ARTICLE

LIGHT AND ELECTRON MICROSCOPIC INVESTIGATION OF SEMELIL (ANGIPARS™) ON THERAPEUTIC PROCESS AFTER CHRONIC MYOCARDIAL INFARCTION IN RABBIT

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ABSTRACT

One of the main therapeutic intentions of modern cardiology is to contrive strategies aimed at decreasing myocardial necrosis and improving cardiac healing following myocardial infarction (MI). This investigation was contrived to study the protective effects of Semelil (ANGIPARS™), a native herbal medicine, on MI in the rabbit model. In this experimental study, Twenty New Zealand white rabbits were utilized. Rabbits were allocated to equal groups: control plus vehicle; sham plus vehicle; ischemia plus vehicle; Seme 10 mg/kg, respectively. MI was created by the complete closure of Left Anterior Descending Coronary Artery (LADC). The animals were treated with Semelil 10 mg/kg daily for 14 days. Electrophysiological, Biochemical, histological and ultrastructural studies were used for detecting protective effects of Semelil. Based on our data, Semelil ameliorated the electrocardiogram (ECG) pattern. Besides, treatment with Semelil improved Creatine Kinase, the complete closure of Left Anterior Descending Coronary Artery (LADC). The animals were treated with Semelil 10 mg/kg daily for 14 days. Electrophysiological, Biochemical, histological and ultrastructural studies were used for detecting protective effects of Semelil. Based on our data, Semelil ameliorated the electrocardiogram (ECG) pattern. Besides, treatment with Semelil improved Creatine Kinase, LDH, and necrosis. Primarily these changes lead to the high sensitiveness of myocardial cells to oxygen shortage. The decrease of heart oxygen supply because of coronary occlusion leads to the reduction of oxygen provision to the mitochondria to uphold oxidative phosphorylation and cardiac ischemia ultimately [4]. Cardiac remodeling subsequent either ischemia or MI primarily develops to recompense for deteriorating cardiac function with the indication of hypertrophy, inflammation, cardio myocyte apoptosis and necrosis. Primarily these changes are advantageous but eventually transition to diminishing cardiac function and eventuate to heart deficiency [5]. The inflammatory responses stimulated by MI play a major impress in the pathogenesis of myocardial ischemia. These inflammatory reactions are found to intensify myocardial damage and remodeling after MI [6, 7], which eventuate to healing and wound formation [8, 9]. Medical trials comparing different myocardial advocating strategies and anti-inflammatory medicines are strongly needed. Clinically, there is noticeably intensifying release of heart damage enzyme markers (creatine kinase, MB-creatine kinase, LDH, et al.) in the serum level of patients with MI injury [10]. In this investigation, we mainly studied on an extract herbal and focused on the intricate mechanisms of their beneficial effects on ischemic heart diseases, especially on myocardial ischemic. Melilotus Officinalis has been produced as a component of a native drug by trade nomination of Semelil (ANGIPARS™). In vivo studies in mice, rabbit and dogs and in vitro studies in several based cell lines have approved its safety [11-13]. Prior examinations have revealed advantageous results of Semelil such as improvement of blood flow, the decrease of inflammation, the improvement of immune system and lymphedema [14-17]. Effects of clinical studies on Semelil revealed its safety and usefulness in human diabetic wound [18]. This extract herbal has been revealed to have the strong antioxidant concoction such as 7 hydroxycoumarin and flavonoids [15, 19]. Despite the clinical procedure of Semelil in Iran, fewer attentions have been paid to the importance of its management on the cardiovascular system. Recently, Joukar showed the pretreatment influence of Semelil on isoproterenol-induced cardiac damage for two days can help to keep the heart contractility and consequently blood pressure balance in myocardial damage conditions [20]. In this investigation, we studied Post-treatment influence of Semelil on permanent closure of LADC for two sequential weeks based on electrophysiological, biochemical, histological and ultra-structural indices.

INTRODUCTION

Cardiovascular disease, prevalently MI, is the prominent cause of mortality worldwide [1]. The decreases in the myocardial bloodstream because of the coronary artery obstruction can significantly conciliation the energy metabolism. The myocardial tissue is aerobic and its metabolism is strictly dependent on oxygen accessibility, which is approved by plenty of myoglobin and mitochondria in the cardio myocytes [2]. Moreover, the high-energy need of cardio myocytes for its contracture is met almost solely by mitochondrial oxidative phosphorylation [3]. These changes lead to the high sensitiveness of myocardial cells to oxygen shortage. The decrease of heart oxygen supply because of coronary occlusion leads to the reduction of oxygen provision to the mitochondria to uphold oxidative phosphorylation and cardiac ischemia ultimately [4]. Cardiac remodeling subsequent either ischemia or MI primarily develops to recompense for deteriorating cardiac function with the indication of hypertrophy, inflammation, cardio myocyte apoptosis and necrosis. Primarily these changes are advantageous but eventually transition to diminishing cardiac function and eventuate to heart deficiency [5]. The inflammatory responses stimulated by MI play a major impress in the pathogenesis of myocardial ischemia. These inflammatory reactions are found to intensify myocardial damage and remodeling after MI [6, 7], which eventuate to healing and wound formation [8, 9]. Medical trials comparing different myocardial advocating strategies and anti-inflammatory medicines are strongly needed. Clinically, there is noticeably intensifying release of heart damage enzyme markers (creatine kinase, MB-creatine kinase, LDH, et al.) in the serum level of patients with MI injury [10]. In this investigation, we mainly studied on an extract herbal and focused on the intricate mechanisms of their beneficial effects on ischemic heart diseases, especially on myocardial ischemic. Melilotus Officinalis has been produced as a component of a native drug by trade nomination of Semelil (ANGIPARS™). In vivo studies in mice, rabbit and dogs and in vitro studies in several based cell lines have approved its safety [11-13]. Prior examinations have revealed advantageous results of Semelil such as improvement of blood flow, the decrease of inflammation, the improvement of immune system and lymphedema [14-17]. Effects of clinical studies on Semelil revealed its safety and usefulness in human diabetic wound [18]. This extract herbal has been revealed to have the strong antioxidant concoction such as 7 hydroxycoumarin and flavonoids [15, 19]. Despite the clinical procedure of Semelil in Iran, fewer attentions have been paid to the importance of its management on the cardiovascular system. Recently, Joukar showed the pretreatment influence of Semelil on isoproterenol-induced cardiac damage for two days can help to keep the heart contractility and consequently blood pressure balance in myocardial damage conditions [20]. In this investigation, we studied Post-treatment influence of Semelil on permanent closure of LADC for two sequential weeks based on electrophysiological, biochemical, histological and ultra-structural indices.
MATERIALS AND METHODS

The animal preparation

In this experimental study, the examination was concurred by the animal Care Committee of Tehran heart Researches Center (EC/THRC/696) ordaining on the care and handling of laboratory animals. We used Twenty-five adult male New Zealand white rabbits (weight range 2–3.2 kg; Pasteur Institute, Tehran, Iran). The rabbits were kept alone in metal cages and organized in a room with the stable temperature (24±2°C) and stable lighting cycle that contain 12 hours light-dark. The rabbits were allocated to equal groups: control plus vehicle (Ethanol 86 %, n=5); sham plus vehicle (n=5); ischemia plus vehicle (n=5); Semelil 10 mg/kg (thirty minutes after LADC obstruction was injected intraperitoneally for two weeks continually, n=5).

Induction of regional myocardial ischemia

The rabbits under deep anesthesia with ketamine and xylazine (100 mg/kg, 10 mg/kg i.m) were intubated and well-ventilated with a combination of O2 and N2O. The body temperature of the rabbits was maintained by using a thermostatically warming plate at 37°C throughout surgery [21]. During the operation, an electrocardiogram (ECG) with six-lead was recorded. The chest was exposed through the fifth intercostal space, and then the pericardium was cut. The root regional of LADC was permanently ligated with silk suture. A successful setup of permanent coronary obstruction was concluded by the occurrence of superficial cyanosis of regional myocardial and typical ST segment elevation. None LADC ligation was managed in the sham operation group.

Drug solution preparation

The Semelil (ANGIPARS™) was prepared by Rose Pharmed Co. (Tehran, Iran) that contained the ethanol extract of Melilotus Officinalis was mixed with different content of selenium, sodium phosphoglycerol, urea, and fructose. The Semelil was diluted in normal saline for using.

Monitoring of the ECG

We screened each New Zealand white rabbit with the ECG in 1st and 30th days after the project under anesthesia by intraperitoneal injection of 50 mg/kg and xylazine 10 mg/kg in all groups. The hair was shaved for the location of electrodes. Electrodes were connected to each leg for limb leads. Six electrodes were connected to chest leads. The presentation of successful coronary obstructions was confirmed by observing the increase of ST-segment elevation and alterations in the QRS wave on the ECG tapes.

Biochemical analysis

The sampling and storage of blood and the evaluation of hemostatic factors have been depicted in detail elsewhere [22]. Blood was taken from the vein of ear rabbits in 1st and 30th days. The blood samples were gathered in sterile tubes and centrifuged at 2500 rpm for 10 minutes. The serums were separated and gathered for biochemical evaluations. Serum was utilized for the test of biochemical factors such as Creatine Kinase (CK) creatine kinase isoenzyme in heart (CK-MB) and Lactate dehydrogenase (LDH) were studied (Roche kit, Germany). The results were declared as U/L for CK, CK-MB, and LDH.

Light microscopic analysis

Rabbits were euthanized with sodium pentobarbitone (100 mg/kg) administrated intraperitoneally. The hearts were removed and cut into five transverse sections from apex to base. The samples were then flushed with normal saline and fixed in 10% buffered formalin for 96 h. The specimens were processed for light microscopy study according to the standard method [23]. Morphologic characteristics of cell necrosis demonstrate cell swelling, disturbance of cell membrane, hyper-eosinophilia and karyolysis [24]. Necrotic cells were enumerated in five different fields applying light microscopy.

Electron microscopic

Ultra structural indices of Cardio cytes were studied in different groups. Left ventricle was cut into small sections (1mm3) and fixed in 2.5% glutaraldehyde in a phosphate buffer (PH 7.4 for 2 h at 4 °C). Then samples were fixed in 1% osmium tetroxide (1 h at 4 °C). After frequent washing with buffer, Samples were dehydrated in ethanol sequence and embedded in Epon 812 (TAAB Laboratories Equipment Ltd, UK). Ultrathin sections were sliced and stained using uranyl acetate and lead citrate [25] and evaluated by transmission electron microscope (Philips, EM 208, Netherlands).

Statistical analysis
The results were evaluated by SPSS, version 21.0 and were presented as means ± SEM. All numerical information in text, tables and figures were studied by one-way ANOVA and the Bonferroni post-hoc test (P<0.05).

RESULTS

Effects of Semelil 10 mg/kg on electrocardiogram

Control group displayed a standard electrocardiograph pattern. Induction of ischemia caused significant changes in ECG differ from the control group at 1st after LADC ligation as presented in [Table 1]. Semelil 10 mg/kg failed to amend ECG disturbances at 30th after LADC ligation [Table 1].

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal</th>
<th>ST flat</th>
<th>T inversion</th>
<th>ST depression</th>
<th>ST elevation</th>
<th>Q wave</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 days after LADC ligation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control vehicle</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham vehicle</td>
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<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ischemia vehicle</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>Semelil 10 mg/kg</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>30 days after LADC ligation</td>
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<td></td>
<td></td>
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<tr>
<td>Control vehicle</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sham vehicle</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ischemia vehicle</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>6</td>
<td>3</td>
<td>1</td>
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<tr>
<td>Semelil 10 mg/kg</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Biochemical parameters

The effects of Semelil 10 mg/kg on serum marker enzymes as LDH, CK, and CK-MB are shown in [Table 2]. LDH decreased significantly (P<0.01) in Semelil 10 mg/kg group compared with the ischemic group at 30th after LADC ligation. CK and CK-MB enzyme significantly decreased (P<0.001) in Semelil 10 mg/kg group compared with the ischemic group at 30th after LADC ligation [Table 2].

<table>
<thead>
<tr>
<th>Time point and parameter</th>
<th>Control vehicle</th>
<th>Sham vehicle</th>
<th>Ischemia vehicle</th>
<th>Semelil 10 mg/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 days after LADC ligation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>1413±42</td>
<td>1605±27.9</td>
<td>8392±244.8</td>
<td>8250±162.5</td>
</tr>
<tr>
<td>CKMB (U/L)</td>
<td>16.9±0.5</td>
<td>19.2±0.63</td>
<td>100.7±2.9</td>
<td>99±3.6</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>76±5.7</td>
<td>106±11.8</td>
<td>3930±133.3</td>
<td>2451±288.3</td>
</tr>
<tr>
<td>30 days after LADC ligation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK (U/L)</td>
<td>1156±23</td>
<td>1389±11.8</td>
<td>7755±323.9</td>
<td>2771±288.3</td>
</tr>
<tr>
<td>CKMB (U/L)</td>
<td>13.8±0.28</td>
<td>16.5±0.41</td>
<td>93.06±3.8</td>
<td>33.2±1.9*</td>
</tr>
<tr>
<td>LDH (U/L)</td>
<td>84±5.3</td>
<td>86±8.8</td>
<td>2852±208</td>
<td>375±38.3*</td>
</tr>
</tbody>
</table>

Values are means ± SD. CK: Creatine Kinase; CK-MB: creatine kinase isoenzyme in heart; LDH: Lactate dehydrogenase; * P < 0.001 compared to control group.

Changes in myocardial structure

Normal morphology of the myocardiocytes was observed with no indication of microscopic modifications in the control plus vehicle and sham plus vehicle groups [Fig. 1 A and B]. In the ischemic group was observed disturbance myocardial fibers, pyknotic nucleus and scar formation [Fig. 1 C and C1].

In Semelil 10 mg/kg group was observed nearly typical cardiac cells with the minimal injury [Fig. 1 D]. Myocardial fibers were organized in neat rows compared to the ischemic group [Fig. 1 D1]. There was a significant difference (P<0.05) of the Necrotic cells in the treatment group compared with ischemic groups [Fig. 2 and Table 3].
**Fig: 1.** Hematoxylin & eosin staining of the pathological structure by light microscopy at 30th after LADC ligation: (A) control (B) sham plus vehicle; muscle fibers and the nuclei form are normal; Arrows show the surviving myocardium. Ischemic group (C and C1); disturbance myocardial fibers, pyknotic nucleus (C) and scar formation (C1); chevrons show the Necrotic cells. Semelil 10 mg/kg group (D and D1); nearly typical cardiac cells (D) and myocardial fibers were organized in neat rows (D1); Chevrons show the cells death and arrows show the surviving myocardial (magnification x400).

**Fig: 2.** The Effect of Semelil 10 mg/kg at 30th after LADC ligation in the heart rabbit; Results are expressed as mean ± S.E.M and data were studied by One-way ANOVA followed by the Bonferroni post-hoc test. ***Significantly different from control.

**Table 3:** Effect of Semelil 10mg/kg on Permanent obstruction of LADC Model in the heart rabbit

<table>
<thead>
<tr>
<th>GROUP</th>
<th>(ALL)*</th>
<th>(DC)**</th>
<th>(DC/ALL×100)***</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vehicle</td>
<td>521±7.81</td>
<td>53.6±2.85</td>
<td>10.28±0.65</td>
</tr>
<tr>
<td>Sham vehicle</td>
<td>528±9.30</td>
<td>60±4.19</td>
<td>11.36±0.87</td>
</tr>
<tr>
<td>Ischemic vehicle</td>
<td>339±10.65</td>
<td>285.8±13.03</td>
<td>84.30±3.33</td>
</tr>
<tr>
<td>Semelil 10 mg/kg</td>
<td>543.4±10.88</td>
<td>284.2±27.28</td>
<td>52.30±5.47</td>
</tr>
</tbody>
</table>

Five groups (n=5) were counted.  
*Average numbers of all the myocytes  
**Average numbers of the degenerated cells (DC)  
***Percent average of the cells death (DC/ ALL×100)  
#P<0.05 for Semelil 10 mg/kg  

Changes in myocardial ultrastructure

Ultrathin Images in the control, sham plus vehicle groups demonstrate regular organization of the myofibrils [Fig 3 B2] and the mitochondria with plentiful regular cristae in between [Fig. 3 A2]. Striations are apparent.
among the well organized myofibrils with H line [Fig. 3 A1]. The Z-line [Fig. 3 A] and intercalated disk [Fig. 3 B] are also seen. The ischemic injury led to lysis of the myofibrils [Fig. 4 C1 and C2] and massive fragmentation. Mitochondria [Fig. 4 C2] display either complete loss or vacuolization of the cristae [Fig. 4 C2]. Disruption and expansion of Z-lines are also seen. Semelil 10 mg/kg group displays organized myofibrillar [Fig. 4 D1] with mitochondria [Fig. 4 D2] in between. The mitochondria maintain a normal organization. Focal areas of myofibrillar loss [Fig. 4 D1] are seen.

**Fig. 3:** Electron micrograph of the left ventricular myocardium of the control (a, a1 and a2; scale bar 200 nm and 640nm) and sham vehicle (b, b1 and b2; scale bar 2.5µm and 640nm) groups at 30th after ladc ligation; displaying z line (a); striations are apparent among the well-organized myofilaments with h line (a1); mitochondria (m) (a2) with plentiful regular cristae in between and intercalated disk (id) (b); regular organization of the myofibrils (myo) (b2).

**Fig. 4:** Electron micrograph of the left ventricular myocardium of the ischemic group at 30th after ladc ligation (c, c1 and c2; scale bar 2.5µm, 400nm, and 640nm) displays lysis of the myofibrils (c1 and c2-black arrows) and massive fragmentation. Mitochondria (m) (c2) display either complete loss or vacuolization of the cristae (c2), disruption and expansion of z lines (white arrows) is also seen. Semelii 10mg/kg group at 30th after ladc ligation (d, d1 and d2; scale bar 2.5µm, 200nm and 640nm) displays organized myofibrils (myo) (d1) with mitochondria (m) (d2) in between. The mitochondria maintain a normal organization, focal areas of myofibrillar loss (d1-black arrow).
DISCUSSION

MI is related to an inflammatory response, which is a prerequisite for therapeutic and scar formation [9]. In addition, ischemia leads to activation of nitric oxide synthesis (NOs) then produces nitric oxide (NO) and several enzymes as LDH, CK, CK-MB from the myocardium is secreted and can be created in peripheral blood almost two hours later [26]. These combinations cause to disturbed mitochondrial, lack of ATP and eventually death of myocardial cells [27]. Electrocardiogram (ECG) is regarded the most prominent clinical device for the diagnosis of some types of MI, specifically for the revelation of ST-segment elevation myocardial infarction (STEMI). In this study, we provided the indications for the cardio protective effects of the Semelil 10 mg/kg and its main constituent on numerous parameters of plasma level of myocardial enzymes and cardiac structure and ultrastructure in the chronic MI model. Semelil 10 mg/kg management significantly ameliorated the ECG pattern that indicating its protective influences on cardiac function. In this examination, treatment with Semelil led to the decrease in the plasma level of enzymes such as LDH, CK, and CK-MB. Structural investigations of this group demonstrated that Semelil 10 mg/kg prevented cell degeneration, myocardial necrosis, and nuclei shrinkage. Ultra structural studies of the treatment group revealed that Semelil 10 mg/kg improved neat organized in myocardial fibers with mitochondria. Semelil is a novel drug product including herbal extract with known useful effects specifically on diabetic foot [18]. Some of its constituents are *Melilotus Officinalis* extract, fructose, urea and selenium prepared by the manufacturer. *Melilotus Officinalis* extract can decline activity of circulating phagocytes and has antioxidants, anti-inflamatory and anti-edematous influences [15, 28]. Selenium is a prominent potent anti-inflammatory and antioxidant medicine that may have cardio protective results [29]. Furthermore, experimental evidence has demonstrated that fructose can prevent the inflammatory response and improve the ischemic injury [30]. Also, the previous examination has revealed that urea could improve cardiac blood flow, oxygenation and have antioxidant and cardio protective effects [31]. The exact protective mechanism of Semelil on MI is not known yet. This examination was just concentrated on the common effect of Semelil on myocardial survival in rabbit heart and not its mechanism. Further investigations focusing on microcirculation of pre & post occlusion LADC and inflammatory factors are needed to find the correlated mechanisms.

CONCLUSION

This study revealed that herbal extract decreased the plasma level of myocardial enzymes, myocardial degeneration and necrosis. Furthermore, this herbal drug prevents disrupted mitochondria, sarcolemma breakage. These protection mechanisms may be associated to antioxidants, anti-inflammatory and anti-edematous influences.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests.

ACKNOWLEDGEMENTS

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FINANCIAL DISCLOSURE

The authors report no financial interests or potential conflicts of interest

REFERENCES


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