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Research Article

PHARMACODYNAMIC INTERACTION OF QUERCETIN AND SILYMARIN AGAINST CYCLOPHOSPHAMIDE INDUCED CARDIO-TOXICITY

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ABSTRACT

The study was designed to investigate the cardioprotective effect of different combination of Silymarin and Quercetin against Cyclophosphamide induced cardiotoxicity in experimental rats. The Albino rats of either sex were divided into five groups of six animals. Group 1 and group 2 served as normal and control group respectively. Other groups treated with Silymarin (60 mg/kg, *p.o.*) alone, high (30 mg/kg, *p.o.*) and low (15 mg/kg, *p.o.*) dose of Silymarin combined with Quercetin (10 mg/kg, *p.o.)*. Myocardial damage was induced by the administration of Cyclophosphamide (200mg/kg, *i.p.*) on the first day and the treatment was conducted for ten days. Twenty-four hours after the last administration, the blood was collected by the retro-orbital puncture method and the serum biomarker level of Lactate Dehydrogenase (LDH), Creatinine Kinase-NAC (CK-NAC), Creatinine Kinase-MB (CK-MB), Aspartate Transaminase (AST), Alanine Transaminase (ALT) and Alkaline Phosphatase (ALP) in serum were evaluated. Electro-cardiograph parameters such as Heart rate, QRS interval, QT segment, PR interval and RR interval also carried out. Compared to the control group all treated group showed significant activity and high and low dose combination group showed profound cardiac protection compare to the Silymarin alone. Among the entire groups high dose combination group (SILLY30+QUE10) showed most effective Cardio-protection and was also responsible for the significant improvement in the ECG parameters.

Keywords: Cardioprotective, Silymarin, Quercetin, Cyclophosphamide.

INDRODUCTION

The heart is a hollow muscular organ of paramount interest which is responsible for pumping oxygenated blood to the different parts of the body and collects the deoxygenated blood through blood vessels for purification by repeated, rhythmic contractions.[1]Because of change in life style there has been an increase in the incidence of heart disease in developed and developing countries. The cardio-toxicity of a drug depends on many different factors related to the drug itself and to the individual patient. The major causes of cardiovascular disease are atherosclerosis, diabetics, antibiotics and cytotoxic drugs leading side effects.[2] The patients with cardiac ischemia, heart failure mild to moderate level hypokalemia are the major cause to the development of cardiac arrhythmias.[3]

Plants based study was used for cardioprotective in traditional Indian medicinal system because of their significant potency and lesser side effect. Herb-drug interaction is produce better effect than the drug-drug interaction due to the presence of more than one single constituent in the herb.[4] Silymarin (SILY) isolated from dried seeds of *Silybum marianum.L.Gaertin* belongs to the family Asteracease *(*compositae*).*[5] It is having potential anti-oxidant activity responsible for various types of action**.** But the problem associated with Silymarin is its poor oral bioavailability.[6] Quercetin(QUE) is a bioactive flavonoid, present in various fruits and vegetables. Quercetin is having wide range of actions due to potential scavenging of oxygen free radicles.[7]Apart from that Quercetin can act as bio enhancer by inhibiting P-gp efflux pump. P-gp inhibitors reverse P-gp-mediated efflux and thus improve the efficiency of drug transport across the membrane, thus resulting in enhanced oral bioavailability and also inhibit some of the enzymes involved in drug metabolism.[8]

Anti-cancer drugs like Cyclophosphamide, Doxorubicin, Epirubicin, Cisplatin are responsible for remarkable damage of cardio-myocyte as a side effect.[9] Cyclophosphamide (CP) belongs to class of alkylating agent responsible for impairment of cellular respiration and also damages the inner mitochondrial membrane of heart leading to the permeability of calcium ions mediated by oxidative stress.[10] CP induced cardio toxicity has been implicated to increase the generation of superoxide radicals and hydrogen peroxide. CP has a small therapeutic index and is associated with significant toxicity. Many case studies report that CP induced cardio toxicity is associated with systemic endothelial damage.[11]

The present study was aimed to investigate the cardioprotective effect of different combination of Silymarin (SILY) and Quercetin (QUE) against Cyclophosphamide (CP) induced cardiotoxicity in experimental rats.

MATERIALS AND METHODS

Pure Quercetin was procured from Yucca enterprises, Mumbai, India. Based on earlier literature review, therapeutic dose of Silymarin is 60mg/kg exerting most of its pharmacological activity without any toxic effects.[12]Quercetin increases bioavailability of most of compounds at the dose of 10mg/kg.[13]So the same dose of were selected. A suspension of Quercetin and Silymarin was

prepared using distilled water and administered orally using gastric incubation using a force feeding needle.

Animals

Experimental rats of either sex weighing 150-250g were housed at 25° ± 5°C, relative humidity 50 ± 5% in a well-ventilated animal house under 12:12 h light dark cycle. All the rats were provided with commercially available standard pellet diet, water *ad libitum*. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in an animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA). The Institutional ethical committee approved the experimental protocol (SDCP/IAEC-02/2013-14).

Chemicals

Cyclophosphamide (ZydusOncosciences, Ahmedabad, India), Ketamine Hcl (Neon Pharmaceutical Ltd, India),Saline (Merck Specialties Pvt Ltd, Mumbai, India), SGPT kits (Robonik India Pvt Ltd, Mumbai), SGOT kits (Robonik India Pvt Ltd, Mumbai)ALP kits (Robonik India Pvt Ltd, Mumbai), CK-MB kits (Lab-Care diagnostic, PvtLtd,Mumbai), CK-NAC kits(Lab-Care diagnostic, PvtLtd,Mumbai), LDH kits (Accurex, India), Xylazine (Indian Immunological, Guntur, India)

Cyclophosphamide Induced Cardiotoxicity:

The Albino rats of either sex were divided into five groups of six animals each. Animals were treated with single injection of Cyclophosphamide (CP) (200 mg/kg) by intra-peritoneal route on first day of experimental period to induce cardiotoxicity and treatments were continued for 10 days.[9]

1 Group-I (Normal control) – Saline for 10 days.
2 Group-II (CP control)-CP (200mg/kg, i.p.) on the first day of experimental period.
3 Group-III (SILY60)- Silymarin (SILY60mg/kg, p.o) for 10 days +CP (200 mg/kg, i.p.) on the first day of experimental period.
4 Group-IV(SILY30+QUE10)-Silymarin (SILY-30mg/kg, p.o) + Quercetin (QUE-10mg/kg, p.o) for 10 days + CP (200 mg/kg, i.p.) on the first day of experimental period.
5 Group-V(SILY15+QUE10)- Silymarin (SILY-15mg/kg, p.o) + Quercetin (QUE-10mg/kg, p.o) for 10 days + CP (200 mg/kg, i.p.) on the first day of experimental period

Group I and Group II rats were treated with Saline for 10 days by intra-peritoneal route and served as normal control and CP control respectively. Group III rats treated with Silymarin (SILY-60mg/kg, p.o), Group IV treated with Silymarin (SILY-30mg/kg, p.o) + Quercetin (QUE-10mg/kg, and Group V rats treated with Silymarin (SILY-15mg/kg, p.o) + Quercetin (QUE-10mg/kg, p.o) dissolved in Saline for 10 days.

Groups II, III, IV &V were treated with single injection of Cyclophosphamide (CP) (200 mg/kg) by intra-peritoneal route on first day of experimental period to induce cardiotoxicity.

Estimation of ECG Parameters

24 hour after the last treatment, the animals were anesthetized with the combination of ketamine (75 mg/ kg, *i.p.*) and xylazine (8 mg/kg, *i.p.*). Leads were attached to the dermal layer of both the front paws and hind legs and recordings were made with the help of digitalphysiograph (Instruments & Chemical Pvt Ltd, Haryana, India). The changes in heart rate, QRS interval, QT interval PR interval and RR interval were determined.

Estimation of biochemical parameter

24 hour after the last administration blood was collected by retro-orbital puncture and the serum was separated by centrifugation at 5000 rpm at 10 min. The isolated serum was subjected for assay of marker enzyme namely Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Creatine kinase isoenzyme MB (CK-MB), Creatinine kinase N-acetyl cysteine (CKNAC) and Lactate Dehydrogenase (LDH) by using automated analyzer(Robonik, Mumbai).

Statistical analysis

Results are expressed as mean ± SE. Statistical significance was assessed using One-way Analysis of variance (ANOVA) followed by Tukey-Karmer multiple comparison tests. $P<0.05$ was considered significant.

RESULTS

Effect on electrocardiographic parameters

As shown in table 1, CP control group reported significant increase in QRS interval, QT segment, RR and PR interval and significant decrease in heart rate compared to normal control. The entire treated group rectified CP induced changes in ECG to normal in dose dependent manner. Both high (SILLY30+QUE10) and low (SILLY15+QUE10) dose combination groups documented significant effect compared to Silymarin alone treated group. Among all the groups high dose combination group was found to be most effective group. (Table 1)

Effect on Serum enzyme biomarkers

As shown in the table 2, It was documented that the CP control demonstrated an extremely significant increase **(**$p<0.001$**)** serum AST, ALT, ALP, CK-MB, CK-NAC, LDH values compared to normal control. All treated group showed significant decrease in the serum enzyme biomarker when compared to CP control group. It was also documented that both high (SILLY30+QUE10) and low (SILLY15+QUE10) dose combination groups showed significant increase in serum biomarker levels compared to Silymarin alone treated group. Among all the groups high dose combination group was found to be most effective group. (Table 2)

DISCUSSION

The present study was aimed to investigate the cardioprotective effect of different combination of Silymarin (SILY) and Quercetin (QUE) against Cyclophosphamide (CP) induced cardiotoxicity in experimental rats.

CP is leads to destruction of myocardial cells because of their direct detrimental effect on myocardial endothelial cell. Back bone molecular mechanisms behind the CP induced cardio-toxicity is increase in myocardial xanthine oxidase activity. Xanthine oxidase catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid. These are the main sources for the generation of reactive oxygen species (ROS). Thus, CP is associated with increase in free radical production and decrease in antioxidant enzymes. The supporting mechanism includes Cyclophosphamide directly release significant amount of Acetyl choline **(**Ach**)** in to the heart and decrease level of anti-oxidant enzymes**.** During the myocardial damage serum biomarker enzymes like Aspartate transaminase (AST), Alanine transaminase (ALT), Alkaline phosphatase (ALP), Creatine kinase isoenzyme MB (CK-MB), Creatinine kinase N-acetyl cysteine (CKNAC) and Lactate Dehydrogenase (LDH) will leakage into serum results

elevating the serum concentration. Estimation of these marker enzymes serves as a diagnostic tool to detect myocardial necrosis.
The present study revealed that Compared to the control group all treated group showed restored the marker enzymes levels and high and low dose combination group showed significant cardiac protection compare to the Silymarin alone. Among the entire groups high dose combination group (SILLY30+QUE10) showed most effective Cardioprotection.
In the present study, CP showed abnormal changes in ECG pattern such as decrease in heart rate and increase in RR, PR and QT intervals and prolongation of QRS interval. Decreased heart rate observed in CP treated animals could be due to release of significant amount of acetylcholine (Ach) which is also linked with the genesis of myocardial damage.
Prolongation of QT interval found in present study with CP may be due to increase in the cellular Na+ content and decrease in K+ content. Hypokalemia is suspected for QT interval elongation. Prolongation of PR interval associated with CP might be due to AV block. Change in parasympathetic tone and conduction system deformation can be the reason for AV block.
All the treated group bring back the ECG parameters towards the normal. Combination of Silymarin and Quercetin group showed significant restores the ECG patterns compared to Silymarin alone group. Among the entire groups high dose combination group (SILLY30+QUE10) showed most effective Cardioprotection.

Table 1: Effect of drugs on Heart Rate and Electrocardiographic parameters in cyclophosphamide induced cardiotoxicity

Treatment	Heart rate	QRS duration (ms)	QT segment (ms)	RR interval (ms)	PR interval (ms)
Normal control	183.52±4.27	120.28±3.52	121.90±5.95	192.80±3.88	79.38±3.40
CP control	88.12±4.02***	267.78±4.64***	244.45±5.91***	303.86±3.36***	178.51±5.50***
SILY60	141.78±1.71***###	213.40±3.16***###	190.45±2.27***###	269.19±1.77***###	141.03±2.87***###
SILY30+QUE10	162.87±2.61**##00	175.82±3.34***###000	143.08±2.27*###000	232.19±5.55***###000	98.52±3.91*###000
SILY15+QUE10	152.81±1.80***###	194.50±3.21***###0	155.61±2.97**###000	252.80±4.84***###	119.36±3.82***##0

All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to CP control and 0P< 0.05, 00P< 0.01,000 P<0.001 when compared to Silymarin (60mg/kg).CP- Cyclophosphomide, SILY- Silymarin, QUE-Quercetin.

Table 2: Effect of drugs on serum level of AST, ALT, ALP, CK-MB, CK-NAC and LDH in Cyclophosphamide induced cardio toxicity

Treatment	Blood Serum level in IU/L					
	AST	ALT	ALP	CKMB	CKNAC	LDH
Normal control	45.87±4.19	36.58±3.04	44.24±3.39	95.68±4.22	72.07±3.85	296.90±4.56
CP control	212.94±6.26***	146.80±5.65***	215.51±4.37***	222.62±5.73***	193.79±3.49***	676.22±4.10***
SILY60	83.79±2.79***###	91.19±5.26***###	177.68±5.58***###	143.62±5.12***###	165.94±6.89***##	385.16±6.37***###
SILY30+QUE10	61.86±1.50*###00	64.86±7.55**###0	140.79±2.87***###000	120.05±2.62**###00	134.79±5.25***###00	350.43±5.07***###00
SILY15+QUE10	71.01±1.44**###0	76.55±6.44**###	154.46±3.71***###00	129.80±2.87***###0	144.11±6.33***###0	364.91±3.44***###0

All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to Normal control, ###P< 0.001 compared to CP control and 0P< 0.05, 00P< 0.01, 000P<0.001 when compared to Silymarin (60mg/kg). CP- Cyclophosphomide, SILY- Silymarin, QUE-Quercetin.

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