METHODS

The test drug was prepared as per the Classic Siddha text, '*Yugi Muni Vaithiya Kaaviyam'.* The ingredients of the test drug along with descriptions regarding their Botanical names, Phyto-chemistry and uses in Siddha Medicine are given below. The prepared medicine was subjected to In-vitro Cox inhibitor studies using THP-1 Cell line.

Ingredients of the test drug:

- Cumin (Cuminum cyminum)
- Black cumin seeds (Nigella sativa)
- Mustard (Brassica juncea)
- Colocynth (Citrullus colocynthis)

Table 1: Information about the ingredients of the Medicine.

S. No	Common name Tamil/English	Botanical name/ Family	Phytochemistry	Actions	Uses in Siddha
1.	Chirakam /Cumin seed	Cuminum cyminum (Umbelliferae)	Cuminaldehyde, Sesquiterpenes, Safrole.	Carminative, Stimulant, Stomachic, Astringent	stomach pain, liver diseases, kidney stones
2.	Karunjchirakam / Black cumin	Nigella sativa (Umbelliferae)	Apiole, n- Decane, Myristicin, Thymoquinone, Carvacrol	Carminative, Diuretic, Emmenagogue, Stomachic, Parasiticide, Emollient.	Cures skin diseases, inflammation, jaundice, ulcer, cough
3.	Kadugu / Black Mustard	Brassica juncea (Brassicaceae)	Diallyl sulfide, eicosane, henelcosane.	Emetic, Stimulant, Rubefacient, Vesicant, Digestive, Diuretic.	Cold, Cough, Diarrhoea, Indigestion, Hiccup.
4.	Artu thumatti / Bitter apple	Citrullus colocynthis (Cucurbitaceae)	Quercetin, Myristic, Oleic, Palmitic, Stearic, Linoleic, Lysine, Methionine, Tryptophan.	Expectorant, Alterative, Hydrogogue, Diuretic, Emetic,	Cures Vatha diseases, Amenorrhea.

ISSN: 0975-8585

Invitro 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) and1.5% sodium bicarbonate. The media was filtered using 0.2µm pore sized cellulose acetate filter (Sartorius) in completely aseptic conditions. The cells were then grown till 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 minutes. 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 minutes at 4°C and enzymes assay was done in pellet suspended in a small amount of supernatant.

Cox - Inhibitory Assay:

Cyclo-oxygenase (COX) is an enzyme that is responsible for the formation of prostanoids. The three main groups of prostanoids - prostaglandins, prostacyclin's, 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 production of leukotrienes leading to inflammation.

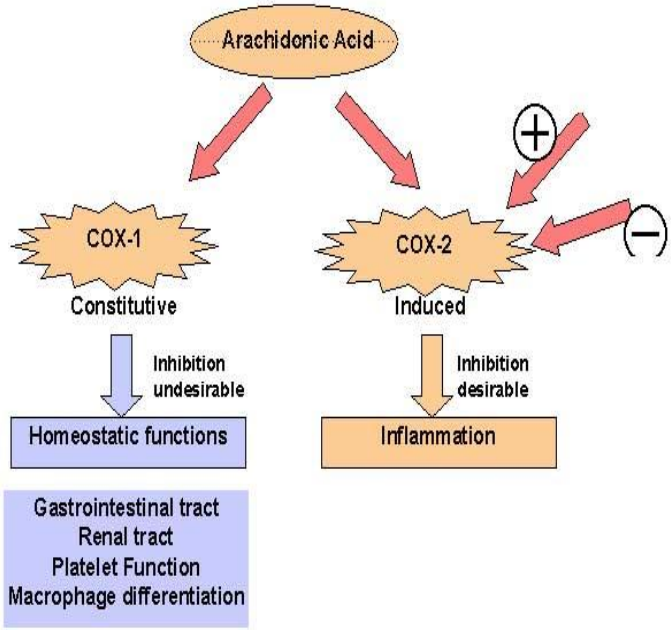

Image 1: In-vitro Anti-inflammatory mechanism.

ISSN: 0975-8585

Assay of Cyclooxygenase (COX):

Reagents used

- Tris Hcl (pH8)- 100Mm
- GSH- 5mM
- Hemoglobin- 5µM
- Arachidonic acid- 200µm
- 10% TCA in Hcl
- 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 37ºc 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. (Lepoivre et. al. 1990) To 0.5 mL of cell lysate, 0.1 mL of sulphosalicylic acid was added and vortexed well for 30 minutes. The samples were then centrifuged at 5,000 rpm for 15 minutes. 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 Cyclooxygenase

Sample concentration(µg/ml)	OD at 632nm	% inhibition
Control	0.559	
10 µg/ml	0.337	39.71
50 µg/ml	0.259	53.66
100 µg/ml	0.211	62.25

ISSN: 0975-8585

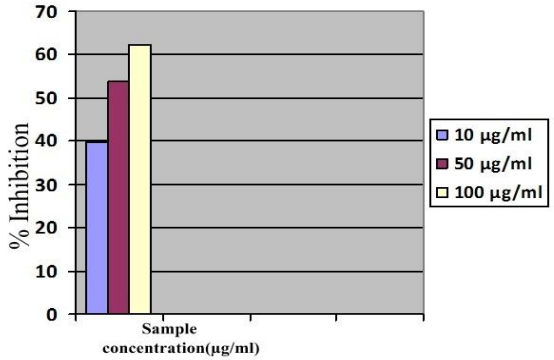

Image 2: Inhibition of test drug

Table 3: Tissue Nitrite Level

Sample concentration(µg/ml)	OD at 540nm	Concentration (µg)
Control	0.1415	721.65
10 µg/ml	0.0861	439.11
50 µg/ml	0.0858	437.58
100 µg/ml	0.0843	429.93

DISCUSSION

The role of Siddha poly herbal medicines in the treatment of painful Musculo-skeletal conditions have been well documented since centuries. In this current scientific world where each and 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. 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).

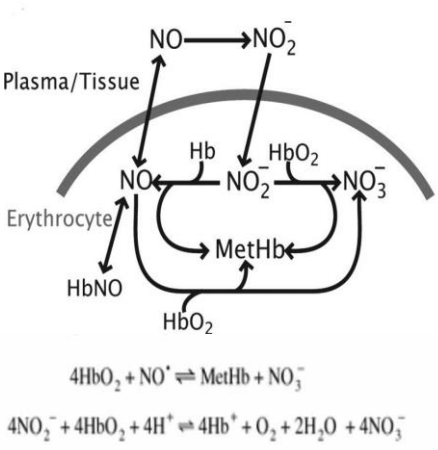

Image 3

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.

From the available results it is inferred that the test drug has potent anti-inflammatory property. The percentage of inhibition increases from 40, 54, 62 in 10, 50 & 100μgm per ml concentration respectively.

The ability of the test drug to lower the tissue nitrite levels are also evident through the concentration ranging from 439,437, 430μgm in 10, 50 & 100μgm /ml concentrations. These concentrations are much lower than the concentration of the control which 722μgm [9-20].

CONCLUSION

It is concluded that the poly herbal Siddha medicine which is being used to treat the *Kudaithiri Vaatham (*a kind of rheumatism*)* since decades exhibits 62.25% of inhibition at 100μgm/ml concentration level. It is also evident that the test drug has lowered tissue Nitrite levels of 430 μgm at100μgm/ml concentration, when compared to the control concentration of 722μgm. This result clearly shows the Anti-inflammatory properties of the test drug along with its efficacy in lowering Tissue nitrite levels.

REFERENCES

[1] RC Mohan. 2002, Yugi Muni Vaithiya Kaaviyam publication of Thamarai Noolagam, Chennai.
[2] Dr Murugesa Muthaliar. 1988, Siddha Materia Medica (Vegetable section), Volume I, Fourth edition, Publisher; Tamilnadu Siddha Medical Council, Chennai.
[3] Dr KM Nadkarani, Indian Materia Medica Vol: I Publisher: Popular Prakash, Mumbai, India.
[4] "The Wealth of India", Vol 10, Publication and Information Directorate, CSIR, New Delhi, 281, (1985).
[5] Neda Hashemian, Abdollah Ghasemi Pirbalouti, Masoud Hashemi, Ahmadreza Golparvar, Behzad Hamedi. AJCS 2013;7(11):1752-1760.
[6] M Akram Khan. Inflammopharmacol 1999;7(1):15-35.
[7] Jimmy C Yu, Zi-Tao Jiang, Rong Li, Sze Man Chan. *J Food Drug Anal 2003;*11(1):22-26.
[8] https://www.researchgate.net/publication/36447396_Catalogue_of_Siddha_medicinal_plants?ev=prf_pub.
[9] S Gurudeepan, K Satyavani, T Ramanathan. Asian J Plant Sci 2010;9(7): 394-401.
[10] Harsh Mohan, 2010. Textbook of Pathology with Pathology Quick Review and MCQS, 6/E. 6 Edition. Jaypee Brothers Medical Publishers (P) Ltd.
[11] TV Sambasivam pillai. 1991, Dictionary Based on Indian Medical science, Second edition, Vol. 2, published by Directorate of Indian Medicine and Homeopathy, Chennai, India.

[12] Merish S,Tamizhamuthu M, and Thomas M Walter. Int J Pharm Bio Sci 2014;5(2):43 – 49.
[13] Speranza L, Franceschelli S, Pesce M, Menghini L, Patruno A, Vinciguerra I, De Lutiis MA, Felaco M, Felaco P, Grilli A. J Biol Regulat Homeostatic Agents 2009, 23(3):189-195.
[14] Ylva Noreen, Therese Ringbom, Premila Perera, Helena Danielson, and Lars Bohlin. *J Nat Prod* 1998, *61,* 2-7.
[15] Lawrence J. Marnett, Scott W. Rowlinson, Douglas C. Goodwin, Amit S. Kalgutkar and Cheryl A. Lanzo. J Biol Chem 1999;274:22903-22906.
[16] Yoshikai Y. Curr Opin Infect Dis 2001; 14(3):257-63.
[17] http://www.webmd.com/rheumatoid-arthritis/an-overview-of-rheumatic-diseases.
[18] George S. Weger,(1993), Arthritis, Rheumatism and Sciatica.
[19] http://www.atsdr.cdc.gov/csem/nitrate/No3cover.html
[20] http://ajpheart.physiology.org/content/293/3/H1508.