

**Original article:**

**MODULATORY EFFECT OF SEMELIL (ANGIPARSTM) ON  
ISOPROTERENOL INDUCED CARDIAC INJURY**

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**ABSTRACT**

Administration of semelil (ANGIPARSTM) has been successful in the treatment of diabetic foot ulcer. Considering the improvement of blood flow and anti-inflammatory effect that are attributed to this drug, we investigated its effect on cardiovascular performance in rabbits with isoproterenol (ISO) induced myocardial injury. Animal groups included: control group; ISO group, received ISO 50 mg/kg s.c. for two consecutive days; S1+ISO, S5+ISO and S10+ISO groups, received semelil 1, 5, and 10 mg/kg/day i.p. respectively, 30 min before ISO. On the 3<sup>rd</sup> day, electrocardiogram (ECG) and hemodynamic parameters were recorded; blood samples were taken and hearts were removed for lab investigations.

ISO induced heart injury, ECG disturbance, raise of cardiac troponin I and significant decrease in LVSP ( $p < 0.05$ ), +dp/dt max ( $p < 0.01$ ), -dp/dt max ( $p < 0.05$ ) along with increase of LVEDP ( $p < 0.01$ ). Semelil had no significant effects on ECG and plasma cardiac troponin I. Impairment of +dp/dt max and -dp/dt max was significantly improved in S5+ISO and S10+ISO groups ( $P < 0.05$  versus ISO). In addition, LVSP and LVEDP was somewhat recovered in these groups, although semelil (1 mg/kg/day) to some extent exacerbated the myocardial lesions induced by ISO ( $P < 0.05$ ).

Therefore, in stressful conditions, semelil may improve myocardial contractility; however, it may aggravate the severity of injury.

**Keywords:** Myocardial injury, heart function, arterial blood pressure, isoproterenol, semelil

**INTRODUCTION**

Cardiovascular disease (CVD) is known as the major cause of disability and early death in both developed and developing countries (WHO, 2007). In this regard, coronary heart disease (CHD) makes up more

than half of all cardiovascular events in American population < 75 years of age (Thom et al., 2001). Diabetes is one of the nine major risk factors that are involved in pathogenesis of CHD (Greenland et al., 2003; Yusuf et al., 2004).

Previous studies demonstrated the beneficial effect of semelil, a formulated extract of *Melilotus officinalis* plant containing important compounds such as 7 hydroxy coumarin and flavonoids, as a new herbal drug in treatment of diabetic foot ulcers (Ebrahimi et al., 2009; Heshmat et al., 2008). Although exact anti-ulcer mechanism of semelil is not clear, this has been attributed to the antioxidant (Hirakawa et al., 2000) and anti-inflammatory properties (Pleşca-Manea et al., 2002) of its components. However, Hemmatabadi et al. (2009) reported that non-antioxidant effects may be involved in the therapeutic action of semelil on diabetic foot ulcers. Improvement in blood flow has been suggested as another possible effect of this agent (Bahrami et al., 2008). Recently, Asadi-Shekaari et al. (2010) showed the neuroprotective role of semelil in rat with focal cerebral ischemia. Despite the clinical use of semelil in Iran, less attention has been paid to the consequences of its administration on the cardiovascular system.

Considering the possible role of this drug in improving blood flow and lack of adequate knowledge about its cardiovascular consequences, in the present study we investigated the pretreatment effect of semelil on cardiovascular performance of animals subjected to experimental myocardial injury based on hemodynamic, electrophysiological, biochemical and histopathological indices.

## MATERIAL AND METHODS

### *Experimental protocol*

All experimental procedures were carried out in accordance with the national guidelines for conducting animal studies (Ethical committee permission No 86/123KA—Kerman University of Medical Sciences, Kerman, Iran) and performed on 36 New Zealand white rabbits weighing 2.5-3.5 kg.

Animals were divided randomly into five groups of control (CTL), ISO, S1+ISO, S5+ISO and S10+ISO. In CTL group, animals received equivalent volume of dis-

tilled water i.p. as vehicle of semelil and ISO solutions for two consecutive days. ISO group received 1 ml/kg distilled water i.p. 30 min before ISO (50 mg/kg s.c) for two consecutive days. This dose of ISO was chosen based on our pilot study in which significant changes in histological, biochemical and electrophysiological indices of heart were observed. S1 + ISO, S5 + ISO and S10 + ISO groups were treated with 1, 5, and 10 mg/kg semelil (i.p.) respectively 30 min before injection of ISO, for two consecutive days. We also tested the dose of 20 mg/kg semelil, but due to the high mortality rate of animals, this dose was excluded.

Surgical preparation and experimental protocol have been explained in the previous studies (Joukar et al., 2010b; Najafipour et al., 2010). Briefly; on the third day of study, animals were anaesthetized by sodium thiopental (50 mg/kg of body weight, i.p.) and the anesthesia level was maintained with 1 % halothane in 30 % O<sub>2</sub>–69 % N<sub>2</sub> mixture during the surgical procedure. The trachea was cannulated, with animals breathing spontaneously throughout the experiment. Left carotid artery and left ventricle were cannulated by two heparinized saline filled (7 units/ml) catheters that were connected to pressure transducers and Physiograph (Beckman R612, USA). At the end of the surgery, the gaseous anesthesia was discontinued. Time window for animal recovery from surgery was 30 min. After this period electrocardiogram (ECG), systolic and diastolic blood pressures, left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) were recorded. The mean arterial pressure (MAP) was calculated by “MAP=Pd + (Ps – Pd)/3 formula”, where Ps and Pd are systolic and diastolic arterial pressures respectively. The maximum velocity of contraction (+dp/dt max) and maximum velocity of relaxation (-dp/dt max) were calculated from the left ventricular pressure trace (Joukar et al., 2012). Thereafter, blood sample was taken for measurement of plasma cardiac troponin I levels as an im-

portant biomarker for estimation of myocardial injury. The hearts were taken out, washed with saline and fixed in 10 % buffered formalin and embedded in paraffin. Slides were prepared and stained with hematoxylin and eosin (H&E) and examined microscopically by two pathologists blinded to animals grouping. The lesions graded as 0 (nil); 1 (minimum, focal myocytes damage); 2 (mild, small multifocal degeneration with slight degree of inflammatory process); 3 (moderate, extensive myofibrillar degeneration and/or diffuse inflammatory process); and 4 (severe, necrosis with diffuse inflammatory process) (Joukar et al., 2010a).

Alterations in ECG, such as T wave changes, ST segment elevation and depression or Q wave were reported by evaluating ECG strips by two cardiologists blinded to animal groups.

### STATISTICAL ANALYSIS

Quantitative data are expressed as mean  $\pm$  SEM and comparisons were performed by one-way ANOVA followed by LSD post hoc test. Changes in ECG variables are presented as the number of animals per group that showed each specific disturbance. Histopathological changes are reported qualitatively as the number of animals with different grades of myocardial lesions in each group and statistical analysis was per-

formed using the non parametric Kruskal-Wallis and pairwise differences by the Mann-Whitney U-test (Joukar et al., 2011). P-values  $< 0.05$  were considered as statistically significant.

## RESULTS

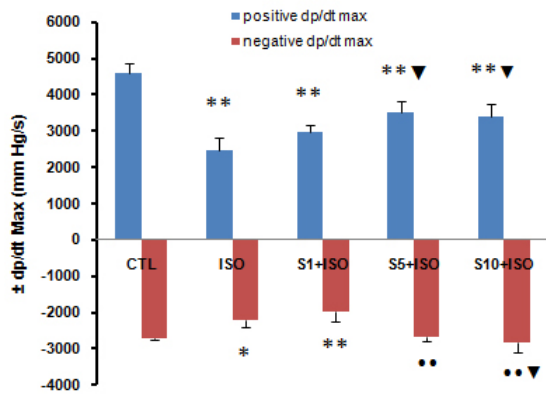
### *Hemodynamic parameters and ventricular function indices*

Non-significant decrease was observed in systolic, diastolic and mean arterial pressures (MAP) of the ISO group. Semelil administration did not show significant effect on these parameters when compared with CTL or ISO groups (Table 1). On the other hand, the non-significant reduction of heart rate in ISO group was aggravated in presence of low dose of semelil (S1+ISO group) ( $P < 0.01$  compared with CTL and  $P < 0.05$  versus other groups) while, the higher doses of semelil had no effect on this index in heart injury conditions (Table 1). Injection of isoproterenol alone was associated with significant decrease in LVSP ( $p < 0.05$ ),  $+dp/dt$  max ( $p < 0.01$ ) and  $-dp/dt$  max ( $p < 0.05$ ) and also increase of LVEDP ( $p < 0.01$ ). These alterations were somewhat improved by higher doses of semelil (i.e. 5 and 10 mg/kg) (Table 1 and Figure 1). However, lowest dose of semelil showed a non significant reduction in  $-dp/dt$  max versus ISO group (Figure 1).

**Table 1:** Hemodynamic parameters and ventricular function of different groups of the study

Groups	Ps	Pd	MAP	HR	LVSP	LVEDP
CTL (n=7)	118 $\pm$ 5	85 $\pm$ 5	96 $\pm$ 4	290 $\pm$ 13	127 $\pm$ 5	3.6 $\pm$ 0.8
ISO (n=8)	107 $\pm$ 9	74 $\pm$ 6	85 $\pm$ 7	262 $\pm$ 18●	102 $\pm$ 8*	14 $\pm$ 3**
S1+ISO (n=7)	101 $\pm$ 7	72 $\pm$ 5	81 $\pm$ 5	224 $\pm$ 5**	108 $\pm$ 5	11 $\pm$ 1**
S5+ISO (n=7)	111 $\pm$ 6	77 $\pm$ 6	88 $\pm$ 6	265 $\pm$ 10●	119 $\pm$ 8	8 $\pm$ 1*
S10+ISO (n=7)	112 $\pm$ 8	81 $\pm$ 6	91 $\pm$ 7	270 $\pm$ 12●	114 $\pm$ 9	7 $\pm$ 1#

n: number of animals, CTL: control, ISO: isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil, Ps: systolic pressure, Pd: diastolic pressure, MAP: mean arterial pressure, HR: heart rate, LVSP: left ventricular systolic pressure, LVEDP: left ventricular end-diastolic pressure. Data are presented as mean  $\pm$  SEM. \* $p < 0.05$  and \*\* $p < 0.01$  versus CTL group, ● $p < 0.05$  versus S1+ISO group



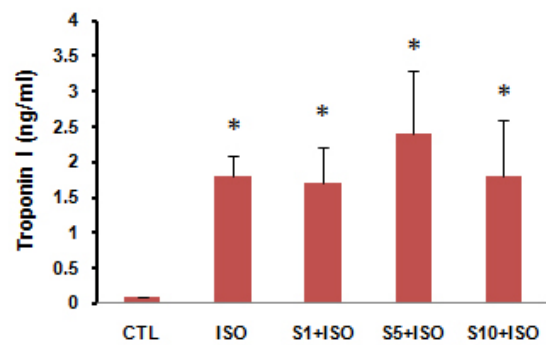
**Figure 1:** Maximum contraction (+dp/dt max) and -relaxation velocity (–dp/dt max) in different animal groups. Values are means  $\pm$  SEM. n: number of animals, CTL: control, ISO: isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil, +dp/dt max: maximum velocity of heart contraction over time, -dp/dt max: maximum velocity of heart relaxation over time. \* $p < 0.05$  and \*\* $p < 0.01$  versus CTL group,  $\blacktriangledown$   $p < 0.05$  versus ISO group, and  $\bullet\bullet$   $p < 0.01$  versus S1+ISO group

### TROPONIN I LEVEL

As shown in Figure 2 the plasma level of cardiac troponin I significantly increased in heart injury conditions ( $p < 0.05$ ). Pre-treatment with three mentioned doses of semelil had no significant effect on troponin I level of animals subjected to myocardial injury.

### ELECTROCARDIOGRAPHIC DATA

CTL group showed a normal electrocardiograph pattern. ISO administration caused significant changes in ECG versus CTL group as shown in Table 2. Different doses of semelil failed to modify ECG disturbances.



**Figure 2:** Plasma levels of troponin I in different animal groups. Values are means  $\pm$  SEM. All groups are significantly different to CTL but there is no significant difference among the four ISO groups. n = 7-8; \*  $p < 0.05$ . CTL: control, ISO: isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil

### HISTOPATHOLOGICAL FINDINGS

In the control group, myocardial cells had normal microscopic appearance (Figure 3). ISO induced mild to moderate heart damage as small multifocal degeneration with slight degree of inflammatory process in 50 % of animals and extensive myofibrillar degeneration and/or diffuse inflammatory process in the other 50 % was seen (Figure 3 and Table 3). Pre-treatment with semelil was somewhat associated with increase of the intensity of leukocyte infiltration and myocardial necrosis but this reached to statistically significant difference only in the S1+ISO group ( $p < 0.5$ ) (Table 3).

**Table 2:** Electrocardiographic disturbance and number of animals per group with relevant changes in ECG

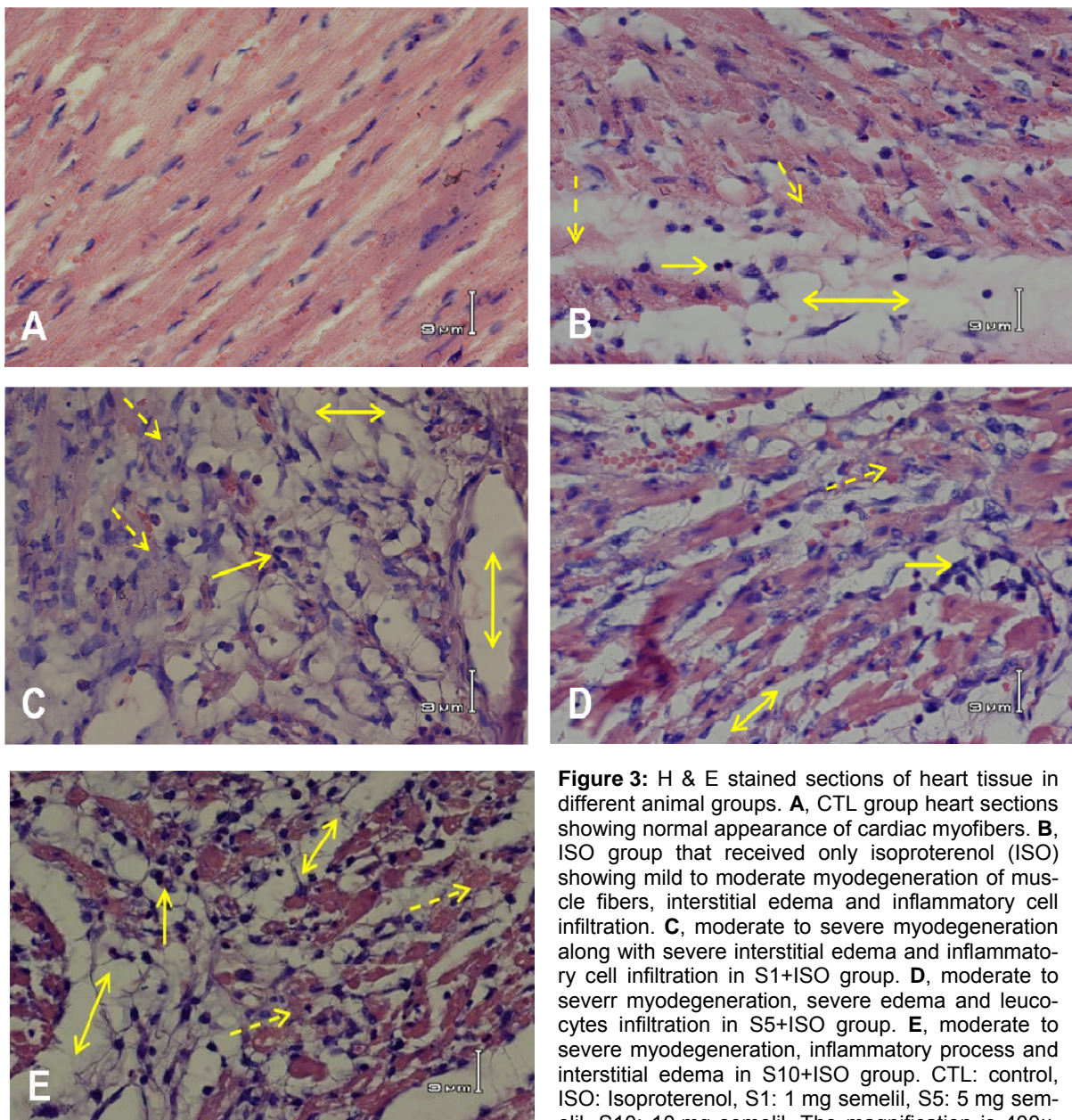
Groups	Normal	ST flat	T inversion	ST depression	ST elevation	Q wave
CTL (n=7)	6	-	-	-	-	-
ISO (n=8)	-	1	1	7	3	1
S1+ISO (n=7)	-	1	4	6	3	-
S5+ISO (n=7)	-	1	3	4	3	-
S10+ISO (n=7)	-	-	4	6	6	1

n: number of animals, ISO: isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil

**Table 3:** Histopathological scores and animals number with different degrees of injury in each group

Groups	Myocardial pathology scores					Mean
	0	1	2	3	4	
CTL (n=7)	7	0	0	0	0	0
ISO (n=8)	0	0	4	4	0	2.5●
S1+ISO (n=7)	0	0	1	2	4	3.43*●
S5+ISO (n=7)	0	0	2	2	3	3.14●
S10+ISO (n=7)	0	0	1	4	2	3●

n: number of animals, CTL: control, ISO: isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil, pathology scores: 0: nil, 1: minimum (focal myocytes damage), 2: mild (small multifocal degeneration with slight degree of inflammatory process), 3: moderate (extensive myofibrillar degeneration and/or diffuse inflammatory process), 4: severe (necrosis with diffuse inflammatory process). The number of animals with severe lesion (score of 4) were more in semelil groups but only significant in S1+ISO group when compared with ISO group. \* p<0.05 compared with ISO group, ● p<0.01 versus CTL group



**Figure 3:** H & E stained sections of heart tissue in different animal groups. **A**, CTL group heart sections showing normal appearance of cardiac myofibers. **B**, ISO group that received only isoproterenol (ISO) showing mild to moderate myodegeneration of muscle fibers, interstitial edema and inflammatory cell infiltration. **C**, moderate to severe myodegeneration along with severe interstitial edema and inflammatory cell infiltration in S1+ISO group. **D**, moderate to severe myodegeneration, severe edema and leucocytes infiltration in S5+ISO group. **E**, moderate to severe myodegeneration, inflammatory process and interstitial edema in S10+ISO group. CTL: control, ISO: Isoproterenol, S1: 1 mg semelil, S5: 5 mg semelil, S10: 10 mg semelil. The magnification is 400×. (↔): interstitial edema, (→): Inflammatory cells, (····): myonecrosis

## DISCUSSION

The present study was conducted to assess the effect of semelil on cardiovascular system in stressful conditions. High doses of isoproterenol, a non selective beta adrenergic agonist, by induction of cardiac stress cause myocardial injury and this method has been accepted as a standard experimental model to induce cardiac dysfunctions and to evaluate following interventions (Rona et al., 1959; Wexler, 1978; Joukar et al., 2010a). In agreement with previous investigations, the present study showed that injection of isoproterenol (50 mg/kg) for two successive days was associated with significant reduction in myocardial function observed by increase in LVEDP and decrease in LVSP and positive and negative dp/dt maxes (see Figure 1 and Table 1). Increase in plasma cardiac troponin I, ECG disturbances and mild to moderate leukocyte infiltration and myodegeneration of the heart muscle was also seen. Blood pressure level was slightly decreased in animals with heart damage (ISO group), but higher doses of semelil was associated with less reduction in mean arterial pressure which may be due to improvement in heart perfusion. Previous studies have reported positive effect of semelil on tissue blood flow (Bahrami et al., 2008). The present study showed that semelil at doses of 5 and 10 mg/kg improved left ventricular function of damaged heart by inducing positive inotropic effect (+dp/dt max, an important index of myocardial contractility) (Koeppen and Stanton, 2008), and positive lusitropic effect (-dp/dt max, indicator of myocardial relaxation). It also reduced the ISO-induced increase in LVEDP (marker of heart failure), in a dose-dependent manner. To our best knowledge, no study in the literature has yet investigated the effects of semelil on the heart function. Increased calcium influx to cardiac cells and prevention of myocardial stunning during heart injury may be two possible reasons for positive inotropic effect of semelil in higher doses. However, confirmation of these possibilities needs future investigations.

Despite the positive effect of semelil on cardiac function, it was associated with more intense myocardial damage based on histology data (Figure 3). Although the improvement of myocardial contractility leads to more stroke volume, more cardiac output and consequently better blood pressure stability (Table 1), this could increase cardiac workload and oxygen demand leading to heart damage in ischemia conditions. In this regard, reduction of myocardial energy demands is one of the several approaches for declining infarct size in patients with myocardial injury (Zipes et al., 2005). More extent and exacerbation of heart lesions in semelil experiments may be due to increased cardiac load and myocardial oxygen supply limitation resulted from increase in contractility that in turn could justify high mortality rate in animals that received the drug in dose of 20 mg/kg. Today, the role of inflammatory processes in the development of cardiovascular disease is well known (Little et al., 2011). Exacerbation of inflammatory process followed by semelil injection may be the negative effect of this drug on heart tissue. The anti inflammatory effect of chronic semelil use has been shown in a previous study (Mousavi-Jazi et al., 2010). In the present study we used semelil only for two consecutive days in each treated groups that can be a reason of difference between our results with others. In addition the heart tissue inflammatory response to semelil may be different from other tissues.

Surprisingly the lower dose of semelil exacerbated the effect of ISO on blood pressure and heart rate. It seems that semelil in this dose has directly suppressed pacemaker activity (S-A node) of the heart because despite the lowering effect on blood pressure (BP) heart rate (HR) was also reduced, while it is anticipated that reduction in BP causes an increase in HR through baro-receptor reflex (sympathetic stimulation). In fact reduction of BP may be the consequence of reduction in HR and hence cardiac output. However, in larger doses, semelil recovered BP through im-

proving myocardial contractility. The evidence for this statement is increase in LVSP and reduction in LVEDP in groups that received larger doses of semelil (Table 1). Therefore semelil should have two different mechanisms of action in low and high doses and this hypothesis needs further investigations to prove.

From ECG point of view, semelil administration had no additional effect on ISO effect alone. This finding may mean that semelil does not affect current injury and conduction properties of the heart. However, the influence of semelil on incidence of cardiac arrhythmia needs complementary studies.

In conclusion the present study showed that semelil administration can help to maintain the heart contractility and hence blood pressure stability in myocardial injury conditions. However this beneficial effect may associate with further cardiac injury due to increased cardiac workload and activation of inflammatory process. In long term periods, this could lead to deterioration of the ischemic heart function. It is noteworthy that present study examined the acute effects of semelil and future studies are needed to elucidate the chronic effects of this drug on cardiovascular system.

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