

In-vitro Evaluation of Anti-Inflammatory Activities of Combined Cinnamon and Lodhra (1:2)

Arun Koteppa Arera¹ and Mohammed Vazir²

M Pharma Student, Department of Pharmacology¹,

Professor & HOD, Department of Pharmacology²,

Hillside College Of Pharmacy & Research Centre, Bangalore, India

Abstract: Inflammation is a biological response of the immune system that can be triggered by variety of factors, including pathogens, damaged cells and toxic compounds. The anti-inflammatory agents are commonly used to reduce and treat the inflammation. The prolonged and untreated inflammation can causes the chronic inflammation. In acute cases the inflammation can be manageable by applying ice and local treatments.

In the presents study mainly focusing on the combination of two herbal anti-inflammatory agents in the particular ration(1:2). The cinnamon and lodhra barks are collected from the herbal store in bangalore, the barks are washed shade dried and coarse powdered, then the cinnamon and lodhra are extracted by maceration method.

Then the samples are evaporated and subjected to phyto-chemical screening.

The in-vitro anti-inflammatory evaluation carried out by protease inhibition method, protein denaturation inhibition method and membrane stabilization method. The aspirin used as the reference drug for the studies.

In the results section the percentage yield and results of phyto-chemical screenings and anti-inflammatory evaluation results are recorded.

Then the conclusion given for the study wheather the anti-inflammation activity present after the combination studies.

Keywords: cinnamon cassia; symplocos racemosa; inflammation; maceration extraction; aspirin.

I. INTRODUCTION

The inflammation is the local response of the body towards the agents like dust, chemicals, pollens and radiations.

The factors or agents may causes the acute or chronic inflammation response in the heart, pancreas, liver and kidney, lungs and brain, potentially leading to tissues damage or disease.

At the tissues level the inflammation is characterized by redness, swelling, heat, pain, and loss of function.[1]

The inflammation can be acute or chronic inflammation

1) Acute inflammation

This is your immune system's response to a sudden injury or illness. Inflammatory cells travel to the site of injury (like a cut on your finger) or infection and start the healing process. A cute inflammation may last for a few hours to a few days, depending on your condition.

2) Chronic inflammation

This is when your body continues sending inflammatory cells even when there's no Danger. This leads to inflammation that comes and goes and can cause severe damage, Chronic inflammation can last for months or years.[2]

Different mediators of inflammation



- Histamine
- Bradykinin
- Prostaglandin
- Leukotrienes
- TNF- α
- IL-1[3]

Signs and symptoms of inflammation

- Rubor(redness)
- Calor(heat)
- Tumor(swelling)
- Dolor(pain)
- Function laesa(loss of function)[4]

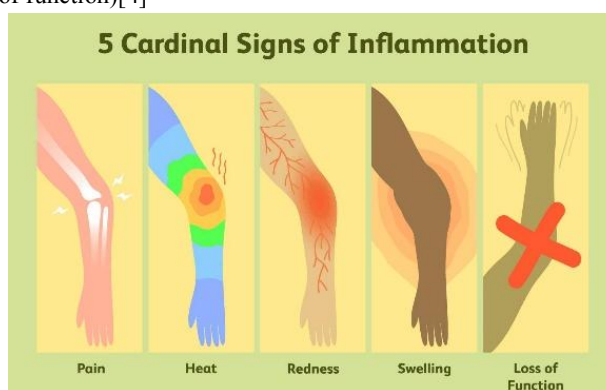


Fig. Cardinal Signs Of Inflammation

Plant profile

a) Cinnamon cassia

- The cinnamon belong to the family lauraceae, one of the most widely used spices gobally.
- It is primarily derived from two species: Cinnamomum zeylanicum and Cinnamomum cassia.
- It contains cinnamaldehyde, cinnamic acid and cinnamte bioactives.
- It widely distributed indonasia, Vietnam and india
- It is a tree of tropical medicine, species of evergreen tree.
- Its cultivated by seed propagation method and after a certain growth the plant harvested and the barks are stripped and collected.
- It has different medicinal properties like anti-inflammation, anti-oxidative, cardiovascular action, and anti-microbial activity.
- The temperature 20-30 celcius and proper irrigation required for the good growth.
- It grows over 20-30feets high with thick scabrous bark and strong branches.[5]





Fig. Cinnamon Plant And Bark

b) Symplocos racemosa

- The symplocos racemosa belongs to the family symplocaceae, known as lodhra in Sanskrit.
- It's a small evergreen tree, found throughout the tropical and sub-tropical countries.
- It is distributed in Bengal, assam, Nagpur, Bangladesh and Thailand.
- The contains loturine, collotune and loturidine active constituents.
- Its has many health benefits in ocular disorders, oncological concerns, respiratory conditions and STIs.
- The lodhra is cultivated by seedling method and the proper sanding and fertilizers are required for the good growth.
- Harvesting done by selectively without damaging.
- The soil should be loamy and sandy with good drainage.[6]



Fig. Lodhra Plant And Bark

Anti-inflammation

Anti-inflammatory agents are drugs or substances that reduce inflammation, characterized by redness, swelling and pain in the body. They work by blocking certain substances responsible for causing inflammation.[7]

The different anti-inflammations agents are,

- **Anti-inflammatory diet:-** olive oil, nuts and high-fiber foods.
- **Corticosteroids:-** cortisons, prednisalones.
- **Non-steroidal anti-inflammatories:-** naproxens, ibuprofen and aspirin.
- **Analgesics:-** paracetamol
- **Herbal medicines:-** lodhra, cinnamon, garlic and ginger.
- **Biologics:-** adalimumab and infliximab.
- **Topical anti-inflammatories:-** diclofenac menthol gel and hydrocortisone cream.
- **Antibiotics:-** amoxicillin and ciprofloxacin etc...[8]



II. AIM AND OBJECTIVES

- The collection of cinnamon cassia and symplocos racemosa barks.
- shade drying the barks, samples are taken separately and made into coarse powder.
- Extraction of the phytoconstituents of each samples by maceration method.
- To carry out the qualitative analysis of cinnamon cassia and symplocos rasemosa.
- To evaluate the in-vitro anti-inflammatory activities of ethanolic extract of combined cinnamon cassia and symplocos racemosa(1:2).
- Calculating percentage inhibition and plotting the graphs for the readings.

III. MATERIALS AND METHODOLOGY

A. Collection And Authentication Of Plant Barks

The barks of cinnamon cassia and symplocos racemosa were collected from the Amruth Kesari depot, No.18, 1st cross, Bbt street, Mamulpet, Bengaluru, 560053.

These barks were shade dried, sliced and coarse powdered by grinding method, then used for the further extraction process. the barks are authenticated by the central ayurveda research institute govt. of india, ministry of Ayush, central council for research sciences #12, uttarahalli manavarthe kaval, kanakapura main road, t halagathapura post, Bangalore-560109. the specimen was authenticated as cinnamon cassia and symplocos racemosa belongs to lauraceae and symplocaceae family respectively with authentication/reference number SMPU/CAR/BNG/2025-26/2818.



Fig. Cinnamon And Lodhra Powder

B) Preparation And Extraction Of Barks

The maceration carried out in room temperature includes the submerging or soaking the barks or samples in the liquid (water, ethanol, hexane ..etc) inside an airtight container.

The maceration works by allowing the solvent to gradually diffuse into the bark powder, drawing out bioactive compounds through a concentration gradient. Active constituents naturally migrate from regions of higher concentration (inside the plant cells) to regions of lower concentration (the solvent) until an equilibrium is reached.

The barks are washed and shade dried with proper hygiene. then they are coarse powdered by using suitable size reduction method and sieved.

The 30g of barks are weighed accurately and transferred into round bottomed flask(RBF). add 300ml of 95% ethanol in the RBF and shake vigorously. the sample and solvent are taken in the 1:10 ratio.

close the RBF with the stopper and shake, keep in the bark for 7days with periodical shaking and mixing so the sedimentation of the bark can be avoided. after 7days filter the extraction content by using whatsmann filter paper and press the marc, collect in the clean and dry container. the evaporation is carried out to concentrate the sample. then the sample stored in the closed contained in the dark. the percentage yield was calculated for the each extracts.[9]





Fig. Reagents Used For Extraction And Maceration Extraction



Fig. Filtration of Cinnamon And Lodhra

C) Phyto-Chemical Screening Of The Extracts

The ethanolic extract was subjected to preliminary phyto-chemical screening for major classes of secondary metabolites. Tests were performed for carbohydrates, alkaloids, tannins and phenolic compounds, saponins, gums or mucilage, flavonoids, triterpenoids, glycosides, steroids and resins using standard qualitative chemical reactions.[10]



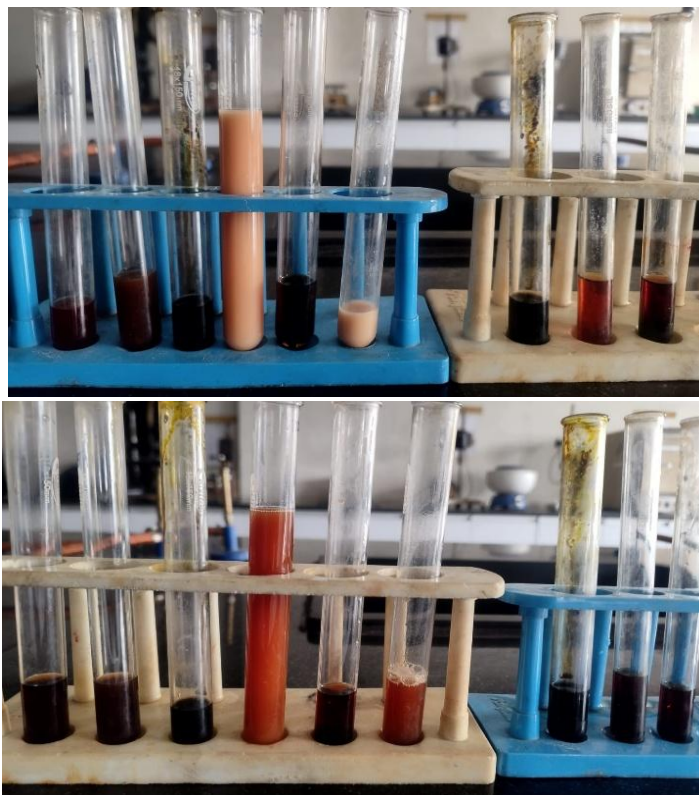


Fig. Phyto-Chemical Screening of Cinnamon And Lodhra

D) Parameters

Preparation of sample: the sample is the mixture of cinnamon cassia and symplocos racemosa in the ratio 1:2 respectively [HS154 = sample]

a) Assay Of Protease Inhibition

Principle

During inflammation, proteolytic enzymes such as trypsin and protease are released from activated leukocytes. These enzymes contribute to tissue damage and inflammation. Compounds that inhibits protease activity may possess anti-inflammatory properties. The protease inhibition assay measures the ability of a test sample to inhibit the proteolytic activity of protease enzyme commonly trypsin, on substrate such as casein.[11]

Procedure

- In this assay different enzymes and proteins are used.
- The reaction mixture contains 0.06mg protease or 0.5ml trypsin, 1ml of 20mM tri HCL buffer (pH 7.4) and 1ml test sample or standard drug, aspirin.
- These test samples are made into different conc. of 100-500µg/ml
- The mixture is incubated for at 37°C for 5min then add 1ml of casein or BSA.
- Again the mixture is incubated for 20min.
- 2ml of 70% perchloric acid or 5% trichloroacetic acid (TCA) is added to terminate the reaction.
- The solution is was allowed to stand for 15min to precipitate undigested proteins.
- Distilled water instead of sample with above mixture is used as control.
- The cloudy suspension is centrifuged at 300rpm for 10min.



- The absorbance of the supernatant is measured at 210nm using UV-visible spectrophotometer or calorimeter.
- The percentage of inhibition of protease is calculated using the equation,
- Percentage inhibition = $\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$
- The graph was plotted for the readings and higher the inhibition indicates better anti-inflammatory activities.[12]



Fig. Protease Inhibition Assay Standard And Sample

b) Protein Denaturation Inhibition Assay

Principle

The protein denaturation is induced by heat, causing changes in the structure of protein such as bovine serum albumin or egg albumin.

The BSA or any other suitable proteins are used for the studies.

Anti-inflammatory agents inhibits this denaturation of protein used.

The degree of inhibition measured calorimetrically or spectrophotometer by comparing the absorbance of treated samples with that of the control.[13]

Procedure

- In this assay the bovine serum albumin is used as protein & denaturation of this protein induced by heating at 70°C in water bath for 10min.
- The reaction mixture contains the various concentration of plant extract of 100-500µg/ml and 0.45ml of BSA.
- Add 1.4ml of phosphate buffer (pH 6.4) solution.
- Distilled water instead of sample with above mixture is used as control.
- The mixture is incubated at 37°C for 15min then at 70°C for 5min to stop the reaction.
- After cooling under running tap water, their absorbance are measured at 660nm using calorimetry or spectrophotometer.
- The aspirin is used as standard drug.
- The percentage of inhibition of protease is calculated using the equation,
- Percentage inhibition = $\frac{\text{absorbance of control} - \text{absorbance of sample}}{\text{absorbance of control}} \times 100$ [14]
- The graph was plotted for the readings and higher the inhibition indicates better anti-inflammatory activities.



Fig. Protein Denaturation Inhibition Assay Standard And Sample



c) Membrane Stabilization Method

Principle

The membrane stabilization assay is based on the principle that anti-inflammatory agents stabilize lysosomal membranes, which are similar to red blood cell (RBC) membranes. Since the rupture of lysosomal membranes releases inflammatory mediators, preventing this rupture indicates anti-inflammatory potential.

The anti-inflammatory agent are capable of stabilizing lysosomal membrane, thereby preventing the release of inflammatory mediators. If the test compound prevents the haemolysis of RBCs under stressful conditions, it is considered as capable of stabilizing biological membrane and may exhibits anti-inflammatory activity.

Thus, the principle of the membrane stabilization method is that anti-inflammatory agents inhibit haemolysis of erythrocytes by stabilizing their membranes, thereby preventing membrane rupture in a manner analogous to stabilization of lysosomal membranes during inflammation.[15]

Procedure

- Fresh human blood will be collected in heparinized centrifuge tubes and centrifuged at 3000rpm for 10min.
- Washed with equal volume of normal saline.
- The volume of blood will be measured and reconstituted as a 10% v/v suspension with normal saline.
- The reaction mixture consists of 1ml of test samples of different concentration 100-500µg/ml and 2ml of phosphate buffer pH-7.4
- Add 1ml of 10% HRBC suspension and two ml of distilled water.
- For control saline will be added.
- The aspirin will be used as a standard drug.
- The sample incubated at 56°C for 30minutes and centrifuged at 2500rpm for 5minutes.
- The absorbance of the supernatant measured at 560nm.
- The percentage of membrane stabilization activity will be calculated by using following formula.
Percentage of Protection = $\frac{\text{absorbance of test}}{\text{absorbance of control}} - 1 \times 100$ [16]



Fig. Membrane Stabilization Method Standard And Sample

IV. RESULTS

A) Extraction By Maceration

The Cinnamon cassia and symplocos racemosa are extracted with 95% ethanol and the yield of the extract are as follows,

SI No.	Plant extract	Percentage Yield (%)
1.	Cinnamon cassia	33.75
	Symplocos racemosa	37.2





Fig :- Cinnamon And Lodhra Extracts

B) Phyto-Chemical Qualitative Analysis

The qualitative screenings are carried out for the extracted samples to determine the presence different Phyto-constituents.

SI No.	Test	<i>Cinnamon cassia</i>	<i>Symplocos racemosa</i>
1.	Alkaloids	+	+
2.	Glycosides	+	+
3.	Flavonoids	+	+
4.	Phenolic compounds	+	+
5.	Tannins	+	+
6.	Saponins	+	+
7.	Terpenoids	+	+
8.	Resins	+	+
9.	Gums or mucilage	-	+

Note:- + indicates presence & - Indicates absence

Table:- Qualitative Phyto-chemical screening

C) Parameters

a) Assay Of Protease Inhibition

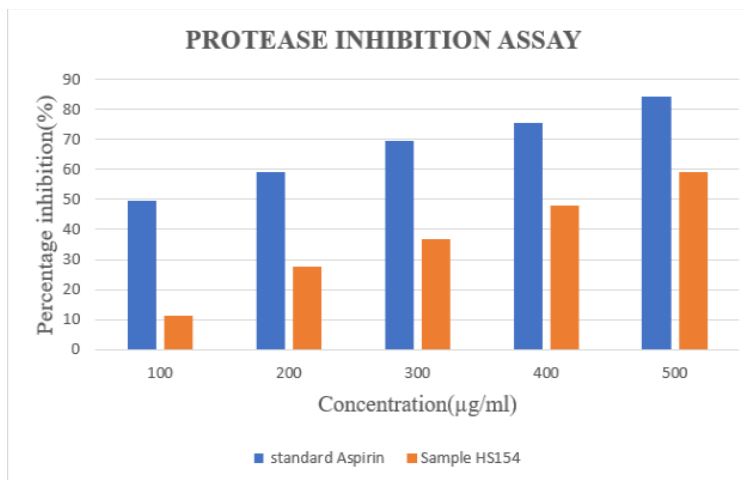
The observed readings for the sample cinnamon cassia and symplocos racemosa(1:2) as follows,

SI No.	Concentration (μg)	Percentage Inhibition of Standard (%)	Percentage Inhibition of Sample (%)
1.	100	49.46	11.41
2.	200	59.24	27.72
3.	300	69.57	36.96
4.	400	75.54	47.83
5.	500	84.24	59.24

Table :- Protease Inhibition Assay

The graph was plotted for the above observation and as below,





Graph : Protease Inhibition Assay

The results showed a dose-dependent increase in protease inhibitory activity with increasing concentrations of HS154. At concentrations of 100, 200, 300, 400, and 500 µg/ml, sample HS154 exhibited approximately 10%, 27%, 36%, 47%, and 59% inhibition, respectively.

The gradual enhancement in protease inhibition by HS154 indicates its ability to stabilize proteins and suppress protease-mediated inflammatory processes.

b) Protein Denaturation Inhibition Assay

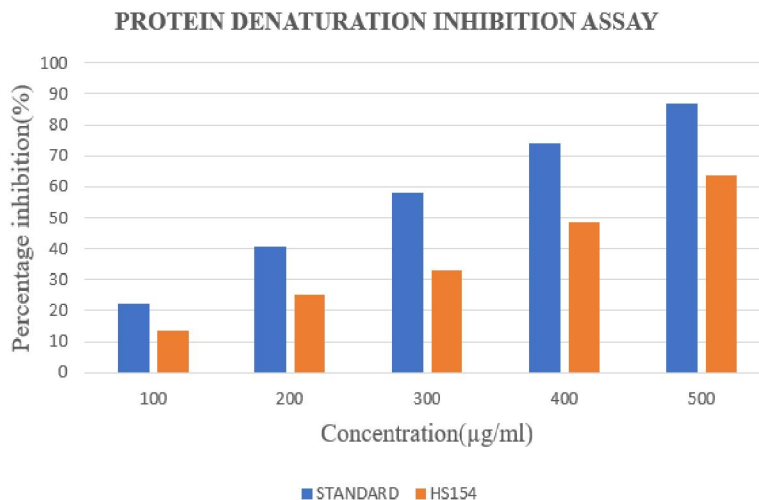
The protein denaturation inhibition assay carried out and the observation for the method are as follows, cinnamon cassia and symplocos racemosa(1:2)

SI No.	Concentration (µg/)	Percentage Inhibition of Standard (%)	Percentage Inhibition of Sample (%)
1.	100	22.08	13.64
2.	200	40.91	25.32
3.	300	57.79	33.12
4.	400	74.03	48.70
5.	500	87.01	63.64

Table :- Protein Denaturation Inhibition Assay

The graph was plotted for protein denaturation inhibition assay using the above observations & as below,





Graph : Protein Denaturation Inhibition Assay

The results demonstrated a concentration-dependent increase in the inhibition of protein denaturation for both the standard and HS154. At concentrations of 100, 200, 300, 400, and 500 µg/ml, HS154 exhibited approximately 12%, 25%, 33%, 49%, and 63% inhibition, respectively, whereas the standard showed higher inhibition values of about 20%, 40%, 57%, 73%, and 86%, respectively.

c) Membrane Stabilization Method

The membrane stabilization method carried out and the observation for the method are as follows, cinnamon cassia and *symplocos racemosa*(1:2)

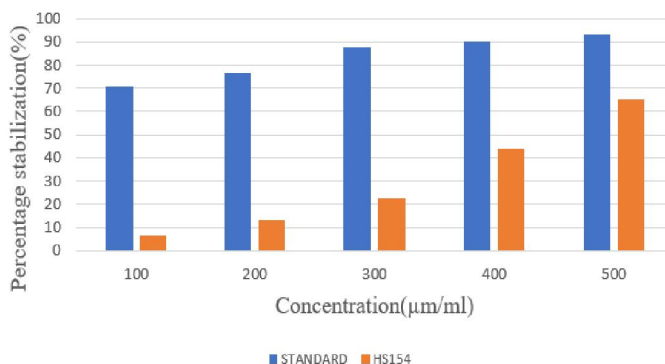
SI No.	Concentration (µg/)	Percentage stabilization of Standard (%)	Percentage stabilization of Sample (%)
1.	100	70.73	6.50
2.	200	76.42	13.01
3.	300	87.80	22.76
4.	400	90.24	43.90
5.	500	93.50	65.04

Table :- Membrane Stabilization Method

The graph was plotted for the membrane stabilization method using observation readings and as below,



MEMBRANE STABILIZATION METHOD



Graph :- Membrane Stabilization Method

HS154 exhibited dose-dependent anti-inflammatory activity in the HRBC membrane stabilization assay. The highest membrane stabilization of 65.04% was observed at 500 µg/mL, demonstrating significant protection against hemolysis. These findings indicate that HS154 possesses promising in-vitro anti-inflammatory properties, although its activity is lower than that of the standard drug Aspirin.

IV. DISCUSSION

Inflammation is a normal part of body's response to injuries and invaders like germs. It promotes the healing and helps feel better. The inflammation also results of harmful stimuli such as physical or chemical injuries, infections (bacterial, viral, fungal), toxic substances and autoimmune disorders(e.g., rheumatoid arthritis).

The inflammation is characterized by redness (rubor), heat (calor), swelling (tumor), pain (dolor), loss of function (functio laesa). In minor cases the inflammation can be manageable and doesn't need treatment.

The qualitative analysis are carried out for the cinnamon cassia and symplocos racemosa. The ethanolic extract of cinnamon cassia contains alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins, terpenoids and resins. These are constituents responsible for the medicinal properties of cinnamon.

The ethanolic extract of symplocos racemosa contains alkaloids, glycosides, flavonoids, phenolic compounds, tannins, saponins, terpenoids and gums, mucilage and resins. These constituents are responsible for the medicinal properties of cinnamon.

The phytochemicals are responsible for plant's characteristic colour, flavour and odour and plays a crucial role in their natural defence system. In-vitro studies are carried out for the combined samples.

The protease inhibition assay, protein denaturation inhibition assay and membrane stabilization assays are carried out, the absorbance are recorded and graphs were plotted to evaluate the anti-inflammatory activities of cinnamon cassia and symplocos racemosa by combining in the ratio 1:2 respectively.

The studies are indicating the combination of cinnamon and lodhra may provide synergistic activity which may or may not greater activity than standard.

V. CONCLUSION

The studies on the cinnamon cassia and symplocos racemosa shown they have many medicinal properties including anti-oxidant, anti-diabetic activity, anti-hypertensive, digestive property, cardioprotective and neurological benefits.

From the present study mainly focusing on the anti-inflammatory activities in combination.



The many studies are concluded cinnamon cassia and symplocos racemosa have anti-inflammatory activity individually, But in this study on ethanolic extract of cinnamon and lodhra combination has showing the extensive anti-inflammatory property.

The effectivity of the combined drugs due to the phytochemicals present mainly flavonoids, tannins and phenolic compounds in the both the samples.

These phytochemicals plays significant role in the medicinal properties

In all the methods of evaluation the sample HS154 showing effective anti-inflammatory activities which almost equal or less than the standard drug.

This study has been able to establish that ethanolic extracts of cinnamon cassia and symplocos racemosa have a statistically significant anti-inflammatory effect in a dose-dependant manner, which supports its extensive anti-inflammatory agent.

Further studies needs to be done to isolate the active components in the different filtrates of cinnamon cassia and symplocos racemosa and to elucidate the full anti-inflammatory profile of cinnamon cassia and symplocos racemosa as well as the mechanism of action.

The high efficiency & suitable method of extraction, collection of phytochemicals and filtration will provides the improved effect of anti-inflammation.

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