A comparative evaluation of cardioprotective activity of two Makandi (*Coleus forskohlii* Willd.) formulations against isoproterenol induced myocardial infarction in hyperlipidaemic rats

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The present study was undertaken to evaluate cardioprotective activity of *Makandi (Coleus forskohlii* Willd.) *Churna* and *Ghanavati* on the basis of electrocardiographic, biochemical, cardiac output and histopathological parameters against isoproterenol (ISO) induced myocardial infarction in diet induced hyperlipidaemic rats. The drug treatment and hyperlipidaemic diets were administered for 20 consecutive days. Myocardial injury was induced by injecting ISO (85 mg/kg) to rats at an interval of 24 h for two days. Forty eight hours after the first dose of ISO injection, parameters like ECG, cardiac output, biochemical and histological observations of the heart tissues were performed. Hyperlipidaemic diet followed by ISO significantly increased heart weight, altered serum lipid profiles, SGPT and ALP activity, caused ST segment elevation, prolonged QT interval and QTc in ECG, increased blood pressure and decreased cardiac output. Histopathologically also, heart showed severe cytoarchitectural disturbances like myonecrosis, fatty changes and endocardial oedema. When administered orally, both the formulations of *Makandi* decreased atherogenic index, almost normalized ST segment elevation, QT interval prolongation and cardiac output. Histopathologically also remarkable protection was observed. Analysis of the results showed that *Makandi* in *Churna* form has both anti-atherogenic potential and cardioprotective activity, while *Ghanavati* has only weak cardioprotective activity.

**Keywords:** Cardioprotective, *Coleus forskohlii*, Hyperlipidaemia, Makandi, Makandi churna, Makandi ghanavati, Myocardial infarction.

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**Introduction**

*Coleus forskohlii* Willd. is popularly known as *Makandi* in Ayurveda belonging to the family Lamiaceae, has been used in traditional medicine since ancient times for treatment of heart diseases, abdominal colic and respiratory disorders¹,². The plant is a rich source of a diterpene, forskolin which is virtually responsible for all reported pharmacological activities. It is an activator of adenylate cyclase³,⁴ and also has positive ionotropic, anti-glaucoma, anti-inflammatory, anti-platelet aggregation, bronchospasmytic actions⁵ and reduce body weight by increasing lean body mass⁶. However, almost all activities have been carried out on active principle forskolin i.e., on the isolated fraction of the drug. Till date no work has been reported on conventional Ayurvedic preparations of this drug.

The majority of the studies show that hyperlipidaemia, independently from the development of coronary atherosclerosis, worsens the outcome of ischaemic injury⁷. These findings emphasize the necessity of lipid lowering therapy and promote the development of new cardioprotective drugs that are capable to reverse the increased susceptibility of hearts to ischaemic stress and to recapture cardiac stress adaptation in hyperlipidaemia. Thus, the present study was undertaken to evaluate comparative cardioprotective effect of two formulations prepared from *Makandi*, viz. *Makandi churna* (powder of roots) and *Makandi ghanavati* (concentrated form of aqueous extract from roots) against isoproterenol induced cardiac injury in hyperlipidaemic diet induced hyperlipidaemic rats.

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Materials and Methods

Test formulations

The fresh tuberous roots of Makandi (C. forskohlii, Plate 1) were procured from local market and authenticated by qualified pharmacognosist of our institute through macroscopical and microscopical characters. The tuberous roots were washed with water, cut into pieces and dried under shade. The Churna (MC) and Ghanavati of Makandi (MG) were prepared from dried roots as per classical procedure.\(^8,9\)

Preliminary phytochemical studies

Both Churna and Ghanavati of Makandi were subjected to preliminary phytochemical studies including quantitative phytochemical investigation through HPLC analysis for important chemical moiety as forskolin.\(^10\) The separation was carried out on a C-18 column (Inertil C18 250 mm × 4.6 mm i.d. with a 5 µm particle size) using Acetonitrile : Water (90:10 v/v) as mobile phase. The eluent was monitored using a UV-Visible detector at 210 nm. Standard Forskolin (87.00 %) was used as marker compound.

Animals

Wistar strain albino rats of either sex weighing 200 ± 30 g were selected from the animal house attached to the institute. They were housed at 25 ± 03°C with constant humidity of 50-70% on a 12 hour natural day and night cycles. They were fed with diet Amrut brand rat pellet food supplied by Pranav Agro Industries, Baroda and tap water was given ad libitum. Institutional Animal Ethics Committee had approved the experimental protocol (Approval number IAEC/09-10/05MD 04) and the care of animals was taken as per the CPCSEA guidelines.

Dose selection and schedule

The clinical dose of Makandi ghanavati and Makandi churna are 3 and 4.2 g, respectively.\(^10\) The dose for experimental animals was calculated by extrapolating the human dose to animals (270 mg rounded to 300 mg/kg and 378 mg rounded to 400 mg/kg, respectively) based on the body surface area ratio by referring to the standard table of Paget and Barnes (1969).\(^11\) The human doses of the formulations were decided on the basis of the clinical experiences, palatability, since the majority of clinical studies have used injected forskolin, it is unclear if oral ingestion of these formulations will provide similar benefits in the amounts recommended by various authors [About 80 g of dried course powder of Makandi gives 14 g of Ghana (aqueous extract)]. The drug solutions were made with distilled water and administered to animals (0.5 ml/100 g body weight) with the help of gastric catheter sleeved to syringe. The drugs were administered to over night fasted animals.

Statistical analysis

The results were presented as Mean ± SEM for six rats in each group. Statistical comparisons were performed by both unpaired student’s t test and one way ANOVA with Dunnets’ multiple t test as post-hoc test by using Sigma stat software (version 3.1) for all the treated groups with the level of significance set at \(P<0.05\).
Experimental protocol

The experimental rats were divided into six groups of 6 animals each and treated as follows:

- **Group I**: Normal control rats received distilled water (NC)
- **Group II**: Cholesterol control rats treated with hyperlipidaemic diet (CC)
- **Group III**: Isoproterenol control rats treated with Isoproterenol (ISO) (85 mg/kg body weight, s.c. in saline).
- **Group IV**: Cholesterol plus Isoproterenol (CCISO) control group
- **Group V**: *Makandi ghanavati* (MG) + cholesterol + Isoproterenol (MG + CCISO)
- **Group VI**: *Makandi churna* (MC) + cholesterol + Isoproterenol (MC + CCISO)

Test drugs and vehicles were administered to respective groups at morning hours and continued for 20 days. The hyperlipidaemic diet was administered to different groups to induce hyperlipidaemia based on previous studies. The hyperlipidaemic diet includes hydrogenated vegetable oil (Vanaspati Ghee-'Raag' brand, Batch No. BA 55, Adani Wilmar Ltd., Gujarat) and cholesterol extra pure powder (Batch No. 14022 Suvidhnath Laboratories, Baroda) made in to 20% suspension in coconut oil (Parachute coconut oil, Batch No. PSO73, Goa). The suspension was administered (0.5 ml/100 g) to the rats daily for 20 days (at evening hours) to all the groups except normal control (Group I) and isoproterenol control (Group II) group. On 20th day, 1 hour after the administration of test drug and vehicle, ISO was administered subcutaneously. Two doses of ISO (85 mg/kg) were administered at 24 hours interval.

Measurement of ECG

At the end of experimental period (after 24 h of second ISO injection) the rats were anaesthetized with ether and ECGs were recorded using a portable electrocardiogram machine (Cardiofax-Medicaid systems). ECG was recorded by using only the four standard (limb) leads attached to four extremities of the animals, the chest leads were not used. In all cases of myocardial infarction, Lead II show the clear, distinct individual waves than Lead I and III. Therefore, ECG was monitored on Lead II only.

Measurement of blood pressure and cardiac output

Systolic blood pressure, diastolic blood pressure, mean blood pressure, pulse rate, cardiac output (CO), left cardiac work (LCW), left ventricular ejection time (LVET), pre-ejection period (PEP) and stroke index (SI) were recorded using impedance cardiographic analysis (ICG) provided with BIOPAC student lab system using Sramek/VEPT method in anaesthetized rats.

Biochemical analysis

After recording the BP and cardiac output, blood was collected from retro-orbital plexus, serum was separated and used for estimation of different serum biochemical parameters. The procedure followed for this was a requisite quantity of serum fed to the auto analyzer (Fully automated Biochemical Random Access Analyzer, BS-200; Lilac medicare Pvt. Ltd., Mumbai) which was automatically drawn in to the instrument for estimating different biochemical parameters like serum total cholesterol (CHOD–PAP, end point), serum HDL cholesterol (Trinder reaction), serum triglyceride (GOP–PAP method, end point), serum alkaline phosphatase (IFCC Method, Kinetic method), SGOT (IFCC method without Pyridoxal Phosphate) and SGPT activity (IFCC method, kinetic without Pyridoxal Phosphate).

Histopathological studies

At the end of the study, all the rats were sacrificed by overdose of ether anaesthesia and the heart was dissected out, washed in ice cold saline. The weight of heart was noted and the tissues were 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 viewed under Trinocular Research Microscope (Germany) at various magnifications to note down the changes in the microscopic features of the tissues studied.

Results and Discussion

Effect on heart weight

The heart weight of rats treated with isoprotrenol significantly increased when compared with the normal control rats (Table 1). Nirmala and Puvanakrishnan (1996) have reported that this increase in heart weight might be due to increased oedema within intramuscular space, massive vascular hemorrhage and/or extensive necrosis of cardiac muscle fibres followed by the invasion of damaged tissues with inflammatory cells. These observations
are in line with the histopathological findings of our study. Both the test drugs did not attenuate the observed cardiac weight gain. This may be due to persistent oedematous condition in the heart in spite of drug treatment.

Effect on serum lipid profiles

High cholesterol diet is regarded as an important factor in the development of cardiac diseases since it leads to development of hyperlipidemia, atherosclerosis and ischemic heart disease\(^2\). In the present study feeding of hyperlipidemic diet not only elevated serum cholesterol level but also elevated BP. Injection of isoprenaline in hyperlipidemic diet given rats produced higher degree of cardiac injury. Though the injury profile was similar to isoprenaline treated but the severity of the injurious lesions were remarkably high (Plate 2d).

Administration of cholesterol and hyperlipidemic diet lead to significant elevation of serum total cholesterol, LDL cholesterol and atherogenic index. The increase in total cholesterol was maintained even after injection of the isoprenaline, in addition significant decrease in serum triglyceride, HDL-cholesterol and VLDL-cholesterol was observed. Both MG and MC failed to attenuate serum lipids elevations. However, CCISO induced atherogenic index which is the ratio of total cholesterol to HDL-cholesterol, was remarkably reversed by both MG and MC treatment (Table 2).

The serum triglyceride levels were found moderately lowered in CC and ISO given groups. When both of them were combined as in case of CCISO group, significant lowering of triglyceride level was observed. This lowering can be attributed to isoprenaline induced modulation of triglyceride metabolism possibly through increased utilization. Since similar decrease was observed in case of VLDL cholesterol also. It implies that metabolism of VLDL may also be modulated. The probable mechanisms of the observed triglyceride lowering can be due to increase in β-oxidative degradation of fatty acids, and lipoprotein lipase activity leading to increased metabolism of triglycerides in VLDL particles and chylomicrons and diversion of fatty acids to muscles for utilization in their activity along with reduction in

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**Table 1 — Effect of Makandi ghanavati and Makandi churna on heart weight**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>CC</th>
<th>ISO</th>
<th>CCISO</th>
<th>MG + CCISO</th>
<th>MC + CCISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart weight (g/100g)</td>
<td>0.325 ± 0.0169</td>
<td>0.312 ± 0.0098</td>
<td>0.453 ± 0.0144***</td>
<td>0.436 ± 0.0152***</td>
<td>0.423 ± 0.0114</td>
<td>0.454 ± 0.0267</td>
</tr>
</tbody>
</table>

The data were expressed as Mean ± SEM; significant differences in each group vs the control is ***\(P<0.001\). (\(^1\) ONE WAY ANOVA-F value 27.133; \(P<0.001\) DMTT- \(P<0.05\) for ISO and CCISO vs Control).

**Table 2 — Effect of Makandi ghanavati and Makandi churna on serum lipid profiles**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>CC</th>
<th>ISO</th>
<th>CCISO</th>
<th>MG + CCISO</th>
<th>MC + CCISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Cholesterol</td>
<td>64.00 ± 2.37</td>
<td>73.50 ± 3.09*</td>
<td>82.50 ± 8.61*</td>
<td>81.00 ± 4.76**</td>
<td>98.33 ± 7.81</td>
<td>86.50 ± 8.01</td>
</tr>
<tr>
<td>Serum HDL cholesterol</td>
<td>36.33 ± 3.04</td>
<td>30.00 ± 1.84</td>
<td>25.17 ± 2.01*</td>
<td>26.83 ± 1.70*</td>
<td>34.33 ± 2.20</td>
<td>36.50 ± 3.27**(\times)</td>
</tr>
<tr>
<td>Serum VLDL cholesterol</td>
<td>20.57 ± 2.75</td>
<td>17.30 ± 1.21</td>
<td>12.87 ± 3.02</td>
<td>11.60 ± 1.12*</td>
<td>10.00 ± 1.37</td>
<td>14.10 ± 2.76</td>
</tr>
<tr>
<td>Serum LDL cholesterol</td>
<td>48.23 ± 2.53</td>
<td>65.40 ± 6.26*</td>
<td>70.20 ± 7.60*</td>
<td>67.77 ± 5.06**</td>
<td>74.00 ± 7.99</td>
<td>64.10 ± 6.60</td>
</tr>
<tr>
<td>Atherogenic index</td>
<td>1.82 ± 0.15</td>
<td>2.68 ± 0.19** (\times)</td>
<td>3.43 ± 0.50** (\times)</td>
<td>3.42 ± 0.32*** (\times)</td>
<td>2.28 ± 0.29(^#) (\times)</td>
<td>2.42 ± 0.31(^#)</td>
</tr>
<tr>
<td>Serum triglyceride</td>
<td>102.83 ± 13.75</td>
<td>86.50 ± 6.07</td>
<td>64.33 ± 15.09</td>
<td>58.00 ± 5.59*</td>
<td>50.00 ± 6.85</td>
<td>70.50 ± 13.82</td>
</tr>
</tbody>
</table>

The data were expressed as Mean ± SEM and unit of each parameter is (mg/dL). Significant differences in each group vs. the normal control are *\(P<0.05\), **\(P<0.01\) and ***\(P<0.001\); vs CCISO are \(4P<0.05\) (\(^1\) ONE WAY ANOVA-F value 5.726; \(P=0.005\) DMTT- \(P<0.05\) for ISO and CCISO vs Control) (\(^2\) ONE WAY ANOVA-F value 4.982; \(P=0.025\) DMTT- \(P<0.05\) for MC + CCISO vs CCISO) (\(^3\) ONE WAY ANOVA-F value 4.240; \(P=0.038\) DMTT- \(P<0.05\) for MG + CCISO vs CCISO)
hepatic production of VLDL. CCISO induced lowering was not antagonized by the test preparations. Instead, further lowering was observed. The reason for this lowering of VLDL was not known.

**Effect on serum enzymes**

Highly significant elevation in both SGOT and SGPT activity was observed in CCISO treated rats (Table 3) in comparison to control group. The increase was 182% in case of SGPT and 138% in case of SGOT. In MG treated CCISO receiving group, slightly higher elevation of SGPT activity was observed where as in MC treated CCISO receiving group only 47% inhibition was observed. This indicates marked reversal of CCISO produced elevation. In case of SGOT activity, in MG treated CCISO receiving group, a moderate 86.75% increase was observed which was moderately less in comparison to CCISO control. In MC treated CCISO receiving group, no decrease was observed. The observed elevation correlates very well with the histopathological changes observed in heart of CCISO combination group indicating the observed elevation is due to drastic tissue damage caused to heart. Though perfect correlation was not obtained in drug treated group the observed decrease indicates that both of them have attenuating effect on CCISO induced tissue injury.

Significant elevation of serum ALP activity was observed in CC and CCISO control group. In MG treated group a further elevation was observed where as in MC treated group a moderate fall of 24% was observed in CCISO control group. This indicates that MC treated CCISO receiving group has moderate to good reversal effect on the CCISO induced toxic effect probably involving bone and cardiac tissue.

**Effect on ECG parameters**

Electrocardiograph abnormalities are the main criteria generally used for the definite diagnosis of myocardial infarction. ECG changes were derived from standard II-lead ECG and the information recorded are PR, QRS, QT and RR intervals, heart rate and QTc interval (Table 4, Fig. 1). The main changes observed were elevation of ST segment (Fig. 1d), prolongation of QT interval and QTc interval in CC and CCISO control group in comparison to normal control group. QT interval provides a measure of ventricular repolarization and is determined by the balance of the repolarizing inward sodium and calcium currents, and the outward potassium and chloride currents. The QT interval correlates with measurements of cardiac autonomic function, with cardiac vagal dysfunction resulting in prolongation of the QT interval. QT interval represents both the dispersion and the lengthening of the AP duration and correlates with the LV mass. The prolongation of this interval is considered as an important parameter to determine cardiac toxicity potential. In humans and large animals, QT interval varies strongly and inversely with heart rate. Consequently, in clinical practice and human pharmacologic studies it is typical to correct the measured QT interval for heart rate in order to obtain measures of QT interval which are heart rate independent (QTc). The prolongation of both these parameters in CC and CCISO control group indicates presence of cardiac injury and aberration in the ventricular repolarization. The fact that both the test formulations were effective in reversing this CCISO induced QT interval prolongation points towards the presence of significant cardioprotective activity in them.

**Effect on blood pressure**

Rats in CC group showed significantly elevated blood pressure in terms of systolic, diastolic and mean blood pressure in comparison to normal control rats (Table 5). Administration of ISO and CCISO leads to

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**Table 3 — Effect of Makandi ghanavati and Makandi churna on serum enzymes**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>NC</th>
<th>CC</th>
<th>ISO</th>
<th>CCISO</th>
<th>MG + CCISO</th>
<th>MC + CCISO</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGPT activity (IU)</td>
<td>49.17 ± 7.77</td>
<td>66.29 ± 10.20</td>
<td>94.60 ± 17.78*</td>
<td>136.20 ± 16.03***</td>
<td>122.17 ± 15.15**</td>
<td>080.50 ± 12.12*</td>
</tr>
<tr>
<td>SGOT activity (IU)</td>
<td>186.50 ± 13.53</td>
<td>190.57 ± 22.55</td>
<td>196.80 ± 33.96</td>
<td>297.17 ± 32.26**</td>
<td>277.33 ± 24.46</td>
<td>337.50 ± 68.02</td>
</tr>
<tr>
<td>Alkaline phosphatase activity (IU/L)</td>
<td>136.83 ± 39.68</td>
<td>301.67 ± 30.42**</td>
<td>223.67 ± 8.74*</td>
<td>276.17 ± 46.34*</td>
<td>323.83 ± 41.63**</td>
<td>208.50 ± 42.19</td>
</tr>
</tbody>
</table>

The data were expressed as Mean ± SEM. Significant differences in each group vs the normal control are *P < 0.05, **P < 0.01 and ***P < 0.001; vs CCISO is #P < 0.05.
moderate non-significant fall in BP in comparison to normal control. This shows that ISO instead of elevating BP has the tendency of lowering it in CC given groups. Both MG and MC failed to antagonize the hyperlipidaemic diet induced BP elevation.

**Effect on cardiac output parameters**

The main and significant change that was observed after feeding rats with cholesterol rich diet and CCISO combination was decrease in cardiac output (Table 6). This decrease was found completely reversed in MC treated group. MG did not reverse this decrease, however a slight further decrease was observed. The left cardiac work was also found to be decreased in CCISO group in comparison to normal control rats. This decrease was also reversed by MC while MG did not produce the reversal. Left ventricular ejection time was found to be moderately prolonged by CCISO combination this moderate prolongation was not influenced by either of the test drugs. Stroke index was found moderately decreased in CCISO group in comparison to normal control. The stroke index was found doubled in MC administered group in comparison to CCISO control whereas in MG treated group, a marginal decrease was observed. Thus, the overall profile indicates that administration of CCISO combination leads to significant reduction in the working capacity of the heart. This incapacitation was completely reversed by MC while MG was not effective.

**Histopathological findings**

No inflammatory cells infiltration was seen in the heart of normal control rats (Plate 2a & b). Isoprenaline injection to rats leads to significant myonecrosis, infiltration of inflammatory cells, fatty changes and endocardial oedema compared to normal control (Plate 2c). In CCISO control group these
Fig.1 — Photographs of ECG leads showing: a-normal ECG pattern of control (NC) group; b-slight elevation of ST segment in CC group; c-elevation of ST segment in ISO group; d-marked elevation of ST segment in CCISO group; e-slight elevation of ST segment in MG treated group; f-elevation of ST segment in MC treated group
pathological features were found further increased involving vast area of the myocardium (Plate 2d). This shows injection of ISO to hyperlipidemic diet given rats further enhances myocardial injury. Administration of MG moderately stemmed these CCISO combination induced myocardioathy (Plate 2e). MC provided marked protection against CCISO induced myocardioathy (Plate 2f). Thus,
Makandi in the form of Churna may be of therapeutic and prophylactic value in the treatment of myocardial infarction. Myocardial infarction is the rapid development of myocardial necrosis caused by critical imbalance between the oxygen supply and the demand of the myocardium. A better understanding of the processes involved in myocardial infarction has stimulated the search for new drugs which could limit the myocardial injury. The major abnormalities noticed in myocardial infarction are lipidaemia, peroxidation and loss of plasma membrane integrity. Further, studies indicate that South Asians have elevated levels of LDL cholesterol and triglycerides, while also suffering from a deficiency in HDL cholesterol. The mechanism by which hyperlipidaemia may influence the severity of myocardial ischaemia is not exactly known, however, accumulation and redistribution of tissue/membrane cholesterol and the resulting changes in sarcolemmal and mitochondrial membrane micro-viscosity rather than the direct effect of high serum lipoprotein levels and coronary atherosclerosis may account for this. A decrease in cardiac NO bioavailability and ecto-5'-nucleotidase activity, an inhibition of the mevalonate pathway, as well as enhanced apoptotic cell death have been also shown to contribute to increased ischaemia/reperfusion injury and loss of preconditioning in hyperlipidaemic animal models.

**Conclusion**

Based on activity profile obtained, it can be concluded that both the formulations of Makandi have good anti-atherogenic potential and the Churna formulation has remarkable cardioprotective activity, while Ghanavati has weak cardioprotective activity. It is more interesting to note the observed differences in the activity profiles of the formulations, despite the fact that both possess the same active ingredient. The HPLC analysis of these formulations showed 4.812 mg of forskolin in 1 g of Makandi ghanavati and 3.51 mg of forskolin in 1 g of Makandi churna. As already stated forskolin is responsible for many pharmacological actions related to cardiovascular system. It was hypothesized that the forskolin present in these two formulations may be responsible for the anti-atherogenic and cardioprotective effects. However, the results obtained did not correlate with the forskolin content. Makandi churna with lower forskolin content showed better cardioprotective activity in comparison to Makandi ghanavati which had higher forskolin content. This indicates that other constituents may also play a major role. Elucidation of the full chemical profile may throw light on the nature of other constituents.

**Acknowledgement**

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**References**

4. Seamon KB and Daly JW, Activation of adenylate cyclase by the diterpene Forskolin does not require the guanine nucleotide regulatory protein, J Biol Chem, 1981, 256, 9799-9801.