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CARDIOPROTECTIVE EFFECT OF BIOFLAVONOIDS AGAINST ISOPROTERENOL INDUCED CARDIOTOXICITY IN RATS.

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ABSTRACT

The present study was undertaken to explore the cardioprotective potential of bioflavonoids against isoproterenol induced cardiotoxicity in rats. Cardiotoxicity was induced by isoproterenol (85mg/kg body weight) in animals. Blood biochemical, hematological parameters, urinalysis and histopathological studies were carried to assess the cardio protective effect. Isoproterenol administration induced significant cardiotoxicity in rats, which was evident from enhanced levels of glucose, cholesterol, triglycerides and calcium except sodium. Pretreatment with quercetin (50 mg/kg dose orally) significantly reversed isoproterenol induced cardiotoxicity than the silymarin (50 mg/kg dose orally). From the obtained results it may be concluded that quercetin exerted a significant cardioprotective effect against isoproterenol induced cardiotoxicity in rats than silymarin ($p<0.001$) for most of the blood biochemical parameters, mean corpuscular hemoglobin count as well as in attenuation of pathological changes in cardiac tissues.

KEYWORDS: **Cardiotoxicity, bioflavonoid, isoproterenol, quercetin, silymarin, cardioprotective**

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INTRODUCTION

Cardiovascular diseases (CVDs) are a group of disorders of the heart and blood vessels and they include: coronary heart disease – disease of the blood vessels supplying the heart muscle; cerebrovascular disease – disease of the blood vessels supplying the brain; peripheral arterial disease – disease of blood vessels supplying the arms and legs; rheumatic heart disease – damage to the heart muscle and heart valves from rheumatic fever, caused by streptococcal bacteria; congenital heart disease – malformations of heart structure existing at birth; deep vein thrombosis and pulmonary embolism – blood clots in the leg veins, which can dislodge and move to the heart and lungs. CVDs are the number 1 cause of death globally: more people die annually from CVDs than from any other cause. An estimated 17.5 million people died from CVDs in 2012, representing 31% of all global deaths. Of these deaths, an estimated 7.4 million were due to coronary heart disease and 6.7 million were due to stroke. Over three quarters of CVD deaths take place in low- and middle income countries. Out of the 16 million deaths under the age of 70 due to non-communicable diseases, 82% are in low and middle income countries and 37% are caused by CVDs. Most cardiovascular diseases can be prevented by addressing behavioral risk factors such as tobacco use, unhealthy diet and obesity, physical inactivity and harmful use of alcohol using population-wide strategies. People with cardiovascular disease or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia or already established disease) need early detection and management using counseling and medicines, as appropriate. Clinical management of cardiovascular diseases is still a nightmare for the cardiologist. Thus far, vasodilators, β adrenergic blockers, antiarrhythmics, thrombolytics, etc. are the mainstay of cardiac therapy. Analgesic agents like morphine have also been used. Most of the currently used therapeutic interventions provide only symptomatic relief. Flavonoids are a large group of compounds (>4000) that occur naturally in fruits, vegetables, nuts, seeds, flowers and other plant matter and as such they are an integral part of the human diet. They all share a common three-ring structure but are subdivided into flavonols, flavons, flavanols and flavanons according to their substituents. Epidemiological studies indicate that diets rich in flavonoids are associated with reduced incidences of several chronic diseases including cardiovascular disease, asthma, type II diabetes and certain types of cancer. The cardioprotective properties of flavonoids are multi-faceted involving antioxidant, anti-hypercholesterolaemia, anti-inflammatory and inhibition of platelet aggregation effects. The antioxidant property of flavonoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases.[1]Silymarin is obtained from the *Silybum marianum* (milk thistle) an edible plant that has been used medicinally for the centuries as an herbal medicine. It is a mixture of mainly three flavonolignans: silybin, silidianin, and silychristine, with silybin being the most active. Silymarin has been used medicinally to treat liver disorders, because of its antioxidant activity and stimulation of ribosomal RNA polymerase and subsequent protein synthesis, leading to enhanced hepatocyte regeneration. [2,3,4] Quercetin a major representative of the flavonol subclass, has received the considerable attention because of its overwhelming presence in foods. Quercetin and its sugar-bond or glucosylated, forms represent to 60-75% of flavonoids intake. Quercetin has displayed the ability to prevent the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions. As a result quercetin may aid the prevention of certain diseases such as cancer, atherosclerosis and chronic inflammation.[5,6,7]

MATERIALS AND METHODS

Animals

Wister rats of either sex, weighing around 200- 250 g. were employed in the present study. They were procured from in house bred animals of Chalapathi Institute of Pharmaceutical Sciences, Guntur. The rats were provided standard laboratory feed and water ad libitum. They were exposed to an alternate light and dark cycle of 12 h and had free access to food and water. The animals were acclimatized to the laboratory conditions for at least 5 days before the cardiotoxicity test. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chalapathi Institute of Pharmaceutical Sciences, Guntur, Andhra Pradesh (Approval No: 09/IAEC/CIPS/2016-17; dt 05/04/2016) and care of the animals was taken as per guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Forests, Environment and Climate Change, Government of India.

Drugs and Reagents

Silymarin, quercetin and isoproterenol was purchased from Sigma Aldrich, Bangalore, India. Isoproterenol was dissolved in distilled water and administered intraperitonially (i.p.) to induce cardiotoxicity in rats. [8,9,10]

Experimental Protocol

Four groups, each comprising of five Wistar rats, were employed in the study.

Group I (Control group): Rats were administered 0.9% w/v normal saline, orally for 14 days.

Group II (Isoproterenol - treated control group): Rats were administered Isoproterenol (85mg/kg, i.p.) on the day 14.[11,12,13]

Group III (Silymarin + isoproterenol - treated group): Rats were treated with silymarin (50 mg/kg, orally) for 14 days. On the 14th day silymarin was administered 60 min prior the administration of isoproterenol.

Group IV (Quercetin + isoproterenol-treated group): Rats were treated with quercetin (50 mg/kg, orally). The remainder of the procedure was similar to that of Group III. The blood samples were collected by cardiac puncture under deep anesthesia (terminal blood sampling using a 23G needle vertically into the sternum)

produced by the administration of ketamine and xylazine. After sufficient exsanguination, the heart of the treatment animals was isolated after cervical dislocation. The blood samples and heart were immediately subjected to blood biochemical, hematological and histopathological studies respectively. The carcass of the dissected animal was disposed by deep burial as per the Standard Operating Procedures of CPCSEA, Government of India. The histopathological study samples were prepared by the hematoxylin and eosin staining techniques.

Parameters Evaluated

Blood biochemical parameters: glucose (mg/dl), calcium (mg/dl), cholesterol (mg/dl), triglycerides (mg/dl), total protein (g/dl).*Haematological parameters:* haemoglobin (mg/dl), total count (mm3), differential count (%), packed cell volume(%), mean corpuscular haemoglobin count (%), platelet count (lac/mm3), red blood cells (million/mm3). *Histopathological studies:* Histological studies of the isolated heart tissues of various treatment groups.

Statistical Analysis

The results are expressed as Mean ± Standard Error of Means (S.E.M.). The data of cardio toxicity results were statistically analyzed by One-way Analysis of Variance (ANOVA) followed by Post hoc Turkey's multiple range test using Graph Pad Prism version 6.0. A p-value <0.05 was considered to be statistically significant.

RESULTS

Various pharmacological interventions employed in the present study did not show any significant signs of toxicity and mortality. Further, no significant difference was observed between the results obtained from rats of either sex.

Effect on blood biochemical and hematological parameters

Isoproterenol (85 mg/kg, i.p.) significantly increased the levels of blood biochemical and hematological parameters when compared to control rats.[14,15,16]Silymarin and quercetin treatment groups showed significant cardioprotective effect ($p<0.05$) against isoproterenol group (Table 1& 2). Administration of isoproterenol (85 mg/kg, i.p.) showed significantly increased levels of all blood biochemical parameters except decreased sodium levels, when compared to normal control group. Administration of silymarin (50 mg/kg, orally) to isoproterenol treated animals showed significant difference in blood glucose, triglycerides, total count, differential count and in PCV. Treatment with quercetin (50 mg/kg, orally) significantly decreased the isoproterenol induced rise in blood biochemical and hematological parameters (Table 1 &2).

Histopathological studies

Histopathological studies showed significant damage in heart tissue by isoproterenol induced cardiotoxicity when compared with normal control group. The significance of p value is <0.05. The histopathological slides of isoproterenol induced group showed congested vessels and necrosis, where as in normal control group no abnormality was observed in heart tissue. The histopathological slides of silymarin treated group showed congested non-specific inflammation where as positive control group showed congested vessels and necrosis in heart tissue. The histopathological slides of silymarin treated group showed congested non-specific inflammation where as quercetin treated group shows significant recovery of heart tissue when compared with silymarin treated group (Fig 1).

Table 1

Blood biochemical parameters of various treatment groups.

S.No.	Parameter	Control	Isoproterenol	Silymarin	Quercetin
1.	Glucose (mg/dl)	22.4±2.99	60.2±2.98	49.6±1.15	32.8±1.15
2.	Calcium (mg/dl)	12.2±0.31	12.1±0.56	13.78±0.18	10.76±0.34
3.	Cholesterol (mg/dl)	41.4±1.07	81.8±6.73	70.6±3.07	68.4±6.64
4.	Triglycerides (mg/dl)	52.6±3.7	110±10.57	99.4±6.75	46.2±5.67
5.	Total protein (g/dl)	9.22±0.36	8.94±0.62	6.9±0.14	7.88±0.45
6.	Sodium (mmol/lit)	135.4±1.5	116.6±5.44	134.8±1.88	132.4±2.29

Table 2

Hematological parameters of various treatment groups.

S.No	Parameter	Control	Isoproterenol	Silymarin	Quercetin
1.	Hemoglobin (mg/dl)	13.56±0.21	16.4±0.39	13.41±0.76	10.55±1.12
2.	Total Count (mm^3)	3800± 291.54	2440± 156.84	1540± 166.13	3500± 164.31
3.	Differential Count (%)				
	a) Polymorphs	30±4.29	36.6±1.28	31.6±2.16	36±1.92
	b) Lymphocytes	62.8±4.72	55±2.05	60.6±3.39	55±1.58
	c) Esinophils	2.2±0.86	4.4±1.02	2.6±0.51	5.8±0.86
	d) Monocytes	2.4±0.81	5±0.71	2.4±0.81	3±0.63
4.	Packed Cell Volume (%)	42.4±0.92	50.2±0.86	38.2±2.31	31±3.57
5.	Mean Corpuscular Hb Count (%)	31±0.32	32.8±0.37	34.4±0.51	30.8±0.37
6.	Platelet Count (lakhs/mm^3)	2.94±0.22	4.86±0.57	3.44±0.65	3.36±0.71
7.	Red Blood Cells (million/mm^3)	7.3±0.26	8.87±0.17	7.22±0.44	5.46±0.72

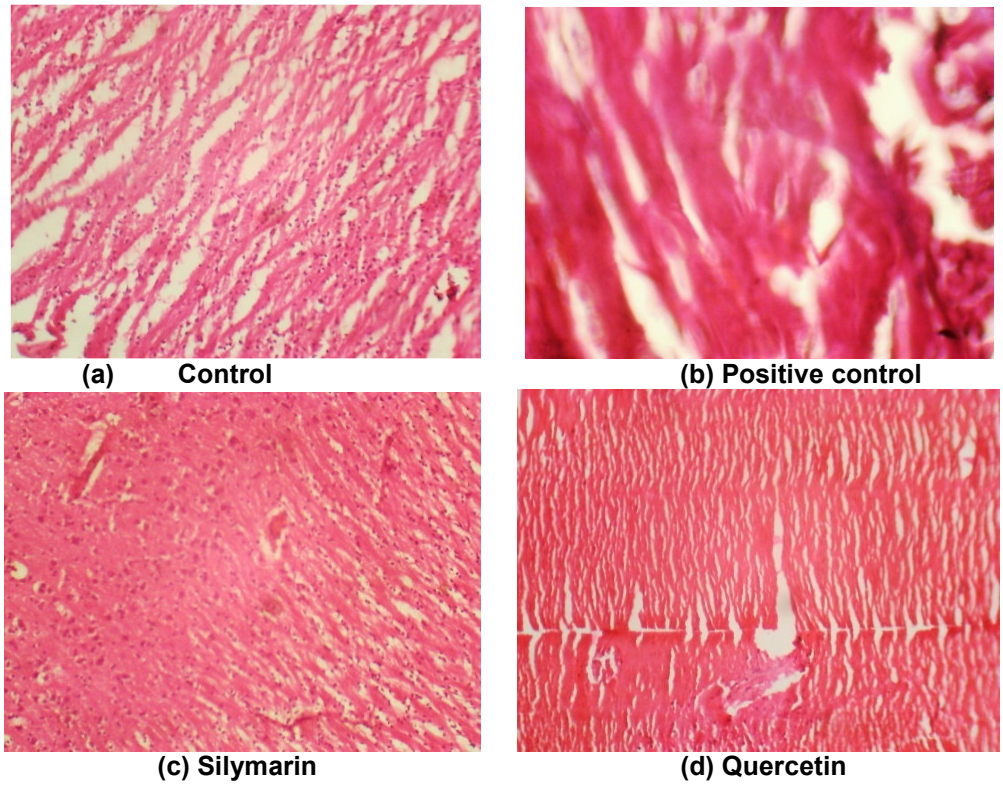

Figure 1

Histology slides of the isolated heart of various treatment groups showing haematoxylin and eosin stained cells.

DISCUSSION

Isoproterenol induced cardiotoxicity employed in the present study is one of the most widely accepted models to evaluate cardiotoxicity of the animals.[17,18,19] In the present study, isoproterenol induced cardiotoxicity showed a significant increase in the levels of blood biochemical parameters except sodium. By administration of isoproterenol, the sodium levels were decreased ($p<0.002$) and also showed significant increase in hematological parameters except lymphocytes ($p <0.271$). In the present investigation, pretreatment of silymarin against isoproterenol induced cardio toxicity with attenuation of isoproterenol associated increase in levels of blood biochemical and hematological parameters. The blood biochemical parameters showed significant difference when compared with isoproterenol except in triglycerides, cholesterol and in total proteins. Hematological parameters showed no significant difference when compared with isoproterenol except total count and packed cell volume. The total count showed a significant decrease ($p<0.0001$) and packed cell volume showed significant decrease ($p<0.001$) when compared to isoproterenol. In hematological parameters silymarin showed no significant difference against quercetin except mean corpuscular hemoglobin count (MCHC). The significant difference of MCHC against quercetin was $p<0.0001$. In blood biochemical parameters the silymarin showed significant difference in glucose ($P<0.0003$), calcium ($p<0.0004$) and triglycerides ($p<0.0002$).The blood biochemical parameters of quercetin showed significant difference when compared with isoproterenol except calcium, cholesterol ($p<0.2439$) and cholesterol ($p<0.124$), it showed no significant difference when compared with isoproterenol. Hematological parameters showed significant difference when compared with isoproterenol except hemoglobin ($p<0.9916$), platelet count ($p< 0.1413$) and differential count ($p<0.4419$). The histopathological slides of quercetin showed the significant recovery of heart tissue. Therefore, from the above findings, it is evident that quercetin abolished isoproterenol-induced cardio toxicity. The antioxidant property of flavonoids was thought, until relatively recently, to underlie the majority of their protective cellular effects. However, it is becoming increasingly apparent that flavonoids also influence cellular function by modulating the activity of many enzymes including the inhibition of protein kinases and lipid kinases. Therefore, it may be concluded that quercetin exerts its beneficial effect in isoproterenol-induced cardio toxicity by virtue of its ability to prevent the oxidation of low density lipoproteins, antioxidative, anti-hypercholesterolemia and anti-inflammatory actions which is in relation with the earlier pharmacological studies reported for the flavonoid.[5,6,7] Nevertheless, further studies are needed to explore the full potential of quercetin in cardio protective effect.

CONCLUSION

From the obtained results it may be concluded that quercetin exerted a significant cardioprotective effect against isoproterenol induced cardiotoxicity in rats than silymarin ($p<0.001$) for most of the blood biochemical parameters, mean corpuscular hemoglobin count as well in attenuation of pathological changes in cardiac tissues.

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