Journal of Pharmaceutical Research Vol. 9, No. 1, January 2010 : 35-38.

CARDIOPROTECTIVE ACTIVITY OF METHANOLIC EXTRACT OF *AEGLE MARMELOS* LEAF EXTRACT AGAINST ISOPROTERENOL INDUCED MYOCARDIAL DAMAGE IN RATS

Khanna P[1], Rawri Rajesh Kumar[2], Asdaq SMB[*1], Nayeem N[2] and Chakraborty M[1]

[1]Department of Pharmacology; [2]Department of Pharmaceutical Chemistry, Krupanidhi College of Pharmacy, Chikkabellandur, Carmalaram Post, Bangalore-560 035, India.

Received on : 22.10.2009 Revised : 09 .01.10 Accepted : 25.01.10

ABSTRACT

The present study was undertaken to evaluate the cardioprotective effect of methanolic extract of *Aegle marmoles* leaves (AMLE) against isoproterenol (ISO) induced myocardial damage in rats. Rats of either sex were administered AMLE (100 and 500 mg/kg) for three weeks and propranolol (PRO, 10 mg/kg) for one week in their respective groups orally. Subsequently myocardium injury was induced by subcutaneous administration of isoproterenol (150 mg/kg) for two consecutive days. The influence of prophylactic treatment was analysed by quantification of biomarkers and antioxidants. The CK-MB activities were fallen in serum and raised in heart tissue of animals treated with low and high doses of AMLE as well as PRO compared to ISO control. However, low dose of AMLE did not demonstrate any significant change in serum and tissue LDH activity when compared to ISO control. Further, high and low doses of AMLE caused significant elevation in SOD and CAT activities compared to ISO control. Furthermore, TBARS level declined in heart tissues of animals treated with PRO and high doses of AMLE, whereas, low dose of AMLE was not equally potent. Hence it is concluded that the low dose of AMLE was less effective than the high dose of AMLE and PRO.

Keywords: *Aegle marmoles*; *Antioxidants; Cardioprotection; Isoproterenol*.

INTRODUCTION

Myocardial infarction is the principle cause of death in developed and developing countries that involves irreversible necrosis of heart tissue[1]. During the last decade, a dramatic increase in the use of herbs and herbal remedies has been witnessed in all around the globe, in particular in Europe and North America. Such products had been used with apparent safety in traditional societies for many centuries[2,3].

Aegle marmelos (Linn.) Correa, commonly known as bael, belongs to the family rutaceae, and is a cosmopolitan plant[4] distributed throughout tropical asia and africa[5]. The principle chemical constituents of leaves extract are tannins, skimmianin, essential oil (mainly caryophyllene, cineole, citral, citronellal, d-limonene and eugenol), sterols and triterpenoids, including lupeol, sitosterol, and amyrin, flavanoids and coumarins including aegeline, marmesin and umbelliferone[6]. The Bael leaves are bitter and used as a remedy for opthalmia, peptic ulcers, dropsy, cholera and beriberi associated with the weakness of heart[7]. A decoction of plant leaves and fruit is used in upper respiratory tract infections and heart ailments[8]. Leaves are also reported for hypoglycaemic effect[9], antioxidant, and anticarcinogenic properties[10], can cause restoration of blood glucose and bodyweight to normal levels[7]. Till now there is no scientific evidence of cardioprotective activity of *Aegle marmelos* compare with any standard synthetic drug. Therefore the present study was designed to explore the myocardial potency of *Aegle marmelos* leaf extract against myocardial damage induced by Isoproterenol (ISO) in rats.

EXPERIMENTAL

Chemicals

Isoproterenol was purchased from Sigma-aldrich, U.S.A. LDH & CKMB Kits for enzyme estimation were purchased from Crest Biosystems, Coral clinical systems, Goa, India. Other chemicals used were obtained from SD fine chemicals Ltd. (Mumbai, India). All chemicals used in the present study were of analytical grade.

Experimental animals

Laboratory bred female Sprague-Dawley (SD) rats weighing 175-250 g were housed at 25° ± 5°C in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Plant material

The shade dried leaves of *Aegle marmelos* were collected from the surroundings of Bangalore (India)

***Correspondence :** basheer_1@rediffmail.com/sasdaq@gmail.com

and Regional Research Institute (Ay), Bangalore authenticated the leaves. The leaves were mechanically grinded and detoxified with the solvent n-butanol for five days with the daily change of the solvent[11]. The detoxified leaves were subjected to exhaustive extraction in a soxhlet apparatus-using ethanol. The extract was concentrated on water bath and stored in a desiccator until further use.

Phytochemical estimations of the extract[12,13]

Hydroaclcoholic extract of Aegle marmelos leaves (AMLE) was subjected to Qualitative analysis to investigate the presence of various phytochemical constituents like alkaloids, carbohydrates, glycosides, phytosterols, proteins, saponins, tannins and flavonoids

Acute toxicity study

The acute oral toxicity study was performed according to the OPPTS guidelines (Office of Prevention, Pesticide and Toxic Substance) following the limit test procedure[14] .The animals were fasted over night prior to the experiment. Test dose of 2 g/kg and 5 g/kg were given orally to mice. Both doses were found to be safe. Hence 1/10th and 1/50th of the maximum safe dose corresponding to 500 and 100 mg/kg orally were selected as high and low doses respectively.

Experimental protocol

The animals were divided into five groups of eight each. Group I and Group II received saline for three weeks and termed as normal control and ISO control respectively; Group III was treated with standard Propranolol (PRO) 10 mg/kg, *p.o* for one week after two week of saline treatment; Group IV and Group V were administered AMLE 100 and 500 mg/kg orally respectively for three weeks.

Isoproterenol (ISO) induced myocardial necrosis in rats[15]

After the treatment of animals for three weeks from group II to V according to the protocol, Isoproterenol (ISO) 150 mg/kg, *s.c* was administered for two consecutive days. Forty-eight hour after the first dose of ISO administration the rats were sacrificed. Blood samples were collected by the retro orbital puncture method. Serum was separated and biochemical markers LDH and CKMB were estimated. Heart tissue homogenate (HTH) was prepared in sucrose solution (0.25 M) and used for estimation of endogenous marker enzymes and biological antioxidants viz., superoxide dismutase (SOD) [16] catalase[17] and thiobarbituric acid reactive species (TBRS)[18,19] activities.

Statistical analysis

Results are expressed as mean ± SEM. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. P<0.05 was considered significant.

RESULTS

Preliminary phytochemical investigation

The preliminary phytochemical investigation of the AMLE extract showed the presence of alkaloids, carbohydrates, flavonoids, cardiac glycosides, proteins, saponnins, tannins and terpenoids. The percentage yield of AMLE was found to be 17%.

Effect on LDH activity

The LDH activity of AMLE-100 and AMLE-500 were compared with normal and ISO control. A significant (p<0.01) increase in the LDH activity in serum and a very significant decrease in LDH activity in heart tissue homogenate was noticed in ISO and AMLE-100 treated groups as compared to normal control. The LDH activities in heart tissue homogenate were significantly (p<0.001) elevated in PRO and AMLE 500 mg/kg compared to ISO control (Table 1).

Table 1 : *Effects on LDH and CKMB level in serum and heart tissue homogenate against Isoproterenol induced myocardial infarction.*

Groups	CKMB ACTIVITY		LDH ACTIVITY	
	Serum (unit/ml)	HTH (unit/gm)	Serum (unit/ml)	HTH (unit/gm)
Normal Control	11±1.4	210±20.7	324±16.8	16.2±2.19
ISO control	92± 2.2	34±10.3	643±3.14	3.40±0.72
PRO	19±5.0	86±1.67	386±40.5	7.26±0.66
AMLE-100	42±6.6	66±13.6	585±86.6	3.40±0.20
AMLE-500	19±2.2	144±1.88	522±24.4	7.36±0.16

*All values are mean ± SEM, n=8; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; ΔP<0.05, ΔΔP<0.01, ΔΔΔ P< 0.001 when compared to ISO(Isoproterenol) control; PRO(Propranolol 10mg/kg); AMLE-100(Aegle marmoles leaves extract 100 mg/kg); AMLE-500(Aegle marmoles leaves extract 500 mg/kg).*

Effect on CK-MB activity

As shown in Table 1, two consecutive doses of ISO resulted in extremely significant elevation in CK-MB activities in serum and fall in heart tissue homogenate. Comparing the treatment groups viz. AMLE-100/AMLE-500/PRO with the ISO group, an extremely significant decrease (p<0.001) in CK-MB activity in serum was observed. In heart tissue homogenate, there was an extremely significant (p<0.001) increase in CK-MB activity were seen in PRO, AMLE-100 and AMLE-500 groups compared to ISO control. An extremely significant (p<0.001) increase in the level of CK-MB in IU/L, in the homogenate in AMLE-500 group compared to ISO group was noticed.

Effect on SOD and Catalase

The SOD and Catalase activity were estimated in the heart tissue homogenate. Subcutaneous administration of ISO caused significant fall in SOD and CAT activities in heart tissue homogenate compared to normal control (Table 2). Prophylactic administration of PRO, AMLE-100 and AMLE-500 resulted in significant (p<0.001) rise in SOD and CAT activities as compared to ISO control.

Table 2 : *Effects on SOD, Catalase and TBARS in Heart tissue homogenate against Isoproterenol induced myocardial infarction*

Groups	HEART TISSUE HOMOGENATE		
	SOD (unit/mg protein)	CATALASE (unit/mg protein)	TBARS (unit/mg protein)
Normal Control	14.4±0.15	6.59±0.38	24.1±2.2
ISO control	4.10±0.07***	0.44±0.05***	72.5±5.0***
PRO	6.90±0.26***ΔΔΔ	4.74±0.28***ΔΔΔ	46.7±2.2***ΔΔΔ
AMLE-100	5.08±0.16***ΔΔΔ	3.27±0.06***ΔΔΔ	57.7±1.4***Δ
AMLE-500	7.10±0.41***ΔΔΔ	6.66±0.02ΔΔΔ	29.8±1.5***ΔΔΔ

*All values are mean ± SEM, n=8; *P<0.05, **P<0.01, ***P< 0.001 when compared to Normal control; ΔP<0.05, ΔΔP<0.01, ΔΔΔ P< 0.001 when compared to ISO(Isoproterenol) control; PRO(Propranolol 10mg/kg); AMLE-100 (Aegle marmoles leaves extract 100mg/kg); AMLE-500(Aegle marmoles leaves extract 500mg/kg).*

Effect on TBARS

TBARS levels increased significantly ($p<0.001$) upon ISO administration and remained high in PRO and AMLE-100 pretreated groups compared to normal control (Table 2). In AMLE-500 group an extremely significant decrease ($p<0.001$) was observed as compared to ISO group.

DISCUSSION

Isoproterenol, a synthetic catecholamine and â-adrenoceptor agonist, is documented to produce myocardial infarction in large doses[20]. Several mechanisms including relative hypoxia, coronary microcirculatory disturbances and catecholamine oxidation products have been proposed, however intracellular calcium load is now the most accepted cause of catecholamine cardio toxicity [21]. Subcutaneous administration of isoproterenol leads to an increase in calcium uptake and energy consumption leading to cell death[22]. The increase in the level of marker enzymes in serum is due to the leakage of enzymes from the heart as a result of isoproterenol-induced necrosis[23]. Isoproterenol also increases the production of cytotoxic free radicals through its auto-oxidation. It has been suggested that the oxidative products of catecholamines produce changes in the myocardium by stimulating lipid peroxidation and cause irreversible damage to the myocardial membrane. This alters membrane permeability, leading to the loss of function and integrity of myocardial membranes[24].

As discussed above ISO induces free radical formation (TBARS) and reduction in antioxidant activities such as superoxide dismutase (SOD) and Catalase and also reduction in marker enzyme such as LDH and CKMB in HTH. Pretreatment of animals with low dose of AMLE (100mg/kg, *p.o.*) produces no significant changes in antioxidant activities compared to ISO group. High dose of AMLE (500mg/kg, *p.o.*) causes significant level of elevation in SOD activities with simultaneous increase in CAT activity, increased SOD activity may lead to intracellular accumulation of H_2O_2 with detrimental effects[25]. Hence simultaneous rise in SOD and CAT scavenge free radicals more effectively.

It has been known that biochemical markers are tissue specific and leak from the damaged tissue. Damage to the membrane induced by the ISO causes release of enzymes in the serum and deficiency of enzymes in HTH reflects the damage to the myocardium. Low and high doses of AMLE (100 & 500 mg/kg, *p.o.*) decreases serum CKMB and the high dose causes only increase in CKMB activities in HTH. Similarly low dose of AMLE was not able to raise LDH activity in HTH confirming its mild nature of efficacy. From our study it may be concluded that the high dose of AMLE (500mg/kg) possess good cardioprotective activity against Isoproterenol (ISO) induced myocardial necrosis in rats but the low dose of AMLE (100mg/kg) failed to show significant cardioprotection.

ACKNOWLEDGEMENT

Authors would like to extend their thanks to Prof. Dr. Suresh Nagpal Chairman and Prof. Dr. Sunil Dhamanigi, Secretary and Prof. Dr. Amit kumar Das, Principal, Krupanidhi institutions for providing essential requirement to complete this research study.

REFERENCES

1. Yasin B, *et al.* The Eurasian Journal of Medicine. 2009; 41: 45.
2. Singh YN. J Ethnopharmacol. 2005; 100: 108.
3. Fugh-Berman A. Lancet. 2000; 355: 134.
4. Jagetia GC, *et al.* Mutagenesis. 2003; 18: 387.
5. Singh R, *et al.* International Journal of Green Pharmacy. 2008; 232-234.
6. Arul V, *et al.* J Ethnopharmacol. 2005; 96: 159.
7. Seema PV, *et al.* Indian J Exp Biol. 1996; 34: 600.
8. Vijaya C, *et al.* Indian J Exp Biol. 2009; 47: 182.
9. Karunanayake EH, *et al.* J Ethnopharmacol. 1984; 11: 223.
10. Singh RP, *et al.* J Pharm Pharmacol. 2000; 52: 991.
11. Kokate CK. In *Practical Pharmacognosy*. 3rd ed. New Delh : VPBN 1991, p107.
12. Finar IL. In *Organic Chemistry*. 4th ed. ELBS: 1993, p518.
13. Mukherjee PK. In *Quality Control of Herbal Drugs* (An approach to evaluation of botanicals). 1st ed. New Delhi: Business Horizons Pharmaceutical Publishers 2002, p246.
14. http://www.epa.gov/opptsfrs, accessed on 12/9/2008.

15. Asdaq SM, *et al.* J Pharmacol Toxicol. 2008; 3(6): 414.

16. Erich F, *et al.* Anal Chem. 1976; 70: 616.

17. Eva ML. Arch Biochem Biophy. 1988; 365: 362.

18. Walter MF, *et al.* J Am Coll Cardiol. 2004; 44: 1996.

19. Sedlakova E, *et al.* Central Eur J of Pharmacol. 2009; 4: 26.

20. Sun DQ, *et al.* Circulation. 1994; 89: 793.

21. Wang S, *et al.* Eur J Pharmacol. 2009; 615:125-132.

22. Takasaku N, *et al.* Basic Res Cardiol. 1988; 83: 567.

23. Sasikumar SC, *et al.* Indian J Pharmacol. 2000; 32: 198.

24. Kumar SH, *et al.* Fitoterapia. 2001; 72: 402. Das DK, *et al.* J Mol Cell Cardiol. 1995; 27: 181-93.

25. Das DK, *et al.* J Mol Cell Cardiol. 1995; 27:181-93.