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RESEARCH ARTICLE

Effect of Hydroalcoholic Extracts of *Tylophora indica* Leaves in Isoprenaline-Induced Myocardial Damage in Rat Heart

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ABSTRACT

The present study was carried out to determine the effect of hydroalcoholic extract of *Tylophora indica* (HETI) on experimentally-induced myocardial infarction (MI) in rats. Albino rats were treated with HETI at doses of 100 mg/kg, (HETI-100) or 200 mg/kg (HETI-200) and propranolol 10 mg/kg (PRO-10) for 30 days orally. MI was induced by subcutaneous administration of isoprenaline (IPL) 150 mg/kg for two consecutive days. Pretreatment of animals with PRO-10 and HETI-200 provided significant myocardium protection from IPL damage as indicated by significant decrease in lactate dehydrogenase (LDH) and creatine phosphokinase-MB (CK-MB) activities in serum and an increase in activities of these enzymes in heart tissue homogenate (HTH). Moreover, HETI-200 significantly increased endogenous antioxidants (SOD and catalase) activities when compared to IPL control as well as normal. The protection offered by HETI could be attributed to the presence of flavanoids which shows antioxidant effect by either inhibiting the release of oxygen free radicals (OFR) or enhancing the synthesis of endogenous antioxidants such as SOD and catalase in IPL-induced cardiotoxicity. These biochemical findings were further confirmed by histological investigations.

Keywords: *Hydroalcoholic extract of Tylophora indica, Propranolol, Isoprenaline, Antioxidants, Myocardial infarction*

Myocardial infarction (MI) and the resultant complication in cardiac function represent the leading cause of morbidity and mortality in developed countries [1]. Moreover, with advanced life style in developing countries, like India, particularly in metropolitan cities, MI is making increasingly important contribution to mortality statistics of such countries [2]. It is well established that MI is a complex phenomenon affecting the mechanical, electrical, structural and biochemical properties of the cardiac system [3]. The use of complementary and alternative medicines is burgeoning globally for MI, especially in developed countries including US [4, 5]. Although many studies identified the increasing prevalence of herbal use throughout the world, only a few reported on how patients perceived the efficacy of this healthcare modality in specific diseases including MI [6, 7].

Epidemiologic studies show an inverse correlation between herbal therapies such as *Tylophora indica* (Asclepiadaceae) and progression of cardiovascular diseases. The leaves of this plant are traditionally used as a folk remedy in certain regions of India for the treatment of bronchial asthma and bronchitis [8]. It was reported to cause myocardial depression and fall of blood pressure in relatively-large doses [9, 10]. The extract of tylophora is known to have anti-oxidant and potent NO scavenging activity [11]. An alcoholic extract from the leaves of the plant *Tylophora indica* were studied for their pharmacological effects on various intact and isolated biological preparations and its protective effect during events of injury has been reported [8]. However, there are no scientific reports on the effect of these leaves in cardiac complication occurring during MI. Hence, the present investigation was undertaken to demonstrate the protective effect of different doses of HETI during isoprenaline (IPL) damage to myocardium in rats.

METHODOLOGY

Chemicals

All chemicals used were of analytical grade and purchased from standard companies. Biochemical kits

like LDH and CK-MB were procured from Crest Biosystems (Goa, India).

Plant extract

The leaves of *Tylophora indica* was purchased from medicinal garden Danvantri vana, Bangalore University, in the month of June 2007. The plant material (Voucher Specimen No.-RRCBI 0691) was authenticated by Regional Research Institute (Ay.), Bangalore. The leaves were shade dried and powdered (moderately coarse). The extraction was carried out with 70% of methanol in soxhlet for about 72 hrs. The obtained syrupy mass was concentrated and dried in hot air oven. The oral doses of HETI were selected on the basis of acute toxicity study, which was carried out as per limit tests of OPPTS guidelines [12]**.** Test dose of 2 g/kg and 5 g/kg were given to mice. Both doses were found to be safe. Hence, $1/25^{th}$ and $1/50^{th}$ of the maximum safe dose corresponding to 200 mg/kg and 100 mg/kg orally were selected as high and low dose respectively.

Phytochemical estimations of the extracts

The hydroalcoholic extracts of *Tylophora Indica L.* were subjected to qualitative analysis for various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids and flavonoids [13, 14].

Experimental animals

Laboratory-bred female Wistar albino rats weighing between 200-250 g were housed at 25 ± 5°C in a well-ventilated animal house exposed to normal day and night cycle. The rats had free access to standard rat chow (Amrut Laboratory Animal feed, Maharashtra, India) containing protein 22.10%, oil 4.13%, fibre 3.15%, ash 5.15%, sand (silica) 1.12% w/w) and water *ad libitum*. There was no significant difference in the body weight of the treated rats when compared with control, either at the beginning or at the end of the study period. Institutional Animal Ethics Committee approved the experimental protocol; animals were maintained under standard conditions in an animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Experimental Protocol

The animals were divided into different treatment groups. The first and second groups served as normal and IPL control respectively. The animals of group III were treated orally for 30 days with propranolol at a dose of 10 mg/kg [15]. The animals of group IV and group V were treated orally for 30 days with two different dose of HETI at 100 mg/kg and 200 mg/kg respectively.

Experimental Procedure

At the end of treatment period, animals of all the groups excluding normal control were administered IPL (150 mg/kg *s.c*) for two consecutive days. Blood was drawn from retro-orbital vein 48 hr after the first dose of IPL under anesthesia and serum was separated by centrifugation for LDH and CK-MB measurement. The heart was isolated from each animal 2 hr after the last dose of the drugs under ketamine (70 mg/kg, i.p) and xylazine (10 mg/kg, i.p) anesthesia and homogenized to prepare heart tissue homogenate (HTH) using sucrose (0.25 M) [16]. The activity of LDH, CK-MB, superoxide dismutase (SOD) and catalase was determined in HTH [17, 18]. Microscopic slides of myocardium were prepared for histopathological studies. The myocardial damage was determined by giving scores depending on the intensity as follows [19]; no changes – score 00; mild – score 01 (focal myocytes damage or small multifocal degeneration with slight degree of inflammatory process); moderate – score 02 (extensive myofibrillar degeneration and/or diffuse inflammatory process); marked – score 03 (necrosis with diffuse inflammatory process).

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance was assessed using One-way analysis of variance (ANOVA) followed by Tukey multiple comparison tests. $p<0.05$ was considered significant.

RESULTS

Phytochemical analysis of the extract

The hydroalcoholic extract of *Tylophora Indica L.* were found to contain various phytoconstituents such as alkaloids, phytosterols, saponins, amino acids and flavonoids.

Effect on LDH and CK-MB activities

The biological activities of endogenous enzymes like LDH and CK-MB were evaluated in serum as well as in heart tissue homogenate (HTH). In serum, high dose of HETI-200 and PRO-10 showed a significant ($p<0.001$) decline in activation of both LDH and CK-MB when compared with IPL control. However, low dose of HETI -100 showed no significant alteration in LDH and CK-MB activities when compared with IPL group (Figs 1-2).

In heart tissue homogenate, high dose of HETI-200 and PRO-10 was found to significantly ($p<0.001$) elevate the enzyme activities compared to IPL. However, HETI-100 fails to show similar significant change in enzyme activities when compared to IPL control. Furthermore, HETI-200 showed no significant change in enzyme activities when compared to PRO-10, whereas, activities of LDH and CK-MB were significantly altered in HETI-200 when compared to HETI-100 (Figs 1-2).

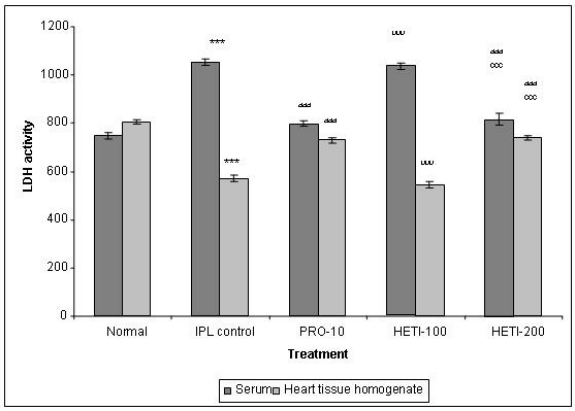


Fig 1. Effect of HETI (hydroalcoholic extracts of *Tylophora indica*)on LDH activity in serum (IU/L) and HTH (IU/g of wet tissue) in IPL-induced MI. HETI-100 and HETI-200 = HETI - 100 mg/kg and 200 mg/kg (30 days treatment, *p.o*); PRO-10 = Propranolol 10 mg/kg (30 days treatment, *p.o*); Values are expressed as mean ± SEM for eight rats in each group; ***significant difference between normal and IPL control $p< 0.001$; aaasignificantly different from IPL control $p< 0.001$; bbbsignificantly different from PRO-10 $p< 0.001$; cccsignificant difference between HETI-100 and HETI-200 $p< 0.001$.

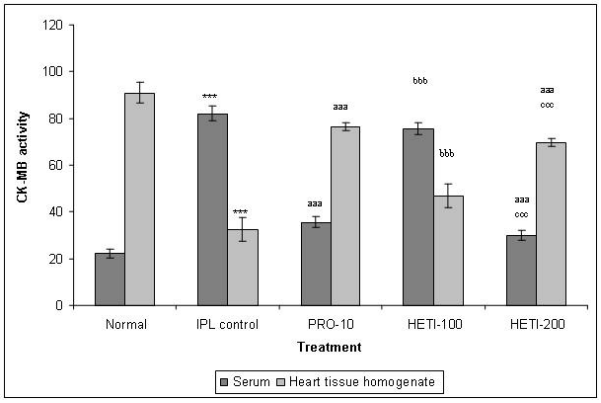


Fig 2. Effect of HETI (hydroalcoholic extracts of *Tylophora indica*) on CK-MB activity in serum (IU/L) and HTH (IU/g of wet tissue) in IPL-induced MI. HETI-100 and HETI-200 = HETI - 100 mg/kg and 200 mg/kg (30 days treatment, *p.o*); PRO-10 = Propranolol 10 mg/kg (30 days treatment, *p.o*). Values are expressed as mean ± SEM for eight rats in each group. ***significant difference between Normal and IPL control $p< 0.001$; aaasignificantly different from IPL control $p< 0.001$; bbbsignificantly different from PRO-10 $p< 0.001$; cccsignificant difference between HETI-100 and HETI-200 $p< 0.001$.

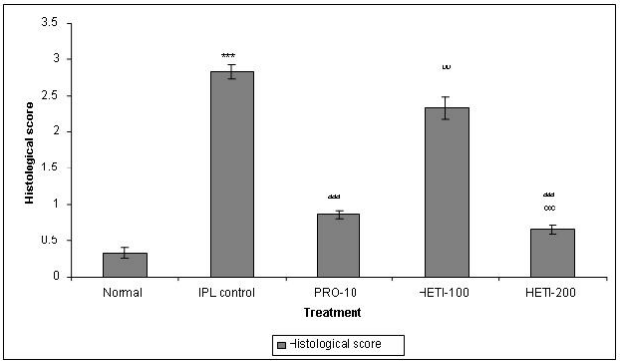


Fig 3. Effect of HETI (hydroalcoholic extracts of *Tylophora indica*) on histological scores of heart tissue in IPL-induced MI.
Values are expressed as mean ± SEM for eight rats in each group; ***significant difference between Normal and IPL control $p< 0.001$. aaasignificantly different from IPL control $p< 0.001$; bbbsignificantly different from PRO-10 $p< 0.001$; cccsignificant difference between HETI-100 and HETI-200 $P< 0.001$.
HETI-100 and HETI-200 = HETI - 100 mg/kg and 200 mg/kg (30 days treatment, *p.o*)
PRO-10 = Propranolol 10 mg/kg (30 days treatment, *p.o*)

Effect on SOD and catalase activity

The SOD and catalase activity in the HTH group were significantly ($p<0.001$) increased after treatment of animals with HETI-200 ($p<0.001$) and HETI-100 ($p<0.01$) when compared to IPL control. It is interesting to note that pretreatment of animals with HETI-200 showed significant increase in antioxidant activity when compared to PRO-10, HETI-100 as well as normal group (Table 1).

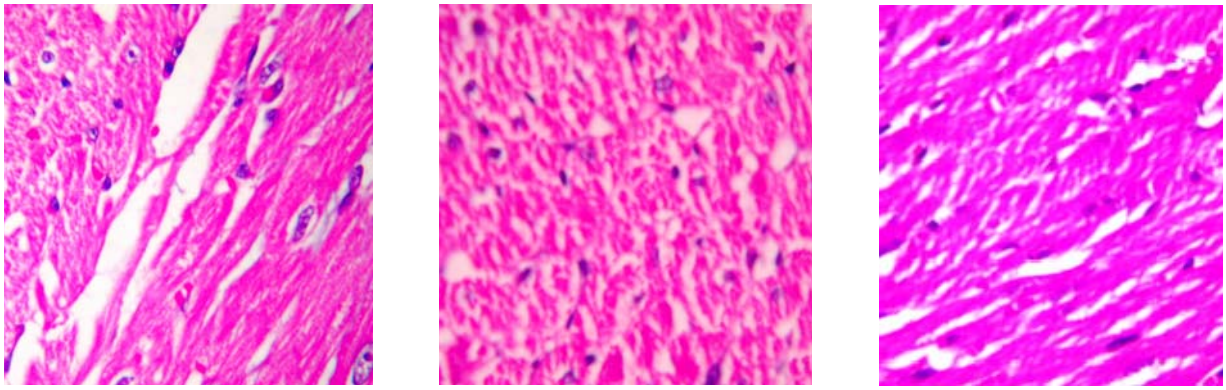
Effect on histological score

Histological examination (Fig 3) of myocardial tissue of the IPL control showed patchy areas of necrosis, hyalinization of muscle fibers with focal cellular infiltrations. The muscle fibers showed vacuolar changes with fragmentation suggestive of necrosis (Fig 4-A). In animals pretreated with PRO-10 (Fig 4-B) and HETI-200 (Fig 4-C), IPL-induced damages were significantly removed. However, HETI-100 fails to provide any protection during IPL damage.

DISCUSSION

The research envisaged was carried out to determine the effect of different doses of HETI and its comparison with PRO-10 during IPL induced myocardial infarction (MI) in rat. The results shows that high dose of HETI-200 and PRO-10 protects the myocardium against IPL damage. However, HETI-100 fails to provide the similar result.

Isoprenaline [1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanolhydrochloride] is a synthetic catecholamine and beta-adrenergic agonist that induces severe stress in the cardiac muscle leading to



(A) **(B)** **(C)**

Fig 4. (A) H&E (×400) stained microscopic section showing nuclear duplication, increased interstitial space and loss of cellular architecture with mild interstitial odema in IPL (150 mg/kg *s.c*)-treated animal. **(B)** H&E (×400) stained microscopic section of heart of animal treated with PRO-10 for 30 days by oral route and subsequently administered IPL (150 mg/kg *s.c*). Normal architecture is visible without infiltration indicating protective effect of PRO-10. **(C)** H&E (×400) stained microscopic section of heart of animal treated with HETI-200 for 30 days by oral route and subsequently administered IPL (150 mg/kg *s.c*). Normal morphology is seen without necrosis indicating cardioprotective role of HETI-200.

development of MI [20]. The MI is produced due to its action on the cardiac β_1-receptors [21, 22]. IPL-induced myocardial necrosis showed membrane permeability alterations, which bring about the loss of function and integrity of myocardial membrane. A number of studies are available that suggest the crucial role of free radicals in pathogenesis of IPL-induced myocardial damage. The pathophysiological changes following IPL administration are comparable to those taking place in human myocardial alterations [19]. Hence IPL-induced myocardial infarction model was used in this study [23]. Animals were pretreated with varying doses of HETI and PRO-10, a known β-adrenergic receptor blocker that is found to have good prophylactic effect [24], before subjecting to MI by IPL.

It is well established that the biological markers like endogenous enzyme are organ-specific and leak from the damaged organ during necrosis [25]. Damage to the cardiac musculature due to IPL results in leakage of cardiac biomarkers such as LDH and CK-MB into the serum with resultant decrease in their activities in HTH and increase in serum [26, 27]. Prophylactic administration of HETI-200 and PRO-10 were found to provide protection by preventing the damage to cardiac musculature during events of ischemia as shown by their decrease activities of enzymes in serum and increase activities in HTH [24]. It can also be emphasized that the damage to myocardium cannot be reversed by low dose of HETI-100 as indicated by increase activity of LDH and CK-MB in serum and decrease activity in HTH.

There is substantial evidence that the associated contractile and rhythmic disturbances involve a contribution from oxygen free radicals (OFRs) [28]. During MI, reactive oxygen species like superoxide and hydrogen peroxide are produced in enormous amount that contribute to myocardial tissue injury [29]. IPL induced myocardial damage is associated with decreased endogenous antioxidants such as superoxide dismutase (SOD) and catalase in HTH which are structurally and functionally impaired by free radicals resulting in damage to myocardium. Inclination in endogenous antioxidant activities in HTH is indication for structural integrity and protection to the myocardium by prior administration of HETI. However, low dose of HETI does not show the similar rise in SOD and

Table 1. Effect of HETI (hydroalcoholic extracts of *Tylophora indica*) on SOD and catalase activities using IPL-induced MI

TREATMENT	SOD (Units/mg protein)	Catalase (Units/mg protein)
Normal control	3.05±0.02	3.14±0.06
IPL-control	2.34±0.08**	2.63±0.08**
PRO-10	2.40±0.03^{aa}	2.77±0.01^{aa}
HETI-100	2.71±0.05^{aab}	3.08±0.03^{aab}
HETI-200	6.24±0.03^{aaabbbccc}	8.02±0.02^{aaabbbccc}

Values are expressed as mean ± SEM for eight rats in each group. ***significant difference between Normal and IPL control $p< 0.001$; ^{aaa}significantly different from IPL control $p< 0.001$; ^{bbb}significantly different from PRO-10 $p< 0.001$; ^{ccc}significant difference between HETI-100 and HETI-200 $p< 0.001$; HETI-100 and HETI-200 = HETI - 100 mg/kg and 200 mg/kg (30 days treatment, *p.o*); PRO-10 = Propranolol 10 mg/kg (30 days treatment, *p.o*)

SOD Units: One enzymatic unit of SOD is the amount in the form of proteins present in 100 µl of 10 % heart tissue required to inhibit the reduction of 24 mM NBT by 50%.

Catalase Units: One international unit of catalase is the amount, which catalyzes the decomposition of 1 mM hydrogen peroxide per minute at 37^{o}C.

catalase when compared to high dose indicating the dose dependent effect of HETI. . It is interesting to note the alteration in SOD is with concomitant fluctuation in catalase after prior treatment of animals with high dose of HETI. Elevated activity of catalase in HTH is more beneficial than increase in SOD activity alone because without a simultaneous increase in catalase activity, increased SOD activity may lead to intracellular accumulation of H_2O_2 with detrimental effects [30]. However, HETI-100 failed to show the beneficial effect probably because low dose failed to reduce the oxidative stress mediated through superoxide and hydrogen peroxide.

It can be speculated from the present study that the PRO-10 mediated cardioprotection is by virtue of its beta blocking property as evident from inability of PRO-10 to increase endogenous antioxidant activity beyond normal.

It is known that PRO-10 causes decrease influx of calcium across the cell membrane leading to dephosphorylation of myosin light chain kinase. It deactivates voltage sensitive calcium channels in the heart via G_s mediated mechanism independent of cAMP concentration. Therefore, pacemaker activity and conduction velocity are decreased with resultant increase in refractory period. As it is known that oxidative phosphorylation is a central site of reactive oxygen species production in the heart [24], by dephosphorylation, generation of OFRs can be drastically reduced. On the basis of the present observation, it is speculated that PRO mediate cardioprotection without significantly elevating SOD or catalase activities in HTH but by scavenging ability towards OFRs. However, HETI-200 inclined the SOD and catalase activities remarkably confirming the cardioprotection via enhanced endogenous antioxidant synthesis due to presence of flavanoids [31].

Damage to cardiac musculature was also demonstrated and confirmed by histopathological parameter i.e histological scores. An increase in this parameter is indicative of myocardial damage [32]. Synthetic β-adrenoceptor agonist isoproterenol leads to development of cardiac hypertrophy as a consequence of increased heart work [33] that is evident from increased histological scores in IPL control group. In the present study, it was found that HETI-200 and PRO-10 showed a remarkable reduction in the histological score when compared to IPL toxic heart. The recovery to myocardium could be attributed to NO scavenging property and generation of endogenous antioxidants due to prior administration of high dose of HETI. Hence it can be speculated that HETI-200 shows antioxidant activity by either inhibiting the release of OFR or enhancing the synthesis of endogenous antioxidants like SOD and catalase in IPL induced cardiotoxicity. The observation of the above manuscript confirms the role of HETI in mediating protection to myocardium during myocardial injury which was recently reported by us in various experimental models of animals [34].

CONCLUSION

HETI in higher doses improves the myocardial recovery from injury induced by IPL. The observations made in the present study showed that prior administration of high dose of HETI prevents oxidative stress and associated structural changes induced by potent cardiotoxic IPL. However, HETI-100 failed to reduce the oxidative stress and hence unable to keep the myocardial integrity. Moreover, further studies are required on ultrastructutal changes in order to validate their use as cardioprotective and explore the possible mechanism of action.

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