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Original Research Article

Cardioprotective and antioxidant effects of *Bougainvillea glabra* against isoproterenol induced myocardial necrosis in albino rats

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Abstract

The present study was executed to evaluate the myocardial protective effect of methanol extract of Bougainvillea glabra against isoproterenol induced myocardial necrosis in rats. Myocardial necrosis was induced by subcutaneous injection of isoproterenol (85mg/kg body weight) on 29th and 30th day at an interval of 24 hours. Myocardial necrosis was evident from the changes of marker enzymes in serum, plasma and heart tissue. The activities of serum cardiac marker enzymes such as lactate dehydrogenase (LDH), creatine kinase myoglobin (CK-MB), serum glutamate oxaloacetate transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), triglycerides (TG), total cholesterol (TC), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and total protein (TP) were estimated. In addition, plasma TBARS and plasma LDH levels were also recorded. Antioxidant parameters viz catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), glutathione peroxidase (GPx) and malondialdehyde (MDA) levels were performed in heart tissue homogenate. The outcome of the study indicated that, pretreatment with methanol extract of Bougainvillea glabra to isoproterenol induced rats significantly prevented the altered serum cardiac marker enzymes, plasma levels and antioxidant parameters to near normal status. The cardioprotective effect was compared with propranolol (10 mg/kg, oral) which was used as the standard. Histopathological findings exposed a reduced degree of necrosis and inflammation succeeding pretreatment with Bougainvillea glabra.Based on these results, it was suggested that methanol extract of Bougainvillea glabra prevents myocardial necrosis and oxidative stress induced by isoproterenol.

Keywords: Myocardial infarction; Bougainvillea glabra; biochemical parameters; propranolol; isoproterenol

Introduction

Generally, cardiovascular diseases (CVD) were found to be primary source of mortality. Developing countries like India are also struggling to manage the impact of CVD along with the growing burden of obesity [1] . Heart disease is any disorder that disturbs the heart's capability to function usually. Heart disease is a general term that refers to a variety of acute and chronic medical conditions that affect one or more of the components of the heart [2] . Myocardial infarction (MI) or acute myocardial infarction (AMI), generally known as a heart attack, happens when the blood supply to portion of the heart is disturbed making some heart cells to die. This is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (like cholesterol) and white blood cells (especially macrophages) in the wall of an artery [3] . Isoproterenol [1-(3, 4- dinydropheny1)-2- isopropylamino ethanol hydrochloride] (ISO) a synthetic catecholamine and β-adrenergic agonist, is documented to cause severe oxidative stress in the myocardium and resulting in infarct like necrosis of the heart muscle [4] . Oxidative stress is a condition in which oxidant metabolites exert their toxic effect because of an increased production or an altered cellular mechanism protection [5] . Increased oxidative stress and the generation of the free oxygen radicals can result in modification of Low- density lipoprotein (LDL) to oxidized Low-density lipoprotein (LDL) that could lead to atheroscle-

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rotic lesions. Many antioxidants like Vitamins C and E and plant polyphenols are efficient tools in oxidative stress and cardiovascular disorders as potential therapeutic agents [6] . Various phytoconstituents like carotenoids, flavonoids, cardiac glycosides and alkaloids were responsible for cardioprotective activity [7] [8] . Bougainvillea glabra is a useful medicinal plant from Nyctaginaceae family finding applications in indigenous systems of medicine. Bougainvillea glabra is an ornamental plant with several medicinal properties. It is distributed everywhere, mostly in warm climatic regions. Bougainvillea glabra is having colorful flowers and the plant is seen in front of houses and in office and on walls and fences [9] . Leaves juice of Bougainvillea glabra is orally taken with rhizome juice of Curcuma caesia for a few days to treat helminthiasis in Bangladesh [10] . Various pharmacological activities like antidiabetic [11] , antilipidemic [12] and anthelmintic [13] were proved from Bougainvillea glabra. The phytochemical analysis of the extract reveals the presence of alkaloids, terpenoids, saponins and cardiac glycosides in Bougainvillea glabra. Five flavonoids were isolated and identified as vitexdin, isovitexin, chrysoeriol, apigenin and luteolin from methanol extract of Bougainvillea glabra [14] . The GC-MS study of Bougainvillea glabra has shown many phytochemicals which contributes to the medicinal activity of the plant. Hence, the present study was dedicated on assessing the cardioprotective effects of methanol extract of Bougainvillea glabra on isoproterenol induced myocardial necrosis in wistar albino rats.

Material and Methods

Chemicals

All chemical substances used in the study stood analytical grade. Thiobarbituric acid, reduced glutathione, oxidized glutathione and propranolol were obtained from SD fine chemicals Ltd. (Mumbai, India). Isoproterenol was procured from Sigma Aldrich chemicals, USA. Serum biochemical parameters like total cholesterol (TC), triglycerides (TG) and high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were analyzed using commercially available reagent methods.

Collection and authentication of plant material

The whole plant of Bougainvillea glabra (Nyctaginaceae) was collected in the month of July from botanical garden, Hanamkonda, Warangal district, Telangana, India. It was shade dried away from sunlight and stored suitably. The plant material was taxonomically identified by Dr. Vatsavaya S Raju, Professor, Plant Systematics Laboratory, Department of Botany, Kakatiya University, Warangal district Telangana, India and a voucher specimen was deposited in the her barium against accession number 4610 for future reference.

Extraction

The whole plant of Bougainvillea glabra was powdered with a mechanical grinder and passed through Sieve no. 40. Powder of Bougainvillea glabra was extracted with methanol by continuous Soxhlet extraction method. The excess solvent was removed by rotary vacuum evaporator; the remaining mass of extract was concentrated and dried. The percentage yield of the extract was calculated.

Experimental animals

Albino rats of Wis tar strain (200-250gm) were procured from Ghost enterprises, Kolkata, India. All the rats were accommodated in polypropylene cages and preserved in a precise environment (28-32°C) with 12-12 hr of light and dark cycle. Each day all the animals were fed a normal laboratory diet ad libitum and had free access to water. The protocol was approved by Institutional Animal Ethical Committee constituted for the purpose. CPCSEA Registration No:- 1287/PO/Re/S/09/CPCSEA. The animals were preserved under customary environments in an animal house as per the guidelines of committee for the purpose of control and supervision on experiments on animals (CPCSEA).

Acute toxicity study

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 of Bougainvillea glabra were administered orally to groups containing three animals of the same age group and weight. The animals were regularly 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 [15].

Experimental protocol

A total of thirty rats were taken for the experiment. After acclimatization, the animals were allocated into five groups containing six animals each. Group I animals received normal saline and termed as control group (1ml/kg/day p.o) for 30 days. Group II animals served as isoproterenol treated and received saline (1 ml/kg, p.o.) daily for 30 days. Group III animals was treated with standard propranolol (10 mg/kg, p.o) for one week after two weeks of saline treatment. Group IV and Group V animals were pretreated with methanol extract of Bougainvillea glabra at 250mg/kg and 500mg/kg BW by orally for 30 days respectively.

Animals from Group II to Group V were administered with isoproterenol (ISO) 85mg/kg, sub cutaneous on 29th and 30th day at an interval of 24 hours [16]. All the animals were sacrificed by decapitation on 31st day of the experiment. The heart was removed, washed in cold saline and stored in liquid nitrogen for further biochemical studies.

Estimation of Biochemical parameters

Preparation of serum from blood

The serum was separated by centrifugation at 2500 rpm at 30°C for 15 min and it was used for the estimation of cardiac marker enzymes like creatine kinase myoglobin (CK-MB), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL).

Preparation of plasma from blood

Plasma was obtained by cold centrifugation of blood samples at 3000 rpm for 10 minutes and used for estimation of plasma LDH, plasma TBARS and total protein.

Preparation of heart homogenate

The heart was dissected out, washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer (50 mm, pH 7.4). The homogenate was centrifuged at 7000 rpm for 15 min and the supernatant was used for the assay of superoxide dismutase (SOD), catalase (CAT), reduced glutathione (GSH), glutathione peroxidise (GPx) and lipid peroxidation (MDA).

Estimation of serum cardioprotective parameters

Estimation of creatine kinase myoglobin (CK-MB) activity

Creatine kinase myoglobin (CK-MB) was estimated spectrophotometrically according to the method of Lamprecht [17] . The sample (50μl) was added to cuvette containing 1 ml of imidazole buffer consisting of adenosine-mono-phosphate (5.2mM), adenosine-di-phosphate (2.1mM), nicotinamide adenine dinucleotide phosphate (2.1mM), glucose-6-phosphate dehydrogenase (1.6 U/l), creatine phosphate (31.2 mm) and N-acetyl cysteine (21 mm). The cuvette containing of sample and imidazole buffer was incubated for 2 min at room temperature. Absorbance was recorded on 340 nm for 180 sec at every 60 sec. One unit of creatine kinase myoglobin isoenzyme is defined as the amount of enzyme that will transfer one μ mol of phosphate from phosphocreatine to adenosine diphosphate per min at pH 7.4 on 30°C.

SGOT & SGPT assay

Serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were determined by the method of Reitman and Frankel [18] . 0.5ml of L-alanine (200mM) and 0.5ml of L-aspartate (200mM) were taken separately and pre incubated with 2mM of α 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 hydra zine (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 NaOH (0.4 N) and the product formed was read at 505nm.

Estimation of triglyceride

The triglyceride (TG) contents was estimated by Foster et al. method [19]. Isopropanol (1.0 ml) was added to 0.1 ml of sample and mixed well, followed by 0.4g of alumina and shaken well for 15 min. Centrifuged at 2000 rpm for 10 min and then 2.0ml of the supernatant was transferred to appropriately labelled tubes. The tubes were placed in a water bath at 65°C for 15 min for saponification after adding 0.6 ml of the saponification reagent. After cooling, 1.0 ml of sodium meta periodate was added followed by 0.5 ml of acetyl acetone reagent, after mixing the tubes at 65°C for 30 min. The contents were cooled and read at 430nm.

Determination of total cholesterol

The serum total cholesterol (TC) level was measured by Zlatkis et al method [20]. To 0.1 ml of the serum, 4.9 ml of ferric chloride precipitation reagent was added and centrifuged for few minutes. The supernatant (2.5 ml) was mixed with ferric chloride diluting reagent (2.5ml) and add 4 ml of concentrated sulfuric acid. Suitable aliquots of the standards were made upto 5 ml with ferric chloride diluting reagent with 4 ml of concentrated sulfuric acid. The optical density was measured at 560 nm. The cholesterol content was expressed as mg/dl of serum.

Estimation of high density lipoprotein

High density lipoprotein (HDL) was determined by Friedewald et al method [21]. Serum (1ml) was mixed with 0.1 ml of phosphotungstate reagent and 50μl of magnesium chloride reagent. The content was centrifuged at ambient temperature for 30 minutes at 1500 rpm. Supernatant (0.1ml) was added to 4.9 ml of ferric chloride precipitating reagent, then it was mixed well and centrifuged. From this, 2.5 ml of supernatant was taken. An ice bath was set. 2.5 ml of diluting reagent and 4 ml of concentrated sulphuric acid were added by thorough mixing. Various concen-

trations of working standard solution were taken to which 5ml diluting reagent, and 4 ml of sulphuric acid were added. A blank was also maintained. The colour developed was read at 560nm.

Estimation of low-density lipoprotein cholesterol

The serum level of low-density lipoprotein cholesterol (LDL) -C was measured according to the protocol of Friedewald et al. [22] using the relationship as follows:

LDL-C = TC-TGL/5 + HDL-C

Where LDL-C is low-density lipoprotein cholesterol, TC is total cholesterol, TGL is triglyceride and HDL-c is high-density lipoprotein. The value was expressed in mg/dl.

Estimation of very low density lipoproteins

The very low density lipoproteins (VLDL) contents were estimated by Friedewald et al method [22]. Serum (1 ml) was mixed with 0.15 ml of sodium dodecyl sulfate (SDS) solution. The contents were mixed well and incubated at 37ºC for 2 hrs. The contents were centrifuged in a refrigerated centrifuge at 10,000 g for 30 min. VLDL C for 2 hrs. VLDL aggregates as pellicle at the top. The supernatant was a mixture containing HDL and LDL fraction. The values were expressed as mg/dl.

$$LDL\ Cholesterol = \underline{Total\ Serum\ Cholesterol} \quad \frac{(Total\ serum\ TGL - HDL\ Cholesterol)}{5}$$

$$VLDL = \frac{Total\ serum\ TGL}{5}$$

Plasma LDH

Plasma LDH was estimated by the method of Moldeus et al. (1978) [16]. Cuvettes (1 ml) in potassium phosphate buffer (pH 7.0) using 20 μl sample. Reduction in NADH was monitored at 340 nm against the appropriate controls every 15 s for 60 s. Data were expressed as mU/mL.

Plasma TBARS

Lipid peroxidation was measured by the method of Liu et al [23]. Reagents acetic acid 1.5 ml (20%, pH 3.5), 1.5 ml of thiobarbituric acid (0.8%) and 0.2 ml of sodium dodecyl sulphate (8.1 %) were added to 0.1 ml of supernatant, then heated at 100°C for 60 min. The above mixture was cooled and 5 ml of n-butanol-pyridine (15:1) mixture, 1 ml of distilled water were added and vortexed vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was parted and absorbance was recorded at 532 nm. The calculation was performed using a molar extinction coefficient of $1.56 \times 10^5 M^{-1}\ cm^{-1}$ and the data was expressed as nM/mL.

Estimation of protein

Estimation of protein was précised by the method of Lowry et al. [24]. Aliquots of the appropriately diluted serum (0.1ml to 10ml by two serial dilutions) was prepared up to 1.0ml with water and 4.5ml of alkaline copper reagent was added to all the tubes comprising blank, containing 1.0ml water and standards containing aliquots of standard BSA and made up to 1ml with water. The tubes were incubated for 10 min at room temperature. 0.5 ml was mixed to all the tubes and incubated for 20 min at room temperature. The blue colour settled was recorded at 640nm.

Biochemical estimation of markers of oxidative stress or cardioprotective activity

Myocardial TBARS

Lipid per oxidation was measured by the method of Liu et al. [23]. It was calculated using a molar extinction coefficient of $1.56 \times 10^5 M^{-1}\ cm^{-1}$ and expressed as nM/gram tissue mass

Myocardial SOD

SOD activity was analyzed by the method described by Kakkaret al. (1984) [25]. Assay mixture contained 0.1 ml of super- natant, 1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052 M), 0.1 ml of phenazine methosulphate (186 μm), 0.3 ml of nitro *blue* tetrazolium, 300 µM, 0.2 ml of nicotinamide adenine dinucleotide reduced disodium salt, (NADH, 750 μM). Reactionwas started by addition of NADH. After incubation at 30° C for 90 sec, the reaction was stopped by the addition of 0.1 ml of glacial acetic acid. Reaction mixture was agitated dynamically with 4.0 ml of n-butanol. Mixture was set a side to stand for 10 min, centrifuged and butanol layer was separated. Colour inten- sity of the chromogen in the butanol was measured at 560 nm by spectrophotometrically and concentration of SOD was expressed as U/mg of protein.

Myocardial catalase

Myocardial catalase parameter was performed by the method of Aebi (1974) [26]. 0.1 ml of supernatant was supplemented to cuvette comprising 1.9 ml of 50 mm phosphate buffer (pH 7.0). Reaction was started by addition of 1.0 ml of freshly prepared 30 mm H_2O_2. The rate of decomposition of H_2O_2 was measured spectrophotometrically at 240 nm. Activity of catalase was expressed as U/mg of protein. Data were expressed as U/mg protein.

Myocardial reduced glutathione (GSH)

Reduced glutathione was measured according to the method of Ellman (1959) [27]. Equal quantity of homogenate was mixed

with 10% trichloroacetic acid and centrifuged to separate the proteins. To 0.01 ml of this supernatant, 2 mL of phosphate buffer (pH 8.4, 0.3 M), 0.5 ml of 5, 5'-dithio, bis (2-nitrobenzoic acid) [DTNB] and 0.4 ml double distilled water was added. Mixture was vortex ed and the absorbance read at 412 nm within 15 min. Data were expressed as μg/gram tissue mass.

Myocardial GPx

GPx activity was determined by the method described by Paglia & Valentine (1967) [28] and modified by Wendel (1981) [29]. The reaction mixture consisted of 400 μl 0.25 M potassium phosphate buffer (pH 7.0), 200 μl supernatant, 100 μl GSH (10 mm), 100 μl NADPH (2.5 mm) and 100 μl glutathione reductase (6 U/ml). Reaction was in progress by adding 100 μl hydrogen peroxide (12 mm) and absorbance restrained at 366 nm at 1-min intervals for 5 min. It was calculated using a molar extinction coefficient of 6.22 x 10^3 $M^{-1}cm^{-1}$. Data was expressed as mU/mg of protein.

Histopathological study

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

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 of extract

The percentage yield of the methanol extract of Bougainvillea glabra was found to be 6.5 % w/w

Acute toxicity study

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

Estimation of serum parameters CK-MB

The activity of serum CK-MB was assessed as marker of cardiac injury. Isoproterenol induced group (Group II) resulted in the significant ($P<0.01$) elevation of CK-MB enzyme activity as compared to the control group (Group-I). Although not normalized, CK-MB activity was significantly ($P<0.05$) reduced by the extracts of both groups and standard, propranolol (Group-III) in the following order. Propranolol (Group-III) > Bougainvillea glabra at 500 mg/kg (Group-V) >Bougainvillea glabra at 250 mg/kg (Group-IV). The result was mentioned in table 1.

SGOT activity

The activity of enzyme marker SGOT was increased significantly ($P<0.01$) in isoproterenol treated rats (group II) when compared to normal control group rats. Rats fed with the Bougainvillea glabra plant extract (group IV & V) and propranolol treated rats (group-III) have shown a significant ($P<0.05$; $P<0.05$) decrease in SGOT levels as shown in table 1.

SGPT activity

The SGPT level was slight significant ($p < 0.05$) reduced in rats fed with Bougainvillea glabra extract at 250 mg /kg body weight (Group IV) when compared to the isoproterenol treated group (Group II). While more significant ($p < 0.01$) decrease was observed in the SGPT level in rats fed with Bougainvillea glabra extract at 500mg/kg body weight (Group V) and in propranolol treated group (Group-III). The result was shown in table 1.

Values are mean ± s.d (n=6). Group-2 (isoproterenol induced) compared with group-1 (control rats). Groups-3 (propranolol), Groups- 4 (methanol extract of Bougainvillea glabra 250 mg/kg), Groups- 5 (methanol extract of Bougainvillea glabra 500 mg/kg) compared with group-2 (isoproterenol induced rats). ** $P< 0.01$, *$P< 0.05$.

Determination of serum triglyceride

The activity of serum triglyceride was increased significantly ($P<0.01$) in isoproterenol treated rats when compared to normal control group rats. There was a significant ($P<0.05$; $P<0.01$) reduction of serum triglyceride levels were observed in two different doses [i.e Bougainvillea glabra extract at 250 mg /kg body weight (Group-IV) and Bougainvillea glabra extract at 500 mg /kg body weight (Group-V)] when compared to the isoproterenol treated rats (Group-II). Noticeable significant ($P<0.01$) reduction in serum triglycerides was seen in propranolol treated rats (Group-III) when compared with high dose, low dose and isoproterenol treated rats (Group-II). The result was tabulated in table 2.

Determination of serum total cholesterol

The activity of serum total cholesterol was increased significantly ($P<0.01$) in isoproterenol treated rats when compared

Table 1 Effectof methanol extract of Bougainvillea glabra on CK-MB, SGOT and SGPT duringisoproterenol induced myocardial necrosis and oxidative stress in rats.

Groups	CK-MB (IU/mg of protein)	SGOT (IU/mg of protein)	SGPT (IU/mg of protein)
Group I (Normal Control)	90.2±9.12	17.54±0.66	24.28±0.89
Group II (Isoproterenol Control)	189.3±2.50**	103.86±0.71**	92.7±1.01**
Group III (Propranolol + Isoproterenol)	123.4±3.67**	55.67±0.91**	47.2±0.81**
Group IV (BG-250mg/kg + Isoproterenol)	168.2±4.67*	29.11±0.18*	29.71±0.85*
Group V (BG-500mg/kg + Isoproterenol)	144.3±1.85**	37.81±0.97**	36.6±0.41**

to normal control group rats. There was a significant ($P<0.05$; $P<0.05$) reduction of serum total cholesterol level was observed when methanol extract of Bougainvillea glabra at two different doses of i.e 250 mg /kg body weight (Group-IV) and 500 mg /kg body weight (Group-V), respectively. Drastic significant ($P<0.01$) reduction was seen in rats treated with propranolol (Group-III) when compared to the isoproterenol treated rats (Group-II). The results was indicated in table 2.

Estimation of high density lipoprotein

The administration of methanol extract of Bougainvillea glabra caused significant ($p<0.05$; $p<0.05$) increase of HDL levels at 500 mg/kg (Group-V) and 250 mg/kg body weight (Group-IV) when compared with isoproterenol treated rats (Group-II). However, the level of HDL was found to be significant ($p<0.01$) and highly increased at the doses 10 mg/kg body weight with propranolol (Group-III) as shown in table 2.

Estimation of low-density lipoprotein

The parameter low-density lipoprotein (LDL) exhibited a significant ($p<0.01$) elevation upon isoproterenol treatment (Group-II). The pretreatment of Bougainvillea glabra was significantly ($p<0.05$) prevented the elevation of these parameters in all the groups of rats. The effect of propranolol (Group-III; $p<0.05$) was more significant ($p<0.01$) and prominent than with Bougainvillea glabra plant extract 250 mg/kg (Group-IV) and 500 mg/kg body weight (Group-V), as shown in table 2.

Estimation of very low-density lipoprotein

The activity of very low-density lipoprotein was increased significantly ($p<0.01$) in isoproterenol treated rats when compared to normal control group rats. Bougainvillea glabra at two different doses of 250 mg/kg body weight (Group IV) and 500 mg/ kg body weight (Group V) were significantly ($p<0.05$; $p<0.01$) reduced the increased levels of very low density lipoproteins. Propranolol (10mg/ kg body weight, Group-III) has shown more significantl ($p<0.01$) effect than the plant extract. The result was shown in table 2.

Values are mean ± s.d (n=6). Group-2 (isoproterenol induced) compared with group-1 (control rats). Groups-3 (propranolol), Groups- 4 (methanol extract of Bougainvillea glabra 250 mg/kg), Groups- 5 (methanol extract of Bougainvillea glabra 500 mg/kg) compared with group-2 (isoproterenol induced rats). ** $P< 0.01$, *$P< 0.05$.

Plasma LDH

Plasma LDH was increased significantly ($p< 0.01$) in isoproterenol induced group (Group-II) in comparison to control (group-1) as shown in figure 4. Significant ($p< 0.01$) reduction of LDH was observed only in group-V (methanol extract of Bougainvillea glabra at 500 mg/kg) and standard drug propranolol treated rats (Group-III) when compared with other groups. However, less significant ($p<0.05$) decrease of plasma LDH was observed with methanol extract of Bougainvillea glabra at 250 mg/kg (group IV). Further, more significant ($p<0.01$) decrease in plasma LDH value was marked in propranolol treated rats. The results were indicated in figure 4.

Plasma TBARS

The methanol extract of Bougainvillea glabra treated groups IV and V were significantly ($p< 0.05$ and $p< 0.001$) decreased the level of plasma TBARS when compared with isoproterenol induced group (Group-II) as shown in figure 2. Isoproterenol induced (group-II) animals showed significantly ($p< 0.001$)

Table 2 Effectof methanol extract of Bougainvillea glabra on TC, TG, HDL, LDL and VLDLduring isoproterenol induced myocardial necrosis and oxidative stress in rats.

Groups	TC (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Group I (Normal Control)	150.63±1.91	132.34±0.98	23.8±2.12	32.79±1.17	5.78±0.67
Group II (Isoproterenol + Control)	287.31±1.03**	209.7±3.89**	10.56±0.78**	88.16±2.04**	11.16±1.04**
Group III (Propranolol + Isoproterenol)	154.23±1.3**	165.76±7.5**	25.69±1.5**	49.72±1.5**	7.56±1.6**
Group IV (BG-250mg/kg + Isoproterenol)	188.55 ±1.5*	197.59±2.12*	16.67±0.72*	62.79±1.80*	9.46±0.88*
Group V (BG 500mg/kg+ Isoproterenol)	162.29±1.89**	172.82±1.34**	21.38±1.25**	54.34±1.56**	8.53±0.24**

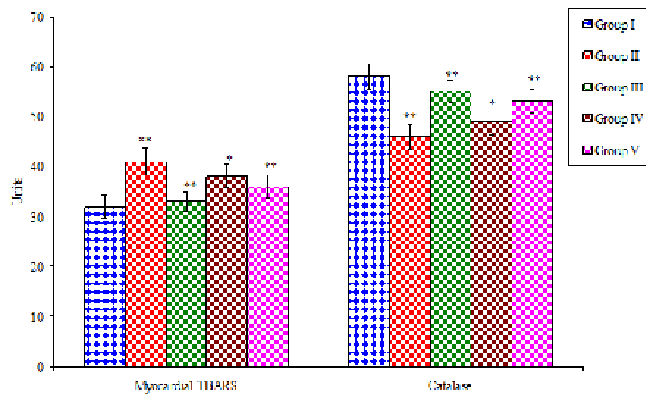

Figure 1 Effect of methanol extract of Bougainvillea glabraon myocardial level of TBARS (nM/g of tissue) and myocardial level of CAT (U/mgof protein) during isoproterenol induced oxidative stress in rats. Values aremean ± s.d (n=6). Group-2(isoproterenol induced) compared with group-1 (control rats). Groups- 3 (Propranolol),Groups- 4 (methanol extract of Bougainvillea glabra 250 mg/kg), 5(methanol extract of Bougainvillea glabra 500 mg/kg) compared withgroup-2 (isoproterenol induced rats). **P< 0.01, *P< 0.05.

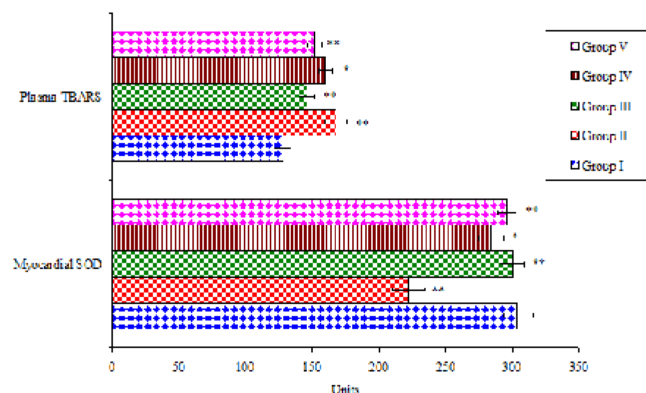

Figure 2 Effect of methanol extract of Bougainvillea glabraon plasma TBARS (nM/mL) and myocardial level of SOD (U/mg of protein) duringisoproterenol induced oxidative stress in rats. Values are mean ± s.d (n=6). Group-2 (isoproterenol induced)compared with group-1 (control rats). Groups- 3 (Propranolol), Group-4(methanol extract of Bougainvillea glabra 250 mg/kg), Group-5 (methanolextract of Bougainvillea glabra 500 mg/kg) when compared with group-2(isoproterenol induced rats). **P< 0.01, *P< 0.05.

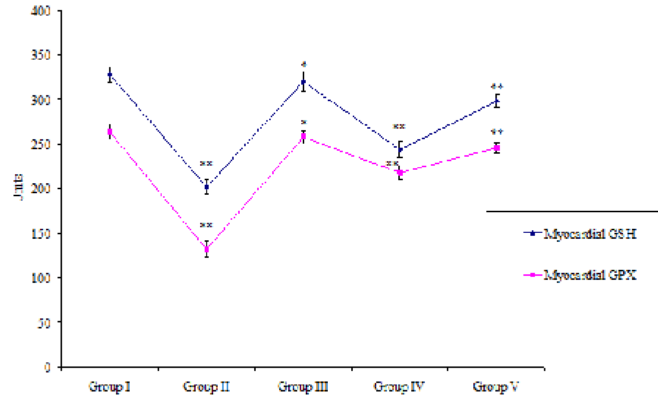

Figure 3 Effect of methanol extract of Bougainvilleaglabra on myocardial level of GSH (μg/ g of tissue) and myocardial level ofGPX (mU/mg of protein) during isoproterenol induced oxidative stress in rats.Values are mean ± s.d (n=6). Group-2(isoproterenol induced) compared with group-1 (control rats). Groups-3(Propranolol), Groups- 4 (methanol extract of Bougainvillea glabra 250mg/kg), 5 (methanol extract of Bougainvillea glabra 500 mg/kg) comparedwith group-2 (isoproterenol induced rats). ** P< 0.01, *P< 0.05.

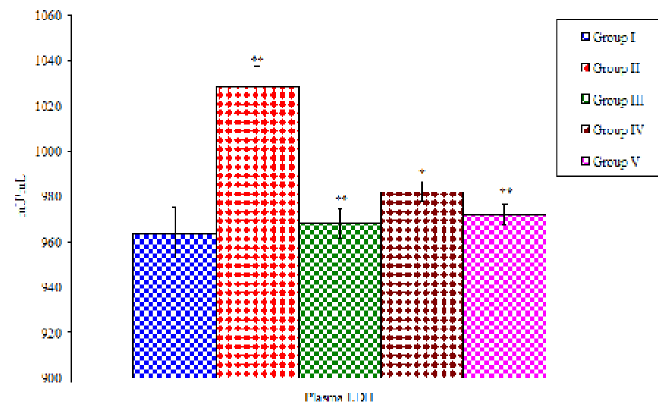

Figure 4 Effect of methanol extract of Bougainvilleaglabra on myocardial level of GSH (μg/ g of tissue) and myocardial level ofGPX (mU/mg of protein) during isoproterenol induced oxidative stress in rats.Values are mean ± s.d (n=6). Group-2(isoproterenol induced) compared with group-1 (control rats). Groups-3(Propranolol), Groups- 4 (methanol extract of Bougainvillea glabra 250mg/kg), 5 (methanol extract of Bougainvillea glabra 500 mg/kg) comparedwith group-2 (isoproterenol induced rats). ** P< 0.01, *P< 0.05.

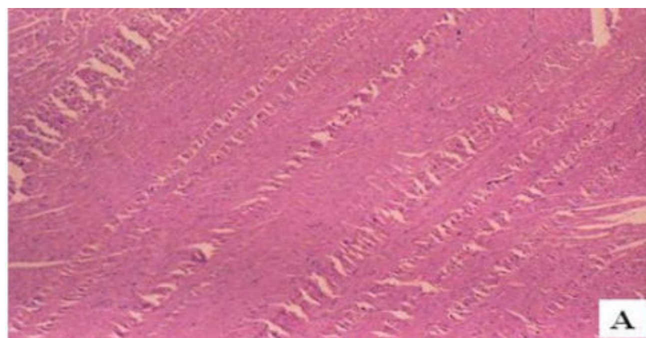

Figure 5 (Normal) (A) Light micrograph of control rat heart. Normal architectureof myocytes (H&E, 10X)

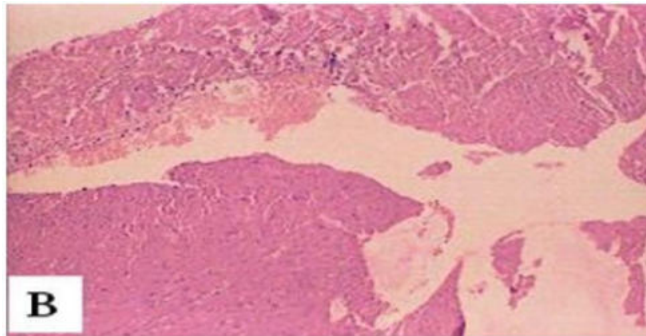

Figure 6 (Induced) (B) Isoproterenol (ISO) group showing focal confluent necrosisof muscle fiber with acute and chronic inflammation and myophagocytosis alongwith extravasation of red blood cells (10X, H&E).

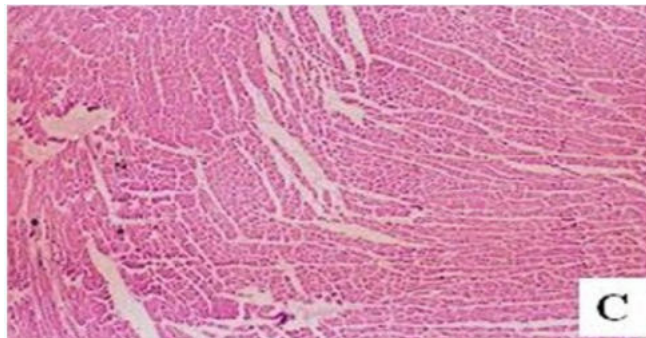

Figure 7 (C) Bougainvillea glabra extract (250 mg/kg) + Isoproterenol(ISO) group, showing focal necrosis of muscle fiber with acute and chronicinflammation (10X H&E)

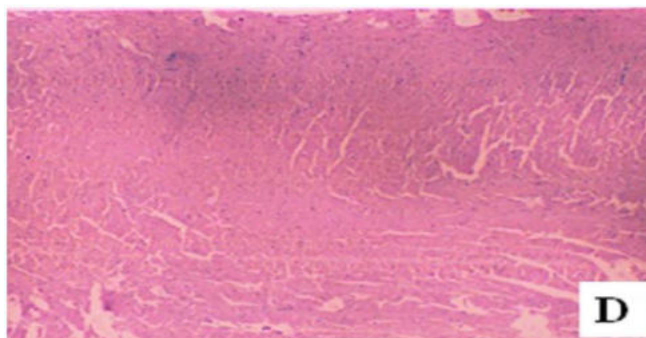

Figure 8 (D)Bougainvillea glabra extract (500 mg/kg) + Isoproterenol (ISO) group,showing occasional loss of muscle fiber with focal acute and chronicinflammation (10X H&E).

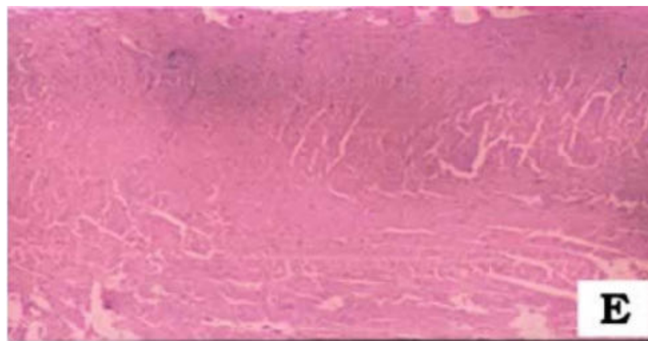

Figure 9 (E)Propranolol, showing occasional loss of muscle fiber with focal acuteand chronic inflammation (10X H&E).

increased plasma TBARS level than the control treated rats (Group-I). Propranolol treated rats (Group-III) has shown more significant ($p<0.01$) decrease in plasma TBARS compared with groups IV and V, respectively. The result was mentioned in figure 2.

Myocardial TBARS

There was significant ($p< 0.001$) increase in myocardial TBARS in the group-II (isoproterenol induced rat) when compared with group-I (control). Significant ($p< 0.01$; $p< 0.05$; $p< 0.01$) decrease in the level of myocardial TBARS was observed in groups III and IV and V in comparison to the isoproterenol induced rats (Group-II). The result was exposed in figure 1.

Myocardial SOD

Significant ($p<0.01$) reduction of myocardial SOD activity was observed in group II (isoproterenol induced rat), when compared to control group (group-I). Myocardial SOD increased significantly ($p<0.01$) in rats treated with propranolol 10 mg/kg (group-III) and 500 mg/kg methanol extract of Bougainvillea glabra (group-V) as shown in figure 2. However, there was less significant ($p< 0.05$) increase in the level of myocardial SOD activity in methanol extract of Bougainvillea glabra at 250 mg/kg (group-IV). The result was mentioned in figure 2.

Myocardial catalase

In isoproterenol induced group of rats (group-II), there was a significant ($p< 0.001$) decrease in myocardial catalase activity compared to control group (Group-I). In group IV, there was less significant ($p<0.05$) increase in the level of myocardial catalase activity compared to isoproterenol induced rat (group-II). However, more significant ($p< 0.01$) increase in myocardial catalase activity was seen in group-V. However, significant ($p< 0.01$) increased level of myocardial catalase was seen propranolol treated rats (group-III), as shown in figure 1.

Myocardial GSH

In group-II, significantly ($p< 0.01$) decreased myocardium GSH was observed in comparison to control rats (group I). There was less significant increase ($p< 0.05$) and change in GSH levels in group-IV (methanol extract of Bougainvillea glabra at 250 mg/kg) following isoproterenol administrations, when compared to group II (isoproterenol induced). However, more significant ($p< 0.01$) increase in myocardial GSH activity was observed in rats administered with propranolol (group-III) and methanol extract of Bougainvillea glabra at 500 mg/kg (group V). The result was indicated in figure 3.

Myocardial GPX

Significant ($p< 0.001$) reduction in GPX activity was observed in isoproterenol-induced rats without treatment of plant extracts (Group-II). Animals of group III exhibited greater significant ($p< 0.05$) increase in myocardial GPX activity when compared to isoproterenol-induced rat group (Group-II). However, there was significant ($p<0.01$) change and increase of GPX activity was observed in group IV and V in comparison to isoproterenol induced rat group. But change was less than the propranolol treated rats (group-III) as shown in figure 3.

Histopathology (Light microscopic study)

Figure 5 (A), shows the H&E light micrograph of control heart showing normal architecture. There was confluent necrosis of cardiac muscle fibers with infiltration of acute and chronic inflammatory cells along with extravasation of red blood cells in isoproterenol induced group [Figure 6 (B)]. In group- II, there was focal necrosis of muscle fibers with acute and chronic inflammation [Figure 7 (C)]. However, there was only occasional loss of myofibers and inflammation was observed in groups III, IV and V with minimal in comparison to other groups [Figure 8 (D)]. Myocardial necrosis was also unremarkable in those groups. [Figure 9 (E)] Propranolol, showing occasional loss of

muscle fiber with focal acute and chronic inflammation (10X H&E).

Discussion

Isoproterenol is a catecholamine it undergoes auto oxidation resulting in the formation of free radicals which causes failure of ATP dependent sodium and potassium channels, calcium overload, attack of cellular proteins, attack on the carbon linkage of polyunsaturated fatty acids [30] [31] . The consequence following a free radical attack is cell death resulting in necrosis of the cardiac tissue. Generation of free radicals by isoproterenol is considered as the causative factor for cardiotoxicity [32] . Thus, the determination of CK-MB isoenzyme is a useful parameter for assessing myocardial damage. Pretreatment with Bougainvillea glabra prevented depletion of CK-MB isoenzyme from heart as compared to isoproterenol group. Therefore, administration of Bougainvillea glabra plant extract reduced the release of CK-MB isoenzyme from myocardium into the systemic circulation, an indicative part of cardioprotective action of Bougainvillea glabra. Myocardial necrosis leads to rise of cardiac serum marker enzymes such as SGOT and SGPT that are liberated from the heart into blood [32] and indicating substantial cardio cellular injury [33] . Administration of methanol extract of Bougainvillea glabra at two different doses lowered the increased levels of the serum enzymes and produced a consequent recovery towards normalization when compared to control group animals. Isoproterenol induced elevation in cholesterol levels could be due to increase in biosynthesis of cholesterol and decrease in its utilization. Isoproterenol induces free radical formation, which may cause cellular cholesterol accumulation in the body by increasing cholesterol biosynthesis, it also causes reduced cholesterol ester hydrolysis and also by reducing cholesterol efflux. Pretreatment with the plant extract of Bougainvillea glabra restored the level of cholesterol in the body and reduces the elevated levels of cholesterol. Increase in lipid profile like triglycerides, LDL, VLDL and decreased HDL level in isoproterenol treated rats indicate that isoproterenol may be interfering with metabolism or biosynthesis of lipids. [34] The increased myocardial triglycerides content observed in ISO induced myocardial infracted rats is because of increased uptake of LDL from the blood by myocardial membranes [35] . A strong positive correlation has been documented between the risk of developing ischemic heart disease and serum LDL level; where as a negative correlation has been reported with HDL cholesterol [36] . Pretreatment with Bougainvillea glabra shows reduction in total cholesterol, triglycerides, LDL, VLDL and increase in HDL was observed. Decreased lipid profiles and increase HDL in Bougainvillea glabra treated groups may be due to presence of some bioactive compounds in plants. LDH has been used traditionally as a nonspecific diagnostic tool for myocardial infarction. A rise in the proportion of LDH can be diagnostic of myocardial infarction [34]. Administration of methanol extract of Bougainvillea glabra at two different doses lowered the increased level of the plasma LDH and produced a consequent recovery towards normalization when compared to control group animals. In isoproterenol induced cardio toxicity, oxidative stress is caused as result of the imbalance between endogenous antioxidants and free radicals of isoproterenol. It is already known that lipids are the most susceptible macro molecules to oxidative stress and our results showed that the level of lipid peroxides, measure in term of TBARS was significantly increased in plasma and heart of isoproterenol treated group. Bougainvillea glabra pretreatment in the present decreases the level of plasma and myocardial lipid peroxides by an apparent direct scavenging of superoxide and hydroxyl radicals and by inactivating the enzyme cyclo-oxygenase [37] . Superoxide dismutase (SOD) is another reactive oxygen species (ROS) defense enzyme present exclusively in the mitochondrial matrix and protects cells against the deleterious effects of super oxide anion derived from the per oxidative process in tissues. The observed enhancement in SOD activity might be due to the scavenging of free radicals by methanol extract of Bougainvillea glabra. However, when treated with, low dose and high dose of Bougainvillea glabra methanol extract the groups showed significant elevation in the level of these enzymes, which denotes the antioxidant activity of the Bougainvillea glabra. Catalase is a peroxisomal enzyme present in the mitochondria of the heart. It is one of the antioxidant defense enzymes which plays an indispensable role in the oxidation of hydrogen peroxide to oxygen and water [26]. In the present study, catalase activity was decreased and then restored to normal levels on administration of methanol extract of Bougainvillea glabra. In case of non enzymic antioxidants, GSH is a critical determinant of tissue susceptibility to oxidative damage. Decrease in cardiac GSH has been correlated with the enhanced toxicity of chemicals, including isoproterenol on living tissues [38]. The increase in myocardial GSH level in the rats treated with, 250 and 500 mg/kg of Bougainvillea glabra methanol extract may be due to synthesis or regeneration of GSH. The glutathione antioxidant system, GPx plays a crucial function in cellular resistance against oxidative stress by continuation 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 configuration of a disulphur bond. The lessening in enzymatic GPx in isoproterenol treated rats might be due to the influence of free radicals on antioxidant arrangement. In current study, GPx levels depleted by isoproterenol

were significantly prominent by methanol extract of Bougainvillea glabra. Methanol extract of Bougainvillea glabra produced cardioprotection at different dose levels. However 500mg/kg was found to be more effective than the lower dose. Histopathology study clearly indicated the extent of myocardial necrosis by showing variations for isoproterenol and other groups treated by two doses of Bougainvillea glabra and propranolol. Degree of myocardial necrosis and loss of muscle fiber were significantly less in methanol extract of Bougainvillea glabra and propranolol treated groups form the light microscopic changes of histopathology study.

Conclusions

In conclusion, the results of the present study indicate that the prior administration of methanol extract of Bougainvillea glabra for 30 days prevents the isoproterenol induced myocardial necrosis in rats. The overall cardioprotective effect of plant extract is probably related to a counteraction of free radicals by its antioxidant property. Histopathological findings further confirmed the cardiac protective effect of Bougainvillea glabra.

Authors' contributions

The complete research work was suggested and designed by Raja Sundararajan (Second Author). The extraction process, cardioprotective and antioxidant activities were carried out by Rakam Gopi Krishna (First author). The draft manuscript was prepared by Rakam Gopi Krishna and the final manuscript was edited by S. Raja (Second Author). Authors read and approved the final manuscript.

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