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ANTI – INFLAMMATORY ACTIVITY OF VAYU KEELAGA ILAKAM BY PROTEIN (ALBUMIN) DENATURATION ASSAY (IN - VITRO)

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ABSTRACT

Inflammatory response is a protective response to injury, irritant or infection and is required to remove the injurious stimuli as well as initiate the healing process. It is a complex process and it associated with increased vascular permeability, protein denaturation and membrane alteration. The common symptoms of arthritis is pain, which is due to the inflammation of joints line. Vayu Keelaga Ilakam is a polyherbal Siddha formulation, indicated in Siddha text Prana Rakshamirtha Sindhu.Which contain 9 ingredients of plant origin. It was mainly indicated for the treatment of Uthira Vatha Suronitham (Rheumatoid Arthritis). The aim of the study is to evaluate the Anti- inflammatory activity of Vayu Keelaga Ilakam by using Protein (Albumin) denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample Vayu Keelaga Ilakam at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of 100 µg /ml. The results of the study showed that the test drug was effective in inhibiting heat induced albumin denaturation. The Concentration range of VKI at 100, 200, 300, 400 and 500 µg/ml produce significant inhibition of protein denaturation in concentration dependent manner. Maximum percentage of protein inhibition about 56.56 ± 7.58 % was observed at 500 µg/ml, when compared to Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition about 98.78 ± 1.364 at the concentration of 100 µg/ml. Hence this study result offers the Vayu Keelaga Ilakam (VKI) possess significant Anti- inflammatory activity in Protein (Albumin) denaturation technique.

KEY WORDS: ***Vayu keelaga ilakam, Uthira vatha suronitham, Rheumatoid arthritis, Anti-inflammatory activity, Protein denaturation***

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1. Introduction

Inflammation is a body defense reaction in order to eliminate (or) limit the spread of injuries agents, followed by removal of necrosed cells and tissues. The response consists of changes in blood flow, an increase permeability of blood vessels, migration of fluid proteins and leukocytes from the circulation to site of tissue damage. The causative agents of inflammations are, Infective agents like bacteria, virus, fungi, parasites and their toxins; Immunological agents like cell mediated antigen, antibody reaction; physical agents like heat, cold, radiation and mechanical traumas; Chemical agents like organic and inorganic poisons, inert materials such as foreign bodies. Signs of inflammation: There is 5 cardinal sign in inflammation, (i,e) rubor (rednes), tumor (smelling), color (heat), dolor (pain), function laesa (less of function). Types of inflammation: Depending upon the defense capacity of the host and duration of response, inflammation is classified into Acute and Chronic. Acute inflammation is an early body response to harmful stimuli and is achieved by increased movement of plasma and Leukocytes (especially granulocytes) from the blood into the injured tissue. Chronic inflammation is a prolonged process in which tissues inflammation and destruction occur at the same time. It is due to recurrent attacks of acute inflammation (or) followed by acute inflammation. The hallmark of chronic inflammation is the infiltration of tissue site by macrophages, lymphocytes and plasma cells. Macrophages are the principle cells involved in chronic inflammation and produce many effects that contribute to the progression of tissue damage and consequent functional damage [1,2].

Rheumatoid arthritis in a chronic, progressive inflammatory autoimmune disease. It affects 0.5 to 1% of population all over the world [3]. RA may be more prevalent in Rural population than in Urban area (R Handa et .al .2016). In a review of RA epidemiology worldwide published in 2006, Almanos et al, compared the incidence and prevalence of RA, based on ACR criteria, across four categories, North America, Northen Europe, Southern Europe and developing countries. In India local survey in Delhi shows prevalence of this disease affecting 0.75 % population. The Mean age onset of the disease in Female is 38± 12.4 years and Male is 44.8 ± 13.12 years [4].

The onset of disease is insidious, beginning with prodrome of fatigue, weakness, joint stiffness, vague arthralgia and myalgia. This is followed by pain and swelling of joints of hands, wrist, feet, ankle and elbow joints. The typical presentation of RA is smaller joints swelling especially proximal Interphalangeal and Metacarbophalangeal joints are affected more severly. The persist inflammation leads to erosive joint damage and functional impairments in vast majority of patients. In later stage, RA produces systemic manifestations such as haematologic, pulmonary, neurological and cardiovascular abnormalities [5-7].

Siddha system of medicine is one of the oldest systems of medicine in South India, especially in Tamil Nadu. The human body is based on the five physical elements i.e. Panja bootham – Earth, Water, Fire, Air and Space and three humors i.e. Vatham, Pitham and Kapham. The causes of all diseases or the pathological conditions of human body are to sought in the abnormalities of these three varieties of materials [8]. Siddhars classified the diseases into 4448 based on Mukkutram. Saint Yugi in Yugi Vaithya Sindhamani, classified 80 types of Vatha diseases. The disease UTHIRA VATHA SURONITHAM is one among them. It is due to the dearrangement of Vatham and Pitham. The signs and symptoms of Uthira Vatha Suronitham may be correlated with Rheumatoid Arthritis in Modern science. In siddha system of medicine many formulations have been indicated for chronic diseases [9-12]. The formulation VAYU KEELAGA ILAGAM is indicated in Siddha text indicated in Siddha text Prana Rakshamirtha Sindhu [13].

2.Materials and Methods

2.1. Plant material

The required raw drugs were purchased from a well reputed country shop in Chennai, Tamilnadu. These drugs were authenticated by the Assistant Professor of Medicinal Botany, National Institute of Siddha, Chennai (Certificte No : NISMB3422018). Then the trial drug was prepared in Gunapadam laboratory, National Institute of Siddha, Chennai.In Vitro Anti – inflammatory (Protein denaturation assay) was carried out by Noble Research Solution, Chennai. Project Id: NRS/AS/0386/05/2019.

2.2. Ingredients of test drug

The ingredients of Vayu keelaga ilakam are following,

1.Parangipattai *(Smila china .Linn)*	Each 3 palam (105gm)
2.VenKodivelivearpattai *(Plumbagozeylanica.Linn)*	
3.Sanganvearpattai (*Azematetrantha. Linn)*	
4.SeemaiAmukkarakizhangu (*Withaniasomnifera .Linn)*	
5.Chukku *(Zingiber officinale .Linn)*	Each 1/2 palam (87.5 gms)
6.Milagu (*Pippernigram .Linn)*	
7.Thippili *(Piper longam. Linn)*	
8.Elakkai *(Elettaiacardamomum .Linn)*	
9.Seeragam *(Cuminumcyminum .Linn)*	
10.Pasunei*(Ghee)*	1/2 padi (670 ml)
11.Thaen (*Honey)*	1/4 Padi (335 ml)
12.Sarkarai (*Palm jaggery*)	8 Palam (280 gm)
13. Neer (Water)	1.12 lit

2.3. Methods of purification

Purification of raw drugs was done as per the methods given in Siddha text Sigitcha Rathna Deepam [14].

2.4. Method of Drug Preparation

The drugs were finely powdered and sieved. The sugar was dissolved in the water and a syrup prepared by gently heating it. Then the dry powder was added into the syrup. When quite warm, ghee was mixed in to the mass. When it cools, honey will be added and mixed. This prepared medicine was stored in air tight glass container and labeled [15].

2.5. In-vitro anti-inflammatory activity of VKI

In-vitro anti-inflammatory activity of VKI was studied using albumin denaturation technique. The reaction mixture consisted of bovine serum albumin (5% aqueous solution) and test sample VKI at varying concentration ranges from 100 to 500 µg/ml and standard Diclofenac sodium at the concentration of 100 µg /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 [16,17].

The Percentage protection from denaturation is calculated by using the formula.

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

2.6. Statistical Analysis

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

3.Results and Discussion

Protein denaturation is a process in which proteins loss their tertiary structure and secondary structure by application of external stress or compound, such as strong acid or base, a concentrated inorganic salt, an organic solvent or heat. Most biological proteins lose their biological function when denatured. Denaturation of proteins is a well-documented cause of inflammation [18]. According to Opie et al 1962, tissue injury during life might be preferable to denaturation of the protein constituents of cell and intracellular substance. Mechanism of denaturation is involved in alteration of electrostatic force, hydrogen, hydrophobic and di-sulphide bonds. Some inflammatory diseases and arthritis like Rheumatoid arthritis, denaturation of protein in tissues may be the causes of production of auto-antigens. So the protein denaturation is the marker for the inflammation and arthritis. The ability of a substance to inhibit the protein denaturation, that substance may be possible to prevent the inflammation (i.e. anti-inflammatory substance) [19,20].

The Concentration range of VKI at 100, 200, 300, 400 and 500 µg/ml produce significant inhibition of protein denaturation in concentration dependent manner. The inhibitory effect of different concentration of VKI on protein denaturation as shown in Table 2. Maximum percentage of inhibition about 56.56 ± 7.58 % was observed at 500 µg/ml when compared to Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition 98.78 ± 1.364 at the concentration of 100 µg/ml. The result obtained from the study clearly indicates that the test drug VKI was effective in inhibiting heat induced albumin denaturation

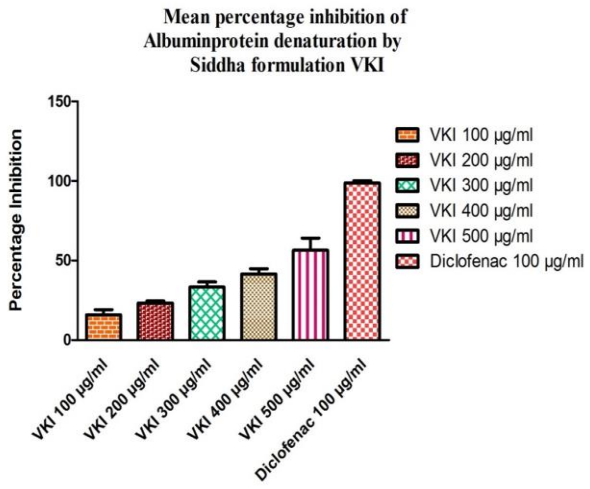

Figure 2: Percentage Inhibition of Protein Denaturation by VKI and Standard

Table: 2 Inhibitory effect of different concentration of VKI and Diclofenac sodium on protein denaturation

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
VKI 100	15.89 ± 3.35
VKI 200	23.36 ± 1.189
VKI 300	33.32 ± 3.29
VKI 400	41.55 ± 3.329
VKI 500	56.56 ± 7.588
Diclofenac sodium (100 µg)	98.78 ± 1.364

Each value represents the mean ± SD. N=3

From the result of the study, it was concluded that the test drug Vayu keelaga ilakam (VKI) possess significant anti-inflammatory property in dose dependent manner. The Anti – inflammatory activity of VKI is may be due to the presence of active principles, such as Polyphenolic compounds, Alkaloids, Tannins and Flavonoids. Hence, it can be used for management of inflammatory diseases.

Inflammation is a part of healing process. However, sometimes inflammation persist for longer time, it produce harmful effect than benefit. Chronic inflammation increase the risk of several serious diseases, such as Rheumatoid arthritis, Asthma, Coronary heart disease, Cancer etc.. There are many drugs used for the treatment of inflammation. NSAID (such as Ibuprofen, Diclofenac, Indomethacin, Aspirin) are commonly used for the management of inflammation.They inhibit the Cyclo-Oxygenase (COX) and Prostaglandin H synthase enzyme, which convert arachidonic acid, derived from membrane phospholipids, to prostaglandin and leucotriens by COX and 5- lipoxygenase pathway respectively. These are very effective for pain and inflammation, they are not thought to have a disease modifying effect in but chronic uses of these drugs produce some adverse effect. The adverse effects are most commonly happening, after the long dose over a long period of usage and/or without prescriptions of physician [21].

Siddha system is a holistic science. In Siddha literature, no of drugs (originated from plants, animals, minerals and metals) are available for chronic inflammation. Among these, plant originated drugs are easily available and low expenses. Vayu keeelaga ilakam is one of the plant originated formulation. All the ingredients of this formulation chiefly indicated for Vatha diseases in Siddha text Gunapadam Mooligai Vaguppu. More over most of the ingredients possess veppa veerium (hot potency), Kaippu (Bitter taste) and Kaarppu (Pungent) suvai, which balance and rectify the deranged Vatham and Pitha dhosham. The herbal drugs also had proven by its Analgesic and Anti – Inflammatory activities. Now the present study, the formulation Vayu keelaga ilakam was scientifically proved for its anti- inflammatory activity [22,23].

4.Conclusion

RA is currently treated with a wide variety of medicines ranging from steroidal/nonsteroidal anti-inflammatory drugs (NSAID and pain killers), to potent biological agents targeting specific immune and inflammatory. But still now there is no proper therapy established for counteracting the symptoms of the RA. Hence exploration of drugs from alternate source seems more reliable. It was observed from the present study that siddha formulation Vayu Keelaga Ilakam (VKI) possess significant Anti- inflammatory activity in Protein (Albumin) denaturation assay whereas further studies needs to be elaborated with proper In-vivo screening before subjecting the drug for clinical application.

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