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CARDIOPROTECTIVE EFFECTS IN ETHANOLIC EXTRACT OF GREWIA UMBELLIFEREA ON ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN ALBINO RATS

ABSTRACT

The present study was designed to investigate whether *Grewia umbellifera*, a natural herb, would attenuate the acute myocardial infarction in isoproterenol (ISP)-treated rat model via maintaining cardiac function and activities of endogenous antioxidant enzymes. Heart tissue enzyme analysis in albino (Wistar) male rats, such as LPO, GSH, GPX, GST, SOD, CAT, CK-MB, MDA and biochemical analysis in serum plasma viz., ALT, AST, LDH, CPK were performed. The lethal doses (LD₅₀) of the plant extract was determined by method of Lorke using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanol extract of the seed at doses of 10, 100 and 1000 mg/kg body weight. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the ethanolic extract at doses of 1000, 1600, 2900 and 5000 mg/kg bodyweight. The median lethal dose (LD₅₀) was calculated using the second phase. Ethanolic extract of the plant at the dose of (100 & 200 mg/kg/p.o) produced significant salvages the heart from isoproterenol induced myocardial ischemic injury. The results indicate that plant administration causes myocardial adaptation by augmenting endogenous antioxidants and protects rat hearts from decline in cardiac function and oxidative stress associated with the plant induced myocardial injury, and justify its potential therapeutic value in the treatment of ischemic heart diseases in albino rats.

KEY WORDS: *Grewia umbellifera*, antioxidant, Isoproterenol, myocardial infarction

INTRODUCTION

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. Isoproterenol (ISP), a synthetic catecholamine and β -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. 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. 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. As few systematic scientific studies are currently available, these medicinal plants need to be investigated scientifically. The study is an effort in the same direction thus the present investigation was undertaken to evaluate the cardioprotective effects of the ethanolic extract of whole plants of *Grewia umbellifera* (Tiliaceae) in isoproterenol induced myocardial infarction in albino (Wistar) male rats.

MATERIALS AND METHODS

Leaves of *Grewia umbellifera* (GU) was collected and identified and authenticated (SMKFITC11/2012) by V.Chelladurai (Research Officer) Botany (C.C.R.A.S) Government of India. Voucher specimen (SMKFITC-01/2012-2013) has been retained in the Dept. of Chemistry, SMK Fomra Institute & Technology, Chennai. Materials were cleaned with water and dried in the shade until a constant weight was obtained. It was extracted with 95% ethanol in a Soxhlet extractor. Extracts were concentrated; the percentage yield for ethanol extract was 7.9% and for pharmacological studies, since the ethanol extract was not soluble in water, it is suspended in 5% gum acacia. Plant material *Grewia umbellifera* is 150-300cm, small gregarious herb, the leaves are dark green, diamond-shaped, arranged alternately along the stem, 4-8cm long, ovate or ovate-oblong, obtuse at apex. The flowers are moderately delicate, flowers occur singly on flower stalks (peduncles) that arise from the area between the stems and leaf petioles, fruits are ribbed capsule, which breaks up into 8-10 segments. The seeds are trigonous, glabrous, tufted-pubescent near the hilum.

EXPERIMENTAL ANIMAL

The institutional animal ethics committee MicroLabs Institute of Technology, Arcot, India approved the experimental design. Albino (Wistar) male rats of 150-200g (weight) were used for the study. Animals were housed in well ventilated room (temperature $23 \pm 2^{\circ}\text{C}$, humidity 65-70% and 12h light/dark cycle). Animals were fed with standard pellet diet and water ad libitum. All studies were conducted in accordance with Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA) norms and the National Institute of Health guidelines "Guide for the Care and use of Laboratory Animals".

DRUGS AND CHEMICALS

Isoproterenol (Isoprin) was purchased from Unichem laboratories, India, ethanol was purchased from CHANGSHU YANGYUAN CHEMICAL, CHINA; other solvents/reagents were analytical grade.

Acute oral toxicity

The lethal doses (LD₅₀) of the plant extract was determined by method of Lorke (15, 23) using 13 rats. In the first phase rats were divided into 3 groups of 3 rats each and were treated with the ethanol extract of the seed at doses of 5, 50 and 500mg/kg body weight intraperitoneal. They were observed for 24 h for signs of toxicity. In the second phase 4 rats were divided into 4 groups of 1 rat each and were also treated with the ethanolic extract at doses of 500, 1000, 1500 and 2000 mg/kg bodyweight (i.p). The median lethal dose (LD₅₀) was calculated using the second phase.

EXPERIMENTAL PROTOCOLS

Male Wistar rats weighing 150–200 g were pre-treated with the oral dose of 100 and 200mg/kg of GU 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 s.c. on two consecutive days (31th & 32nd day). Symptoms and mortality in each group were recorded and compared with those of rats given isoproterenol alone. 48 h 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. 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 [8], GSH [10], GPX [11], GST [12], SOD [13], CAT [14] and CK-MB [15].

Groups/ Parameters	ALT($\mu\text{mol/L}$)	AST($\mu\text{mol/L}$)	LDH($\mu\text{mol/L}$)	CPK($\mu\text{mol/L}$)
Group I (Normal control)	81.4 \pm 7.42	94.12 \pm 6.74	111.4 \pm 9.64	104.24 \pm 8.48
Group II (Isoproterenol control)	314.14 \pm 27.4***	298.25 \pm 22.6***	260.2 \pm 18.6***	286.14 \pm 23.4***
Group III (GU 100mg/kg/po)	136.04 \pm 21.3***	144.21 \pm 19.4***	152.31 \pm 22.6***	178.1 \pm 14.6***
Group IV (GU 200mg/kg/po)	92.04 \pm 7.64***	111.4 \pm 6.14***	121.9 \pm 8.64***	124.02 \pm 8.76***

Table- 1 : Results of biochemical analysis in serum

RESULTS

Acute toxicity study (LD₅₀)

The sign of toxicity were first noticed after 4 - 8 h of extract administration. There was decreased locomotor activity and decreased in sensitivity to touch. Also there was decreased feed intake, and prostration after 16 h of extract administration. There is no any mortality rate after administered 2000 mg/kg. The median lethal dose (LD₅₀) in rats was calculated to be greater than 2000 mg/kg body weight.

Values are expressed as the mean \pm 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.

Groups/ Parameters	LPO	GSH	GPX	GST	SOD	CAT	CK-MB	MDA
Group I (Normal control)	1.14± 0.05	4.11±0.12	2.76±0.12	1224±88	5.62±0.23	8.12±0.21	152.2±2.6	67.5±2.6
Group II (Isoproterenol control)	2.38± 0.13*	2.14±0.14	1.91±0.07 **	729±56***	2.01±0.04** *	3.25±0.09** *	56.4±7.2***	97.6±1.9** *
Group III (GU 100mg/kg/p o)	1.91± 0.12*	3.84±0.14**	2.20±0.22 **	924±36**	3.02±0.21**	5.44±0.16** *	98.7±2.6***	64.9±7.5**
Group IV (GU 200mg/kg/p o)	1.11± 0.03*	4.14±0.12***	2.91±0.21 **	1237±85***	4.45±0.04** *	8.28±0.24** *	132.2±1.6***	61.2±4.6** *

Table – 2: Results of Tissue enzyme analysis – Heart

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 (H₂O₂ 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: Malonaldehyde; CK-MB: Creatine phosphokinase-MB isoenzyme. One unit of CK-MB transfers 1mmol of phosphate from phosphocreatine to ADP per min at pH 7.4 at 30°C. The values of 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<0.01 moderately significant, * = P<0.05 significant.

DISCUSSION

The plant extracts are characterized by a very low degree of toxicity. The acute toxicity LD₅₀ of Hibiscus sabdariffa l. seed extract in albino rats was found to be above 5000 mgkg⁻¹ according to the method of Lorke (23).

Isoproterenol (ISP), a synthetic β -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, viz. altered membrane permeability, increased turnover of nor-epinephrine and generation of cytotoxic free radicals. In addition, ISP administration reduces blood pressure that triggers reflex tachycardia, thereby increases myocardial oxygen demand [16-18]. In the present study, ISP-induced model of myocardial necrosis was used to investigate the cardio-protective effects of GU by lipid peroxidation, 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. [19, 20] ISP treated rats showed extensive necrosis due to lipid peroxidation the leakage of enzymes from the heart [19]. Reduced necrotic changes in GU 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 [20]. Large number of studies has demonstrated that free radicals initiate lipid peroxidation resulting in alteration of membrane integrity, fluidity and permeability. 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 O₂ and H₂O₂ before interacting to form the more reactive hydroxyl radical (OH \cdot). 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 infarcted 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 [21], which indicated that the tissue antioxidant status was being operated at diminished level in isoprenaline induced myocardial infarction condition. Depletion of GSH results in enhanced lipid peroxidation and excessive lipid peroxidation can cause increased GSH consumption as observed in the present study. Lowered activities of these prime antioxidant enzymes may lead to the formation of O₂ - and H₂O₂, which in turn can form hydroxyl radical (OH \cdot) and bring about a number of reactions harmful to the cellular and subcellular 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.

Pre-treatment with orally administered GU led to the retention of near normal activities of the clinical marker enzymes in the serum and cardiac tissue. Pretreatment with GU was associated with a decreased release of enzymes from the cardiac cell fractions, which could be due to the membrane stabilizing effect of GU on the cardiac cell membrane. GU 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 ethanolic extract of *Grewia umbellifera* scavenging for oxygen free radicals, resulting in the

preservation of cellular viability serving, secondarily, to preserve cardiac cell and thereby, retaining near normal functioning of the cardiac cell thus preventing myocardial necrosis.

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