

PHARMACODYNAMIC INTERACTION OF QUERCETIN WITH SILYMARIN AGAINST PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

Objective: To investigate the interactive effect of Quercetin on hepatoprotective effect of Silymarin against paracetamol induced hepatotoxicity in rats.

Method: Albino rats of either sex were divided into six groups and treated for 5 days. Group 1 and 2 served as normal and toxic control and other groups were treated with Silymarin(100 mg/kg), Quercetin (100mg/kg), high and low dose combination of Silymarin (100 and 50mg/kg) + Quercetin (100 and 50mg/kg) respectively. On 5th day paracetamol (2 g/kg, *p.o.*) was administered after dilution with 40% w/v sucrose. 48 hrs after the administration of paracetamol, blood samples were collected by retro-orbital puncture method and levels of alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase(ALP) and bilirubin in serum were evaluated. Histopathology of liver was also carried out.

Results: The combination of Silymarin(100mg/kg) + Quercetin(100mg/kg) has got more potent hepatoprotective activity than Silymarin(100mg/kg) alone treated group. This was evident from the reduction in elevated serum enzyme and bilirubin levels. This was also supported by histopathological studies of liver sections.

Conclusion: From the present study it can be concluded that Quercetin has got a synergistic effect on hepatoprotective action of Silymarin against paracetamol induced hepatotoxicity .

Keywords: Hepatoprotective, Silymarin, Quercetin, Paracetamol, Histopathology, Retro-orbital puncture

INTRODUCTION

The liver is the largest internal organ in our body and plays an important role in regulating the homeostasis of the body. However because of its complex nature, it is susceptible to many adverse effects from wide variety of hepatotoxins, hence liver diseases remain one of the major threats to public health and are a worldwide problem. In spite of the tremendous development in modern medicine, most of the synthetic drugs used in liver disease are less effective or has got serious side effects. Hence plant based drugs with their significant potency and lesser side effects is an alternative therapeutic option for liver diseases.[1,2]

Silymarin is a mixture of flavonolignans obtained from *Silybum marianum*. Even though it is a potent antioxidant and safest hepatoprotective drug, the problem associated with its use is poor oral bioavailability, hence dose of silymarin given needs to be large in order to achieve therapeutic plasma levels.[3] Quercetin is a bioactive flavonoid present in various edible fruits and vegetables and is a potent antioxidant and exhibits a wide range of biological functions.[4] Quercetin improves blood levels and efficacy of number of drugs since it is P-gp inhibitor and also inhibits drug metabolising enzymes.[5]

Therefore the present study was carried out to evaluate the interactive effect of Quercetin in hepatoprotective activity of Silymarin against paracetamol 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 100mg/kg *p.o.* and 50mg/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 150-250g were used for the study. The animals were randomized into experimental, normal and toxic

groups, housed individually in sanitized cages containing paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. The approval of Institute Animal Ethics Committee was obtained. 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), Paracetamol (National chemicals, Vadodara, Gujarat), Sucrose (Merck specialities Pvt Ltd, Mumbai), ketamine (Neon Labs, India) all the chemicals used was of analytical grade and the enzyme kits used were ALT (Robonik India Pvt Ltd, Mumbai), AST (Robonik India Pvt Ltd, Mumbai), ALP (Robonik India Pvt Ltd, Mumbai), Bilirubin (total and direct) (Robonik India Pvt Ltd, Mumbai).

Paracetamol (PCM) induced liver toxicity in rats[9]

The animals were divided into 6 groups consisting of six animals each. The animals were then subjected to either one of the following treatments for 5 days. The PCM (2 g/kg, *p.o.*) was administered after dilution with 40% w/v sucrose on day 5. Food was withdrawn 12 hours before paracetamol administration to enhance liver damage in animals of all groups except normal control. 48 hrs after the administration of paracetamol, blood samples were collected by retro-orbital puncture method and levels of alanine aminotransferase(ALT), aspartate aminotransferase(AST), alkaline phosphatase(ALP) and bilirubin in serum were evaluated. Then the animals were sacrificed and the liver from each group were isolated and subjected for histopathological studies.

The treatment groups were as follows;

1. Group I- Vehicle; 1ml/250 g, *p.o.* (Normal control)
2. Group II- PCM (2g/kg, *p.o.*) (Toxic control)
3. Group III- Silymarin (100 mg/kg/day, *p.o.*) for 5 days + PCM (2 g/kg, *p.o.*) on 5th day. (Standard)
4. Group IV- Quercetin (100mg/kg/day, *p.o.*) for 5 days+ PCM (2g/kg, *p.o.*) on 5th day

5. Group V- Silymarin (50mg/kg/day, *p.o.*) + Quercetin (50mg/kg/day, *p.o.*) for 5 days + PCM (2g/kg, *p.o.*) on 5th day
6. Group VI- Silymarin (100mg/kg/day, *p.o.*) + Quercetin (100mg/kg/day, *p.o.*) for 5 days + PCM (2g/kg, *p.o.*) on 5th day

Estimation of biochemical parameters

48hrs after the administration of PCM, blood samples were collected by retro-orbital puncture method into plain tubes and then serum was isolated by centrifuging at 5000rpm for 10min. 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 (H&E) for observing microscopic changes in the liver.

Statistical Analysis

Results are expressed as Mean \pm 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.

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(100mg/kg) + Quercetin(100mg/kg) compared to Silymarin (100mg/kg) alone treated group.

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

Treatment	ALT (U/L)	AST (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Direct Bilirubin (mg/dl)
Vehicle control	61.48 \pm 3.36	144.96 \pm 3.97	212.60 \pm 4.33	15.93 \pm 1.10	0.33 \pm 0.01
Toxic control(PCM)	315.48 \pm 3.05***	497.66 \pm 6.69***	410.90 \pm 2.74***	45.87 \pm 1.61 ***	11.12 \pm 0.56***
Silymarin (100mg/kg)	152.10 \pm 4.34***[00] ⁰	258.33 \pm 4.41***[00] ⁰	262.73 \pm 4.47***[00] ⁰	27.53 \pm 0.86***[00] ⁰	2.54 \pm 0.15***[00] ⁰
Quercetin(100mg/kg)	112.16 \pm 6.67***[00] ⁰	202.66 \pm 5.36***[00] ⁰	230.80 \pm 1.32*[00] ⁰	20.27 \pm 0.89[00] ⁰	0.12 \pm 0.01[00] ⁰
Silymarin(50mg/kg)+ Quercetin(50mg/kg)	148.44 \pm 1.78***[00] ⁰	245.00 \pm 2.88***[00] ⁰	259.25 \pm 2.43***[00] ⁰	26.13 \pm 0.46***[00] ⁰	2.08 \pm 0.15**[00] ⁰
Silymarin(100mg/kg)+ Quercetin(100mg/kg)	77.25 \pm 2.58[00] ^{0###}	175.00 \pm 2.88**[00] ^{0###}	203.46 \pm 4.28[00] ^{0###}	17.44 \pm 0.73[00] ^{0###}	0.38 \pm 0.03[00] ^{0###}

All values are mean \pm SEM, n=6,* $P < 0.05$, ** $P < 0.01$,*** $P < 0.001$ when compared to vehicle control, [00]⁰ $P < 0.001$ compared to toxic control and ### $P < 0.001$ when compared to Silymarin(100mg/kg).

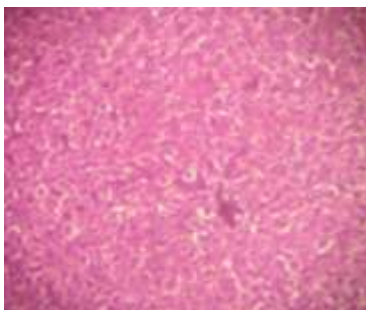


Fig. 1a: Vehicle control.
1. Normal texture of liver tissue.

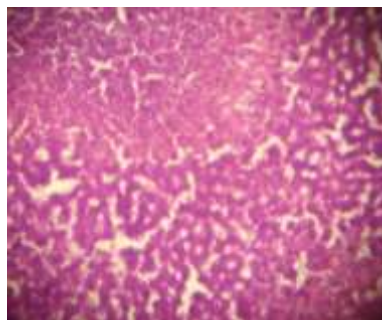


Fig. 1b: Toxic control (PCM)
1. Severe tissue degranulation.

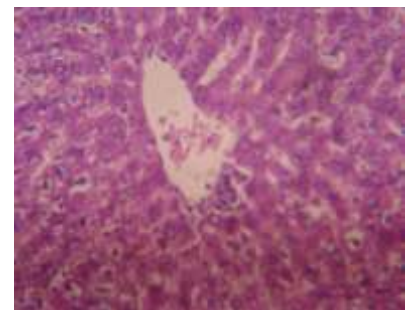


Fig. 1c: Silymarin(100mg/kg).
1. Mild to moderate liver tissue degranulation.

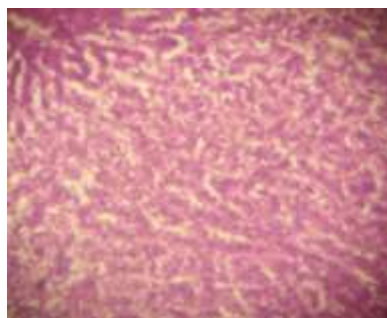


Fig. 1d: Silymarin(100mg/kg) + Quercetin(100mg/kg)
1. Mild tissue degranulation

Fig. 1: Haematoxylin and eosin (H&E) stained section of liver in PCM induced liver toxicity. Photographed at magnification 100X

DISCUSSION

The present study was aimed to investigate the possible interaction of Quercetin in hepatoprotective effect of Silymarin in paracetamol (PCM) induced hepatotoxicity in rats.

In case of paracetamol induced hepatotoxicity, the liver damage is due to its toxic metabolite. Paracetamol is normally eliminated mainly as sulfate and glucuronide. Upon administration of toxic doses of paracetamol the sulfation and glucuronidation routes become saturated and hence, higher percentage of paracetamol molecules is oxidized to highly reactive N-acetyl-p-benzoquinoneimine (NAPQI) by cytochrome-450 enzymes. Semi Quinone, a radical obtained by one electron reduction of NAPQI, can covalently bind to macromolecules of cellular membrane and increases the lipid peroxidation resulting in the tissue damage. Higher dose of paracetamol and NAPQI can alkylate and oxidize intracellular GSH and protein thiol group, which results in the depletion of liver GSH pool subsequently, leads to increased lipid peroxidation and liver damage. PCM treatment significantly increased the serum enzyme levels, namely ALT, AST and ALP indicating chemical induced hepatocellular toxicity. When the hepatocellular plasma membrane is damaged, the enzymes normally present in the cytosol are released into the blood stream. This can be quantified to assess the type and extent of liver injury. The liver injury due to toxins can result in defective excretion of bile by hepatocytes which are reflected as their increased levels in serum.[10,11]

Documented results suggested that the combination of Silymarin (100mg/kg) and Quercetin (100mg/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 PCM. 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. Quercetin being a P-gp inhibitor reverse P-gp-mediated efflux and thus improves the efficiency of drug transport across the epithelia. Quercetin also inhibits some of the enzymes involved in drug metabolism and this can also result in enhanced activity.[5] This enhanced hepatoprotective effect was also supported by histopathological report.

CONCLUSION

With the findings of the present study it can be concluded that the combination of Silymarin (100mg/kg) and Quercetin (100mg/kg) has demonstrated significant increase in hepatoprotective effect against Paracetamol (PCM) induced hepatotoxicity in rats compared to Silymarin (100mg/kg) alone treated group. The reported activity may be due to improved bioavailability of Silymarin and Quercetin effect. The improvement in the bioavailability of Silymarin by

Quercetin can be due to inhibition of P-gp efflux pump, thus resulting in enhanced oral bioavailability. The enhanced activity could also be due to the contribution of Quercetin to decrease the metabolism of Silymarin by inhibiting some of the enzymes involved in drug metabolism.

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