IJBPAS, April, 2020, 9(4): 755-767

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF CARDIO-PROTECTIVE ACTIVITY OF *IPOMOEA BILOBA* LEAVES AGAINST THE ISOPROTERENOL STIMULATED MYOCARDIAL INFARCTION IN RATS

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Received 19th Oct. 2019; Revised 14th Nov. 2019; Accepted 24th Dec. 2019; Available online 1st April 2020

https://doi.org/10.31032/IJBPAS/2020/9.4.5016

ABSTRACT

The main aim of this investigation is to investigate the cardioprotective effect of ethanolic extract of *Ipomoea biloba* on isoproterenol induced myocardial infarction in rats. 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) through sub cutaneous injection to induce the myocardial infarction. Group III and IV were pretreated with ethanolic extract of *Ipomoea biloba* leaf (100mg/kg, 200mg/kg respectively) before to the subcutaneous injection of isoproterenol (20mg/kg, b.w) for 2 consecutive days. Group V received ethanolic extract of *Ipomoea biloba* (200mg/kg b.w) alone for 28 days excluding the treatment procedures. After the completion of experimental period, the levels of cholesterol, triglycerides, phospholipids, and lipoproteins were estimated. The myocardial damage was assessed by quantifying the serum levels of cardiac marker enzymes (AST, ALT, LDH, CK, and TP) uric acid, and ceruloplasmin. The results of this experiment revealed that the Isoproterenol induced rats showed a significant increase in the levels of triglycerides, total cholesterol and phospholipids in both serum and heart homogenate. A rise in the levels of LDL, VLDL with significant decrease in the level of HDL was also observed in the serum of isoproterenol-intoxicated rats. Interestingly our findings were proved that the treatment with

the ethanolic extract of *I.biloba* leaf was showed the noticeable decrease in the lipid profiles and myocardial marker enzymes in the experimental rats. The extract treatment also prevented the elevation of cholesterol, triglycerides and phospholipids in myocardial infarction induced rats. Hence, the findings of this work was clearly proved that the *I.biloba* leaf was appreciably prevented the isoproterenol induced myocardial infarction in rats.

Keywords: Cardiopreotective activity, isoproterenol, myocardial infarction, *Ipomoea biloba*

INRODUCTION

Myocardial infarction is a clinical disorder which is characterized by insufficient blood (without oxygen) supply from the coronary arteries and it results in damage or destroy of heart muscle **[1-3]**. The numerous previous studies showed that the oxidative stress produced by the generation of reactive oxygen species (ROS) plays a major role in the development of Myocardial infarction. It may sometimes damages cell membrane and metabolic alterations leads to cardiac dysfunction and damage **[4, 5]**. In developing countries due to lifestyle changes myocardial infarction makes an increase in mortality and morbidity rate **[6, 7]**. Isoproterenol (ISO) is a synthetic catecholamine and β-adrenergic agonist; on oxidation due to its free radicals it produces stress in the myocardium and cause damage to the myocardial membrane **[8]**.

Medicinal plants were demonstrated to play an important role in the management of various diseases in humans; including cardiovascular diseases **[9]**. *Ipomoea* is the largest genus in the flowering plant family Convolvulaceae, with more than 500 species. Some of the species of *Ipomoea* are frequently used in Indian traditional medicine. The *Ipomea biloba* is an important medicinal plant India with the numerous pharmacological activities. Many previous reports highlighted the biological activities of *Ipomea biloba* plant **[10, 11]**. However, still now there were no any scientific reports to claim the cardioprotective actions of *I.biloba* leaves. Hence this present investigation was aimed to investigate the cardioprotective activity of ethanolic leaf extract of *I.biloba* leaves against the ISO induced myocardial infarction in rats.

MATERIALS AND METHODS

Collection of plant

The fresh and matured leaves of *Ipomoea biloba* were collected from the Kolli hills of Namakkal district. 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 20gm of *I.biloba* leaf powder was mixed with 100 ml of water. Then it was heat macerated at the 85°C for 30 minutes and then the suspension was filtered by using the whatman 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 adult male Wistar albino rats were used for the study and the same was collected from the Institutional animal house. 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 *Ipomea 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 *I.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 cholesterol, triglycerides, phospholipids, lipoproteins, myocardial marker enzymes, and other biochemical studies.

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.

Estimation of cholesterol

The total cholesterol in both serum and heart tissue sample were estimated according to the previous method described by **[12]**. The 0.1ml of the samples was

added to the 4.9 ml of ferric chloride precipitation reagent and then centrifuged for 6 minutes at 7000rpm and then the resulting supernatant was collected. The 2.5 ml of supernatant was added to the 2.5 ml of ferric chloride diluting reagent and along with the 4 ml of concentrated sulphuric acid. The reaction mixture was incubated for 10 minutes at room temperature and finally optical density was measured at 560 nm. The cholesterol content was expressed as mg/dl of serum.

Determination of triglyceride

The triglycerides level in the serum and heart tissue homogenate was estimated by the method of **[13]**. Briefly, the 1.0 ml of isopropanol was added to 0.1 ml of each sample and the 4g of alumina was added and then the suspension was shaken well for 15 mins. Then this suspension was centrifuged at 6000 rpm for 7 minutes then 2.0ml of the supernatant was placed to the properly labelled vials. Then the tubes were located in the water bath at 70°C for 20 min for saponification after adding 0.6 ml of the sapponification reagent. Finally, the suspension was cooled to room temperature then 1.0 ml of sodium metaperiodate was added subsequently to the 0.5 ml of acetyl acetone reagent. The contents were cooled and the absorbance was taken at 430nm.

Determination of phospholipids

The previously described method by **[14]** was employed to determine the phospholipids in the samples. For this, the 0.1 ml of each sample was treated with the 0.2 ml of perchloric acid and this digestion was continued till the reaction solution becomes colourless. After that, the liberated phosphorus was estimated adding 4.3 ml of H_2O to the digested sample and 0.5 ml of ammonium molybdate. Then the tubes were shaken well and maintained for 20 minutes incubation. Finally the developed blue colour in the reaction solution was determined at 620 nm.

Assay of lipoproteins level

The levels of lipoproteins such as, high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) in the serum as well as heart tissue homogenate of the experimental animals were estimated according the previous described method by **[15]**. Briefly, the 1 ml serum and heart tissue homogenate was added separately to the 0.1 ml of phosphotungstate reagent and 50µl of magnesium chloride reagent. The reaction content was centrifuged at optimum temperature for 30 minutes at 3000 rpm. Then the 0.1 ml of resulted supernatant was added to the 4.9 ml of ferric chloride precipitating reagent, then it was mixed well and again centrifuged at 3000rpm for 10 minutes. After that, 2.5 ml of resulting supernatant was taken and mixed with the 2.5 ml of diluting reagent and 4 ml of concentrated sulphuric acid by

mixing thoroughly. Subsequently, the blank was also maintained for the results interpretations. The colour developed was read at 560nm.

Biochemical analysis

Cardiotoxicity was assessed by quantifying the serum levels of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), creatine kinase (CK), total protein (TP), uric acid, and the ceruloplasmin, by using the commercially procured assay kits (Sigma aldrich, USA).

RESULTS

Effects of ethanolic extract of *Ipomea biloba* leaves on cholesterol, triglycerides, and phospholipids level in the experimental rats

The myocardial infarction was induced to the experimental animals by the injection of ISO by subcutaneously to examine the therapeutic potential of ethanolic leaf extract of *I.biloba*. The subcutaneous injection ISO to the rats (Group II) showed the drastic increase in the cholesterol and triglycerides level in the serum as well as heart tissue homogenate when compared to the control rats (Group I). The difference in the levels of cholesterol and triglycerides in the control and myocardial infarction induced rats were contrasts each other. Interestingly, the treatment with the ethnolic extract of *Ipomea biloba* leaves (100 and 200mg/kg) to the myocardial infarction induced rats showed the noticeably decreased level of the both cholesterol and triglycerides **(Figure 1)**. The extract alone treated animals were showed completely similar kind of results to the control. There were no any significant variations between control and plant extract treated experimental animals.

Effects of ethanolic extract of *Ipomea biloba* leaves on lipoproteins level in the experimental rats

In this present study, ISO induced myocardial infarction in rats were demonstrated the significantly elevated levels of low density lipoprotein (LDL), very low density lipoprotein (VLDL), and decreased the high density lipoprotein (HDL) in the serum as well as heart tissue homogenate of the myocardial induced experimental rats when compared to the control group. This result showed the severe effects of ISO induced myocardial infarction in rat model. Whereas, the 100 and 200mg/kg of ethnolic extract of *Ipomea biloba* leaves (100 and 200mg/kg) treatment to the myocardial infarction induced rats demonstrated the appreciably reduced levels low density lipoprotein (LDL), very low density lipoprotein (VLDL), and amazingly increased level of high density lipoprotein (HDL) when compared to the myocardial induced experimental rats **(Figure 2)**. This result

was proved the beneficial activity of ethanolic extract of *Ipomea biloba* leaves against the cardio-protection. There were no any significant variations between control and plant extract treated experimental animals. The extract alone treated animals were showed completely similar kind of results to the control.

Effects of ethanolic extract of *Ipomea biloba* leaves on marker enzymes level in the experimental rats

As depicted in the **Figure 3**, the levels of aspartate transaminase (AST), alanine transaminase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK) was drastically elevated and also the reduced level of total proteins were noted in the serum and heart tissue homogenate of the myocardial infarction stimulated experimental animals when compared to the untreated control animals. This severs reduction in the myocardial markers enzymes in the experimental rats were revealed the serious myocardial infarction in the experimental animals. Interestingly the treatment with the 100 and 200mg/kg of ethnolic extract of *Ipomea biloba* leaves (Group III and IV) to the myocardial infarction induced rats showed the amazingly reduced levels of myocardial marker enzymes such as, AST, ALT, LDH and CK in the serum as well as heart tissue homogenate when compared to the myocardial infarction induced experimental rats. The extract treatment also revealed the significantly increased levels of total proteins in the serum and heart tissue homogenate of the myocardial infarction induced experimental rats **(Figure 3)**. The extract alone treated animals did not show any alterations in the myocardial marker enzymes and it was quite similar to results of the control. There were no any significant variations were noted between control and plant extract treated experimental animals **(Figure 3)**.

Effects of ethanolic extract of *Ipomea biloba* leaves on the uric acid and ceruloplasmin level in the experimental rats

Figure 4 revealed that the level of uric acid was harshly increased and the level of ceruloplasmin protein was drastically decreased in the serum of myocardial infarction induced (Group II) experimental animals when compared to the untreated normal control animals (Group I). The severe increase of the uric acid in the serum of myocardial infarction induced rats revealing the declined activity and kidneys. On the other hand, the treatment with the ethanolic extract of the *Ipomea biloba* leaves (100 and 200mg/kg) to the myocardial infarction induced experimental animals (Group III and IV) were showed the significantly reduced level of uric acid and increased level of ceruloplasmin in the serum of myocardial

infarction induced experimental animals than the diseased animals (Group II). The extract treatment appreciably regained the ceruloplasmin level and reduced the uric acid level in the serum of experimental animals **(Figure 4)**. There were no any significant variations were noted between control and plant extract treated experimental animals. The extract alone treated animals did not show any alterations in the uric acid and ceruloplasmin levels in the serum of experimental animals.

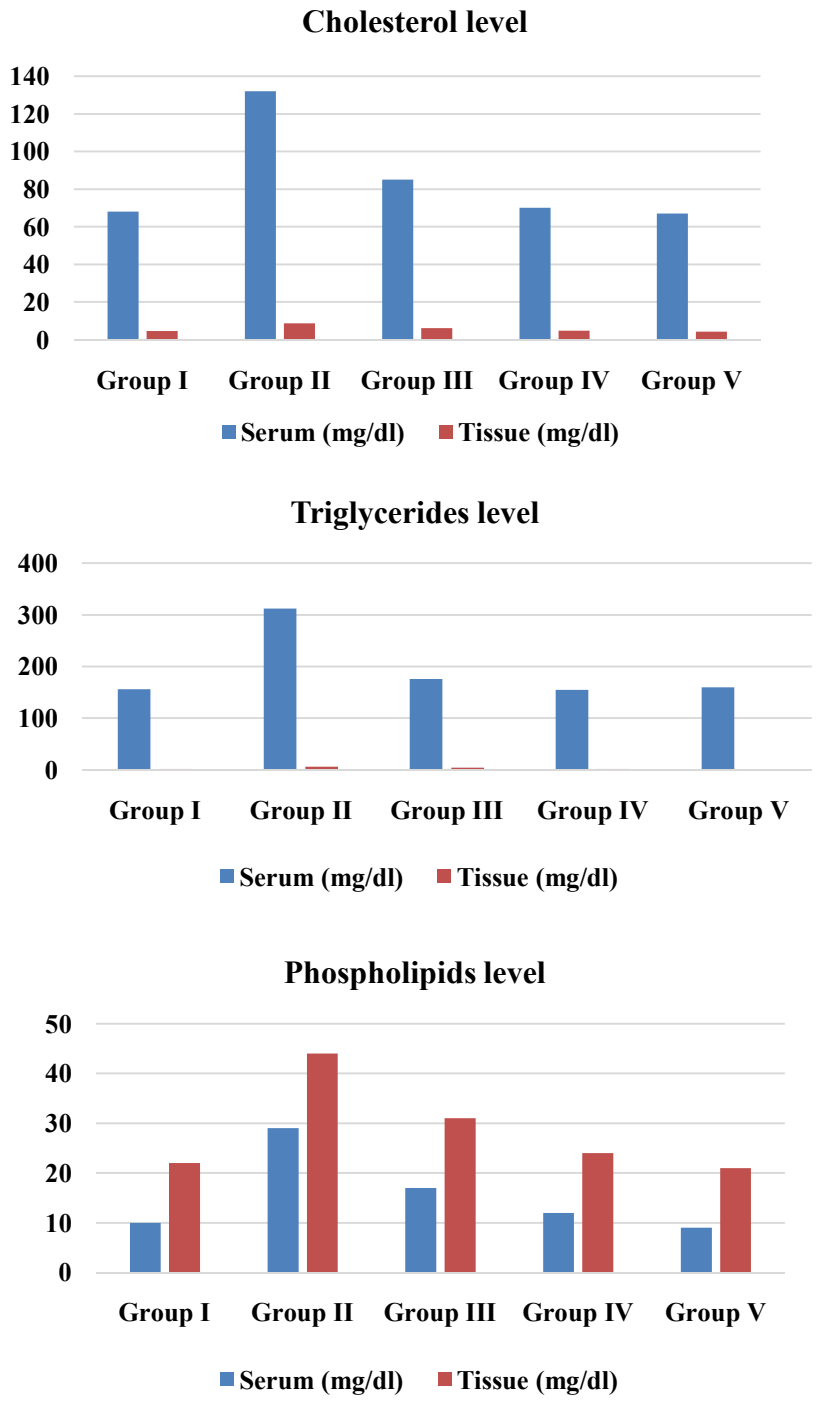


Figure 1: Effects of ethanolic extract of *Ipomea biloba* leaves on the levels of cholesterol, triglycerides, and phospholipids in the experimental rats

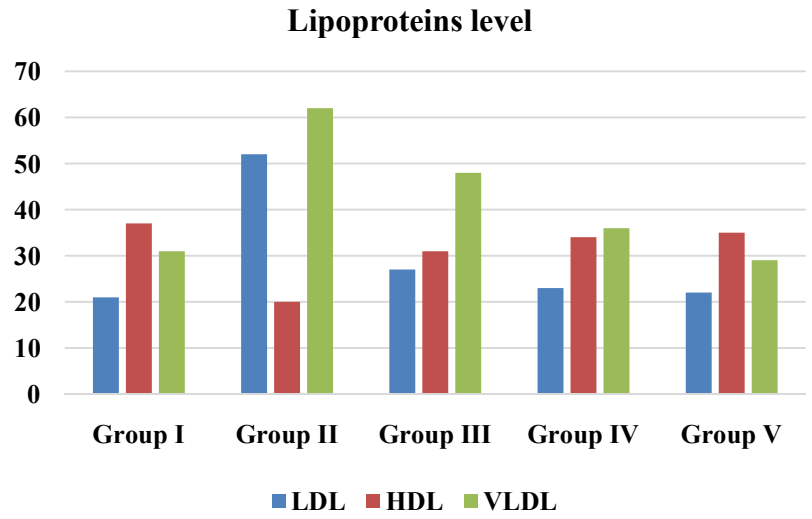
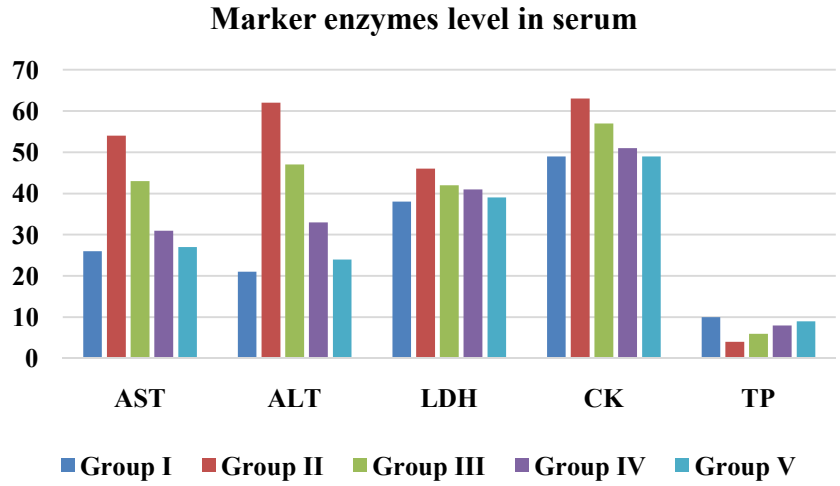


Figure 2: Effect of ethanolic extract of *Ipomoea biloba* leaf on the lipoproteins level in the experimental rats



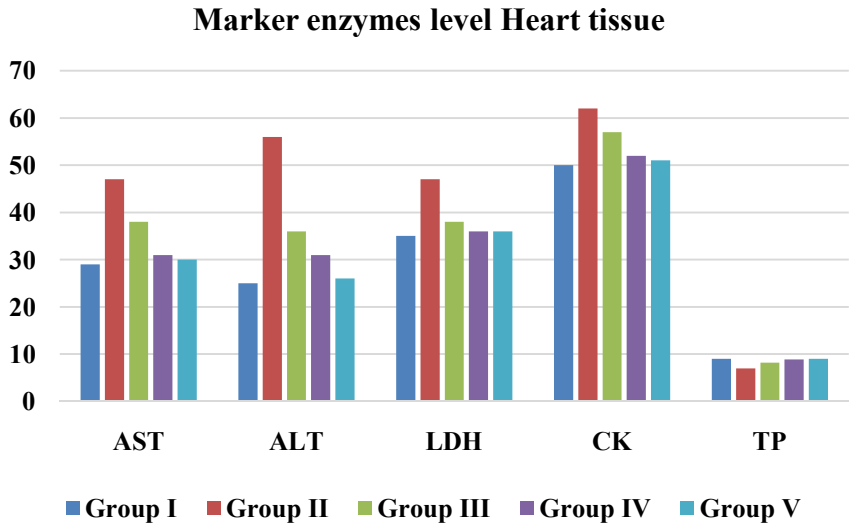


Figure 3: Effect of ethanolic extract of *Ipomoea biloba* leaf on the myocardial marker enzymes level in the experimental rats

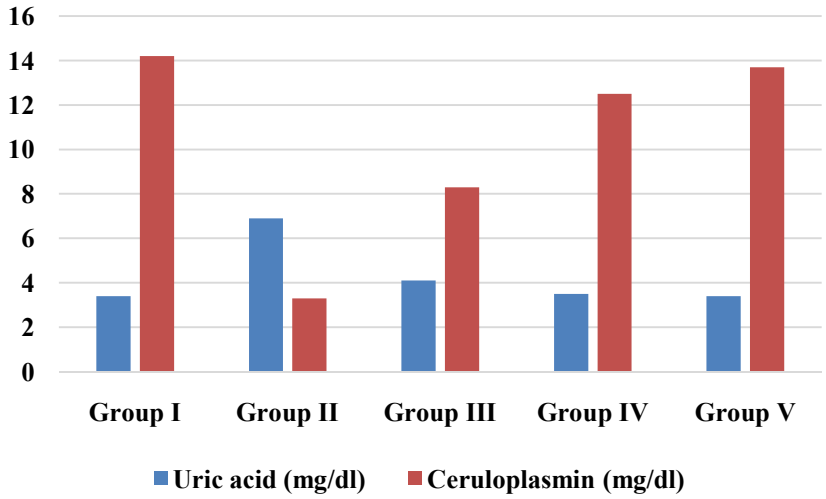


Figure 4: Effect of ethanolic extract of *Ipomoea biloba* leaf on uric acid and ceruloplasmin levels in the serum of experimental rats

DISCUSSION

Myocardial infarction is characterized by altered cardiac function due to imbalance between oxygen supply and demand. Oxidative stress induced by reactive oxygen species (ROS) plays a major role. ROS mediate various signaling pathways that results into vascular inflammation and atherogenesis. Oxidative stress by the reactive oxygen species is mainly responsible for myocardial infarctio. Isoproterenol is responsible for the free radical formation which may cause cellular cholesterol accumulation by increasing the cholesterol accumulation and decrease in its utilization **[16, 17]**. In this present study, the findings were exactly similar to this statement. The results of this study showed that the isoproterenol injection to the experimental animals were demonstrated the gradual increase levels of cholesterol, triglycerides and phospholipids in the serum as well as heart tissues of the myocardial infarction induced experimental animals. This causes the severe damage to the myocardial membrane. Interestingly, the treatment with the ethnolic extract of *Ipomea biloba* leaves to the myocardial infarction induced rats showed the noticeably decreased level of the both cholesterol and triglycerides **(Figure 1)**. There were no any significant variations between control and plant extract treated experimental animals.

The Myocardium contains the abundant of cardiac enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and creatine kinase (CK). These enzymes are some of the ideal markers for the indication of myocardial infarction. The drastic elevation of these marker enzymes levels in the serum, denoting the severe injury to the

myocardium. The injury to the cardio tissue may release these marker enzymes to the serum. In this study, we also noted the exact outcomes. The isoproterenol induced animals (Group II) showed the severely elevated levels of these marker enzymes such as AST, ALT, LDH, and CK in the both serum and heart tissues. This elevation in these marker enzymes may due to the administration of isoproterenol, which may leads to the insufficient supply of oxygen to the cardio cells that further leads to cell cellular damage and/or tissue necrosis. This phenomenon allows the cardiac marker enzymes to leak out in to the blood stream **[18]**. The findings of our present study were revealed that the pre-treatment with ethanolic extract of *Ipomoea biloba* leaves were significantly decreases the levels of cardio marker enzymes in the serum of experimental animals and showed the appreciable cardio-protection.

A few previous research reports were confirmed that the uric acid is prime indicator of the kidney function that directly associated with the heart diseases. In the case of myocardial infarction, the uric acid level in the serum was regarded as the foremost risk factor. The uric acid is usually produced from the xanthine. During the hypoxic conditions, the tissues are disturbed due to the increased oxidation of essential-SH-groups of the enzyme xanthine dehydrogenous and it was converted to xanthine oxidase. Xanthine oxidase enzymes normally catalyze the conversion of hypoxanthine to xanthine, uric acid and superoxide. The uric acid can also stimulate the granulocytes adherence to the endothelium and peroxide and superoxide free radical liberation **[19, 20]**. These free radicals liberation eventually produces the increased stress to the myocardium and causes cellular damage and/or cell death. In this investigation, we also noted the drastically increased level of the uric acid in the serum of myocardial infarction induced experimental animals than the untreated normal control animals. Interestingly, the treatment with the ethanolic extract of the *Ipomea biloba* leaves to the myocardial infarction induced experimental animals were showed the appreciably reduced level of uric acid in the serum of myocardial infarction induced experimental animals **(Figure 4)**. There were no any significant variations were noted between control and plant extract treated experimental animals.

The ceruoplasmin is an extracellular antioxidant and acute phase protein. Some previous findings were demonstrated that the ceruloplasmin protects the intima against free radical stimulated tissue injury. The antioxidant property of ceruloplasmin is through its ferroxidase activity catalyzing the oxidation of Fe^{2+} to Fe^{3+}. Thus it inhibits ferrous ion stimulated lipid

peroxidation. Since it has both ferroxidase and copper binding capacity, it is used to neutralize the excess amount of free radicals in the body **[21, 22]**. With the above note, in this study we have also observed the significantly decreased levels of ceruloplasmin in the serum of ISO-induced myocardial infarction rats. The findings of this present study were revealed that the pre-treatment with ethanolic extract of *Ipomoea biloba* leaves were noticeably augmented the ceruloplasmin protein level in the serum of experimental animals.

CONCLUSION

In conclusion, the novel findings of this present investigation was 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 cholesterol, triglycerides, myocardial marker enzymes, and low density lipoproteins levels in both serums as well as heart tissue. These results were proved the cardio-protective action of the ethanolic extract of *I.biloba* leaves. Hence, it was concluded that the *I.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 *I.biloba* leaves against the myocardial infarction.

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