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Research Article

EFFECT OF QUERCETIN ON HEPATOPROTECTIVE ACTIVITY OF SILYMARIN AGAINST THIOACETAMIDE INTOXICATED RATS

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ABSTRACT

The study was designed to investigate the interactive effect of Quercetin on hepatoprotective effect of Silymarin against thioacetamide induced hepatotoxicity in rats. Albino rats of either sex were divided into six groups and treated for 7 days. Group 1 and 2 served as normal and toxic control and other groups were treated with Silymarin (100 mg/kg), Quercetin (100 mg/kg), high and low dose combination of Silymarin (100 and 50 mg/kg) + Quercetin (100 and 50 mg/kg) respectively. Liver damage was induced in by administering thioacetamide (100 mg/kg, s.c) on 7th day. 48 h after the administration of thioacetamide blood samples were collected by retro-orbital puncture method and levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase and bilirubin in serum were evaluated. Histopathology of liver was also carried out. The combination of Silymarin (100 mg/kg) + Quercetin (100 mg/kg) demonstrated significant reduction in enzyme and bilirubin level in serum compared to Silymarin (100 mg/kg) alone treated group which was supported by histopathological study. It can be concluded that Quercetin has got a synergistic effect on hepatoprotective action of Silymarin against thioacetamide induced hepatotoxicity.

Keywords: Hepatoprotective, Silymarin, Quercetin, Thioacetamide, Histopathology.

INTRODUCTION

The liver is one of the major organs in the body responsible for maintaining the homeostasis of body. However liver is one of the most frequently injured organs in the body. Most of the synthetic drugs used in liver disease are less effective or has got serious side effects. Plant based drugs with their significant potency and lesser side effects are alternative therapeutic option[1]. Silymarin, which is the oldest and safest hepatoprotective drug, is a mixture of flavonolignans obtained from the seeds of *Silybum marianum*. Even though Silymarin is a potent antioxidant it has got poor oral bioavailability[2]. Quercetin, a bioactive flavonoid present in various edible fruits and vegetables, is a potent antioxidant and exhibits a wide range of biological functions[3]. Quercetin being a P-gp inhibitor reverse P-gp-mediated efflux and thus improves the efficiency of drug transport across the epithelia and also being able to inhibit some of the enzymes involved in drug metabolism increases the bioavailability, blood levels and efficacy of number of drugs[4]. The pathological lesions produced by hepatotoxins (like alcohol, thioacetamide, CCl4, etc) may be similar to many forms of liver disease, which helps in evaluation of potential hepatoprotectants[5]. Therefore the present study was carried out to evaluate the interactive effect of Quercetin in hepatoprotective activity of Silymarin against thioacetamide (TAA) induced hepatotoxicity in rats.

MATERIALS AND METHOD

Procurement of Quercetin and its dose selection

Pure quercetin was procured from Yucca Enterprises, Mumbai, India in the month of December, 2012. Based on earlier reported study the high and low dose of Quercetin[6] and Silymarin[7,8] was selected as 100 mg/kg p.o. and 50 mg/kg p.o. respectively. A suspension of Quercetin and Silymarin was prepared using Carboxy methyl cellulose (1%) and administered orally using gastric intubation using a force feeding needle.

Animals

Albino rats of either sex weighing 150-250 g were housed at 25° ± 5°C, relative humidity 50 ± 5% in a well-ventilated animal house under 12:12 h light dark cycle. Institutional Animal Ethics Committee approved the experimental protocol. The animals were maintained under standard conditions in the animal house as per the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Chemicals

Sodium chloride (RFCL Limited, New Delhi, India), Carboxy Methyl Cellulose (PRS Pharma, Private Limited, Salem, India), Thioacetamide (Sigma Aldrich, U.S.A), ketamine (Neon Labs, India) all the chemicals used was of analytical grade and the enzyme kits used were ALT (Robonik India Pvt Ltd, Mumbai, India), AST (Robonik India Pvt Ltd, Mumbai, India), ALP (Robonik India Pvt Ltd, Mumbai, India), Bilirubin (total and direct) (Robonik India Pvt Ltd, Mumbai, India).

Thioacetamide induced liver necrosis in rats

Rats were divided into six groups of six animals each. The animals were then subjected to either one of the following treatments for 7 days. On 7th day thioacetamide (TAA) was administered.[9]

The groups were as follows;

Group I- Vehicle; 1 ml/250 g, p.o. (Normal control)

Group II- TAA (100 mg /kg, s.c.) (Toxic control)

Group III- Silymarin (100 mg/kg/day, p.o.) for 7 days + TAA (100 mg/kg, s.c.) on seventh day. (Standard)

Group IV- Quercetin (100 mg/kg/day, p.o.) for 7 days + TAA (100 mg /kg, s.c.) on seventh day.

Group V- Silymarin (50 mg/kg/day, p.o.) + Quercetin (50 mg/kg/day, p.o.) for 7 days + TAA (100 mg /kg, s.c.) on seventh day.

Group VI- Silymarin (100 mg/kg/day, p.o.) + Quercetin (100 mg/kg/day, p.o.) for 7 days + TAA (100 mg /kg, s.c.) on seventh day.

Estimation of biochemical parameters

48 h after the administration of thioacetamide, blood samples were collected by retro-orbital puncture method into plain tubes and then serum was isolated by centrifuging at 5000 rpm for 10 min. The isolated serum was then subjected for the assay of marker enzymes, namely, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and serum bilirubin (total and direct) levels using reagent kits of Robonik (India) private limited and by using a automated analyzer.

Histopathological study

After the blood samples were collected, animals were sacrificed and the liver from each group were isolated and preserved in 10% formalin solution. After paraffin embedding, tissues were sectioned and stained with hematoxylin and eosin (HandE) for observing microscopic changes in the liver.

Statistical Analysis

Results are expressed as Mean ± SEM. Statistical significance was assessed using One-Way Analysis of Variance (ANOVA) followed by Turkey-karmer multiple comparison test. P<0.05 was considered significant.

Table 1: Effect of Silymarin and Quercetin on serum ALT, AST, ALP, bilirubin (total and direct) in TAA induced liver necrosis in rats

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Vehicle control	61.48 ± 3.36	144.96 ± 3.97	212.60 ± 4.33	15.93 ± 1.10	0.33 ± 0.01
Toxic control (TAA)	514.07 ± 9.65***	859.66 ± 9.16***	1095.83 ± 5.98***	65.78 ± 2.48***	75 ± 0.02***
Silymarin (100 mg/kg)	120.43 ± 2.71***000	251.03±10.65***000	294.30 ± 6.95***000	32.73 ± 0.81***000	51 ± 0.01***000
Quercetin (100 mg/kg)	97.66 ± 3.52**000	210.46±8.19***000	235.00 ± 2.88000	24.81 ± 0.89**000	0.37 ± 0.01000
Silymarin(50 mg/kg) + Quercetin(50 mg/kg)	110.00 ± 2.88***000	253.00 ± 5.82***000	300.00± 5.77*** 000	32.10 ± 0.63***000	0.47 ± 0.00*** 000
Silymarin(100 mg/kg) + Quercetin(100 mg/kg)	78.33± 4.05000###	152.04±6.05000###	214.00 ± 2.08000###	18.45 ± 0.58000###	0.28± 0.00000###

All values are mean ± SEM, n=6, *P< 0.05, **P< 0.01, ***P< 0.001 when compared to vehicle control, 000P< 0.001 compared to toxic control and ###P<0.001 when compared to Silymarin (100 mg/kg)

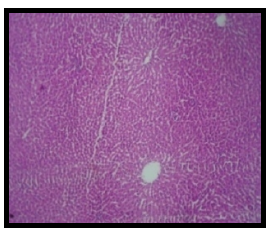

Figure 1a: Vehicle control
Normal texture of liver tissue

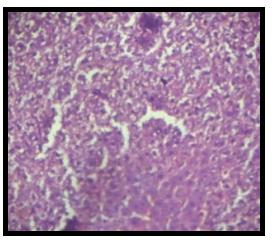

Figure 1b: Toxic control (TAA 100 mg/kg)
Severe tissue degranulation

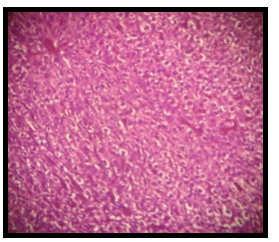

Figure 1c: Silymarin (100mg/kg)
Mild to moderate liver tissue degranulation

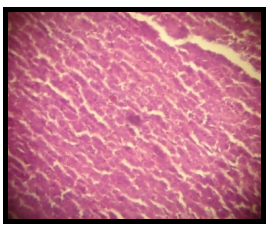

Figure 1d: Silymarin (100 mg/kg) + Quercetin (100 mg/kg)
Mild tissue degranulation

Figure 1: Haematoxylin and eosin (Hand E) stained section of liver in TAA induced acute liver toxicity. Photographed at magnification 100X

RESULTS

As shown in Table 1, it was documented that the toxic control demonstrated an extremely significant increase (p<0.001) in serum ALT, AST, ALP and bilirubin levels when compared to normal control indicating liver damage. All other prophylactic groups showed extremely significant (P<0.001) decrease in biomarker level when compared to toxic control group. It was also documented that biomarkers levels reduced significantly in the group pre-treated with combination of Silymarin (100 mg/kg) + Quercetin (100 mg/kg) compared to Silymarin (100 mg/kg) alone treated group.

DISCUSSION

The present study was aimed to investigate the possible interaction of Quercetin in hepatoprotective effect of Silymarin in thioacetamide induced hepatotoxicity in rats. Hepatotoxins initially damage the centrilobular regions of liver where there are high levels of cytochrome P450 oxidases which mediate their conversion to toxic intermediates, followed by reactive oxygen species (ROS) production, lipid peroxidation and release of pro-inflammatory cytokines Thioacetamide (TAA) induces liver damage after its metabolism to thioacetamide sulphene and sulphone, via CYP4502E1-mediated biotransformation. TAA thus cause centrilobular necrosis followed by apoptosis and periportal inflammatory cell infiltration in rat liver. TAA generates reactive oxygen species (ROS) by binding covalently to liver macromolecules. During hepatic damage cellular enzymes like Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP) and bilirubin will leak into the serum resulting in elevating their serum concentrations. Serum levels of these enzymes are very sensitive markers employed in the diagnosis of liver diseases.[5] Documented results suggested that the combination of Silymarin (100 mg/kg) and Quercetin (100 mg/kg) has more potent hepatoprotective effect than other prophylactic groups which is evident by the reduction in the elevated marker enzyme level which is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damages caused by thioacetamide. The exact mechanism for the enhanced activity is not known. However

the enhanced activity could be due to the ability of Quercetin to inhibit P-gp efflux pump and some of the enzymes involved in drug metabolism.[4] this enhanced hepatoprotective effect was also supported by histopathological report.

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