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Cardioprotective Effects in Methanolic Extract of *Evolvulus Alsinoides* Linn on Isoproterenol - Induced Myocardial Infarction in Albino Rats

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Abstract – The present study was performed to investigate *Evolvulus alsinoides*. Linn a natural herb, would attenuate the acute myocardial infarction in isoproterenol [ISP]-treated rat model maintaining cardiac function and activities of endogenous antioxidant enzymes. Heart tissue enzyme analysis in *albino* male rats, such as LPO, GSH, GPX, GST, SOD, CAT, CK-MB, MDA and biochemical analysis in serum plasma viz., ALT, AST, LDH, and CPK were performed. Methanolic extract of *Evolvulus alsinoides [EA]* at the dose of [100 & 200 mg/kg/p.o] showed significant cytoprotection in the heart from isoproterenol induced myocardial ischemic injury. The results indicate that *Evolvulus alsinoides [EA]* administration causes myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from oxidative stress associated with ISP induced myocardial injury, and justify its potential therapeutic value in the treatment of ischemic heart diseases in albino rats.

Keywords – *Evolvulus alsinoides [EA]*, antioxidant, Isoproterenol, myocardial infarction, Serum biochemistry, heart enzymes.

1. Introduction

A myocardial infarction occurs when an atherosclerotic plaque slowly builds up in the inner lining of a coronary artery and then suddenly ruptures, causing catastrophic thrombus formation, totally occluding the artery and preventing blood flow downstream. Cardiac hypertrophy is a general term signifying an increased workload and is characterized with an increase in cardiac mass in response to applied stimulus [26]. Prolongation of this process leads to congestive heart failure [HF] defined as a progressive syndrome that appears as the final phase of most cardiac diseases [23]. Myocardial infarction [MI] is manifested with the impaired systolic and diastolic function, ventricular dilatation and ultimately with congestive HF [25].

For decades, the major causes of death in many developed countries have been diseases of the heart and blood vessels [the venous system], collectively known as cardiovascular disease [CVD].

The use of herbal medicines has been steadily increasing over the past decade to cure some of the disorders in human. Epidemiologists in India and international agencies such as the World Health Organization [WHO] have been sounding an alarm on the rapidly rising burden of CVD for the past 15 years. The reported prevalence of coronary heart disease [CHD] in adult surveys has risen four-fold in 40 years and even in rural areas the prevalence has doubled over the past 30 years. It is estimated that by 2020, CVD will be the largest cause of disability and death in India [2].

Isoproterenol [ISP], a synthetic catecholamine and ß-adrenergic agonist that causes severe stress in myocardium and infarct-like necrosis of the heart muscles. ISP induced myocardial injury involves membrane permeability alterations, which brings about the loss of functions and integrity of myocardial membranes . ISP induced myocardial necrosis is a well-known standard model to study the beneficial effect of many drugs on cardiac dysfunction [4] several medicinal plants have been found to possess antioxidant properties and have beneficial effects in pathological conditions like cancer, liver diseases, cataract and myocardial ischemia . The use of herbal medicines has been steadily increasing over the past decade [8].

A considerable number of these plants/plant based products have been widely used. Therefore, interest in the examination of plants as potential sources of new drugs is increasing. In India, medicinal plants are traditionally used in the treatment of cardiovascular disease, as they are inexpensive, efficacious and safe [3].

Evolvulus alsinoides [EA] [Convolvulaceae] is well known for its memory enhancement, antiepileptic and immune modulatory properties in the traditional Indian system of medicine, Ayurveda. In view of the increasing attention towards plants offering nonspecific resistance [adaptogens] towards stress, we have evaluated crude ethanolic extract of EA for its adaptogenic and memory enhancing properties in rodents [16]. The study is an effort in the same direction thus the present investigation was undertaken to evaluate the cardioprotective effects of the methanolic extract of whole plants *Evolvulus alsinoides* [EA] on isoproterenol induced myocardial infarction in *albino [Wistar*] male rats[1].

2. Materials and methods

2.1. Drugs and Chemicals

Isoproterenol [Isoprin] was purchased from Unichem Laboratories India, methanol was purchased from SD

Fine chem and other solvents/reagents were analytical grade.

2.2. Plant Material

Fresh mature whole plant was collected from natural source in rural parts of South Karnataka regions of India. Routine pharmacognostic investigations were carried out to confirm authenticity of this material. The identification was carried out in our P.G Dept of Biotechnology. The Arial parts were dried and cut into small pieces and crushed to a coarse powder using mixie. Coarse powder was subjected to extraction in Sox let apparatus [in ratio of 1:5 to the quantity of raw material] for 6 h under reflux condenser at 45^{o} C using thermostat [6]. This extract was cooled to room temperature and evaporated using condenser then filtered through filter cloth [#100 mesh size], to get methanolic extract of *Evolvulus alsinoides* [EA]. The resulting extracts were stored in well-packed container at room temperature for future use. The yield of dried extract was approximately 40 %. [22].

2.3. Experimental Animal

The *Albino [Wistar*] male rats of 150-200g [weight] were used for the study. Animals were housed in well ventilated room [temperature 23 ± 2^{o} C, humidity 65-70% and 12h light/dark cycle] obtained from the Central Animal facility. Sri Raghavendra Enterprises. Bangalore. India [Reg. no. 941/5/11/CPCEA], were used for the study. The animals were housed in polypropylene cages at a population density of six per cage, under controlled Environmental conditions of temperature [27 + 30^{0}C] with normal pellet diet and water was provided ad libitum [7].

2.4. Experimental Protocol

Male Wistar rats weighing 150–200 g were pre-treated with the oral dose of 100 and 200mg/kg of *Evolvulus alsinoides* [EA] for 30 days. At the end of treatment period, animals of all groups excluding group I received 5.25 and 8.5 mg/kg isoproterenol on two consecutive days [31 th & 32 nd day]. [9] Symptoms and mortality in each group were recorded and compared with those of rats given isoproterenol alone. 48 hs after the first dose of ISP administration, rats were sacrificed by cervical decapitation method under Xylazine + Ketamine [16 + 100 mg/kg i.m.], blood samples were collected *via* abdominal aorta puncture using sodium citrate [3.8%w/v] as anticoagulant and the serum separated were used for the determination of diagnostic marker enzymes[10].The marker enzymes ALT, AST, LDH and CPK were assayed in serum using standard kits supplied from Swemed diagnostics, Bangalore, India. The heart tissue was excised immediately, washed with chilled isotonic saline, tissue homogenates were prepared in ice cold 0.1 M Tris-HCl buffer [pH 7.2], used for the assay of clinical marker enzymes LPO & MDA , GSH , GP, GST, SOD, CAT and CK-MB [15].

Table 1. Serum Biochemical analysis

Groups/ Parameters	**ALT[µmol/L]**	**AST[µmol/L]**	**LDH[µmol/L]**	**CPK[µmol/L**
Group I [Normal control]	78.5 ± 6.34	87.26 ± 6.74	102.7 ± 8.54	110.12 ± 3.8
GroupII [Isoproterenol control]	312.50 ± 21.2***	287.37 ± 34.8***	255.5 ± 18.6***	284.56 ± 31.4**
Group III [*Evolvulus alsinoides* [EA] 100mg/kg/po]	124.02 ± 18.4***	128.86 ± 21.6***	158.26 ± 16.2***	172.2 ± 12.4**
Group IV [*Evolvulus alsinoides* [EA] 200mg/kg/po]	89.02 ± 6.54***	96.4 ± 4.26***	119.4 ± 3.56***	115.30 ± 4.96**

ALT-Alanine aminotransferase
AST-Aspartate aminotransferase
LDH-Lactate aminotransferase
CPK-Creatine phosphokinase

Values are expressed as the mean ± SEM from 6 animals in each group, differences in means were estimated by ANOVA followed by Dunnet's post Hoc test.

The values of serum ALT, AST, LDH and CPK values of Group IV, III was compared with Group II; Group II was compared with Group I; *** = P<0.001 highly significant.

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Table 2. Analysis of Heart enzyme

Groups/ Parameters	**LPO**	**GSH**	**GPx**	**GST**
Group I [Normal control]	1.12 ±0.02	4.21 ±0.62	2.54±0.24	1246±62
Group II [Isoproterenol control]	2.26 ±0.20*	2.18 ±0.14*	1.68±0.20	768 ±56***
Group III [Evolvulus alsinoides [EA]100mg/kg/po]	1.86 ±0.19**	3.36± 0.18**	2.36±0.76**	935±48* *
Group IV [Evolvulus alsinoides [EA]200mg/kg/po]	1.18 ±0.05***	4.24±0.32***	2.89±0.19**	1254±76***

LPO- Lipid peroxides
GSH- Glutathione
GPx- Glutathione peroxidase
GST- Glutathione-S transferase

Table 3.

Groups/ Parameters	SOD	CAT	CK-MB	MDA
Group I [Normal control]	4.46±0.34	8.42±0.18	160.6±1.22	58.2±4.2
Group II [Isoproterenol control]	690±52* *	1.98±0.14***	4.24±0.06***	46.4±6.4***
Group III [Evolvulus alsinoides [EA]100mg/kg/po]	3.22±0.20	4.82±0.72***	102.4±1.8***	68.8±4.2**
Group IV [Evolvulus alsinoides [EA]200mg/kg/po]	4.28±0.12***	8.14±0.82***	128.6±2.4***	71.2±3.8***

SOD- Superoxide dismutase
CAT-Catalyze
CK-MB- Creatine phosphokinase
MDA- Malonaldihyde

Values expressed: Levels of lipid peroxides [LPO]-nmol malondialdehyde released/mg protein; Reduced glutathione [GSH]-µmol [oxidized min-1 mg-1 protein];Glutathione peroxidase [GPx]-nmol [Oxidized min-1 mg-1 protein]; Glutathione-S transferase [GST]-µmol [1-chloro-2, 4-dinitrobenzene conjugate formed min-1 mg-1 protein];Catalase [CAT]-nmol [H_2O_2 decomposed min-1 mg-1];Superoxide dismutase [SOD] -[one unit of the SOD activity is the amount of protein required to give 50% inhibition of epinephrine autoxidation. MDA [nmol/g tissue], CK-MB [IU/mg protein] MDA: Malonaldihyde; CK-MB: Creatine phosphokinase-MB isoenzyme. One unit of CK-MB transfers 1mmol of phosphate from phosphor creatine to ADP per min at pH 7.4 at 30°C [11].The values of Heart tissue LPO, GSH, GPx, GST, SOD, CAT, CK-MB and MDA values of Group IV, III was compared with Group II; Group II was compared with Group I; *** = P<0.001 highly significant; *** = P<0.001 highly significant, **= P<0.01 moderately significant, *= P<0.05 significant.

3. Results and Discussion

Isoproterenol [ISP], a synthetic ß -adrenergic agonist, by its positive inotropic and chronotropic actions increases the myocardial oxygen demand that leads to ischemic necrosis of myocardium in rats similar to that seen in human myocardial infarction. A number of patho-physiologic mechanisms have been outlined to explain the ISP-induced myocardial damage, altered membrane permeability, increased turnover of nor-epinephrine and generation of cytotoxic free radicals[5]. In addition, ISP administration reduces blood pressure that triggers reflex tachycardia, thereby increases myocardial oxygen demand [12]. In the present study, ISP-induced model of myocardial necrosis was used to investigate the cardio-protective effects of SRE by lipid per oxidation, myocyte injury marker and mechanism of its cardio protective effect. ALT, AST, LDH and CPK were present in cardiac muscle, injury to these tissues results in the release of the enzyme of the blood stream. Increased levels are found in myocardial infarction. Increased levels of ALT, AST, LDH and CPK in blood 'the diagnostic markers', were due to the leakage of these enzymes as a result of necrosis induced by ISP in rats [13]. ISP treated rats showed extensive necrosis due to lipid peroxidation the leakage of enzymes from the heart. Reduced necrotic changes in *Evolvulus alsinoides* treated animals could be the reason for the decreased activities of the marker enzymes in Group III & IV animals. In the present study, ISP administration in rats resulted increased generation of cytotoxic free radicals is one among the several mechanisms proposed to explain the ISP-induced myocardial necrosis. Large number of studies has demonstrated that free radicals initiate lipid peroxidation resulting in alteration of membrane integrity, fluidity and permeability [14].

Free radical scavenging enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase are the first line cellular defence against oxidative injury, decomposing O_2 and H_2O_2 before interacting to form the more reactive hydroxyl radical [OH]. The equilibrium between these enzymes is an important process for the effective removal of oxygen stress in intracellular organelles. Glutathione plays an important role in the regulation of variety of cell function and in cell protection from oxidative injury. In the present study, significant reduction in the activities of glutathione-dependent antioxidant enzymes [GPx and GST] and antiperoxidative enzymes [SOD and CAT] with a concomitant decline in the level of reduced glutathione was observed in the heart tissue of Group II myocardial infracted rats as compared to Group I normal control animals, reflecting an increased oxidative stress in ISP induced myocardial injury. This is in accordance with previous investigations , which indicated that the tissue antioxidant status was being operated at diminished level in isoprenaline induced myocardial infarction condition [20]. Depletion of GSH results in enhanced lipid peroxidation can cause increased GSH consumption as observed in the present study [17]. Lowered activities of these prime antioxidant enzymes may lead to the formation of O_2 and H_2O_2 , which in turn can form hydroxyl radical [OH] and bring about a number of reactions harmful to the cellular and sub cellular membranes in the heart tissue. Reduction noticed in the activities of the anti peroxidative enzymes in isoprenaline -induced myocardial infarction might be due to the increased generation of reactive oxygen radicals such as superoxide and hydrogen peroxide, which in turn lead to the inactivation of these enzyme activities. The ethanolic extract of *E. alsinoides* as having antibacterial properties when used in vitro against many common bacteria, including *E. coli*. In one of the study demonstrated antibacterial activity that was comparable to a standard antibiotic [21]. The ethanolic extract of *E. alsinoides* also displays adaptogenic and anti-amnesiac activity in rodents. Nootropic and anti-inflammatory activity in *E. alsinoides* is also well known. *In vivo* studies have also verified the anti-ulcer and anti-catatonic abilities of the herb [19]. A review study sought to verify many of the traditional

claims laid out by the traditional usage of *E. alsinoides*. One study verified that *E. alsinoides* acted as an anthleminitic, antibacterial, anti-oxidant and immunomodulatory with potential use as a gastroprotective. The large doses of *E. alsinoides* may cause drowsiness and lowered mobility, but there is no toxic effect on the body. Pre-treatment with orally administered *Evolvulus alsinoides* extract led to the retention of near normal activities of the clinical marker enzymes in the serum and cardiac tissue. Pretreatment with Evolvulus *alsinoides* extract was associated with a decreased release of enzymes from the cardiac cell fractions, which could be due to the membrane stabilizing effect of *Evolvulus alsinoides* extract on the cardiac cell membrane[18] .

Methanolic extract of *Evolvulus alsinoides* has been reported to possess phenolic compound and flavanoids which exhibit Lipid peroxidation, antioxidant and free radical scavenging properties. The antioxidant property is due to in Methanolic extract of *Evolvulus alsinoides* scavenging for oxygen free radicals [24], resulting in the preservation of cellular viability serving, secondarily, Hence with biochemical and histopathological profiles considerably preserve cardiac cell and thereby, retaining near normal functioning of the cardiac cell thus preventing myocardial necrosis.

4. Conclusion

Evolvulus alsinoides could be a potent Cardioprotective herbal extract with beneficial therapeutic response.

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