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Evaluation of In-vitro Anti-inflammatory activity of Kadukkai mathirai by inhibition of protein denaturation assay

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Abstract

Background:
Kadukkai mathirai is a Siddha herbo-mineral formulation indicated in fatty liver disease. Scientific evaluation is required to validate its traditional use.

Objective:
To assess the in-vitro anti-inflammatory activity of Kadukkai Mathirai by inhibition of Egg albumin protein denaturation.

Methods:
Kadukkai mathirai was prepared using purified annabedhi, *kadukkai thol, milagu and vellai karisalai* according to the classical Siddha procedures. The anti-inflammatory activity was evaluated using the Egg albumin protein denaturation assay at concentrations of 300 – 500 µg/ml. Ibuprofen (100 µg/ml) was used as the standard drug. Absorbance was measured at 280 nm, and percentage inhibition was calculated. Data were expressed as mean ± SD and analyzed using one-way ANOVA followed by Dunnett's test.

Results:
Kadukkai mathirai exhibited a concentration-dependent inhibition of protein denaturation, ranging from 37.76 % at 300 µg/ml to 59.22 % at 500 µg/ml. The standard drug ibuprofen showed significantly higher inhibition ranging from 50% at 300 µg/ml to 68 % at 500 µg/ml.

Conclusion:
Kadukkai mathirai demonstrates moderate in-vitro anti-inflammatory activity, supporting its traditional use in inflammatory disorders. Further in-vivo and clinical studies are warranted to establish its therapeutic efficacy.

Keywords: Kadukkai mathirai, anti -inflammatory activity, In vitro, protein denaturation, fatty liver.

Introduction

Kadukkai mathirai is a Siddha herbo-mineral formulation indicated for Anemia and liver disorders like fatty liver that is mentioned in the textbook "Hospital pharmacopeia of Indian medicine". [1] Abnormal accumulation of lipids in the liver is called fatty liver or hepatic steatosis. Non-alcoholic fatty liver disease (NAFLD) is defined by macrovesicular steatosis in ≥5% hepatocytes, in the absence of a secondary cause such as alcohol or drugs. [2] NAFLD has a strong association with obesity, Type 2 diabetes and hypertension, and is therefore considered the hepatic manifestation of the metabolic syndrome. Inflammation is a key driver of disease progression and fibrosis development, evidenced by a strong correlation between the presence of NASH and stage 4 fibrosis. Inflammatory mechanisms are involved along the entire spectrum of NAFLD but particularly at more advanced disease stages, including cirrhosis and during the transition to Hepatocellular Carcinoma .Hepatic steatosis develops because of increased liver triglyceride storage and hepatic *de novo* lipogenesis resulting in lipotoxicity. NASH onset is linked to oxidative stress, reactive oxygen species, mitochondrial dysfunction and endoplasmic reticulum stress resulting in hepatocellular metabolic dysfunction and injury.[3] The present study aims to evaluate the in-vitro anti-inflammatory activity of KM by egg albumin protein denaturation assay.

Principle of protein denaturation assay:

Physical or chemical triggers such as stress, pH level, heat will damage the three-dimensional structure of proteins. This process is called denaturation. Protein denaturation leads to loss of biological function and inflammatory conditions where altered proteins act as auto-antigens. In this assay, a standard protein such as albumin is subjected to heat-induced denaturation. When a test substance is added, its ability to inhibit the denaturation is measured spectrophotometrically. A lower absorbance indicates greater protection against denaturation [4].

Materials and Methods

Table:1 Ingredients of KK:

Name of the drug	Part used	Quantity
1.Annabedhi	powder	- 0.9 grms
2.Kadukkai thol	Fruit	- 0.3 grms
3.milagu	Seed	- 0.3 grms
4.Vellai karisalai	Leaves	- 3 kg

Source of raw drugs:

The raw drugs were procured from a reputed raw drug store and authentication was obtained from the Head of the Pharmacology department. The raw drugs were purified as mentioned in the Siddha text, *Marundhu Sei Iyalum Kalayum* [5].

Purification of raw drugs:

Name of the drug	Part used	Purification procedure
1. purified Annabedhi	Chendooram	Annabethi will be dissolved in water and filtered through the muslin cloth in order to remove the dust and other impurities. To the filtrate 10 drops of sulphuric acid was added and this mixture was kept Under sunlight until evaporated completely and formation of solids.
2.Kadukkai thol	fruit	Kadukkai will be soaked in kazhu neer and the yellowish water will be filtered and seed will be removed and dried well.
3.milagu	seed	Milagu will be soaked in buttermilk for 3 hours and will be dried well.

Preparation of Kadukkai Mathirai :

Purified Annabethi was taken and ground with lemon juice, and villai was prepared. The villai was placed in an agal, covered with a suitable lid, and the margins were sealed with clay cloth and dried thoroughly. After complete drying, the chenduram was incinerated by pudam and then collected.

Purified Kadukkai thol was crushed well, powdered using a mortar and pestle, and sieved to obtain a fine powder. Purified milagu was similarly crushed and powdered using a mortar and pestle.

Fresh Vellai Karisalai leaves were cleaned, crushed using a mortar and pestle, and the juice was filtered and collected.

The powdered Kadukkai, milagu, and Annabethi chenduram were mixed and ground well by adding Vellai Karisalai juice. The mixture was then made into small pills of sundaikkai size (approximately 500 mg) and dried in shade.

Preparation of Extraction:

About 2g of Kadukkai mathirai were crushed and added into a conical flask Containing 100ml of distilled water. It was kept at room temperature for 15 min and later it was kept at 80 degrees Celsius for 20 min. The Extract was filtered using a muslin cloth.

Procedure of protein (albumin) denaturation assay:

The final volume of all samples was 5 ml. The reaction mixture was made up of 2.8 ml of phosphate-buffered saline (pH 7.4) and 0.2 ml of egg albumin from a fresh hen's egg. Then 2 ml of Kadukkai Mathirai extract at three different concentrations (300 µg/ml, 400 µg/ml, and 500 µg/ml) were added to this and mixed it gently. The reference drug, Ibuprofen, which was the positive control, was treated in the same way. The negative control was distilled water. The reaction mixtures were put in a water bath at 37 ± 2°C for 15–20 minutes and then heated to 70°C for 5 minutes to make the proteins lose their shape. A UV spectrophotometer was used to measure the absorbance of each sample at 280 nm after they had cooled down. The percentage protection against protein denaturation was calculated using the formula,

Percentage of inhibition % =

$$\left[\frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100\right]$$

Statistical analysis:

The values are expressed as Mean ± SD. The difference between experimental groups were evaluated by one -way ANOVA (Analysis of Variance) followed by Dunnett's test.

Results

Test drug – Kadukkai mathirai (280 nm)

Concentration in µg/ml	Absorbance of control	Absorbance of sample	Percentage of inhibition	Ic50 value
KM 300 µg	0.233	0.145	37.76 %	25.439
KM 400 µg	0.233	0.012	48.49 %	34.75
KM 500 µg	0.233	0.095	59.22 %	44.07

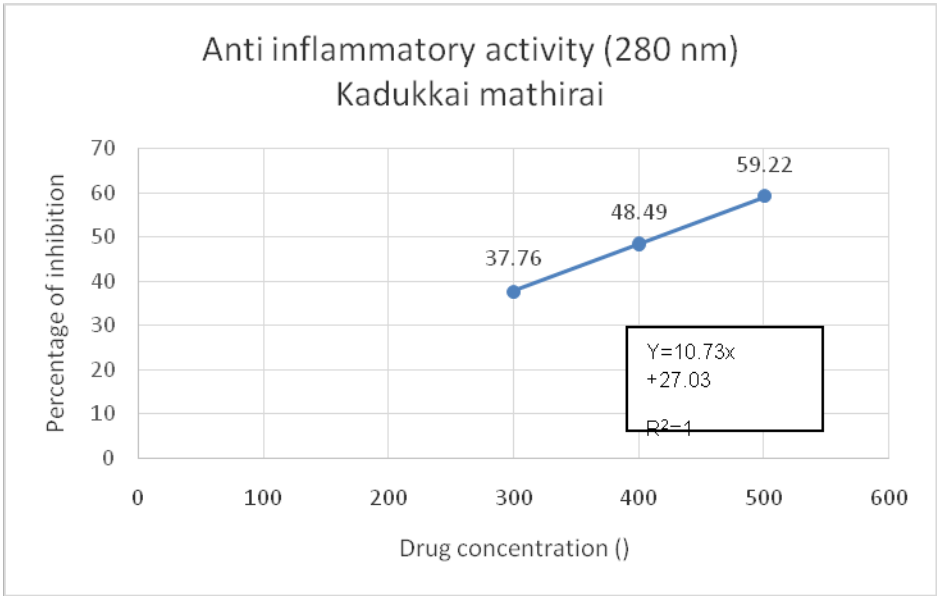
Standard drug – Ibuprofen (220 nm)

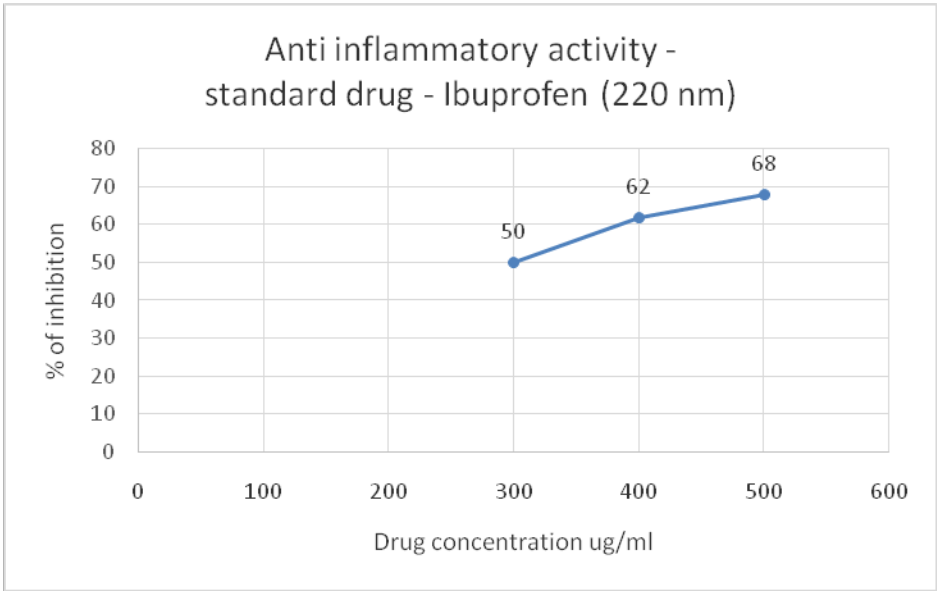
Concentration µg/ml	Absorbance of control	Absorbance of sample	% of inhibition	Ic50 value
300 µg/ml	0.05	0.025	50 %	28.66
400 µg/ml	0.05	0.019	62 %	39.77
500 µg/ml	0.05	0.016	68 %	50.88

Discussion

Kadukkai mathirai (KM) is a Siddha herbo-mineral formulation indicated for Anemia and liver disorders. This study evaluated the in-vitro anti-inflammatory activity of KM by inhibition of protein denaturation assay. The present study evaluated the in-vitro anti-inflammatory activity of *Kadukkai Mathirai* using the egg albumin protein denaturation assay. Protein denaturation is a well-known mechanism involved in inflammatory conditions, where structural alteration of proteins leads to the formation of auto-antigens and subsequent inflammatory responses. Therefore, the ability of a compound to inhibit protein denaturation is considered an important indicator of its anti-inflammatory potential.

In this study, *Kadukkai Mathirai* demonstrated a concentration-dependent inhibition of protein denaturation. The percentage of inhibition of standard drug Ibuprofen reveals 50% at 300 µg/ml concentration and 62% at 400 µg/ml and 68 % at 500 µg/ml. The percentage inhibition of test drug kadukkai mathirai increased progressively from 37.76% at 300 µg/ml to 59.22% at 500 µg/ml. This gradual increase clearly indicates that the formulation possesses significant anti-inflammatory activity. The decrease in absorbance values of the test sample compared to the control further confirms its protein stabilizing effect. The IC_{50} value, which represents the concentration required to inhibit 50% of protein denaturation, was observed within the concentration range of 400–500 µg/ml. This suggests that the formulation exhibits moderate to good anti-inflammatory activity in the in-vitro model.





The observed anti-inflammatory activity may be attributed to the presence of bioactive constituents in the formulation. *Kadukkai* is well known for its anti-inflammatory properties., *Milagu* contains piperine, which has been reported to exhibit anti-inflammatory effects by modulating cytokine production. *Vellai Karisalai* further enhances the hepatoprotective and anti-inflammatory potential due to its bioactive phytoconstituents.

Inflammation plays a crucial role in the progression of non-alcoholic fatty liver disease, where lipid accumulation induces oxidative stress and inflammatory cytokine release, leading to hepatocellular damage. Therefore, the anti-inflammatory property exhibited by *Kadukkai Mathirai* suggests its potential therapeutic role in preventing or managing inflammatory conditions associated with NAFLD. Overall, the findings of this study provide scientific support for the traditional use of this Siddha formulation. However, further studies, including in-vivo models is necessary to confirm its efficacy, safety, and mechanism of action.

Conclusion

Kadukkai mathirai showed concentration-dependent increase in the percentage inhibition of protein denaturation. Kadukkai mathirai exhibited a moderate anti-inflammatory activity compared to the standard drug. Further in-vivo studies and clinical studies are necessary to validate its therapeutic potential.

References

1. Hospital Pharmacopea of Indian medicines, by v.narayana swamy, pg no34.Tamilnadu siddha medical board.
2. James Maurice, Pinelopi Manousou, Non-alcoholic fatty liver disease, Clinical Medicine, Volume 18, Issue 3, 2018, Pages 245-250, ISSN 1470-2118,
3. Moritz Peiseler, Robert Schwabe, Jochen Hampe, Paul Kubes, Mathias Heikenwälder, Frank Tacke, Immune mechanisms linking metabolic injury to inflammation and fibrosis in fatty liver disease – novel insights into cellular communication circuits, Journal of Hepatology, Volume 77, Issue 4,2022, Pages 1136-1160, ISSN 0168-8278,
4. Acharya V, Chaudhuri P. Modalities of protein denaturation and nature of denaturants. Int J Pharm Sci Rev Res. 2021; 69:19–24. doi:10.47583/ijpsrr. 2021. v69i02.002.
5. Deva Aashirvadam Samuel. Marundhu Sei Iyalum Kalaiyum. Chennai: Department of Indian Medicine and Homoeopathy, Government of Tamil Nadu; p. 285, 295.
6. Thiyagarajan R. Gunapaadam Thathu Seeva Vaguppu. Chennai: Department of Indian Medicine and Homoeopathy; 1952. p. 53.
7. M. Ibne Jami, Z. Sultana, M. Ali, M. Begum and M. Haque, "Evaluation of Analgesic and Anti-Inflammatory Activities on Ethanolic Extract of *Terminalia chebula* Fruits in Experimental Animal Models," American Journal of Plant Sciences, Vol. 5 No. 1, 2014, pp. 63-69. doi: 10.4236/ajps.2014.51010.

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