International Journal of Research in Phytochemical and Pharmacological Sciences

Cardioprotective effect of *pulicaria wightania* against isoproterenol induced myocardial infarction in experimental rats

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ABSTRACT

The current study was carried out to evaluate the cardio protective activity of *Pulicaria Wightiana* against Isoproterenol (ISO) induced myocardial infarction (MI). Pretreatment to the different groups were given for 30 days and ISO was administered at last two days with an interval of 24 hrs. Due to chronic ionotropy ISO induces MI. Blood was collected at the last day of experimental period and biomarkers were observed. The results indicate that the extract exhibited significant cardioprotective activity.

Keywords: Abutilon indicum, Medicinal, plant.

ISSN: Awaiting
Research Article

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Article Info

Received on: 11-06-2019

Revised on: 30-056-2019

Accepted on: 01-07-2019

DOI: <https://doi.org/10.33974/ijrpps.v1i1.106>

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INTRODUCTION

Myocardial Infarction (MI) is the acute condition of necrosis of the myocardium that occurs a result of imbalance between coronary blood supply and myocardium demand. Myocardial infarction normally known as a heart attack, happens when flow of blood reduces or stops to a part of the heart, then causes damage to the muscle [1].

Isoproterenol drug (ISO) is a artificial catecholamine which is a β -adrenergic agonist; on high concentrations it depletes the endogenous stores, energy reserves of cardiac myocytes and results in biochemi-

cal, structural changes which are responsible for irreversible damage. Chemically, ISO is an L-B-(3, 4-dihydroxyphenyl)-or-isopropyl amino ethanol hydrochloride [2].

The pathophysiological changes as induced by ISO mimics to a larger extent with those occurring in humans [3]. ISO is a β -adrenergic receptor agonist that increases cytosolic cAMP [4]. The drug hormone receptor complex initiates enzyme adenylyl cyclase on the internal surface of the plasma membrane of the specific cells. This accelerates the intracellular formation of cyclic adenosine monophosphate (cyclic AMP), the second "messenger" which then stimulates or inhibits various metabolic or physiological processes [5], [6]. ISO increase the activities of Raf-1 kinase and MAP kinase, which accelerate phenylalanine incorporation into proteins [7], leading to cardiomyocyte hypertrophy [8].

ISO causes increase in oxidative stress resulting in increase free radical activity. ISO induced biochemical & histopathological alterations observed in animal model are similar to human myocardial infarction. ISO was administered at a dose of 85 mg/kg s.c. body weight [7].

Herbal drugs exhibit medicinal properties in the treatment of heart ailments and need to explores to identify their potential application in prevention and therapy of human ailments and also used to treat myocardial infarction. While herbs like *Moringa olifera*, *Withania somnifera* [9] etc are helpful in the treatment of cardiovascular disorders.

WHO currently encourages, recommend and promote conventional as well as natural remedies in the national health care programmes, as they available

easily at low cost, comparatively safe, and are culturally acceptable. The usage of herbs in myocardial infarction as they are safe and alternative medicine [10]. Therefore the aim is to evaluate the potent cardio protective activity of Ethanolic Extract of *Pulicaria Wightiana* (EEPW) in ISO induced cardiac necrosis using an *invivo* rodent model.

MATERIALS AND METHODS

Plant material:

Collection of Leaves: Fresh leaves of *Pulicaria Wightiana* were collected from Botanical garden of PRRM College of Pharmacy, Kadapa, and Andhra Pradesh and authenticated by Asst. Prof. Dr. K. Madava Chetty of the Department of Botany, S.V. University, Tirupathi, Andhra Pradesh.

Preparation of extract: The dried leaves of *Pulicaria Wightiana* were taken, powdered in the grinder – mixer then obtain a coarse powder and then passed through 40 mesh sieve. About 200gms of powder was extracted by using 70% ethanol by maceration process up to 24 hrs. The solution was filtered through with the whatmann filter paper and the resultant filtrate was distilled under reduced pressure for recovery of solvent. Then the obtained dried extract was kept in a desiccator and used for the further experiments.

Experimental Animals: Male wistar albino rats (180-230gm) procured from Raghavendra enterprises (Bangalore), were used in present study. The animals housed in the clean propylene cages and maintained the standard conditions (25±2°C such as relative humidity 44 - 56% and 12 hours light and dark cycles respectively) and fed with the standard rat diet (Mysore feeds, Bangalore) and purified drinking water *ad libitum* for every 1 week before and during the experiments.

Institutional Animal Ethical Committee (IAEC) of P. Rami Reddy Memorial College of Pharmacy (1423/PO/Re/s/11/CPCSEA/102/03/2017) approved the present study.

Assessment of cardio-protective activity: The experimental animals randomly divided into 5 groups (n=6) and treated for the duration of 30 days as per treatment schedule given in table no.3. MI was treated by administration of ISO (85 mg/kg s.c.) for 29th & 30th days with an interval of 24 hrs^[11].

Collection of blood samples: The blood samples were collected from retro-orbital venous plexus of the rats without any agent for partition of serum, at end of the experimental period. After collecting blood in eppendorf tubes they were kept for 1 hour time at the room temperature and the serum was separated by centrifugation at 2000 rpm for 15 min and they stored until analyzed for the different biochemical parameters.

Statistical analysis: All the data was expressed as the mean ± S.E.M. Statistical significance between

more than two groups was tested using one way ANOVA followed by the Tukey test using computer based fitting program (Prism graph pad 7.0). Statistical significance was set accordingly.

RESULTS

Serum cardiac markers:

Effect on CK -MB: The result had shown the effect of EEPW on serum CKMB levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum CKMB levels in ISO control group when compared with normal group. The standard group receiving Propranolol (5 mg/kg) shown more significant decrease in serum CK-MB levels and the results were parallel with normal. Amazingly the test-2 group receiving EEPW (400 mg/kg) showed significance decrease in ($p < 0.01$) serum CK-MB levels than test-1 which was receiving EEPW (200 mg/kg).

Figure 1: Effect of EEPW on CK –MB

Effect of Lactate dehydrogenase (LDH): The result had shown the effect of EEPW on serum LDH levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum LDH levels in ISO control group when compared with normal group.

The test-1 which was receiving EEPW (200 mg/kg) and test-2 which was receiving EEPW (400 mg/kg) showed significant and dose dependent decrease in the significance ($p < 0.01$) of serum LDH levels when compared to ISO control group.

Figure 2: Effect of EEPW on LDH

Effect on Aspartate transaminase (AST)/ SGOT: The result had shown the effect of EEPW on serum AST levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum AST levels in ISO control group when compared with normal group.

The test-1 which was receiving EEPW (200 mg/kg) had shown less significance ($p < 0.05$) but test-2 which was receiving EEPW (400 mg/kg) ($p < 0.01$) showed significant and dose dependent decrease in Serum AST levels when compared to ISO control group.

Figure 3: Effect of EEPW& EEPW on SGOT

Effect on Alanine transaminase (ALT)/ SGPT: The result had shown the effect of EEPW on serum ALT levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum ALT levels in ISO control group when compared with normal group.

Surprisingly the test-2 which was receiving EEPW (400 mg/kg) had shown more significance ($p < 0.001$) than test-1 which was receiving EEPW (200 mg/kg) ($p < 0.01$) when compared to ISO control group.

Figure 4: Effect of EEPW& EEPW on SGPT

Effect on Alkaline phosphatase (ALP): There was significant ($p < 0.001$) increase in serum ALP levels in ISO control group when compared with normal group. The result had shown the effect of EEPW on serum ALP levels in normal and experimental groups.

Figure 5: Effect of EEPW& EEPW on ALP

Amusingly the test-2 group which was receiving both EEPW (400 mg/kg) had shown significant ($p < 0.001$) decrease in the serum ALP levels when compared to ISO control group than Test-1 group which was receiving EEPW (200 mg/kg).

LIPID PROFILES

Effect on serum cholesterol: The obtained result pretense the effect of EEPW on serum cholesterol levels in the normal and experimental groups. Administration of ISO causes fibrotic changes and accumulation of fat, this can be observed in control group rats because there was significant ($p < 0.001$) increase in the serum cholesterol levels in ISO control group when they compared with the normal group.

The test-1 and test-2 which were receiving EEPW showed decrease in the serum cholesterol levels. But test-2 group which had shown significant ($p < 0.001$) decrease in the serum cholesterol levels when compared to ISO control group.

Figure 6: Effect of EEPW& EEPW on Cholesterol

Effect on serum triglycerides (TG): The result shows the effect of EEPW on serum triglycerides levels in normal and experimental groups. ISO causes increase in triglyceride synthesis, so when it is administered in control group it leads to significant ($p < 0.001$) increase in serum triglycerides levels.

Flavonoids present in both EEPW caused reduction of triglycerides. Test-2 had shown more significant ($p < 0.001$) decrease in the serum triglycerides levels when compared to ISO control group than test-1 which was receiving EEPW (200 mg/kg).

Figure 7: Effect of EEPW& EEPW on Triglycerides

Effect on serum HDL-cholesterol: The result had shown the effect of EEPW on serum HDL levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum HDL levels in ISO control group when compared with normal group.

The test-1 and test-2 which were receiving EEPW showed decrease in the serum HDL levels when compared to ISO control group than individual administration. But test-2 which were receiving EEPW (400 mg/kg) showed less significance ($p < 0.05$) in the serum HDL levels.

Figure 8: Effect of EEPW& EEPW on HDL

Effect on serum LDL levels: The result shows the effect of EEPW on serum LDL levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum levels LDL in ISO control group when compared with normal group.

The test-1 and test-2 which were receiving EEPW (200 mg/kg) and EEPW (400 mg/kg) respectively had shown significant decrease in the serum LDL levels. Interestingly test-2 group showed significant ($p < 0.001$) decrease in the serum LDL levels when compared to ISO control group.

Figure 9: Effect of EEPW& EEPW on LDL

Effect on serum VLDL levels: The result shows the effect of EEPW on serum VLDL levels in normal and experimental groups. There was significant ($p < 0.001$) increase in serum VLDL levels in ISO control group when compared with normal group.

The test-2 group which was receiving EEPW (400 mg/kg) had shown significant ($p < 0.001$) decrease in the serum VLDL levels when compared to ISO control group than test-1 which was receiving EEPW (200 mg/kg).

Figure 10: Effect of EEPW& EEPW on VLDL

Effect on weight of heart: The result shows the effect of EEPW on heart weight in normal and experimental groups. There was significant ($p < 0.001$) increase in heart weight in ISO control group due to edema when compared with normal group.

The test-1, 2 group animals which were receiving EEPW (200 mg/kg & 400 mg/kg) respectively had shown decrease in heart weight when compared to ISO control group. Interestingly test-2 group showed significant ($p < 0.001$) decrease in the heart weight when compared to ISO control group.

From the above observation it is noted that due to presence of polyphenols, catechins, flavonoids and other constituents in *Pulicaria Wightiana* it leads to decrease in ischemia, necrosis and increase in the antioxidant levels.

So it is confirmed that the administration *Pulicaria Wightiana* had shown decreased incidence of MI and increase in the cardio protective activity when compared with ISO treated group. Further investigations like exploring mechanism of actions as well as isolations of responsible phytochemicals may help for better understanding.

Figure 11: Effect of EEPW& EEPW on Heart Weight

DISCUSSION

The present study was carried out to evaluate the combined cardio protective activity of *Pulicaria Wightiana* against Isoproterenol (ISO) induced myocardial infarction (MI). Pretreatment to the different groups were given for 30 days and ISO was administered at last two days with an interval of 24 hrs. Due

Table 1: Treatment schedule for assessing the benefits of EEPW against ISO induced MI

S. No.	Group	No. of Animals	Treatment	Treatment period (Days)
1	Normal	6	Vehicle (1% CMC, p.o.)	30
2	Control	6	Isoproterenol (85 mg/kg rat, s.c.)	30
3	Standard	6	Propranolol (5 mg/kg, p.o.) + Isoproterenol (85 mg/kg rat, s.c.)	30
4	Test-1	6	EEPW (200 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	30
5	Test-2	6	EEPW (400 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	30

S.C. - Subcutaneous, P.O. – Per oral, EEPW- Ethanolic extract of Pulieria Wightiana

Table 2: Effect of EEPW on Serum biomarkers

Groups	Treatment	ON 31 ST DAY				
		CK-MB(IU/L)	LDH(IU/L)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
Normal	Vehicle (1% CMC, p.o.)	79.80 ± 2.53	348.8 ± 8.57	29.83 ± 1.19	28.83 ± 1.24	53.17 ± 1.30
Control	Isoproterenol (85 mg/kg rat, s.c.)	197.2 ± 2.07 ^{###}	485.3 ± 13.36 ^{###}	77.5 ± 2.04 ^{###}	47.0 ± 2.12 ^{###}	122.8 ± 2.49 ^{###}
Standard	Propranolol (5 mg/kg, p.o.) + Isoproterenol (85 mg/kg rat, s.c.)	93.04 ± 2.24 ^{***}	350.2 ± 6.23 ^{***}	33.83 ± 1.35 ^{***}	30.50 ± 0.42 ^{***}	55.3 ± 3.94 ^{***}
Test-1	EEPW (200 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	179.9 ± 6.0 [*]	400.2 ± 19.31 ^{**}	43.00 ± 5.80 ^{***}	39.17 ± 2.21 [*]	105.0 ± 4.93 ^{***}
Test-2	EEPW (400 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	101.7 ± 2.06 ^{***}	359.2 ± 9.77 ^{***}	36.00 ± 1.03 ^{***}	31.67 ± 0.42 ^{***}	65.00 ± 1.71 ^{***}

All values are shown in mean ± SEM and n=6, ^{###}, indicates p<0.001, when compared with normal group, ^{***} indicates p<0.001, ^{**} indicates p<0.01, ^{*} indicates p<0.05, when compared to control group.

to chronic ionotropy ISO induces MI. Blood was collected at the last day of experimental period and biomarkers were observed.

ISO is a synthetic catecholamine. It is a β -adrenergic agonist when it binds to β_1 receptors which are present in heart, produces stimulation in receptors and production of calcium ions results in positive ionotropic and chronotropic effects. On higher doses it causes increase in preload and after load which increases ischemia leading to decrease in blood supply to myocytes. Due to hypoxia the myocytes tends to die on prolonged ischemia it leads to necrosis of tissue (Sharma and Sharma 2007). Upon administration of ISO, the oxygen demand of the heart increases with increase in ionotropic effect in the heart, resulting in prolonged ischemia and glucose deprivation. The cells are damaged with increased muscle contractility, which results in increasing the cell membranes permeability allowing cardiac enzymes to leak out into the bloodstream.

Elevation of cardiac markers in plasma is one of the criteria being used for the diagnosis of acute myocardial infarction. The cardiac markers should be present in high concentration in myocardium and should be absent from non-myocardial tissues. It should be rapidly released into the blood stream at the time of myocardial injury and there should be a direct relation between the plasma level of the marker and the extent of myocardial injury. The plasma diagnostic marker enzymes are of important because of their catalytic activity and tissue specificity. The myocardial cells contain many cardiac enzymes like CK-MB and lactate dehydrogenase.

CK-MB is localized predominantly in the heart and this makes it a valuable diagnostic tool for MI since damage specific to the myocardium would result in elevation of CK-MB Cardiac biomarkers are protein components of cell structures released into the blood stream when myocardial injury occurs and they can be measured in the systemic circulation^[12].

Table 3: Effect of *Pulicaria Wightiana.L* on serum lipid profile

Groups	Treatment	ON 31 ST DAY				
		CK-MB(IU/L)	LDH(IU/L)	ALT(IU/L)	AST(IU/L)	ALP(IU/L)
Normal	Vehicle (1% CMC, p.o.)	79.80 ± 2.53	348.8 ± 8.57	29.83 ± 1.19	28.83 ± 1.24	53.17 ± 1.30
Control	Isoproterenol (85 mg/kg rat, s.c.)	197.2 ± 2.07 ^{###}	485.3 ± 13.36 ^{###}	77.5 ± 2.04 ^{###}	47.0 ± 2.12 ^{###}	122.8 ± 2.49 ^{###}
Standard	Propranolol (5 mg/kg, p.o.) + Isoproterenol (85 mg/kg rat, s.c.)	93.04 ± 2.24 ^{***}	350.2 ± 6.23 ^{***}	33.83 ± 1.35 ^{***}	30.50 ± 0.42 ^{***}	55.3 ± 3.94 ^{***}
Test-1	EEPW (200 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	179.9 ± 6.0*	400.2 ± 19.31 ^{**}	43.00 ± 5.80 ^{***}	39.17 ± 2.21*	105.0 ± 4.93 ^{***}
Test-2	EEPW (400 mg/kg p.o) + Isoproterenol (85 mg/kg s.c.)	101.7 ± 2.06 ^{***}	359.2 ± 9.77 ^{***}	36.00 ± 1.03 ^{***}	31.67 ± 0.42 ^{***}	65.00 ± 1.71 ^{***}

All values are shown in mean ± SEM and n=6, ^{###}, indicates p<0.001, when compared with normal group, ^{***} indicates p<0.001, ^{**} indicates p<0.01, ^{*} indicates p<0.05, when compared to control group.

LDH plays an important role in cellular respiration, the process by which glucose (sugar) from food is converted into usable energy for cells. LDH isoenzymes are five kinds of the LDH enzyme that are found in specific concentrations in different organs and tissues. LDH-1 is found in the heart and in RBC. Levels of LDH peak at 3–4 days and remain elevated for up to 10 days in MI. Tissue break down releases.

The results were showed a significantly increase of serum CK-MB and LDH levels in ischemic control when compared with normal group. *Pulicaria Wightiana* treated individually as well as in combination had shown significant and dose dependently decrease in serum CK-MB and LDH levels when compared with ischemic control group. Interestingly, effect of combination was found more significant than individual

treatments. Treatment with standard drug i.e. Propranolol (5 mg/kg p.o.) found to be restores serum CK-MB and LDH levels to normal level. These observations are in line with previous studies done on ISO induced ischemic rats treated with *Andrographis paniculata*^[13], *Curcuma longa*^[14].

The serum enzymes namely AST, ALT and ALP serve as sensitive indices to assess the severity of myocardial infarction. All these parameters were found to be elevated in ISO control group when compared normal group. *Pulicaria Wightiana* individual and combination treatment showed significant decrease of these parameters, and values were found parallel with normal group.

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. Hypercholesterolemia, high concentration of low-density lipoprotein cholesterol, hyper triglyceridemia and low high-density lipoprotein are accepted as independent risk factors for atherosclerotic cardiovascular disease and mortality^[15,16]. Significant changes in the fatty acid composition of serum triglycerides, cholesterol ester and phospholipids were also reported in acute myocardial infarction condition^[17].

Pulicaria Wightiana treatment tends to decrease the levels of lipids. But test -3 showed potentiation effect on decrease in the cholesterol, TG, LDL, VLDL and increase in the HDL levels as compared to individual treatments. EEPW had shown significant decrease in cholesterol, TG, LDL and VLDL levels.

Rats treated with ISO have been reported to undergo increase in heart weight due to increase in water content and development of edema in intramuscular spaces culminating in extensive necrotic changes and invasion of inflammatory cells. The test-2 group EEPW (400mg/kg) had shown significant decrease in heart weight due to presence of antioxidants and the results of test-2 group coincide with standard group.

Flavonoids (catechins) the most common group of polyphenols plays an important role as antioxidant. Flavonoids decrease the free radical which cause oxidative stress, scavenge superoxide radicals and hydrogen peroxide produced by ISO and reduces myocardial damage. *Premna serratifolia* rich in flavonoids showed cardioprotective effect in ISO induced MI.

From the above observation it is noted that due to presence of polyphenols, catechins, flavonoids and other constituents in *Pulicaria Wightiana* it leads to

decrease in ischemia, necrosis and increase in the antioxidant levels.

So it is confirmed that the administration *Pulicaria Wightiana* had shown decreased incidence of MI and increase in the cardio protective activity when compared with ISO treated group.

CONCLUSION

The present research reveals that ISO treatment resulted in marked elevation in the level of cardiac marker enzymes like CK-MB, AST, ALT, LDH and ALP in serum, serum lipid profiles such as LDL, VLDL, TGA, and Cholesterol and decreased levels of HDL were observed. But treating with the extract of *Pulicaria wightiana* may alter these cardiac marker enzymes and lipid profiles and helps to treat the coronary artery diseases. Scientists proved that catechins and flavonoids possess many of the structural components that contribute to their antioxidant property. From the above observation it was confirmed that *Pulicaria Wightiana* were safe and highly effective in preventing cardiovascular dysfunction in rats, possibly due to anti-oxidative and cardioprotective properties. *Pulicaria Wightiana* was enriched with flavanoids, polyphenols and antioxidants acts by scavenging free radicals and reduces the risk of oxidative stress, myocardial infarction. Thus *Pulicaria Wightiana* can ameliorate the effects of ISO. Present efforts are directed to isolate the active constituents from various extracts of plant and elucidation of mechanism of action.

REFERENCES

1. Boudina, S., Laclau, M. N., Tariosse, L., Daret, D., Gouverneur, G., Bonoron-Adèle, S., ... & Dos Santos, P. (2002). Alteration of mitochondrial function in a model of chronic ischemia in vivo in rat heart. *American Journal of Physiology-Heart and Circulatory Physiology*, 282(3), H821-H831.
2. Swahn, E. (1998). The care of patients with ischaemic heart disease from a gender perspective. *European heart journal*, 19(12), 1758-1765.
3. Ravichandran, L. V., & Puvanakrishnan, R. (1993). Collagen levels in isoproterenol induced myocardial infarction in rats. *Indian journal of experimental biology*, 31(10), 825-830.
4. Lopez-Jimenez, (2000) F. Heavy meals may trigger heart attacks. *American Heart Association Scientific Sessions*. New Orleans. *Science News*, 158: 366.
5. Robinson, G. A., Butcher, R. W., & Sutherland, E. W. (1968). 140. Cyclic AMP. *Ann. Rev. Biochem*, 37, 149-174.
6. Motulsky, H. J., & Insel, P. A. (1982). Adrenergic receptors in man: direct identification, physiologic regulation, and clinical alterations. *New England Journal of Medicine*, 307(1), 18-29.
7. Yamazaki, T., Komuro, I., Zou, Y., Kudoh, S., Mizuno, T., Hiroi, Y., & Takahashi, T. (1997). Protein Kinase A and Protein Kinase C Synergistically Activate the Raf-1 Kinase/Mitogen-activated Protein Kinase Cascade in Neonatal Rat Cardiomyocytes. *Journal of molecular and cellular cardiology*, 29(9), 2491-2501.
8. Zou Y, Komuro I, Yamazaki T, Kudoh S, Uozumi H, Kadowaki T, Yazaki Y. Both Gs and Gi proteins are critically involved in isoproterenol induced cardiomyocyte hypertrophy. *J Biol Chem*. 1999; 274: 9760-9770.
9. Hina, S., Rehman, K., Dogar, Z. U. H., Jahan, N., Hameed, M., Khan, Z. I., & Valeem, E. E. (2010). Cardioprotective effect of gemmotherapeutically treated *Withania somnifera* against chemically induced myocardial injury. *Pak J Bot*, 42(2), 1487-1499.
10. Alschuler, L. (1998). Green tea: healing tonic. *Am. J. Natur. Med.*, 5, 28-31.
11. Parmar, N. S., & Prakash, S. (2006). *Screening methods in pharmacology*. Alpha Science International Limited.
12. Apple, F. S. (1999). Tissue specificity of cardiac troponin I, cardiac troponin T and creatine kinase-MB. *Clinica chimica acta*, 284(2), 151-159.
13. Ojha, S., Bharti, S. A. U. R. A. B. H., Golechha, M. A. H. A. V. E. E. R., Sharma, A. K., Rani, N., Kumari, S., & Arya, D. S. (2012). *Andrographis paniculata* extract protect against isoproterenol-induced myocardial injury by mitigating cardiac dysfunction and oxidative injury in rats. *Acta Pol Pharm*, 69, 269-278.
14. El-Sayed, E. M., El-azeem, A. S. A., Afify, A. A., Shabana, M. H., & Ahmed, H. H. (2011). Cardioprotective effects of *Curcuma longa* L. extracts against doxorubicin-induced cardiotoxicity in rats. *J Med Plants Res*, 5(17), 4049-58.
15. Wood, D., De Backer, G., Faergeman, O., Graham, I., Mancini, G., & Pyörälä, K. (1998). Prevention of coronary heart disease in clinical practice: Recommendations of the Second Joint Task Force of European and other Societies on Coronary Prevention1, 2. *Atherosclerosis*, 140(2), 199-270.
16. Gotto AM Jr, Assmann G, Carmena R, Davignon J, Fruchart J C, Kastelein JJP, Paoletti R. Tonkin A., (2000) *The IL-IB lipid handbook for clinical practice: blood lipids and coronary heart disease*, International Lipid Information Bureau. 2nd edn. New York.

17. Padma, V. V., Devi, C. S., & Ramkumar, K. M. (2006). Modulatory effect of fish oil on the myocardial antioxidant defense system in isoproterenol-induced myocardial infarction. *Journal of basic and clinical physiology and pharmacology*, 17(1), 1-16.