

SYNTHESIS AND ANTIHEPATOTOXIC ACTIVITY OF SOME NEW CHALCONES CONTAINING 1, 4 - DIOXANE RING SYSTEM

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ABSTRACT

Silybum marianum is a medicinal plant used widely for treating liver diseases. The silymarin, a mixture of three flavolignan isomers namely silybin (1), silydianin (2), and silychristin (3) is an active constituent of the plant. However, silybin containing 1, 4-dioxane ring is the most potent antihepatotoxic agent. In contrast, other isomers do not possess 1, 4- dioxane ring, and thus do not exhibit a significant activity. We, therefore, thought that 1, 4-dioxane ring plays an important role in displaying antihepatotoxic activity, and have prepared some chalcones containing 1, 4-dioxane ring. The synthesized compounds were evaluated for antihepatotoxic activity against carbon tetrachloride induced hepatotoxicity in albino rats. The degree of protection was measured by using biochemical parameters like serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT), alkaline phosphatase (ALKP), total protein (TP) and total albumin (TA). The compounds namely 2-hydroxy- 4-methoxy- 3',4'-(2''- hydroxy methyl-1'', 4'' - dioxano) chalcone (9b) and 2-hydroxy- 4, 6 -dimethoxy-3', 4'-(2''- hydroxy methyl-1'', 4''- dioxano) chalcone (9c) exhibited a potent activity in comparison to standard drug silybon-70. The other compounds also showed moderate activity.

Keywords: *Silybum marianum*, chalcones, antihepatotoxic activity.

INTRODUCTION

Liver disease is a leading cause of death in many countries which occurs due to malnutrition, alcohol consumption, continuous exposure to environmental pollutants and due to variety of drugs, chemicals, toxins, bacteria, viruses and parasites (Davies, 1985) leading to various disorders such as acute viral hepatitis, chronic viral hepatitis and liver cirrhosis. So far no effective measures are available for the treatment of liver diseases except some naturally occurring medicinal plants (Handa *et al*, 1986), which are widely used in alternative system of medicine. Silymarin isolated from seeds of *Silybum marianum* commonly known as *Milk thistle* has been used as a potent antihepatotoxic agent against a variety of toxicants (Flora *et al*, 1998). It is a mixture of three isomers (Khan *et al*, 2003) namely, silybin (1), silydianin (2) and silychristin (3). Silybin is the most active component containing 1, 4 dioxane ring system, whereas other isomers do not possess 1, 4 -dioxane ring, and thus do not display significant activity (Ahmed *et al*, 2003). We, therefore, thought that 1, 4 dioxane unit plays an important role in exhibiting antihepatotoxic activity and consequently have prepared some new chalcone derivatives possessing 1, 4 -dioxane ring system. These compounds were screened for antihepatotoxic activity in albino rats using CCl₄ as toxicant and conducting the estimation of liver enzymes such as SGOT, SGPT, total proteins, total

albumin and alkaline phosphatase. Some of the compounds namely 2-hydroxy- 4-methoxy-3',4'-(2''- hydroxy methyl-1'', 4'' - dioxano) chalcone (9b) and 2-hydroxy- 4, 6 -dimethoxy-3',4'-(2''- hydroxy methyl-1'', 4''- dioxano) chalcone (9c) showed a potent antihepatotoxic activity, whereas other compounds exhibited moderate activity with respect to standard drug silybon-70.

MATERIALS AND METHODS

General experimental part

Melting points were determined in capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Hitachi IR- 270-300 spectrometer (cm⁻¹). ¹H NMR were recorded on 300 MHz (Bruker model DRX-300 NMR spectrometer) in CDCl₃ and DMSO-d₆ using TMS as an internal reference (chemical shift in δ ppm). Purity of the compounds was checked on silica gel G plates using iodine vapours as visualizing agent.

Preparation of chalcones containing 1, 4 – dioxane ring system

Synthesis of Resacetophenone (5a): Freshly fused ZnCl₂ (16.5 g) dissolved in glacial acetic acid (15.8 ml) was heated on a sand bath at 140°C. Resorcinol (4a) (11.0 g, 100 mmol) was added portionwise with constant stirring, and heated to just boiling point 152°C. The reaction mixture was allowed to stand at room temperature. The contents were then diluted with mixture of conc. HCl and distilled water (1:1).

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The resulting dark red solution was cooled to 5°C in ice bath, the orange red solid that settled down was filtered and washed five times with 20 ml portions of dil. HCl. The product was crystallized with dil. HCl (1:11) to give orange red crystals; m.p. 139°C; R_f 0.69 (benzene: ethyl acetate, 9:1); yield 76 %.

Synthesis of Phloroacetophenone (5b): Phloroglucinol (**4b**) (2.52 g, 20 mmol), anhydrous acetonitrile (2.09 ml, 40 mmol), sodium dried ether (100 ml) and powdered zinc chloride (5 g) were placed in a buchner funnel fitted with a wide gas inlet tube. The flask was cooled in an ice salt mixture and a stream of dry HCl was passed through the solution for 2 h with occasional shaking. The reaction was completed as reported (Vogel, 1988), which on standing overnight afforded pale yellow needles of phloroacetophenone; m.p. 217-219°C; R_f 0.72 (benzene: acetone, 9:1); yield 81 %.

Synthesis of methyl ether of Resacetophenone (6a): A solution of resacetophenone (7.6 g, 50 mmol) in dry acetone (120 ml) was refluxed with anhydrous K_2CO_3 (4.5 g) and dimethyl sulphate (4.76 ml, d 1.33) on a heating mantle for 8 h under anhydrous conditions, and on usual work up afforded colorless needles; m.p. 52°C; R_f 0.47 (benzene: ethyl acetate 9:1); yield 64%; $IR_{\nu_{max}}$ (KBr): 3380 (OH), 1743 (C=O), 1459, 1378, 1241, 1160, 1100, 1045 (C-O), 759 and 668 cm^{-1} ; 1H NMR: δ 2.45 (3H, s, -COCH₃), 3.79, 3H, s, -OCH₃), 6.10 (3H, brm, -aromatic protons).

Synthesis of dimethyl ether of phloroacetophenone (6b): A solution of phloroacetophenone (5.0 g, 30 mmol) in dry acetone (50 ml), anhydrous K_2CO_3 (18.0 g) and dimethyl sulphate (10.0 ml, d 1.33) was refluxed, and on usual work up afforded colorless needles; m.p. 52°C; R_f 0.43 (benzene: ethyl acetate, 9:1); yield 51 %; $IR_{\nu_{max}}$ (KBr): 3400 (OH), 1750 (C=O), 1510 (C=C), 1462, 1372, 1210, 1165, 1106, 1035 (C-O), 756 and 628 cm^{-1} ; 1H NMR: δ 2.31 (3H, s, -COCH₃), 3.79 (6H, s, 2 x OCH₃), 6.10 (2H, d, J=1.5 Hz, H-3, H-5).

Synthesis of benzo 1, 4 dioxane -6- aldehyde (8a): A solution of KOH (3.68 g, 66 mmol) in water (30 ml) was added to 3, 4-dihydroxy benzaldehyde (**7**) (2.76 g, 20 mmol) and 1, 2-dibromo ethane (8.46 g, 45 mmol) in water (20 ml) with stirring. After 20 h reflux and usual work up, a yellowish solid obtained which was recrystallized from methanol; m.p. 53°C; R_f 0.49 (benzene : methanol 4:1); yield 53 %.

Synthesis of 2''- hydroxy methyl benzo 1, 4-dioxane-6-aldehyde (8b): 3, 4-dihydroxy benzaldehyde (1.38 g, 10 mmol) dissolved in aqueous ethanol (30 ml of alcohol (95%) in 17.1 ml of water) containing NaOH (0.5 g) and epichlorohydrin (8.0 ml, 9 mmol) was heated under reflux

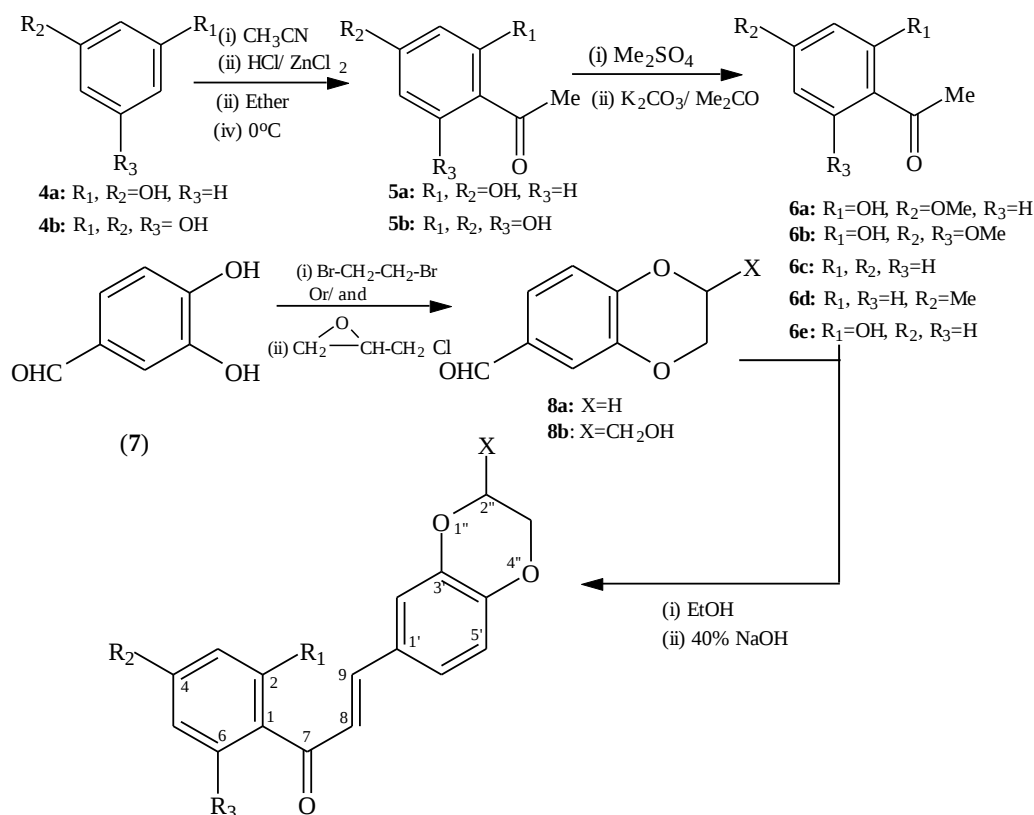
at 75°C for about 2 h with stirring and on usual work up yielded a solid, crystallized from aqueous ethanol; m.p. 62°C; R_f 0.65 (benzene: ethanol 9:1); yield 70%.

Synthesis of 2- hydroxy - 4- methoxy 3', 4' (1'', 4''-dioxano) chalcone (9a): The mono methyl ether of resacetophenone (**6a**) (1.66 g, 10 mmol) dissolved in oxygen free ethanol (20 ml) was added to benzo 1, 4-dioxane-6-aldehyde (**8a**) (1.64 g, 10 mmol) in a solution of NaOH (40%, 8 ml) in oxygen free water drop-wise with constant stirring at 0°C. The solution was stirred for 2 h on a magnetic stirrer and then acidified with dil. HCl. A yellow colored solid separated out which was filtered, washed with sodium bicarbonate solution (2%) and then consequently with ice cold water and dried. It was recrystallized from methanol; m. p. 171°C; R_f 0.51 (benzene: ethanol (9:1); yield 39%; $IR_{\nu_{max}}$ (KBr): 3430 (OH), 1754 (C=O), 1499 (C=C), 1223, 1165, 1041 (C-O), 907, and 601 cm^{-1} ; 1H NMR: δ 3.86 (3H, s, -OCH₃), 4.22 (2H, brm, -CH₂), 4.34 (2H, brm, -CH₂), 6.44 (1H, d, J=7.8, Hz, H-6), 6.64 (1H, q, J=1.8, 7.2, Hz, H-5), 6.88 (1H, d, J=1.8, Hz, H-3), 6.95 (1H, d, J=16.7, Hz, H- α), 7.03 (1H, d, J=16.7, Hz, H- β), 7.40 (1H, q, J=2.0, 8.2, Hz, H-6'), 7.53 (1H, d, J=8.0, Hz, H-5'), 7.84 (1H, d, J=1.8, Hz, H-2').

Synthesis of 2-hydroxy-4-methoxy 3', 4' (2''-hydroxy methyl-1'', 4''-dioxano) chalcone (9b): The mono methyl ether of resacetophenone (**6a**) (1.66 gm, 10 mmol) (20 ml) was treated with 2-hydroxy methyl benzo 1, 4 dioxane-6-aldehyde (**8b**) (1.94 gm, 10 mmol) in a usual manner to yield **9b**; m.p. 131°C; R_f 0.46 (benzene: methanol 9:1); yield 42 %; 1H -NMR: δ 3.59 (3H, s, -OCH₃), 3.69 (2H, brm, -CH₂OH), 3.90 (2H, brm, -CH₂), 4.38 (1H, brm, -CH), 6.41 (1H, d, J=8.0, H-6), 6.62 (1H, q, J=2.0, 8.0, H-5), 6.93 (1H, d, J=1.8, H-3), 6.98 (1H, d, J=17.0, H- α), 7.1 (1H, d, J=17.0, H- β), 7.43 (1H, q, J=2.0, 8.2, H-6'), 7.59 (1H, d, J=8.2, H-5'), 7.89 (1H, d, J=1.8, H-2').

Synthesis of 4, 6-dimethoxy-2-hydroxy-3', 4' (2''-hydroxy methyl -1'', 4''-dioxano) chalcone (9c): Prepared from dimethyl ether of phloroacetophenone (**6b**) (1.96 gm, 10 mmol) and 2-hydroxy methyl benzo 1,4 dioxane-6-aldehyde (**8b**) (1.94 gm, 10 mmol); m.p. 103-104 °C; R_f 0.39 (benzene: methanol, 9:1); yield 41 %; $IR_{\nu_{max}}$ (KBr): 3405 (OH), 1628 (C=O), 1502 (C=C), 1275, 1198, 1112, 1033 (C-O), 825, 724 and 624 cm^{-1} ; 1H -NMR: δ 3.59 (6H, S, 2 x -OCH₃), 3.63 (2H, brm, H-5''-CH₂OH), 3.82 (2H, brm, H-3''-CH₂), 4.34 (1H, brm, H-2''-CH), 6.25 (1H, d, J=2.0, H-3), 6.28 (1H, d, J=2.0, H-5), 6.87 (1H, d, J=15.9, H- α), 6.93 (1H, d, J=8.4, H-5'), 7.10 (1H, d, J=15.9, H- β), 7.18 (1H, dd, J=2.0, 8.0, H-6'), 7.25 (1H, d, J=2.0, H-2').

Synthesis of 3', 4' (1'', 4''dioxano) chalcone (9d): Prepared from acetophenone (1.20 gm, 10 mmol) and benzo 1,4 dioxane-6-aldehyde (1.64 gm, 10 mmol); m.p. 119-120°C; R_f 0.43 (benzene:methanol, 9:1); yield 54 %; $IR_{\nu_{max}}$ (KBr):



S.No.	R ₁	R ₂	R ₃	X
9a	OH	OCH ₃	H	H
9b	OH	OCH ₃	H	CH ₂ OH
9c	OH	OCH ₃	OCH ₃	CH ₂ OH
9d	H	H	H	H
9e	H	H	H	CH ₂ OH
9f	H	CH ₃	H	CH ₂ OH
9g	OH	H	H	CH ₂ OH

Scheme-1

1684 (C=O), 1540, 1516 (C=C), 1457, 1395, 1297, 1175, 1024 (C-O), 922, 823, 773 and 599 cm⁻¹; ¹H NMR: δ 3.24 (2H, ddd, J=2.1,9, 3.3,2''-CH₂), 3.35 (2H, ddd, J=2.4,8.4,2.7,3''-CH₂, J=6.95 (1H, d, J=8.2, H-5'), 7.08 (1H, d, J=7.8, H-6'), 7.28 (1H, d, J=8.2, H-2'), 7.36 (1H, d, J=14.7, H-α), 7.51 (1H, d, J=14.7, H-β), 7.77 (2H, d, J=7.8, H-5, 6), 7.82 (2H, d, J=7.8, H-2, 3).

Synthesis of 3', 4'-(2'' hydroxy methyl -1'', 4''-dioxano) chalcone (9e): Prepared from acetophenone (6c) (1.20 gm, 10 mmol) and 2-hydroxy methyl benzo 1, 4 dioxane-6-aldehyde (8b) (1.94 gm, 10 mmol); m.p. 93-94°C; R_f 0.36

(benzene: methanol, 9:1); Yield 63 %; IR_vmax (KBr): 3231 (OH), 1689 (C=O), 1588, 1529 (C=C), 1487, 1395, 1107, 1026 (C-O), 930, 828 and 712 cm⁻¹; ¹H NMR: 3.646 5H, brm, -2''-CH₂, 5'-CH₂OH, 3'-CH₂), 7.268 (1H, d, J =8.2, H-5'), 7.317 (1H, d, J=8, -6'), 7.42 (1H, d, J=7.8, H-2'), 7.602 (1H, d, J=15.6, H-α), 7.73 (1H, d, J=15.6, H-β), 7.78 (2H, d, J=7.8, H-5, 6), 8.03 (2H, d, J=7.8, H-2, 3).

Synthesis of 4- methyl- 3', 4' (2'' hydroxy methyl -1'', 4''dioxano) chalcone (9f): Prepared from 4-methyl acetophenone (6d) (1.34 gm, 10 mmol) and 2-hydroxy methyl benzo 1,4 dioxane-6-aldehyde (8b) (1.94 gm, 10

Table 1: Estimation of biochemical parameters of synthesized compounds

Groups	Treatment	Dose mg/kg/ b.w	SGOT±S.E units/ml	SGPT±S.E units/ml	ALKP±S.E units/ml	TP± S.E g/dl	TA± S.E g/dl
I	Normal	----	36.28±1.19	30.3±0.196	15.8±0.37	5.32±0.156	3.32±0.168
II	Toxic	1 ml/ Kg	71.52±1.36	59.4±0.354	46.34±1.043	4.306±0.48	4.30±0.075
III	Silybon-70	10	53.84±0.65***	45.8±0.62***	28.88±0.23***	6.219±0.186**	3.76±0.13**
IV	Chalcone- 9a	10	68.4±0.846	53.38±0.596***	33.56±0.50***	4.56±0.087*	4.16±0.043
V	Chalcone- 9b	10	57.62±0.494***	48.22±0.638***	34.18±0.0912***	5.24±0.135**	3.68±0.026***
VI	Chalcone- 9c	10	58.3±0.398***	47.44±0.916***	38.76±0.46***	5.33±0.034***	3.70±0.049**
VII	Chalcone- 9d	10	64.02±1.01**	51.50±0.179***	37.42±0.216***	4.76±0.069***	4.022±0.029**
VIII	Chalcone- 9e	10	63.98±1.35**	51.60±0.152***	38.08±0.494***	4.62±0.044**	3.87±0.041***
IX	Chalcone- 9f	10	61.54±0.32***	51.40±0.551***	38.56±0.292**	4.93±0.045***	3.95±0.079*
X	Chalcone- 9g	10	60.38±0.521**	50.96±0.505***	35.74±0.85**	4.81±0.056	3.95±0.045**

n= 5 animal per group; SGOT- serum glutamic oxaloacetic acid transaminase; SGPT-Serum glutamic pyruvic transaminase; ALKP- Alkaline Phosphatase; TP- Total Protein; TA- Total Albumin; *P<0.1, **P<0.01, ***P<0.001 vs CCl₄; Student's *t* test

mmol) as usual; m.p. 147-149°C; *R_f* 0.37 (benzene: methanol, 9:1); yield 29 %; IR_{vmax} (KBr): 3282 (OH), 2062, 1640 (C=O), 1511 (C=C), 1406, 1285, 1142, 1051 (C-O), 937, 756, 700 and 611 cm⁻¹; ¹H-NMR: δ 3.696 (5H, *brm*, 5"-CH₂OH, 2"-H, 3"-CH₂), 6.92 (1H, *d*, J=8.4, H-5), 7.29 (1H, *d*, J=7.8, H-6'), 7.52 (1H, *d*, J=8.4, H-2'), 7.65 (1H, *d*, J=15.2, H-α), 7.82 (1H, *d*, J=15.2, H-β), 7.97 (2H, *d*, J=7.8, H-5, 6), 8.13 (2H, *d*, J=7.8, H-2, 3).

Synthesis of 2- hydroxy- 3', 4' (2" hydroxy methyl -1", 4"dioxano) chalcone (9g): Prepared from 2-hydroxy acetophenone (**6e**) (1.36 gm, 10 mmol) and 2-hydroxy methyl benzo 1,4 dioxane-6-aldehyde (**8b**) (1.94 gm, 10 mmol); m.p. 210-211 °C; *R_f* 0.51(benzene:acetone, 9:1); yield 46 %; IR_{vmax} (KBr): 3410 (OH), 2890 (CH₂), 1685 (C=O), 1508 (C=C), 1275, 1031 (C-O), 758 and 621 cm⁻¹; ¹H-NMR: δ 4.15 (5H, *brm*, -OCH-CH₂OH, -CH₂), 6.93 (1H, *q*, J=1.2, 8.5, H-6), 9.96 (1H, *q*, J= 1.2, 8.5, H-7), 7.03 (1H, *q*, J=1.2, 8.5, H-8), 7.22 (1H, *q*, J=1.2, 8.4, H-5), 7.28 (1H, *d*, J=8.4, H-2'), 7.49 (1H, *d*, J=7.8, H-5'), 7.59 (1H, *d*, J= 15.6, H-α), 7.88 (1H, *d*, J=15.6, H-β), 7.98 (1H, *q*, J=1.2, 8.1, H-6').

Pharmacological screening

Experimental animals: The antihepatotoxic studies of compounds (**13a- 13g**) were carried out on Wistar albino rats (150-200 g) of either sex. 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 standard environmental conditions (temp. 25-28°C and 12h light/dark cycle). They were divided into fifteen groups of five animals each in all sets of experiments. Carbon tetrachloride mixed with liquid paraffin (1:1) was used as hepatotoxic agent. The drugs

were administered for seven days after CCl₄ administration, in the form of aqueous suspension made from carboxymethyl cellulose. On the last day, four rats from each group were taken for biochemical evaluation.

Treatment schedule: **Group I** (Normal control) was neither given CCl₄ nor any drug. **Group II** (Toxic control) was treated with CCl₄ (1.0 ml/Kg) for the first day of study to produce toxicity in the liver. **Group III** (Silymarin treated) was given a single dose of CCl₄ (1.0 ml /Kg) on the first day and then silymarin (silybon-70, 10 mg/Kg, daily) was given for seven days. Groups IV to X were administered with a single dose of CCl₄ (1.0 ml/Kg) on the first day followed by oral treatment with a daily dose (10.0 mg/kg) of chalcones **9a** to **9g** respectively for seven days.

Assessment of the liver function: The biochemical parameters such as serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvate transaminase (SGPT) (Reitman and Frankel, 1957), alkaline phosphatase (ALKP) (Kind and King, 1954), total protein and total albumin (Wooton, 1964) were analyzed according to the reported methods.

STATISTICAL ANALYSIS

The results of the biochemical estimations are reported as mean ± S.E. Total variation, present in a set of data was estimated by one-way analysis of variance (ANOVA) and Student's 't' test was used for determining the significance (Woolson, 1987).

RESULTS AND DISCUSSIONS

The results of the synthesized compounds and their pharmacological screening have been summarized in table

1. The compounds were obtained in good yield and were characterized by their spectral and chemical data. The levels of enzymes like SGOT, SGPT, ALKP were enhanced on administration of CCl₄ by 71.52, 59.4 and 46.34 units/ml in comparison to normal values of 36.28, 30.3 and 15.8 units/ml respectively. The administration of the compounds under investigation have decreased the enzyme levels in the range of 57.62 - 68.4 units/ml in case of SGOT, 47.44 - 53.38 units/ml in case of SGPT and 33.56 - 38.76 units/ml in case of ALKP, which were found to be comparable to the enzyme levels reduced by standard drug silybon-70 (53.84, 45.8 and 28.88 units/ml respectively). The most potent compounds, which exhibited almost similar antihepatotoxic activity as that of standard drug silybon-70 were found to be 2-hydroxy-4-methoxy-3',4'-(2''-hydroxy methyl-1'', 4''-dioxano) chalcone **9b** (57.62, 48.22 and 34.18 u/ml respectively) and 2-hydroxy-4,6-dimethoxy-3',4'-(2''-hydroxy methyl-1'', 4''-dioxano) chalcone **9c** (58.3, 47.44 and 38.76 u/ml respectively).

The toxicant CCl₄ also reduced the level of total protein (4.306 gm/dl) and increased the level of total albumin (4.3 gm/dl) in comparison to normal values (5.32 gm/dl, 3.32 gm/dl respectively). The administration of test compounds enhanced the reduced level of total protein in the range of 4.56 - 5.33 gm/dl, and decreased the elevated values of total albumin in the range of 3.68 - 4.16 gm/dl in comparison to standard drug silybon-70 (6.219 and 3.76 gm/dl respectively). The most potent compounds 2-hydroxy-4-methoxy-3',4'-(2''-hydroxy methyl-1'', 4''-dioxano) chalcone **9b** (5.24 and 3.68 g/dl respectively) and 2-hydroxy-4,6-dimethoxy-3',4'-(2''-hydroxy methyl-1'', 4''-dioxano) chalcone **9c** (5.33 and 3.70 g/dl respectively) displayed these values comparable to standard drug silybon-70, whereas other derivatives showed inferior results.

It was also observed that both the potent compounds (**9b** and **9c**) possess 2-hydroxy methyl group at position 2 of the dioxane ring of chalcone derivatives, which has also indicated that the presence of hydroxy methyl group at position 2 in dioxane ring possesses a significant role in exhibiting the antihepatotoxic activity. This is in accordance with the view that silybin too possess the same group at the same position.

The substitution in the aromatic ring of chalcones have no significant role in exhibiting antihepatotoxic activity. However, it was observed that the substitution at position 4 and 6 with methoxyl groups in the chalcone produced better pharmacological effects.

CONCLUSION

The above studies have shown that such compounds which contain both 1, 4 dioxane ring and 2-hydroxy methyl group in the molecule could exhibit a potent antihepatotoxic

activity and in turn could be used for the treatment of various ailments of liver. The synthesized compounds are simple, low molecular weight and thus could be prepared easily. On the other hand silybin is a complex molecule and thus can not be prepared easily. Furthermore, the synthesized compounds are expected to be easily metabolizable in comparison to silymarin, being simple and low molecular weight. In addition, some more compounds can also be synthesized to find out comparatively superior molecule possessing a better antihepatotoxic activity.

ACKNOWLEDGEMENTS

The authors are thankful to the Head, Department of Pharmaceutical Chemistry for providing the necessary research facilities, and Dr. (Mrs) Shaukat Shah, Jamia Hamdard, for providing rats. Dr. Bahar Ahmed (Principal Investigator) is also thankful to CSIR, New Delhi for financing the research project along with a post of SRF, which was awarded to SAK through a selection committee.

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Received: 23-8-2006 – Accepted: 10-10-2006