



IN-VITRO ANTI-INFLAMMATORY ACTIVITY AND QUALITATIVE BIOCHEMICAL ANALYSIS OF VATHATHUKKU KASHAYA CHOORANAM - A SIDDHA POLYHERBAL FORMULATION

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ABSTRACT

“Health is wealth”. Healthy living is the most essential thing in everyone’s life. Disease is the barrier for this achievement. Every medical system has its own way to treat diseases. Siddha system of medicine not only cures the disease but also postulated ways to attain eternity. Siddha medicine deals with plants, metals and minerals. Herbal medicine used in siddha to treat illness and to assist bodily function, are safe drug than metal and mineral formulation. The concept of herbalism means of presenting health rather than curing disease lead to much emphasis on dietetics. **Background:** Vathathukku kashaya chooranam is a polyherbal drug used for the treatment of Vatha noi (Arthritis). **Aim:** The main aim of this study is to analyse the in-vitro anti inflammatory activity and bio chemical analysis of Vathathukku kashaya chooranam (VKC). **Methodology:** Vathathukku kashaya chooranam as its reference in Agathiyar ayul vedham 1200. The present study was taken to evaluate the anti – inflammatory activity by using albumin denaturation assay. **Result:** The study result was concluded that Vathathukku kashaya chooranam has significant Anti-inflammatory activity.

KEYWORDS: Lumbar spondylosis, Korai vadham, Vathathukku kashaya chooranam, Anti-inflammatory activity.

1. INTRODUCTION

Siddhars classified disease into 4448 types .Among those 80 types of vadham are mentioned in yugimuni vaithiya kaviyam , one among them is korai vadham. This signs and symptoms are correlated with lumbar spondylosis in Modern. Lumbar spondylosis is a age related wear and tear of lumbar vertebrae.It is a degenerative arthritis of lumbar spine.It cause various health problems ranging from back pain to neurological issues. The space between the vertebra will be narrowed and formation of osteophytes. Major risk factors are age factor,obesity,improper posture. Many number of medicines mentioned in Siddha literature for vatha disease. Polyherbal medicines are potent, effective and devoid of severe side effects. This current study was carried out to prove the anti – inflammatory activity of Vathathukku kashaya chooranam by invitro – assay and biochemical analysis.

2. MATERIALS AND METHODS

2.1 Drug selection

The siddha polyherbal formulation has taken from the classical siddha literature “Agathiyar Ayul Vedham 1200 (Page. No. 42)” for vatha disease.

2.2 Ingredients of Vathathukku kashaya chooranam:

1. Amanakku vaer (Ricinus communis)
2. Aavarai (Cassia auriculata)
3. Kandangkattari (Solanum surtense)
4. Neermulli (Hygrophila auriculata)
5. Kandathipilli / Thippili ver (Piper longum)
6. Chukku (Zingiber officinale)
7. Karunjchirakam (Nigella sativa)
8. Kadugu (Brassica juncea)
9. Chathakuppai (Anethum graveolens)

2.3. Collection of Raw Drugs

The drugs are purchased from ASN herbal drug shop, Melapalayam, Tirunelveli.

2.4. Authentication of Raw Drugs

The identification of polyherbal drugs are authenticated by faculties of Department of Gunapadam, Government siddha medical college and Hospital, Palayamkottai.

2.5. Method of Purification

All the raw drugs are purified as per the methods mentioned in Siddha literature.

2.6. Method of preparation

The above purified ingredients were powdered individually and mixed together and stored in air tight container. 5gm of VKC was weighted accurately and 120ml of water added and boiled, filtered into 30ml of decoction and took concentrated sample weight per ml.

2.7. Anti-inflammatory activity evaluation using Albumin Denaturation Assay

Procedure

In-vitro anti-inflammatory activity of the sample VKC was studied using albumin denaturation technique. The reaction mixture consisting of bovine serum albumin (5% aqueous solution) and the test sample VKC at varying concentration ranges from 100 to 500 µg/ml along with 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. The Percentage protection from denaturation is calculated by using the formula.

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

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.

2.7. Biochemical Analysis of Vathathukku kashaya chooranam:

Preparation of the extract

5 gms of the Vathathukku kashaya chooranam was weighted accurately and placed in a 250 ml clean beaker then 50 ml of distilled water is added and dissolved well. Then it is boiled well for about 10 minutes. It is cooled and filtered in a 100 ml volumetric flask and it is made to 100 ml with distilled water. This fluid is taken for analysis.

3. RESULTS

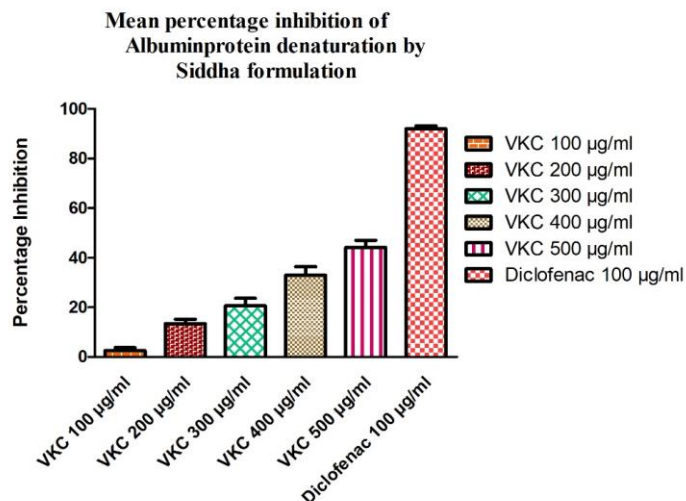
Anti-inflammatory activity evaluation using Albumin Denaturation Assay

FINAL RESULT

Concentration in µg/ml	Percentage Inhibition of Protein Denaturation
VKC 100	2.579 ± 1.136
VKC 200	13.46 ± 1.746
VKC 300	20.6 ± 3.007
VKC 400	32.92 ± 3.552
VKC 500	44.11 ± 2.881
Diclofenac sodium (100 µg)	92.07 ± 1.192

Each value represents the mean ± SD. N=3

Percentage Inhibition of Protein Denaturation by VKC and Standard



Biochemical Analysis

S.NO	EXPREMENTS	OBSERVATION	INFERENCE
1	Test for calcium 2ml of the above prepared extract taken in aclean test tube.to this add 2ml of 4% ammonium oxalate solution.	A white precipitate is formed	Indicates the presence of calcium
2	Test for sulphate 2ml of the extract is added to 5%barrium	A white precipitate is formed	Indicates the presence of Sulphate

	chloride solution.		
3	Test for chloride The extract is treated with silver nitrate solution.	No white precipitate is formed	Absence of chloride
4	Test for carbonate The substance is treated with concentrated HCL.	No brisk effervescence is formed	Absence of carbonate
5	Test for starch The extract is added with weak iodine solution.	Blue colour is formed	Indicates the presence of Starch
6	Test for ferric iron The extract is acidified with glacial acetic acid and potassium Ferro cyanide.	No blue colour is formed	Absence of ferric iron
7	Test for ferrous iron The extract is treated with concentrated nitric acid ammonium thiocyanate solution.	Blood red colour is formed	Indicates the presence of ferrous iron
8	Test for phosphate The extract is treated with ammonium molybdate and concentrated nitric acid.	No yellow precipitate is formed	Absence of phosphate
9	Test for albumin The extract is treated with esbach's reagent.	No yellow precipitate is formed	Absence of Albumin
10	Test for tannic acid The extract is treated with ferric chloride.	No blue black precipitate is formed	Absence of Tannic acid
11	Test for unsaturation Potassium permanganate solution is added to the extract.	It gets decolourised	Indicates the presence of unsaturated compound
12	Test for the reducing sugar 5ml of benedict's qualitative solution is taken in a test tube and allowed to boil for 2 minutes and add 8 to 10 drops of the extract and again boil it for 2 minutes	Colour change occurs	Indicates the presence of reducing sugar
13	Test for amino acid One or two drops of the extract is placed on a filter paper and dried well. After drying, 1% ninhydrin is sprayed over the same and dried it well.	Violet colour is formed	Indicates the presence of amino acid
14	Test for zinc The extract is treated with potassium Ferro cyanide.	No white precipitate is formed	Absence of zinc

4. DISCUSSION

Result Analysis of VKC on response of albumin denaturation assay

The result obtained from the present clearly indicates that the test drug VKC was effective in inhibiting heat induced albumin denaturation. Maximum percentage inhibition of about $44.11 \pm 2.881\%$ was observed at 500 µg/ml, when compare to that of the Diclofenac sodium, a standard anti-inflammatory agent with the maximum inhibition $92.07 \pm 1.192\%$ at the concentration of 100 µg/ml.

Biochemical analysis reveals

VKC contains calcium, sulphate, starch, ferrous iron, unsaturated compounds, reducing sugar, amino acids.

5. CONCLUSION

From this study we concluded that VATHATHUKKU KASHAYA CHOORANAM possess promising anti inflammatory activity. So it is the most promising drug

for vadha disease. In future invivo study can be done and more promising result may be expected.

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