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# Cardioprotective potential of methanol extract of *polygonum glabrum* on isoproterenol Induced myocardial necrosis in Rats

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## Abstract

The aim of present study was to evaluate the cardioprotective efficacy of *Polygonum glabrum* on isoproterenol induced myocardial necrosis in rats. Methods: Experimental rats were treated orally with methanol extract of *Polygonum glabrum* at two doses (200 mg and 400 mg/kg) for 30 days. Isoproterenol (85 mg/kg, s.c.) was administered on 29$^{th}$ and 30$^{th}$ day to induce myocardial necrosis. At the end of the experiment, serum cardiac marker enzymes [creatine kinase muscle brain (CK-MB), lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (SGOT)], serum glutamate pyruvate transaminase (SGPT) and total protein (TP) were estimated. Plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels were also recorded. Further, antioxidant parameters viz catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GRD) and malondialdehyde (MDA) levels were evaluated in heart tissue homogenate. Results: The results of the study indicated that, methanol extract of *Polygonum glabrum* showed greater cardio protection by restoring the cardiac marker enzymes and attenuated the level of plasma lipid profiles along with an increase in HDL. Additionally, level of myocardial antioxidants significantly increased along with a reduction in the content of malondialdehyde. The cardio protective effect was compared with propranolol (10 mg/kg, oral) which was used as the standard. Histopathological findings revealed a decrease in the degree of necrosis and inflammation following pretreatment with *Polygonum glabrum*. Conclusion: The present investigation indicates that *Polygonum glabrum* could protect myocardium from isoproterenol induced necrosis.

**Keywords:** *Polygonum glabrum*, Isoproterenol, Necrosis, Marker enzymes.

## Introduction

From a global health perspective, ischemic heart disease turns out to be the leading cause of death annually. Myocardial impairment occurs when insufficient amount of oxygenated blood is supplied to the myocardium through coronary arteries. Since the relative demand of myocardium is not balanced, a condition called acute myocardial ischemia occurs [1]. The major consequences of this condition are arrhythmia, contractile disfunction and myocardial necrosis. A cursory examination by the world health organization revealed that ischemic heart disease was one of the major causes for annual deaths worldwide [2]. Certain factors such as high blood pressure, hyper cholesterol, diabetes and living a sedentary life style may increase the incidence of myocardial necrotic attack [3]. When myocytes are exposed to inherently damaging agents such as toxins, trauma and various physical and chemical agents, the cell chooses necrosis as the common pathway to cell death [4]. The infarcted myocardium begins to undergo coagulative necrosis shortly hours after cell death. Morphological changes associated with necrosis are disintegration of total nuclei, cytoplasmic cross-striations and infiltration of inflammatory cells in first three days, pursued by fragmentation of cardiomyocytes in the first week. In the next week, the granulation tissue advances and matures as a dense collagen deposit. Finally a faint scar is formed at the second month [5].

Isoproterenol (ISO) is a synthetic bronchodilator which produces classic site specific cardiac necrosis by increasing the amplitude and frequency of cardiac contractions leading to greater cardiac output [6]. The overload imbibed on cardiomyocytes results in excessive activation of calcium dependent ATPases impairing mitochondrial oxidative phosphorylation. Since isoproterenol is a catecholamine it also undergoes auto oxidation to aminochromes and free radicals causing a severe oxidative stress in the

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myocardium associated with necrosis of the left ventricular heart muscle [7]. Conventional treatment includes use of synthetic drugs like beta blockers, anticoagulants, calcium channel blockers, antithrombolytics, angiotensin converting enzymes (ACE) inhibitors and surgeries (cardiac catheterization, angioplasty). Since these drugs were associated with limitations like side effects, cost, non availability, resistance development etc. herbal treatments were tried to substitute them. Several plants (*Ocimum gratissimum, Terminalia chebula, Oroxylum indicum* etc) and herbo mineral formulations were useful in the treatment of myocardial necrosis, congestive heart failure and renal failure [8].

*Polygonum glabrum* is a semi aquatic perennial plant. Traditionally, it is used as a cardiotonic, astringent, anthelmentic and for the treatment of jaundice, rheumatism and piles [9]. Pharmacologically, oral administration of methanol extract produced significant nephro protection, hypolipidaemic and cytotoxic effect [10,11]. The methanol extract showed maximum antimicrobial activity against pathogens such as *Staphylococcus aureus, Micrococcus luteus, Candida tropicalis* etc [12]. Substantial literature survey suggested that many phytoconstituents such as (2)-2-methoxy-2-butenolide-3-cinnamate, beta-hydroxyfriedalanol, 3-hydroxy-5- methoxystilbene, pinocembrin, sitosterol-(6 -O-palmitoyl)-3-O-β-Dglucopyranoside, pinocembrin-5-methyl ether and sitosterol-3-O-βDglucopyranoside were isolated from *Polygonum glabrum* [13]. Other flavonoids (avicularin, rhamnetin, diosmetin, cyanidin 3 5-diglucoside, delphinidin 3,5, diglucoside) and sesqueterpene diesters were isolated from methanol extract of leaves and flowers of *Polygonum glabrum* [14,15]. As far as our knowledge, the potential of *Polygonum glabrum* as a cardio protective agent was not demonstrated earlier. Hence, present study was focused on evaluating the cardioprotective effect of methanol extract of *Polygonum glabrum* on rats intoxicated with isoproterenol.

## Experimental

# Materials and methods

## Chemicals

Thiobarbituric acid, reduced glutathione and propranolol were obtained from SD fine chemicals Ltd. (Mumbai, India). ROCHE diagnostic kit was used for CK-MB enzyme estimation. Total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were analyzed using EXCEL diagnostic kit. Isoproterenol was procured from Sigma chemicals, USA.

## Plant collection

*Polygonum glabrum* was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, Professor, Botany department, Sri Venkateswara University, Tirupati. The plant material with Voucher specimen No - 1916 was preserved in the herbarium of GITAM Institute of Pharmacy, GITAM University, for future reference. The whole plant was washed, air-dried, homogenized to fine powder and stored under ambient conditions.

## Extraction

About 500 gm of plant powder was extracted with methanol in a Soxhlet apparatus. The extraction was done continuously till a clear solvent was observed in the thimble. The excess solvent was removed from methanol extract using a rotary vacuum evaporator and later on concentrated on a water bath. The percentage yield of the extract was calculated. Finally dried extract was stored in desiccators for cardio protective study.

## Safety evaluation

Acute oral toxicity study in experimental rats was carried out as per OECD-423 guidelines. Four doses (10, 50, 300, 2000 mg/kg body weight) of methanol extract were administered orally to groups containing three animals of the same age group and weight. The animals were monitored for 1 hour continuously and then hourly for 4hr and finally after every 24hr up to 15 days for any symptoms of toxicity and mortality [16].

## In-vivo cardio protective activity

## Experimental animals

Albino rats of wistar strain of either sex (200-250gm) procured from Ghosh enterprises was used. The animals were maintained in standard laboratory conditions. They were fed with standard pellet diet and water ad libitum. The study protocol was reviewed and approved by the Institutional Animal Ethical Committee (IAEC) and experiments were conducted as per the guidelines of CPCSEA. Reg. Number: 1287/PO/Re/S/09/CPCSEA.

## Experimental design

A total of thirty rats were divided into5 groups of 6 rats each. Group I was treated as normal control and received only saline (1ml/kg/day p.o) for 30 days. Group II was treated with saline (1 ml/kg, p.o.) daily for 30 days and in addition received ISO (85 mg/kg, s.c.) on 29$^{th}$ and 30$^{th}$ day at an interval of 24 hours. Group III was treated with standard propranolol (10 mg/kg, p.o) for one week after two week of saline treatment and in addition received ISO (85 mg/kg, s.c.) on the 29$^{th}$ and 30$^{th}$ day at an interval of 24 hours. Group IV was treated with methanol extract of *Polygonum glabrum* (200 mg/kg, p.o.) daily for 30 days and in addition received

ISO (85 mg/kg, s.c.) on the 29th and 30th day at an interval of 24 hours. Group V was treated with methanol extract of *Polygonum glabrum* (400 mg/kg, p.o.) daily for 30 days and in addition received ISO (85 mg/kg, s.c.) on the 29th and 30th day at an interval of 24 hour. All rats were weighed and sacrificed by cervical dislocation 24hrs after the last subcutaneous injection of ISO treatment.

## Biochemical parameters

The blood samples were collected by cardiac puncture and subjected to clot for 30 min at room temperature. Serum was separated by centrifugation at 2500 rpm at 30 C for 15 min and used for estimation of cardiac marker enzymes [creatine kinase myoglobin (CK-MB), serum glutamate oxaloacetate transaminase (SGOT), lactate dehydrogenase (LDH)], Serum glutamate pyruvate transaminase (SGPT) and total protein (TP). Blood samples were collected in vials precoated with Trisodium citrate. From the samples, plasma was obtained by cold centrifugation of blood at 3000 rpm for 10 minutes and used for estimation of lipid parameters like TG, TC, HDL, LDL and VLDL.

## Preparation of heart homogenate

The heart was excised following the sacrifice of animals. The removed heart was washed with ice cold saline and weighed. A portion of the heart was used for preparation of 10% (w/v) homogenate in phosphate buffer (50mM, pH 7.4). The tissue homogenates were centrifuged at 7000 g for 10 min at 4 C. The supernatant obtained was used for the estimation of catalase (CAT), superoxide dismutase (SOD), reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GRD) and malondialdehyde (MDA). The remaining part of heart was fixed in 10% buffered formalin and used for histological study.

## Estimation of serum cardio protective parameters

Analysis of serum CK-MB was done by using ROCHE diagnostic kit.

## SGOT & SGPT assay

Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) were determined by the method of Reitman and Frankel [17]. 0.5ml of L-alanine (200mM) and 0.5ml of L-aspartate (200mM) were taken separately and pre incubated with 2mM of ketoglutarate for 5 min at 37 C. To this 0.1ml of serum was added and the volume was adjusted to 1.0ml with sodium phosphate buffer (pH 7.4; 0.1M). The reaction mixture was incubated for 30 and 60 min for SGPT and SGOT respectively. A 0.5ml of 2, 4-dinitrophenyl hydrazine (1mM) was added to the reaction mixture and left for 30 min at room temperature. Finally, the color was developed by the addition of 5ml Na OH (0.4 N) and the product formed was read at 505nm.

## Lactate dehydrogenase assay

Lactate dehydrogenase (LDH) activity was estimated in serum by the standard method [18]. A buffered substrate was prepared by dissolving lithium lactate in glycine buffer. To 1.0 ml of this buffered substrate, 0.2 ml of serum was added and the tubes were incubated at 37ºC for 15 min. To this 0.2 ml of nicotinamide adenine dinucleotide ($NAD^+$) solution was added and the incubation was continued for 30 min. Further, 1.0 ml of DNPH (2-4-dinitrophenyl hydrazine) reagent was added and the tubes were incubated at 37ºC for 15 min. Finally, 7.0 ml of 0.4N NaOH was added and the colour developed was measured at 420nm in a UV spectrophotometer against the reagent blank.

## Total protein assay

This assay was carried out by the method of Lowry et al [19]. To 0.3 ml of tissue homogenate solution, 2 ml of alkaline copper sulphate reagent was added. This solution is incubated at room temperature for 10 mins. Then 0.2 ml of Folin ciocalteau solution was added to each tube and incubated for 30 min. Absorbance was measured at 660 nm. Bovine serum albumin was used as the standard.

## Estimation of lipid parameters

Activity levels of Plasma total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoproteins (VLDL) and were analysed using EXCEL diagnostic kit.

## Estimation of antioxidant parameters

## Catalase assay

The principle of this assay was based on the scavenging of hrdrogen peroxide radicals by catalase. The technique was carried out according to the method of Aebi [20]. The reaction mixture contained 0.1ml of supernatant and 1.8ml of 50mM phosphate buffer (pH 7.0). The reaction was started by the addition of 1.0ml of 30mM of hydrogen peroxide. The rate of the decrease in absorbance of hydrogen peroxide was measured spectrophotometrically at 240 nm. Activity of catalase was expressed as $Umg^{-1}$ of protein.

## Superoxide dismutase (SOD) assay

This was analyzed by the method described by Rai et al [21]. The principle of this assay is that the activity of SOD was inversely

proportional to the concentration of adrenochrome (oxidation product), The reaction mixture contained 0.1ml of supernatant, 1.2ml of sodium pyrophosphate buffer (pH 8.3; 0.052M), 0.1ml of phenazine methosulfate (186 mM), 0.3ml of nitroblue tetrazolium (300 mM), and 0.2ml of NADH (750 mM). Reaction was initiated by adding NADH. After incubation at 30ºC for 90s, the reaction was stopped by the addition of 0.1ml of glacial acetic acid. 4.0mL of n-butanol was added to the reaction mixture. Absorbance was measured spectrophotometrically at 560nm and the concentration of SOD was expressed as $Umg^{-1}$ of protein.

## Reduced glutathione assay

Reduced glutathione (GSH) was measured according to the method of Ellman [22]. The heart tissue homogenate was mixed with 10% trichloroacetic acid containing 1mM EDTA and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2ml of phosphate buffer (pH 8.4), 0.5 ml of Ellman reagent [5''5-dithio, bis (2-nitrobenzoic acid)] and 0.4ml double distilled water was added. Mixture was stirred vigorously and the absorbance read at 412nm. The concentration of glutathione was expressed as μ g/mg of protein.

## Glutathione reductase assay

Glutathione reductase (GRD) activity was assayed by the method of Mohandas et al [23]. The assay mixture contained 1.65ml sodium phosphate buffer (0.1M; pH 7.4), 0.1mL EDTA (0.5 mM), 0.05mL oxidized glutathione (1mM), 0.1mL NADPH (0.1 mM), and 0.05mL supernatant in a total mixture of 2ml. The enzyme activity was quantified by measuring the decrease of NADPH at 340nm at 30s intervals for 3min. The activity was calculated using a molar extinction
coefficient of 6.22 X $103M^{-1}cm^{-1}$ and was expressed as nanomoles of NADPH oxidized $min^{-1}$ $mg^{-1}$ protein.

## Glutathione peroxidase assay

Glutathione peroxidase (GPx) activity was determined by the method described by Wendel [24]. In a test tube, 0.4ml of 0.25M potassium phosphate buffer (pH- 7.0), 0.2 mL tissue supernatant, 0.1ml of GSH (10 mM), 0.1ml NADPH (2.5mM), and 0.1ml GRD (6UmL-1 were taken). Reaction was started by adding 0.1ml of hydrogen peroxide (12mM) and absorbance was measured at 366nm at 1min intervals for 5 min using a molar extinction coefficient of 6.22X 103 $M^{-1}cm^{-1}$. Data was expressed as mU $mg^{-1}$ of protein.

## Lipid per oxidation assay

Lipid peroxidation (LPO) was measured by the method of Liu et al [25]. Acetic acid 1.5mL (20%; pH 3.5), 1.5 of TBA (0.8%), and 0.2mL of sodium dodecyl sulfate (8.1%) was added to 0.1ml of supernatant and heated at 100oC for cooled and 60 min. Mixture was cooled, and 5mL of nbutanol: pyridine (15:1) mixture and 1mL of distilled water was added and vortexed vigorously. After centrifugation at 1200rpm for 10min, the organic layer was separated and the absorbance was measured at 532nm using a spectrophotometer. Malonyldialdehyde (MDA) was an end product of LPO, which reacts with TBA to form pink chromogen TBA reactive substance. It was calculated using a molar extinction coefficient of 1.56 X $105M^{-1}$ $cm^{-1}$ and it was expressed as nanomoles of TBARS $mg^{-1}$ of protein.

## Histopathological studies

Paraffin sections of buffered formalin fixed heart samples were stained with hematoxyline and eosin. The sections were examined under a light microscope.

## Statistical analysis

The data were presented as mean ± SEM and analyzed using the one-way analysis of variance (ANOVA) procedure of Statistical analysis system followed by Dunnett's Multiple Comparison test to identify significant differences of treated groups from the control ($p < 0.001$), ($p<0.01$), ($p<0.05$).

# Results

## Percentage yield

The percentage yield of methanol extract of *Polygonum glabrum* was found to be 7.6%w/w.

## Toxicity study

In acute toxicity study, no mortality or any signs of behavioral changes were observed throughout the 15 day period after single oral administration of *Polygonum glabrum* up to the dose levels of 2000 mg /kg.

## Serum cardio protective parameters

Table-1 illustrates the effects of isoproterenol and methanol extract of *Polygonum glabrum* on cardiac marker enzymes including CK-MB, LDH, SGOT, SGPT and total protein level in serum. The activities of enzyme markers CK-MB (189.3±2.50), LDH (183.56±4.54), SGOT (252±3.43) and SGPT (176±17.3) were increased significantly along with a decrease in protein level

(4.6±0.06) in isoproterenol treated rats when compared to normal control group rats. Similarly, the standard (propranolol) treated group also significantly decreased the level of CK-MB (123.4±3.67), LDH (106.45±2.87), SGOT (98±2.52) and SGPT (51±16.23) with an increase in protein (8.2±0.62). Further, methanol extract of PG-(200mg/kg) pretreatment in isoproterenol treated animals significantly decreased cardiac marker CK-MB (168.2±4.67), LDH (166.42±6.87), SGOT (189±7.38) and SGPT (116±11.45) concurrently increasing protein (5.7±0.55) level when compared to untreated isoproterenol group. Moreover, pretreatment with methanol extract of PG-(400mg/kg) in isoproterenol treated group attenuated myocardial necrosis by decreasing the elevated levels of marker enzyme CK-MB (145.3±2.45), LDH (136.65±6.76) SGOT (129±9.11) and SGPT (63±31.87) besides decreasing level of protein (7.8±0.13) significantly when compared to untreated isoproterenol group.

**Table 1.** Effect of methanol extract of *Polygonum glabrum* on cardiac serum parameters

| Groups | CK-MB (IU/mg of protein) | LDH (IU/mg of protein) | SGOT (IU/L) | SGPT (IU/L) | Total protein (mg/dl) |
|---|---|---|---|---|---|
| Group I (Normal Control) | 90.2±9.12 | 96.32±8.61 | 103.2±9.50 | 44.8±11.4 | 9.4±0.15 |
| Group II (Isoproterenol) | 189.3±2.50### | 183.56±4.54### | 252±3.43### | 176±17.3### | 4.6±0.06### |
| Group III (Propranolol +ISO) | 123.4±3.67 | 106.45±2.87 | 98±2.52 | 51±16.23 | 8.2±0.62 |
| Group IV (PG 200mg/kg+ISO) | 168.2±4.67** | 166.42±6.87* | 189±7.38** | 116±11.45* | 5.7±0.55* |
| Group V (PG 400mg/kg+ISO) | 145.3±2.45*** | 136.65±6.76** | 129±9.11** | 63±31.87** | 7.8±0.13** |

Data are given as mean SD of six animals. # (P<0.05), ##(P<0.01), ###(P<0.001) when normal compared with isoproterenol group, * (P<0.05), ** (P<0.01), *** (P<0.001) when PG extracts compared with isoproterenol group.

## Plasma lipid parameters

The control group recorded plasma lipids level as TC (60.94±1.91), TG (44.34±0.98), HDL (25.8±2.12), LDL (30.79±1.27) and VLDL (5.98±0.87) as shown in table 2. Isoproterenol (IP) group showed significant elevation (P<0.05) in plasma TC (102.31±2.03), TG (112.7±3.89), LDL (89.16±2.04) and VLDL (13.16±2.04) accompanying a significant decline in HDL level (11.46±0.78) compared to control group. The standard treated group also decreased the plasma TC (75.23±1.5), TG (67.76±7.5), LDL (53.72±1.5), VLDL (8.56±1.5), along with elevation in HDL level (22.69±1.5). Pretreatment with methanol extract of PG (200mg/kg) mitigated the cardiac damage by a significant decrement in plasma TC (86.25 ±1.5), TG (89.59±2.12), LDL (64.79±1.80), VLDL (11.46±0.68), along with elevation in HDL level (17.67±0.72) when compared to untreated isoproterenol group. Similarly, pretreatment with methanol extract of PG (400mg/kg) showed reduced lipoproteins level as plasma TC (80.29±1.99), TG (70.82±1.34), LDL (59.34±1.56), VLDL (9.53±0.54), along with a significant (p<0.01) increase in HDL level(20.38±1.25) when compared with untreated isoproterenol group.

**Table 2.** Effect of methanol extract of *Polygonum glabrum* on lipid parameters

| Groups | TC (mg/dl) | TG (mg/dl) | HDL (mg/dl) | LDL (mg/dl) | VLDL (mg/dl) |
|---|---|---|---|---|---|
| Group I (Normal Control) | 60.94±1.91 | 44.34±0.98 | 25.8±2.12 | 30.79±1.27 | 5.98±0.87 |
| Group II (Isoproterenol) | 102.31±2.03### | 112.7±3.89### | 11.46±0.78### | 89.16±2.04### | 13.16±2.04### |
| Group III (Propranolol +ISO) | 75.23±1.5 | 67.76±7.5 | 22.69±1.5 | 53.72±1.5 | 8.56±1.5 |
| Group IV (PG 200mg/kg +ISO) | 86.25 ±1.5* | 89.59±2.12* | 17.67±0.72** | 64.79±1.80* | 11.46±0.68* |
| Group V (PG 400mg/kg+ISO) | 80.29±1.99** | 70.82±1.34** | 20.38±1.25** | 59.34±1.56* | 9.53±0.54** |

Data are given as mean SD of six animals. #(P<0.05), ##(P<0.01), ###(P<0.001) when normal compared with isoproterenol group, * (P<0.05), ** (P<0.01), *** (P<0.001) when PG extracts compared with isoproterenol group.

## Cardiac antioxidant parameters

Table-3 depicted that the activities of CAT (188.6±0.06), SOD (58.4±6.5), GSH (9.1±07), GRD (17.6±1.39) and GPx (276.6±0.99) in isoproterenol treated group declined significantly along with significant elevation in lipid peroxidation (4.9±03) level (expressed as MDA) than that of normal group. Standard (propranolol) treated group significantly increased the the activities of CAT (178.4±15), SOD (80.8±0.45), GSH (8.7±08), GRD (16.2±0.04), and GPx (259.2±18) with significant decline in lipid peroxidation (5.7±03) when compared to isoproterenol group. Co-administration of methanol extract of *Polygonum glabrum* at a dose of 200 mg/kg distinctly prevented these isoproterenol induced alteration and maintained enzymes level of CAT (47.5± 0.3), SOD (63.5±1.05), GSH (8.2±2.7), GRD (10.4±1.7), GPx (206.1±3.4) and lipid peroxidation (8.5±1.05) near to normal values. Pretreatment with methanol extract of *Polygonum glabrum* at a dose of 400 mg/kg elevated the levels of cardiac CAT (52.7±0.5), SOD content (74.9±1.1), GRD (14.8±0.11), GSH (10.9±1.2) and GPx (216.2±5.9) along with reduced lipid peroxidation (6.1±04) when compared to isoproterenol group.

**Table 3.** Effect of methanol extract of *Polygonum glabrum* on antioxidant parameters

| Treatment | CAT (U/mg of protein) | SOD (U/mg of protein) | GSH (µg/mg of protein) | GRD(nanomoles of NADPH Oxidized/min/mg protein) | GPx (mU/mg of protein) | MDA(nanomoles of TBARSmg/of protein |
|---|---|---|---|---|---|---|
| Group-I (Normal Control) | 188.6±0.06 | 58.4±6.5 | 9.1±07 | 17.6±1.39 | 276.6±0.99 | 4.9±03 |
| Group-II (Isoproterenol) | 45.5±07$^{###}$ | 22.6±0.34$^{###}$ | 3.3±09$^{###}$ | 6.4±1.16$^{###}$ | 167.4±0.85$^{###}$ | 13.6±02$^{###}$ |
| Group-III (Propronolol +ISO) | 178.4±15 | 80.8±0.45 | 8.7±08 | 16.2±0.04 | 259.2±18 | 5.7±03 |
| Group-IV (PG-200mg/kg +ISO) | 47.5±0.3 * | 63.5±1.05* | 8.2±2.7* | 10.4±1.7** | 206.1±3.4* | 8.5±1.05* |
| Group-V (PG-400mg/kg +ISO) | 52.7±0.5** | 74.9±1.1** | 10.9±1.2** | 14.8±0.11** | 216.2±5.9** | 6.1±04** |

Data are given as mean SD of six animals. $^{#}$(P<0.05), $^{##}$(P<0.01), $^{###}$(P<0.001) when normal compared with isoproterenol group, * (P<0.05), ** (P<0.01), *** (P<0.001) when PG extracts compared with isoproterenol group

## Histopathological study of cardiac tissue

Examination of heart tissue in normal rat exhibited intact histioarchitecture. The nucleus and cytoplasm were not disturbed or degenerated. The blood vessels and vacuoles of cytoplasm appeared intact. There was no visible necrotic damage to the myocytes (Figure-1.a). The heart section of isoproterenol treated rats (Figure-1.b) showed degeneration of cytoplasm, penetration of mononuclear inflammatory cells, presence of focal myelin fibres, accumulation of lipid material, faded nucleus and cellular swelling. Figure-1.c illustrated the histopathology of standard treated group showing normal cardiomycytes with considerable reduction in necrosis. Figure 1.d depicted heart sections from rats treated with methanol extract of *Polygonum glabrum* (200mg/kg) showed less change in the morphology of myocytes with changes in cytoplasmic vacuoles, swelling of cells and infiltration of macrophages and lymphocytes. When compared to the former dose, methanol extract of *Polygonum glabrum* (400mg/kg) showed relatively less faded basophilia of chromatin in nucleus, mild infiltration of inflammatory cells and less necrotic area (Figure-1.e).

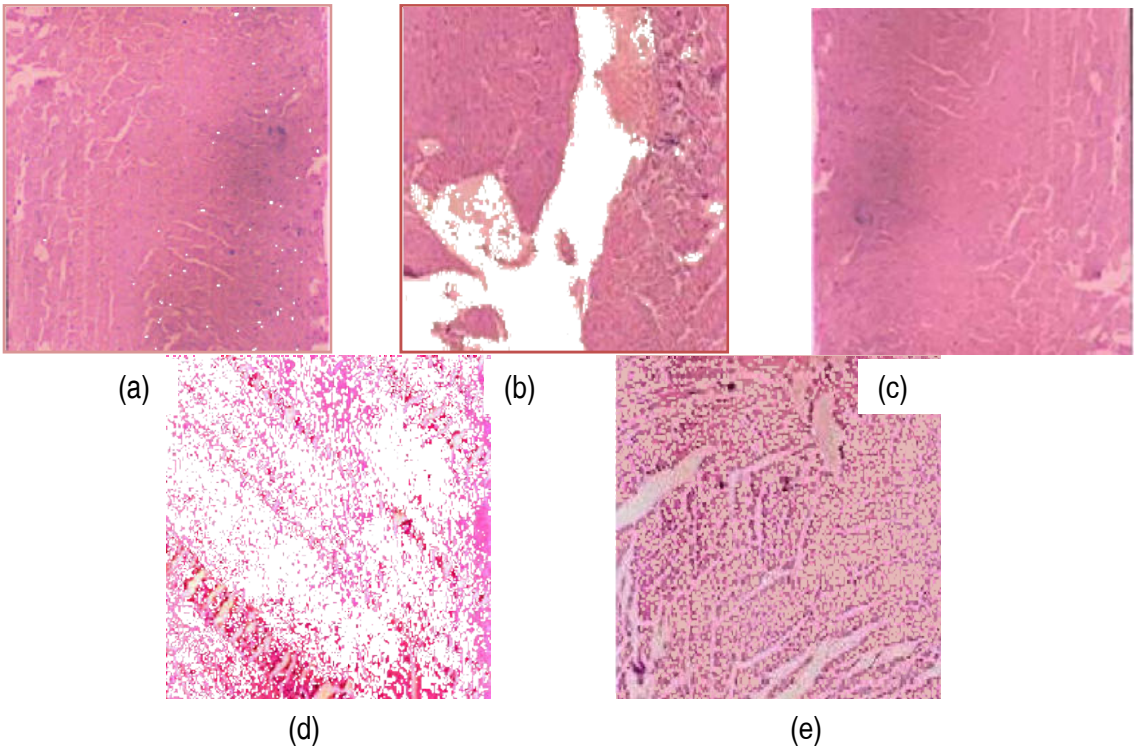


**Figure-1.** Photo micrograph of rat cardiomyocytes. (a) Normal architecture of cardio myocytes (b) Rats treated with isoproterenol showed degeneration of cytoplasm, presence of focal myelin fibres, accumulation of lipid material and faded nucleus. (c) Propranolol treated rats showed normal cardiomycytes with reduced necrosis. (d) Rats treated with methanol extract of *Polygonum glabrum* (200mg/kg) showed less change in the morphology of myocytes with changes in cytoplasmic vacuoles and swelling of cells. (e) Methanol extract of *Polygonum glabrum* (400mg/kg) showed relatively less faded basophilia of chromatin in nucleus and less necrotic area. H&E (10 X)

## Discussion

The result of present study revealed an increase in free radical mediated oxidative stress leading to cardiac necrosis and methanol extract of *Polygonum glabrum* showed a significant cardioprotective effect against such oxidative stress. Myocardial necrosis can result indirectly due to disturbance in the blood supply to the heart or directly by any chemical insult to the myocyte. When the injury to myocyte is severe the enzymes present in lysosomes leak out of it and enter the cytoplasm. Thus a promising approach to detect cardiac injury involves monitoring and estimation of certain cytoplasmic enzymes, as they can be detected in blood serum [26]. CK-MB, LDH, troponins and SGOT, are some of the diagnostic cardiac markers of myocardial infarction. According to Robbins, the mitochondrial membrane, plasma membrane and lysosomal membrane are more prone to attack in the event of cell injury. Damage to mitochondrial membrane leads to decline in production of ATP resulting in necrosis [27]. Similarly an injury to lysosomes leads to emptying of its enzymes in to intracellular fluid. Creatine kinase is a cytoplasmic enzyme which is a dimer with two subunits namely M and B. One of its isoenzyme, CK-MB (creatine kinase-muscle brain) is abundant in myocardium. Any elevation of the ck-mb fraction in serum is a reliable indication of myocardial infarction. Scrupulous study on the effect of isoproterenol induced myocardial damage has reported elevated levels of this marker enzyme in serum [28]. However, pretreatment with methanol extract of *Polygonum glabrum* groups recorded a decrease in activity levels of enzyme CK-MB. Lactate dehrdrogenase is an enzyme comprising of zinc along with two subunits namely H (heart) and M (muscle). The elevation of LDH in the blood stream serves as an indication of tissue breakdown. The present study is also in accordance with the previous reports since an increase in LDH and SGOT enzymes are observed in isoproterenol treated groups. However, pretreatment with methanol extract of *Polygonum glabrum* groups recorded a decrease in activity levels of enzymes (SGOT, LDH, SGPT) suggesting the cardio protective potential of the *Polygonum glabrum* extract. Additionally, In 2014, Khan et al repoted that methanol extract of leaves of *Polygonum glabrum* and its kupchan fractions exhibited better cytotoxic, membrane stabilizing and thrombolytic activities [10]. Since methanol extract of *Polygonum glabrum* consisted of array of phytoconstituents such as triterpenes [beta-hydroxyfriedalanol], phenols [3-hydroxy-5-methoxystilbene], steroids [sitosterol - (6-O-palmitoyl)- 3-O-β-D

glucopyranoside and sitosterol-3-O-β-D glucopyranoside], sesqueterpenes [2,3-dihydroxy isodrimeninol] etc, administration of this extract might prevent the leakage of biomarkers in to blood stream by stabilizing the membranes [13].

The quantity and composition of lipids in the myocardium is also a determinative factor for development of cardiovascular disease. *Isoproterenol is a synthetic* catecholamine that *stimulates beta adrenergic receptors and* adenylate cyclase which results in the elevation of cAMP [29]. Also, free radicals produced from catecholamines activates cAMP-dependent protein kinases, I and II (PKAI and -II,) resulting in lipid accumulation in the myocardium. Free radicals also alter the structure of PK (phosphokinase enzyme) enzymes by causing a loss of a tryptophan residue from site A of phosphokinase enzyme [30]. On the whole, isoproterenol leads to augmentation in the levels of myocardial lipids signifying its hyperlipidemic effect [31]. This is in line with the present study where a hike in serum TC, TG, VLDL, LDL levels and decrement in level of HDL in isoproterenol treated groups were observed. Pretreatment with methanol extract of *Polygonum glabrum* significantly altered the levels of TC, TG, LDL and VLDL by decreasing their levels. Similarly it increased the levels of HDL in pretreated group. This modification in lipid profile might be due to the presence of terpenoids in methanol extract of *Polygonum glabrum.* The anti-hyperlipidemic properties of terpenoids from plant extracts were studied by many researchers [32]. Hence the ability of methanol extract of *Polygonum glabrum* to curb the changes of TC, TG, LDL, VLDL, HDL and restore it to normalcy might be attributed to the presence of sesqueterpene diesters (2 alpha 3 beta diangeloyloxy isodrimeninol, 2 alpha angeloyloxy 3 beta 2'methyl butanoyloxy isodrimeninol) and triterpenoids (beta-hydroxyfriedalanol) in the extract [15].

Catalase is a peroxisomal enzyme present in mitochondria of heart. It is one of the antioxidant defense enzymes which play a indispensable role in the oxidation of hydrogen peroxide to oxygen and water. Previous studies reported that cardiotoxicity was reduced in transgenic mice by 60-100 fold overexpression of catalase citing the contribution of catalase in detoxification of hydrogen peroxide [33]. Similarly, SOD is another ROS defense enzyme present exclusively in the mitochondrial matrix and its prime function is to assist in dismutation of superoxide radicals. In the current study, isoproterenol treated groups exhibited a depletion or decrease in the amount of catalase and SOD resulting in intense myocardial necrosis [34]. Administration of methanol extract of *Polygonum glabrum* to isoproterenol challenged rats reinstated the antioxidant enzyme levels to normalcy. This enhancement in antioxidant support system of myocardium might be due to the scavenging of free radicals by methanol extract of *Polygonum glabrum.*

The glutathione antioxidant system including GSH, GPx and GRD plays a crucial role in cellular defense against oxidative stress by upholding a cascade of reactions. Glutathione peroxidase (GPx) is a selenoprotein that oxidizes two molecules of glutathione (GSH) into oxidized glutathione (GSSG). This oxidation is favoured by the formation of a disulphur bond. Glutathione reductase catalyse the reduction of GSSG in to glutathione. Thus glutathione is rapidly oxidized and regenerated in the cell [35]. The reduction in non enzymatic GSH and enzymatic GPx and GRD in isoproterenol treated rats might be due to the influence of free radicals on antioxidant system. In present study, glutathione levels depleted by isoproterenol were significantly elevated by methanol extract of *Polygonum glabrum.* It was understood that increased levels of GSH, GPx and GRD could be because of presence of flavonoids in methanol extract of *Polygonum glabrum.*

In the present study, elevation of lipid peroxidation in the heart of rats treated with isoproterenol was observed. The increase in malondialdehyde levels in heart indicates excessive lipid peroxidation [36]. Free radicals produced from isoproterenol led to irreversible tissue damage in heart by activation of lipid peroxidation. Treatment with methanol extract of *Polygonum glabrum* significantly decreased the lipid peroxidation induced membrane damage. The cardioprotective effect of the methanol extract of *Polygonum glabrum* was further approved by histopathological examinations. Methanol extract of *Polygonum glabrum* furnished cardio protection at different dose levels. Altogether, morphological and histological changes such as decrease in swelling, lesser accumulation of fatty acids and lesser degree of karyolysis rendered considerable evidence for the cardio protective activity of *Polygonum glabrum.*

## Conclusion

From the study, it was observed that methanol extract of *Polygonum glabrum* possessed cardio protective activity against chemical induced myocardial necrosis. The data obtained from the study are consistent with the concept that free radicals formed from isoproterenol play a major role in inducing myocardial necrosis. Methanol extract of *Polygonum glabrum* restored the levels of cardiac enzymes (CK-MB, LDH, SGOT, SGPT and TP), lipid parameters (TC, TG, HDL, LDL, VLDL) and antioxidant parameters (CAT, SOD, GRD, GSH, GPx and MDA) levels to normalcy when compared with propranolol. Hence, it can be concluded that the active constituents present in methanol extract of *Polygonum glabrum* could be the reason behind the counter active action against oxidative stress by myocytes.

### Author's contribution

The complete research work was suggested and designed by RS. The manuscript was edited by RS. Extraction and cardioprotective activity was carried out by RI. The manuscript was drafted by RI. Authors read and approved the final manuscript.

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## Conflict of interest

Authors declare no conflict of interest.

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