INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

ANTI-PLATELET AGGREGATION STUDY ON *RASAM*: A SOUTH INDIAN TRADITIONAL FUNCTIONAL FOOD

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ARTICLE INFO

Article history
Received 29/03/2017
Available online
30/04/2017

Keywords
Chaaru,
Saaru,
Spices.

ABSTRACT

Rasam, is a common South Indian traditional spice soup. The ingredients used in the preparation of *rasam* are medicinally claimed for anti-platelet aggregation activity. Hence, *rasam* was studied for its anti-platelet aggregation potential beyond its culinary and nutritional effect. Adenosine diphosphate induced platelet aggregation study for four stage wise samples in the preparation of *rasam* (RS1, RS2, RS3 and RS4) were studied. RS4, the final product of rasam showed higher percentage of platelet aggregation inhibition and IC_{50} value of 93.43% and 10.75 µL/mL respectively than other samples. The percentage of platelet inhibition of *rasam* was found to be directly proportional to the concentration. *Rasam* is a traditional functional food and can be an anti-platelet aggregation inhibitor on chronic use.

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*Please cite this article in press as **Agilandeswari Devarajan** et al. Anti-Platelet Aggregation Study on Rasam: A South Indian Traditional Functional Food. Indo American Journal of Pharmaceutical Research.2017:7(04).*

INTRODUCTION

The approach of traditional functional food is that, food can have an expanded role that goes well beyond providing a source of nutrients. Traditional functional foods can help to prevent chronic disease or optimize health, therefore reducing health care costs, improving the quality of life. *Rasam*, is a common South Indian traditional spice soup and also called as *chaaru* or *saaru* in different South Indian languages. *Rasam*, is a traditional functional food as, its ingredients are medicinally claimed for various ailments. The main spices used in the preparation of *rasam* are tamarind, turmeric, chili pepper, cumin, garlic, black pepper, black mustard, curry leaves, coriander and asafoetida [1].

Pathogenesis of many cerebro-vascular disorders (CVD) is due to platelet aggregation [2]. Anti-platelet therapy is recommended by The American Heart Association (AHA) as a primary prevention strategy in those with increased cardiovascular risk [3]. Platelet aggregations in fact, also play a key role in increasing ischemic risk in diabetic subjects [4, 5].

Turmeric [6], chili pepper [7], cumin [8], garlic [9], black pepper [10], curry leaves [11] and coriander [11] have been reported for anti-platelet aggregation activity. All the above quoted spices are ingredients of *rasam*. Daily consumption of *rasam* can prevent the incidence of platelet aggregation beyond its culinary and nutritional effect. Hence, it was studied for anti-platelet aggregation activity.

MATERIALS AND METHODS

Materials

All ingredients of *rasam* were purchased from Arokya organic shop, Vellore, Tamil Nadu. All utensils used for the preparation of *rasam* were of Stainless Steel 316 grade (SS 316). Trisodium citrate, adenosine diphosphate (ADP) and phosphate buffered saline (PBS) were procured from Sigma Aldrich (Bangalore, India). All other chemicals and solvents were obtained from SD Fine Chemicals (Mumbai, India) and were of analytical grade.

Preparation of *rasam*

Rasam was prepared in five stages as mentioned below;

1. Tamarind fruit pulp mixture (T1): 6.88 g of tamarind fruit pulp was immersed in 450 mL of water for 10 min, then it was hand crushed for 45 times and strained. The strained liquid was rinsed with 5 mL water, to which 0.4 g of turmeric powder and 4 g of sea salt was added.
2. Tomato fruit mixture (T2): 82.44 g of fresh tomato fruits were cut and hand crushed for 60 times. The crushed fruit was rinsed with 5 mL of water.
3. Spice mixture (T3): 1.33 g of pepper drupes was crushed in a SS 316 mortar and pestle for 85 times. 2.67 g of cumin fruits was added over to the crushed pepper drupes and crushed for 100 times. To the above crushed mixture 0.82 g of chili pepper was added and crushed for 50 times. To the above mixture 9.63 g of garlic cloves was added and crushed for 90 times.
4. All mixture (T4): Tomato fruit mixture (T2) was rinsed with 10 mL of water and spice mixture (T3) was rinsed with 10 mL of water. Both rinsing were added to tamarind fruit pulp mixture (T1), which was designated as sample RS1.
5. Final product (T5): 4 ml of Indian sesame oil was heated at 60 °C for 2 min. After 5 seconds 0.82 g of mustard seeds were added. After 3 seconds 1.53 g of whole chili pepper was added. After 2 seconds 0.61 g of curry leaves was added, which was designated as sample RS2. Immediately all mixture (T4) was rinsed with 20 mL of water and added. The whole liquid was allowed to boil for a 5 min. After 5 min 1.50 g of coriander leaves was added, which was designated as sample RS3. When the liquid frothed, 0.05 g of asafoetida was added and the heating was switched off to yield the final product, which was designated as sample RS4.

The stage wise samples RS1, RS2, RS3 and RS4 in the preparation of *rasam* were studied to evaluate the significance of the traditional processing.

Anti-platelet aggregation study [12]

Preparation of platelet rich plasma (PRP):

Blood was collected invacutainer containing $1/10^{th}$ volume of trisodium citrate (3.8% w/v). PRP was prepared by centrifugation at 200 g for 10 min at room temperature. PRP was collected and used for anti-platelet aggregation assay at a count of 200-250 x 10^3/µL.

Preparation of platelet aggregator:

Adenosine diphosphate (CDH chemicals) was prepared in PBS (pH 7.4) at a final concentration of 20 µM and used as a platelet aggregator.

Preparation of samples:

Test samples, RS1, RS2, RS3 and RS4 were diluted in PBS to a final concentration of 10, 100, 500 and 1000 mL/mL and used in the assay.

Procedure:

Five microliters of different dilutions of samples RS1, RS2, RS3 and RS4 or PBS (control) was added to 285 µL of PRP in 5 mL plastic vials and preincubated for 2 min at 37 °C. 15 µL of ADP was added to preincubate PRP vials. The absorbance of samples was recorded after 5 min incubation in a Tecan plate reader at 630 nm and % of transmittance was calculated. Platelet aggregation was calculated based on % transmittance in PBS treated control and ADP treated PRP tubes. The percentage inhibition of platelet aggregation was calculated as follows;

Percentage inhibition = (Platelet aggregation of ADP treated control- platelet aggregation of ADP with sample)/platelet aggregation of ADP treated control X 100

RESULTS AND DISCUSSION

The anti-platelet aggregation activity of most *rasam* ingredients, ascertains it as a traditional functional food. The biological source of the ingredients and its quantity used in the preparation of *rasam* is shown in Table 1.

Table 1. Biological source of the ingredients and its quantity used in the preparation of *rasam*.

Common names	Morphological part used	Nature of the material	Botanical name	Family	Quantity
Tamarind	Ripped fruit pulp	Dried	*Tamarindus indica* L.	Fabaceae	6.00 g
Turmeric	Rhizome powder	Dried	*Curcuma longa* L.	Zingiberaceae	0.40 g
Sea salt	NA	Solid	NA	NA	4 g
Tomato	Ripped fruit	Fresh	*Solanum lycopersicum* L.	Solanaceae	82.44 g
Chili pepper	Crushed fruit of long chilli pepper	Dried	*Capsicum annuum* L.	Solanaceae	0.82 g
Cumin	Ripped fruit	Dried	*Cuminum cyminum* L.	Apiaceae	2.67 g
Garlic	Cloves	Dried	*Allium sativum* L.	Amaryllidaceae	9.63 g
Black pepper	Unripe drupe	Dried	*Piper nigrum* L.	Piperaceae	1.33 g
Indian sesame oil	Seed	Oil	*Sesamum indicum* L.	Pedaliaceae	4 mL
Black mustard	Seed	Dried	*Brassica nigra* L.	Brassicaceae	0.82 g
Chili pepper	Whole fruit of long chili pepper	Dried	*Capsicum annuum* L.	Solanaceae	1.53 g
Curry leaves	Leaves	Fresh	*Murraya koenigii* (L.) Sprengel	Rutaceae	0.61 g
Portable water	NA	Liquid	NA	NA	500 mL
Coriander	Leaves	Fresh	*Coriandrum sativum* L.	Apiaceae	1.50 g
Asafoetida	Dried latex (oleogum resin) exuded from the rhizome or tap root	Powder	*Ferula assa-foetida* L.	Apiaceae	0.05 g

RS4, the final product of *rasam* showed higher percentage of platelet aggregation inhibition and IC_{50} value of 93.43% and 10.75 µL/mL respectively than other samples of *rasam* (Table 2). While, RS3 showed 85.80% and 95.12 µL/mL respectively. RS1 and RS2 showed an IC_{50} value higher than 1000 µL/mL. The percentage of platelet inhibition of RS1, RS2, RS3 and RS4 was found to be directly proportional to the concentration of all the samples.

Table 2. Effects of *rasam* samples RS1, RS2, RS3 and RS4 on platelet aggregation.

Samples	% transmittance	% platelet aggregation	% platelet aggregation inhibition	IC_{50} (µL/mL)
Negative Control (PRP+PBS)	33.93±1.05	0	0	0
Control (ADP +PBS)	26.33±0.92	22.40	0	0
RS1+ADP				
10 (µL/mL)	27.54±1.12	18.83	15.94	> 1000
100 (µL/mL)	28.12±0.87	17.12	23.57	
500 (µL/mL)	29.45±1.03	13.20	41.07	
1000 (µL/mL)	29.85±0.94	11.94	46.70	
RS2+ADP				
10 (µL/mL)	27.23±1.21	19.75	11.83	> 1000
100 (µL/mL)	28.64±1.08	15.60	30.36	
500 (µL/mL)	28.99±0.94	14.60	34.82	
1000 (µL/mL)	29.38±1.02	13.41	40.13	
RS3+ADP				
10 (µL/mL)	29.25±0.91	13.70	38.83	95.12
100 (µL/mL)	30.25±0.76	10.85	51.56	
500 (µL/mL)	31.35±0.88	7.60	66.07	
1000 (µL/mL)	32.85±1.15	3.18	85.80	
RS4+ADP				
10 (µL/mL)	30.13±0.69	11.23	49.87	10.75
100 (µL/mL)	30.76±0.90	9.34	58.30	
500 (µL/mL)	31.96±1.22	5.81	74.06	
1000 (µL/mL)	33.43±0.81	1.47	93.43	

Experiments were conducted in triplicate; values are expressed as Mean±SD; statistical analysis was done by using Graphpad Instat Version 4 software.

Apart from the anti-platelet aggregation activity of all spices, tomato and sesame oil used in the preparation of *rasam*, are also known for their anti-platelet aggregation [13, 14]. The other ingredients used in preparation of *rasam* like tamarind [15] and asafoetida [16] are clinically advised to avoid with anti-platelet drugs. Stage wise preparation analysis shows, 100 fold increase in platelet aggregation inhibition in RS4 compared to that of RS1, RS2 and RS3. It is evident that, the process in preparation of *rasam* plays an important role in increasing the anti-platelet aggregation activity of the final product (RS4) (Figure 1). The IC_{50} values of *rasam* may not be very significant comparable to active pharmaceutical agents which, are administered in a fixed dose but, consuming *rasam* as daily diet would reach its therapeutic dose in time to ensure preventive effect.

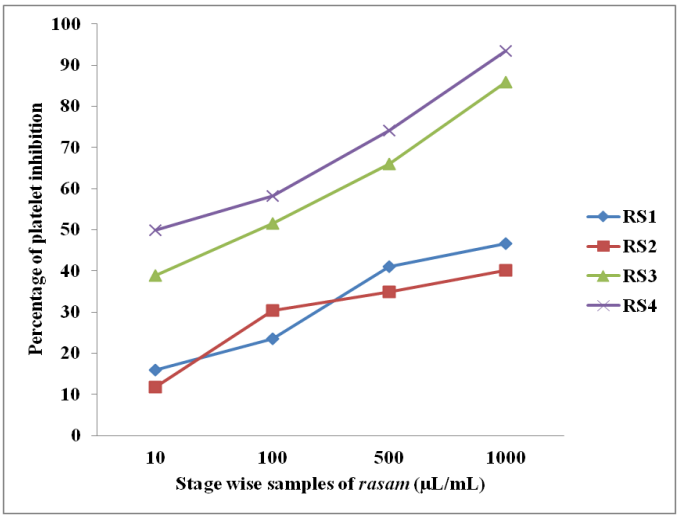

Figure 1. Effect of *rasam* samples RS1, RS2, RS3 and RS4 on platelet inhibition.

The processing in the formulation of *rasam* involves heating the spices in water and oil. This processing provides tremendous opportunity for a completely altered/different chemical composition of the finally formulated *rasam*. Loss of active principles or synergetic effect or breakdown of inactive metabolite to an active one or formation of new chemical entities (NCEs) is a real possibility. Hence, accurate mechanism for such an anti-platelet aggregation effect of *rasam* needs further evaluation.

Moreover, a better understanding of chemistry of constituents during traditional formulation processes will provide;

1. New leads based on structure activity relationship (SAR) studies of chemical constituents.
2. Interesting leads can be obtained on the novel chemical structure of NCEs from the formulation.
3. Possibility of deriving new knowledge on the mechanism of action, which in turn may help in better understanding of the etiopathogenesis and the course of diseases.
4. Interactions between chemical constituents of different ingredients will provide unique combination for studying interaction between organic and inorganic constituents.

CONCLUSION

Rasam is a traditional functional food and can be an anti-platelet aggregation inhibitor on chronic use. The real challenge lies not in proving whether *rasam* is a traditional functional food having health benefits, but in defining what these benefits are and developing the methods to expose them by scientific means. Recommend future research.

Authors' Statements

The authors declare that there is no conflict of interests.

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