INTERNATIONAL JOURNAL OF CURRENT RESEARCH IN BIOLOGY AND MEDICINE ISSN: 2455-944X www.darshanpublishers.com Volume 4, Issue 4 - 2019

Original Research Article

DOI: http://dx.doi.org/10.22192/ijcrbm.2019.04.04.003

Scientific Evaluation of Anti-Inflammatory effect of siddha herbal drug *Karappan Nei* using Protein (Albumin) denaturation Assay *-In Vitro* study

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Abstract

Inflammation followed by pain is highly prevalenced nowadays. Many siddha medicinal herbal formulations were being in practice to treat inflammation from ancient times. *Karappan Nei* is one among them was indicated in sastric Siddha literatures to treat various diseases. The ingredients of this herbal formulation have a wide range of active components to treat inflammation. Though these kind of formulations are being in use with high therapeutic efficacy, it has to be scientifically evaluated for safe global use. The anti inflammatory effect of *Karappan Nei* was subjected into assessment using protein denaturation assay and diclofenac was used as standard drug. The drug possessed significant In- vitro anti-inflammatory effect against the denaturation of protein. The drug showed 55.83 % inhibition at 500 μ g/ml. The research findings showed promising anti inflammatory activity. If further clinical trial will be followed during this process, it will help to save the human society from pain.

Keywords: Siddha, Herbals, Karappan, Medicated Ghee, Protein denaturation

Introduction

Human immune system plays an important role to save human society from harmful infections, Tissue damage, irritants etc, Inflammation is a biological tissue process which indicates as a stimuli to defence body from diseases. Many modernanti inflammatory drugs are available worldwide nowadays. Sometimes there are many harmful issues arising day by day due to modern anti inflammatory drugs.

Many herbals having anti inflammatory potential without causing any adverse effects¹.Usage of herbals

during inflammatory conditions are being in practice in siddha medical system since time immemorial. Herbals such as *Delonix elata*, *Zingiber officinale*, *Sida cordifolia*, *Vitex negundo* etc., having significant anti inflammatory potential. *Karappan Nei* is a best herbal formulation which was indicated to treat various diseases in Sastric siddha texts².

An attempt was made to evaluate the antiinflammatory efficacy of siddha drug *Karappan Nei* using in Vitro protein denaturation assay. This assessment will helps to determine the therapeutic potential of *Karappan Nei* to treat inflammation.

Materials and Methods

Preparation of siddha formulation Karappan nei

The plants were collected in and around Kanchipuram district. All the ingredients were authenticated by Botanist in National Institute of Siddha. The drug was prepared according to siddha sastric text.

Albumin Denaturation Assay Procedure

In-vitro anti-inflammatory activity *Karappan Nei* was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample *Karappan Nei* at varying concentration ranges from 100 to 500 μ g/ml and standard Diclofenac sodium at the concentration of100 μ g /ml of final volume. pH was adjusted by using a small amount of 1N Hydrochloric acid.

The samples were incubated at 37°C for 20 min and then heated at 57°C for 3 min. After cooling the sample, 2.5 ml of phosphate buffer solution was added into each test tube. Turbidity developed was measured spectrophotometrically at 660 nm, for control distilled water was used instead of test sample while product control tests lacked bovine serum albumin. The experiment was performed in triplicate^{3,4}.

The Percentage protection from denaturation is calculated by using the formulae

$$\left[\frac{(A)_{\rm control} - (A)_{\rm sample}}{(A)_{\rm control}}\right] \times 100.$$

Statistical analysis

Results are expressed as Mean \pm SD. The difference between experimental groups was compared by One-Way Analysis Of Variance (ANOVA) followed by Dunnet Multiple comparison test.



Figure 1: Preparation of Test and control



Figure 2: Absorbance of reaction mixture – Test Sample

Experimental section:



Figure 3: Absorbance of reaction mixture - Control and Standard



Graph 1: Absorbance Range of test and standard at Trial 1



Graph 2: Absorbance Range of test and standard at Trial 2





Results and Discussion

Table 1 Effect of KN on heat induced protein denaturation (their absorbance)

Concentration in µg/ml	Absorbance
Control	$0.92 \hspace{0.1 cm} \pm 0.07$
KN 100	$0.76 \hspace{0.1 cm} \pm \hspace{0.1 cm} 0.02$
KN 200	0.63 ± 0.064
KN 300	$0.52 \hspace{0.1 cm} \pm 0.02$
KN 400	$0.43 \hspace{0.1 in} \pm 0.51$
KN 500	0.34 ± 0.6
Diclofenac sodium (100 µg)	0.010 ± 0.08

Table 2: Effect of KN on proteinase inhibitory action

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
KN 100	10.46 ± 3.36
KN 200	24.5 ± 3.30
KN 300	35.65 ± 3.91
KN 400	45.4 ± 2.20
KN 500	55.83 ± 3.26
Diclofenac sodium (100 µg)	91.45 ± 1.41

Each value represents the mean ± SD. N=3

The result obtained from the present research work clearly indicates that the test drug KN was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about 55.83 % was observed at 500 μ g/ml when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 91.45 % at the concentration of 100 μ g/ml.

Conclusion

The results confirms that the siddha formulation *Karappan Nei* has potential anti inflammatory effect in protein denaturation assay. This is probably due to active molecular components of the ingredients and their synergistic interactions. Even Herbal medicines also have to be scientifically evaluated for global acceptance in our recent scenario⁵. This scientific pharmacological research work will lead a way to explore a wonderful anti inflammatory siddha drug *Karappan Nei* to medical world for the great benefit of human kind.

Acknowledgment

We acknowledge our thanks to National Institute of Siddha, The Vice chancellor, The Tamilnadu Dr. M.G.R Medical University, Chennai.

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How to cite this article:

Bakkiyadevi M, Suresh K, Meenashisundaram M, Banumathi V. (2019). Scientific Evaluation of Anti-Inflammatory effect of siddha herbal drug *Karappan Nei* using Protein (Albumin) denaturation Assay -*In Vitro* study. Int. J. Curr. Res. Biol. Med. 4(4): 15-19. DOI: http://dx.doi.org/10.22192/ijcrbm.2019.04.04.003