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CARDIOPROTECTIVE EFFECT OF METHANOL EXTRACT OF BUDDLEJA *ASIATICA* ON ISOPROTERENOL INDUCED MYOCARDIAL NECROSIS IN RATS

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ABSTRACT

Inadequate blood supply to the heart leads to infarction followed by irreversible necrosis of myocardial tissue. Buddleja asiatica is a medicinal plant screened for antihepatotoxic, hypotensive, anticancer and antioxidant activities. The current experimental research was designed to evaluate the cardioprotective efficacy of Buddleja asiatica against isoproterenol induced cardiac damage in rats. Methanol extract of Buddleja asiatica was administered orally for 30 days at two doses (500 mg and 1000 mg/kg). 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. 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. The results of the study indicated that, methanol extract of Buddleja asiatica showed myocardial salvage by restoring the cardiac marker enzymes and lessened the level of plasma lipid profiles along with an increase in HDL. Additionally, level of myocardial antioxidants increased along with a reduction in the content of malondialdehyde. The cardioprotective 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 Buddleja asiatica. The present investigation indicates that Buddleja asiatica exerts cardioprotection to rats intoxicated with isoproterenol.

***Key Words:** Buddleja asiatica, Isoproterenol, Necrosis, Antioxidant, CK-MB.*

INTRODUCTION

Choosing a healthy life style is important as it encompasses our overall well being. A scrupulous examination by the world health organization revealed that ischemic heart disease was one of the major causes for annual deaths worldwide [1]. Heart

disease is greatly influenced by age, diet, body weight, smoking and other stress levels. Any impairment in the supply of oxygenated blood to the myocardium through coronary artery may lead to oxidative stress related problems. The major consequence of this stress is overproduction of free radicals. There are much evidence indicating the role of free radicals in diseases such as coronary heart disease, stroke, diabetes mellitus and carcinogenesis [2]. Subjection of cardiomyocytes to inherently damaging agents such as toxins, trauma and chemical agents, impel the cell to choose necrosis as the common pathway to cell death [3]. Once the cell dies, the infarcted myocardium begins to undergo coagulative necrosis shortly. Unlike apoptosis, there is swelling of cell organelles followed by early disruption of plasma membrane in necrosis. Infiltration of macrophages inside the cell and emptying of cytosol into extracellular fluids happen consequently [4].

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 [5]. 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 autooxidation to aminochromes and free radicals causing a severe oxidative stress in the myocardium associated with necrosis of the left ventricular heart muscle [6]. 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 [7].

Buddleja asiatica commonly called as butterfly bush is a tender deciduous plant [8]. It is endemic to Asia and found in most parts of India. Traditionally, it is used in the treatment of many health disorders such as inflammation, rheumatism, skin disease, malaria etc [9,10]. In view of pharmacological aspect, Buddleja asiatica was screened for antihepatotoxic, antibacterial, hypotensive, anticancer, antifungal, antimalarial and antioxidant activities [11, 12]. More than 80 compounds including phenyl propanoids, flavonoids, Phenyl ethanoid glycosides, phenylpropanoid esters, non-phenolic compounds, triterpene saponins, iridoid glucosides, benzoates, triterpenoids, monoterpenes, acetogenins, steroids, shikimates as well as other trace elements were identified in Buddleja asiatica [13, 14]. To our knowledge, the potential of Buddleja asiatica as a cardioprotective agent was not demonstrated. Hence, present study was focused on evaluating the cardioprotective effects of methanol extract of Buddleja asiatica on rats intoxicated with isoproterenol.

MATERIALS AND METHODS

Chemicals

Isoproterenol was procured from Sigma chemicals, USA. 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.

Plant collection

Buddleja asiatica was collected from Tirupati (Andhra Pradesh) and further identified, confirmed & authenticated by Dr. Madavchetty, professor, Botany department, Sri Venkateswara University, Tirupati. The plant was preserved in the herbarium of GITAM Institute of Pharmacy, GITAM University, for future reference (Voucher specimen No -1751). 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 cardioprotective study.

Safety evaluation

Acute oral toxicity study in experimental rats was carried out as per OECD-423 guidelines and Barkatullah *et al* [15]. 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.

***In-vivo* cardioprotective activity**

Experimental animals

Albino rats of wistar strain of either sex (200-250gm) procured from Ghosh enterprises were 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 received saline (1 ml/kg, p.o.) daily for 30 days and in addition received isoproterenol (85 mg/kg, s.c.) on 29th and 30th 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 29th and 30th day at an interval of 24 hours. Group IV was treated with methanol extract of *Buddleja asiatica* (500 mg/kg, p.o.) daily for 30 days 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 *Buddleja asiatica* (1000 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 cardioprotective 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 [16]. 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 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 NaOH (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 [17]. 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 [18]. 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 [19]. 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 [20]. 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 30°C 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 [21]. 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 [22]. 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 103$M^{-1}cm^{-1}$ 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 [23]. 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 peroxidation assay

Lipid peroxidation (LPO) was measured by the method of Liu et al [24]. 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 study

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 *Buddleja asiatica* was found to be 6.6%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 *Buddleja asiatica* up to the dose levels of 2000 mg /kg.

Serum cardioprotective parameters

(Table 1) illustrates the effects of isoproterenol and methanol extract of *Buddleja asiatica* 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, BA-(500mg/kg) pretreatment in isoproterenol treated animals significantly decreased cardiac marker CK-MB (170.2±4.67), LDH (168.42±6.87), SGOT (190±7.38) and SGPT (118±11.45) concurrently increasing protein (6.7±0.55) level when compared to untreated isoproterenol group. Moreover, pretreatment with methanol extract of BA-(10000mg/kg) in isoproterenol treated group attenuated myocardial necrosis by decreasing the elevated levels of marker enzyme CK-MB (148.3±2.45), LDH (138.65±6.76) SGOT (132±9.11) and SGPT (66±31.87) besides decreasing level of protein (7.1±0.13) significantly when compared to untreated 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 BA (500mg/kg) mitigated the cardiac damage by a significant decrement in plasma TC (88.25 ±1.5), TG (91.59±2.12), LDL (67.79±1.80), VLDL (13.46±0.68), along with elevation in HDL level (19.67±0.72) when compared to untreated isoproterenol group. Similarly, pretreatment with methanol extract of BA (1000mg/kg) showed reduced lipoproteins level as plasma TC (82.29±1.99), TG (73.82±1.34), LDL (61.34±1.56), VLDL (11.53±0.54), along with a significant (p<0.01) increase in HDL level(24.38±1.25) when compared with untreated isoproterenol group.

Table 1. Effect of methanol extract of *Buddleja asiatica* 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(BA-500mg/kg +ISO)	170.2±4.67**	168.42±6.87*	190±7.38**	118±11.45*	6.7±0.55*
Group V(BA1000mg/kg+ISO)	148.3±2.45***	138.65±6.76**	132±9.11**	66±31.87**	7.1±0.13**

Note: 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 BA extracts compared with isoproterenol group.

Table 2. Effect of methanol extract of *Buddleja asiatica* 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(BA-500mg/kg +ISO)	88.25 ±1.5*	91.59±2.12*	19.67±0.72**	67.79±1.80*	13.46±0.68*
Group V(BA1000mg/kg+ISO)	82.29±1.99**	73.82±1.34**	24.38±1.25**	61.34±1.56*	11.53±0.54**

Note: 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 BA 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 *Buddleja asiatica* at a dose of 500 mg/kg distinctly prevented these isoproterenol induced alteration and maintained enzymes level of CAT

(48.5± 0.3), SOD (61.5±1.05), GSH (7.2±2.7), GRD (9.4±1.7), GPx (203.1±3.4) and lipid peroxidation (7.5±1.05) near to normal values. Pretreatment with methanol extract of *Buddleja asiatica* at a dose of 1000 mg/kg elevated the levels of cardiac CAT (50.7±0.5), SOD content (72.9±1.1), GRD (12.8±0.11), GSH (9.9±1.2) and GPx (212.2±5.9) along with reduced lipid peroxidation (6.8±04) when compared to isoproterenol group.

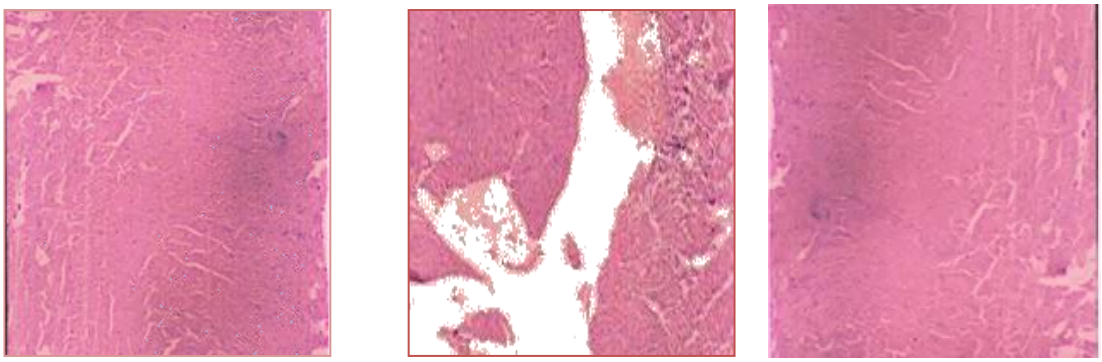
Table 3. Effect of methanol extract of *Buddleja asiatica* on antioxidant parameters.

Treatment	CAT(Umg-1 of protein)	SOD (Umg-1 of protein)	GSH (µ g/mg of protein)	GRD (nanomoles of NADPH oxidized min-1 mg-1 protein)	GPx (mU mg-1 of protein)	MDA (nanomoles of TBARS mg-1 of
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-1V(BA-500mg/kg +ISO)	48.5±0.3 *	61.5±1.05*	7.2±2.7*	9.4±1.7**	203.1±3.4*	7.5±1.05*
Group-V(BA-1000mg/kg +ISO)	50.7±0.5**	72.9±1.1**	9.9±1.2**	12.8±0.11**	212.2±5.9**	6.8±04**

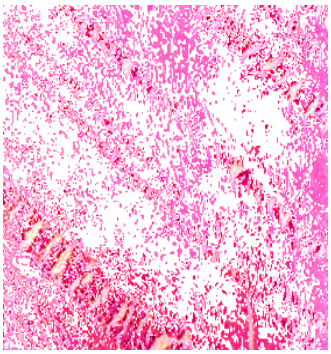
Note: Data are given as mean SD of six animals. # (P<0.05), ## (P<0.01), ### (P<0.001) when normal compared with isoproterenol group, * (P<0.05), ** (P<0.01), *** (P<0.001) when BA extracts compared with isoproterenol group.

Histopathological study of cardiac tissue

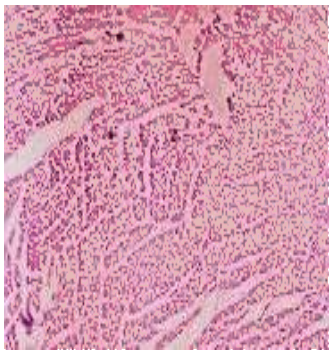
In (Figure 1a), the nucleus and cytoplasm were not disturbed or degenerated. The blood vessels and vacuoles of cytoplasm appeared intact. There was no visible necrotic damage to the myocytes in normal rat. (Figure 1b) illustrated the heart section of isoproterenol treated rats showed degeneration of cytoplasm, penetration of mononuclear inflammatory cells, presence of focal myelin fibres, accumulation of lipid material, faded nucleus and cellular swelling. Standard treated group showed normal cardiomycytes with considerable reduction in necrosis (Figure 1c). (Figure 1 d) showed heart sections from rats treated with methanol extract of *Buddleja asiatica* (500mg/kg) with less change in the morphology of myocytes, less changes in cytoplasmic vacuoles, swelling of cells and infiltration of macrophages and lymphocytes. When compared to the former dose, methanol extract of *Buddleja asiatica* (1000mg/kg) showed relatively less faded basophilia of chromatin in nucleus, mild infiltration of inflammatory cells and less necrotic area.(Figure 1e).



(a) (b) (c)



(d)



(e)

DISCUSSION

The present investigation was carried out to assess cardioprotective effect of methanol extract of *Buddleja asiatica* on isoproterenol induced cardiotoxicity in rats. Generation of free radicals by isoproterenol is considered as the causative factor for cardiotoxicity. Since isoproterenol is a catecholamine it undergoes autooxidation 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 [25]. The consequence following a free radical attack is cell death resulting in necrosis of the cardiac tissue.

Myocardial necrosis leads to rise of cardiac serum marker enzymes such as CK-MB, SGOT, SGPT and LDH that are liberated from the heart into blood [26]. The present study revealed a significant elevation in the activities of CK-MB, SGOT, SGPT and LDH levels indicating substantial cardiocellular injury. This was found to be in line with the previous reports [27]. Administration of methanol extract of *Buddleja asiatica* at two different doses (500 and 1000 mg/kg) lowered the increased levels of the serum enzymes, caused by isoproterenol and produced a consequent recovery towards normalization when compared to control groups animals.

Figure1. 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 *Buddleja asiatica* (500mg/kg) showed less change in the morphology of myocytes with changes in cytoplasmic vacuoles and swelling of cells. (e) Methanol extract of *Buddleja asiatica* (1000mg/kg) showed relatively less faded basophilia of chromatin in nucleus and less necrotic area. H&E (10 X)

Methanol extract of Buddleja asiatica produced cardioprotection at different dose levels. However 1000mg/kg was found to be more effective than the lower dose. In isoproterenol induced cardiotoxicity, oxidative stress is caused as result of the imbalance between endogenous antioxidants and free radicals of isoproterenol. 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 [28]. On the whole, isoproterenol leads to augmentation in the levels of myocardial lipids signifying its hyperlipidemic effect [29]. 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 *Buddleja asiatica* significantly altered the levels of TC, TG, LDL and VLDL by decreasing their levels besides increasing the level of HDL.

In the current study, isoproterenol treated groups exhibited a depletion or decrease in the amount of CAT, SOD, GRD, GSH and GPx resulting in intense myocardial necrosis. Pre treatment with methanol extract of *Buddleja asiatica* at a dose of 500 and 1000 mg/kg for 30 days markedly arrested isoproterenol induced alteration and preserved the antioxidant enzymes level near to normal values. Standard (propranolol) treated group also significantly increased the level of CAT, SOD, GRD, GSH and GPx in rats intoxicated with isoproterenol group. Catalase is a peroxisomal enzyme present in mitochondria of heart. It is one of the antioxidant defense enzymes which plays a indispensable role in the oxidation of hydrogen peroxide to oxygen and water [30]. In the present study, catalase activity was decreased and then restored to normal levels on administration of methanol extract of *Buddleja asiatica*. SOD is another ROS defense enzyme present exclusively in the mitochondrial matrix and protects cells against the deleterious effects of super oxide anion derived from the peroxidative process in tissues. The observed enhancement in SOD activity might be due to the scavenging of free radicals by methanol extract of *Buddleja asiatica.* The diminished activity of GRD and GPx observed specifies cardiac damage in the rats administered with isoproterenol [31]. Nevertheless, when treated with, 500and 1000mg/kg of Buddleja asiatica methanol extract the groups showed significant elevation in the level of these enzymes, which denotes the antioxidant activity of the *Buddleja asiatica.* 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 [32]. The increase in myocardial GSH level in the rats treated with, 500 and 1000 mg/kg of *Buddleja asiatica* methanol extract may be due to synthesis or regeneration of GSH. 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 [33]. Free radicals produced from isoproterenol led to irreversible tissue damage in heart by activation of lipid peroxidation. Treatment with methanol extract of *Buddleja asiatica* significantly decreased the lipid peroxidation induced membrane damage. The cardioprotective effect of *Buddleja asiatica* methanol extract was further supported by the histoarchitectural examinations. In general, morphological and histological changes such as decrease in swelling, lesser accumulation of fatty acids and lesser degree of karyolysis rendered considerable evidence for the cardioprotective activity of *Buddleja asiatica*.

CONCLUSION

The methanol extract of *Buddleja asiatica*, ameliorated the biochemical (SGPT, SGOT, TB, ALP, LDH and TP) and antioxidant parameters (CAT, SOD, GRD, GSH, GPx and MDA) levels significantly, which were comparable with prpranolol. From the experimental studies, *Buddleja asiatica* administered at two different doses (500mg/kg and 1000mg/kg) showed dose dependent cardioprotective activity. On the basis of the study it can be concluded that the anti oxidant potential might be instrumental for cardioprotective activity of methanol extract of *Buddleja asiatica.*

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

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