Corrected Research Article

Protective effect of *Ipomoea biloba* on myocardial antioxidant status in Isoproterenol Induced Myocardial Infarction male albino Rats

Abstract: The protective effect of ethanolic leaf extract of *Ipomoea biloba* in isoproterenol (ISO)-induced cardiotoxicity and the antioxidant activity involved in this protection were investigated in rats. Myocardial infraction was produced in rats with 20 mg/kg b.wt of ISO administered subcutaneously twice at an interval of 24 h. Effect of *EEIB* oral treatment for 28 days at two doses (100 mg and 200 mg/kg body weight) was evaluated against ISO – induced cardiac necrosis. Level of enzymatic (SOD, CAT, GPx and GST), non-enzymatic (GSH, Vitamin C and E) and of membrane bound ATPases (Na^+K^+ATPase, Mg^{2+}ATPase and Ca^{2+}ATPase) were assayed in heart homogenate. Significant myocardial infraction, depletion of endogenous antioxidants enzymatic and non-enzymatic were observed in ISO-treated animals when compared with the normal animals. Rats induced with ISO, showed a significant ($P<0.05$), decrease in the activities of GSH, Vitamin C and Eon comparison with normal rats. *EEIB* elicited a significant cardioprotective activity by elevated the levels of GSH, SOD, CAT, GPx and GR. A significant decrease in the activity of Na^+/K^+ ATPase and a corresponding increase in the activities of Ca^{2+} ATPase and Mg^{2+} ATPase were observed in isoproterenol induced rats when compared to normal control rats. Pretreatment with *EEIB* was able to efficiently prevent the increase in activity of Mg^{2+} ATPase and maintain the activities of Na^+/K^+ ATPase and Ca^{2+} ATPase at near normality. There is no significant difference between the control and plant alone treated rats. The aim of this investigation is to evaluate the antioxidant effects on the main cardioprotective activity of ethanolic leaf extract *Ipomoea biloba.*

Key words: Antioxidant, *EEIB*, Isoproterenol, *Ipomoea biloba*, Myocardial infarction

Introduction

Antioxidants are substances that prevent oxidation, a chemical reaction that can produce free radicals and chain reactions that can damage an entity's cells. Antioxidants like thiols and ascorbic acid can stop these processes from happening. Plants and mammals maintain sophisticated systems of releasing antioxidants, such as glutathione, to balance oxidative stress. (Dabelstein *et al., 2007*).

Many foods, particularly fruits and vegetables, are high in antioxidants, which help to counteract the detrimental effects of oxidation on cells throughout the body (Tracey Roizman 2018). Antioxidant substances reduce reactive oxygen species (ROS), which causes free radicals to decay. When the ROS benefit is questioned, there is a greater vulnerability to atopic or disease consequences due to disruption to the Thymus-1 immune response chain's attack-kill-present-respond activity. Free radicals are unbalanced molecules that form as consequences of the conversion of food into energy in your body. They also release when you are exposed to

pollutants in the environment. By altering the structure of biological DNA, free radicals can cause harm. They can also harm cellular membranes, altering their appearance and threatening cell survival. Free radical damage could speed up the ageing process and raise the risk of heart disease, cancer, and other disorders. Antioxidants help free radicals by altering their chemical structure and converting them to harmless molecules (Kandhan *et al.,* 2017,). Excessive antioxidant consumption may consequently result in antioxidative stress, in which antioxidants may reduce or stop adaptive stress responses, resulting in severe health conditions and injury (Poljsak, and Milisav 2012).

Antioxidative stress occurs when the immune system's ability to balance pathogenic threats is hampered by an overabundance of bioactive antioxidant molecules. The primary contrast is oxidative stress, which can result in diseases like acute myocardial infarction or cancer (Valko,*et al.*, 2006). Among other disorders, oxidative stress is a major active factor in the onset and progression of cancer, diabetes mellitus, cardiovascular diseases, neurodegenerative diseases, and inflammatory diseases (Arika *et al.,* 2019*)*. Hence this present investigation was aimed to investigate the Antioxidant activity against ethanolic leaf extract of *Ipomoea biloba* leaves against the ISO induced myocardial infarction in rats.

MATERIALS AND METHODS

Chemicals and reagents

Isoproterenol was acquired from the Sigma Aldrich (USA). All other additional chemicals and reagents were used in the analytical grade and the same was purchased from the Himedia chemicals, USA.

Collection of plant

The fresh and matured leaves of *Ipomoea biloba* were collected from Kolli Hills, Namakkal district, Tamil Nadu coastal area. Then the collected leaves were washed completely with distilled water add the same was processed and dehydrated under the shady cabin.

Preparation of plant material

The shade dried plant leaves were powdered with an electrical blender and then the 10gm of *Ipomoea biloba* leaf powder was mixed with 100 ml of ethanol. Then it was heat macerated at

the 85°C for 30 minutes and then the suspension was filtered by using the Whatman No.1 filter paper. Then the resulted plant extract was powdered by vacuum evaporation process and finally the powder was used for the further investigations.

Experimental animals

The Institutional animal ethics committee (Reg.no.1416/PO/a/11/CPCSEA & 7 MARCH 2011), Muthayammal College of Arts and Science, Rasipuram, Tamilnadu, India approved the experimental design. Male Wistar albino rats weighing 170-200 g were obtained from Small Animal Breeding centre, Bangalore. The animals were maintained in a ventilated room with temperature 23±2o C, humidity 60-70% and 12 hours light/dark cycle. Animals were fed with standard pellet and water ad libitum. All the studies were conducted in accordance with committee for the purpose of control and supervision of experiments on animals (CPCSEA) norms and the National Institute of Health Guidelines "Guide for the care and use of laboratory animals". All the experimental animals were maintained under the standard laboratory conditions. All experimental animals were acclimatized for 7 days in prior to the starting of experiments and during that period animals were fed with standard pelleted rat chow and water ad libitum.

Experimental design

The rats were divided into five groups of six animals each.

Group I: Served as a normal control.

Group II: Rats were administered isoproterenol (20mg/kg) by the subcutaneous injection to induce the myocardial infarction.

Group III and IV: Rats were pretreated with the ethanolic extract of *Ipomoea biloba* leaf extract (100 and 200mg/kg, respectively) for a period of 28 days subsequently to the subcutaneous injection of isoproterenol (20mg/kg, b.w) for 2 consecutive days.

Group V: Rats were received the ethanolic extract of *Ipomoea biloba* (200mg/kg b.w) alone for 28 days without any experimental treatments.

After the experimental period, blood and heart tissue samples were collected and serum was separated and used for estimation of Superoxide Dismutase (SOD), Catalase (CAT),

Glutathione Peroxidase (GPx), Glutathione-S-transferase (GST), Reduced Glutathione (GSH), Calcium dependent Adenosine Triphosphatase (Ca^{2+}-ATPase), Magnesium dependent ATPase (Mg^{2+}-ATPase), Vitamin C and Vitamin E.

Preparation of heart tissue homogenate

After the completion of experiments, all the experimental animals were anesthetized by chloroform administration and sacrificed by cervical decapitation. After the animal scarification, the heart tissues were excised washed thoroughly in ice-cold phosphate buffered saline to remove the excess blood. Ten percent of homogenate was prepared in 0.1M Tris HCl buffer (pH-7.4). Then the homogenate was centrifuged at 6000 rpm for 20 min at 4°C and the supernatant was used for the further biochemical assays.

Determination of Antioxidant activities

The level of antioxidant activities of plasma and heart tissue were estimated by following methods superoxide dismutase enzyme Das *et al.*, 2000, catalase enzyme by the method of Sinha, (1972), glutathione level in the heart cell lysate was assayed Glutathione peroxidase activity was estimated by the method of Ellman, 1959,Glutathione-s-transferase activity was estimated by method of Habig *et al*., 1973, Reduced Glutathione (GSH) was estimated by the method of Beutler, 1984, activity of cytochrome C-oxidase was assayed by the method of Pearl *et al.,* (1963). ascorbic acid were estimated by the method of Omaye *et al*,(1979) and vitamin E were estimated by the methods of Varley *et al*., 1981.

Statistical analysis

Statistical analysis all conclusions happen to be demonstrated as Mean ± SD. in each group for six animals. All the compiled data were statistically examined utilizing SPSS10 software. Theory Hypothesis test approaches included one-way variance analysis (ANOVA) followed by the least significant difference (LSD) test. The significance level at alpha=0.05 was considered to statistical significance.

Results

Levels of enzymatic antioxidants in the plasma of control and experimental animals are shown in Table 1 and Figure 1. SOD, catalase, Glutathione Peroxidase (GPx), Glutathione-S-

transferase (GST), Reduced Glutathione (GSH) activities were significantly lowered due to the myocardial infraction in Group II rats. *Ipomoea biloba* administration successfully prevented the decrease in the activities of these enzymes in Group III & IV animals.

ISO-induced myocardial necrosis produced a significant depletion in activities of antioxidant enzyme such as SOD, GPx and GR compared to normal animals. *Ipomoea biloba* 200mg/kg treatment to myocardial necrotic rats significantly restored the activities of CAT, GPx and GR. *Ipomoea biloba* 100mg/kg, however, could only restore the ISO depilated activities of CAT and GPx significantly.

Ipomoea biloba 100mg/kg and *Ipomoea biloba* 200mg/kg treatments to ISO intoxication rats augmented the SOD levels decreased by ISO but not to a significant extent.

Table 1 Levels of enzymatic antioxidants in ISO induced rats against *Ipomoea biloba* leaf extract

GROUPS	Group I – Control	Group II - ISO induced	Group III - ISO + EEIB (100 mg/kg b.wt)	Group IV - ISO + EEIB (200 mg/kg b.wt)	Group V - EEIB (200 mg/kg b.wt)
SOD	115.13±8.95	63.58±5.21	102.67±7.17	110.84±8.18	112.55±7.08
CAT	7.83±1.68	2.33±0.93	6.95±0.32	7.12±1.97	7.76±1.64
GPx	42.31±3.69	22.57±3.04	32.55±4.12	39.44±3.97	40.12±4.01
GST	12.27±0.08	8.43±0.17	10.79±1.03	10.42±0.04	12.18±1.02

ISO- Isoproterenol, EEIB- Ipomoea biloba leaf extract

SOD: Superoxide dismutase (*Inhibition of 50% nitrite formation/min/mg protein*), CAT: Catalase (*micromoles of H_2O_2 decomposed/min/mg protein*), GPx: Glutathione peroxidase (*micromoles of glutathione consumed/min/mg protein*), GST: Glutathione-S-Transferase (*1 μM of CDNB conjugate formed/min/mg protein*)

Figure 1**. Levels of enzymatic antioxidants in ISO induced rats against *Ipomoea biloba* leaf extract**

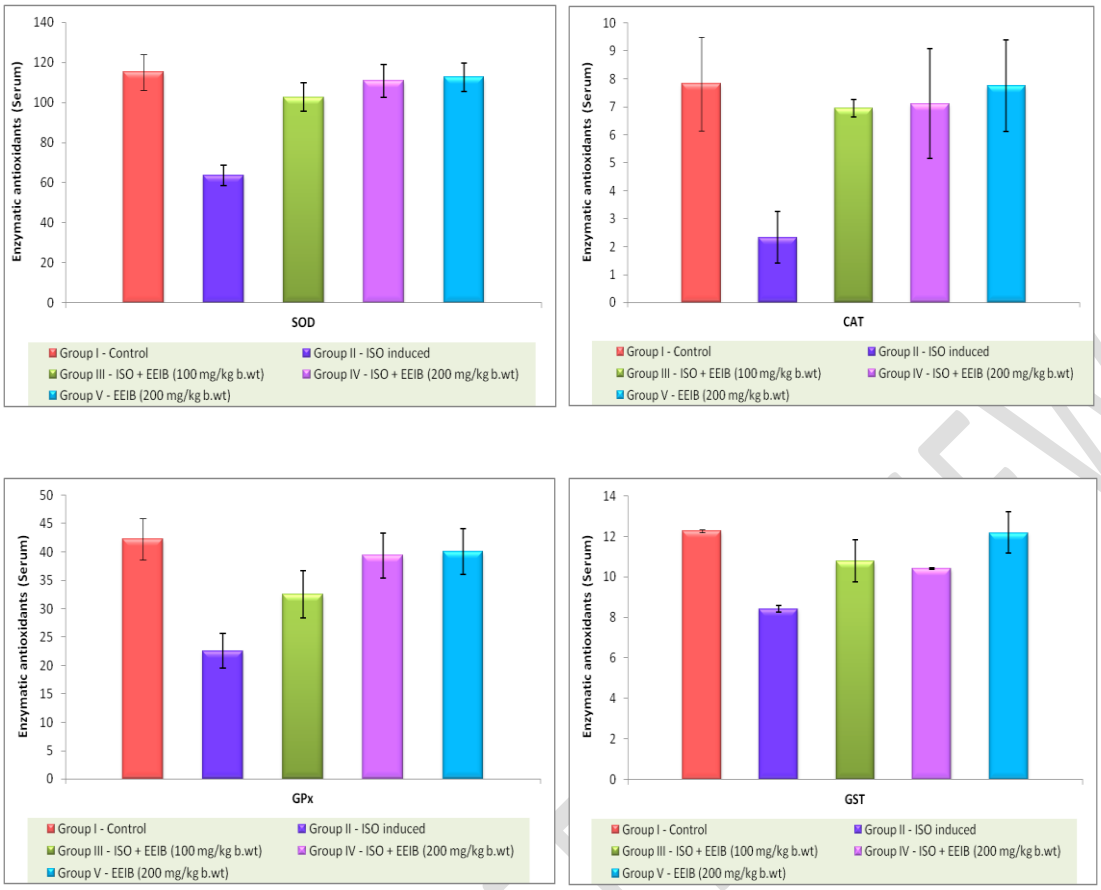


Table 2 and Figure 2 show that significant decline in myocardial GSH was observed in ISO control group as compared to the normal group. *Ipomoea biloba* 100mg/kg significantly prevent the ISO – induced decline. *Ipomoea biloba* 100mg/kg also insignificantly elevated the decreased GSH levels.

Isoproterenol administered rats (Group II) showed a significant decrease in reduced glutathione, α-tocopherol and ascorbic acid levels when compared with control rats (Group I). In *Ipomoea biloba* pretreated and isoproterenol administered animals (Group III & IV), these levels were significantly increased when compared to Group II animals. Group III animals had significantly elevated reduced glutathione, α-tocopherol and ascorbic acid when compared with Group II animals. Group IV animals had significantly elevated reduced glutathione, α-tocopherol and ascorbic acid when compared with Group II animals.

Oral feeding of *Ipomoea biloba* 100mg/kg as well as 200mg/kg to rats did not adversely affect the basal levels of GSH, CAT, GPx and GR, nor were they significantly elevated in

comparison to the normal rats. However, *Ipomoea biloba per se* feeding to rats at both doses elevated GSH level higher than normal.

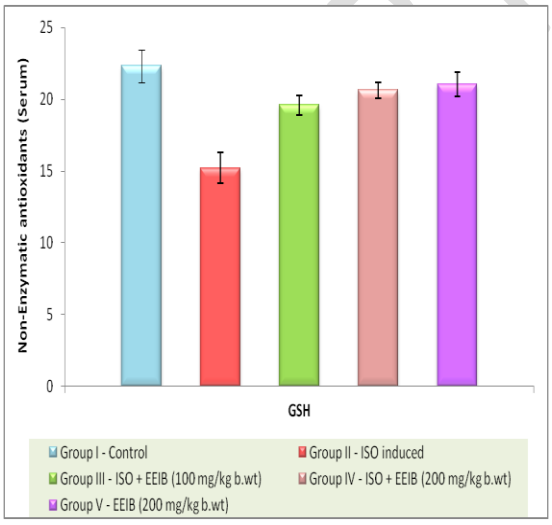
Table 2 Levels of non-enzymatic antioxidants in ISO induced rats against *Ipomoea biloba* leaf extract

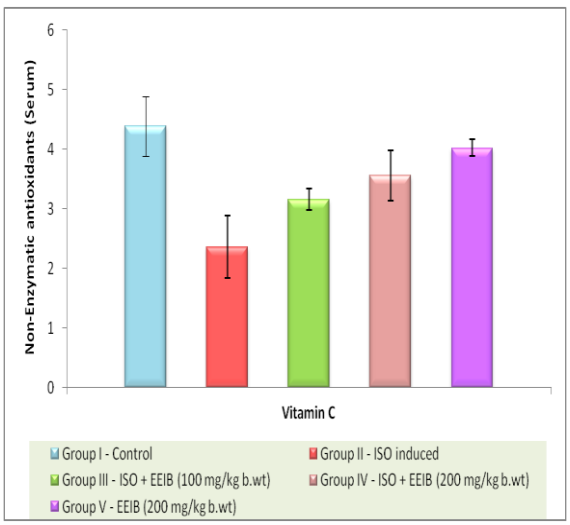
GROUPS	GSH		Vitamin C		Vitamin E	
	Serum	Tissue	Serum	Tissue	Serum	Tissue
Group I – Control	22.32±1.12	10.74±2.38	4.39±0.5	2.05±0.31	2.95±0.61	1.33±0.23
Group II - ISO induced	15.21±1.06	3.66±1.29	2.36±0.52	0.93±0.19	1.45±0.13	0.67±0.19
Group III - ISO + *EEIB* (100 mg/kg b.wt)	19.57±0.69	9.21±0.08	3.16±0.18	1.44±0.23	2.13±0.08	0.83±0.21
Group IV - ISO + *EEIB* (200 mg/kg b.wt)	20.63±0.57	9.72±1.95	3.56±0.42	1.81±0.25	2.67±0.15	0.98±0.25
Group V - *EEIB* (200 mg/kg b.wt)	21.06±0.86	10.23±0.31	4.02±0.14	1.92±0.33	2.49±0.17	1.06±0.18

ISO- Isoproterenol, EEIB- Ipomoea biloba leaf extract

GSH – The Reduced Form of Glutathione

Figure 2. Levels of non-enzymatic antioxidants in ISO induced rats against *Ipomoea biloba* leaf extract





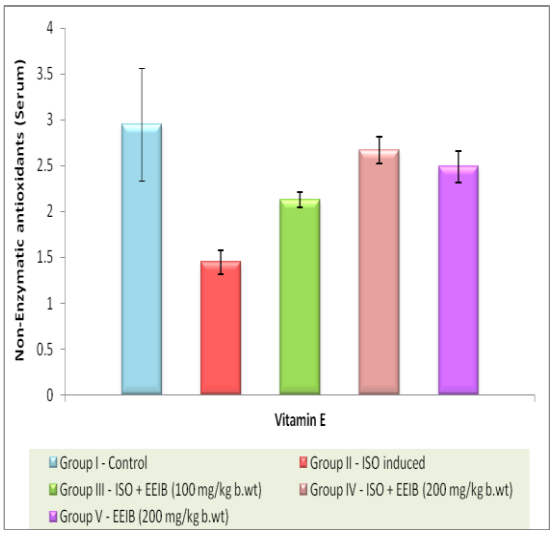


Table 3 The activity of membrane bound ATPases such as Na^+K^+ATPase, Mg^{2+}ATPase, Ca^{2+}ATPase were assayed in the heart homogenate of control and experimental animals

GROUPS	Na^+K^+ATPase	Ca^{2+} ATPase	Mg^{2+} ATPase
Group I – Control	0.58±0.07	0.73±0.08	0.53±0.04
Group II - ISO induced	0.23±0.05	1.93±0.06	0.98±0.11
Group III - ISO + *EEIB* (100 mg/kg b.wt)	0.38±0.06	1.74±0.09	0.73±0.14
Group IV - ISO + *EEIB* (200 mg/kg b.wt)	0.45±0.05	1.56±0.19	0.67±0.08
Group V - *EEIB* (200 mg/kg b.wt)	0.51±0.07	0.71±0.11	0.59±0.10

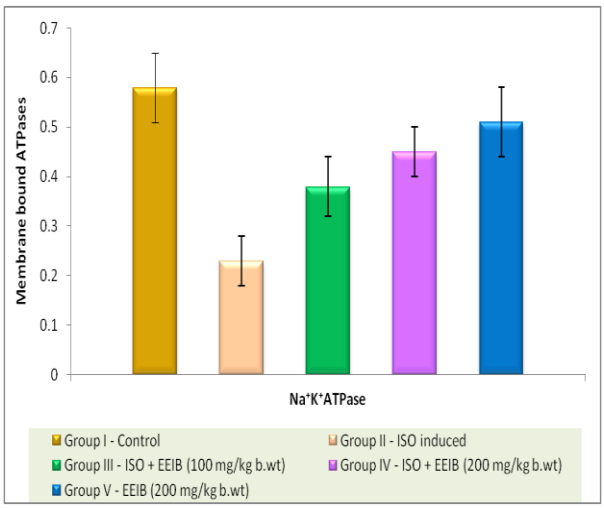
Values are expressed as mean ± SD of six animals.

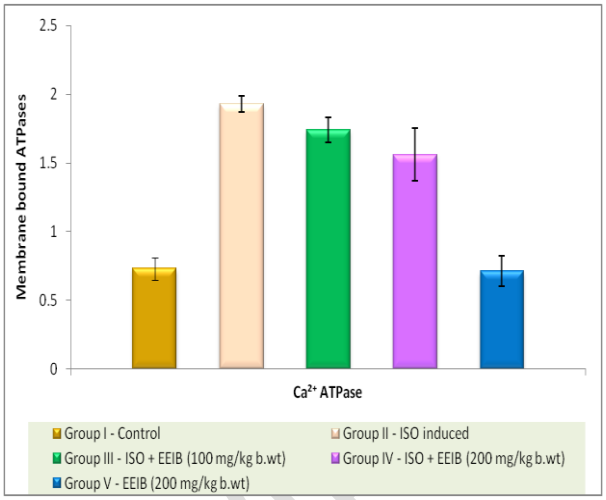
ISO- ISoproterenol, EEIB- Ipomoea biloba leaf extract

Na^+K^+ATPase: Sodium potassium-dependent ATPase; Ca^{2+} ATPase: Calcium-dependent ATPase; Mg^{2+} ATPase: Magnesium-dependent ATPase

Units : *µmoles of Pi liberated/min./mg protein*

Figure-3 The activity of membrane bound ATPases such as Na^+K^+ATPase, Mg^{2+}ATPase, Ca^{2+}ATPase were assayed in the heart homogenate of control and experimental animals





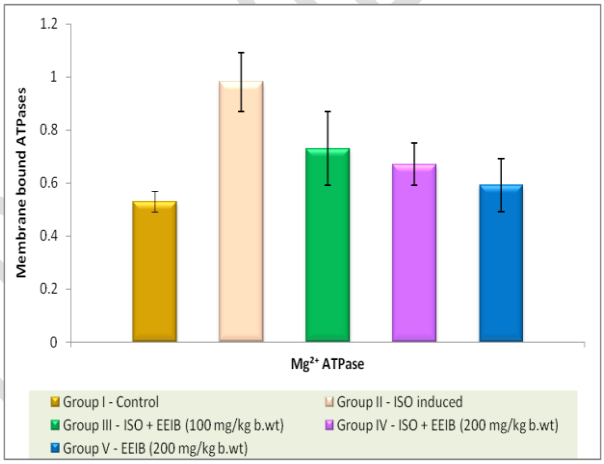


Table 3 and Figure 3 shows the activities of membrane bound enzymes (Na^+/K^+ -ATPase, $Ca^{2+}$and Mg^{2+} -ATPases) in normal and ISO-induced rats. The activity of Na^+/K^+ -ATPase was decreased significantly and the activities of Ca^{2+} and Mg^{2+}ATPase were increased significantly in the heart of ISO-induced rats when compared with normal control rats. Pretreatment with *Ipomoea biloba* to ISO-induced rats significantly decrease the activities of Ca^{2+} and Mg^{2+} ATPases in the heart when compared to ISO-alone induced control rats.

Discussion

When animals are given high doses of ISO, a powerful synthetic catecholamine, the heart develops "infarct-like" lesions, similar to those found in acute myocardial infraction (AMI) and sudden death in humans (Anand *et al.*,2011).

Antioxidant enzymes (GPx and GST) activity is reduced by isoproterenol treatment (Table 1). Reduced availability of their substrate, GSH, which was depleted on isoproterenol, could explain the lower activity of GPx and GST in the isoproterenol intoxicated group. Treatment with Ipomoea biloba leaves raised GSH levels as well as GPx and GST activity in the heart. According to reports, higher GPx levels make the heart more resistant to myocardial infarction (Yoshida, 1996).

α-tocopherol is a chain-breaking antioxidant that sequesters free radicals. In the myocardial infracted rats, a decrease in tissue-tocopherol should have resulted in increased lipid peroxidation, leading to heart injury. *Ipomoea biloba* leaves extract preserved α-tocopherol levels in the rat heart and hence protected it from isoproterenol-induced injury (Table 2). Ascorbic acid is said to augment the antioxidant action of α-tocopheryl radical to α-tocopherol (Leung *et al.,* 1981). *Ipomoea biloba* leaves extract probably assisted the above process indirectly, since the flavonoids in it have an ascorbic acid-sparing property (Middleton, 1984).

Isoproterenol treatment resulted in a decrease in mitochondrial antioxidant levels (Table 2). GSH is a tripeptide that is essential for cell survival. GSH can protect cells from lipid peroxidation when used alone or in combination with other proteins (Tirmenstein and Reed, 1988). Mitochondrial GSH is important for sustaining cell viability because it regulates mitochondrial inner membrane permeability by keeping sulphydryl groups in a decreased condition. GSH is drawn from the cytosolic pool and transported into the mitochondrial matrix (Fernandez – checa *et al*., 1991). Glutathione biosynthesis enzymes are not found in mitochondria (Meister 1991). Mitochondria use a system that includes a high affinity transporter to transfer GSH from the cytoplasm (Martensson *et al*.,1990).

Glutathione (GSH) and glutathione enzymes such as glutathione peroxidase, glutathione-S-transferase (GST), glutathione reductase, catalase, and superoxide dismutase (SOD) effectively scavenge harmful free radicals (Poliodoro *et al*., 1984). The mitochondrial membrane is protected from peroxidative damage by GPx, an antioxidant enzyme. When GPx activity is reduced, mitochondria become more sensitive to isoproterenol-induced cardiac injury, resulting in a shift in mitochondrial function. GPx has been observed to be inactivated in the presence of severe oxidative stress (Litov *et al*.,1981). The activity of GPx requires adequate quantities of glutathione and NADPH, and reduced glutathione availability, as seen in isoproterenol-

administered rats (Table 2), and NADPH resulted in decreased GPx activity (Condell and Tappel, 1983). Similarly, decreased glutathione availability causes GPx and GST activity to decrease. Our findings revealed that rats given isoproterenol had lower GPx activity (Table 2). The activity of GPx was sustained after pretreatment with *Ipomoea biloba* leaves extract.

Reduced glutathione is a non-enzymatic antioxidant biomolecule that is abundant in the body (Meister, 1984). It effectively scavenges free radical species such as H_2O_2, superoxide anions, and alkoxy radicals when combined with GPx, GR, and CAT-SOD couples.

It protects cellular constituents from the detrimental effects of ROS and peroxides generated during metabolism as a substrate for antioxidant enzymes GPx and glutathione transferase (GST). Reduced GSH levels in ISO-intoxicated rats could be owing to its increased usage for enhancing GPx and GST activities.

Glutathione levels depleted by ISO were significantly elevated by *Ipomoea biloba* 200mg/kg treatment. It may be understood that increased level of GSH could either be because of its enhanced synthesis or due to improved GR activity in presence of *Ipomoea biloba.*

SOD, CAT, and GPx create a mutually supporting enzyme system that serves as the first line of defense against oxidative injury, decomposing O_2 and H_2O_2 before they interact to form more dangerous hydroxyl radicals (Vandana Panda and Suresh 2008).

SOD activity reduced considerably in the ISO group of rats in this study, possibly due to an increased production of superoxide anions. The elimination of superoxide anions can be harmed by a decrease in SOD activity, which can be damaging to the myocardium (Sharma *et al*., 2001). After ISO treatment, the activity of the H_2O_2 scavenging enzymes CAT and GPx also fell dramatically. Excessive superoxide anions may inactivate SOD, resulting in inactivation of the H_2O_2 scavenging enzymes, which could explain the fall in enzyme levels. The administration of *Ipomoea biloba* to ISO-challenged rats successfully avoided the decrease in SOD, CAT, and GPx activities, which could be attributed to *Ipomoea biloba's* ability to scavenge radicals and so protect these enzymes (Vandana Panda and Suresh 2008).

GR is an antioxidant enzyme that aids in the conversion of GSSG (a GPx end product) to GSH. There was a significant decrease in GPx activity in ISO-treated cells, which resulted in a decrease in substrate availability for GR and, as a result, a decrease in GR activity. *Ipomoea*

biloba administration to ISO myocardial infraction rats restored GR activity, speeding up the conversion of GSSG to GSH.

Reduced GSH levels caused membrane integrity loss, cardiac contractile failure, and myocyte toxicity in ISO-induced rats, eventually leading to myocardial necrosis (Marikannan and Darlin Quine, 2012). In the heart of ISO-induced rats, pretreatment with *Ipomoea biloba* dramatically boosted the activities of mitochondrial SOD, CAT, GPx, and GST, as well as the levels of mitochondrial GSH. This could be attributed to *Ipomoea biloba's* direct effect on lipid peroxidation levels and indirect effect on lipid levels (Ganapathy and Rajadurai 2015).

Protection of membrane integrity by *Ipomoea biloba* leaves extract in isoproterenol-induced myocardial infraction

The activity of ATPases was dramatically reduced in membrane tissues treated with isoproterenol (Table 3) Na^+ and K^+ ATPase is regarded to be a necessary component of animal cell plasma membranes, as it is involved in the active transport of Na^+ and K^+ ions. Ca^{2+} is a second messenger in the cell, and an increase in cytosolic Ca^{2+} ATPase pumps Ca^{2+} out of the cytosol at a rapid rate. Intracellular Ca^{2+} overload can arise as a result of changes in the membrane Na^+, K^+ ATPase, and Ca^{2+} pump activities, leading to cardiomyopathy. Na^+ K^+ ATPase is known to be inhibited by high cholesterol levels. Malondialdehyde, a thiobarbituric acid reactive result of lipid peroxidation, is formed more frequently when Ca^{2+} ATPase is reduced in the membrane. Pretreatment with *Ipomoea biloba* leaves extract reduces ATPase activity and keeps it at a normal level (Jayalakshmi and Niranjali, 2005).

In this work, we found that ISO-induced rats had lower Na^+/K^+ ATPase activity and higher Ca^{2+} and Mg^{2+} ATPase activity. Since Na^+/K^+ ATPase is a ‘SH' group containing enzyme and lipid-dependent, inactivation could be attributed to increased lipid peroxidation by free radicals on ISO-induction (Paritha and Devi,1997). ISO activates adenylate cyclase, which results in increased Ca^{2+} ATPase in ISO-induced rats. During ischemia, calcium overload in cardiac cells activates the membrane's Ca^{2+}-dependent ATPase, depleting high energy phosphate reserves and indirectly blocking Na^+ and K^+ transport and inactivating the Na^+/K^+ ATPase (Rajadurai and Stanely, 2007). In ISO-induced rats, pretreatment with *Ipomoea biloba* boosted the activity of Na^+/K^+ ATPase while decreasing the activities of Ca^{2+} and Mg^{2+} ATPase. This may be related to *Ipomoea biloba's* capacity to preserve the 'SH' groups from oxidative

degradation by inhibiting peroxidation of membrane lipids. This impact could be attributed to *Ipomoea biloba's* ability to stabilize membranes.

The activity of membrane-associated enzymes such as ATPases can be measured to see if there is a change in the membrane under pathological situations. The plasma membrane is intricately related with ATPases, which contribute in the energy-demanding translocation of sodium, potassium, calcium, and magnesium (Mourelle and Franco.1991). The Na^{+}-Ca^{2+} exchange pathway in the myocardium can be activated by inhibiting Na^{+}/K^{+}ATPase. This Na^{+}-Ca^{2+} exchange pathway could be involved in calcium regulation in cells (Rajadurai & Stanely, 2007). The active calcium transport protein Ca^{2+} ATPase is responsible for maintaining proper intercellular calcium levels in a range of cell types.

CONCLUSIONS

In conclusion, the novel findings of this present investigation were proved that the ethanolic extract of *Ipomoea biloba* leaves were showed the appreciable cardio protection against the ISO-induced myocardial infarction in experimental rats. The ethanolic extract treatment noticeably reduced the enzymatic and non-enzymatic antioxidation activity in both plasma as well as heart tissue. These results were proved the cardioprotective action of the ethanolic extract of *Ipomoea biloba* leaves. Hence, it was concluded that the *Ipomoea biloba* leaves may play a significant role in the development of novel cardio-protective drugs in future. However, the additional researches were still needed in future to elucidate the exact curative mechanism of the *Ipomoea biloba* leaves against the myocardial infarction.

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