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Anandhi
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Doss VA
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Sowndarya R
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Correspondence
Doss VA
Department of Biochemistry, PSG College of Arts & Science, Coimbatore, Tamil Nadu, India

Cardioprotective activity of *Euphorbia hirta* in Isoproterenol induced Myocardial Infarction in Rats

Anandhi, Doss VA and Sowndarya R

Abstract
Myocardial infarction is a major public health concern and the leading cause of death throughout the World. Considering the creditable medicinal benefits of *Euphorbia hirta,* the present study aimed to elucidate the cardioprotective effects of *E. hirta* leaf extract on isoproterenol induced myocardial infarction in rats. Prior administration of hydroethanolic leaf extract at 250 mg/kg of body weight was found to diminish the effect of isoproterenol on the levels of Total cholesterol, Triglycerides and LDL with a parallel rise in the level of HDL. The extract effect was compared with standard drug propranolol which also offered similar protection and it was further confirmed by histological changes of the heart. Thus our present study clearly indicated a significant cardioprotective activity of leaf extract due to the presence of phytochemicals and antioxidant properties.

Keywords: *Euphorbia hirta*, Isoproterenol, CVD, HDL, LDL, TGL

Introduction
Myocardial infarction, commonly known as heart attack is a disease that occurs when the blood supply to a part of the heart is interrupted, causing death of heart tissue. It means necrosis of a region of myocardium caused by an interruption in the supply of blood to the heart usually as a result of occlusion of a coronary artery also called as cardiac infarction [1].
Reperfusion of the ischemic myocardium is accompanied by the generation of reactive oxygen species (ROS) that can cause vascular and microvascular injury, endothelial cell dysfunction, myocyteedema, increases myocyte apoptosis, increased myocyte necrosis and cardiac contractile dysfunction [2].
There are different ways of preventing and treating cardiovascular disease. Besides drug therapy and life style changing, dietary modification and supplementation play an increasingly important role in the conservative treatment of cardiovascular diseases [3]. Many medicinal plants have been found to possess beneficial effects in pathological condition like cancer, liver diseases, cataract and myocardial ischemia [4]. A considerable number of these plants and plant based products have been widely used [5].
Euphorbia hirta is commonly known as asthma plant (or) snake weed. The common name is Amman pacharisi. It is a medicinal rhizomatous herb distributed in southern Western Ghats of India and Northern east coast of Tamilnadu [6]. *Euphorbia hirta* has a long history as a medicinal herb in china. Different formulations are used including crude drug, decoction, infusion, lotion and powder. It plays a very important role in traditional Chinese medicine, especially in folk medicine because of its wide range of biological and pharmacological properties [7].

Materials and Methods
Animals
Adult male wistar rats (120-150g) procured from the laboratory animal house at KMCH, Coimbatore, India were used for the study. The ethical clearance (CPCSEA/NO.352/2017/IAEC) for the handling of experimental animals was obtained from the Institutional Animal Ethics Committee (IAEC) of PSGIMSR. The animals were maintained under standard laboratory conditions with controlled temperature and humidity where they were acclimatized to standard laboratory diet and filtered water. They were kept at constant room temperature at 37 ^{0}C, 12 hours day and night cycle.

The place where the experiments were conducted was kept in very hygienic conditions.

Chemicals

Isoproterenol was purchased from Sigma chemical Co., USA. Propranolol (standard drug-Inderal 10 mg/kg) was purchased from a pharmacy.

Preparation of *Euphorbia hirta* extract

1 Kg of coarse powder of *Euphorbia hirta* leaves were soaked in 50% ethanol cold macerated for three days. The suspension was filtered through a fine muslin cloth. The residue was removed. The filtrate was taken in round-bottom glass flask and the sample was evaporated to dryness at a low temperature in a rotary evaporator. When needed residual extracts were dissolved in distilled water and to check the cardioprotective activity.

Experimental setup

The rats were randomly divided into 4 groups of 6 animals each in group

Group 1	Normal rats
Group 1	Cardiac toxicity induced –85 mg/kg of isoproterenol
Group 2	10 mg/kg of propranolol + Cardiac toxicity induced
Group 3	250 mg/kg of treated Leaves extract + Cardiac toxicity induced

Induction of myocardial infarction using isoproterenol

At the end of treatment period, all the animals, except the normal untreated rats that served as the control group, were administered isoproterenol (ISO) 85 mg/kg, intraperitoneal injection for two consecutive days on the 29 and 30 day at an interval of 24 hour to induce myocardial injury.

After 30 days of experimental period, on the next day, animals of all the groups were anaesthetised with ketamine. Blood was collected via cardiac puncture method and allowed to clot for 1 hour at room temperature. Serum was separated centrifuged at 4000 rpm for 20 minutes and stored at 80^0C for further biochemical analysis.

Estimation of lipid profiles

Lipid profile in serum Triglycerides (TGL) [8], Total cholesterol [9], HDL and LDL were assayed in serum by using autospan diagnostic kits as per manufacturer protocol.

Histological studies

The lower portion of heart tissue was subjected to histological evaluation. Fresh heart tissues were excised and then fixed in 10% formalin for 24 hours. The fixative was removed by washing through running tap water for overnight. After dehydration through a graded series of alcohols, the tissues were cleared in two changes of xylene, one hour each and embedded in paraffin wax. Sections were cut into 5 µm thickness and stained with hematoxylin and eosin. After repeated dehydration and cleaning, the sections were mounted and observed under light microscope with magnification of 100x for histological changes.

Statistical analysis

All the data obtained was expressed as mean ± SD. Statistical analysis was performed by using the method of distribution statistics (standard descriptive analysis) and analysis of means (Student t test) using R - Statistical Computing and Graphical Tools (formerly AT & T, Lucent technology). A probability of $P<0.05$ was considered significant.

Results and discussion

Lipid profile is a group of tests comprising total cholesterol, triglycerides, HDL and LDL. The lipid profile is used together with other risk factors, to assess a person's risk of cardiovascular disease (CVD).

Table 1: Effect of *Euphorbia hirta* levels on total cholesterol, LDL and HDL in serum of control and experimental rats.

GROUPS	TC	TGL	LDL	HDL
Normal	173.33 ± 4.179	135 ± 2.449	61.67 ± 2.161	49.32 ± 20176
Isoproterenol	276.36 ± 3.678*	182 ± 7.91*	70 ± 2.867*	31.67 ± 2.921*
Drug treated	167.3 ± 2.867*	132 ± 2.942*	39.42 ± 3.407*	47.476 ± 0.973*
Plant treated	198.66 ± 1.67*	124 ± 3.741*	57 ± 4.546*	43.33 ± 2.189*

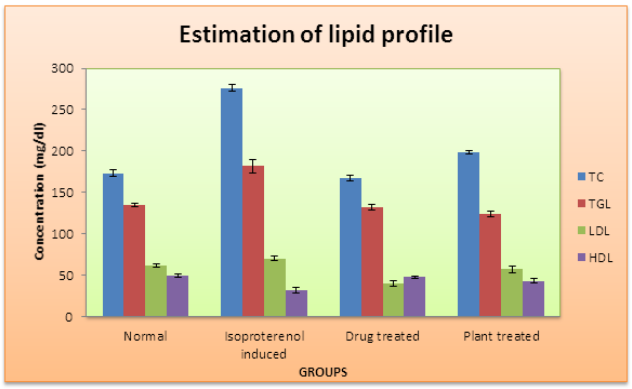

Fig 1: Effect of *Euphorbia hirta* levels on total cholesterol, Triglycerides, LDL and HDL in serum of control and experimental rats

The data represented in the Table 1 and Figure 1 depicts that in Isoproterenol induced rats there was a significant increase in total cholesterol that was due to lipid metabolism which plays an important role in myocardial necrosis produced by ischemia [10]. Treatment with *Euphorbia hirta* extract produced a significant decrease in serum total cholesterol level.

The significant acute myocardial infarction was indicated by the levels of Triglycerides (TGL) and LDL in ISO induced rats. In the treated group IV, the levels were reduced nearest to the normal values, because of the action of plant extract.

The levels of HDL reduced in ISO induced rats reflecting the reduction of good cholesterol. But in the treated group IV the HDL level increased significant which was comparable with group III.

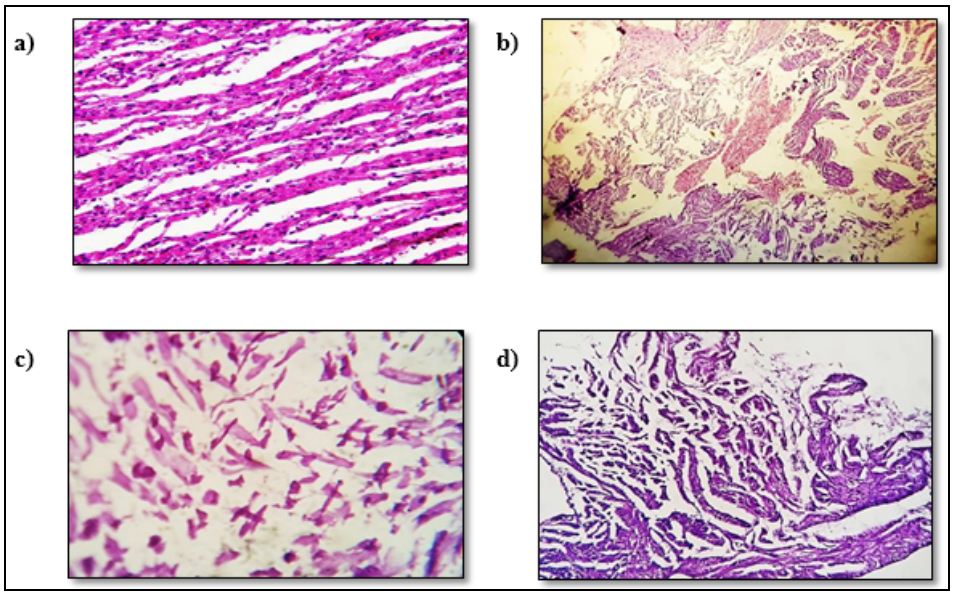

Fig 3: Histological examination of heart tissues of control and experimental animals. a) Normal rat showing normal cardiac muscle tissue; b) ISO induced rat showing necrotic cells and destruction of cardiac muscle fibre; c) Rat treated with propranolol and isoproterenol showing normal heart with mild inflammation; d) Pretreated with *Euphorbia hirta* showing regeneration of normal cardiac muscle.

Histological studies showed no pathological changes of myocardium in group Iwhereas in ISO induced rats, microscopic examination of heart tissue showed necrosis of myofibrils with inflammatory mononuclear collections and edema. Pretreatment with *Euphorbia hirta* reversed these changes in accordance with group III showing normal heart with mild inflammation.

Conclusion

The present study demonstrates that *Euphorbia hirta* has potential to protect against myocardial infarction by preserving the levels of TC, TGL, LDL and restoring the levels of HDL and was confirmed by histological changes. The potent cardioprotective activity of the plant was due to the presence of phytochemicals and antioxidant properties. In conclusion, further studies are needed to identify the active principles responsible for the cardioprotective activity and to evaluate its exact mechanism of action.

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