Evaluation of Cardioprotective effect of *Coleus forskohlii* against Isoprenaline induced myocardial infarction in rats

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**ABSTRACT**

*Coleus forskohlii* is an important ancient root drug of Indian origin, commonly known as *gander* in Indian ayurvedic system of medicine. A lot of research work has been done on *Coleus forskohlii* regarding various cardiovascular disorders but no work has been done to find out its cardioprotective activity. Wistar albino rats were divided into five main groups having 5 animal each: Group 1 termed as Normal control (NC) received 0.5ml of normal saline throughout experimental period and served as control. Group 2 termed as Isoprenaline group (ISO) received 0.5ml of normal saline for 28 day and received Isoprenaline (85mg/kg, s.c.) on 29th and 30th day at an interval of 24 hours. Group 3 termed as Standard group (STD) received Metoprolol (pure) (10 mg/kg/day, p.o.) for 28 day and received Isoprenaline (85mg/kg, s.c.) on 29th and 30th day at an interval of 24 hours. Group 4 termed as Test group 1 (TG 1) & Group 5 termed as Test group 2 (TG 2) received *Coleus forskohlii* (50 mg/kg/day, p.o.) (100 mg/kg/day, p.o.) for 28 day and received Isoprenaline (85mg/kg, s.c.) on 29th and 30th day at an interval of 24 hours respectively. The experiment was terminated on 31st day and animal were sacrificed by cervical decapitation after an overnight fast. Blood was collected for estimation of biochemical parameter and heart was dissected out for grading, heart/weight ratio and histopathological examination. The level of marker enzyme in serum as AST, ALT, LDH, CK, Troponin-I were significantly decreased (P<0.001) in rats pretreated with *Coleus forskohlii* when compared to that of group which received isoprenaline alone. Further, histopathological examination showed the reduction of necrosis, edema and inflammation following *Coleus forskohlii* pretreatment. Based on present findings, it is concluded that *Coleus forskohlii* may be a potential preventive and therapeutic agent against the myocardial necrosis associated ischemic heart disease.

1. Introduction

Myocardial infarction (MI) is a clinical problem defined as acute necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardial demand[1]. Ischemia caused due to reduced blood supply to heart causes several biochemical alterations which may lead to cardiac dysfunction and ultimately cell death [2]. It is well recognized that free radicals generated in ischemic tissues causes metabolic stresses which results in degradation of tissue defence system, leading to myocardial damage and necrosis[3]. The development of myocardial ischemia and infarction is a dynamic process with the widespread occurrence of coronary atherosclerosis and involvement of oxidative stress in the humans. Among several pharmacological interventions to protect heart against oxidative stress, the use of antioxidants is most promising. Epidemiological, clinical and experimental studies have provided compelling evidence that MI is largely preventable by antioxidant intervention via suppression of free radical generation and/or augment endogenous antioxidant[4].
half of the volume, a waxy material separate out. The water bath. When extract was concentrated by evaporation to recover. Then conc. extract is subjected to evaporation on remove its volatile oil component. The drug was extracted with was then messed up by petroleum ether (40-60) in order to small pieces convenient for the purpose of extraction. The drug was extracted with a voucher specimen (Ref No. slab/cif/0359/02) of the Coleus, *Coleus forskohlii* (Willd.) Briq. (synonym C. barbatus (Andr.) Benth.), a member of the family Lamiaceae, is an ancient root drug in Ayurvedic materia medica10. Forskolin showed positive effects against a wide range of conditions such as asthma[11], glaucoma[12], hypertension[13, 14], cancer[15], heart diseases[16], diabetes[17] and obesity[18]. It also showed increase in the rate of sensory nerve regeneration in freeze-lesioned sciatric nerves[19], stimulation of water and cation permeability in aquaporin 1 water channels[20] and direct alteration of gating of a single class of voltage-dependent potassium channels from a clonal pheochromocytoma (PC12) cell line independent of adenylate cyclase activation[21]. Its foliage is also employed in treating intestinal disorders and used as a condiment since long. Flavonoids are present in both the roots and tubers of *C. forskohlii* as flavonoids are known to act as antioxidant.

### 2. Material and method

#### 2.1 Plant material

The Coleus, *Coleus forskohlii* was donated by Sami labs ltd, Bengaluru. The drug was authenticated by Division of Taxonomy, Central instrumentation facility, Sami labs ltd, Bengaluru, with a voucher reference (Ref No. slab/cif/0359/02). A specimen sample of the same was preserved in the section of the Faculty of Pharmacy, Integral University, Lucknow, with the voucher No. slab-cif-0359, for future reference.

#### 2.2 Preparation of extract

The clean fresh roots of the *Coleus forskohlii* were cut in to small pieces convenient for the purpose of extraction. The drug was then mixed up by petroleum ether (40-60) in order to remove its volatile oil component. The drug was extracted with 80% ethanol in a soxlet apparatus. The excess of ethanol was recovered. Then conc. extract is subjected to evaporation on water bath. When extract was concentrated by evaporation to half of the volume, a waxy material separate out. The concentrated extract was weighed according to body weight, and accurately dissolves in CMC (0.5%) for dose treatment daily.

#### 2.3 Animals

Albino Wistar rats of 125-150 g of either sex were used for the study. The inbred species of rats were obtained from animal house of Central drug research institute (CDRI), Lucknow for experimental purpose. The animals were maintained under controlled conditions of temperature (23 ± 2°C) before the study. The animals were randomized into experimental, normal and control groups, housed individually in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water ad libitum. Animals were habituated to laboratory conditions for 48 hours prior to experimental protocol to minimize if any of non-specific stress. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Faculty of Pharmacy, Integral University, Lucknow (Registration No. IU/Pharm/M.pharm/CPCSEA/12/10), India.

#### 2.4 Chemicals

Normal saline (0.9%) (Albert David Ltd, Ghaziabad), Isoprenaline Hydrochloride, Metoprolol Tartrate (Pure drug) (Sigma Chemicals, USA), Petroleum Ether (40-60) (S.D.Fine Chemicals, Mumbai), Ethanol (Jiangsu Huaxi Ltd, China), Formaldehyde (Fisher scientific Ltd, Navi Mumbai), Serum ALAT diagnostic kit, Serum ASAT diagnostic kit, Serum Creatinine diagnostic kit (Span Diagnostics Ltd, Surat), Serum LDH diagnostic kit (Accurex Biomedical Pvt Ltd Thane, Mumbai), all the chemicals used was of analytical grade.

#### 2.5 Experimental Procedure

**Grouping**

Group I – Termed as normal control group (NC group), received distilled water (0.5ml, po) daily for 30 days.

Group II – Termed as Isoprenaline group (ISO group), received distilled water (0.5ml, po) daily for 30 days, in addition exported injections of ISO (85 mg/kg, sc) at an interval of 24 hours on 29th and 30th day.

Group III – Termed as standard group (STD group), received metoprolol (pure) (10 mg/kg/day, p.o.) daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

Group IV – Termed as Test group 1(TG1), *Coleus forskohlii* extract (50 mg/kg/day, p.o.) administered daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

Group V – Termed as Test group 2 (TG2), *Coleus forskohlii* extract (100 mg/kg/day, p.o.) administered daily for 30 days and in addition received ISO (85 mg/kg, sc) on the 29th and 30th days at an interval of 24 hours.

ISO = Isoproterenol

*Each group contains 5 animals (either sex)*

#### 2.6.1 Induction of myocardial infarction

Myocardial infarction was induced by Isoproterenol hydrochloride 85mg/kg body weight, dissolved in normal saline given through subcutaneous injection for two consecutive days (29th and 30th) [22]. Rats were weighed and put down 24 hours after the final subcutaneous injection of ISO.
Blood collection was done by adhering to Good Laboratory Practices. Blood was collected through retro-orbital plexus from the inner canthus of the eye using capillary tubes and cardiac puncture under light ether anesthesia and allowed to clot for 30 minutes at room temperature. The serum was separated by centrifugation at 3000 rpm at 30°C for 15 minutes and used for the estimation of marker enzymes, including aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and creatine phosphokinase (CPK).

All animal were sacrificed by cervical decapitation. The hearts were dissected out immediately, weighed, and then the heart was fixed in 10% buffered neutral formalin solution[23].

2.5.2 Estimation parameters

I. Gross examination of rat heart

The dissected hearts was washed with Ice-cold saline. The visually examined the Inflammation, redness, capillary dilatation, Scar formation, colour, in all part of heart and grading was performed[9].

II. Heart weight: body weight ratio

The mouse was euthanized, weighed and recorded total body weight. Mouse was placed on its back and pinned onto board with extended extremities (inner side of hands and foot). The mouse was wiped or wetted with 70% ethanol to control hair and dander. Removal of the heart was performed by dissecting the aortic root immediately above the aortic valves and the superior vena cava above the atria. Adjacent mediastinal fat pads were removed from the excised heart carefully with forceps. Heart blood was removed from heart by tapping the heart on a kim wipe (absorbent pad) or surgical compress. This process was repeated until the heart was totally dry. The dry heart was weighed and recorded. Then the heart was place in fixative[24].

III. Biochemical estimations

At the end of experimental period the blood samples were taken and serum was separated for analysis of different enzymes related to myocardial infarction such as lactate dehydrogenase (LDH), creatine kinase-MB fraction (CK-MB), aspartate transaminase (AST), alanine transaminase (ALT). All the analyses were performed with commercially available kits purchased from Span Diagnostics Ltd, Surat, Accurex Biomedical Pvt Ltd Thane, Mumbai and measured spectrophotometrically, Shimadzu. Release of TROPONIN-I was estimated by Troponin –I Rapid test kit commercially purchased from Reckon diagnostic Pvt Ltd[23].

IV. Statistical analysis

Statistical analysis was performed using one way ANOVA followed by Dunnett t test (GraphPad Instat, USA).

V. Histopathological study

At the end of study, the heart was isolated, washed with ice cold saline. The tissue was fixed in 10% buffered neutral formalin solution. After fixation tissues were embedded in paraffin-wax and five micrometer thick sections were cut and stained with hematoxylin and eosin. The slides were observed under light microscope and photomicrograph was taken.

3. Results

1. Gross examination of heart

The visually examination of the heart showing inflammation, redness, capillary dilatation, Scar formation, colour, was performed and grading of the heart has been done.

Grading of heart parameter

Grade 0 = No Lesion
Grade 1 = Inflammation, redness, capillary dilations.
Grade 2 = Edema, yellowish ventricle portion
Grade 3 = Atrium & ventricle turns yellow, scar formation
Grade 4 = Diffuse heart, absolute scar formation, increased necrosis portion. Figure 1 shows the gross examination of heart.

![Gross Examination of Heart](image)
Figure 1: Visual photographs of dissected rat heart: a- (NC) group showing no scar, oedema, capillary dilation; b- (ISO) group showing hypertrophy, oedema, and change in colour (circle and arrow); c- (STD) group showing with no oedema, capillary dilation but showing inflammation and redness (circle); d- (TG1) group showing redness, inflammation near ventricle (circle); e- (TG2) group showing no redness, but shows minor inflammation (arrow)

Table 1: Observation table for grading of heart

<table>
<thead>
<tr>
<th>Groups</th>
<th>Grading of cardiac damage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>Grade 0</td>
</tr>
<tr>
<td>Isoprenaline (ISO)</td>
<td>Grade 4</td>
</tr>
<tr>
<td>Standard (STD)</td>
<td>Grade 1</td>
</tr>
<tr>
<td>Test group 1 (TG 1) (50mg/kg)</td>
<td>Grade 3</td>
</tr>
<tr>
<td>Test group 2 (TG 2) (100mg/kg)</td>
<td>Grade 2</td>
</tr>
</tbody>
</table>

The isoprenaline (ISO) group showed marked inflammation, scar formation, diffused heart when compared with Normal control (NC) group. The standard (STD) group showed marked reduction in edema, capillary dilation, and scar formation, with little redness when compared with Isoprenaline (ISO) group. The test group 2 (TG2) (100mg/kg) showed remarkable decrease in inflammation, redness, capillary dilatation and scar formation as compared to test group 1 (TG1) (50mg/kg) when both of the extract have been compared with isoprenaline (ISO) group. (Table 1)

2 - Heart weight/ body weight ratio

Table 2: Body weight, heart weight, heart: body weight ratio

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>Heart/body weight ratio (x 10^3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control (NC)</td>
<td>208</td>
<td>0.81</td>
<td>3.8</td>
</tr>
<tr>
<td>Isoprenaline (ISO)</td>
<td>191</td>
<td>1.08</td>
<td>5.6</td>
</tr>
<tr>
<td>Standard (STD)</td>
<td>216</td>
<td>0.88</td>
<td>4.0</td>
</tr>
<tr>
<td>Test group 1 (TG 1) (50mg/kg)</td>
<td>220</td>
<td>1.01</td>
<td>4.5</td>
</tr>
<tr>
<td>Test group 2 (TG 2) (100mg/kg)</td>
<td>225</td>
<td>0.98</td>
<td>4.3</td>
</tr>
</tbody>
</table>

The heart weight and heart weight/body weight ratio was analysed in various treatment group. The isoprenaline (ISO) group showed marked increase in heart weight due to hypertrophy; when compared with Normal control (NC) group. The standard (STD) group showed reduction in heart weight and heart/body weight ratio when compared with Isoprenaline (ISO) group. Test group 2 (TG 2) (100 mg/kg) demonstrated significant decrease in heart weight and decrease in heart/body weight ratio when compared with Isoprenaline group. When both test groups (50mg/kg, 100mg/kg) was compared with Isoprenaline (ISO) group, the highest dose (TG2) (100mg/kg) showed remarkable reduction in heart weight and heart/body weight ratio as compared to lowest dose (TG1) (50mg/kg). (Table 2).
3 - Biochemical estimations

Table 3: Effect of ethanolic extract of Coleus forskohlii on CK–MB, LDH, AST and ALT levels in rat by Isoprenaline induced cardiac toxicity.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>AST (IU/L)</th>
<th>ALT (IU/L)</th>
<th>LDH (IU/L)</th>
<th>CK (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>155.137±24.5</td>
<td>103.630±2.41</td>
<td>107.780±2.11</td>
<td>1.034±0.020</td>
</tr>
<tr>
<td>ISO</td>
<td>285.911±24.9ª</td>
<td>255.144±16.9ª</td>
<td>307.743±22.44ª</td>
<td>1.260±0.044ª</td>
</tr>
<tr>
<td>STD</td>
<td>161.277±23.4ª</td>
<td>105.226±22.8ª</td>
<td>111.106±4.27ª</td>
<td>1.068±0.029ª</td>
</tr>
<tr>
<td>TG1</td>
<td>190.386±20.3ª</td>
<td>198.153±5.98ª</td>
<td>229.117±14.98ª</td>
<td>1.105±0.018ª</td>
</tr>
<tr>
<td>TG2</td>
<td>172.549±9.64ª</td>
<td>143.178±13.18ª</td>
<td>171.802±22.85ª</td>
<td>1.085±0.002ª</td>
</tr>
</tbody>
</table>

Values are mean ± SEM for five animals in each group
ªP < 0.001 When ISO group is compare with NC group; ³P < 0.001 When standard group is compare with ISO group.
ªP < 0.01 When experimental group is compare with ISO group.

The effects of ethanolic extract of Coleus forskohlii oral treatments on serum marker enzymes AST, ALT, LDH, and CPK for 30 days are outlined in Table 3. Rats treated with ISO showed a highly significant increase (p<0.001) in activities of serum marker enzymes compared with the normal rat (NC) group. Rats pretreated with metoprolol (STD group) when compared with Isoprenaline (ISO) group showed a highly significant (p<0.001) reduction in cardiac marker enzyme.

Pretreatment with Coleus forskohlii high dose (TG2) (100 mg/kg) to rats for 30 days, followed by ISO subcutaneous injection on the 29th and 30th days, elicited a highly significant (p<0.001) reduction in the ISO-induced increased activities of AST, ALT, LDH, and CPK. The low dose of coleus forskohlii (TG1) (50 mg/kg) when compared with Isoprenaline (ISO) group was significant (p<0.01) in lowering ISO elevated serum enzyme activities, (Table 3)

![Figure 2: Bar diagram showing effect of various treatment groups on release of cardiac enzyme marker AST, ALT, LDH, CK in Isoprenaline treated cardiac necrosis](image)

Table 4: Release of TROPONIN–I in various treatment groups

<table>
<thead>
<tr>
<th>Animal no.</th>
<th>Normal Control</th>
<th>Isoprenaline (85mg/kg)</th>
<th>Standard (10mg/kg)</th>
<th>Test extract 50mg/kg</th>
<th>Test extract 100mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>2</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>3</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
<tr>
<td>4</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
<td>-ve</td>
</tr>
<tr>
<td>5</td>
<td>-ve</td>
<td>+ve</td>
<td>+ve</td>
<td>-ve</td>
<td>+ve</td>
</tr>
</tbody>
</table>

+ve - Presence of troponin in serum; -ve - Absence of troponin in serum
The release of troponin-I was estimated by Rapid test kit after 4 hours of infarction. When Isoprenaline (ISO) group compared with normal control (NC) group, all animal of ISO group showed presence of troponin in serum. When Standard group was compared with Isoprenaline group (ISO) more than half animal showed absence of troponin in serum while in rest of them troponin was found to be present. When both test groups (TG1, TG2) (50mg/kg, 100mg/kg) was compared with (ISO) group, the highest dose (TG2) (100mg/kg) showed remarkable reduction in release of troponin as compared to lowest dose (TG1) (50mg/kg) (Table 4).

4. Histopathological examination

The myocardial tissue was immediately fixed in 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin and serial sections were cut and each section was stained with hematoxylin and eosin. The slides were examined under light microscope and microphotographs were taken.

![Photomicrograph of heart section](image-url)

**Figure 3:** Photomicrograph of heart section (10x, 10x10): a- Normal group (NC) showing normal cytoarchitecture; b- Isoprenaline (ISO) group shows shows focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium vacuolar changes and prominent edema along with chronic inflammatory cells are present; c- Standard (STD) group showing very lesser degree of myonecrosis, myophagocytosis and lymphocytic infiltration, edema and very little infiltration of inflammatory cells; d- Test group (TG 1) showing decreased degree of myonecrosis and lesser infiltration of inflammatory cells but myophagocytosis and subendocardium vacuolar changes are present; e- Rat heart Pretreated with Test group 2 (TG 2) (100 mg/kg) treated group showing little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagocytosis and subendocardium vacuolar changes are present.
Photomicrograph of rat heart of normal control group shows, the endocardium, myocardium, and epicardium as well as papillary muscles and vasculature were all normal and healthy in both the groups. There was no muscular hypertrophy or evidences of myositis (necrosis and/or round cell infiltrates), clearly visible in 10x (prominently) (Fig 1.a). Isoprenaline treated (ISO) group shows focal myonecrosis with myophagocytosis and lymphocytic infiltration. In subendocardium vacuolar changes and prominent oedema along with chronic inflammatory cells are clearly visible in 10x (prominently) (Fig 1.b). Rat heart pretreated with the Standard (STD) Metoprolol (10mg/kg) showing very lesser degree of myonecrosis, myophagocytosis and lymphocytic infiltration, oedema and very little infiltration of inflammatory cells are clearly visible in 10x (prominently) (Fig 1.c). Photomicrograph of rat heart pretreated with Coleus forskohlii (TG1) (50mg/kg) group showing decreased degree of myonecrosis and lesser infiltration of inflammatory cells but myophagocytosis and subendocardium vacuolar changes are present & clearly visible in 10x (prominently) (Fig 1.d). Pretreated rat heart with Coleus forskohlii (TG 2) (100 mg/kg) treated group showing little degree of myonecrosis and lesser infiltration of inflammatory cells as well as a decreased myophagocytosis and subendocardium vacuolar changes are present & clearly visible in 10x (prominently) (Fig 1.e).

4. Discussion

Cardiovascular disease is major global health problem reaching epidemic proportion in Indian subcontinent[25] and low and middle income countries accounting for 78% of all death[26]. Myocardial cell protection and prevention of cell ischemia or necrosis have been therapeutics targets for a long time. New therapies are needed to treat ischemia because current treatment has only a limited impact on survival and annual cost[27].

The present investigation is aimed to explore and evaluate the cardioprotective effect of Coleus forskohlii root on isoprenaline induced myocardial infarction in rats. Myocardium contains an abundant concentration of diagnostic marker enzyme of myocardial infarction viz., AST, ALT, LDH, CK-MB and TROPONIN once myocardium is damaged, releases of its content into the extra cellular fluid serves as the diagnostic enzyme marker of myocardial damage tissue[28].

In our study we observed significant increase in the level on marker enzyme (AST, ALT, LDH, and TROPONIN) in serum of isoprenaline treated rats. It is noticeable that isoprenaline induced rat showed increased level of AST, ALT, LDH and CK-MB when compared to control rats. This finding could be a consequence of a reduction in the number of viable myocytes due to enhanced cell death in heart as these animals showed the highest level of AST, ALT, LDH and CK-MB[29]. Among both dose of Coleus forskohlii, treatment with the highest dose (TG2) (100mg/kg) decreased the level of these marker enzyme quite significantly when compared to low dose (TG1) (50mg/kg) pointing clearly that Coleus forskohlii could be a cardioprotective against the MI.

The isoprenaline (ISO) group showed marked increase in heart weight due to hypertrophy, when compared with Normal control (NC) group. The standard (STD) group showed reduction in heart weight and heart/body weight ratio when compared with Isoprenaline (ISO) group showing significantly less hypertrophy. Test group (TG2) (100 mg/kg) demonstrated more significant decrease in heart weight and decrease in heart/body weight ratio than Test extract (TG1) (50mg/kg) when compared with isoprenaline group, showing marked reduction in hypertrophy, change in shape of heart.

Histopathological examination of myocardial tissue obtained from normal control (NC) group exhibited clear integrity of myocardial membrane. Normal control (NC) rats showed cardiac fibers without any infarction. The heart sections obtained from Isoprenaline (ISO) treated rats showed disruption of several subcellular elements including myonecrosis, myophagocytosis and lymphocytic infiltration, oedema, loss of myofibrils, swelling of mitochondria, vacuolization of the cytoplasm, formation of lysosomal bodies and dilation of the sarcotubule and dilation of the sarcotubular system[30]. Treatment with test extract 2 (TG2-100 mg/kg, restores the architecture of the heart near to normal as compared to test extract 1 (TG1- 50mg/kg) when both group are compared with the Isoprenaline (ISO) group. The lesions produced by ISO in rat heart are similar to those found in myofibrillar degeneration in human ischemic heart disease (IHD) [31]. Hence, the study of ISO-induced myocardial necrosis and its underlying mechanisms might provide better insight and new leads on the pathogenesis of IHD.

In conclusion the result of present study indicated that the prior administration of ethanolic extract of Coleus forskohlii attenuates isoprenaline induced MI. The cardioprotective activity of Coleus forskohlii is probably related to its ability to strengthen the myocardial membrane by its membrane stabilizing action.

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Conflict of interest
No conflict of interest among author.

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