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Cardioprotective Potential of *Wedelia chinensis* on Isoproterenol Induced Myocardial Rat

¹M.S.S. Monisha, ²B. Ragavan and ¹D.A.B. Rex

¹Department of Biotechnology, Dr. G.R. Damodaran College of Science, Coimbatore, India

²Department of Biochemistry, PSG College of Arts and Science, Coimbatore, 641014, Tamil Nadu, India

Corresponding Author: M.S.S. Monisha, Department of Biotechnology, Dr.G.R. Damodaran College of Science, Avinashi Road, Civil Aerodrome Post, Coimbatore, 641014, Tamil Nadu, India Tel: +91 9843120004 Fax: +914222210187

ABSTRACT

Wedelia chinensis is a deciduous tree traditionally considered as cardio tonic but has not been proven scientifically. Therefore, the present study aimed to evaluate the cardio-protective effect of *W.chinensis* on isoproterenol induced myocardial infarction in rat. The experimental albino rats were divided into four groups. The hydro-ethanolic extract of *W.chinensis* was orally administered to group 3 and 4 rats for 28 days. Isoproterenol (20 mg/100 g) subcutaneously injected twice at an interval of 24 h to the group 3 rats on 29 and 30th day. At the end, rats were sacrificed and collected serum and heart samples for biochemical and histopathological studies. Significant ($p < 0.05$) results were observed on protein, urea, creatinine, serum lipid profile, cardiac marker enzymes and antioxidants in group 3 rats nearer to normal. Histopathological observation clearly showed the cardioprotective action of the study plant. Further studies will identify the bioactive molecule responsible for the cardioprotective activity.

Key words: Alanine transaminase, cardiovascular diseases, hematoxylin, isoproterenol

INTRODUCTION

Cardiovascular diseases (CVD) are one of the leading causes of deaths even though several advancements in the medical interventions. According to World Health Organization (WHO) nearly 16.7 million people died each year owing to heart attacks. It would constitute the major disease burden and more deaths will be in India by 2020 (Niccoli *et al.*, 2001). Among the ischemic heart diseases, AMI is the death of heart muscle due to sudden blockage of coronary artery by a blood clot, followed by formation of free radicals, accumulation of lipids and macrophages in the wall of artery. It causes changes in electrical, mechanical and structural biochemical properties of heart muscle tissues (Mallinson, 2010; Ohnishi *et al.*, 1982). AMI are caused by non atherosclerotic factors like hyperlipidemia, diabetes, family history, smoking, hypertension which are common in patients with coronary artery disease. As well as, the different clinical, electrocardiographic, biochemical and pathological studies defined the perspective of AMI (Thygesen *et al.*, 2007). Majority of Indian population depends on modern medicine, due to their undesirable effects attention has been drawn towards Herbal Medicines (HM) (Wexler and Greenberg, 1978). It is a common element in ayurvedic, homeopathic, naturopathic, traditional and Native American Indian system of medicine which play a vital role in world market (Ebenezar *et al.*, 2003; Arora *et al.*, 2002).

Wedelia chinensis is a large deciduous tree belongs to Asteraceae family. Traditionally, aerial parts of the plant used to treat cephalalgia, skin diseases, hair growth, alopecia, bites, fever, infection, uterine haemorrhage and menorrhagia. The leaf extracts are used in treatment of kidney dysfunction, cold, wounds and amenorrhea (Verma *et al.*, 2008). Decoction used in menorrhagia, dyeing hair, inflammations, helminthic diseases and liver disorders (Nomani and Kotnala, 2013). Some of the scientific studies proved the wound healing antioxidant, analgesic, anti-inflammatory and lipid-peroxidation inhibitory activity of alcoholic leaf extracts in rats (Suresh *et al.*, 2010). Conventionally it is considered as cardiogenic to reduce mental tension and induce sleep but not yet proven scientifically. Hence the present study aimed to evaluate the cardioprotective potential of *W. chinensis* extract on isoproterenol induced acute myocardial infarction experimental rats.

MATERIALS AND METHODS

Preparation of plant material: *W. chinensis* were collected in and around Coimbatore, identified and authenticated by Dr. Sudhakar, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India. About 3 kg leaves of *W. chinensis* were dried over polythene cover in shade drying method at 21°C and pulverized using a mixer grinder. The coarse powder was used for the preparation of extract.

Extraction: One kilogram powdered material of *W. chinensis* was cold macerated with 3000 mL of hydroethanolic mixture for 3 days, with occasional stirring. After 3 days, the suspension was filtered using fine muslin cloth and the residues were removed. The filtrate was taken in a round - bottom flask and evaporated to separate the distilled alcohol fraction. Then the water portion of this sample was evaporated and dried at 40°C under reduced pressure in a rota-evaporator. The final residual extract was dissolved in distilled water and used for experimental study.

Experimental animals: Male albino rats of wistar strain weighting about 130-150 g procured from the Animal House Facility, PSG Institute of Medical Science and Research, Coimbatore, Tamil Nadu, India. The rats were housed in well ventilated 12±1 h day night schedule in large spacious hygienic cages during the experimental period, fed with pellets supplied by Hindustan Lever Limited, Bangalore, India. The water and libitium rats described as fasted deprived of food for at least 16 hours but allowed free access to water. The experimental study was approved by the Institutional animal Ethics committee of PSG Institute of Medical Science and Research (Prop. No.:101, Reg.No.:158/99/CPCSEA).

Induction of myocardial infarction: Freshly prepared Isoproterenol hydrochloride in sterile normal saline was used to induce myocardial infarction (AMI) subcutaneously in rats at a dose of 20 mg/100 g b.wt.

Experimental design: The experimental rats were divided into four groups of six animals in each group. The animals were fasted overnight with free access of water:

Group 1: Rats received standard pellet diet for 28 days

Group 2: Rats were injected with isoproterenol (20 mg/100 g of rat) subcutaneously twice at an interval of 24 h dissolved in normal saline

Group 3: Rats administered with HEEWC (300 mg kg⁻¹ b.wt.) orally and Isoproterenol injection (20 mg/100 g of b.wt.) subcutaneously twice at an interval of 24 h on the 29th and 30th days

Group 4: Rats administered with HEEWC (300 mg kg⁻¹ b.wt.) for a period of 28 days

Preparation of serum and tissue homogenate: At the end of experimental period, rats were sacrificed by cervical decapitation method under ketamine (24 mg kg⁻¹ i.m.). The heart were excised immediately and thoroughly washed with ice-cold physiological saline, blood samples were collected *via* abdominal aorta puncture using sodium citrate (3.8%w/v) as anticoagulant. Serum separated by centrifugation at 2500×g was used for the biochemical studies. One gram of heart tissue was taken and homogenized with 0.1 M cold Tris buffer (pH 7.4) in a potter homogenizer fitted with Teflon plunger at 600 revolutions for 3 min. The homogenate was used for various biochemical assays. The protein, urea and creatinine were estimated by colorimetric method (Lowry *et al.*, 1951). Marker enzymes such as ALT, AST, LDH, total cholesterol and triglycerides were assayed in serum using standard kits supplied from Kamineni Life Science Pvt. Ltd, Hyderabad. The prepared heart tissue was used for the antioxidant assay viz., TBARS (Nieuhas and Samuelson, 1968), Superoxide dismutase (Kakkar *et al.*, 1984; Sinha, 1972).

Histopathological studies: The myocardial heart tissue from dissected rat was 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 examined under light microscope and photographs were taken.

Statistical analysis: The results were expressed in Mean±SD for each parameter under four different groups. The significance of difference in means of two groups was tested using student t-test at 1, 5% levels. The result is significant at 1% level if p<0.01 and significant at 5% level if p<0.05.

RESULTS

25 g of dark brown colored crystals of hydroethanolic extract of *W. chinensis* was obtained as final yield. The level of protein in serum and heart tissues of all experimental groups was represented in Table 1. After 28 days, protein level was significantly increased (p<0.01) in serum and decreased in heart tissue of Group 2 rats. In the case HEEWC treated myocardial rats, the protein in serum (54.65±1.80b) was decreased and increased in heart tissue (95.05±2.68) indicates the protective action of *W. chinensis* against on oxidative damage. There was no changes were observed in group 4 rats. Table 2 represents the level of urea and creatinine in all experimental rats. Urea (48.23±0.92) and creatinine (1.36±0.03) were significantly elevated in myocardial rats when compared to control. It shows an impaired renal function in myocardium. In the case of HEEWC treated myocardial rats, there was a significant decrease in level of serum urea (28.41±0.84 mg dL⁻¹) and creatinine (0.62±0.03 mg dL⁻¹) it showed the restored renal function in rats. Table 3 represents the serum lipid profile of all experimental groups. The level of total cholesterol, triglyceride, VLDL and LDL in myocardial control rats were significantly (p<0.01)

Table 1: Effect of HEEWC on serum, heart tissue protein levels in experimental rats

Groups	Protein levels	
	Serum (g dL ⁻¹)	Heart (mg g ⁻¹ tissue)
I	21.33±0.69	106.86±2.64
II	62.49±1.80 ^{***}	84.63±1.60 ^{***}
III	54.65±1.80 ^{***}	95.05±2.68 ^{***}
IV	23.54±3.35 ^{ns}	112.79±0.37 ^{ns}

Values are expressed by Mean±SD of six samples, (a) Group I vs. Group II, (b) Group II vs. Group III, (c) Group I vs. Group IV, Statistically significant at *p<0.05, **p<0.01, ns: Not significant

Table 2: Effect of HEEWC on Urea and Creatinine levels in serum of experimental rats

Groups	Serum urea (mg dL ⁻¹)	Serum creatinine (mg dL ⁻¹)
I	13.08±0.59	0.52±0.08
II	48.23±0.92 ^{***}	1.36±0.03 ^{***}
III	28.41±0.84 ^{b**}	0.62±0.03 ^{b**}
IV	16.91±1.04 ^{ns}	0.71±0.08 ^{ns}

Values are expressed by Mean±SD of six samples, (a) Group I vs. Group II, (b) Group II vs. Group III, (c) Group I vs. Group IV, Statistically significant at *p<0.05, **p<0.01, ns: Not significant

Table 3: Effect of HEEWC on serum lipid profile levels in experimental rats

Groups	Triglycerides (mg dL ⁻¹)	Cholesterol (mg dL ⁻¹)	HDL (mg dL ⁻¹)	LDL (mg dL ⁻¹)	VLDL (mg dL ⁻¹)
I	26.3±0.90	85.23±2.94	18.30±2.78	36.36±0.70	16.42±0.44
II	42.51±1.06 ^{***}	125.68±1.09 ^{a**}	13.11±0.87 ^{a**}	46.27±2.94 ^{a**}	34.10±2.64 ^{***}
III	28.10±1.76 ^{b**}	93.85±1.26 ^{b**}	16.73±0.30 ^{b**}	36.78±1.10 ^{b**}	23.01±1.10 ^{b**}
IV	20.88±0.59 ^{ns}	82.33±0.70 ^{ns}	20.71±0.89 ^{ns}	35.21±0.76 ^{ns}	17.87±0.39 ^{ns}

Values are expressed by Mean±SD of six samples, (a) Group I vs. Group II, (b) Group II vs. Group III, (c) Group I vs. Group IV, Statistically significant at *p<0.05, **p<0.01, ns: Not significant

increased compared to normal rats and HDL was significantly (p<0.01) decreased in myocardial control rats. In group 3 more significant reduction the level of triglycerides (28.10±1.76 dL⁻¹), cholesterol (93.85±1.26), LDL (36.78±1.1), VLDL (23.01±1.10) and increased in HDL (16.73±0.30) than compared to the group 2 (Isoproterenol treated). There was no significant change in group 4 when compared with group 1 rats. Levels of serum and heart tissue marker enzymes of experimental rats were showed in Fig. 1. There was a significant elevation (p<0.01) in transaminases (AST and ALT) and LDH level in isoproterenol injected animals in both serum and heart tissues compared to the control. In group 3 there was a significant reduction in the AST, ALT, LDH enzyme leakage were observed in both serum (37.12±0.75, 15.65±1.03, 81.38±0.76) and heart tissues (42.62±0.48, 26.05±0.54, 114.29±0.9) than compared with isoproterenol administered rats (group 2). The reducing effect of the HEEWC restores the injury during myocardial infarction. No significant change in serum and heart marker enzyme levels was observed in normal and plant control. There was a significant decrease in antioxidant enzyme SOD (3.96±0.48), CAT (35.21±0.56) and LPO (5.78±0.1) in isoproterenol injected animals in both serum and heart tissues compared to the control. On treatment with HEEWC daily for a period of 28 days, a significant (p<0.05) increase in the activities of antioxidant was observed in both serum and heart tissues

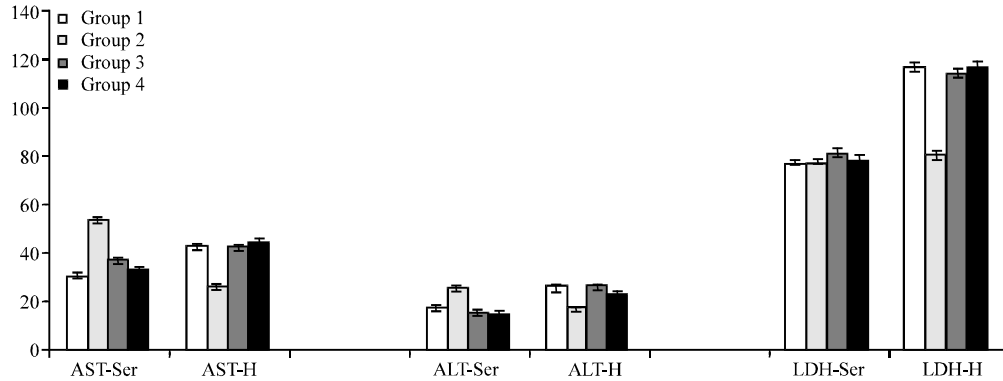


Fig. 1: Effect of HEEWC on cardiac marker enzymes levels in experimental rats, Values are expressed by Mean±SD of six samples, (a) Group I vs. Group II, (b) Group II vs. Group III, (c) Group I vs. Group IV

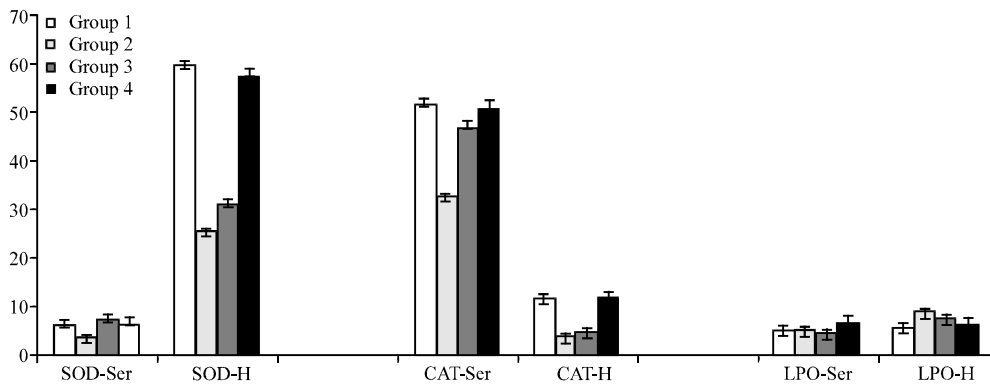


Fig. 2: Effect of HEEWC on serum, enzymatic antioxidant levels in control and experimental rats, Values are expressed by Mean±SD of six samples, (a) Group I vs. Group II, (b) Group II vs. Group III, (c) Group I vs. Group IV

of isoproterenol-treated rats when compared with the isoproterenol induced rats. No significant change in serum and heart tissue antioxidant enzyme level was observed between normal and group 4 (Fig. 2).

Histopathological observation of heart tissues of all experimental groups were showed in Fig. 3. In group 1, H and E sections of heart showed normal structure of heart muscle with no obvious abnormality. In group 2, H and E sections of heart muscle showed destructive focal areas of muscle bundles, surrounding inflammatory cells. The entire apex of the heart showed large, grossly visible, confluent areas of necrosis found in the left and right ventricles. The infarcted areas consisted of large pools of mucoproteinaceous material. In group 3, H and E sections shows normal architecture of the heart muscle with less destruction of muscle bundles and inflammation. Also marked improvement in ISO induced alterations such as vascular changes, edema, capillary dilation and leukocyte infiltration when compared to ISO administered groups. In group 4, H and E sections shows structure of heart muscle showing no obvious abnormality.

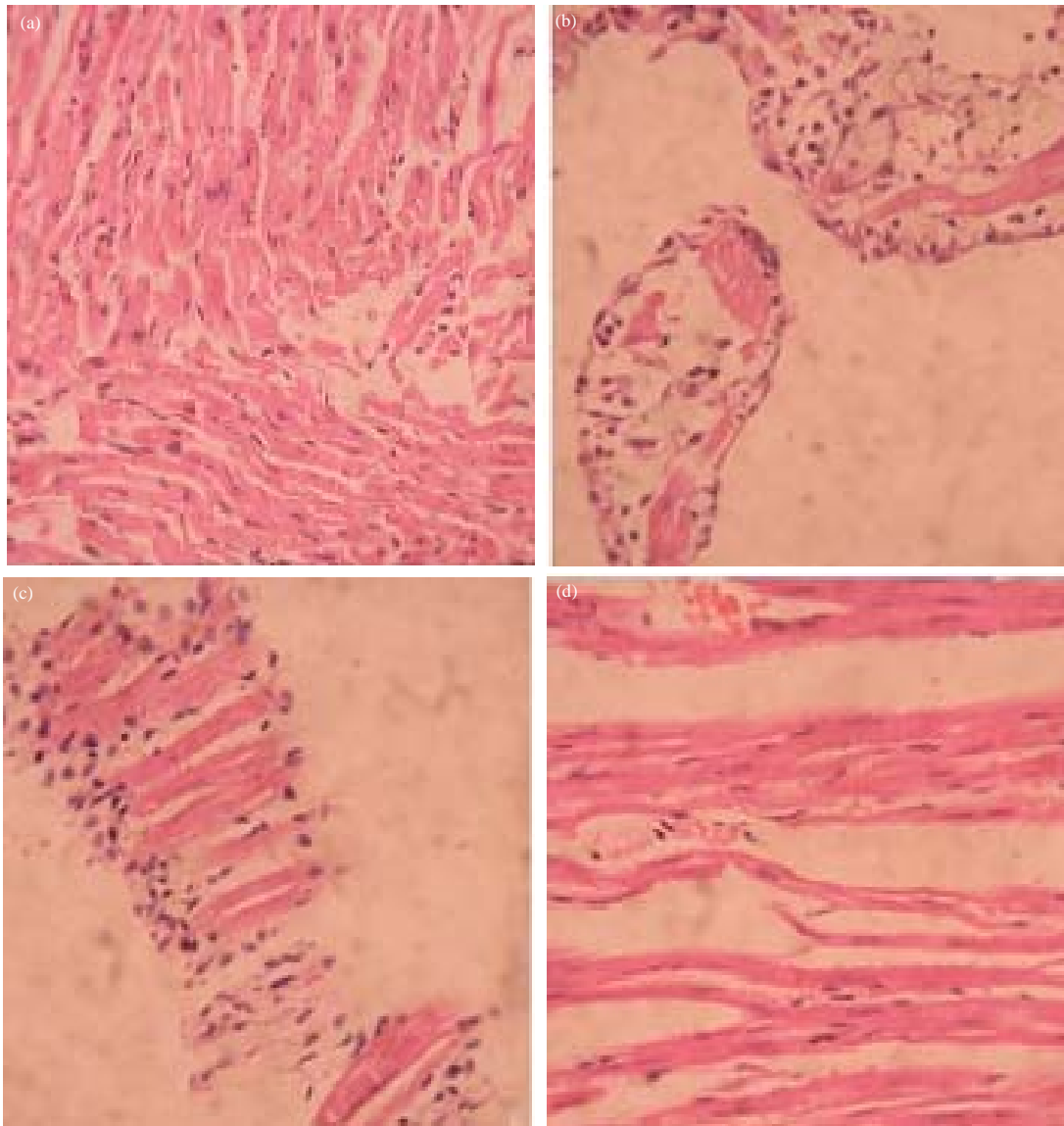


Fig. 3(a-d): Histopathological examination in heart tissues of experimental rats, (a) 40X necrotic cells of normal vehicle treated rat, (b) 40X destruction of muscles of ISO treated rat, (c) 40X regeneration of normal muscle cells of HEEWC treated on ISO induced rat and (d) 40X normal muscular tissues of HEEWE treated rat

DISCUSSION

In the present study, an increased level of serum protein was observed in myocardium rats. This may be the damage in myocardial tissues and simultaneous release of cytosolic proteins to blood stream quickly resulting in a rapid peak of serum proteins (Fleckenstein *et al.*, 1974). The concentration of tissue protein was found to be decreased in the myocardial rats, may be due to consequences of cell injury through lipid peroxides caused by Isoproterenol (Ganesan *et al.*, 2007). Earlier studies

also supported the oral administration of *Daucus carota* restore the level of protein in experimental rats (Farvin *et al.*, 2008). Renal dysfunction envisages the increased mortality in cardiovascular diseases. Creatinuria occurs when the serum concentrations exceed the normal value. In group 2, serum creatinine concentrations significantly increased ($p < 0.01$) because of direct leakage of low molecular mass of creatinine from myocardial cells of infarcted zone. Subsequent to acute cellular injury, the renal excretion rate of creatinine theoretically depends on the glomerular filtration rate and difference between serum concentration and renal threshold, also creatinine release during acute myocardial infarction, unstable angina and cardiac surgery (Hu *et al.*, 2006).

High level of circulating cholesterol and its accumulation in heart tissue are fighting with cardiovascular damage (Manjula *et al.*, 1992). Subcutaneous administration of isoproterenol (ISO) raised LDL cholesterol level (46.27 ± 2.94) in serum. High levels of LDL cholesterol have positive and high level of HDL cholesterol have negative relation with myocardial infarction. In the present study, HDL level was high observed in the HEEWC treated rats. This represent HEEWC may inhibit the uptake of LDL by arterial wall and also facilitates the transport of cholesterol from peripheral tissue to the liver. Hyper-triglyceridemia noticed in isoproterenol injected rats (42.51 ± 1.06 mg dL⁻¹). Generally, this condition was observed in ischemic heart disease due to decrease in activity of lipoprotein lipase in the myocardium resulting in decreased uptake of triglycerides from circulation (Priscilla and Prince, 2009). Pretreatment with hydroethanolic extract of *W. chinensis* in myocardial induced rats reduced triglycerides, total cholesterol, VLDL and HDL and increasing HDL significantly ($p < 0.01$). It might be due to the hypocholesterolaemic and heart protective effect of the plant.

Enzymes are the best marker to represents tissue damage because of their specificity and catalytic activity to the tissue. The release of cellular enzymes reflects nonspecific alterations in the membrane integrity and permeability as a response to β -adrenergic stimulation (Sivakumar *et al.*, 2007). The cytosolic marker enzymes AST and ALT activities are reflecting necrosis when they are released into the blood after cell membrane damage (Sangeetha and Quine, 2006). Lactate dehydrogenase is involved in inter conversion of lactate to pyruvate of body tissues and fluids. It reflects the NAD⁺/NADH ratio indicated by the acetate to pyruvate ratio of hepatocyte cytosol. In the present study ISO injection significantly decreases in the activities of AST, ALT and LDH in the myocardial tissue, subsequently in serum. This might be the damage in heart muscle, rendering the leakage of enzymes in to serum (Sangeetha and Darlin, 2006). Treatment with HEEWC significantly prevented the ISO induced elevation in levels of diagnostic marker enzymes. This is probably the protective effect of *W. chinensis* on the myocardium. The reduced extent of myocardial damage and there by restricted the leakage of the enzymes from the myocardium (Gnanapragasam *et al.*, 2007). ISO also causes significant reduction in the activities of serum and heart antioxidant enzymes in myocardial rats. This might be the increased utilization of antioxidants. Increased activity of SOD and CAT indicates increased removal of Superoxide radicals by reducing myocardial damage caused by free radicals (Karthick and Prince, 2006). Krishnaswami (1998) reported the resorted activity of SOD from radiation induced damage using *W.chinensis*. HEEWC in myocardial induced rats, significantly reversed the enzymatic antioxidants nearer to normal and control the free radical formation. It is probably one of the most effective defenses systems against the disease (Punithavathi and Prince, 2009).

Lipid peroxidation plays an important role in heart, liver and kidney toxicity (Prince *et al.*, 2008). ISO increased levels of thiobarbituric acid reactive substance indicate the excessive

formation of free radicals and activation of lipid peroxidation which cause heart damage in animals (Rajesh and Latha, 2004). The present findings clearly indicated the ISO induced myocardial ischemia and curative effect of HEEWC. Histopathological studies provide evidences over the morphological changes, evidencing that toxicity correlates with changes in heart tissue and cell morphology of a scale that can be visualized using light microscopy.

CONCLUSION

In conclusion, the present study reveals that administration of HEEWC proved to be more effective in reducing the extent of myocardial damage and significantly counteracted the oxidative stress during isoproterenol-induced myocardial infarction in rats. Further studies will be carried out to find out the biomolecule and its pharmacokinetic action to develop a cost effective and non toxic cardioprotective drug from *W. chinensis*.

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