

Hepatoprotective Activity Of Some Quercetin Derivatives

C Roy, A Das

Citation

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Abstract

The study was designed to evaluate the hepatoprotective activity of some quercetin derivatives 6-Bromoquercetin (Comp 1), 6,8-Dibromoquercetin (Comp 2), 2',5',6,8-Tetrabromoquercetin (Comp 3) and ,5',6,6',8-Pentabromoquercetin (Comp 4) in acute experimental liver injury induced by carbon tetrachloride and paracetamol. The effects observed were compared with a known hepatoprotective agent, silymarin. Four quercetin derivative was prepared by bromination, 6-Bromoquercetin (Comp 1), 6,8-Dibromoquercetin (Comp 2), 2',5',6,8-Tetrabromoquercetin (Comp 3) and ,5',6,6',8-Pentabromoquercetin (Comp 4) respectively. In the acute liver damage induced by different hepatotoxins, quercetin derivatives 6-Bromoquercetin (Comp 1) and 6,8-Dibromoquercetin (Comp 2) significantly reduced the elevated serum levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and bilirubin whereas other quercetin derivatives 2',5',6,8-Tetrabromoquercetin (Comp 3) and 2',5',6,6',8-Pentabromoquercetin (Comp 4) did not show significant reduction in elevated serum levels when compared to standard silymarin (100mg/kg body weight). Histological examination of the liver tissues supported the hepatoprotection. It is concluded that the quercetin derivatives possesses good hepatoprotective activity.

INTRODUCTION

Natural flavonoids are distinguished by the breadth of their therapeutic action and their low toxicity. They exert an influence on the enzyme and immune systems of the organism [Kubo et al.,1992, Carte BK et al., 1991] and on metabolic processes [Petrushkova AM et al. 1992], and exhibit antiarrhythmic [Babaskin VS et al. 1992], antimicrobial [Ankhwinala MD et al., 1991, Joshi V et al., 1991, Aumente Rubio AD et al. 1988], antiviral [Dombi G, 1989, Shier W et al. 1992], antioxidant [Faur M et al., 1990, Beck G et al., 1990, Budzianowski J et al. 1991], and antitumoral and radioprotective [Kabiev OK et al. 1975] properties. With the aim of expanding the arsenal of drugs with a physiological action we have carried out a directed synthesis of bromine derivatives of a natural hydroxyflavone - quercetin, 3,5,7,3',4'-pentahydroxyflavone (1).The bromination of aromatic compounds has been studied and provides a logical theoretical basis for analyzing the reaction mechanism and the structures of the compounds obtained [P. Sykes, 1970, Mabry TJ et al. 1970].

We studied bromination in organic solvents. To find the optimum conditions for obtaining mono- and dibromo derivatives of quercetin, we varied the molar ratio of quercetin and bromine from 1:1 to 1:4, the reaction temperature from 20 to 85°C, and the reaction time from 1 to

72 h. 6-Monobromoquercetin (2) was obtained in 2 h at 20-25°C by the action of an equimolar amount of bromine in dioxane, while 6,8-dibromoquercetin (3) was obtained in acetic acid at 35-40°C in 1 h. Compound (2) can be used both as the final product and as an intermediate for the introduction of new prognosticated groups. It must be mentioned that in the dynamics of the bromination of quercetin a decisive role is played by the temperature regime; in particular, elevated temperatures lead to an intensification of electrophilic substitution with the formation of a mixture of bromine derivatives. The main objective of the study is to find out the hepatoprotective potency of the synthesized derivatives.

MATERIALS AND METHODS

EXPERIMENTAL

Preparation of 6-Bromoquercetin (Derivative 1): A solution of 0.2 ml (0.0039 mole) of bromine in 10 ml of dioxane was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of quercetin (1) in 80 ml of dioxane. The reaction was continued at 20-22°C for 2 h. The resulting precipitate was washed with water and dried (Nagimova AD et al. 1996).

Preparation of 6,8-Dibromoquercetin (Derivative 2): At 35-40°C, 0.4 ml (0.0078 mole) of bromine was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of 6-

Bromoquercetin in 100 ml of glacial acetic acid. After an hour's vigorous stirring, a yellow-green precipitate deposited. The reaction mixture was concentrated, and the precipitate was washed with water and dried (Nagimova AD et al. 1996).

Preparation of 2',5',6,8-Tetrabromoquercetin (Derivative 3) and 2',5',6,6',8-Pentabromoquercetin (Derivative 4): At 75-80°C, 0.8 ml (0.0154 mole) of bromine in 40 ml of dioxane was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of 6-Bromoquercetin in 100 ml of dioxane. After eight hours' vigorous stirring, the reaction mixture was evaporated to dryness. The residue was dissolved in water and the solution was brought to a boil and filtered. On cooling, it deposited a precipitate of 2',5',6,8-Tetrabromoquercetin. The residual reaction mixture was separated by preparative paper chromatography on FN-11 paper in system 1 to get 2',5',6,6',8-Pentabromoquercetin (Nagimova AD et al. 1996).

Experimental animals - Wistar albino rats weighing 175-250 g of either sex were used. The experimental protocol was approved by the Institutional Animal Ethics Committee and animals were maintained under standard conditions in the animal house approved by Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA).

Evaluation of hepatoprotective activity - Hepatoprotective activity was evaluated using acute hepatic injury models induced by carbon tetrachloride, paracetamol or thioacetamide. The effect on chronic hepatic injury was evaluated using carbon tetrachloride induced chronic liver damage model.

ACUTE HEPATITIS MODELS

>(i) Carbon tetrachloride (CCl₄) induced acute toxicity: The CCl₄ was diluted with liquid paraffin (1:1) before administration. The animals were divided into 5 groups consisting of 6 animals for each. The animals were then subjected to either one of the following treatments for 9 days.

Group 1: distilled water (1 ml/kg, p.o)

Group 2: distilled water for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Group 3: silymarin (100mg/kg /day, p.o) for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Group 4: derivative 1 for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Group 5: derivative 2 for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Group 6: derivative 3 for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Group 7: derivative 4 for 9 days + CCl₄ (1ml/kg, p.o) on ninth day

Food was withdrawn 12 hr before carbon tetrachloride administration to enhance the acute liver damage in animals of groups 2, 3, 4, 5, 6 and 7. The animals were sacrificed 24 hr after the administration of CCl₄. Blood samples were collected and the serum was used for assay of marker enzymes such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and serum bilirubin. The liver was immediately isolated and washed with normal saline, blotted with filter paper and weighed. The liver was then subjected to histopathological examination (Matsuda H et al. 1970).

(ii) Paracetamol (PCM) induced liver toxicity: The same procedure as mentioned above was followed except that the liver was damaged using PCM (1g/kg, p.o) diluted with sucrose solution (40% w/v). PCM was administered in 3 divided doses on day 9 and animals were sacrificed 48 hr after administration of PCM (Yoshigurki M et al. 1992).

STATISTICAL ANALYSIS

The statistical significance was assessed using one way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. The values are expressed as mean + SEM and P<0.05 was considered significant.

RESULTS

SYNTHESIS OF 6-BROMOQUERCETIN (DERIVATIVE 1):

Yield: 1.08 gms

Colour: yellow brownish.

State: solid.

Odour: Slight pungent.

Melting Point: 270-272 C

Rf value: 0.78.

IR spectra (KBr, ν , cm^{-1}): 610 (C-Br), 1600, 1490, 1470 (Ar), 1680 (C=O), 3300 (Ar-OH).

NMR Spectra: (100 MHz, DMSO, ppm): 6.47 (s, H-8), 6.77 (d, H-5'), 7.59 (d, H-2'), 7.77

(d, H-6').

Mass Spectra: C 47.89, 47.75; H 2.94, 2.63; Br 21.85, 21.02; $\text{C}_{15}\text{H}_9\text{O}_7\text{Br}$.

SYNTHESIS OF 6,8-DIBROMOQUERCETIN (DERIVATIVE 2):

Yield: 1.2 gms

Colour: yellow green.

State: solid.

Odour: Slight odour.

Melting Point: 254-256 C

Rf value: 0.79.

IR spectra (KBr, ν , cm^{-1}): 690 (C-Br), 1560, 1530, 1470 (Ar), 1640 (C=O), 3480 (Ar-OH).

NMR Spectra: (100 MHz, DMSO, ppm) 6.71(d, H-5'), 7.56(d, H-2'), 7.64(d, H-6').

Mass Spectra: C 39.3, 38.9; H 1.8, 1.53; Br 33.50, 33.92; $\text{C}_{15}\text{H}_8\text{Br}_2$.

SYNTHESIS OF 2',5',6,8-TETRABROMOQUERCETIN (DERIVATIVE 3):

Yield: 1.00 gms

Colour: nearly colourless.

State: solid.

Odour: Slight odour.

Melting Point: 248-250 C

Rf value: 0.87.

IR spectra (KBr, ν , cm^{-1}): 650 (C-Br), 1545, 1515, 1485 (Ar), 1650 (C=O), 3400 (Ar-OH).

NMR Spectra: (100 MHz, DMSO, ppm): 8.09 (s, H-6').

Mass Spectra: C 28.07; H 1.24; Br 51.01; $\text{C}_{15}\text{H}_6\text{Br}_4$.

SYNTHESIS OF 2',5',6,6',8-PENTABROMOQUERCETIN (DERIVATIVE 4)

Yield: 0.9 gms

Colour: nearly colourless.

State: solid.

Odour: Slight odour.

Melting Point: 135-137 C

Rf value: 0.90.

IR spectra (KBr, ν , cm^{-1}): 650 (C-Br), 1545, 1515, 1485 (Ar), 1650 (C=O), 3400 (Ar-OH).

NMR Spectra: No signals reported.

Mass Spectra: C 24.1; H 0.82; Br 56.9; $\text{C}_{15}\text{H}_5\text{O}_7\text{Br}_5$.

PHARMACOLOGICAL STUDIES:

CARBON TETRACHLORIDE (CCL) INDUCED ACUTE HEPATIC INJURY

Serum Biochemical markers and liver weight:

A significant difference in biochemical markers was observed between normal and CCl_4 control groups. Comparative analysis on the effect of ALT, AST and ALP and between the quercetin derivatives revealed that quercetin derivatives (Comp 1 and Comp 2) have almost similar activity compared to the standard silymarin (100mg/Kg body weight). Comparative analysis revealed that Comp 3 and Comp 4 prevented the increase in biomarker levels but were not significant when compared to standard silymarin, a known hepatoprotective agent. Significant reduction of liver weight was also seen in animals treated with Comp 1 and Comp 2, whereas other quercetin derivatives (Comp 3 and Comp 4) did not show significant reduction in the liver weight (Figure 1 and 2). Significant reduction in bilirubin level was seen in silymarin, Comp 1 and Comp 2 whereas the other two compounds did not show much decrease in bilirubin level. (Figure 3).

PARACETAMOL (PCM) INDUCED ACUTE HEPATIC INJURY:

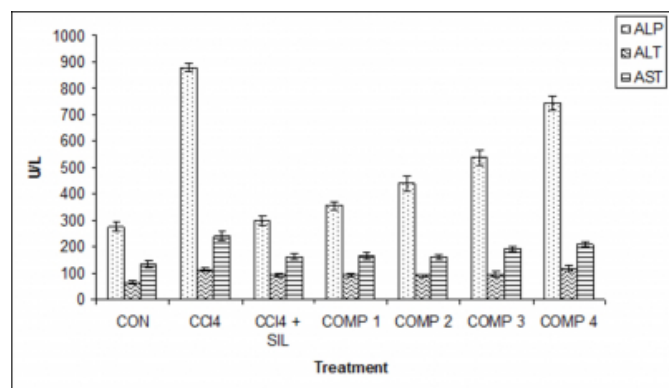
SERUM BIOCHEMICAL MARKERS AND LIVER WEIGHT:

Forty eight hours after treatment with paracetamol, the parameters ALT, AST, ALP and bilirubin levels in the serum increased remarkably. A significant difference in biochemical markers was observed between normal and

PCM control groups. Comparative analysis on the effect of ALT, AST and ALP and between the extracts revealed that quercetin derivative Comp 1 and Comp 2 have almost similar activity compared to the standard silymarin (100mg/Kg body weight). The other derivatives Comp 3 and Comp 4 did not show significant activity when compared to standard silymarin. Significant reduction of the liver weight was observed in groups administered with standard silymarin, Comp 1 and Comp 2 whereas the other extracts did not show any reduction of the liver weights (Figure 4 and 5). Significant reduction in bilirubin levels were seen in silymarin, Comp 1, Comp 2 whereas Comp 3 and Comp 4 did not show much reduction in bilirubin levels. (Figure 6).

Figure 1

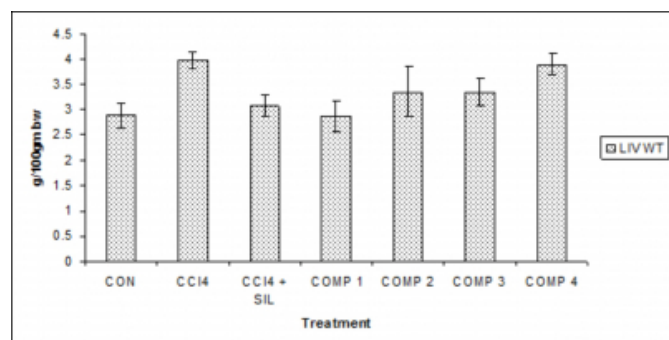
Figure 1: Effect of Silymarin and different quercetin derivatives on Serum ALT, AST and ALP level in CCl₄ induced acute hepatitis in rats.



Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001 vs. CCl₄ treated control.

Figure 2

Figure 2: Effect of silymarin and various quercetin derivatives on Liver weight in Carbon tetrachloride (CCl₄) induced acute hepatotoxicity in rats.

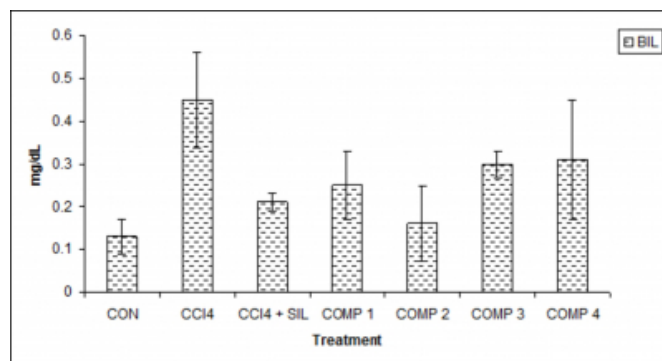


Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05,

*p<0.05, **p<0.01, ***p<0.001 vs. CCl₄ treated control.

Figure 3

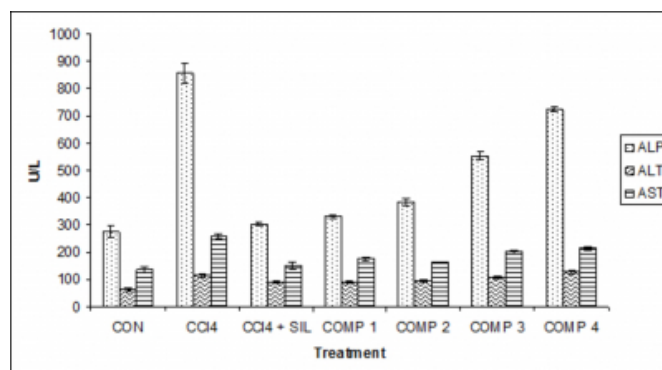
Figure 3: Effect of silymarin and different quercetin derivatives on bilirubin in CCl₄ induced acute hepatotoxicity in rats.



Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001 vs. CCl₄ treated control.

Figure 4

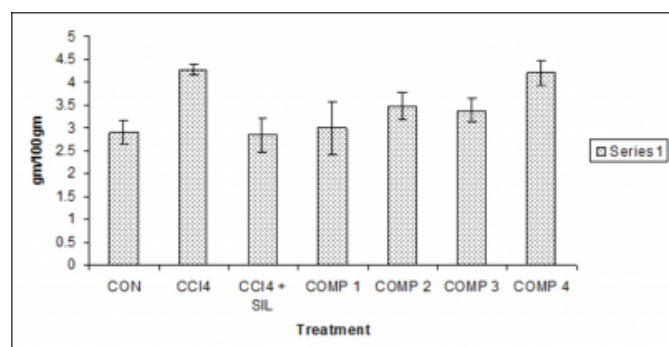
Figure 4: Effect of silymarin and different quercetin derivatives on serum ALT, AST and ALP level in paracetamol (PCM) induced acute hepatotoxicity in rats.



Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05, *p<0.05, **p<0.01, ***p<0.001 vs. PCM treated control.

Figure 5

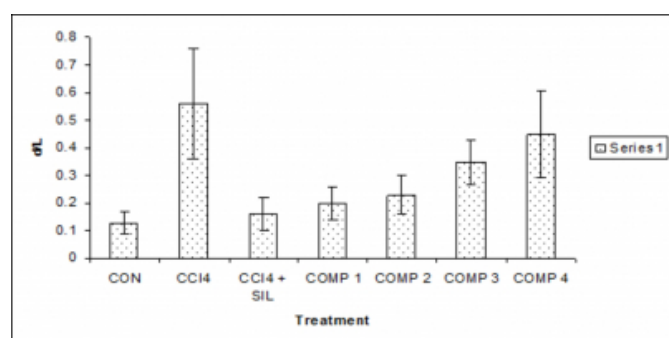
Figure 5: Effect of silymarin and various quercetin derivatives on Liver weight in Paracetamol (PCM) induced acute hepatotoxicity in rats.



Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05, * p<0.05, ** p<0.01, *** p<0.001 vs. PCM treated control.

Figure 6

Figure 6: Effect of silymarin and different quercetin derivatives on bilirubin in CCl₄ induced acute hepatotoxicity in rats.



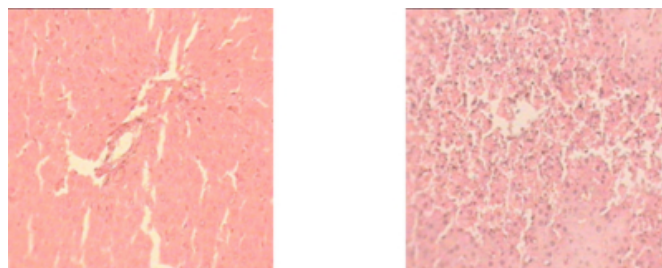
Values are mean \pm S.E.M, n = 6, ^a p<0.001 vs. vehicle control, ^b p<0.01, ^c p>0.05 vs. vehicle control, ^{ns} p>0.05, * p<0.05, ** p<0.01, *** p<0.001 vs. CCl₄ treated control.

HISTOPATHOLOGY

Histological examination of the liver tissue from CCl₄ treated animals revealed that CCl₄ had produced profound inflammation and congestion especially in the sinusoids. Hydropic degeneration and steatosis in the periportal region was also observed (fig 7a). In animals pretreated with silymarin, and derivative 1 found to reduce the inflammation, degenerative changes and steatosis (fig 7b).

Figure 7

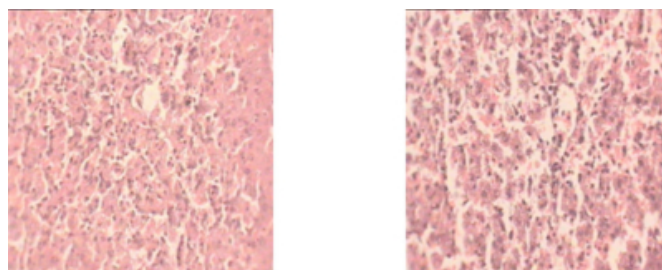
Fig 7a/7b



Histopathological examinations of the liver tissues showed severe congestion of blood vessels, mild hydropic degeneration, pyknosis of nucleus and occasional necrosis in PCM treated animals (fig 8a). Silymarin reduced the pyknosis of hepatocytes when compared to PCM treated control. Animals treated with derivative 1 showed mild hydropic degeneration and there was no pyknosis or congestion (fig 8b).

Figure 8

Fig 8a/8b



DISCUSSIONS

Derivatives were prepared by bromination of quercetin, four compounds were synthesized Derivative 1 (6-Bromoquercetin), Derivative 2 (6,8-Dibromoquercetin), Derivative 3 (2',5',6,8-Tetrabromoquercetin) and Derivative 4 (2',5',6,6',8-Pentabromoquercetin). 6 bromoquercetin was prepared by a solution of 0.2 ml (0.0039 mole) of bromine in 10 ml of dioxane which was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of quercetin (1) in 80 ml of dioxane. The reaction was continued at 20-22°C for 2 h. The resulting precipitate was washed with water and dried. 6,8-Dibromoquercetin was prepared at 35-40°C where 0.4 ml (0.0078 mole) of bromine was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of 6-Bromoquercetin in 100 ml of glacial acetic acid. After an hour's vigorous stirring, a yellow-green precipitate deposited. The reaction mixture was concentrated, and the precipitate was washed with water and dried. 2',5',6,8-Tetrabromoquercetin was prepared at 75-80°C where 0.8 ml (0.0154 mole) of bromine in 40 ml of

dioxane was slowly added dropwise to a solution of 1.0 g (0.0033 mole) of 6-Bromoquercetin in 100 ml of dioxane. After eight hours' vigorous stirring, the reaction mixture was evaporated to dryness. The residue was dissolved in water and the solution was brought to a boil and filtered. On cooling, it deposited a precipitate of 2',5',6,8-Tetrabromoquercetin. 2',5',6,6',8-Pentabromoquercetin was prepared by separating it with paper chromatography on FN-11 paper in system 1 to get 2',5',6,6',8-Pentabromoquercetin.

Pharmacological activity of the synthesized derivatives (Comp 1, Comp 2, Comp 3 and Comp 4) revealed that Comp 1 (Derivative 1) and Comp 2 (Derivative 2) has significant hepatoprotection when compared to silymarin (100mg/kg body weight) which is a standard hepatoprotective agent.

In carbon tetrachloride induced hepatotoxic model significant difference in biochemical markers was observed between normal and CCl₄ control groups. Comparative analysis on the effect of ALT, AST and ALP and between the quercetin derivatives revealed that quercetin derivatives (Comp 1 and Comp 2) have almost similar activity compared to the standard silymarin (100mg/Kg body weight). Comparative analysis revealed that Comp 3 and Comp 4 prevented the increase in biomarker levels but were not significant when compared to standard silymarin, a known hepatoprotective agent. Significant reduction of liver weight was also seen in animals treated with Comp 1 and Comp 2, whereas other quercetin derivatives (Comp 3 and Comp 4) did not show significant reduction in the liver weight. Significant reduction in bilirubin level was seen in in silymarin, Comp 1 and Comp 2 whereas the other two compounds did not show much decrease in bilirubin level.

In paracetamol (PCM) treated acute hepatic injury a significant difference in biochemical markers was observed between normal and PCM control groups. Comparative analysis on the effect of ALT, AST and ALP and between the extracts revealed that quercetin derivative Comp 1 and Comp 2 have almost similar activity compared to the standard silymarin (100mg/Kg body weight). The other derivatives Comp 3 and Comp 4 did not show significant

activity when compared to standard silymarin. Significant reduction of the liver weight was observed in groups administered with standard silymarin, Comp 1 and Comp 2 whereas the other extracts did not show any reduction of the liver weights. Significant reduction in bilirubin levels were seen in silymarin, Comp 1, Comp 2 whereas Comp 3 and Comp 4 did not show much reduction in bilirubin levels.

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Author Information

Chanchal K Roy

Krupanidhi College of Pharmacy

Amit K Das

Krupanidhi College of Pharmacy