CARDIOPROTECTIVE PROPERTIES OF PYRAZOLONE DERIVATIVES AGAINST ISOPROTERENOL INDUCED MYOCARDIAL ISCHEMIC INJURY

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Summary

The present study investigates the cardioprotective effect of pyrazolone derivatives (PYZ1-PYZ10) on plasma lipid profile, serum marker enzymes, endogenous enzymatic and non-enzymatic antioxidants in cardiac tissues against isoproterenol (ISO) induced myocardial ischemic injury in rats. Pretreatment with the pyrazolone derivatives at 10 mg/kg body weight for 5 days prevented the elevation of serum marker enzymes namely lactate dehydrogenase (LDH), aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in myocardial injured rats. ISO-induced animals exhibited decreased levels of superoxide dismutase (SOD) and glutathione (GSH) in the heart, which were restored to near normal levels following treatment with pyrazolone derivatives. These derivatives also attenuated lipid peroxidation (LPO) in the heart and improved the imbalance in lipid profile (TG, LDL, VLDL, HDL) caused by ISO. These findings revealed the cardioprotective effect of pyrazolone derivatives against isoproterenol induced myocardial injury.

Key words: plasma marker enzymes, cardioprotective, reperfusion injury, pyrazolone

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Introduction

Ischemic heart disease (IHD) is one of the leading causes of deaths worldwide, accounting for 16.7 million deaths per annum [1, 2]. It occurs when blood supply is insufficient to the myocardium as a result, death of myocardial muscle occurs and such condition is known as ischemia. The prolonged ischemia of the myocardium leads to necrosis, which is referred as myocardial infarction [3]. Drugs such as beta-blockers and angiotensin converting enzyme inhibitors (ACEIs) had significantly improved the survival rate of IHD patients. However, attempts are made globally to get complementary and alternative medicines for IHD since no sufficient therapy for this obstinate illness is available. Pyrazolone ring systems represent an important class of compounds not only for their theoretical interest but also for their anti-inflammatory, analgesic, antipyretic [4], hypoglycemic agent [5], fungicide [6], antimicrobial [7] and some of them have been tested as potential cardiovascular drugs [8] including hypertension, hypercholesterolemia, atherosclerosis, myocardial infarction, angina pectoris, and heart failure[9]. Moreover, it has been proved that these molecules have preventive effects on myocardial injury following ischemia and reperfusion in the rat heart [10] and in patients with acute myocardial infarction [11]. Pyrazolone derivatives have been used in patients with acute brain infarction since April 2001 in Japan [12]. These derivatives have been shown to be effective against brain edema after ischemia and reperfusion injury in animal models [13] and in stroke patients [14]. Some animal studies using acute myocardial ischemia-reperfusion models have suggested the protective effects of pyrazolone derivatives on myocardial damage. By taking these research findings as evidence, the present study is focused to explore the cardioprotective properties of pyrazolone derivatives against isoproterenol induced myocardial ischemic injury.

Material and methods

Chemicals and Drugs

Isoproterenol hydrochloride (ISO) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sodium chloride, magnesium chloride, adenosine triphosphate and trichloroacetic acid (TCA) were purchased from Himedia Laboratories Private Ltd. (Mumbai, India). Nitroblue tetrazolium, phenazine methosulphate and nicotinamide adenine dinucleotide (NAD) were purchased from Sisco Research Laboratories Ltd. (Mumbai, India). The chemicals used in this study were of analytical grade.

Test compounds

Pyrazolone derivatives investigated in the present study were synthesized,characterized in Department of PharmaceuticalChemistry, Himalayan Pharmacy Institute, Sikkim. The physiochemical data of the synthesized compounds have been given in Table.1

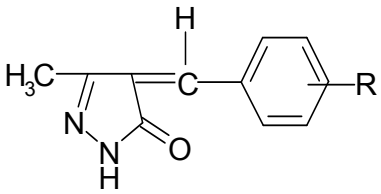
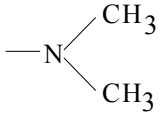


Table 1: Physical data of the synthesized compounds

Compound code	-R	Molecular formula	Mol. weight
PYZ1		$C_{13}H_{15}N_3O$	239
PYZ2	phenyl ethenyl	$C_{13}H_{12}N_2O$	212
PYZ3	$-OCH_{3(2,3)}$	$C_{13}H_{14}N_2O_3$	246
PYZ4	$-NO_2$	$C_{11}H_9N_3O_3$	231
PYZ5	Cl	$C_{11}H_9N_2OCl$	221
PYZ6	H	$C_{11}H_{10}N_2O$	186
PYZ7	$-CH_3$	$C_6H_8N_2O$	128
PYZ8	4-OCH_3	$C_{12}H_{12}N_2O_2$	216
PYZ9	4-OCH_3	$C_{12}H_{12}N_2O_2$	216
PYZ10	- OH	$C_{11}H_{10}N_2O_2$	202

Diagnostic Kits

Total Cholesterol, Total Protein, HDL, VLDL, LDL, TG, AST (SGOT), ALT (SGPT) and Alkaline Phosphatase were purchased from Span Diagnostics Ltd., Surat, India.

Animals

Rats (sprague-dawley or wistar, 100-150 g) were used as an experimental animals. They were housed hygienically under standard conditions of temperature (24±1ºc), relative humidity (65±10%) and 12 light/dark cycle environment. During the study period, guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Institutional Animals Ethics Committee (IAEC) were followed for the maintenance of animals. The research work was approved by IAEC No: HPI/09/60/IAEC/0075.

Experimental Procedure

Seventy two male rats were completely randomized into twelve groups of six animals in each group. Group 1: normal control (distilled water p.o.), Group 2: ISO-treated (5.5 mg and 8.5 mg/ kg, s.c.) at an interval of 24 h for two days . Groups 3 to 12 were administered with 10 mg/kg body weight/ day p.o. of Pyrazolone derivatives (PYZ1-PYZ10) for 5 days followed by ISO treatment at an interval of 24 h for two days.

24 hours after the second injection of ISO, the rats were sacrificed by ether anaesthetization and the heart was dissected out. The neck area was quickly cleared of fur to expose the jugular vein. The vein, after being slightly displaced, was sharply cut with sterile surgical blade and an aliquot (5 ml) of the blood was collected and centrifuged at 10000g for 5 mins. The serum was carefully aspirated with a Pasteur pipette into sample bottles for biochemical analysis.

Plasma lipid profile

Plasma total cholesterol (TC), Total Protein, Creatinine, triglycerides (TG) and high density lipoprotein (HDL) were analysed using commercially available kits (Reckon diagnostics, Baroda, India).Very low density lipoproteins (VLDL) and low density lipoprotein (LDL) were calculated as per Friedewald et al. [15].

Plasma cardiac specific injury markers

Activity levels of creatine phospokinase (CPK), lactate dehydrogenase(LDH), alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphate (ALP) in plasma were estimated using commercially available kits (Eve's Diagnostics, Baroda, India).

Estimation of cardiac endogenous antioxidant

Cardiac tissue pieces from control and treated groups were weighed and homogenized (10% w/v) in chilled Tris buffer (10 mM, pH 7.4), centrifuged at 10,000g for 20 min in high speed cooling centrifuge (0 1C). Clear supernatant was used for assaying superoxide dismutase (SOD); [16], catalase (CAT); [17] and reduced glutathione (GSH); [18]

Statistical Analysis

All the results were expressed as mean±SEM. The results were analysed by one way analysis of variance (ANOVA) followed by Dunnett's test through the computer program Graph Pad Instat 3. P value <0.05, $P<0.01$ was considered statistically significant.

Results and discussion

There were alterations in serum lipid profile of the rats administered with pyrazolone derivatives when compared with the Isoproterenol control. The Isoproterenol raised the serum levels of total cholesterol, Total Protein, LDL, VLDL LDL, triglycerides; and decreased HDL level. Pretreatment with the pyrazolone derivatives for 5 days however, restored the lipid profile to near normalcy and improved the cardiac damage caused by isoproterenol (Table 2 and Table 3). Lipid metabolism plays an important role in myocardial injury produced by ischemia [19]. Isoproterenol causes hyperlipidemia and it increases the LDL cholesterol in the blood, which in turn leads to harmful deposits in the arteries thus favoring coronary heart diseases (CHD) [20]. In the present study also, ISO administration caused a significant raise in the serum lipids thereby increases lipid biosynthesis and lipid peroxidation. Rats treated with pyrazolone derivatives showed decreased concentration of serum total cholesterol, triglycerides, LDL cholesterol indicates the beneficial effects of pyrazolone derivatives in reducing hyperlipidemia caused by Isoproterenol.

The administration of Isoproterenol in rats resulted significant increase in the serum levels of heart marker enzymes including LDH, AST, ALT and ATP However, pretreatment with Pyrazolone derivatives reduced the activities of these enzymes to near normal levels (Table 2).

Table 2: Effect of pyrazolone derivatives on marker enzymes in ISO induced myocardial injury in rat model

Compd code	SGOT (IU/ml)	SGPT (IU/ml)	ALP (IU/ml)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Control	152.56±0.15	40.56±0.31	141.81±.71	43.87±.01	99.56±.11	8.05±1.25
ISO (8.5 mg/kg)	203.26±.02	81.23±0.43	213.89±0.81	20.58±1.2	186.87±.13	30.22±.01
PYZ1	191.52±0.61^{b}	79.01±0.26^{b}	210.56±0.42^{ns}	23.54±1.65^{a}	165.25±.56^{b}	23.33±.11^{b}
PYZ2	190.12±0.51^{b}	79.99±0.11^{ns}	208.20±0.34^{b}	23.99±.11^{a}	165.66±.23^{b}	23.01±.51^{b}
PYZ3	181.84±0.63^{b}	76.44±0.13^{b}	207.32±0.62^{b}	24.89±.31^{b}	154.84±.29^{b}	20.38±.65^{b}
PYZ4	184.91±0.71^{b}	76.00±0.01^{b}	200.89±.10^{b}	25.63±.84^{b}	153.22±.55^{b}	19.99±2.63^{b}
PYZ5	182.38±0.02^{b}	74.55±0.71^{b}	198.36±0.52^{b}	27.00±.67^{b}	146.40±.10^{b}	18.85±1.85^{b}
PYZ6	180.01±0.41^{b}	72.36±0.10^{b}	195.39±1.20^{b}	28.56±.24^{b}	136.55±.61^{b}	15.84±1.95^{b}
PYZ7	179.85±0.53^{b}	70.85±0.63^{b}	193.95±1.90^{b}	29.66±.01^{b}	134.66±.01^{b}	14.55±2.55^{b}
PYZ8	164.22±0.87^{b}	61.02±0.45^{b}	176.95±.97^{b}	31.11±.03^{b}	130.58±.33^{b}	12.86±.01^{b}
PYZ9	162.00±0.72^{b}	55.00±.06^{b}	175.00±.15^{b}	32.33±.21^{b}	125.98±.78^{b}	10.62±.11^{b}
PYZ10	158.01±1.01^{b}	54.62±0.14^{b}	172.83±1.57^{b}	36.98±.02^{b}	120.88±.91^{b}	10.01±.02^{b}

Values are expressed as mean±SEM, (N=6); ns non significant; ^{a}P <0.05, ^{b}P<0.01 compared with ISO control (ANOVA followed by Dunnett's test). All the test compounds were administered at 10 mg/kg.

Table 3: Effect of pyrazolone derivatives on lipids profile in ISO induced myocardial injury in rat model

Compd code	Total Protein (g/L)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Creatinine (mg/dL)	Lactose Dehydrogenase (IU/L)
Control	39.80±0.98	79.04±3.97	117.15±2.02	2.26±0.04	120.07±0.54
ISO(8.5 mg/kg)	86.86±0.25	186.47±0.34	201.65±7.56	3.93±0.08	198.71±0.29
PYZ1	56.46±.050[b]	135.43±3.18[b]	168.61±0.63[b]	2.81±0.007[b]	134.25±0.58[b]
PYZ2	66.33±0.28 [b]	122.32±0.70[b]	162.75±0.74[b]	2.92±0.01[b]	142.70±0.51[b]
PYZ3	53.00±4.70 [b]	180.58±4.44[b]	153.95±0.81[b]	2.65±0.18[b]	148.45±0.41[b]
PYZ4	40.43±0.85 [b]	152.94±4.29[b]	133.46±0.58[b]	2.39±0.03[b]	158.39±0.47[b]
PYZ5	39.23±0.92 [b]	158.33±3.74[b]	132.14±0.73[b]	2.65±0.01[b]	138.55±0.52[b]
PYZ6	58.07±0.86 [b]	123.33±0.61[b]	118.04±0.37[b]	2.67±0.04[b]	128.83±0.71[b]
PYZ7	38.08±0.79 [b]	124.66±0.94[b]	120.59±0.53[b]	2.67±0.07[b]	123.68±0.95[b]
PYZ8	48.36±0.66 [b]	80.07±0.37[b]	121.01±0.73[b]	2.78±0.07[b]	121.80±0.50[b]
PYZ9	52.20±0.77[b]	81.20±0.35[b]	122.89±0.45[b]	2.56±0.0 [b]	127.76±0.59[b]
PYZ10	53.06±0.79[b]	82.95±0.75[b]	123.82±0.64[b]	2.45±0.10[b]	127.25±1.8 [b]

Values are expressed as mean±SEM, (N=6);
[b]P<0.01 compared with ISO control (ANOVA followed by Dunnett's test). All the test compounds were administered at 10 mg/kg.

Pyrazolone derivatives pre-treatment improves cardiac antioxidant status in ISO induced myocardial injury by effective scavenging of free radicals generated during oxidation of lipids thus collectively contributing to its overall antioxidant and anti ischemic activity. ISO induced myocardial injury has been reported to alter membrane permeability [19] and to cause leakage of marker enzymes of cardiac damage (LDH, CPK, AST, ALT and ALP) into the blood stream[21]. Significantly elevated levels of these marker enzymes have been recorded in ISO induced myocardial damage [22]. However, PYZ treated group controlled the elevation in activity levels of these enzymes suggesting that with PYZ pretreatment ISO induced leakage of marker enzymes can be prevented.

Table 4: Effect of pyrazolone derivatives on endogeneous antioxidant enzymes in ISO induced myocardial injury in rat model

Compound code	SOD U/mg of protein	CAT IU/mg of tissue	GSH µg /mg wet tissue	CPK IU/ml
Control	5.38±0.21	73.05±.02	5.63±.02	110.25±0.01
ISO(8.5 mg/kg)	1.99±0.01	40.11±.10	1.69±0.12	194.45±0.25
PYZ1	2.71±0.02[a]	43.25±.21[a]	2.01±0.11[ns]	190.85±0.74[a]
PYZ2	2.91±0.14[b]	43.00±.06[a]	2.65±0.21[ns]	188.65±0.46[b]
PYZ3	3.01±0.04[b]	44.55±.11[b]	2.97±0.14[b]	184.29±1.01[b]
PYZ4	3.51±0.13[b]	46.88±.56[b]	3.86±0.18[b]	180.11±0.08[b]
PYZ5	3.80±0.02[b]	49.57±.32 [b]	3.88±0.19[b]	179.86±0.87[b]
PYZ6	3.91±0.14[b]	50.22±.55[b]	3.15±0.25[b]	175.97±1.56[b]
PYZ7	3.99±0.31[b]	50.00±1.21[b]	3.6±0.3[b]	168.52±1.50[b]
PYZ8	4.01±0.42[b]	53.85±1.38[b]	3.55±0.15[b]	160.28±0.05[b]
PYZ9	4.49±0.02[b]	54.92±.07[b]	4.02±0.49[b]	158.87±0.24[b]
PYZ10	4.99±0.11[b]	58.87±1.02[b]	4.09±0.28[b]	158.77±0.08[b]

Values are expressed as mean±SEM, (N=6); ns. non significant

[a]P<0.05, [b]P<0.01 compared with ISO control (ANOVA followed by Dunnett's test). All the test compounds were administered at 10 mg/kg.

As reported in Table 4, there was significant decrease in GSH levels in the heart of ISO-treated rats. Pretreatment with pyrazolone derivatives resulted in marked improvement in these indices at the end of the experiment and were reverted back to normalcy. In addition, the significantly decreased activities of enzymic antioxidants (CAT,GSH and SOD) observed in the heart of ISO-treated rats were improved following pretreatment with pyrazolone derivatives for 5 days. The present study has clearly demonstrated that the pyrazolone derivatives have antioxidant activity which could prevent the occurrence of heart related diseases. Significantly elevated activities levels of SOD and CAT recorded in PYZ treated group could be due to its potent free radical scavenging ability. GSH scavenges singlet oxygen, superoxide and peroxy radicals to form oxidised glutathione and other disulfides [23]. Also, antioxidant compounds have been shown to increase glutathione reductase activity that maintains GSH in a reduced state [22]. The elevated GSH content observed in PYZ treated groups may be due to its enhanced synthesis.

ISO treatment is also known to create an imbalance between enzymatic as well as non-enzymatic antioxidant defence system leading to production of free radicals that induce myocardial injury and LPO [24]. The significant decrement in LPO in pyrazolone derivatives treated group further justifies the role of pyrazolone derivatives as a potent antioxidant and free radical scavenger. These results are in conformity with reports that have demonstrated modulation of cellular antioxidant by treatment with pyrazolones[25]. It can be summarized that PYZ pre-treatment to ISO treated rats provide cardioprotection by inhibiting the formation of free radicals generated during oxidation of lipids thus inhibiting peroxidation of membrane lipids and preventing subsequent leakage of soluble enzymes. Also, pyrazolone derivatives pre-treatment appears to improve the status of enzymatic antioxidants that further contributes to its overall cardioprotective property. Hence, it can be concluded that pyrazolone derivatives pretreatment provides cardioprotection against ISO induced myocardial injury via multiple mechanisms.

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