IN VITRO ANTI-PLATELET AGGREGATION ACTIVITY OF A CLASSICAL HRIDYA (CARDIOPROTECTIVE) YOGA OF BHAVAMISRA

Vidhya Unnikrishnan*, K. Nishteswar2, B. R. Patel3, Mukesh Nariya4

1Ph. D Scholar, Dept. of Dravyaguna IPGT & RA, Jamnagar, Gujarat.
2Ex-Professor and HOD Dept. of Dravyaguna IPGT & RA, Jamnagar, Gujarat.
3Asst. Professor, Dept. of Dravyaguna IPGT &RA, Jamnagar, Gujarat.
4Head Pharmacology Laboratory, IPGT &RA, Jamnagar, Gujarat.

ABSTRACT
Cardioprotection includes all mechanisms and means that contribute to the protection of the heart by reducing or even preventing myocardial damage. Antiplatelet drugs reduce the incidence of cardiovascular events by about 20-25% in people with established cardiovascular diseases or at high risk of cardiovascular diseases. In the present experiment, the impact of three different concentrations (i.e. 10mg/ml, 5mg/ml and 2.5mg/ml) of Hridya yoga [formulation consisting of seven drugs namely Haritaki (Terminalia chebula Retz.), Vacha (Acorus calamus Linn.), Rasna (Alpinia galanga (L.) Willd.), Pippali (Piper longum Linn.), Sunthi (Zingiber officinale Roscoe), Shati (Hedychium spicatum Sm. in A.Rees.) and Pushkaramoola (Inula racemosa Hook.f.)] methanolic extract has been assessed on inhibition of platelet aggregation in Rat blood. The study was conducted using Platelet Aggregometer (turbidimetric method), in platelet rich plasma in response to Adenosine Diphosphate. The results obtained indicate that all the concentrations of hridya yoga extract showed effective antiplatelet aggregation activity and the maximum activity was observed at 10mg/ml concentration.

KEYWORDS: Antiplatelet, Hridyayoga, Cardioprotection.
INTRODUCTION
Cardiovascular diseases cause more than 17.5 million deaths in the world each year, according to the World Health Organization.[1] Platelet aggregation is a precipitating event in cardiovascular disease and antiplatelet therapy is one of the most effective therapies for treatment of atherothrombotic disease. Though aspirin is a well established antiplatelet drug and provides effective secondary prevention of ischemic cardiovascular disorders, it produces severe hemorrhagic events and upper gastrointestinal bleeding.[2] Several antiplatelet drugs have been developed to inhibit platelet activity in acute thrombotic situations as well as to prevent adverse events. Extensive researches are conducted recently, to explore the potential of herbal drugs for their antiplatelet and antithrombotic activities. Scientific evidences are available on the usefulness of several Ayurvedic drugs in cardiovascular diseases. Several drugs like Arjuna[3] (Terminalia arjuna), Haritaki[4] (Terminalia chebula), Pushkaramoola[5] (Inula racemosa), Pippali[6] (Piper longum) are proved to have hypotensive, hypocholestremic, anti-platelet and thrombolytic activities which play a crucial role in the management of cardio-vascular and cerebrovascular disorders. In the present study one of the reputed formulations of Bhavaprakasasamhita[7] is taken up to assess the antiplatelet activity.

MATERIALS AND METHODS
Preparation of the test drug extract
The individual drugs of the classical hridya yoga were collected personally, and were identified. Individual powder microscopy was done at Pharmacognosy unit, IPGT&RA, Jamnagar to prove the authenticity of the drug. All the drugs were powdered separately and the powder was sieved through mesh size #85. All the seven drugs were taken in equal quantity and mixed together to make the formulation and stored in airtight containers and kept at room temperature. About 5g of the test drug (formulation) was macerated with methanol (100ml) in a closed flask for 24 hours with initial shaking frequently during first 6hrs and kept it for 18 hrs. After 24hours it was filtered using Whatman No. 1 filter paper. The filtrate was evaporated to dryness and the dried substance was stored in air tight bottles until required.
Table 1: Composition of the Test drug.

<table>
<thead>
<tr>
<th>No.</th>
<th>Drugs</th>
<th>Botanical source</th>
<th>Part Used</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Haritaki</td>
<td>Terminalia Chebula</td>
<td>Fruit rind</td>
<td>All drugs in equal quantity</td>
</tr>
<tr>
<td>2</td>
<td>Vacha</td>
<td>Acorus calamus</td>
<td>Rhizome</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Rasna</td>
<td>Alpinia galanga</td>
<td>Root</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Pippali</td>
<td>Piper longum</td>
<td>Fruit</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Sunthi</td>
<td>Zingiber officinale</td>
<td>Rhizome</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Shati</td>
<td>Hedychium spicatum</td>
<td>Rhizome</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pushkaramoola</td>
<td>Inula racemosa</td>
<td>Root</td>
<td></td>
</tr>
</tbody>
</table>

Standard drugs and platelet aggregation analysis

The study was carried out at Accuprec Research Labs, Ahmedabad. For the present study, Aspirin (5 μM) was used as an anti-platelet drug and Adenosine Diphosphate (5 μM) as a platelet aggregating agent. Siliconised glass cuvettes were used with Teflon coated stir bars. The cuvettes were dipped in 0.5% silicone oil emulsion and dried in hot air oven at 120°C for 1 hour before use. The analysis was performed using turbidimetric aggregometry method on Platelet aggregometer. Turbidimetric aggregometry is based on the concept of passing light through a stirred turbid suspension of platelets. The presence of platelets in suspension causes the light to be scattered in such a manner that a reduced proportion of light passes through the platelet suspension unobstructed. The amount of transmitted light is recorded and gives a measure of optical density of the platelet suspension. On addition of an aggregatory agent, platelets form clumps, as a result of which the amount of light that is scattered is reduced since it passes unobstructed through the suspension. Thus, as platelets aggregate, the optical density of the suspension is reduced.

The potential ability of the test drug extract to prevent platelet aggregation was investigated on rat platelets. Platelet rich plasma was obtained from the collected blood as a supernatant by centrifugation (1000 rpm for 10 min at 25°C) and the remaining blood was centrifuged (4000 rpm for 15 min at 25°C) to obtain platelet poor plasma (PPP). Platelets in the PRP were counted using a platelet counter and the count was adjusted to 2x10^7/μl for dilution (working plasma). The working plasma (0.5 ml) was incubated with 0.5ml of Normal Saline (vehicle), (0.5 ml) positive control (Aspirin- 5μM) and the (0.5 ml) herbal extracts of different concentrations for 3 mins at 37°C. Adenosine Diphosphate (0.5 ml) was then added and platelet aggregation was studied using platelet aggregometer. The percentage inhibition shown by Aspirin and the different concentrations of Herbal extract was then calculated by the formula:
% Inhibition = 1 - MPA of Herbal extract or aspirin /MPA of Vehicle X 100
(MPA- Mean platelet aggregation)

RESULTS AND DISCUSSION

Table 2: Percentage aggregation and inhibition of Hridya yoga extract

<table>
<thead>
<tr>
<th>Study group</th>
<th>% aggregation (Mean±SD) (n=3)</th>
<th>% inhibition (Mean)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle control (D/W)</td>
<td>65.99±3.01</td>
<td>------------</td>
</tr>
<tr>
<td>Std Drug Aspirin (5μM)</td>
<td>32.29±2.91</td>
<td>51.05</td>
</tr>
<tr>
<td>Test Drug (10mg/ml)</td>
<td>37.45±6.47</td>
<td>43.24</td>
</tr>
<tr>
<td>Test Drug (5mg/ml)</td>
<td>42.64±2.31</td>
<td>35.38</td>
</tr>
<tr>
<td>Test Drug (2.5mg/ml)</td>
<td>47.33±2.78</td>
<td>28.28</td>
</tr>
</tbody>
</table>

Graph 1: Percentage aggregation and Percentage inhibition activity of Hridya yoga extract

In the present experiment, the impact of three different concentrations (i.e. 10mg/ml, 5mg/ml and 2.5mg/ml) of the hridya yoga methanolic extract has been assessed on inhibition of platelet aggregation in Rat blood. The mean percentage aggregation and inhibition obtained in different concentrations are 37.45, 43.24, 42.64, 35.38 and 47.32, 28.28 respectively. The results obtained indicate that all the concentrations of herbal extract showed effective antiplatelet aggregation activity and the maximum activity was observed at 10mg/ml concentration. The standard drug aspirin showed aggregation 32.29% and inhibition 51.05% at a concentration 5 μM. Even though the antiplatelet activity obtained is less compared to the positive control aspirin, the drug showed maximum activity with increasing concentrations.

Drugs that inhibit platelet aggregation are called antiplatelet drugs. Due to defective metabolism (impaired function of agni) morbid accumulation of kapha and medas (sanghata/obstruction) occurs in the Rasaraktavaha srotas (circulating channels). Due to this
flow gets obstructed. Shonitasanghatabhedana is the term used to describe the drugs which remove the sanghata (obstruction) and facilitates free movement in the raktavahasrota (blood vessels/ circulating channels). Katu rasa (Pungent taste) is ascribed with the property of “shonitasanghatabhedana”. Acharya Sushruta in Sonitavarnaniya adhyaya describes a group of drugs which facilitates the free flow of blood. The drugs included are Elettaria cardamomum, Cinnamomum camphora, Saussurea lappa, Valeriana wallichii, Cissampelos pariera, Cedrus deodara, Embelia ribes, Plumbago zeylanica, Piper longum, Piper nigrum, Zingiber officinale, Curcuma longa, Calotropis procera and Pongamia pinnata. The internal use of these drugs may be useful in dissolving the obstruction (clot) and facilitating the free flow of blood. Kshara dravyas (alkalis derived from herbs) described also helps in removing the obstruction in the srotas. Charaka advises the use of ksharas of Utpalanala (Nymphaea stellata), Nelumbo nucifera, Butea monosperma, Pterocarpus marsupium, Callicarpa macrophylla, Glycyrrhiza glabra with honey and ghee in dissolving the blood clots (kaphanubhanda grathita rakta).

Among the seven drugs of the hridya yoga, Pippali, Sunthi and Vacha are included under katurasa skanda dravyas (group of drugs having pungent taste) which possess the property of sonitasanghatabhedana. According to Acharya Sushruta, Haritaki is the best drug to be used in santarpanotha vikaras (diseases due to impaired fat metabolism). It is deepana (stimulates the gastric fire/secretions), vibandhahara (removes obstructions) and anulomana (channel obstruction remover). Vacha which is included in Lekhaneya dasamani by Charaka, acts as kaphamedohara and by its pramathi action it removes the accumulated doshas (waste products) from the srotas. Rasna is the best vatahara (pacifies vata) drug and helps in amapachana. Pippali is ushna (hot), tikshna (sharp), kaphavatahara (alleviates kapha and vata) and deepana. When used along with madhu removes kapha and medas. Sunthi is kaphavatahara and vibandhabhedana (breaks down the obstruction). Shati is laghu (light) and teekshna. Pushkaramoola possesses katu, thikta (bitter) rasa, is vatakaphahara and specially indicated in parswasoola (pain in the flanks) and hritsoola (cardiac pain). The combination of these drugs by virtue of their katu rasa (pungent taste), ushna virya (hot potency), tikshna guna (sharp nature), deepana, vibandhahara (removes obstructions) and lekhana (scrapes/ dries up the kapha and medas) karmas removes the obstruction in the srotas and may act like antiplatelet drugs. Among the seven drugs of the
hridayayoga four drugs namely, Haritaki\textsuperscript{[17]}, Rasna\textsuperscript{[18]}, Pippali\textsuperscript{[19]} and Sunthi\textsuperscript{[20]} are experimentally proven for their antiplatelet activity. According to Chakrapani all the measures intended to subdue vata and kapha may cure Hridroga.\textsuperscript{[21]} All the drugs of the hridya yoga possess kaphavatahara property. Agents that prevent the oxidation of low density lipoproteins reduce the development and progression of atherosclerosis. Five drugs namely Haritaki\textsuperscript{[22]}, Rasna\textsuperscript{[23]}, Pippali\textsuperscript{[24]}, Sunthi\textsuperscript{[25]} and Shati\textsuperscript{[26]} have more significant antioxidant activity and by their Rasayana (rejuvenating) activity they may act as cardiotonic or cardioprotective (Hridya) drugs.

CONCLUSION

In the present experiment, the impact of three different concentrations (i.e.10mg/ml, 5mg/ml and 2.5mg/ml) of hridya yoga extract has been assessed on inhibition of platelet aggregation in Rat blood. The study was conducted using Platelet Aggregometer (turbidimetric method developed by Born, 1962), in platelet rich plasma in response to ADP. Even though the antiplatelet activity obtained is less compared to the positive control aspirin, the drug showed maximum activity with increasing concentrations. However further studies are needed to confirm the mode of action and efficacy of the drug extracts in platelet aggregation.

REFERENCES


16. Bhavamisra, Bhavaprakashanighantu, Dr G.S Pandey (eds), Chowkambha bharathi Academy, Varanasi, 2010; 7,42,44,15,13,236,91


18. Chudiwal AK, Jain DP, Somani RS,Alpinia galanga willd An overview on phytopharmacological properties, Indian journal of natural products and resources, 2010; 1(2); 143-149.