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Short Communication

ANTIHEPATOTOXIC ACTIVITY OF TWO NEW QUERCETIN DERIVATIVES IN CARBON TETRACHLORIDE INDUCED HEPATOXICITY IN RATS

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ABSTRACT

Quercetin, a bioflavonol is widely found in nature and possesses diverse pharmacological properties. A heterocyclic 1,4 dioxane nucleus was incorporated in Quercetin structure to obtain two structural analogues of silybin. The aim of the study was to antihepatotoxic potential of Quercetin derivatives containing 1,4 dioxane heterocyclic ring in Carbon tetrachloride (CCl_4) induced hepatotoxicity in female Wistar Albino rats. Two Quercetin derivatives (QD) were synthesized by reported method. QD were administered orally at dose of 10 mg/kg, once daily for 7 days to Wistar Albino rats. A single dose of CCl_4 (1mL/kg) was used for inducing liver damage. Antihepatotoxic activity was evaluated by measuring levels of total proteins (TP), total albumin (TA), alkaline phosphatase (ALKP) and liver enzymes such as serum glutamate oxaloacetate (SGOT) and serum glutamate pyruvate transaminase (SGPT).QD exhibited potent antihepatotoxic activity with respect to standard drug silybon-70. However, it was observed that the Quercetin derivative having $-CH_2OH$ group in the dioxane ring exhibited better activity in comparision to unsubstituted 1,4 dixoane ring derivative.The exact mechanism by which QD protects the liver is unknown however the observed effects could be attributed to presence of 1,4 dioxane ring and due to the significant antioxidant activity of Quercetin flavone.

Key words: Antihepatotoxic activity, Quercetin; Silymarin, 1,4 dioxane ring, CCl_4

INTRODUCTION

Liver is an important organ regulating homeostasis in the body. It is involved in the detoxification and excretion of various endogenous and exogenous substances. It protects against the harmful effects of drugs and chemicals and thus frequently exposed to variety of toxicants resulting in liver diseases (Meyer and Kulkarni, 2001). Liver disease, a leading cause of death in many countries, is a major concern throughout the world. The different medical, surgical and therapeutic methods available at present for the treatment of liver diseases are inadequate with generally poor results (Davies, 1985). Therefore, it is essential to search for newer drugs for the treatment of liver diseases.

It is well known that some naturally occuring medicinal plants are potent hepatoprotective drugs and are widely used in alternative system of medicine through out the world to treat liver ailments (Handa *et al*., 1986). Silymarin isolated from the seeds of *Silybum marianum* commonly known as milk thistle, is used as a potent antihepatotoxic agent against variety of toxicants in modern medicine (Morazzoni and Bombardelli, 1995). It is a complex mixture of three flavolignan isomers namely, silybin, silydianin and silychristin (Flora *et al*., 1998) (Figure 1). Silybin contains 1,4 dioxane ring system in its structure and is the most active component, whereas Silychristin and Silydianin do not possess 1, 4 –dioxane ring, and thus do not display significant activity (Ahmed *et al*., 2003). We, therefore, proposed that 1, 4 dioxane unit plays an important role in exhibiting antihepatotoxic activity and thus evaluated the antihepatotoxic potential of many natural and synthetic products after incorporating 1,4 dioxane ring system in their

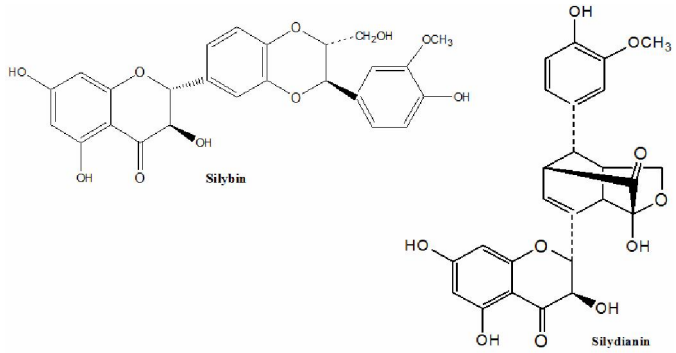

Figure 1. Chemical structures of silybin, (1a), silydianin (1b) and silychrystin (1c)

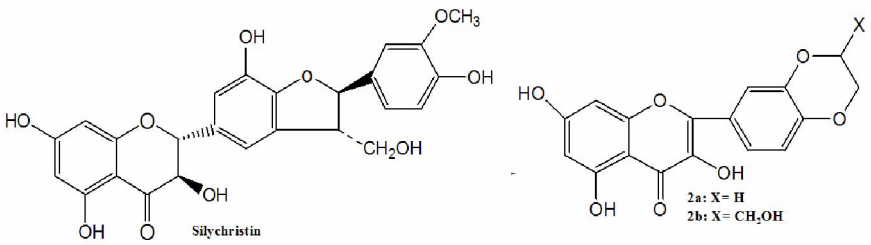

Figure 2. Chemical structures of quercetin derivatives

skeleton in search of effective and safer antihepatotoxic agents (Ahmed *et al.*, 2003; Khan *et al.*, 2006).

Quercetin (2-(3,4-dihydroxyphenyl)-3,5,7 -trihydroxy-4*H*-chromen-4-one), a polyhydroxy flavone is found in many herbal drugs and foods and demonstrates diverse biopharmacological properties (Boots *et al.*, 2008) Quercetin prevents the oxidation of low-density lipoproteins (LDL) by scavenging free radicals and chelating transition metal ions and helps in the prevention of certain diseases, such as cancer, atherosclerosis, and chronic inflammation etc (Hollman and Katan, 1997; Muraota and Terao, 2003). 1,4 dioxane ring was incorporated into the Quercetin structure by condensing 3,4 dihydroxy phenyl group of Quercetin with ethylene bromide or epicholorohydrin as per reported method to yield two new Quercetin derivatives (Figure 2) (Ahmed *et al.*, 2003). The QD (2a and 2b) possessing H and CH_2OH group at 2nd position of benzodioxane ring were evaluated for anticipated antihepatotoxic activity in carbon tetrachloride induced hepatotoxicity in rodents.

METHODOLOGY

Experimental animals

The antihepatotoxic studies of compounds (2a and 2b) were carried out on the female Wistar albino rats (150-200 g). The study was approved by an institutional animal ethics committee and ethical norms were strictly followed during all the experimental procedures. The rats were bred in a colony in the Central Animal House of Jamia Hamdard. They were fed with a standard pellet diet (Gold Mohar, Lipton India Ltd., Calcutta) and water *ad libitum*. Before and during the experiment, the rats were kept in a standard environmental conditions (temp. 25-28° C and 12 h light/dark cycle).

Treatment schedule

Animals were divided into five groups of five animals each in all the sets of experiments. CCl_4 mixed with liquid paraffin (1:1) was used as hepatotoxic agent. The drugs were administered for seven days after CCl_4 administration, in the form of an aqueous suspension made from carboxymethyl cellulose as per the following treatment schedule;

Group I (Normal control) was given only distilled water for 7 days; Group II (Toxic control) was treated with CCl_4 (1.0 mL/kg) for the first day of study to produce toxicity in the liver; Group III (silybon-70 treated) was given a single dose of CCl_4 (1.0 mL/kg) on the first day and then silybon-70, 10 mg/kg, daily) was given for seven days; Groups IV and V were administered with a single dose of CCl_4 (1.0 mL/kg) on the first day followed by an oral treatment with a daily dose (10 mg/kg) of dioxino quercetin 2a and 2b respectively for seven days.

On the last day, blood was collected directly from the retro plexus orbital of four rats from each group and serum was separated for biochemical analysis.

Biochemical analysis

The biochemical parameters such as SGOT (Rietman and Frankel, 1957), SGPT (Kind and King, 1954) , ALKP (Wooton, 1964) , TP and TA were analyzed according to the reported methods (Khan and Ahmed, 2011).

Statistical analysis

The results of the biochemical estimations are reported as mean ± S.E.M. Statistical analysis was done by one-way analysis of variance (ANOVA) followed by Student's '*t*' test by using 13th version of SPSS software. P values <0.05 were considered as significant.

RESULTS AND DISCUSSION

The effect of QD on serum biomarker enzymes is presented in table no 1. The levels of SGOT, SGPT, ALKP, TA were markedly elevated, and that of TP decreased after a single dose of CCl_4 (1 mL/kg) in toxic control group as compared to the normal animals, indicating hepatocellular damage. The activities of the bio marker enzymes for liver such as SGOT, SGPT, AKLP and TA were elevated on administration of CCl_4 to 71.52, 59.4, 46.34 units/mL and 4.30 g/dL respectively in comparison to normal values of 36.28, 30.30, 15.8 units/mL and 3.32g/dL respectively. Treatment of the rats with Silybon-70, a standardized extract of silymarin and two synthesized QD at dose levels of 10 mg/kg body weight, prevented CCl_4-induced elevation of SGOT, SGPT, ALKP, TA and also prevented decrease in TP. Both QD prevented the CCl_4 induced hepatotoxicity as they decreased the levels of SGOT (55.84 and 53.7 units/mL), SGPT (46.82 and 46.28 units/mL), ALKP (37.8 and 32.6 units/mL), TA (3.57 and 3.63 g/dL) and increased the level of TP (5.43 and 5.66 g/dL), which were found to be comparable to the effect produced by standard drug Silybon-70 (53.84, 45.8 and 28.88 units/mL for SGOT, SGPT and ALKP respectively, 6.29 and 3.26 g/dL for TP and TA respectively).

CCl_4 is one of the most commonly used hepatotoxins in experimental studies of liver diseases (Johnson and Kroening, 1998). The hepatotoxic effects of CCl_4 are largely due to its active metabolite, trichloro methyl (CCl_3) radical (Srivastava *et al*., 1990) that disrupts the structure and function of lipid and protein macromolecules in the membrane of the cell organelles. The elevated levels of serum bio marker enzymes are indicative of cellular leakage and loss of functional integrity of the cell membrane due to toxicity produced by CCl_4. Significant rise in serum enzymatic concentration viz. SGOT and SGPT could be taken as an index of liver damage.

Silymarin, the active ingredient of Silybon-70, is a well established hepato-protective drug capable of decreasing the elevated levels of liver enzymes in various drug induced hepatotoxicity through multiple actions. The antioxidant property and cell-regenerating functions as a result of increased protein synthesis are considered as the most important (Kosina *et al.,* 2002). QD are structural analogs of silybin, so expected to protect against CCl_4 induced hepatotoxicity by the similar antioxidant mechanism. In addition to structural resemblance, QD are of low molecular weight and thus can be easily metabolized *in vivo* as compared to complex isomeric mixture of silymarin.

The QD with hydroxyl methyl group at 2nd position on the 1,4 dioxane ring (2b) showed better antihepatotoxic activity than the quercetin derivative with unsubstituted 1,4 dioxane ring (2a). Compound 2b significantly reversed the hepatotoxicity and exhibited almost similar activity comparable to standard drug silybon- 70 by bringing back the enzyme

Table I. Results of biochemical parameters of QD in CCl_4 induced hepatotoxicity in wistar albino rats

Treatment Groups	SGOT units/mL	SGPT units/mL	ALKP units/mL	TP g/dL	TA g/dL
Normal control	36.28±1.19	30.3±0.19	15.8±0.37	5.32±0.15	3.32±0.17
Toxic	71.52±1.36	59.4±0.35	46.34±1.1	4.306±0.5	4.30±0.08
Silybon-70	53.84±0.65*	45.8±0.62*	28.8±0.23*	6.29±0.18*	3.76±0.13*
2a	55.84±1.4*	46.824±0.7*	37.8±0.34*	5.43±0.12*	3.57±0.06*
2b	53.7±1.37*	46.28±0.25*	32.6±0.27*	5.66±0.07*	3.63±0.04*

Values are Mean±S.E.M. (n= 5 animal per group); SGOT- Serum glutamic oxaloacetic acid transaminase; SGPT-Serum gluatamic pyruvic transaminase; ALKP- Alkaline Phosphatase; TP- Total Protein; TA- Total Albumin; *P<0.05 vs CCl_4; Student's *t* test

levels to near normal. This could be due to the fact that compound **2b** resembles structurally and chemically to silybin, the most active isomer of silymarin. Both are poly hydroxyl flavones possessing the $–CH_2OH$ group at the same position on the dioxane ring. The outcome of this study is in accordance with the previously reported results from our lab (Ahmed *et al*., 2003; Khan *et al*., 2006; Khan and Ahmed, 2011).

CONCLUSION

In the present study, administration of QD containing 1,4 dioxane ring system significantly protected against CCl_4 induced hepatotoxiciy in rats. Compound **2b** having a hydroxymethyl group on the 1,4 dioxane ring was found to be more potent than compound **2a** with unsubstituted 1,4 dioxane ring. The exact mechanism by which it protects the liver is unknown however the effect could be due to the significant antioxidant activity of Quercetin (Harborne, 1999) and due to the presence of 1,4 dioxane ring. Further detailed studies are needed to probe their role as antihepatotoxic agent and to ascertain the exact mechanism of action.

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