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Original Research Article

Cardioprotective and Antioxidant Potential of *Phoenix dactylifera* Fruit Extract on Isoproterenol Induced Myocardial Damage in Animal Model

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Abstract: Myocardial infarction and related cardiovascular complications are the main causes of deaths throughout the world. The use of herbal antioxidants is increasing as defensive agents against number of cardiovascular abnormalities. The bioactive agents from natural sources have gained fundamental importance in modern system of medicines, reducing the risks of cardiac ailments by scavenging the free radicals formation. *Phoenix dactylifera L.* is a fruit bearing tree with a lot of prospects. Its fruits, and seeds otherwise known as pit and by products are made up of nutritional and medicinal potentials. The ethanolic extract of *Phoenix dactylifera* fruit was studied for the cardio protective and antioxidant activity against Isoproterenol induced myocardial infarction in wistar albino rats. *Phoenix dactylifera* showed significant cardio protective effect by reversing the cardio biomarkers (CK-MB, LDH) and oxidative stress markers (SOD, GSH, GPX, CAT, LPO) induced by Isoproterenol. The findings of cardio biomarkers from serum and oxidative stress markers from myocardial tissue in the present work are comparable with the reference control Vitamin E. The results conclude that ethanolic extract of *Phoenix dactylifera* showed cardio protective activity and the effect may be mediated by its antioxidant potential.

Keywords: *Phoenix dactylifera*, Isoproterenol, Cardio biomarkers, Antioxidant and Myocardial Infarction.

INTRODUCTION

Nature is a very important source for finding new drugs that leads to the treatment of diseases. Herbs have been used as medical treatments since the beginning of civilization and some derivatives have become mainstays of human pharmacotherapy. For cardiovascular diseases, herbal treatments have been used in patients with congestive heart failure, systolic hypertension, angina pectoris, atherosclerosis, cerebral insufficiency, venous insufficiency, and arrhythmia [1]. Welknown drugs from herbal and plant sources include aspirin from the Salix alba L. tree, digoxin (cardiac glycoside) from *Digitalis purpurea*, ephedrine from *Ephedra sinica*, lovastatin from *Monascus purpureus* L., taxol from *Taxusbre vifolia*, reserpine from *Rauvolfia serpentina*, and many others [2-4].

According to the World Health Organization (WHO) statistics, cardiovascular diseases (CVDs) were responsible for the highest number of deaths in 2019 [5]. Increasing and aging populations further complicate the situation, and 22.2 million CVD-related deaths are expected to occur in 2030 [6].

The use of traditional medicinal plants has rapidly expanded in recent years. Medical plant research is no longer limited to chemical composition and pharmacology and now encompasses the study of metabolomics and underlying mechanisms of action [7]. Finding safe and effective drugs derived from natural products are a hot topic in the CVD field [8]. Medicinal plants have great advantages for the treatment of cardiovascular disease owing to their safety profiles [9]. Favourable effects of medicinal plants have been described for diseases such as hypertension, hyperlipidemia, atherosclerosis, and chronic heart failure, as well as for the overall reduction of cardiovascular risk [10]. Accumulating evidence suggests that flavonoids, phenolics, and saponin from medical plants could reduce oxidative stress [11].

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Phoenix dactylifera L. is considered as one of the oldest and main staple and ancient crops in Southwest Asia and North Africa. Besides, dates can be grown in Australia, Mexico, South America, southern Africa, and the United States, especially in southern California, Arizona, and Texas [12]. Date palm tree belongs to *Arecaceae* family (Angiosperms, monocotyledon) consisting of about 200 genera and more than 2,500 species. *Phoenix* (*Coryphoideae phoeniceae*) is one of the genera with approximately 14 species, which are native to the tropical or subtropical regions of southern Asia or Africa, including *Phoenix dactylifera* L [13]. The name of the species *dactylifera* means "finger-bearing" which refers to the fruit clusters produced by this plant. *Dactylifera* is a grouping of the Greek word *dactylus*, means "finger," and the Latin word *ferous*, mean "bearing" [14]. The fruits of *Phoenix dactylifera* is rich in phytochemicals such as carotenoids, polyphenols (e.g., phenolic acids, isoflavons, lignans, and flavonoids), tannins, and sterols [15]. Preclinical studies have shown that the date fruits possess free radical scavenging, antioxidant, antimutagenic, antimicrobial, anti-inflammatory, gastroprotective, hepatoprotective, nephroprotective, anticancer and immunostimulant activities [16]. Consumption of date palm fruits to lower the danger of liver, cancer, and cardiovascular diseases [17]. In addition, dietary oils, rich in unsaturated fatty acids have been reported to prevent cardiovascular and inflammatory diseases [18]. In order to substantiate the ethanomedical claim, present study was conducted to evaluate the cardioptotective activity of ethanolic fruit extract of *Phoenix dactylifera* against Isoproterenol induced myocardial infarction in rats.

MATERIALS AND METHODS

Chemicals

Isoproterenol was obtained from the Sigma Chemical Company, St. Louis, MO, USA. All the other chemicals and reagents used were of analytical grade.

Plant Material

The fruits of *Phoenix dactylifera*, Linn. werelocal market of Erode. Tamilnadu. The fruit was authenticated and identified as *Phoenix dactylifera* by the Scientist D, Botanical Survey of India, Southern Regional Center, Agricultural University, Coimbatore, India. The voucher specimen (BSI/SRC/12/42/2019-20/Sci/147) has been deposited in Herbarium for further reference.

Preparation of Extract

The fruits were washed with water and chopped in to small pieces. The pieces were dried in sunlight for one hour and then it was dried under shade. With the help of mechanical blender the dried fruits were powered to get coarse. Dried course powders of the fruits of *Phoenix dactylifera* were extracted with 70% ethanol by cold maceration for 7 days. The extracts were concentrated by rotary evaporator, dried and stored in desiccators.

Animals and Ethical Considerations

Healthy male Sprague–Dawley (SD) rats weighing 180-200 gm were used in the study. The animals were obtained from Kerala Veterinary and Animal Science University, Thiruvazhamkunnu, Kerala. On arrival, the animals were accustomed to the animal house condition of Nandha College of Pharmacy, Erode for 14 days by placing the animals in polypropylene cages with paddy husk as bedding. Animals were housed at a temperature of 24±2^{o}C and relative humidity of 30–70%. A 12:12 light: day cycle was followed. All animals were allowed to free access to water and fed with standard commercial pelleted rat chaw (M/s. Hindustan Lever Ltd, Mumbai). All the experimental procedures and protocols used in this study were reviewed by the Institutional Animal Ethics Committee (688/PO/Re/S/02/CPCSEA) and were in accordance with the Institutional ethical guidelines.

Treatment Schedule

The animals were randomized into five groups of six animals each. Groups I and II were administered with 0.5% carboxymethyl cellulose solution (10 ml/kg b.wt/day, p.o), group III was administered with Vitamin E (60 mg/kg. b.wt/day, p.o), once daily for 15 days and groups IV and V were administered with *Phoenix dactylifera* (250 and 500 mg/kg b.wt/day, p.o) respectively once daily for 15 days. Groups II to V were subcutaneously injected with Isoproterenol Hydrochloride (85 mg/kg b.wt) on day 14th and 15th twice at an interval of 24 hrs, to induce myocardial necrosis. After 24 hrs of Isoproterenol Hydrochloride administration, blood was collected from the overnight fasted rats through retro-orbital puncture under Pentobarbitone (45mg/kg, b.wt, i.p) anaesthesia and was immediately processed for further biochemical evaluations. The animals were then euthanized by briefly exposing to CO_2 gas and heart was rapidly dissected and processed for further biochemical evaluations.

Estimation of Cardiac Biomarkers

Whole blood was collected from the right carotids of the overnight fasted rats (with simultaneous monitoring of the hemodynamic parameters) in commercially heparinized red-top tubes. It was clotted by leaving it undisturbed for 2 hrs at room temperature and was then centrifuged at 1000-2000xg for 10 min at 4°C. The clot-free supernatants containing serum were quantified for the levels of the two cardiac biomarkers: CK-MB and LDH, by adopting the standard protocols described in CK-MB and LDH biochemical assay kits and their levels were expressed in units/L [19, 20]. Absorbance was detected at their respective wavelength using Semi- Analyzer.

Estimation of Oxidative Stress Markers

The hearts were rapidly dissected from the euthanized rats and were thoroughly washed with ice-cold physiological saline. They were used to prepare 10% homogenates in phosphate buffer (50 mM, pH 7.4) using a tissue homogenizer and the homogenates were centrifuged at 2500 rpm for 10 min at 4°C. The precipitate-free supernatants were quantified for the total protein content by Lowry's method and then for the levels of the five oxidative stress markers: Superoxide Dismutase (SOD), Reduced Glutathione (GSH), Glutathione Peroxidase (GPX),Catalase (CAT) and Lipid Peroxidation (LPO) by adopting the standard protocols [21-24]. Absorbance was detected at their respective wavelengths using UV Spectrophotometer and expressed as Units/mg of Protein.

Statistical Analysis

The results were expressed as mean ± standard error of the mean (SEM). One-way Analysis of Variance (ANOVA) was applied for the statistical analysis, followed by Dunnet's *'t'* test for post-hoc analysis. Statistical analyses were performed using GraphPad prism 5.0 and a p-value <0.05 was considered to be statistically significant.

RESULTS

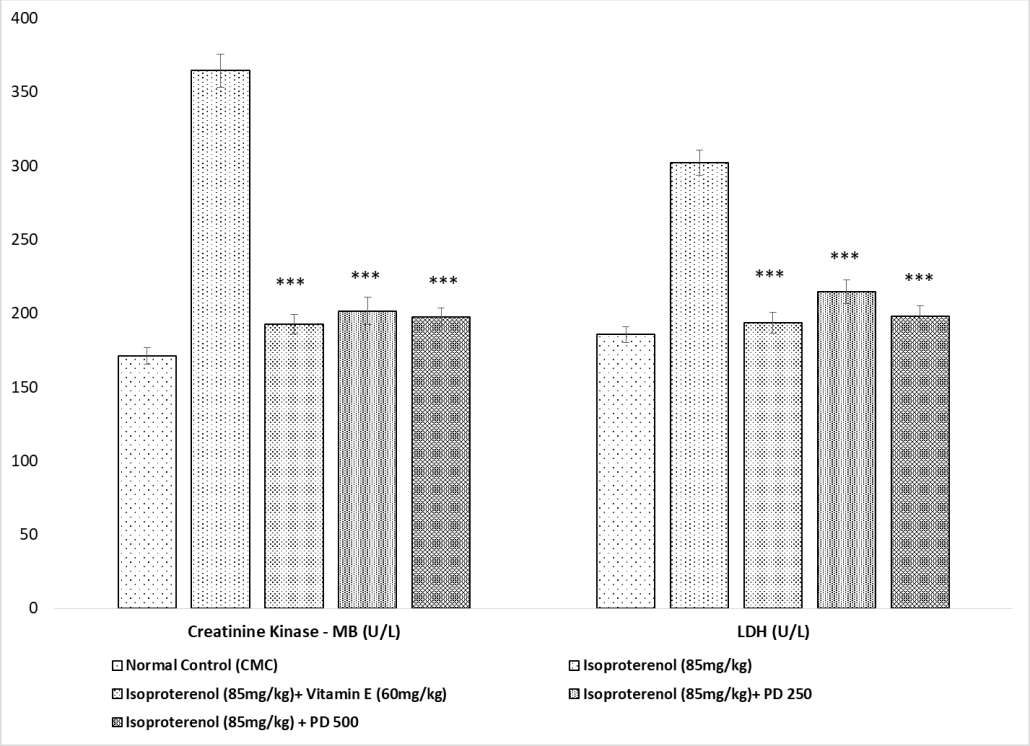

Figure 1: Effect of *Phoenix dactylifera* fruit extract on cardiac biomarkers (Creatinine Kinase – MB and LDH) against Isoproterenol induced myocardial infarction in rats

Values are in Mean±SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Isoproterenol

Effect of *Phoenix dactylifera* fruit extract on cardiac biomarkers (Creatinine Kinase – MB and LDH) against Isoproterenol induced myocardial infarction in rats were given in the fig 1. Administration of Isoproterenol, elevates both cardiac biomarkers, Creatinine Kinase – MB (364.57± 11.24) and LDH (302.21±8.44) levels when compared with normal control which received vehicle CMC. Significant (P<0.001) decrease in the levels of both cardiac biomarkers, Creatinine Kinase – MB (192.63±6.75) and LDH (193.64±7.25) were observed with the animals pre-treated with Vitamin E in Isoproterenol administered group. Similarly, both the doses of PD significantly (P<0.001) decreased the levels of Creatinine Kinase – MB and LDH in the groups administered with Isoproterenol.

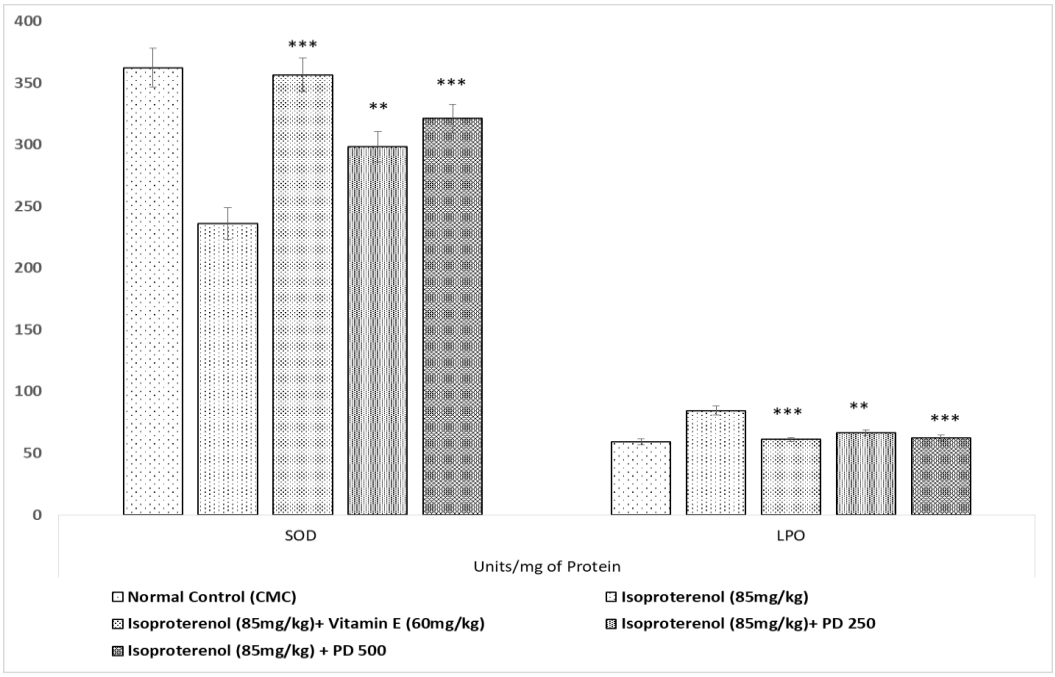

Figure 2: Effect of *Phoenix dactylifera* fruit extract on oxidative stress markers (Superoxide dismutase and Lipid peroxidase) against Isoproterenol induced myocardial infarction in rats

Values are in Mean±SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Isoproterenol

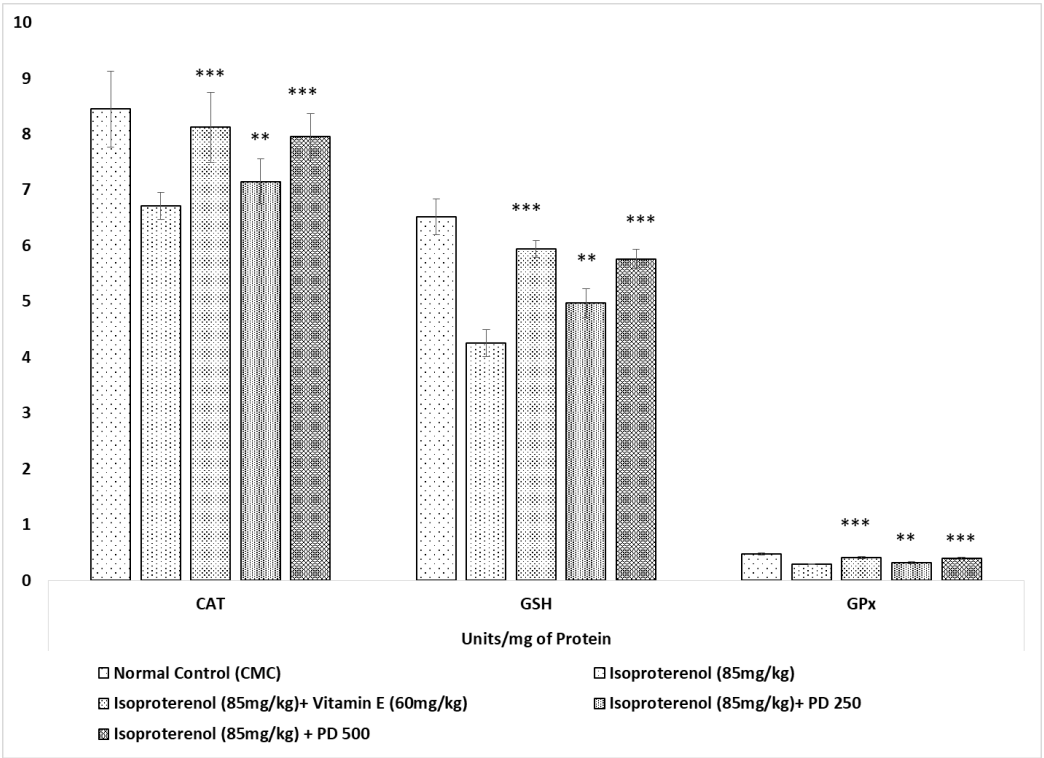

Figure 3: Effect of *Phoenix dactylifera* fruit extract on oxidative stress markers (Catalase, Reduced Glutathione and Glutathione Peroxidase) against Isoproterenol induced myocardial infarction in rats

Values are in Mean±SEM (n=6)

*P<0.05, **P<0.01, ***P<0.001 Vs Isoproterenol

Effect of *Phoenix dactylifera* fruit extract on oxidative stress markers (SOD, LPO, CAT, GSH and GPx) against Isoproterenol induced myocardial infarction in rats were given in fig 2 & 3. Administration of Isoproterenol decreases all the oxidative stress markers except LPO compare to vehicle control. Pre-treatment of Vitamin E significantly (P<0.001) elevated SOD, CAT, GSH, GPx and

significantly (P<0.001) decrease the LPO, in Isoproterenol administered group compare to Isoproterenol alone treated groups. Pre-treatment of low dose of *Phoenix dactylifera* fruit extract significantly (P<0.01) elevated SOD, CAT, GSH, GPx and significantly (P<0.01) decrease the LPO, in Isoproterenol administered group compare to Isoproterenol alone treated groups. Pre-treatment of high dose of *Phoenix dactylifera* fruit extract significantly (P<0.001) elevated SOD, CAT, GSH, GPx and significantly (P<0.001) decrease the LPO, in Isoproterenol administered group compare to Isoproterenol alone treated groups. Among 250 and 500mg/kg of *Phoenix dactylifera* fruit extract, high dose showed marked antioxidant activity and it was similar to the reference control Vitamin E.

DISCUSSION

Phoenix dactylifera can be regarded as a promising edible fruit owing to its therapeutic, nutritive and bioactivity potentials. *Phoenix dactylifera* has a great medicinal value as it has been reported to have versatile phytochemical including phenolics, sterols, carotenoids, anthocyanins, procyanidins, flavonoids, minerals and vitamins. *Phoenix dactylifera* possess various pharmacological effects like antibacterial, anti-inflammatory, antidiabetic, antiasthamatic, nephroprotective, hepatoprotective and aphrodisiac etc. In the present study, an attempt was made to validate the ethnobotanical claim of *Phoenix dactylifera* fruit for its cardioprotective activity against Isoproterenol induced cardiac damage in rats.

Isoproterenol is a β- adrenergic receptor agonist, which widely used to induce myocardial damage experimental animal models, resulting in biochemical changes in the heart [25]. Clinically, Creatinine Kinase – MB and LDH are released into the blood during myocardial injury, and there is a significantly positive correlation between elevated Creatinine Kinase – MB and LDH in ischemic myocardial injury [26]. So, Creatinine Kinase – MB and LDH are often used as indicators to measure the extent of ischemic heart damage. In our findings, the levels of Creatinine Kinase – MB and LDH were significantly increased in Isoproterenol group when compared to the levels in the control group. Both the doses of *Phoenix dactylifera* significantly reversed the levels of Creatinine Kinase – MB and LDH, elevated by Isoproterenol. The dose dependant decrease in cardiac biomarkers by *Phoenix dactylifera* fruit extract, suggest that it protects myocardia from Isoproterenol induced cardiac damage. The endogenous antioxidant enzymes plays a major role in the neutralization of free-radical-mediated oxidative stress [27]. SOD, CAT and GPX are the chief hydroxyl-radical scavenging antioxidant enzymes that decompose oxygen and hydrogen peroxide, prior to their fusion to form highly-reactive hydroxyl-radicals [28]. In our study, Isoproterenol significantly reduced the tissue levels of these enzymes in the rats when compared with the levels of normal control rats. The reduced levels of these enzymes can be attributed to their increased uptake for neutralizing the free-radicals and/or their reduced expression due to excessive Isoproterenol oxidation [29]. Pre-treatment with *Phoenix dactylifera* significantly reversed the tissue levels of these enzymes at their respective normal levels. These results imply that *Phoenix dactylifera* imparts cardioprotectivity by maintaining the expression levels of theseenzymes such that their cytoplasmic reserves will equalize to normal, despite the utilization and degradation of a fraction of these enzymes for scavenging the free-radicals.

CONCLUSION

The results from this study it was concluded that, the ethanolic extract of *Phoenix dactylifera* fruit has a protective effect on isoproterenol induced Myocardial Infarction. The cardio protective activity of *Phoenix dactylifera* may be due to its free radical scavenging property. Further studies to be step up in order to record its exact mechanism of action.

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