Evaluation of synergistic potential of *Tridax procumbens and Boerhavia diffusa* in isoproterenol induced myocardial injury

A. Shanmugapriya[1] and S. Maneemegalai[2]

[1]Department of Biochemistry, Shrimati Indira Gandhi College, Tiruchirappalli, Tamil Nadu - 620 002, India

[2]Department of Biochemistry, Government Arts and Science College (Women)

[Formerly Bharathidasan University Constituent College for Women], Orathanadu – 614 625, Thanjavur District, Tamil Nadu, India.

(*Received: September 2019 Revised: October 2019 Accepted: November 2019)*

Corresponding author: **S. Maneemegalai**. Email: maneedevi@yahoo.co.in

ABSTRACT

Introduction and Aim: Combinative action of medicinal plants has earned an important place in the herbal medicinal field. The combination therapeutics may provide higher activity against various diseases in a synergistic manner. In the present research work the ethanolic leaf extracts of *Tridax procumbens* and root extracts of *Boerhavia diffusa* were studied for the assessment of synergistic cardioprotective activity through animal model.

Materials and Methods: Wistar male albino rats were treated with combinative ethanolic extract (150 +150 mg/kg BW) to isoproterenol-induced myocardial infarcted rats. Cardio-protection was investigated by estimating the activities of inflammatory markers like CRP, myeloperoxidase, homocysteine, xanthine oxidase and HMG CoA reductase in serum, plasma and tissue sample.

Results: The activities of CRP in serum and tissue myeloperoxidase were significantly elevated in isoproterenol-induced rats. Parameters like plasma homocysteine, tissue xanthine oxidase and HMG CoA reductase of isoproterenol administered rats also showed significant elevation. Combinative ethanolic leaf extract of *Tridax procumbens* and root extracts of *Boerhavia diffusa* treatment showed a marked difference in the altered parameters by preventing myocardial necrosis.

Conclusion: The present study concludes that co-treatment with ethanolic extract of *Tridax procumbens* and *Boerhavia diffusa* confirmed the protective and inhibitory effect against isoproterenol induced myocardial infarction and proved to be more beneficial.

Keywords: Isoproterenol; myocardial infarction; *Tridax procumbens*; *Boerhavia diffusa*; ethanol extract.

INTRODUCTION

Myocardial infarction (MI) is the most important killer disease responsible for death and disability throughout the world (1). It is characterized by inadequate oxygen due to low supply of blood, which leads to myocardial injury. Oxidative stress was found to play a major role in the development of myocardial infarction (2). Development of free radicals leads to hemo-dynamic, biochemical and histopathological alterations which results in membrane damage, diminished endogenous antioxidants and escape of cardiac markers into systemic circulation (3). Even though various medications are available to treat MI, nature has provided excellent source of medicinal agents that find applications in various field. Phytochemicals present in medicinal plants play a vital role in fighting against deadly diseases in particular MI. Medicinal plants are the best choice for a preventive approach that is cost effective with fewer side effects (4). Several reports are focused on medicinal plants individually and in combination. Bioactive compounds present in different plants exert synergistic functions in combination by interacting with one another (5).

Tridax procumbens is a weed that is found throughout India along fields, roadside etc., (6). *T. procumbens* belongs to Asteraceae family called as "*Vettukayathalai"* in Tamil that has significant wound healing activity (7). It is well known for excellent medicinal properties like antidiabetic, antioxidant and cardiovascular role. Its leaves are well known medicine for liver disorders (8), hypocholesterolemic, hypo-tensive and weight reducing properties (9).

Boerhavia diffusa belongs to Nyctaginaceae family called as "*Mookerettai keerai*" in Tamil, "*Punarnava*" in Hindi, "*Varshabhu*" in Sanskrit (10). It is a creeping weed found in fields and wastelands (11). Various parts of *B. diffusa* provide significant medicinal activity. The roots of *B. diffusa* have numerous medicinal properties. It is used to treat kidney disorders, cardiac disorders, and cancer (12).

The present research work is focused on the evaluation of the cardioprotective efficacy of *T. procumbens* and *B. diffusa*, individually and in combination, against isoproterenol induced MI.

MATERIALS AND METHODS

Collection on medicinal plants

Fresh leaves and roots of *T. procumbens* and *B. diffusa*, respectively, were collected from the local villages around Tiruchirappalli and was authenticated by John Britto Rapinat Herbarium, Department of Botany, St. Joseph's College, Tiruchirapalli. The collected parts of the plants were washed and shade dried. The shade dried plant materials were ground into coarse powder separately and stored in clean containers at room temperature.

Preparation of the plant extracts

T. procumbens leaves (100 g) and *B. diffusa* root (100 g) powder were mixed with 500ml of ethanol for 24 hours. It was filtered and the filtrates were then evaporated with the help of rotary evaporator. The extracts were stored in sterile glass bottle at room temperature.

Selection and maintenance of experimental animals

Male Wistar albino rats weighing 150-200 g were selected for the study. The animals were housed in well-ventilated neat cages, lined with sterile husk and fed with standard pellet rodent diet and water *ad libitum*. The rats were acclimatized to laboratory conditions for 10 days before the commencement of the experiments. All procedures described were reviewed and approved by the Institutional Animal Ethical Committee.

Grouping of experimental animals

The animals were divided into nine groups. Each group consisted of 6 rats (n=6).

Group I: Control (normal rats received 0.9 % saline)

Group II: received isoproterenol subcutaneously twice (85 mg/kg SC) at an interval of 24 hours.

Group III: received isoproterenol (85 mg/kg SC) and *T. procumbens* (100 mg/kg body weight orally) for 15 days

Group IV: received isoproterenol (85 mg/kg SC) and *T. procumbens* extract (200 mg/kg body weight orally) for 15 days

Group V: received isoproterenol (85 mg/kg SC) and *T. procumbens* extract (300 mg/kg body weight orally) for 15 days

Group VI: received isoproterenol (85 mg/kg SC) and *B. diffusa* (100 mg/kg body weight orally) for 15 days

Group VII: received isoproterenol (85 mg/kg SC) and *B. diffusa* extract (200 mg/kg body weight orally) for 15 days

Group VIII: received isoproterenol (85mg/kg SC) and *B. diffusa* extract (300 mg/kg body weight orally) for 15 days

Group IX: received isoproterenol (85 mg/kg SC) in combination with *T. Procumbens* (150 mg/kg body weight orally) and *B. diffusa* extracts (150 mg/Kg body weight orally) for 15 days.

The animals were sacrificed by cervical decapitation and blood was collected and separated. The heart was dissected and washed in ice cold saline, homogenized and used for experiments.

Assessment of biochemical parameters in serum

Blood samples from animals were taken. Serum and plasma were separated for the analysis of CRP, myeloperoxidase and homocysteine by standard procedure (13, 14).

Assessment of biochemical parameters in tissue

The heart tissue of the experimental animals was taken and homogenized in 10% ice-cold phosphate buffer and the mixture was centrifuged. The homogenate thus obtained was used for the estimation of xanthine oxidase (15).

Estimation of HMG-CoA reductase in rat liver

The ratio between 3-hydroxy-3-methylglutaryl-CoA and mevalonate concentrations in tissues in terms of absorbance was taken as an index of the activity of HMG-CoA reductase. The level of HMG-CoA reductase was estimated in rat liver homogenate (16).

Statistical analysis

Statistical significance was evaluated by One-way analysis of variance (ANOVA) using SPSS version (17.0) and the individual comparisons were obtained by the Duncan's multiple range test (DMRT). A value of $p<0.05$ was considered as a significant difference between groups.

RESULTS

Development of atherosclerosis is considered to be a significant inflammatory event (17). The effect of plant extracts on serum / tissue levels of inflammatory markers (CRP, myeloperoxidase) in normal and isoproterenol-injected rats are shown in figure 1 and 2. Rats injected with isoproterenol showed a significant ($p<0.05$) increase in serum CRP and tissue MPO activity as compared to the control group. Treatment with the leaf extracts of *T. procumbens* and the root extracts of *B. diffusa* individually and in combination

in isoproterenol injected rats showed a significant ($p<0.05$) decrease in serum CRP levels, and MPO activity.

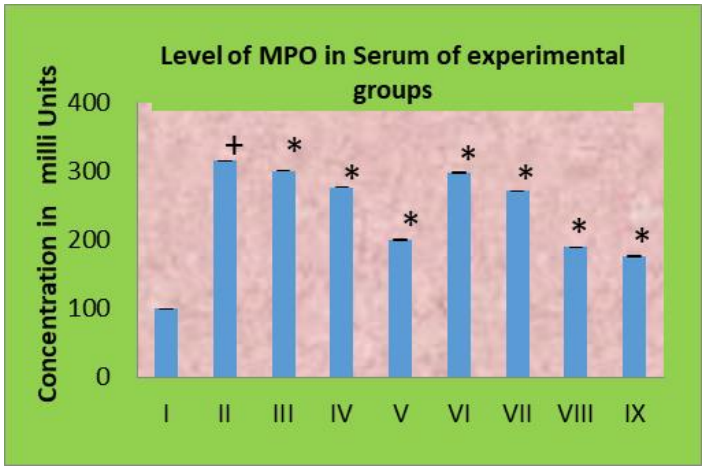

Fig. 1: Level of myeloperoxidase in serum of experimental groups

Values are mean ± SD; n = 6; Statistically significant difference at $P < 0.05$ + group I Vs group II, * group II Vs group III, IV, V, VI, VII, VIII, IX.

Group descriptions:

Group I - Control rats;
Group II - isoproterenol induced rats;
Group III - isoproterenol with *T. procumbens* (100mg);
Group IV - isoproterenol with *T. procumbens* (200mg);
Group V- isoproterenol with *T. procumbens* (300mg);
Group VI - isoproterenol with *B. diffusa* (100mg);
Group VII - isoproterenol with *B. diffusa* (200mg);
Group VIII - isoproterenol with *B. diffusa* (300mg);
Group IX - isoproterenol with *T. procumbens* and *B. diffusa* (150 mg + 150 mg).

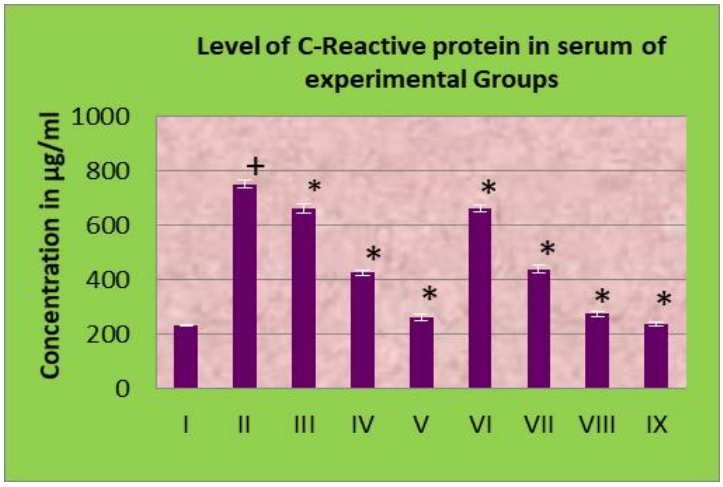

Fig. 2: Level of C - reactive protein in serum of experimental groups

Values are mean ± SD; n = 6; statistically significant difference at $p< 0.05$ + group I Vs group II, * group II Vs group III, IV, V, VI, VII, VIII, IX.

Group descriptions:

Group I – Control rats;
Group II – isoproterenol induced rats;
Group III - isoproterenol with *T. procumbens* (100mg);
Group IV - isoproterenol with *T. procumbens* (200mg);
Group V - isoproterenol with *T. procumbens* (300mg);
Group VI - isoproterenol with *B. diffusa* (100mg);
Group VII - isoproterenol with *B. diffusa* (200mg);
Group VIII - isoproterenol with *B. diffusa* (300mg);
Group IX - isoproterenol with *T. procumbens* and *B. diffusa* (150 mg + 150 mg).

Homocysteine, a non-essential thiol containing amino acid is derived from methionine metabolism may generate partially reduced ROS that are able to stimulate the LPO in the atherosclerotic process (18). Significant ($p<0.05$) elevation in the level of homocysteine was noted in plasma of Group II isoproterenol-administered rats compared to Group I control rats (Fig. 3). *T. procumbens* and *B. diffusa* treated rats individually in increasing concentrations and in combination normalized the level of homocysteine.

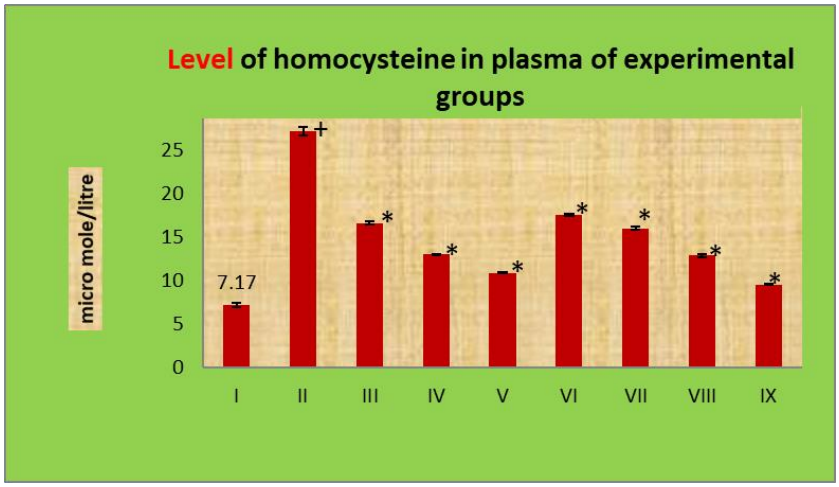

Fig. 3: Level of homocysteine in plasma of experimental groups

Values are Mean ± SD; n = 6; statistically significant difference at p< 0.05 + group I Vs group II, * group II Vs group III, IV, V, VI, VII, VIII, IX.

Group descriptions:

Group I – Control rats;
Group II – isoproterenol induced rats;
Group III - isoproterenol with *T. procumbens* (100mg);
Group IV - isoproterenol with *T. procumbens* (200mg);
Group V- isoproterenol with *T. procumbens* (300mg);
Group VI - isoproterenol with *B. diffusa* (100mg);
Group VII - isoproterenol with *B. diffusa* (200mg);
Group VIII - isoproterenol with *B. diffusa* (300mg);
Group IX - isoproterenol with *T. procumbens* and *B. diffusa* (150 mg + 150 mg)

Table 1 shows the level of HMG-CoA reductase in the liver of experimental rats. A significant (p<0.05) increase in HMG-CoA reductase in isoproterenol administered rats were observed when compared to control groups. Isoproterenol administered rats treated with plant extracts showed significant decrease in the activity of HMG-CoA reductase.

Table 1: Comparison of HMG-CoA activity in the liver tissue homogenate of the experimental groups

Groups	HMG CoA reductase (Units)
I	3.50 ± 0.01
II	6.24 ± 0.02^{+}
III	5.07 ± 0.03*
IV	4.45 ± 0.02*
V	4.00 ± 0.02*
VI	5.13 ± 0.04*
VII	5.01 ±0.05*
VIII	4.15 ± 0.01*
IX	3.83 ± 0.03*

Values are Mean ± SD; n = 6; statistically significant difference at p < 0.05 + group I Vs group II, * group II Vs group III, IV, V, VI, VII, VIII, IX.

Group descriptions:

Group I - Control rats;
Group II - isoproterenol induced rats;
Group III - isoproterenol with *T. procumbens* (100mg);
Group IV - isoproterenol with *T. procumbens* (200mg);
Group V- isoproterenol with *T. procumbens* (300 mg);
Group VI - isoproterenol with *B. diffusa* (100 mg);
Group VII - isoproterenol with *B. diffusa* (200 mg);
Group VIII - isoproterenol with *B. diffusa* (300 mg);
Group IX - isoproterenol with *T. procumbens* and *B. diffusa* (150 mg + 150 mg).

Xanthine oxidase is a vital cause of free radical generation. Xanthine oxidase act on xanthine and hypoxanthine with the resultant production of oxygen free radicals and increased uric acid level . Figure 4 depicted the level of xanthine oxidase in heart tissue of experimental rats. There was a significant increase in xanthine oxidase level in heart tissue of

isoproterenol-injected rats compared to control rats. Plant extract treated rats in combination significantly reversed the increased levels of xanthine oxidase.

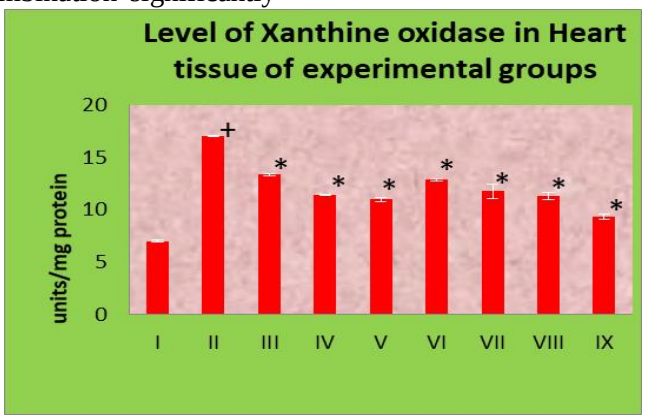

Fig. 4: Level of xanthine oxidase in heart tissue of experimental groups

Values are Mean ± SD; n = 6; statistically significant difference at $p < 0.05$ + group I Vs group II, * group II Vs group III, IV, V, VI, VII, VIII, IX.

Group descriptions:

Group I - Control rats;
Group II - isoproterenol induced rats;
Group III - isoproterenol with *T. procumbens* (100mg);
Group IV - isoproterenol with *T. procumbens* (200mg);
Group V- isoproterenol with *T. procumbens* (300 mg);
Group VI - isoproterenol with *B. diffusa* (100 mg);
Group VII - isoproterenol with *B. diffusa* (200 mg);
Group VIII - isoproterenol with *B. diffusa* (300 mg);
Group IX - isoproterenol with *T. procumbens* and *B. diffusa* (150 mg + 150 mg).

DISCUSSION

C- reactive protein is frequently studied as an inflammatory marker and sensitive predictor of cardiovascular events. Myeloperoxidase (MPO) has emerged as a new inflammatory marker, which is involved in oxidative stress and is a leading marker for the assessment of atherosclerosis (19).

Myeloperoxidase cause oxidative modification of low density lipoprotein (LDL) is considered as a key event in the promotion of athero-genesis and in the initiation and progression of cardiovascular diseases (20). An elevated concentration of C-reactive protein is an indicator of inflammation. C-reactive protein is predominantly made in the liver and is secreted in increased amounts within 6 hours of an acute inflammatory stimulus. Co-treated plant extracts based on different dosage and in combination prevented the inflammation and modification caused by LDL (21).

Elevation of homocysteine is used as an independent risk factor for MI (22). The plant extracts might have inhibited the production of homocysteine from methionine that are vital for free radicals inducement. Investigations by Subashini and Rajadurai (2011) indicated that *Nelumbo nucifera* leaf extracts effectively decreased the levels of homocysteine in isoproterenol treated rats (23).

HMG-CoA reductase is the rate limiting enzyme in the cholesterol biosynthesis. Inhibitors of 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase are the most effective and best-tolerated drugs to treat elevated levels of low-density lipoprotein cholesterol. Increase in the level of HMG CoA might be due to higher demand for energy by the β-adrenergic receptor stimulated myocardium and lipid peroxidation in isoproterenol-induced rats. The plant extracts in combination significantly decreased the activity of this enzyme in the liver of myocardial infarcted rats due to the regulation of β-adrenergic receptor stimulation and its anti-lipid peroxidative activity. The results of present study are in accordance with the previous reports of Shameela *et al.*, (2015), who reported that ethanolic extract of *B. diffusa* significantly decreased the activity of HMG-CoA reductase in isoproterenol treated myocardial infarcted rats (24).

Increased xanthine oxidase level indicates that myocardial ischemia has a specific relationship with xanthine oxidase activity. During ischemic conditions, the adenosine nucleotide group is catabolized to hypoxanthine and xanthine, in association with the conversion of xanthine dehydrogenase to xanthine oxidase (25). In the present study, rats treated with the combination of plant extracts had significant decrease in the myocardial necrosis. Studies also showed that serum uric acid concentrations are higher in patients with established coronary heart disease compared with healthy control.

CONCLUSION

Screening the synergistic combinations of medicinal plants is an ongoing challenge in research field. The present study clearly concludes that *T. procumbens* and *B. diffusa* in combination exhibited significant cardioprotective activity when compared to individual

extracts. The combinative treatment altered the changes exerted by isoproterenol by proving as powerful cardioprotective agent. Thus, the combination of *T. procumbens* and *B. diffusa* can be used as an alternative effective drug for treating MI due to the excellent synergistic potential and due to enriched polyphenolic contents.

REFERENCES

1. Kloner, R. A. New observations regarding post ischemia/ reperfusion myocardial swelling. J. Am. Coll. Cardiol. 2015; 65: 324-326.
2. Prince, P. S. M., Dhanasekar, K., Rajakumar, S. Vanilic acid prevents altered ion pumps, ions, inhibits FAS receptor and caspase mediated apoptosis-signaling pathway and cardiomyocyte death in myocardial infarcted rats. Chem. Biol. Interact. 2015; 232: 68-76.
3. Hemalatha, K., Prince, P. Preventive effects of Zingerone on altered lipid peroxides and non-enzymatic antioxidants in the circulation of isoproterenol induced myocardial infarcted rats. J. Biochem. Mol. Toxicol. 2015; 29: 63-69.
4. Visioli, F., Borsani, L., Galli, C. Diet and prevention of coronary heart disease: The potential role of phytochemicals. Cardiovasc Res. 2000; 47: 419-425.
5. Wang, X. L. Potential herb drug interaction in the prevention of cardiovascular diseases during integrated traditional and western treatment. Chinese Journal of Integrative Medicine. 2015; 21(1): 3-9.
6. Mundada, S., Shivhare, R. Pharmacology of *Tridax procumbens* a weed: Review, International Journal of Pharma Tech Research. 2010; 2(2): 1391-1394.
7. Pai, C., Kulkarni, U., Borde, M., Murali, S., Mrudula, P., Deshmukh, Y. Antibacterial Activity of *Tridax procumbens* with special reference to Nosocomial Pathogens. 2011; (4): 164-173.
8. Bhagwat, D. A., Killedar, S. G., Adnaik, R. S. Antidiabetic activity of leaf extract of *Tridax procumbens.* International. Journal of Green Pharmacology. 2008; 2: 126-128.
9. Catherine, C., Ikewuchi., Jude, C., Ikewuchi., Mercy, O. Phytochemical Composition of *Tridax procumbens* Linn Leaves: Potential as a Functional Food. Food and Nutrition Sciences. 2015; 6: 992-1004.
10. Ayurvedic Pharmacopoeia of India. MHFW, Department of AYUSH, Govt. of India, Delhi. 2005; 1(1): 40.
11. Rajpoot, K., Mishra, R. N. *Boerhaavia diffusa* roots (Punarnavamool) – Review as Rasayan (Rejuvenator / Antiaging). International Journal of Research in Pharmaceutical and Biomedical Science. 2011; 2(4): 1451-1460.
12. Kirtikar, K. R., Basu, B. D. Traditional Medicinal Plants Used by Local People at the Village Sabgram under Sadar Upazila of Bogra District, Bangladesh. Research in Plant Sciences. 2015; 3(2): 31-37.
13. Ridker, P. M., Hennekens, C. H., Buring, J. E. C-reactive protein and other markers of inflammation in the prediction of cardiovascular disease in women. The New England Journal of Medicine. 2015; 342: 836-843.
14. Nishioka, T., Maki, K., Kimura, M., Takahama, U. Determination of salivary peroxidase activity in human mixed whole saliva. Archives of Oral Biology.2003; 48: 397-400.
15. Roussos, G. G. Xanthine oxidase from bovine small intestine. In: Grossman L, Moldave K, editors. New York (NY): Academic Press; 1967; 5-16.
16. Rao, A. V., Ramakrishnan, S. Indirect assessment of hydroxymethylglutaryl-CoA reductase (NADPH) activity in liver tissue. Clinical Chemistry. 1975; 21(10): 1523-1525.
17. Ross, R. Atherosclerosis–an inflammatory disease, The New England Journal of Medicine. 1999; 340: 115-126.
18. Toborek, M., Kopieczna., Grzebieniak, E., Drozdz, M., Wieczorek, M. Increased lipid peroxidation as a mechanism of methionine-induced atherosclerosis in rabbits. Atherosclerosis. 1995; 115: 217-224.
19. Zang, R., Raman, K., Mariswamy, J. Association between myeloperoxidase levels and risk of coronary artery disease. The Journal of the American Medical Association. 2001; 286(17): 2136-2142.
20. Klebanoff, S. J. Myeloperoxidase: friend and foe. Journal of Leukocyte biology. 2005; 77: 598-625.
21. Wells, B. J., Manious, A. G., Everett, C. J. Association between dietary arginine and C - reactive protein. Nutrition. 2005; 21(2): 125-130.
22. Mayer, E. L., Jacobsen, D. W., Robinson, K. Homocysteine and coronary atherosclerosis. Journal of the American College of Cardiology.1996; 27: 517-527.
23. Subashini, R., Rajadurai, M. Evaluation of cardio-protective efficacy of *Nelumbo nucifera* leaf extract on isoproterenol induced myocardial infarction in Wistar rats. International Journal of Pharmacology and Bio Sciences. 2011*;* 2(4): 285-294.
24. Shameela, S., Priyadarsini, I., Devi, L. Evaluation of *Boerhaavia diffusa* for Hepatoprotective Activity in Experimental Wistar Rats. International Journal of Pharmacy and Biological Sciences. 2015; (1): 115-122.
25. Bhakuni, P., Chandra, M., Misra, M. K. Oxidative stress parameters in erythrocytes of post reperfused patients with myocardial infarction. Journal of Enzyme Inhibition and Medicinal Chemistry. 2005; *20: 377*-381.