ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD

Volume - 01 Issue - 06 NOV-DEC 2013

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

EFFECT OF *AEGLE MARMELOS* ON HISTOPATHOLOGICAL AND BIOCHEMICAL CHANGES IN ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RAT

J.Sathish[1], M.Thirumagal[1], M.Ayesha Sabeen[2], M.Syed Muzammil[3*].

[1]PG & Research Department of Biochemistry, K.M.G. College of Arts & Science, Gudiyattam **Tamilnadu-India.**
[2]PG & Research Department of Biochemistry, Marudhar Kesari Jain College for Women Vaniyambadi, **Tamilnadu-India.**
[3]PG & Research Department of Biochemistry, Islamiah College (Autonomous) Vaniyambadi **Tamilnadu-India.**

Received: 27 December. 2013 ***Revised and Accepted: 03Jan 2014***

ABSTRACT

From the very beginning of human existence, man has familiarized himself with medicinal plants and used them in a variety of ways throughout the ages. In view of the above considerations the present study was designed to investigate the effect of Aegle marmelos aqueous leaves extract on isoproterenol induced myocardial infarction and its phytochemical screening. In search of food and to cope successfully with human suffering, primitive man began to distinguish those plants suitable for nutritional purpose from others with definitive pharmacological action. The present investigation is part of continuing programme related to the phytochemical screening of Aegle marmelos (herbal extracts) and on Myocardial Infarction and their enhanced potential.

Keywords: *Isoproterenol, Aegle marmelos, Myocardial infarction, Lipid peroxidation.*

INTRODUCTION

Myocardial infarction is the acute condition of myocardial necrosis that occurs as a result of imbalance between coronary blood supply and myocardial demand. Patient experiences significant disability or die [1]. Clinical studies have shown that there is increased generation of reactive oxygen species such as superoxide anion ($\cdot O2^-$) and hydroxyl radicals ($\cdot OH$) in heart failure, which involved in formation of lipid peroxides, destruction of antioxidative defense system and damage of cell membranes.

*For Correspondence:
M.Syed Muzammil
Assistant Professor & Research Supervisor
PG & Research Department of Biochemistry
Islamiah College (Autonomous)
Vaniyambadi – 635 752,
Vellore District, Tamilnadu, INDIA.
Mail id: syed_bio2004@yahoo.co.in

Therapeutic intervention via suppression of free radical generation and enhancement of endogenous antioxidant enzymes may limit the infarct size and attenuate myocardial dysfunction [2]. Isoproterenol, a β-adrenergic agonist, is a well-known inducer of myocardial hypertrophy [3] and its supramaximal dosages produce acute myocardial necrosis and interstitial fibrosis [4]. The present study was designed to evaluate the cardioprotective activity of *Aegle marmelos* on isoproterenol induced myocardial damage in rats.

Aegle marmelos is naturally distributed in India, Myanmar and Sri Lanka and widely cultivated in Southeast Asia and Tropical Africa, commonly known as Bael, belonging to the family Rutaceae, is a sacred tree for Hindu Religon, is used in different system of medicine, especially Ayurveda, Unani and Homeopathy. In English it is known as Bengal

quince, stone apple and golden apple. It is known as *bael* and *bilwa* in Sanskrit and by different names such as *maredu* (Andhra Pradesh) *bael* (Bengal), *bil* (Gujarat), *bael, bil* (Himachal Pradesh), *bilpatra, kumbala, malura* (Karnataka), *kuvalam, vilwam* (kerala), *kuvalum* (Tamilnadu).

Every part of the plant has medicinal properties. The root is an important ingredient of the '*Dasmula*' (ten roots) recipe. It is a medium sized deciduous thorny tree with its roots, bark, leaves and fruits of high medicinal value and is cited as one of the red-listed medicinal species of South India, due to its overexploitation in Ayurvedic medicines. The decoction of the root and root bark is useful in intermittent fever, hypo-chondriasis, and palpitation of the heart.

The leaves and bark have been used in medicated enema. The leaves are also used in diabetes mellitus. The greatest medicinal value, however, has been attributed to its fruit and the unripe fruit is said to be an excellent remedy for diarrhoea and is especially useful in chronic diarrhoea. The constituents of Aegle are used in heart diseases, inflammatory and wound healing. Leaves of A. *marmelos* have been reported as hypoglycemic effect. The essential oil from the leaves of A. *marmelos* is known to exhibited antifungal properties.

MATERIALS AND METHODS

CHEMICALS

All the fine chemicals and media were purchased from Sigma Chemical Co., USA. All other chemicals used were of Good Quality and Analytical Grade.

PLANT COLLECTION AND EXTRACTION

Aegle marmelos leaves extract got as a gift from the Siddha Maruthuva Salai, Vellore, Tamilnadu. All the plants were identified taxonomically by our Botany Professor.

200gms of coarsely powdered shade dried *Aegle marmelos* leaves were mixed with 1200ml of water, boiled and reduced to one third volume. The filtrate obtained in paste form was subjected to pre-clinical screening.

PHYTOCHEMICAL ANALYSIS

Qualitative analysis of phytonutrients was done for the aqueous extract.

Test for carbohydrates

A small quantity of extract was dissolved separately in 5 ml of distilled water and filtered. The filtrate was tested to detect the presence of carbohydrates.

Molisch's test:

i) To 2ml of extract, 2 ml of Molisch's reagent was added. Then, 2 ml of concentrated sulphuric acid was added along the sides of the test tubes. Disappearance in color on the addition of excess solution indicated the presence of carbohydrates.

Benedict's test:

i) To 0.5 ml of extract, 5 ml of Benedict's reagent was added. The mixture is then boiled for 5 minutes. Presence of a bluish green precipitate indicated the presence of carbohydrates.

Test for Glycosides:

i) To 2ml of extract 1ml of aqueous NaOH solution was added. The appearance of a yellow color indicated the presence of glycosides.

Test for Proteins and Amino acids

Ninhydrin test:

i) A small quantity extract solution was boiled with 0.2% solution of Ninhydrin. Purple color indicated the presence of free amino acids

Test for Phytosterols and Triterpenoids

Salkowski test:

i) To 2 ml of the extract, 1 ml of concentrated Sulphuric acid added. Chloroform was added along the sides of the test tube. A red color produced in the chloroform layer indicated the presence of Phytosterols or if it is yellow in

color at the lower layer indicated the presence of triterpenoids.

Zinc hydrochloride reduction test:

i) The extract was treated with mixture of zinc dust and concentrated hydrochloric acid. Red color indicated the presence of flavanoids.

Test for Alkaloids

i) A small portion of the solvent free extract was stirred separately with a few drops of dilute hydrochloric acid and filtered. The filtrate was tested with Mayer's reagent (Potassium mercuric iodide solution). The cream precipitate indicates the presence of alkaloids.

ii) Dried powder of herb treated with 5% Ammonical Ethanol and the test carried out after 48 hours, the fraction was treated with Mayer's, Wagner's and Dragndroff's reagent.

Test for Tannins

Gelatin test:

i) To 5ml of extract, few drops of 1 % lead acetate were added. Absence of a yellow or red precipitate indicated the absence of tannins.

ii) 5gm of extract in 50ml water and boiled for 45 min. in waterbath and 2% gelatin solution is added dropwise.

Test for Saponins:

i) To 5 ml of the extract, a drop of sodium bicarbonate was added. It is shaken vigorously and kept undisturbed for 3 minutes. Appearance of a honey comb like froth indicated the presence of saponins.

Proanthocyanidin:

i) 2ml of extract add 5ml of 2N HCL and kept in water bath for 30 minutes. Then the mixture was cooled and shaken with amyl alcohol.

Iridoids:

i) 1ml of extract add 5ml of aqueous HCl and kept for 3-6 hours, 0.1 ml of extract is decanted and macrate and treated with trim Hill reagent.

Flavonoids:

i) 1ml of extract add 10ml of 95% ethanol and kept in boiling waterbath for 15 minutes and after filtration mg ribbon were added along with 2-3 drops of HCl.

Steroids:

i) 1ml of extract was extracted with methanol for 15minutes and then Libermann Burchard reagent was added drop wise.

ANIMALS

Male albino rats weighing 150-200 g were used in the present study. All rats were kept at room temperature of 20°C and were kept on standard rat chow with free access to water *ad libitum*, in rooms with controlled temperature and humidity, under a 12-hr light-dark cycle, 8 rats, included for the study, were divided into 4 groups, each consisting of two animals. All procedures with animals were conducted strictly in accordance with guidelines approved by the Institutional Animal Ethics Committee.

SEGGREGATION OF EXPERIMENTAL GROUPS

Group 1: Control given only saline (2ml/kg for 14 days)
Group 2: Isoproterenol induced (treated) (85mg/kg of ISO subcutaneous 24 hrs for 2 days, on 13^{th} & 14^{th} day)
Group 3: Rats treated with *Aegle marmelos* extract (300 µg/day, 2 weeks)
Group 4: Rats treated with *Aegle marmelos* and Isoproterenol
(300 µg/day, 12 days & 85mg/kg of ISO 13^{th}-14^{th} day).

INDUCTION OF EXPERIMENTAL MYOCARDIAL INFARCTION

Isoproterenol of 85 mg/kg was dissolved in normal saline and injected subcutaneously to rats at an interval of 24 h for 2 days to induce Myocardial infarction [5].

EXPERIMENTAL DESIGN

8 rats, included for the study, were divided into 4 groups, each consisting of two animals.

Group 1: control rats, Group 2: Isoproterenol treated, Group 3: *Aegle marmelos* was administrated subcutaneously at a dosage of 300 µg/day per rat for 2-weeks, Group 4: rats were injected by subcutaneous 300 µg/day/rat of *Aegle marmelos* for 12 days and then 85 mg/kg of Isoproterenol for two days for our study. All the rats were anesthetized with sodium pentobarbital (35 mg/kg, intraperitoneal) and sacrificed by cervical decapitation. Serum and plasma samples were separated for enzymes assays. The excised heart tissues were rinsed in ice-cold physiological saline and homogenized in 100 mM Tris-HCl buffer (pH 7.4) to give a 10% homogenate. Aliquots of the tissue homogenate is processed for antioxidant studies, biochemical assays and lipid peroxidation. Aliquots of heart tissues were set aside for histopathological processing.

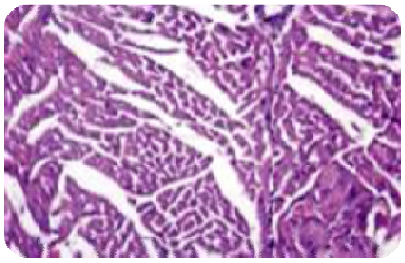
Experimental rats were anesthetized with sodium pentabarbitol and sacrificed by survical decapitation. Serum, plasma and heart tissues were separated for enzymes assays. Creatine kinase (CK), Lactate dehydrogenase (LDH) aminotransferases (AST & ALT) activities were increased in serum and decreased in heart tissue of Isoproterenol group and are normalized by *Aegle marmelos* pretreated rats. Glutathione peroxidase (GPx), Superoxide dismutase (SOD), catalase (CAT) and reduced glutathione (GSH) activity are abnormal in the Isoproterenol induced rats, and this was minimized by *Aegle marmelos* pretreatment. Increased level of thiobarbituric acid reactive substances (TBARS) in plasma and the heart of Isoproterenol treated rats, pre subcutaneous injected with *Aegle marmelos* to Isoproterenol induced rats decreased the levels of TBARS. Restoration of cellular normalcy accredits *Aegle marmelos* has protective role against Isoproterenol induced cardiotoxicity.

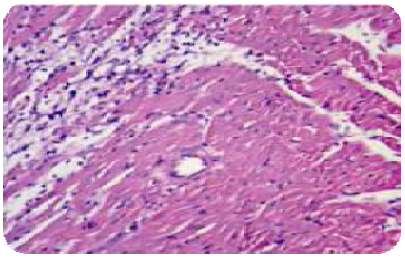
ASSESSMENT OF OXIDATIVE STRESS IN TISSUES

Creatine kinase was assayed in the serum and heart tissues [6], LDH [7], AST and ALT were determined and expressed in terms of µmoles of pyruvate liberated/min/mg of protein at 37ºC [7]. Protein estimations [8]. Lipid peroxidation in the heart tissues [9], and malondialdehyde (MDA) produced during peroxidation of lipids, served as an index of lipid peroxidation, MDA reacts with thiobarbituric acid to generate a coloured product which absorbs at 532 nm. The peroxidation system contained 10 mM ferrous sulfate and 0.2 mM ascorbate as inducers [10]. The degree of inhibition of the autoxidation of pyrogallol at an alkaline pH by superoxide dismutase (SOD) was used as a measure of the total enzyme activity [11]. Glutathione peroxidase (GPx) activity was assessed in terms of utilization of glutathione [12] and based on the reaction between glutathione (present in the reaction mixture), remaining after the action of GPx (present in the tissue homogenate aliquot), and 5,5' –dithio-bis(2-nitrobenzoic acid) resulting in a complex that absorbs maximally at 412 nm. Total reduced glutathione was estimated in the cardiac tissues [14], Catalase activity [13], the enzymes in tissue homogenate aliquot was allowed to split hydrogen peroxide (H_2O_2) for different time periods. The reaction was stopped at specific time intervals by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 was determined by colorimetric measurement of the resulting chromic acetate after heating the reaction mixture.

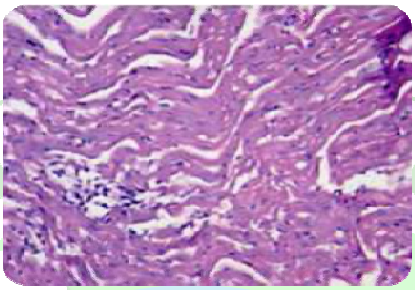
HISTOLOGICAL STUDIES

10% formalin was used as a fixative for heart tissues and were washed, dehydrated in the descending grades of isopropanol and cleared in xylene solution. Tissues were embedded in molten paraffin wax of 5 µm thickness were sectioned, cut, stained with haematoxylin and eosin and were viewed in light microscope (320-X) for histopathological changes.





(1) (2)



(3)

Figure 1.

Histology of Rat Heart

Group 1: Normal control
Group 2: Isoproterenol induced (Fragmentation of myocardial fibers and greater focal interstitial inflammatory response)
Group 3: *Aegle marmelos* treated
Group 4: *Aegle marmelos* and isoproterenol treatment (Reduced focal interstitial inflammatory response)

STATISTICAL ANALYSIS

Statistical analysis was performed using one-way analysis of variance (ANOVA) using SPSS Software 9.05 followed by Duncun's multiple range test (DMRT). Results were expressed as mean ± S.D. from 2 rats in each group and triplicate estimates in each rat. *P* values <0.05 were considered as significant.

Table 1. Alterations in serum and tissue enzyme activities in the early phase Isoproterenol induced cardiotoxicity and effect of *Aegle marmelos* treatment.

Enzyme Assays (U/mg Protein)	Group 1 (Control)	Group 2 (Isoproterenol)	Group 3 (*Aegle marmelos*)	Group 4 (*Aeglemarmelos* + Isoproterenol)
Serum				
CK	250.32±0.17	385.45±0.28^{a***}	241.20±0.02	253.06±0.02^{b***}
LDH	156.30±0.17	253.01±0.02^{a***}	150.46±0.02	170.10±0.02^{b***}
AST	77.06±0.17	105.22±0.33^{a***}	75.07±0.17	83.08±0.01^{b***}
ALT	64.07±0.33	85.01±0.02^{a***}	63.30±0.02	69.38±0.03^{b***}
Heart				
CK	66.04±0.33	60.0.2±0.17^{a***}	68.35±0.17	64.04±0.12^{b***}
LDH	135.0.3±0.17	100.77±0.02^{a***}	144.17±0.02	138.20±0.17^{b***}
AST	84.52±0.17	75.03±0.17^{a***}	88.30±0.17	79.08±0.02^{b***}
ALT	91.02±0.17	84.10±0.02^{a***}	93.04±0.17	78.18±0.17^{b***}

Enzyme units: CK: µmoles x 10^{-3} of phosphorus liberated/min; LDH: µmoles x 10^{-1} of pyruvate liberated/min; AST, ALT: µmoles x 10^{-2} of pyruvate liberated/min. *** *P* <0.05, (DMRT)
(values are expressed as mean ± S.D. for 2 animals in each group)

Table 2. Estimation of Antioxidant status in the Isoproterenol induced and *Aegle marmelos* treated groups compared with the controls.

Antioxidant Activity	Group 1	Group 2	Group 3	Group 4
Enzymatic Antioxidants				
SOD	8.05±0.17	8.43±0.23 ^{a***}	10.32±0.47	5.30±0.03^{b***}
CAT	21.04±0.02	14.06±0.14 ^{a***}	22.52±0.63	21.12±0.30^{b***}
GPx	6.12±0.17	6.00±0.25 ^{a***}	5.25±0.15	4.29±0.03^{b***}
Non-Enzymatic Antioxidant (Plasma) GSH	5.29±0.63	09.59±0.30 ^{a***}	5.48±0.43	14.21±0.47^{b***}
Non-Enzymatic Antioxidant (Heart) GSH	09.16±0.03	7.45±0.43 ^{a***}	12.32±0.13	10.56±0.03 ^{b***}

Enzyme activities are expressed as follows SOD: U/mg protein (1 U = amount of enzyme that inhibits the autoxidation reaction by 50%), Catalase: µmol of H_2O_2 consumed/min/mg protein.
Non-enzymatic antioxidant is expressed as: GSH: µg/mg protein. GPx: µg of reduced glutathione utilized/min/mg protein. *** P <0.05, (DMRT). (values are expressed as mean ± S.D. for 2 animals in each group)

Table 3. Effect of *Aegle marmelos* on the levels of TBARS in plasma and the heart in normal isoproterenol induced myocardial infarction (MI) in rats.

GROUPS	Heart TBARS (mM/100g wet tissue)	Plasma TBARS (nM/ml)
Control	4.60±0.30	4.55±0.03
Group 2	12.23±0.47^{a}	5.73±0.30^{a}
Group 3	4.05±0.47	3.59±0.30
Group 4	6.22±0.02^{b}	4.31±0.11^{b}

Mean ± S.D. for 2 rats in each group. Values not sharing a common letter differ significantly at P <0.05 (DMRT).

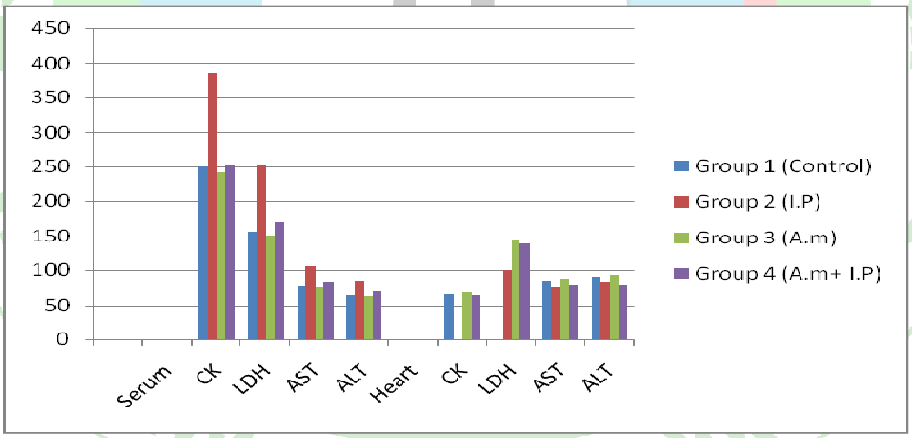


Fig: 1. Isoproterenol induced cardiotoxicity and changes in serum and tissue enzymes

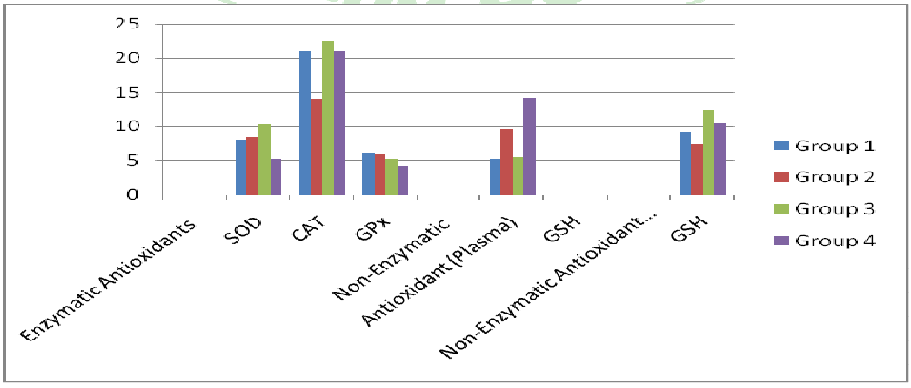


Fig: 2. Antixoxidant ststus in isoproterenol induced and *Aegle marmelos* treated

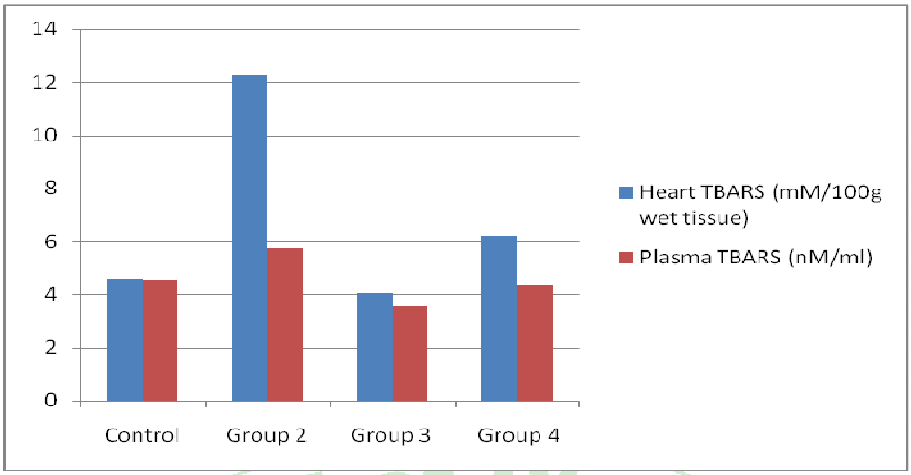


Fig: 3. *Aegle marmelos* levels in TBARS levels in Heart and Plasma levels.

RESULTS

In isoproterenol induced myocardial infarction rats there was a significant ($p<0.05$) increase in Isoproterenol group with respect to control (P <0.05). In isoproterenol group markers enzymes were determined both in serum and tissues (Table.1). *Aegle marmelos* pretreatment of Isoproterenol induced rats resulted in near normal activities of these enzymes. The dosage of *Aegle marmelos* in our study was non toxic to heart, the activities of enzymes in the *Aegle marmelos* control (Group 3) did not reveal any significant changes when compared with the normal control group. Groups I and III represents normal cardiac histology. Cardiac muscle fibre destruction and hypertrophy. In the photomicrograph, the swollen and flabby muscle fibres of the Isoproterenol induced group (Group 2) can be clearly observed. *Aegle marmelos* plus Isoproterenol treatment (Group 4), wherein the changes are less marked and an almost normal cardiac muscle fibre picture is presented.

Isoproterenol induced group, exhibited a significant (P <0.05) decrease in the activity of antioxidant enzymes in the heart tissue when compared with control rats. The activities of SOD, catalase and GPx in heart of normal and experimental rats are depicted in Table 2. Pretreatment with *Aegle marmelos* (300µg/day) to Isoproterenol induced group significantly (P <0.05) and increased in antioxidant enzymes compared to isoproterenol induced group. Table 2 shows the assessment of non-enzymatic antioxidant status of GSH in the Isoproterenol induced and *Aegle marmelos* in plasma and heart rats. Isoproterenol induced group, showed a significant (P <0.05) decrease in antioxidant enzyme and the levels of GSH on comparison with control (normal) rats. Subcutaneous injection of *Aegle marmelos* (300 µg/day) to Isoproterenol induced group significantly (P <0.05) increased the activities antioxidant enzymes and GSH level compared with Isoproterenol induced alone. Isoproterenol induced group showed a significant (P <0.05) increase in TBARS levels in plasma and heart compared to normal. Table 3 shows the levels of TBARS in plasma and heart of normal and induced. Pre subcutaneous injected (group 4) with *Aegle marmelos* (300 µg/day) to Isoproterenol induced group for 2-weeks significantly (P <0.05) decreased the TBARS in plasma and heart tissue when compared to Isoproterenol alone induced group.

DISCUSSION

Our study reveals the significant elevation in the levels of enzymes (LDH, CK, ALT & AST) decrease level in serum and heart of Isoproterenol induced groups. This may be due to necrotic damage to myocardial membrane. When myocardial cells are damaged or destroyed due to the deficiency of oxygen supply the cell membrane becomes permeable or rupture and results in leakage of marker enzymes. Cellular enzymes discharge reflects alterations in integrity of plasma membrane or permeability as a response to β-adrenergic

stimulation. Pretreatment with *Aegle marmelos* to Isoproterenol induced group, enzymes increase in heart and decreased levels of enzymes in serum. Hence forth its due to reduction in damage of the myocardium by *Aegle marmelos*, reduces the release of enzymes from myocardium. Lipid peroxidation, oxidative degeneration a type of damage of polyunsaturated fatty acids has been linked with altered membrane structure, enzyme inactivation and act as an indication of severity of Isoproterenol induced damage to heart. Activated lipid peroxidation a pathogenic event in myocardial infarction, with increased levels of TBARS reflecting the major stages and complication of the disease. Increase in the levels of serum and heart TBARS in Isoproterenol induced group showed excessive formation of activation of lipid peroxidation and free radicals. Pretreatment with *Aegle marmelos* to Isoproterenol induced rats decreased levels of TBARS in heart and serum. Decrease in CAT, SOD, GPx and the levels of GSH in heart Isoproterenol induced groups.

During myocardial infarction, SOD and Catalase are functionally and structurally impaired by free radicals resulting in myocardial damage and is due to involvement of superoxide and hydrogen peroxide free radicals in myocardial cell damage mediated by isoproterenol. Reduction in GSH activity in Isoproterenol induced group also reduced activity of GPx upon administration of Isoproterenol. Inactivation of GPx in the heart leads to accumulation of oxidized glutathione which in turn inactivates many enzymes containing the SH group and inhibits protein synthesis. *Aegle marmelos* pretreatment to the Isoproterenol induced groups prevented the buildup of oxidative stress. This in turn prevents the depletion of antioxidant molecules, namely SOD, catalase, GPx and GSH. GAG exerts anti-free radical effects and restores the antioxidant balance to normalcy. The rise in the activities of the primary enzymatic antioxidant defenses SOD, catalase GPx and GSH in the *Aegle marmelos* treated Isoproterenol induced group highlight the protection rendered by the heparin derivative in combating the oxidative insult.

Our study reports that small amounts of *Aegle marmelos* enhanced the antioxidant activity of SOD and contribute to inhibition of free radical mediated tissue injury. Hence we prove from our study that the protective effect of *Aegle marmelos* against damage of cardiac by Isoproterenol, on the basis of biochemical assessment and oxidative stress management confirmed by histopathological examination. Following treatment with *Aegle marmelos* usefulness in pharmacology can be tested by *in vitro* also in near future.

CONCLUSION

Our findings might be rational to understand the beneficial effects of *Aegle marmelos* extract on cardioprotection against myocardial injury. *Aegle marmelos* also possess cardioprotective potentials, which could be used to treat different cardiac problems. The recovery of the heart and restoration of biochemical and histopathological alterations may be due to the presence of phytochemicals and bioactive compounds present in the *Aegle marmelos*. Further isolation, characterization and purification of the active constituents and further experimentation would be necessary to elucidate the exact mechanism of action of *Aegle marmelos*.

ACKNOWLEDGEMENTS

We are grateful to the faculty members of the colleges, PG & Research Department of Biochemistry, Islamiah College, K.M.G College, Gudiyattam and Marudhar Kesari Jain College for Women,Vaniyambadi, Principal, Secretary & Correspondent, Islamiah College, General Secretary and VMES for their encouragement and support in carrying out the work.

REFERENCES

1. *F.Alla, F.Zannad, G.Filippatos, Epidemiology of acute heart failure syndromes. Heart Fail Rev 2007; 12: 95.*
2. *T.Nakamura, H.Nishi, Y.Kokusenya, K.Hirota, Y.Miura. Mechanism of antioxidative activity of fluvastatin determination of the active position. Chem Pharm Bull 2000; 48:237.*
3. *M.O.Boluyt, X.Long, T.Eschenhagen, U.Mende,W.Schmitz, M.T.Crow, E.G.Lakatta. Isoproterenol infusion induces alterations in expression of hypertrophy associated genes in rat heart. Am J Physiol 1995; 269:H647.*

4. *I.J.Benjamin, J.E.Jalil, L.B.Tan, K.Cho, K.T.Weber, W.Clark. Isoproterenol induced myocardial fibrosis in relation to myocyte necrosis. Clrc res 1989; 65:670.*
5. *P.R.Deepa, P.Varalakshmi. Protective effect low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem Biol Intr 2003;146:210.*
6. *Okinaka S, Kumogai H, Ebashi S, Sugita H, Momoi H, Toyakura Y, Fujie Y. Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease. Arch Neurol 1961; 4: 525.*
7. *King J. Practical clinical enzymology, In: Van D (ed.), Nostrand Company Limited, London; 1965. 208.*
8. *Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with folinphenol reagent. J Biol Chem 1951; 193: 275.*
9. *Hogberg J, Larson RE, Kristoferson A, Orrenius A. Biochem Biophys Res Commun 1974; 56: 842.*
10. *Devasagayam TPA. Lipid peroxidation in rats uterus. Biochem Biophys Acta 1986; 876: 514.*
11. *Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyogalol and a convenient assay of superoxide dismutase. Eur J Biochem 1974; 47: 474.*
12. *Rotruck JT, Pope AL, Ganther HE. Selenium: Biochemical role as a component of glutathione peroxidase. Sci 1973; 179: 590.*
13. *Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972; 47:395.*
14. *Moron MS, Defierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione-S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979; 582: 78.*
15. *A.K.Sinha. Colorimetric assay of catalase. Anal Biochem 1972;47:395.*
16. *D.L.Dinwoody, J.E.Anselt. Heparins, low molecular weight heparins, and pentasaccharides. Clin Gerlatr Med 2006;22:15.*
17. *F.W.A. Verheugt, F.A.Liem, Felix Zijlstra, R.C.Marsh, F.G.Veen, J.G.F.Bronzwaer. High dose bolus heparin as initial therapy before primary angioplasty for acute myocardial infarction. Results of the heparin in early potency (HEAP) pilot study. JACC 1998; 31(2):293.*
18. *H.R.Raschke. Heparin and low molecular weight heparin-The seventh ACCP conference on antithrombotic and thrombocytic therapy. Chest 2004; 126:203s.*
19. *J.Hogberg, R.E.Larson, A.Kristoferson, A.Orrenius. Biochem Biophys Res Commun 1974; 56:842.*
20. *J.King. Practical clinical enzymology, In: D.Van (ed.), Nostrand Company Limited, London; 1965.208.*
21. *J.T.Rotruck, A.L.Pope, H.E.Ganther. Selenium : Biochemical role as a component of glutathione peroxidase. Sci 1973; 179:590.*
22. *L.De Biase, P.Pignatelli, G.Tocci, F.Piccioni, S.Riondino, F.M.Pulcinelli, S.Rubattu, M.Volpe, F.Violi. Enhanced TNF alpha and oxidative stress in patients with heart failure: effect of TNF alpha on platelet O2- production. Thromb Haemost 2003; 90:325.*
23. *M.Shanmugam, K.H.Moody. Heparinoid active sulfated polysaccharides from marine algae as potential anticoagulant agents. Curr Sci 2004; 79:1683.*
24. *M.S.Moron, J.W.Defierre, B.Mannervik. Levels of glutathionine –S-transferase activities in rat lung and liver. Biochem Biophys Acta 1979;582:78.*
25. *O.H.Lowry, N.J.Rosebrough, A.L.Farr, R.J.Randall. Protein measurement with folin-phenol reagent. J Biol Chem 1951; 193:275.*
26. *R.Saravanan, S.Sambasivam, A.Shanmugam, D.Sathish Kumar, T.Tamil vanan, R.A.Nazeer. Isolation, purification and biochemical characterization of conotoxin from Conus figulinus Linnaeus (1758), Ind J Biotechnol 2009;8:271.*
27. *R.Saravanan,S.Vairamani, A.Shanmugam. Glycosaminoglycans from marine clam Meretrix meretrix (Linne.) are an anticoagulant. Prep Biochem Biotechnol 2010;40:315.*
28. *R.Saravanan, A.Shanmugam. Preventive effect of low molecular weight glycoaminoglycan from Amusslum pleuronectus (Linne) on oxidative injury and cellular abnormalities in isoproterenol induced abnormalities in wistar rats. Appl Biochem Biotechnol 2010;162:51.*
29. *S.Okinaka, H.Kumogai, S.Ebashi, H.Sugita, H.Momoi, Y.Toyakura, Y.Fujie. Serum creatine phosphokinase activity in progressive muscular dystrophy and neuromuscular disease. Arch Neurol 1961; 4:525.*
30. *S.Marklund, G.Marklund. Involvement of the superoxide anion radical in the auto-oxidation of pyogalol and a convenient assay of superoxide dismutase. Eur J Biochem 1974;47:474.*
31. *T.P.A.Devasagayam. Lipid peroxidation in rats uterus. Biochem Biophys Acta 1986;876:514.*
32. *W.S.Arnonow, Epidemiology, pathophysiology, prognosis, and treatment of systolic and diastolic heart failure. Cardiol Rev 2006; 14: 124.*