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CARDIO PROTECTIVE EFFECT OF *AEGLE MARMELOS* ON ISOPROTERENOL INDUCED MYOCARDIAL INFARCTION IN RATS

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ABSTRACT

In the present study scientific evaluation was carried out by using petroleum ether, ethanol and aqueous extract of leaf, stem and root extract of *A. marmelos* to prove their cardioprotective potential. Among the various extracts of *A. marmelos* treated animals, the animal group treated with aqueous extract of leaf exhibited significant to more significant cardioprotective effect as compared with that of standard drug and the petroleum ether and ethanol extract of leaf, petroleum ether, ethanol and aqueous extract of stem and root of *A. marmelos* failed to exhibit cordioprotective effect against Isoproterenol induced Myocradial infarction. Study provides experimental evidence that aqueous extract of *A. marmelos* has significant cardioprotective activity. This finding may be rational to understand the beneficial effects of aqueous extract of *A. marmelos* in cardioprotection against Isoproterenol induced myocardial injury, in which oxidative stress was known to contribute to the pathogenesis.

Keywords: Petroleum Ether Extract, Ethanol Extract, Aqueous Extract and Myocardial Injury

INTRODUCTION

In spite of spectacular advances in synthetic drugs in recent years, some of the drugs of plant origin have still retained their importance. The use of plant based drugs of the western world is increasing and this is because of the belief that many herbal medicines are known to be free from side effects. Furthermore, the fact that the discovery of the new synthetic drug is time consuming and expensive affair. Despite the vast availability of medicinal plants, *Aegle marmelos* plant is selected in our study and the case for the collection of material and its unique importance in this area. Plants have always been an exemplary source of drugs and many of the currently available drugs have been derived directly or indirectly from them.

A. marmelos belongs to the family Rutaceae. Its common name is Bilwa; it has many Indian names, depending on the geographical region or the language. In English language it is called by different names: Bengal quince, golden apple, stone apple and *Elisabetta ghermandi* etc **[1]**. It is a medium sized tree (average height is 8.5 metres tall), with spines on the branches and very aromatic. It is useful in treating pain, fever, inflammation, respiratory disorders, cardiac disorders, dysentery and diarrhea **[2] [3]**. More than 30 identified compounds from the leaves of *Aegle marmelos* have been reported **[4] [5] [6]**. Fresh aqueous and alcoholic extracts of the leaves of *Aegle marmelos* have been reported to have a cardiotonic effect like digitalis and to decrease the requirement of circulatory stimulants **[7]**. Isoproterenol (ISO) induced myocardial necrosis is a well known standard model to study the beneficial effect of many drugs on cardiac dysfunction **[8]**. ISO induced myocardial injury involves membrane permeability alterations, which bring about the loss of functions and integrity of myocardial membranes **[9]**. ISO induced myocardial injury in rats has been shown to be accompanied by hyperglycemia, hyperlipidemia, increase in serum creatine phosphokinase, alanine aminotransferace, aspertate aminotransferase and lactate dehydrogenase activities **[10]**. It is now well recognized that isoproterenol [1-(-3, 4-dihydroxyphenyl-2-isopropyl amino ethanol) hydrochloride] a synthetic catecholamine in large doses produces myocardial infarction **[11]**.

In view of this, the present study was designed to investigate in detail on the cardioprotective effect of the plant extracts of *A. marmelos* by isoproterenol induced myocardial infarction in rats.

MATERIALS AND METHODS

Plant Material and Preparation of the Extract

The leaves stem bark, flowers and root of *A. marmelos* were collected in and around the Kuvempu University campus. The plant was authenticated by comparing with the herbarium voucher specimen deposited at Kuvempu University herbaria. The plant materials of both the species were shade dried, powdered mechanically (sieve No. 10/44), 200g of powdered material was soaked in 100ml of petroleum ether and ethanol separately for 48 h. Simultaneously 1 kg of the powdered leaf, stem bark and roots of *A. marmelos* were boiled in distilled water for 30 min, kept for 3 days with intermittent shaking and filtered to get the aqueous extract **[12]**. It was filtered by using Whatman no.1 filter paper. The solvent was distilled out completely from the filtrate under the reduction pressure in Rota vapour.

Phytochemical Screening

The preliminary phytochemical analysis of petroleum ether, ethanol and aqueous extracts was carried out using the methods as described in **[13] [14]**.

Animal Collection

Adult male Albino Wistar rats weighing 150 – 200g were acclimatized in a well ventilated animal house condition and were fed with commercial feed. There were no significant differences in the body weights of the treated rats when compared with control, either at the beginning or at the end of the study period. Institutional animal ethics committee (IAEC) approved the experimental protocol and care of the animals was taken as per guidelines of CPCSEA Department of animal welfare, Govt. of India.

Induction of Myocardial Injury

Total of 72 rats were used. The rats were divided into 12 groups with 6 rats in each group as shown in **Table 1**. Myocardial injury was induced in experimental rats by injection of Isoproterenol hydrochloride 200mg/kg body weight, subcutaneously, twice at an interval of 24 hours at the end of experimental period of 14 days.

Electrocardiography (ECG)

ECGs of rats were recorded using computerized data acquisition system (Biopac, MP 35, Santa Barbara, California) under mild anesthesia. Recordings were made on the bipolar standard Leads that is I, II and III. At the end of experimental period, after 12 hours of second ISO injection, the animals were anaesthetized with pentobarbitone sodium (35mg/kg body weight I.P). All the rats were sacrificed by

cervical decapitation. Blood was collected and serum separated was used for the assay of marker enzymes.

Biochemical Assay

The activities of aspertate aminotransferase (AST) and alanine aminotransferase (ALT) in serum were determined spectrophotometrically by the method of Mohur and Cook, (1975) **[15]**. The absorbance was measured at 520 nm and enzyme activity was expressed as U/L. The lactate dehydrogenase (LDH) activity in serum was assayed according to the method of King, 1965 **[16]**. The absorbance was measured 520 nm and the enzyme activity was expressed as U/L. The creatine phosphokinase (CPK) activity in serum was determined by the method of Okinaka *et al.*, 1961 **[17]**. The absorbance was measured at 640 nm and the enzyme activity was expressed as IU/L.

Histopathological Studies

Myocardial tissue after removal was immediately fixed in 10% buffered neutral formalin solution. After fixation was complete, tissue was processed and then embedded in paraffin and serial thin sections were cut using Microtome. Each section was stained with hematoxylin and eosin. The sections were examined under light microscope and photographs were taken

Statistical Analysis

Results are expressed as mean ± S.E.M for six rats in each group. One way ANOVA was carried out, and the statistical comparison among the groups were performed with Dunnet't' test using statistics package program Graph pad prism. A value of $P<0.05$ was considered statistically significant.

RESULTS

Phytochemical Screening

The petroleum ether, ethanol and aqueous extracts of leaf, stem and root of *Aegle marmelos* were subjected to preliminary phytochemical screening. The various groups of phytochemical constituents present in these extracts are presented in **Table 2**

Cardioprotective Effect

Isoproterenol, a catecholamine was administered in the present study; there was significant increase in the levels of diagnostic marker enzymes ALT, AST, LDH and CPK in the serum of these rats as compared to control rats. The prior administration of petroleum ether, ethanol and aqueous extracts of leaf, stem and root of A. *marmelos* for 14 days maintained the

levels of these diagnostic marker enzymes at close to normal levels compared to Isoproterenol alone treated group. Among the various extracts of *A. marmelos* treated animals the animal group treated with aqueous extract of leaf exhibited significant cardioprotective effect (AST 335±12.2, ALT 103.8±4.40, LDH 2578±138.73 and CPK 1004±34.22) as compared with that of standard drug (AST 328±10.00, ALT 101.2±3.52, LDH 2356±51.25, CPK 982±18.48). The petroleum ether and ethanol extract of leaf, petroleum ether, ethanol and aqueous extract of stem and root failed to exhibit cardioprotective effect against Isoproterenol induced Myocardial infarction **(Table 3)**.

Electrocardiographic Results

Among the various extracts of *A. marmelos* treated animals, the animal group treated with aqueous extract of leaf exhibited significant to more significant cardioprotective effect. (PR interval 0.03789±0.001138, QRS interval 0.03790±0.001072, QT interval 0.08860±0.001538, RR interval 0.1589±0.004963, Cardiac cycle 0.1237±0.003871, Heart rate 348.0±8.640) as compared with that of standard drug (PR interval 0.03753±0.001421, QRS interval 0.03775±0.001088, QT interval 0.08833±0.001549, RR interval 0.1559±0.005173, Cardiac cycle 0.1221±0.004010, Heart rate 345.0±9.346). Pretreatment with aqueous extract of leaf of *A. marmelos* and Isoproterenol induced group rats exhibited normal ECG pattern with a slight elevation in ST segment and heart rate was maintained near normal. The data of the experimental animals, including the P wave, QRS complex, QT interval, RR interval, Cardiac cycle and Heart rate are presented in **Table 4**.

Table 1: Induction of Myocardial Injury

GROUP	COMPOSITION	
	Type of extract	Isoproterenol hydrochloride (mg/kg)
Normal control rats	-	-
Negative control	-	200
Positive control (Metoprolol)	-	2
Group 4	Pet. ether extract of leaf	200
Group 5	Ethanol extract of leaf	200
Group 6	Aqueous extract of leaf	200
Group 7	Pet. ether extract of stem	200
Group 8	Ethanol extract of stem	200
Group 9	Aqueous extract of stem	200
Group 10	Pet. ether extract of root	200
Group 11	Ethanol extract of root	200
Group 12	Aqueous extract of root	200

Table 2: Phytochemical Detection of Bioactive Components in *A marmelos*

Test	Leaf			Stem			Root		
	Pet. Ether ext.	Ethanol ext.	Aqueous ext.	Pet. Ether ext.	Ethanol ext.	Aqueous ext.	Pet. Ether ext	Ethanol ext.	Aqueous ext.
Steroids	+	+	+	+	+	+	−	+	−
Alkaloids	+	+	+	+	+	+	+	+	+
Glycosides	+	+	+	+	+	+	+	+	+
Phenols	+	+	+	+	+	+	+	+	+
Carboxylic acid	−	−	−	−	+	−	−	+	−
Reducing sugars	+	+	+	+	+	−	−	+	+

+ Positive, − Negative

Table 3: Cardioprotective Effect of *A. marmelos* on Isoproterenol Induced Myocardial Infarction in Rats

Group (n)	Cardiac enzyme levels (Mean ±SEM)			
	AST	ALT	LDH	CPK
Normal control	315±18.3	97.00±4.97	2347±48.32	1010±21.86
Negative control (ISO 200mg/kg)	408±16.2	134.4±9.83	3324±162.50	1180±17.40
Positive control (Metoprolol 2mg/kg)	328±10.0**	101.2±3.52**	2356±51.25**	982±18.48**
Pet. ether ext. of leaf + ISO	404±13.3	128.6±8.80	3110±73.48	1193±21.64
Ethanol ext. of leaf + ISO	410±10.0	130.5±7.62	2996±122.34	1154±16.50
Aqueous ext. of leaf + ISO	335±12.2**	103.8±4.40**	2578±138.73**	1004±34.22**
Pet. ether ext. of stem ISO	412±8.0	124.3±5.56	3340±150.21	1177±20.82
Ethanol ext. of stem + ISO	410±13.2	119±8.23	2870±86.53	1098±28.43
Aqueous ext. of stem+ ISO	405±6.70	133.8±7.38	3218±57.49	1167±12.10
Pet. ether ext. of root + ISO	407±5.53	137.2±8.32	3314±60.54	1155±14.82
Ethanol ext. of root + ISO	418±9.84	129.7±5.35	3328±118.50	1162±23.64
Aqueous ext. of root + ISO	402±6.30	126.3±6.55	3007±144.37	1182±16.64

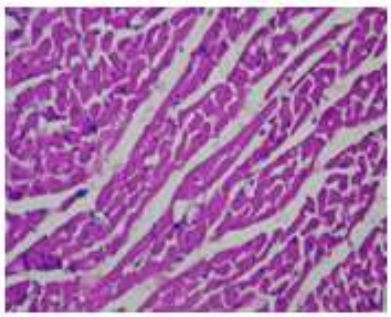
Activities of AST, ALT and LDH are expressed as units/liter, whereas, CPK is expressed as U/liter. Non significant (P>0.05), *Significant (P<0.05), ** More significant (P<0.01), n=6 number of animals in each group.

Table 4: Cardioprotective Effect of *A. marmelos* on Isoproterenol Induced Myocardial Infarction in Rats ECG Parameters

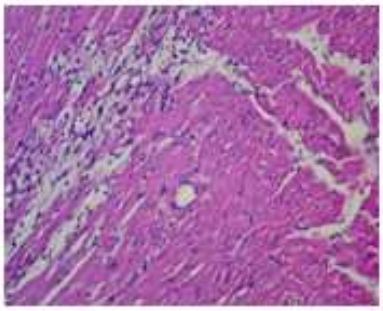
Group	P – R Interval	QRS Interval	Q T Interval	RR Interval	Cardiac cycle	Heart rate
Normal control	0.03750±0.001306	0.0375±0.001151	0.08500±0.003314	0.1879±0.00340	0.1213±0.003699	321.2±5.871
Negative control (ISO 200mg/kg)	0.03278±0.008078	0.03944±0.001002	0.09944±0.001944	0.1367±0.003005	0.1361±0.003310	417.9±6.901
Positive control (Metoprolol 2mg/kg)	0.03753±0.001421**	0.03775±0.001088**	0.08833±0.001549*	0.1559±0.005173*	0.1221±0.004010**	345.0±9.346**
Pet. ether ext. of leaf + ISO	0.03284±0.006830	0.03957±0.001105	0.09730±0.002516	0.1392±0.03202	0.1358±0.003231	412.6±7.10
Ethanol ext. of leaf + ISO	0.3460±0.005923	0.03918±0.001143	0.09273±0.001860	0.1358±0.004328	0.1379±0.004130	419.3±8.31
Aqueous ext. of leaf + ISO	0.03789±0.001138*	0.03790±0.001072**	0.08860±0.001538*	0.1589±0.004963*	0.1237±0.003871*	348.0±8.640**

Pet. ether ext. of stem + ISO	**0.03502±0.005633**	**0.03923±0.001107**	**0.009927±0.001232**	**0.1370±0.03268**	**0.1364±0.004070**	**417.6±6.75**
Ethanol ext. of stem + ISO	**0.03513±0.005317**	**0.03976±0.001043**	**0.9842±0.002460**	**0.1390±0.03108**	**0.1387±0.003842**	**421.2±7.39**
Aqueous ext. of stem + ISO	**0.03306±0.005239**	**0.3990±0.01137**	**0.9732±0.005230**	**0.1395±0.003104**	**0.1383±0.003214**	**417.8±6.58**
Pet. ether ext. of root + ISO	**0.03517±0.004730**	**0.03914±0.001108**	**0.9960±0.002134**	**0.1374±0.003256**	**0.1378±0.004032**	**423.1±8.46**
Ethanol ext. of root + ISO	**0.03363±0.001834**	**0.03982±0.001068**	**0.9624±0.002615**	**0.1308±0.004637**	**0.1366±0.003784**	**408.0±10.32**
Aqueous ext. of root + ISO	**0.3497±0.008360**	**0.3846±0.01053**	**0.9563±0.002104**	**0.1387±0.003890**	**0.1378±0.003304**	**415.0±5.48**

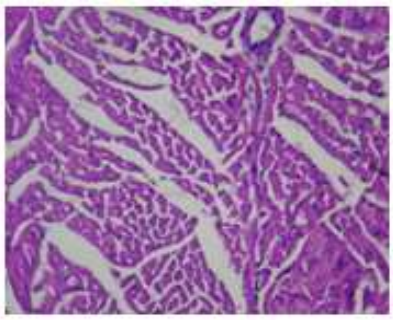
Non significant (P>0.05), *Significant (P<0.05), ** More significant (P<0.01).



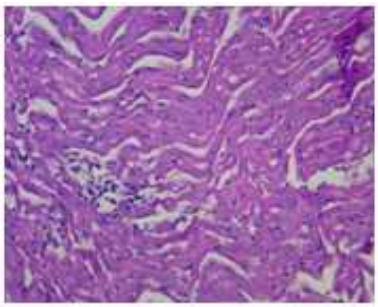
Normal control



ISO treated



Metaprolol treated



Aqueous extract of leaf treated

Figure 1: Histopathological Studies of Myocardial Infarction

DISCUSSION

Consistent with the previous reports in the current study isoproterenol administration in rats leads to increased lipid peroxidation and extensive necrosis of cell membranes **[18]**. Free radical generation & lipid peroxidation could be involved in isoproterenol induced cardiac damage **[19]**. As a result of myocardial cell necrosis, in the present study, the levels of diagnostic marker enzymes of myocardial infarction such as ALT, AST, LDH, and CPK are increased in the serum. Aqueous leaf extract of *A. marmelos* pretreatment for 14 days decreased the activities of these enzyme markers. This could be due to the free radical scavenging property of the extract in the presence of phytochemicals such as sterols and amino acids like arginine, leucine, lysine, methionine and phenylalanine. Early diagnosis of myocardial infarction has done by Electrocardiogram **[20]**. Significant alterations of ECG patterns were observed in isoproterenol administered rats when compared to normal control rats. The characteristic findings were reductions in the P-R Interval, R-R intervals and significant elevation in the ST segment and a higher heart rate, prolongation of QT interval & cardiac cycle. No change was observed in QRS complex when compared to normal control. The appearance of Q wave & ST segment elevation are some of the indicative signs of ischemia.

In the present study we did not observe pathological Q wave due to conditions of ischemia. The prominent Q wave were seen only on severe ischemia, infarction and in patients with severe heart diseases. In agreement with the previous reports isoproterenol induction increase the heart rate & significant elevation in ST segment was observed when compared to normal control **[21]**. The consecutive loss of cellular membrane damage due to oxidative stress might be characterized by ST elevation **[22]**.

Aqueous extract of leaf of *A. marmelos* administration showed a protective effect against isoproterenol induced altered ECG patterns and presuming the free radical mediated myocardial cellular membrane damage. Electrocardiograph and biochemical findings were further confirmed by histopathological studies. Histopathological examination of myocardial tissue in control depicted clear integrity of the myocardial cell membrane. No inflammatory cell infiltration was seen in the rat heart of control. In isoproterenol administered group, focal lesions in many sections consisting of molted staining and fragmentation of muscle fibers with confluent retrogressive lesions, hyaline

necrosis, and sequestrating mucoid edema were observed in **Figure 1**. Pretreatment with aqueous extract of leaf of *A. marmelos* demonstrated reversal of focal lesions, fragmentation of muscle fibers and retrogressive lesions with hyaline necrosis seen with isoproterenol treated group. Inflammatory cells were seen with reduced density in aqueous extract of leaf of *A. marmelos* treated group confirming further the cardioprotective activity exerted by aqueous extract of leaf of *A. marmelos.*

CONCLUSION

In conclusion, by carefully examining the cardioprotective activity of *A. marmelos,* the present work provides evidence that aqueous extract of *A. marmelos* has significant cardioprotective activity. Hence further investigations using more experimental paradigms are warranted for further confirmation of the treatment of various ailments, diseases and disorders.

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