Effects of Indian herbal formulation Body Revival on human platelet aggregation and myocardial ischemia in rats

Tapas Kumar Sur, Biswajit Audy, Dipankar Bhattacharyya
Department of Pharmacology, Institute of Postgraduate Medical Education and Research, West Bengal University of Health Sciences, Kolkata 700020, West Bengal, India

Objective: To study the effect of Body Revival (BR), a compound traditional Indian herbal medicine, on human platelet aggregation and isoproterenol (IS)-induced myocardial ischemia (MI) damage in male Wistar rats.

Methods: BR suspension 10, 20 and 30 μg was mixed with platelet-rich plasma and incubated at 37°C for 30 min, respectively. Then, adenosine diphosphate (ADP, 20 mmol/L) or collagen (2 μg) was added in the mixture and the aggregation was observed against platelet-poor plasma mixed with equal volume of suspension of the same test samples. Wistar rats divided into 4 groups were used to investigate BR’s effects on IS-induced MI. Levels of serum creatinine kinase (CK), aspartate transaminase (AST) and alanine transaminase (ALT) were estimated by standard commercial biological kits. Serum nitric oxide (NOX) was also measured. The lipid peroxides (LPO) and protein concentrations in heart tissues were measured.

Results: BR could inhibit ADP- or collagen-induced human platelet aggregation dose-dependently. Moreover, it could protect MI caused by IS in rats. BR reduced the levels of serum CK, AST, ALT and NOX dose-dependently and also lowered LPO in heart tissues in comparison with the MI control (P<0.01).

Conclusion: BR can inhibit human platelet aggregation and protect MI caused by IS in rats.

Keywords: platelet aggregation inhibitors; myocardial ischemia; lipid peroxidation; nitric oxide; plant extracts; rats

Cardiovascular disorders (CVDs) like hypertension, ischemic heart disease, cardiac arrhythmia and cerebrovascular disorders like stroke are responsible for a high incidence of mortality and

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<td>Correspondence: Dipankar Bhattacharyya, MD, Professor. Tel: +91-33-22235181; E-mail: <a href="mailto:ddhdhrks@gmail.com">ddhdhrks@gmail.com</a></td>
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morbidity worldwide. It has been projected by the World Bank Health Sectoral Priority Review that in India, CVDs alone will account for 33.5% of deaths at all ages by 2015. In modern medicine, the treatment for CVDs involves expensive drug therapy or equally expensive interventional procedures, such as thrombolytic therapy and surgical recanalization. Many herbal secondary metabolites, chemical compounds and herbal formulations have been studied for their biological actions related to preventing human diseases by using models such as adenosine diphosphate (ADP) or collagen-induced platelet aggregation and isoproterenol (IS)-induced myocardial infarctions. Body Revival (BR) is a compound medicinal herbal formulation prepared based on traditional Indian medicine, containing cardioprotective, lipid-lowering, antihypertensive, anti-inflammatory, antioxidant and immunomodulatory properties. The rationale behind such formulations is provided by modern research, which documents that cholesterol, hypertension and vascular injury play a predominant role in the atherosclerosis or infarction that predominantly leads to progression of the disease and its secondary complications. BR is a suspension of *Aegle marmelos* (fruit pulp), *Acorus calamus* (rhizome), *Saussurea lappa* (roots), *Blumea lacera* (whole plant), *Rumex vesicatorius* (leaves), *Rubia cordifolia* (root), *Cucumis melo* (seed), *Symplocos racemosa* (bark) and honey. The medicinal properties of these herbs have been reviewed. In the present context, BR was studied to find out its role in human platelet aggregation and IS-induced myocardial ischemia (MI) in rats.

1. Materials and methods

1.1 Animals Wistar strain male albino rats (body weight (150±10) g) were used in the study. In the present experiment, recommended guidelines for the care and use of the animals were strictly followed and permission from the Institutional Animal Care and Use Committee (IACUC) was also obtained. Rats were housed in groups in polypropylene cages with steel nozzle water bottles. The cutting straw was used as matting substances and was changed every day. The room temperature was maintained at (25±2) °C and humidity between 40% and 60%. The light cycle was also maintained (a 12 h light/dark cycle). The rats were fed with supplementary balanced diet feed for animal and water ad libitum.

1.2 Test drugs and reagents ADP, collagen, IS, sodium nitrite and malondialdehyde were procured from the Sigma Chemical Company (St. Louis, MO, USA) and commercial kits of creatinine kinase (CK), aspartate transaminase (AST) and alanine transaminase (ALT) from the Coral Clinical Systems (Goa, India). All the other chemicals used were of analytical grade. BR suspension (5 mL) consists of *Aegle marmelos* (150 mg), *Acorus calamus* (175 mg), *Saussurea lappa* (325 mg), *Blumea lacera* (115 mg), *Rumex vesicatorius* (240 mg), *Rubia cordifolia* (200 mg), *Cucumis melo* (200 mg), *Symplocos racemosa* (95 mg) and honey. All the components (except honey) were extracted with hydro-ethanol (volume ratio 1:1), dried out to powder and mixed proportionately to make the suspension. The atomic absorption study exhibited that BR was free from arsenic, lead, cadmium and mercury. Moreover, the microbiological study showed absences of harmful bacterial contaminations (Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa) or mould contents. BR suspension was suspended in required amount of distilled water prior to use.

1.3 Safety evaluation BR was weighed and dissolved in distilled water and was given orally to rats in single dose in a graded manner (0.5, 1 and 2 g/kg body weight) and the lethality was observed up to 72 h. Further, increment of doses could not be possible due to insolubility of BR on required volume given. No lethality was noted within 72 h up to 2 g/kg orally fed rats.

1.4 Platelet aggregation The test has been developed originally by Born and standardized by Sur et al. and is used to evaluate quantitatively the effect of compounds on induced platelet aggregation *in vitro*. In this study, 6 volunteers of either sex were selected from the medical out patients’ department of the Institute of Postgraduate Medical Education & Research, Kolkata, India after the written consents were obtained. The study was approved by the institutional human ethical committee. A careful drug history was taken from the subjects. Patients not receiving drugs like aspirin, sulfinpyrazone, chlorpromazine, amitriptyline, furosemide or penicillin and its derivatives for last two weeks, which will interfere with the platelet aggregation activity, were selected for the present research program. Specimens of blood samples were collected using 3.2% sodium citrate at the ratio 1:9 with the blood in plastic container with minimum trauma or stasis at the venipuncture site. Testing was performed 30 min after venipuncture at room temperature. The platelet-rich plasma (PRP) was prepared by centrifuging the blood at 100 × g under 4 °C for 15 min. The platelet-poor plasma (PPP) was prepared by centrifuging the blood at approximately 2,400 × g for 20 min. The platelet count was adjusted to 2×10⁹ to 3×10⁹/mm³ by diluting PRP with normal saline. Samples were maintained at 37 °C before testing. The test substance, BR suspension 10, 20 and 30 μg was mixed with PRP and incubated at 37 °C for 30 min, respectively. Then, ADP (20 mmol/L) or collagen (2 μg) were added in the incubation mixture and the aggregation was observed against PPP mixed with equal volume of suspension of the same test samples. The
optical density due to platelet aggregation was recorded in a Chrono-Log optical platelet aggregometer (Model 490, USA). The light transmission was set at 0% with PRP and at 100% with PPP.

1.5 IS-induced MI Wistar strain male albino rats (body weight (150±10) g) were used in this study. The rats were divided into 4 groups each containing 6 rats. Group I served as normal control, group II as ischemic model control and group III and group IV as test drug-treated groups. Test compound BR was orally administered for 7 d to rats of group III (200 mg/kg) and group IV (400 mg/kg), while group I and II received an equivalent amount of distilled water. At day 5, ischemia was induced by an intraperitoneal injection of IS (85 mg/kg) for 2 d[19]. BR treatment was continued on days 6 and 7 along with IS injection. After 48 h from the first injection of IS, all rats were sacrificed and blood and heart were taken for analysis of biochemical studies. Levels of serum CK[20], AST and ALT were estimated by standard commercial biological kits[21]. Serum nitric oxide (NOx) was measured by using Gress’s method[22]. The lipid peroxides (LPO)[23] and protein[24] concentrations were measured in heart tissues.

1.6 Statistical analysis Data were expressed as X±sX. The statistical significance was determined by one-way analysis of variance followed by Newman-Keuls multiple comparison tests. P < 0.05 was considered statistically significant.

2 Results

2.1 Platelet aggregation There was 23.8% inhibition in human platelet aggregation against ADP with a dose of 10 µg BR, which gradually increased the inhibition as 40.3% for 20 µg BR and 46% inhibition for 30 µg BR in comparison with the ADP-induced aggregation control (Table 1). Also, 10 µg BR inhibited 17.2% platelet aggregation against collagen, 20 µg BR inhibited 32.9% and 30 µg BR inhibited 38.3% in comparison with the collagen-induced aggregation control (Table 2).

### Table 1 Effects of BR on ADP-induced platelet aggregation (X±sX, %)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>ADP-induced platelet aggregation (%)</th>
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<tbody>
<tr>
<td>ADP control</td>
<td>6</td>
<td>86.23±0.51</td>
</tr>
<tr>
<td>BR 10 µg</td>
<td>6</td>
<td>65.66±0.23 **</td>
</tr>
<tr>
<td>BR 20 µg</td>
<td>6</td>
<td>51.41±0.45 **</td>
</tr>
<tr>
<td>BR 30 µg</td>
<td>6</td>
<td>46.53±0.48 **</td>
</tr>
</tbody>
</table>

** P < 0.01, vs ADP control.

### Table 2 Effects of BR on collagen-induced platelet aggregation (X±sX, %)

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Collagen-induced platelet aggregation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>6</td>
<td>72.10±0.49</td>
</tr>
<tr>
<td>BR 10 µg</td>
<td>6</td>
<td>59.68±0.38▲▲</td>
</tr>
<tr>
<td>BR 20 µg</td>
<td>6</td>
<td>48.37±0.31△△</td>
</tr>
<tr>
<td>BR 30 µg</td>
<td>6</td>
<td>44.51±0.28△△</td>
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▲▲ P < 0.01, vs collagen control.

2.2 IS-induced MI IS markedly enhanced the levels of serum CK, AST and ALT compared with the normal control rats, due to infarct-like myocardial lesions in cardiac muscles in rats. However, the test drug BR (200 and 400 mg/kg) showed dose-dependent reduction of CK, AST and ALT production in serum when compared with the ischemic model control rats (Table 3). Moreover, IS enhanced the NOx level within 48 h. BR treatment showed dose-dependent reduction of NOx level in rats (Table 3). Furthermore, in the ischemic model control rats, the increased activities of LPO confirmed the onset of myocardial necrosis when compared with the normal control. Pretreatment with BR significantly reduced the levels of LPO (P < 0.01).

### Table 3 Effects of BR on IS-induced myocardial ischemia in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Serum level</th>
<th>Heart tissue LPO (nmol/L)</th>
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<tr>
<td></td>
<td></td>
<td>CK (U/L)</td>
<td>AST (U/L)</td>
</tr>
<tr>
<td>Normal control</td>
<td>6</td>
<td>88.10±3.69</td>
<td>38.01±1.22</td>
</tr>
<tr>
<td>Ischemic model control (IS 85 mg/kg)</td>
<td>6</td>
<td>268.50±8.26 ▲▲</td>
<td>90.65±2.57 ▲▲</td>
</tr>
<tr>
<td>IS (85 mg/kg) + BR (200 mg/kg)</td>
<td>6</td>
<td>193.50±4.42 ▲ ▲</td>
<td>62.16±1.47 ▲ ▲</td>
</tr>
<tr>
<td>IS (85 mg/kg) + BR (400 mg/kg)</td>
<td>6</td>
<td>159.65±5.76 ▲ ▲</td>
<td>50.51±1.25 ▲ ▲</td>
</tr>
</tbody>
</table>

▲ ▲ P < 0.01, vs normal control.

3 Discussion

The present investigation is aimed to evaluate and explore the cardioprotective effect of BR, a compound traditional Indian herbal medicine, on human platelet aggregation and IS-induced MI in rats. Infarct-like myocardial lesions in rat induced by IS have been described by many researchers[33]. IS, a non-selective β-adrenergic agonist, has been reported to cause oxidative stress in the myocardium resulting in infarct like necrosis of the cardiac muscles and increase in the levels of lipids in the myocardium. Free radical generation and lipid peroxidation could be involved in IS-induced
cardiac damage\cite{25}. The pathophysiological changes during IS induction are comparable to those taking place in human myocardial infarction, due to alteration of lipid metabolism\cite{26}. Many herbal secondary metabolites, chemical compounds and herbal formulations have been studied for their biological actions related to preventing human diseases by using models such as IS-induced MI\cite{7, 29}. It is well documented that, all the components of BR, like Aegle marmelos, Acorus calamus, Sauussurea lappa, Blumea lacera, Rumex vesicarius, Rubia cordifolia, Cucumis melo, Symplocos racemosa and honey have several medicinal properties, particularly antioxidant, anti-inflammatory, hypolipidemic, hypotensive, anti-thrombotic and detoxifying actions during pathophysiological situations\cite{14-16}. Pervious reports suggested that Aegle marmelos\cite{7} is one of the most important components of BR which has the ability to combat IS-induced MI.

In the present study, IS markedly enhanced the levels of serum CK, AST and ALT due to infarct-like myocardial lesions in cardiac muscles in rats. Wildenthal et al\cite{27} showed that in CVDs, phospholipase and acid phosphatase levels were elevated due to lysosomal membrane destruction. BR treatment showed marked reduction in serum CK, AST and ALT in rats with IS-induced MI. This results suggest that BR may prevent the damage to lysosomes induced by IS and hence avoid leakage of these enzymes, which means it may act by stabilizing the structure of biological membranes. The study also reveal that BR, like other natural products namely, guggulip, guggulsterone and coleolol and compound herbs like Abana could be employed as a potential cardioprotective agent\cite{20, 22}.

During the last two decades, numerous studies have been done that focused on the roles of NOX in the pathogenesis progress and pharmacological intervention of MI\cite{28}. NOX may cause cytotoxicity through formation of iron-NOX complexes with several enzymes including electron transport chain, oxidation of protein sulfhydryls and DNA nitration and potent activator of lipid peroxidation\cite{29}. In the present study, it is noted that IS enhanced the endogenous NOX levels within 48 h. BR treatment showed dose-dependent reduction of NOX levels in serum and thereby it is hypothesized that it may have the ability to diminish the genesis of high amount of NOX radicals by preventing membrane bound tissue damage. The significant increase observed in the levels of LPO in rats with IS-induced MI compared with the normal control, was in accordance with the observation of previous reports\cite{28}. While, BR-treated rats showed a significant decrease in LPO level in cardiac tissues compared with the MI model rats. Previous investigations have shown that herbal formulations could exhibit cardioprotective effect against MI injury by inhibiting LPO and thus enhancing the recovery of cardiac function\cite{28, 30}. In hypertensive patients, the platelets are hyperactive and responsible for thrombogenesis and if left untreated may aggravate and complicate the hypertensive disorders\cite{31}. Platelet aggregation is enhanced in presence of ADP, collagen or adrenaline in vascular bed and may cause fatalities\cite{32}. ADP is contained within the platelet in storage granules and released from the platelet during formation of primary haemostatic plug and thereby could induce further platelet aggregation\cite{18}. In the present study, it indicates that BR has the ability to reduce platelet aggregation induced by ADP and also by collagen. The active principles of the plants present in BR have been reported to possess antioxidant, anti-inflammatory and cardioprotective properties\cite{14-15}. The present study of platelet aggregation reflect that BR may at least partially inhibit prostaglandins synthesis pathway.

The therapeutic effect of BR may be due to its antioxidant, antilipidperoxidative, free radical-scavenging, immunomodulatory and cardioprotective property that could have prevented IS-induced tissue injury. Thus it could be concluded that BR could protect experimental MI and platelet aggregation which merit further detailed studies to develop it as a cardioprotective formulation.

4 Acknowledgements

The authors sincerely acknowledge M/s Health Reactive (Baddi, Solan, Himachal Pradesh, India) for financial help.

5 Competing interests

The authors declare that they have no competing interests.

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印度草药方 Body Revival 对人类血小板聚集及大鼠心肌缺血的作用

Tapas Kumar Sur, Biswajit Auddy, Dipankar Bhattacharyya
Department of Pharmacology, Institute of Postgraduate Medical Education and Research, West Bengal University of Health Sciences, Kolkata 700020, West Bengal, India

目的：观察印度草药方 Body Revival (BR) 对人类血小板聚集及异丙肾上腺素引起的大鼠心肌缺血的作用。

方法：BR 悬浮液 (分别含 BR 10, 20 和 30 µg) 与富血小板血浆混合后，37 °C 下培养 30 min，分别将二磷酸腺苷 (20 mmol/L) 或胶原蛋白 (2 µg) 加入混合液中，与混合了等体积的 BR 悬浮液的血小板血浆对比观察 BR 对于血小板聚集的作用。Wistar 大鼠分为 4 组以检测 BR 对于异丙肾上腺素引起的心肌缺血的作用。使用标准的商业化试剂盒测量大鼠血清中肌酸激酶、天冬氨酸氨基转移酶及丙氨酸氨基转移酶的水平，同时检测大鼠血清中氨基氧化物的含量及大鼠心肌中脂质过氧化物及蛋白质的含量。

结果：BR 能够剂量依赖性地抑制二磷酸腺苷或胶原蛋白引起的人类血小板聚集。此外，BR 对于异丙肾上腺素引起的大鼠心肌缺血具有保护作用。与对照组比较，BR 能够显著降低大鼠血清中肌酸激酶、天冬氨酸氨基转移酶及丙氨酸氨基转移酶和氨基氧化物的含量以及大鼠心肌中脂质过氧化物的含量 (P<0.01)。

结论：BR 能够抑制人类血小板聚集并对异丙肾上腺素引起的大鼠心肌缺血具有保护作用。

关键词：血小板聚集抑制剂，心肌缺血，脂质过氧化反应，一氧化氮，植物提取物，大鼠