Amelioration of inflammation by phenolic rich methanolic extract of
Ocimum sanctum Linn. leaves in isoproterenol induced myocardial infarction

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Myocardial infarction (MI) is one of the leading causes of death worldwide. Oxidative stress and inflammation play vital role in the development of MI. The Indian basil or Tulsi (Ocimum sanctum Linn.), owing to its antioxidant potential, is used in the traditional system of Indian medicine to treat various disorders. We evaluated methanolic extract of O. sanctum (Tulsi) leaves on inflammation in isoproterenol (ISP) induced MI in rats. ISP-induced MI increased the levels of cardiac markers, phospholipases and phospholipid content. However, the same were reduced on pre-treatment with methanolic extract of O. sanctum leaves. The activities of 5-lipoxygenase and cycloxygenase-2 and levels of leukotriene B4 and thromboxane B2 were also elevated in ISP-treated rats, which were significantly decreased (P <0.001) in extract pre-treated rats. The enhanced mRNA expressions of nuclear factor kappa-B, 5-lipoxygenase activating protein and receptor for leukotriene B4 on MI induction, were considerably reduced (P <0.001) on extract pre-treatment. Histopathological analysis also confirmed the findings. The results also revealed the high phenolic content of methanolic extract of O. sanctum leaves. The study demonstrated that methanolic extract of Tulsi leaves can decrease inflammation in the cardiac tissue of ISP-induced MI in rats and its effect may be through downregulation of oxidative stress and arachidonic acid pathway. This cardioprotective effect may be due to the high phenolic content of methanolic extract of O. sanctum leaves.

Keywords: Arachidonic acid, Herbal, Holy basil, Oxidative stress, Tulsi.
been proved as good antioxidant compounds. The antioxidant activity of phenolic compounds is mainly attributable to their redox properties, which exerts their effects by absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides.

The synthetic catecholamine isoproterenol (ISP) is a β-adrenergic agonist which produces severe oxidative stress in the myocardium, causing infarct-like necrosis of the heart muscle. Repeated subcutaneous administration of ISP produces myocardial necrosis in rats because of its iatrogenic effect and hence serves as a good model for acute MI. A number of studies have been conducted using this animal model to study the cardioprotective effects. Further, it has also been reported that leaves are non-toxic and safe for human use. In the present study, we evaluated the impact of 50% methanolic extract of O. sanctum leaves on inflammation in ISP-induced MI in rats.

Materials and Methods

Preparation of leaf extract—The leaves of Ocimum sanctum were collected from Trivandrum, India and were authenticated by Dr Valsaladevi, Curator, Department of Botany, Kerala University. The identified and authenticated specimen was deposited in the herbarium of the Department of Botany, University of Kerala (Plant No: KUBH5840). Fresh leaves were collected, washed and dried in shade. 10 g of dried powdered leaves were mixed with 100 ml of 50% methanol. This was refluxed in a water bath for 1½ h at 60-65°C. The whole extract was defatted using petroleum ether and it was concentrated using a rotary flash evaporator. The yield of methanolic extract of O. sanctum leaves was 15.16%. The total phenolic content of the yield was 160 mg/g of the extract.

Experiment I (Dose response study)—Male albino rats (Sprague Dawley strain) weighing between 200-250 g, bred and reared in our animal house were used for the experiment. The rats were divided into 12 groups of 6 rats each. The groups were as follows: Group I, Control; Group II, ISP control (100 mg/kg body wt.); Groups III, V, VII, IX & XI received methanolic extract of O. sanctum leaves @ 50, 100, 150, 200 and 250 mg/kg body wt., respectively; and Groups IV, VI, VIII, X & XII received extract same as Groups III, V, VII, IX & XI, respectively + ISP @ 100 mg/kg body wt. each group.

Experiment II (Impact of extract on Arachidonic acid pathway)—A total of 24 male albino rats (Sprague Dawley strain, weighing between 200-250 g) were divided into 4 groups of 6 rats each. The groups were as follows: Group I, Control; Group II, methanolic extract of O. sanctum leaves @150 mg/kg body wt.; Group III, ISP control (100 mg/kg body wt.); and Group IV received extract @150 mg/kg body wt. + ISP (100 mg/kg body wt.).

Animals were housed in polypropylene cages kept in a room maintained at 28-32°C and 12 h light:dark cycle. The animals were handled using laboratory animal welfare guidelines. Rats were fed with standard laboratory diet supplied by Ashirwad Pvt Ltd., India and water was given ad libitum. The methanolic extract of O. sanctum leaves suspended in distilled water was given by gastric intubation for 30 days. ISP was given subcutaneously on the 29th and 30th day of the experiment at an interval of 24 h to induce MI. The dose of ISP was taken from previous studies. Animal experiments were approved by the Institutional Animal Ethics Committee [IAEC No. KU-13-2011-BC-MI (30)]. On the 31st day, the animals were sacrificed. The heart was dissected out and blood collected in ice cold containers for analysis of various biochemical parameters.

Biochemical analysis—The activities of cardiac markers, creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) were assayed using kits from Reckon diagnostics Pvt Ltd., India. Superoxide dismutase (SOD) was assayed by the method of Kakkar et al. Thiobarbituric acid reactive substances (TBARS) were estimated by the method of Ohkawa et al. Phospholipase A (PLA) activity by Rimon & Shapiro; phospholipase C (PLC) by Kleiman & Lands; phospholipase D (PLD) by Bergmayer et al.; phospholipids by Zilversmith & Davis; monocytes isolation as described by Huch et al.; COX-2 activity by TBA method of Shimizu et al.; and 5-LOX by Axelrod et al. Level of high sensitive C-reactive protein (hsCRP) was estimated by kit from Spinreact, Spain; leukotriene B₄ (LTB₄) by ELISA kit from R & D systems (Cat No.KGE006B), USA; and thromboxane B₂ (TXB₂) by ELISA kit from Cayman chemicals (Cat No. 519031), USA. The total phenolic content of methanolic extract of O. sanctum leaves was estimated by the method of Singleton & Rossi. Protein estimation was done by the method of Lowry et al. Total RNA isolation—Total RNA was isolated from the heart using TRI Reagent (Sigma Aldrich) as described by Chomczynski & Sacchi. Reverse Transcription PCR—The isolated RNA was used for reverse transcriptase-polymerase chain reaction.
reaction (RT-PCR) to quantify the expression. Total RNA was reverse transcribed and PCR was performed using Eppendorf RT-PCR kit with gene-specific primers. Primer sequences for glyceraldehyde-3-phosphate dehydrogenase (GAPDH), NFκB, 5-lipoxygenase activating protein (FLAP) and receptor for Leukotriene B₄ (BLT₁) are given in Table 1. The PCR mixture contained 10 mM Tris (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, dNTP at 20 mM each, gene-specific primers at 0.5 mM each and 0.025 units/µl Taq polymerase. After an initial denaturation step at 94°C, 35 amplification cycles were performed. A final extension step of 5 min at 72°C was performed in order to complete the PCR reaction.

PCR mixture was resolved on 2% agarose gel containing ethidium bromide, subjected to densitometric scanning (Bio-Rad Gel Doc, California, USA) to determine the OD of each, and normalized against GAPDH (internal control) using quantity one imaging software.

Histopathological analysis—Heart tissue was dissected out and immediately fixed in buffered neutral formalin solution (40%). The fixed tissues were embedded in paraffin and serial sections were cut. Each section was stained with haematoxylin and eosin. The sections were examined for pathological changes under the light microscope and photomicrographs were taken.

Statistical analysis—The results were analysed using a statistical programme SPSS/PC+, Version 17 (SPAA Inc., Chicago, IL, USA). One way ANOVA was employed for comparison among the groups. Duncan’s post-hoc multiple comparison tests of significant differences among groups were determined, P < 0.05 was considered to be significant.

Results

Experiment I (Dose response study)—The activities of CK-MB and LDH (Table 2) in the serum were increased significantly in MI-induced group and were decreased in all MI-induced rats pre-treated with methanolic extract of *O. sanctum* leaves. Maximum effect was observed in group VII (@ 150 mg/kg body wt.). The concentration of TBARS in the heart tissue increased significantly in MI-induced rats, whereas the activity of antioxidant enzyme SOD was significantly reduced (Table 3). All rats pre-treated with the extract showed reduced level of TBARS and increased SOD activity. Maximum effect was shown by rats treated with the extract @ 150 mg/kg body wt.

<table>
<thead>
<tr>
<th>Groups</th>
<th>LDH (IU/L)</th>
<th>CK-MB (IU/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>156.26±5.84⁴</td>
<td>175.10±6.52⁴</td>
</tr>
<tr>
<td>II</td>
<td>230.06±8.57⁵</td>
<td>351.40±13.09⁶</td>
</tr>
<tr>
<td>III</td>
<td>155.38±5.79⁴</td>
<td>174.82±6.51⁴</td>
</tr>
<tr>
<td>IV</td>
<td>223.47±8.33⁵</td>
<td>307.24±11.44⁵</td>
</tr>
<tr>
<td>V</td>
<td>153.19±5.71⁴</td>
<td>174.17±6.48⁴</td>
</tr>
<tr>
<td>VI</td>
<td>215.12±8.01⁴</td>
<td>273.69±10.20⁴</td>
</tr>
<tr>
<td>VII</td>
<td>151.75±5.65⁴</td>
<td>172.61±6.43⁴</td>
</tr>
<tr>
<td>VIII</td>
<td>194.40±7.24⁵</td>
<td>254.76±9.49⁵</td>
</tr>
<tr>
<td>IX</td>
<td>151.96±5.66⁴</td>
<td>172.87±6.44⁴</td>
</tr>
<tr>
<td>X</td>
<td>202.92±7.55⁵</td>
<td>266.22±9.91⁵</td>
</tr>
<tr>
<td>XI</td>
<td>152.42±5.68⁴</td>
<td>173.64±6.47⁴</td>
</tr>
<tr>
<td>XII</td>
<td>210.70±7.85⁵</td>
<td>270.97±10.09⁵</td>
</tr>
</tbody>
</table>

Mean values with same superscript do not differ significantly, P < 0.05

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (units/mg protein)</th>
<th>TBARS (mM/100g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>9.67±0.36⁴</td>
<td>0.450±0.018²</td>
</tr>
<tr>
<td>II</td>
<td>4.76±0.18⁴</td>
<td>0.599±0.024²</td>
</tr>
<tr>
<td>III</td>
<td>9.73±0.36⁴</td>
<td>0.446±0.018³</td>
</tr>
<tr>
<td>IV</td>
<td>5.20±0.19⁴</td>
<td>0.580±0.022²</td>
</tr>
<tr>
<td>V</td>
<td>9.77±0.37⁴</td>
<td>0.442±0.018³</td>
</tr>
<tr>
<td>VI</td>
<td>6.31±0.24⁴</td>
<td>0.554±0.021⁴</td>
</tr>
<tr>
<td>VII</td>
<td>9.98±0.37⁴</td>
<td>0.422±0.016⁴</td>
</tr>
<tr>
<td>VIII</td>
<td>7.46±0.27⁴</td>
<td>0.464±0.018⁴</td>
</tr>
<tr>
<td>IX</td>
<td>9.89±0.37⁴</td>
<td>0.428±0.017⁴</td>
</tr>
<tr>
<td>X</td>
<td>7.16±0.27⁴</td>
<td>0.475±0.018⁴</td>
</tr>
<tr>
<td>XI</td>
<td>9.73±0.38⁴</td>
<td>0.435±0.017⁴</td>
</tr>
<tr>
<td>XII</td>
<td>6.48±0.24⁴</td>
<td>0.484±0.019⁴</td>
</tr>
</tbody>
</table>

Mean values with same superscript do not differ significantly, P < 0.05

*Enzyme concentration required to inhibit the chromogen production by 50% in one min.*
Experiment II—The mRNA expression of NFκB (Fig. 1A) evaluated by RT-PCR revealed significant increase in the expression in the heart of MI-induced rats compared to the control, whereas it was significantly decreased in the extract pre-treated rats. The level of hsCRP (Table 4) in the serum was elevated significantly in MI-induced rats. However, it was decreased \( (P < 0.001) \) to near normal levels in rats pre-treated with leaf extract.

The activities of phospholipases A, C and D in the heart tissue also showed significant increase in the MI-induced group compared to the control. However, there was a significant reduction in the activities of these enzymes in MI-induced rats pre-treated with the leaf extract of *O. sanctum*. No significant differences in the activity of these three enzymes were seen in the group treated with extract alone compared to the control. The level of phospholipids in the heart tissue of MI-induced rats showed significant decrease compared to the control. However, there was a significant increase in the phospholipid content in rats pre-treated with the leaf extract compared to the MI-induced rats (Table 4).

The activities of COX-2 and 5-LOX showed significant increase in the monocytes of MI-induced group compared to the control. There was a significant reduction \( (P < 0.001) \) in the activities of these enzymes in the extract pre-treated group (Table 5).

Similarly, concentration of LTB\(_4\) and TXB\(_2\) also increased in the serum of MI-induced rats compared to the control rats. However, it was decreased

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**Fig. 1**—mRNA expression of (A) NFκB; (B) FLAP; and (C) BLT\(_1\) in heart tissue. [(C, Gr.I; O, Gr.II; I, Gr.III; & O+I, Gr.IV).](1) The relative amount of NFκB, FLAP and BLT\(_1\) mRNA were estimated by semi-quantitative RT-PCR. The PCR products were quantified by densitometry and standardised to their respective GAPDH controls. Results are expressed as average of quadruplicate experiments ± SEM. Different letter indicates values statistically significant at \( P < 0.05 \).]

<table>
<thead>
<tr>
<th>Groups</th>
<th>hsCRP (mg/dl)</th>
<th>PLA*</th>
<th>PLC*</th>
<th>PLD*</th>
<th>Phospholipids (mg/100g wet tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.237±0.009(^a)</td>
<td>0.919±0.036(^a)</td>
<td>0.307±0.012(^a)</td>
<td>0.49±0.019(^a)</td>
<td>2660.42±93.49(^a)</td>
</tr>
<tr>
<td>II</td>
<td>0.240±0.009(^a)</td>
<td>0.901±0.035(^a)</td>
<td>0.301±0.012(^a)</td>
<td>0.48±0.019(^a)</td>
<td>2671.14±104.6(^a)</td>
</tr>
<tr>
<td>III</td>
<td>0.904±0.02(^b)</td>
<td>2.24±0.088(^b)</td>
<td>0.692±0.027(^b)</td>
<td>1.46±0.057(^b)</td>
<td>2034.18±79.68(^b)</td>
</tr>
<tr>
<td>IV</td>
<td>0.450±0.014(^c)</td>
<td>1.56±0.061(^c)</td>
<td>0.459±0.018(^c)</td>
<td>0.82±0.032(^c)</td>
<td>2376.57±93.04(^c)</td>
</tr>
</tbody>
</table>

Mean values with same superscript do not differ significantly, \( P < 0.05 \). *milliequivalents of ester hydrolysed/min/mg protein. \(^a\)millimoles of phospholipid formed/min/mg protein. \(^b\)millimoles of choline formed/min/mg protein.
significantly ($P < 0.001$) in MI-induced group pre-treated with the *O. sanctum* leaf extract. No significant changes were observed in the level of these metabolites in the extract alone treated group compared to the control (Table 6).

The mRNA expressions of FLAP (Fig. 1B) and BLT$_1$ (Fig. 1C) in the heart were evaluated by RT-PCR. In MI-induced group, the levels of these expressions showed increase when compared to the control. The treatment with the leaf extract reduced the level of expression of these genes ($P < 0.001$). There was no significant change in the expression of these two genes in the extract alone treated group compared to the control.

The histopathological analysis (Table 7) of heart tissues of control group (Fig. 2A) showed normal myocytes with vesicular nuclei and well preserved muscle architecture. The rats treated with leaf extract alone also showed normal muscle architecture similar to control group (Fig. 2B). In MI-induced group, the myocytes were hyalinized and in some areas of interstitium there were moderate (30-60%) inflammation and haemorrhage (Fig. 2C). Swollen and necrotic myocytes were also seen. However, the MI-induced group pre-treated with the leaf extract showed only mild change in the muscle architecture when compared to ISP alone treated group (Fig. 2D). There was mild (<30%) level of inflammation found and oedema in that group.

**Discussion**

The dose response study using various doses of methanolic extract of *O. sanctum* leaves showed a marked increase in the activities of LDH and CK-MB.

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**Table 5—Activities of COX-2 and 5-LOX in monocytes**

<table>
<thead>
<tr>
<th>Groups</th>
<th>COX-2 ($\mu$mol of MDA liberated/mg protein)</th>
<th>5-LOX (OD shift/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.35±0.01$^a$</td>
<td>2.87±0.11$^a$</td>
</tr>
<tr>
<td>II</td>
<td>0.33±0.01$^a$</td>
<td>2.82±0.11$^a$</td>
</tr>
<tr>
<td>III</td>
<td>0.73±0.06$^b$</td>
<td>6.48±0.25$^b$</td>
</tr>
<tr>
<td>IV</td>
<td>0.45±0.02$^c$</td>
<td>3.22±0.13$^c$</td>
</tr>
</tbody>
</table>

Mean values with same superscript do not differ significantly, $P < 0.05$.

*Values are expressed as mean ± SEM of 6 animals in each group.

**Table 6—Levels of LTB4 and TXB2 in serum**

<table>
<thead>
<tr>
<th>Groups</th>
<th>LTB4 (pg/ml)</th>
<th>TXB2 (ng/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>2.64±0.104$^a$</td>
<td>23.4±0.92$^a$</td>
</tr>
<tr>
<td>II</td>
<td>2.61±0.102$^a$</td>
<td>23.1±0.89$^a$</td>
</tr>
<tr>
<td>III</td>
<td>8.95±3.52$^b$</td>
<td>67.28±2.63$^b$</td>
</tr>
<tr>
<td>IV</td>
<td>4.91±0.190$^c$</td>
<td>41.92±1.64$^c$</td>
</tr>
</tbody>
</table>

Mean values with same superscript do not differ significantly, $P < 0.05$.

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**Table 7—Histopathology of heart**

<table>
<thead>
<tr>
<th>Features</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Architecture of muscle fibres</td>
<td>Well preserved</td>
<td>Well preserved</td>
<td>Altered</td>
<td>Well preserved</td>
</tr>
<tr>
<td>Nuclear details of myocytes</td>
<td>Vesicular</td>
<td>Vesicular</td>
<td>Hyperplastic</td>
<td>Vescular</td>
</tr>
<tr>
<td>Cytoplasmic Staining reaction</td>
<td>Normal (pink )</td>
<td>Normal (pink )</td>
<td>Eosinophilic (red)</td>
<td>Eosinophilic (red)</td>
</tr>
<tr>
<td>Degree of inflammation</td>
<td>Nil</td>
<td>Nil</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>Intersitial Oedema</td>
<td>Nil</td>
<td>Nil</td>
<td>Moderate</td>
<td>Mild</td>
</tr>
<tr>
<td>Tissue Necrosis</td>
<td>Nil</td>
<td>Nil</td>
<td>Mild</td>
<td>Nil</td>
</tr>
<tr>
<td>Intersitial haemorrhage</td>
<td>Nil</td>
<td>Nil</td>
<td>Mild</td>
<td>Nil</td>
</tr>
</tbody>
</table>

Grading scheme: mild-<30%, moderate-30-60%, severe->60%.

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Fig. 2—Histopathology of heart. Hematoxylin and Eosin stained sections of rat myocardium with original magnification (40X). (A) Control; (B) *O. sanctum* extract alone treated; (C) ISP treated; (D) *O. sanctum* extract + ISP treated. [Nu-nucleus, MC-normal myocyte, N-necrosis, OE-Oedema, HMC- hyalinised myocytes, I-inflammation]
in ISP-treated rats. Due to myofibril degeneration and myocyte necrosis these cardiac biomarkers are released from heart into the blood during myocardial damage\textsuperscript{40}. The MI-induced rats pre-treated with the extract showed a decline in the activities of these markers, with maximum decrease in the group treated with the extract @ 150 mg/kg body wt. This is in agreement with the report by Suanarunsawat \textit{et al.}\textsuperscript{24} in which \textit{O. sanctum} fixed oil normalized the high serum levels of LDH and CK-MB in rats fed with high fat diet. This may be due to the effect of the herb in reducing myocyte damage by necrosis during MI. The anti-apoptotic activity of \textit{O. sanctum} was previously demonstrated by Mohanty \textit{et al.}\textsuperscript{42} in experimentally induced myocardial ischemic-reperfusion injury in rats.

Increased production of ROS associated with oxidative stress in pathophysiological conditions may play an important role in heart failure\textsuperscript{43}. ROS can react with unsaturated lipids thereby initiating a chain of self-perpetuating lipid peroxidation reactions in the membranes\textsuperscript{44}. The antioxidant capacity gets exhausted by the action of ROS and the ensuing lipid peroxidation\textsuperscript{45}. A significant increase in the concentration of TBARS, which are products of lipid peroxidation and significant decrease in the activity of antioxidant enzyme SOD was observed in the MI-induced rats. But these parameters were brought to near normal levels in all \textit{O. sanctum} extract pre-treated rats with more effect in rats those received a dose of 150 mg/kg body wt. This is in agreement with Panda \textit{et al.}\textsuperscript{23} in which the hydroalcoholic extract of \textit{O. sanctum} leaves normalized the levels of SOD and lipid peroxidation in MI-induced rats.

The protective effect of the methanolic extract of \textit{O. sanctum} leaves was proportional with the dose strength. However, higher doses \textit{i.e.}, 200 and 250 mg/kg body wt. showed less increase in the activity of SOD and less reduction in the CK-MB and LDH activities, and TBARS levels as compared to the other doses. This may be due to the pro-oxidant activity of the leaf extract at higher doses. Hence, the leaf extract @150 mg/kg body wt. was selected as the optimal dose for further investigation.

The transcription factor NFkB is activated by ROS and this enhanced activity can be modulated by antioxidants\textsuperscript{46}. In our study also the mRNA expression of NFkB in the heart of MI-induced rats was increased significantly compared to the control. This may be due to the oxidative stress developed in the heart as a consequence of MI. The leaf extract pre-treatment significantly reduced the expression of this transcription factor. Lo \textit{et al.}\textsuperscript{47} reported that \textit{O. sanctum} contains ursolic acid and carnosol components which downregulate NFkB.

Elevation in markers of inflammation particularly CRP are associated with increased susceptibility of future cardiovascular events in healthy subjects, in patients with stable or unstable coronary artery disease and AMI\textsuperscript{48,49}. hs-CRP, the inflammatory marker, is considered to be better than CRP in predicting the development of heart failure in AMI patients\textsuperscript{49}. In our study also, the serum level of hsCRP is increased significantly in ISP-treated rats. But rats pre-treated with \textit{O. sanctum} leaf extract showed significant reduction in hsCRP level, indicating reduction in inflammation.

Arachidonic acid (AA), a polyunsaturated fatty acid found in the phospholipids of cell membranes, on oxygenation gets transformed into multiple products mediating inflammatory reactions\textsuperscript{50}. The liberation of AA from membrane phospholipids by phospholipase \textit{A} \textit{2} (PLA\textsubscript{2}) is the key reaction in AA cascade\textsuperscript{51}. There are evidences that PLC and PLD also play critical role in the inflammatory responses\textsuperscript{52,53}. In this study, the activities of PLA, PLC and PLD were increased significantly in MI-induced rats which were reverted to near normal levels in leaf extract pre-treated rats. The increased activities of phospholipases might have caused the liberation of AA.

The observed decrease in the phospholipid content in the heart tissue seen in MI-induced rats confirms the hydrolytic cleavage of phospholipids. Ischemic injury-related alterations in lipid composition of myocardial tissue have been reported to occur because of destruction of the myocardial membrane lipid bilayer\textsuperscript{24}. The phospholipid content increase in the extract pre-treated rats observed here indicates the maintenance of membrane integrity by methanolic extract of \textit{O. sanctum} leaves.

COX-2 and 5-LOX are the critical enzymes of AA metabolism which catalyze AA to PGS, TXs and LKBs, major lipid mediators involved in various inflammatory disease\textsuperscript{54}. Activities of these two enzymes were significantly increased in the monocytes of MI-induced rats. However, the tulsi leaf extract reduced the activities of these enzymes. The anti-inflammatory activity of fatty acids of \textit{O. sanctum} fixed oil was previously demonstrated by Singh & Majumdar\textsuperscript{55}. 


The sequential actions of 5-LOX, FLAP, and leukotriene A4 hydrolase produce LTB$_4$, a potent lipid mediator of inflammation\textsuperscript{36}. TXB$_2$ is the stable metabolite of TXA$_3$, a product of COX-2 pathway\textsuperscript{57}. Serum levels of these two AA metabolites were found to be increased significantly in MI-induced rats. However, this was decreased significantly in rats pre-treated with the tulsi leaf extract, which may be due to the decreased activity of COX-2 and 5-LOX.

FLAP is necessary for the activation of 5-LOX and therefore for the production of leukotrienes\textsuperscript{58}. In our study, the ISP induced MI increased the mRNA expression of FLAP. Helgadottir \textit{et al.}\textsuperscript{59} reported that the gene encoding FLAP is associated with twice the risk of myocardial infarction. Biomarkers associated with increased risk of MI were found to be suppressed significantly by inhibitor of FLAP in a dose-dependent manner\textsuperscript{60}. A decreased mRNA level of FLAP was observed in the extract pre-treated rats. It indicates that tulsi leaf extract can downregulate the production of leukotrienes.

BLT$_1$ is a G-protein coupled receptor to which LTB$_4$ binds with high affinity and mediates its potent biological actions\textsuperscript{61}. Early protection against development of atherosclerotic lesions in mice was evidenced by deletion of BLT$_1$ gene\textsuperscript{62}. In the present study also the mRNA expression of BLT$_1$ was increased significantly in MI-induced inflammation in the myocardium. Pre-treatment of rats with tulsi leaf extract reduced the expression of BLT$_1$, which might have downregulated the biological actions of LTB$_4$.

The histopathological analysis revealed that on MI-induction with ISP, the myocytes become hyaline and in some areas of interstitium showed focal inflammation and haemorrhage. Myocytes became swollen and its nucleus showed changes of degeneration. However, the tulsi leaf extract pre-treatment protected the myocardium from severe damage and maintained the architecture of the cells. The reduced inflammation observed in the illustration (Fig. 2D) confirmed our biochemical results.

The estimation of total phenolic content in extract revealed 16% phenolic compounds. Various phenolic antioxidants have been reported to scavenge radicals, and hence are viewed as promising therapeutic drugs for free radical pathologies\textsuperscript{63}. Datta \textit{et al.}\textsuperscript{64} demonstrated the role of polyphenols from \textit{Ipomoea aquatica} against pesticide toxicity\textsuperscript{64}. Nandave \textit{et al.}\textsuperscript{65} attribute the cardioprotective effect of picrorhiza root extract to its antioxidant, anti-peroxidative and myocardial preservative properties\textsuperscript{65}. Here, in \textit{O. sanctum}, the cardioprotective effects of the leaf extract could be due to its high content of phenolics.

In conclusion, the methanolic extract of \textit{O. sanctum} leaves possess cardioprotective effects. The mechanism of cardioprotection may be by reduction of oxidative stress, and thereby causing reduction in NFkB expression and catabolism of AA, with subsequent reduction in inflammation. The observed cardioprotective effect may be due to the presence of high content of phenolics in the leaf extract.

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Conflict of interest

The authors declare that there is no conflict of interest.

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