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HYPOLIPIDEMIC AND ANTI INFLAMMATORY ACTIVITY OF BOERHAAVIA DIFFUSA IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTED RATS

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ABSTRACT

Cardiovascular disease (CVD) or diseases of the circulatory system can be described as all diseases relating to the heart and blood vessels. The World Health Organization (WHO) estimates there will be about 20 million CVD deaths in 2015, accounting for 30 percent of all deaths worldwide. The present study was designed to evaluate the cardioprotective role of whole plant extract of Boerhaavia diffusa on isoproterenol induced myocardial infarction in wistar albino rats. The rats were divided into four groups of six animals each. Group I served as a normal control, Group II rats were administered isoproterenol (85mg/kg, *i.p*) at the end of experimental period on the45th and 46th days. Group III received an ethanolic extract of *Boerhaavia diffusa* 150mg/kg b.w for 45 days. Group IV rats were pretreated with *Boerhaavia diffusa* 150mg/kg b.w for a period of 45 days and received intraperitonial injection of isoproterenol (85 mg/kg, b.w) at the end of experimental period for 2 consecutive days. After the experimental period, blood was collected and serum was separated and used for the estimation of cholesterol, triglycerides, phospholipids, and lipoproteins and the assay lipid peroxidation and protein oxidation. The heart homogenate was used for the assay lipid profile, lipid peroxidation and protein oxidation. Isoproterenol induced rats showed a significant increase in the levels of triglycerides, total cholesterol and free fatty acids in both serum and heart homogenate. A rise in the levels of LDL, VLDL with a significant decrease in the level of HDL was also observed in the serum and heart tissues of isoproterenol treated rats. But the phospholipid content was found to be significantly increased in serum and decreased in heart tissue. The values of atherogenic index and C/P ratio were significantly increased in the ISPH treated group. The oral administration of ethanolic extract of *Boerhaavia diffusa* 150/kg b.w) to isoproterenol-induced rats daily for a period of 45 days proved the protective role of *Boerhaavia diffusa*. The levels of the biochemical parameters in the plant treated groups were nearly the same as that of the normal control.

KEY WORDS: Boerhaavia Diffusa, Isoproterenol, Lipid Peroxidation, Atherogenic Index

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INTRODUCTION

Cardiovascular disease (CVD) remains the leading cause of death and disability in developed countries and was predicted by the WHO to become the major cause of mortality in developing countries by 2020. The world health organization (WHO) estimates that 17 million people die from cardiovascular disease annually [1]. CVDs accounts for ~30% of death worldwide, including nearly 40% in high-income countries and 28% in low and middle-income countries. By 2030, researchers project that non-communicable diseases will account for more than three-quarters of deaths worldwide; CVD alone will be responsible for more deaths in low income countries than infectious diseases (including HIV/AIDS, tuberculosis, and malaria), maternal and perinatal conditions, and nutritional disorders combined[2]. India is already the 'Death Capital' of the world and is projected to have the highest number of individuals suffering from atherosclerotic CVD by the year 2020[3]. CVD, a group of disorders of the heart and the vasculature, includes high blood pressure, coronary heart disease, congestive heart failure, stroke and congenital heart defects, cerebrovascular disease, peripheral arterial disease, rheumatic heart disease, deep vein thrombosis and pulmonary embolism. The medical term for heart attack is Myocardial infarction (MI). In which "Myocardia" means heart muscle and "Infarction" refers to the permanent damage to the part of heart tissue resulting from lack of oxygen and blood supply, which occurs by a process of hardening of the arteries (commonly called " atherosclerosis") in coronary vessels [4]. The resultant abnormalities of Myocardial infarction in cardiac function are well predictable that, it is a compound event of distressing the electrical, physical, mechanical, and biochemical properties of heart. Consequences of MI include hyperlipidemia, peroxidation of membrane lipids and loss of plasma membrane integrity [5]. Most heart attacks are caused by atherosclerosis (stiffening and narrowing of the arteries).Atherosclerosis results from the formation of plaques inside the artery which is composed of high blood fats (triglycerides) and LDL or "bad" cholesterol, narrowing the passage and reducing the amount of blood that can flow through blood vessels [6]. LPO is a natural metabolic process under normal conditions. Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids (PUFA) of membranes and are capable of further lipid peroxidation by a Free radical chain reaction [7]. LPO of biological membranes increases their leakiness to ions and causes damage to transmembrane proteins such as receptors and enzymes. The levels of LPO expressed as MDA, which is the product of major chain reactions leading to definite oxidation of polyunsaturated fatty acids such as linoleic acid and linolenic acid, saves as a reliable marker of LPO [8]. LPO of biological membranes increases their leakiness to ions and causes damage to transmembrane proteins such as receptors and enzymes. Chemically isoproterenol (ISPH) is an L-β-(3, 4-dihydroxyphenyl)-α isopropyl amino ethanol hydrochloride. Isoproterenol acts as synthetic catecholamine β-adrenergic agonist and its intra peritoneal administration generates unaltered acute myocardial injury in rats that resembles the myocardial infarction of human beings [9]. It induces myocardial necrosis by a cascade mechanism [10]. The cardiotoxic action of isoproterenol is principally due to the peroxidation of endogenous lipids [11]. Isoproterenol-induced myocardial infarction is generally attributed to the formation of the highly reactive hydroxyl radical (OH'), stimulator of lipid peroxidation and source for the destruction and damage to cell membranes [12].The plant kingdom represents an enormous reservoir of biologically active molecules and so far only a few plants with medicinal activity have been evaluated. Nearly 50% of drugs used in medicine are of plant origin [13]. In addition, herbal drugs are extensively used to treat various diseases due to their effectiveness, minimal side effects and relatively low cost [14]. Several plant extracts have been employed in the management of cardiovascular and cerebrovascular diseases [15,16] of which plasma lipid profile form part of risk factors. In these circumstances one of the most vital, easily available, medicinal and edible plant is *Boerhaavia diffusa. Boerhaavia diffusa* (Hogweed in English) belongs to the family Nyctaginaceae, is mainly a diffused perennial herbaceous creeping weed of India (known also under its traditional name as

Punarnava) and of Brazil (known as *Erva tostao).* Various parts of *Boerhaavia diffusa* are used for the treatment of numerous disorders in different parts of India. In the Ayurvedic herbal medicine, the whole plant of *B. diffusa* is widely used for the treatment of various disorders. The root is primarily used to treat gonorrhea, internal inflammation of all kinds, dyspepsia, oedema, jaundice, menstrual disorders, anaemia, liver, gallbladder and kidney disorders, enlargement of spleen, abdominal pain, abdominal tumours, and cancers. The juice of *B. diffusa* leaves serves as a lotion in opthalmia. It is also administered orally as it is a blood purifier and to relieve muscular pain. Though the plant has so much of microbial, therapeutical, pharmacological, medicinal and nutritive value, no studies were undertaken regarding its beneficial effect in preventing isoproterenol-induced myocardial infarction. So the present study was designed to evaluate the cardio protective role of the ethanolic extract of *B. diffusa* in Wistar strains of albino rats.

MATERIALS AND METHODS

PLANT MATERIAL

The whole plant of fresh *Boerhaavia diffusa* was collected in the month of august at bramhadevum village of Anantapur district, Andhrapradesh. The plant is thoroughly washed for 3 times under tap water and finally with distilled water. The plants were shade dried, cut into small pieces and coarsely powdered. The coarse powder was used for extraction with ethanol.

PREPARATION OF ETHANOLIC EXTRACT

The shade dried powder from whole plant of *B. diffusa* Linn., (3.0kg) was extracted with ethanol at room temperature for 16 h for 5 times (5 X 4L). The combined extracts were evaporated under vacuum using rotavapor at 40^0C. A weighed portion of the extract was dissolved in 20% dimethyl sulfoxide (DMSO) prior to oral administration to animals.

SOURCE OF CHEMICALS

The chemicals used in the present study were of analytical reagent grade. It was purchased from SD fine chem., Himedia and Qualigens, India

EXPERIMENTAL MODEL

Wistar albino rats, weighing 150 – 190g, procured from the small animal breeding centre, sri venkateswara enterprises, Bangalore, India were used. The study was approved by Animal Ethics Committee of S.K. University, Anantapur (Reg. No 470/01/a/CPCSEA, dt.24th Aug 2001). Animals were acclimatized under standard laboratory conditions at 25°± 2°C. 60 ± 15% room humidity and normal photoperiod (12 h light: dark cycle) for seven days. The animals were fed with commercial rat pellet diet and water *ad libitum*.

INDUCTION OF MYOCARDIAL INFARCTION

Myocardial infarction was induced by intraperitoneal (i.p.) injection of isoproterenol hydrochloride 85 mg/kg body weight, dissolved in physiological saline, for two consecutive days (45^{th} and 46^{th} day) [17].

EXPERIMENTAL DESIGN

The three months age of wistar albino rats, weighing 150 – 190g were divided into four groups, each comprising six animals. Group I served as a control, Group II rats were administered with isoproterenol (85mg/kg body weight administered subcutaneously twice at an interval of 24h) dissolved in normal saline at the end of the experimental period. Groups III and IV animals were pretreated with ethanolic extract of *Boerhaavia diffusa (BDEEWP)* (150mg/kg body weight) for a period of 45 days and then the group IV is administrated with isoproterenol (85 mg/kg body weight administered intraperitonial twice at an interval of 24 h) at the end of the treatment period on the 45th and 46th day.

EXTRACTION AND ESTIMATION OF HEART TISSUE LIPIDS

From the samples of heart tissue homogenate the total lipids were extracted by the method of [18]. A known volume of suspension was mixed with 10 ml of chloroform– methanol (2:1 v/v) and shaked vigourously. Then it was filtered through Whatman filter paper (No. 42) into a separating funnel. The filtrate was mixed with 0.2 ml of physiological saline and the mixture was kept overnight undisturbed. The lower phase containing the lipid was drained off into pre weighed beakers. The upper phase was re extracted with more of chloroform–methanol

mixture, the extracts were pooled and evaporated to dryness. The lipids were re-dissolved in 1.0 ml of chloroform–methanol (2:1) mixture and aliquots were taken for the estimation of lipid components. The aliquots were dried in a rota evaporator to evaporate the solvent before use.Total cholesterol [19], triglyceride [20], free fatty acids [21], and phospholipids [22] and HDL[23]were assayed. LDL and VLDL cholesterol were calculated using formula of [24]. Atherogenic index (AI) is the ratio of total cholesterol to HDL-C, and calculated by the method given by [25]. Atherogenic Index (AI) = Total cholesterol – HDL-C/H DL-C Lipid peroxidative extent was measured by the formation of malondialdehyde (MDA) by using the method of [26].

STATISTICAL ANALYSIS

All results were expressed as means ± SE of a six individual observations Duncan's Multiple Range (DMR) test was performed to know the level of significance among all the experimental groups [27].

RESULTS

1.CHANGES IN THE LEVELS OF SERUM LIPOPROTEINS

Lipids being insoluble in water need a transport system made up of lipoproteins such as chylomicrons, VLDL-C, LDL-C and HDL-C. Estimation of these lipoproteins is used as an index to measure the levels of lipids present in the serum. Table 1 shows the effect of *B.diffusa* on lipoproteins (VLDL, LDL and HDL) of control and experimental animals. Circulating levels of LDL-C (170.04%) and VLDL-C (40.22%) was significantly (P<0.05) increased followed by parallel decrease in HDL–C (48.47%) in isoproterenol treated rats (group 2) as compared to the control rats (group 1). Nevertheless, the levels were statistically similar in control (group 1) and BDEEWP alone treated (group 4) rats. In BDEEWP + Isoproterenol treated rats (group 3), a fall in the HDL-C level is partially prevented (63.10%) and further increase in the levels of LDL-C and VLDL-C is inhibited by 87.42% and 26.28% respectively when compared to ISPH administered (group 2) rats. Rats treated with BDEEWP (group 4) for 45 days did not change in value of AI compared to control rats (group 1), but administration of ISPH (group 2) enhanced this value by 386.94% which is statistically significant (P<0.05). Rats pretreated with BDEEWP followed by ISPH administration (group 3) showed moderate decrease in this level by 87.37% compared to control rats (group 1).

2.CHANGES IN THE LEVELS OF SERUM LIPID PROFILE

Table 2 depicts the levels of TC, TG, FFA, PL and C/P ratio in serum of control and experimental rats. Intraperitonial administration of Isoproterenol (group 2) caused a significant (P<0.05) increase in the levels of cholesterol (71.28%), TG (59.47%), FFA (28.54%), PL (16.38%) and C/P ratio (47.38%) in serum when compared with control rats. In BDEEWP pretreated rats (group 4) alteration was minimized in the levels of cholesterol (90.89%), TG (50.15%), FFA (175.67%), PL (101.91%) and C/P ratio (84.83%) when compared with Isoproterenol alone injected rats (group 2). The activities of tissue levels of triglyceride, cholesterol, free fatty acids and phospholipids were near to control rats treated with BDEEWP (group 3) as compared to untreated control rats (group 1).

3.CHANGES IN THE LEVELS OF HEART LIPOPROTEINS

Data in table 3 represents the effect of BDEEWP on heart lipoprotein levels in normal control and ISPH treated rats. ISPH administered (group 2) rats showed a significant increase (P<0.05) in the concentrations of heart LDL-C and VLDL-C (295.29% and 79.46%) and a significant decrease (P<0.05) in the concentration of HDL-C (44.31%), when compared with those of normal (group1) rats. Pretreatment with BDEEWP to ISPH treated rats (group 3) decrease the levels of LDL-C and VLDL-C (78.95% and 180.35%) significantly (P<0.05), whereas the levels of HDL-C (78.87%) of ISPH induced myocardial infracted rats increased when compared with untreated ISPH induced myocardial infarcted (group 2)rats.The activities of lipoproteins (VLDL, LDL and HDL) were near to control rats treated with BDEEWP (group 3) as compared to untreated control rats (group 1).

4.CHANGES IN THE LEVELS OF HEART TISSUE LIPID PROFILE

Table 4 shows the effect of *Boerhaavia diffusa* on tissue lipid metabolism (triglyceride, cholesterol, free fatty acids and phospholipids) of control and experimental animals. Tissue levels of triglyceride (79.84%), cholesterol (61.84%), free fatty acids (42.46%) and C/P ratio (158.33%) were significantly ($P<0.05$) increased except phospholipids (37.71%), which show a significant decreased levels in isoproterenol treated rats (group 2) as compared to the control rats (group 1), whereas the activity of triglyceride (90.18%), cholesterol (82.13%), free fatty acids (87.56%) and C/P ratio (81.13%)were significantly ($P<0.05$) decreased except phospholipids (60.56%), which showed increased levels in BDEEWP + isoproterenol treated rats (group 4) as compared to untreated isoproterenol treated rats (group 2). The activities of tissue levels of triglyceride, cholesterol, free fatty acids and phospholipids were near to control (group1) rats treated with BDEEWP (group 3).

5.CHANGES IN THE COMPONENTS OF LIPID PEROXIDATION AND PROTEIN OXIDATION

The effect of *Boerhaavia diffusa* on the extent of lipid peroxidation (LPO) and protein oxidation (PO) in heart of control and experimental groups are summarized in the table 5. After the end of the experiment, ISPH induced group showed significantly enhanced LPO in heart (51.3 %), when compared to corresponding values of control group. Oral pre administration of BDEEWP to ISPH induced myocardial infracted rats prevented the increased tissue LPO to almost normal was observed. However, administration of BDEEWP to normal rats showed slight decrease in tissue LPO levels when compared to the corresponding values of control group.

Table 1

Effect Of Bdeewp Treatment And Pretreatment On Plasma Lipoprotein Cholesterol And Atherogenic Index In Control And Experimental Groups.

PARAMETER	CONTROL	ISPH	CON+BDEEWP	ISPH+BDEEWP
HDL (mg %)	38.71 ± 0.37^{a}	19.95 ± 0.22^{d}	34.81 ± 0.26^{b}	31.77 ± 0.19^{c}
VLDL (mg %)	9.87 ± 0.11^{a}	13.84 ± 0.02^{c}	9.87 ± 0.05^{a}	12.80 ± 0.04^{b}
LDL (mg %)	48.12 ± 0.72^{a}	129.95 ± 0.78^{c}	48.85 ± 0.16^{a}	58.42 ± 0.29^{b}
AI*	1.51 ± 0.03^{a}	7.34 ± 0.10^{c}	1.69 ± 0.02^{a}	2.24 ± 0.02^{b}

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other. * AI = Total Cholesterol – LDL-c/HDL-C

Table 2

Changes in the serum lipid profile of control and experimental rats.

PARAMETER	CONTROL	ISPH	CON+BDEEWP	ISPH+BDEEWP
TC (mg %)	96.71 ± 0.58^{a}	165.64 ± 0.78^{c}	93.53 ± 0.28^{a}	102.99 ± 0.22^{b}
TG (mg %)	49.35 ± 0.56^{a}	78.693 ± 0.15^{c}	49.35 ± 0.26^{a}	63.98 ± 0.19^{b}
FFA (mEq/L)	34.17 ± 0.60^{a}	43.93 ± 0.01^{c}	31.89 ± 0.22^{a}	26.79 ± 0.25^{b}
PL (mg %)	94.63 ± 0.83^{a}	110.13 ± 0.04^{b}	94.36 ± 0.23^{a}	94.33 ± 0.17^{a}
C/P RATIO	1.02 ± 0.01^{a}	1.50 ± 0.01^{c}	0.99 ± 0.00^{a}	1.09 ± 0.00^{b}

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other.

Table 3

Effect of BDEEWP treatment and pretreatment on Heart lipoprotein cholesterol and Atherogenic Index in Control and Experimental groups

PARAMETER	CONTROL	ISPH	CON+BDEEWP	ISPH+BDEEWP
HDL (mg %)	1.99 ± 0.00^{a}	1.11 ± 0.00^{c}	2.01 ± 0.01^{a}	1.81 ± 0.02^{b}
VLDL (mg %)	0.75 ± 0.00^{a}	1.34 ± 0.00^{b}	0.75 ± 0.01^{a}	0.27 ± 0.00^{c}
LDL (mg %)	0.85 ± 0.01^{a}	3.36 ± 0.00^{c}	0.95 ± 0.04^{a}	1.38 ± 0.01^{b}
AI*	0.80 ± 0.00^{a}	4.24 ± 0.00^{c}	0.85 ± 0.02^{a}	1.21 ± 0.01^{b}

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other. * AI = Total Cholesterol – LDL-c/HDL-c

Table 4
Changes in the Heart tissue lipid profile of control and experimental rats

PARAMETER	CONTROL	ISPH	CON+BDEEWP	ISPH+BDEEWP
TC (mg %)	3.59±0.01[a]	5.81±0.01[c]	3.70±0.03[a]	3.99±0.01[b]
TG (mg %)	3.72±0.01[c]	6.69±0.00[a]	3.73±0.03[c]	4.01±0.01[b]
FFA (mEq/L)	5.02±0.01[a]	7.15±0.02[c]	5.05±0.02[a]	5.28±0.02[b]
PL (mg %)	15.06±0.03[a]	9.38±0.06[c]	15.25±0.02[a]	12.82±0.03[b]
C/P RATIO	0.24±0.00[c]	0.62±0.00[a]	0.24±0.00[c]	0.31±0.00[b]

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other

Table5
changes in the components during lipid peroxidation and protein oxidation

PARAMETER	CONTROL	ISPH	CONC+BDEEWP	ISPH+ BSEEWP
µmoles of MDA	11.43±0.12[a]	18.27±0.10[c]	12.56±0.19[a,b]	13.70±0.28[b]
Protein oxidation	3.33±0.01[a]	4.24±0.01[c]	3.16±0.01[b]	3.37±0.00[a]

Values are mean ± S.E.M for six rats in each group. Values in the same row not sharing a common superscript (a-c) differ significantly P<0.05 with each other.

DISCUSSION

Cardiovascular disease is a major global health problem reaching epidemic proportions in the Indian subcontinent [28] and low and middle income countries, accounting for 78% of all deaths[29]. Myocardial cell protection and prevention of cell ischemia or necrosis have been therapeutic targets for a long time. Lipids play an important role in cardiovascular disease, not only by way of hyperlipidemia and the development of atherosclerosis leading to myocardial infarction, but also by modifying the composition, structure and stability of cellular membranes. The relationship between lipid levels and myocardial infarction studied in detail and it has contributed enormously to the literature. Hypercholesterolemia, high concentration of low-density lipoprotein cholesterol and hyper triglyceridemia are accepted as independent risk factors for atherosclerotic cardiovascular disease and mortality[30,31]. High blood cholesterol concentration is one of the important risk factors for cardiovascular disease[32].Significant changes in the fatty acid composition of serum triglycerides, cholesterol ester and phospholipids were also reported in acute myocardial infarction condition [33]. The lipid abnormalities seen in myocardial infarction appear to correlate with changes in cellular and cell membrane functions.In the current study, pretreatment with *B.diffusa* significantly ($p< 0.05$) prevented the isoproterenol-induced elevation in total cholesterol, triglycerides and free fatty acids in serum and heart tissue of BDEEWP + Isoproterenol treated (Group 4) rats as compared to that of isoproterenol treated (Group 2) rats. It also maintained the level of LDL-cholesterol and HDL-cholesterol in serum at a concentration comparable to that of Group I rats. The cardioprotective effect of *B.diffusa* is related to its ability to inhibit the increased accumulation of lipids both in systemic circulation and in myocardium by its antilipidemic property. LDL-receptors play an important role in the regulation of serum LDL cholesterol levels [34]. Hence, it might be the mechanism of action of *B.diffusa* in exerting hypolipidemic activity in experimentally induced myocardial infarction condition. Plasma concentration of atherogenic LDL-cholesterol is regulated by the production of rate of VLDL and the utilization of LDL-cholesterol by LDL receptors. The protective role of HDL cholesterol is attributed to its involvement in reverse cholesterol transport, its antioxidant and anti-thrombotic properties [35]. In the present study, there was a significant ($p< 0.05$) elevation noticed in the levels of cholesterol, triglycerides and free fatty acids in serum and heart tissue of Isoproterenol induced rats (Group 2) rats as compared to Group1 control rats, which is an indication of severity of isoproterenol-induced

hyperlipidemic condition. The level of LDL cholesterol was significantly ($p< 0.05$) higher in myocardial infarction induced (Group 2) rats, whereas HDL cholesterol levels were significantly lower compared to control (Group 1) animals. These findings are in accordance with earlier reported studies [36, 37]. There was an increase in the mobilization of LDL-cholesterol from the blood into the myocardial membranes, resulting in abnormal cholesterol deposition in the myocardium, which showed that the free fatty acids liberated from adipose tissue enters into the myocardium, and the process is proportional to the free fatty acid concentration in the coronary sinus. Though heart utilize free fatty acids for its energy requirements, the excess free fatty acid may be used for the synthesis of triglycerides which ultimately leads to hypertriglyceridemia condition, as observed in the present study.The AI is thought to be an important risk factor for atherosclerosis, and was significantly lowered in the BDEEWP pretreated rats. This decrease in the AI is however another positive change resulting from the BDEEWP treatment. The ratio of difference between TC and HDL-C to HDL-C, greater than 4.5 is considered a powerful predictor of CAD[38].There was a significant ($p< 0.05$) depletion noticed in the levels of phospholipids in heart tissue of Group 2 animals compared of Group 1 rats. This is in line with earlier reported studies [36,39,40], which indicated that ischemic injury related alterations in lipid composition of myocardial tissue appeared to occur due to destruction of myocardial membrane lipid bilayer. Hence, the significant elevation noticed in the levels of free fatty acids in plasma and heart tissue of isoproterenol-induced rats might be due to enhanced breakdown of membrane phospholipids both in adipose tissue and myocardium by the lipolytic action of phospholipase A; [41,42]. The results of the current investigation showed that prior administration of *B.diffusa* significantly ($p< 0.05$) prevented the isoproterenol-induced degradation of membrane phospholipids in heart tissue of Group 4 rats compared to Group 2 rats, establishing its membrane stabilizing effect. It probably did so by decreasing isoproterenol-induced calcium overload in the myocardium. Hence, it is postulated that *B.diffusa* may also protect myocardial cell membrane from necrotic damage by its membrane-stabilizing action and antioxidant property. BDEEWP pretreatment significantly prevented the degradation of phospholipid levels and decreasing the ratio of cholesterol / phospholipids (C/P). it might be due to decreased membrane lipolysis by decreasing the action of phospholipase A2 through the depletion of intracellular calcium levels. The results clearly demonstrate the antiatherogenic nature of the BDEEWP against ISPH action. Lipid peroxidation in vivo has been identified as one of the basic deteriorative reaction in cellular mechanisms of the myocardial ischemia [43,44.] Significantly, increased extent of lipid peroxidation and protein oxidation observed in heart of diseased groups (2 and 4) indicates the induction of oxidative stress by isoproterenol. Lipid peroxidation has been implicated in the pathogenesis of a number of diseases include atherosclerosis, cancer etc., This was in agreement with the reports of Nirmala & Puvanakrishnan[45] which indicated that a lack of antioxidant defense might lead to an increase in lipid peroxidation and subsequent deleterious effects on the myocardial membrane in isoproterenol-induced myocardial infarction condition. It is now generally accepted that lipid peroxidation and its product play an important role in liver, kidney, heart and brain toxicity[46]. Lipid peroxidation has probably been the most extensively investigated process induced by free radicals. Lipid peroxides are derived from the oxidation of polyunsaturated fatty acids of membranes and are capable of further LPO by a free radical chain reaction [7]. All bio macromolecules are faced with oxidative stress including proteins. Protein oxidation is defined as the covalent modification of a protein induced either directly by reactive ROS or indirectly by reactions with secondary by products of oxidative stress. There are several pieces of evidence for the role of protein oxidation in atherosclerosis. In the atherosclerotic plaques besides lipids and DNA, proteins are also prevalent targets for reactive oxygen species (ROS) mediated oxidative damage. Structural differences between oxidized and native phospholipids affect their function and mode of interaction with cells, proteins, elements of the immune

system, and role in the atherosclerotic process[47]. Oxidation of protein is a common phenomenon mediated by highly reactive agents in myocardial infarction condition and oxidized proteins are in turn capable of inducing oxidative stress, a potential mediator of the pathogenesis. This protein oxidation might also be a possible reason for the decline noted in the protein and glycoprotein levels in the heart tissue of isoproterenol-injected rats. The disaggregation of polyribosomes might be associated with the inhibition of protein synthesis and the decreased protein synthesis in turn might have led to reduced glycoprotein synthesis. Pretreatment with BDEEWP resulted in near normal levels of protein and glycoprotein components. It probably did so by preventing the isoproterenol~induced necrotic damage to the myocardial cell membrane by inhibiting the disaggregation of polyribosomes or by attenuating the isoproterenol-induced oxidation of myocardial proteins.

CONCLUSION

The present study established that ethanolic extract of *Boerhaavia diffusa* exhibited hyolipidemic and anti inflamatory effects in isoproterenol induced MI rats. Therefore, BDEEWP could be used as hypolipidemic and anti inflammatory agent in the management of CVD associated with abnormalities of lipid profiles. Further research is needed to purify and identify the specific bioactive compounds that are responsible for the cardioprotective action of *Boerhaavia diffusa*.

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