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Research Article

EFFECT OF AQUEOUS EXTRACT OF *PUNICA GRANATUM* FLOWER ON BIOMARKERS AND ECG CHANGES IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT
The present study was designed to investigate the effect of aqueous extract of *Punica granatum* flower (PG) against isoproterenol (ISO) induced myocardial infarction (MI) in rats by studying cardiac markers and electrocardiographic changes. MI was induced in rats by subcutaneous injection of ISO (150mg/kg b.w) at an interval of 24 hours for 2 days. ISO treated rats showed significant increase in cardiac markers such as Lactate dehydrogenase (LDH), Creatine kinase (CK-MB), Alanine aminotransferase (ALT), Aspartate aminotransferase (AST) in serum. Altered ECG pattern with ST segment elevation, which conforms the MI. Oral pretreatment with PG at 400mg/kg and Vitamin E (100mg/kg as a standard) daily for a period of 28 days showed significant decrease in serum cardiac marker enzymes level and ECG alterations. Thus the result suggests that PG has cardioprotective effect in ISO induced myocardial infracted rats.
KEYWORDS: *Punica granatum* flower, isoproterenol, myocardial infarction, vit-E, rat.

INTRODUCTION

Cardiovascular diseases remain the principal cause of death in both, developed and developing countries accounting for roughly 20% of all deaths worldwide per year[1]. Cardiovascular diseases will be the most important cause of morbidity in India by the year of 2015[2]. Myocardial ischemic reperfusion injury is a common accompaniment and is a cause of morbidity in ischemic heart disease, where oxidative stress plays an important role[3].

MI is the acute condition of necrosis of the myocardium that occurs as a result of imbalance between coronary blood supply and myocardium demand[4]. It is well recognized that there is increasing generation of reacting oxygen species such as superoxide anion and hydroxyl radicals and other reactive species in ischemic tissue, bringing about oxidative damage of membrane lipids, proteins, carbohydrates and DNA. Hence therapeutic intervention with antioxidant may be useful in preventing these deleterious changes[5].

ISO induced MI is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction[6]. ISO, a synthetic catecholamine and ß-adrenergic agonist, that causes severe stress in myocardium and necrotic lesions in the heart muscles[6,7]. ISO has been reported to show many metabolic and morphological aberrations in the heart tissue on the experimental animals similar to those observed in human MI[8].

The synthetic drugs like organic nitrates, calcium channel antagonist and β-Blockers are recently used to treat MI but they are not free from side effects like hypotension, bradycardia and dizziness etc[9]. Herbal medicines are increasingly gaining greater acceptance from the public and medical profession due to greater advances in the understanding of the mechanisms by which herbs positively influence health and quality of life[10]. Common belief that, herbal formulations are safer than modern drugs has lead to increasing use of herbal preparation. The prophylactic and therapeutic effect of many plant extracts such as Withania somnifera[11], Allium sativum[12], Cladosiphon okamuranus[13], Azadirachta indica[14] etc, in reducing cardiovascular diseases have been reported.

Punica granatum Linn. (family -Punicaceae) is commonly known as pomegranate tree in English, anar in Hindi and dadam in Gujarati[15]. In traditional medicinal system *Punica granatum* used as diuretic,

cardiotonic and in vomiting[16]. A number of biological activities such as antitumour[17], antibacterial[18], antidiarrhoeal[19], antifungal[20], antiulcer[21], anti-diabetic[22] and anti plasmodial[23] have been reported with various extracts/constituents of different parts of this plant.

It is reported that the flowers of *Punica granatum* possess a very high content of polyphenols, which are responsible for antioxidant activity[24]. It also contains reducing sugars, cardiac glycosides, saponins, tannins and Vit-C. The aim of the present study is to explore the cardioprotective activity of aqueous extract of *Punica granatum* flower in ISO induced MI in Wistar rats.

MATERIAL AND METHOD

Collection of plant material

The *Punica granatum* flower were collected from Satara district, Maharashtra, India and identified and authenticated by Dr. Harsha Hegde Taxonomist, Regional Medical Research Centre (RMRC), Belgaum, where the herbarium of the specimen is deposited (Voucher no. RMRC-510).

Preparation of extract

The shade dried flowers of *Punica granatum* were ground with a mechanical grinder. The grounded material (500gm) was macerated in distilled water for 7 days. The extract was concentrated to dryness under reduced pressure in a rotary evaporator to yield dried aqueous extract and that was stored at 4^0 c for further experimental study.

Chemicals

ISO was obtained from sigma chemicals (USA) and Vit-E is a generous gift from Merck pharmaceuticals, Goa. All other chemicals used were of analytical grade.

Experimental animals

The complete course of experiment was carried out using healthy male Wistar rats weighing between 150-200 gm, were procured from Sri Venkateshwara enterprises Bangalore. They were housed in standard laboratory condition at room temperature along with 12 h light/dark cycle. The animals were provided with standard pelleted diet obtained commercially from the manufacturer (Amrut Laboratories, sangli) and water ad libitum. After seven days of acclimatization period, they were randomly selected for different experimental groups. Ethical clearance was obtained from Institutional Animal Ethics Committee (IAEC/CPCSEA), before conducting the experiment.

Acute toxicity studies

The acute oral toxicity study was carried out using female Wistar rats (150-200gm) as per guidelines set by organization for Economic Co-operation and Development (OECD-420). The PG was orally administered to different groups of rats at the dose of 50, 300, 1000, 2000mg/kg body wt. respectively. Animals were observed for 24 hr to study the general behaviour of animals, sign of discomfort and nervous manifestations till 14 days. The PG was found devoid of mortality of animals at the dose of 2000 mg/kg body wt. Hence the $1/5^{th}$ (400mg/kg) was selected for the screening of cardioprotective activity.

Experimental protocol

Animals were divided into six groups of six each.

Group I (Normal control) - Normal saline (5ml/kg b.wt).

Group II (Disease control) - Normal saline + ISO (150mg/kg b.wt).

Group III – 400 mg/kg b.wt of PG + ISO.

Group IV - 100 mg/kg b.wt of vitamin E + ISO.

All the animals were pretreated with normal saline or PG for 28 days. At the end of the treatment period, animals of all groups excluding Group - I was administered ISO (150 mg/kg body wt. s.c)[25] for two consecutive days at the interval of 24hr.

Biochemical analysis

48 hrs after the first dose of ISO animals were anaesthetized with Thiopentone sodium (50mg/kg). The blood was collected from retro-orbital sinus. Serum was separated by centrifugation. Serum Creatine kinase isoenzyme (CKMB), Lactate dehydrogenase (LDH), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured kinetically at 340 nm according to standard methods by using commercially available diagnostic kits from ERBA, Germany.

Examination of electrocardiogram (ECG)

Cardio physiology parameters were estimated by using ECG. 48 hrs after the first dose of ISO Lead II ECGs of all animals were recorded using Biopac Student Lab PRO 3.7 software (model no. MP-35) make BIOPAC systems, Inc.42 Aero Camino, Goleta, CA93117. For each ECG tracing QT interval, QRS complex and heart rate were measured.

Statistical analysis

Results were expresses as Mean ± S.E.M. the statistical significance of any difference in each parameter among the groups was evaluated by one–way ANOVA, using Dunnets multiple comparison test as post hoc test.

RESULTS

Acute administration of ISO (150 mg/kg s.c for two consecutive days) induces myocardial infarction and showed significant increase in the levels of serum cardio biomarker enzymes viz., LDH, CK-MB, ALT, AST

when compared to normal rats (P< 0.0001). The increased concentration of serum enzymes is a well accepted quantitative index of myocardial damage caused by ISO treatment. Pretreatment with PG at 400mg/kg b.wt. p.o. significantly (P<0.001) reduced the elevated serum enzyme levels when compared to ISO treated rats. (Table 1).

ECG alterations

ISO treated group showed significant changes in the repolarization phase of the ECG: Significant prolongation of QT interval and elevation of ST segment, with no significant effect on QRS complex as compared to normal group. In addition a significant increase in heart rate of ISO treated rats was observed as compared to normal group. Pretreatment with PG at 400mg/kg significantly (P<0.001) reduced the ECG alterations when compared to ISO induced myocardial infracted rats. (Table.2).

DISCUSSION

The myocardial infarction was induced by administration of Isoproterenol[1-(3,4-dihydroxyphenyl)-2-isopropylamino-ethanolhydrochloride][26], which is a synthetic catecholamine and β–adrenergic agonist that induces severe stress in the cardiac muscle leading to development of myocardial necrosis. ISO induced MI 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 ISO induced MI[26].

The serum marker enzymes viz. LDH, CKMB, AST and ALT serve as sensitive index to assess the severity of MI[27]. In ISO treated rats, the increased activities of the serum marker enzymes confirmed MI.

ECG-abnormalities are the main criteria generally used for the definite diagnosis of MI. ST-segment elevation, significant increase in heart rate and QT-interval serve as definite diagnostic markers of MI[28]**.** These alterations could be due to the consecutive loss of cell membrane in injured myocardium. In the present study, we observed an elevation of ST-segment, increased heart rate and QT-interval in ISO treated rats, and pretreatment with PG markedly inhibited ISO induced ECG alterations.

Literature survey revealed that the aqueous extract of pomegranate flower possess free radical scavenging, antioxidant and anti diabetic activities. And it is capable of protecting lipids and proteins against oxidative damage and also increases/maintains the levels of antioxidant enzymes in vivo. PG contains a high amount of polyphenolics, which may be responsible for antioxidant activity[22].

Extent of cardioprotection offered by the drug is associated with significant attenuation of serum LDH, CKMB, ALT, AST levels. In the present study, A significant (P<0.001) reduction in the serum biomarker enzymes and ECG alterations were observed in the myocardial infracted rats pretreated with PG at 400mg/kg b.wt dose.

From the present study it is difficult to establish mechanism of action for cardioprotection against ISO induced MI. Phyto-constituents like polyphenols, saponins, tannins and Vit-C may play a vital protective role against oxidative stress induced damage.

CONCLUSION

The present study suggests that pretreatment with aqueous extract of *Punica granatum* flower decreased the level of cardiac marker enzymes and normalized the ECG pattern in ISO induced myocardial infracted rats. The flower was found to be most effective in restoration of biochemical and ECG alterations in ISO induced MI. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Punica granatum* flower.

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Table 1: Effect of aqueous extract of *Punica granatum* flower on serum ALT, AST, LDH, CK-MB in ISO induced MI in rats

GROUPS	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	CK-MB (IU/L)
Group I	22.37±1.948	31.05±0.77	109.3±2.87	82.49±1.874
Group II	45.71±2.482$^{\#\#\#}$	67.71±3.064$^{\#\#\#}$	189.7±2.35$^{\#\#\#}$	243±6.212$^{\#\#\#}$
Group III	30.12±1.866**	34.24±2.447***	125.3±2.49***	129.5±3.369***
Group IV	24.50±1.071***	31.87±1.893***	112.5±1.017***	101.4±1.821***

Compared with Normal control: $^{\#}$P<0.01, $^{\#\#}$P<0.001, $^{\#\#\#}$P<0.0001.
Compared with Disease control: *P<0.01, **P<0.001, ***P<0.0001.

Table 2: Effect of aqueous extract of *Punica granatum* flower on ECG parameters in ISO induced MI

GROUPS	HEART RATE (BPM)	QRS COMPLEX (Sec)	QT INTERVAL (Sec)
Group I	352.5±18.45	0.055±0.0018	0.090±0.0012
Group II	435.8±16.167$^{\#\#\#}$	0.040±0.0028$^{\#\#}$	0.1075±0.0021$^{\#\#\#}$
Group III	364.2±15.724***	0.0525±0.003*	0.095±0.0028*
Group IV	349.5±18.49***	0.060±0.0012***	0.090±0.0022**

Compared with Normal control: $^{\#}$P<0.01, $^{\#\#}$P<0.001, $^{\#\#\#}$P<0.0001.
Compared with Disease control: *P<0.01, **P<0.001, ***P<0.0001.

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