

Flavonoids and Hepatoprotective Activity of Leaves of *Senna Surattensis* (Burm.f.) In CCl₄ Induced Hepatotoxicity in Rats

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Abstract: The flavonoidal content, as well as the antioxidant, cytotoxic and hepatoprotective activities of leaves of *Senna surattensis* (Caesalpiniaceae) were investigated. Quercetin, quercetin 3-O-glucoside 7-O-rahmnoside and rutin were isolated from the ethyl acetate extract. The structure elucidation was performed by UV, ¹HNMR and ¹³CNMR spectroscopy. The cytotoxic activity of the ethanol extract (Up to 10 mg/ml) was tested against five human tumor cell lines namely; cervix (Hela), lung (H460), breast (MCF7), brain (U251) and liver (HepG2). The extract showed the highest activity against Hela cell line. The antioxidant activity was determined by radical scavenging activity of DPPH free radical using electron spin resonance (ESR) spectroscopy. 0.5 mg/ml of the 80% ethanol extract resulted in 99% inhibition of the peak area of DPPH. For the investigation of the hepatoprotective activity, liver damage was induced to male albino rats by intraperitoneal injection of CCl₄. Liver damaged rats were pretreated with daily oral dose of 100 mg/kg of 80% ethanol extract for 1 month. Administration of the extract was continued after liver damage for another month. Silymarin (25mg/kg taken orally) was used as a positive control. Followed by an overnight fast, whole blood was obtained from the retro orbital venous plexus through the eye canthus of anesthetized rats. Blood samples were collected at zero time and 1 month before liver damage, and 72 hours and one month after liver damage. Serum ALT, AST and ALP were measured. The relative potencies of the extract with respect to silymarin in reducing serum AST, ALT and ALP after 30 days of administration were 90%, 85% and 87%, respectively. The significant reduction in AST, ALT and ALP in plasma indicates the efficacy of *S. surattensis* leaf extract as a hepatoprotective.

Key words: Antioxidant, Cytotoxic, Flavonoids, Hepatoprotective, *Senna surattensis*.

INTRODUCTION

Senna surattensis (Synonyms: *Senna glauca*, *Cassia glauca*) is an ornamental plant belonging to family Leguminosae (Fabaceae), subfamily Caesalpinoideae. It is native to Asia (India, Thailand, Vietnam, Indonesia, Malaysia, Laos, Ceylon, Polynesia and the Philippine Islands) and Australia.

Two anthraquinones (chrysophanol and physcion) were isolated from the ethyl acetate extract of the bark (Tiwari and Misra, 1993). Phytochemical investigation of the pods leads to the isolation and structural elucidation of a new flavonol glycoside, 5, 7-dihydroxy-4'-methoxyflavonol-3-O-β-D-galactopyranoside, along with chrysophanol, physcion, kaempferide and quercetin (Rai *et al.*, 1997). Luteolin-7-O-β-D-glucopyranosyl-(1-4)-O-α-L-arabinopyranoside (Salpekar and Khan, 1996), γ-sitosterolin and digitolutein were isolated from the seeds (Khare *et al.*, 1994). Phytochemical investigation of the stem leads to the isolation of a new anthraquinone glycoside; 8-hydroxy-6-methoxy-3-methylanthraquinone-1-O-α-L-rhamnopyranosyl (1-6)-β-D-glucopyranoside (Rai and Roy, 1991), as well as, chrysophanol, physcion, stearic acid, β-sitosterol and β-sitosterol-β-D-glucoside (Hemlata and Kalidhar, 1994). *S. surattensis* is used as antihypertensive (with a group of plants). It is used in folk medicine as antihyperglycemic (Chopra *et al.*, 1956).

In this study, the flavonoidal content of *S. surattensis* leaves was investigated, accompanied with the evaluation of the antioxidant, cytotoxic and protective effect of the alcoholic extract on CCl₄-induced hepatotoxicity through biochemical methods.

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MATERIAL AND METHODS

Plant Material:

The fresh leaves of *S. surattensis* were collected from Garden of National Research Centre, Egypt, and was identified by Mrs. Treeze Labib, specialist in plant taxonomy. A voucher specimen of the plant (voucher number S-195) was kept in Pharmacognosy Department, National Research Centre, Egypt.

Experimental Animals:

Adult male albino rats weighing 130-150 g. were obtained from the animal house of National Research Centre, Egypt. They were kept under the same hygienic conditions and well balanced diet and water.

Experimental Diet:

Normal diet: vitamin mixture 1%, mineral mixture 4%, corn oil 10%, sucrose 20%, cellulose 0.2%, casein (95% pure) 10.5% and starch 54.3%. Doses of the drugs were calculated according to (Paget and Barner, 1964), and were administered orally by gastric tube. Ethical issue was followed as reported by Ney *et al.* (1988).

Biochemical Kits and Reagents:

Transaminase kits (Biomerieux Co.): Biochemical kits used for the assessment of serum Aspartate Amino Transferase (AST), Alanine Amino Transferase (ALT), Alkaline Phosphatase (ALP).
Silymarin : (SEDICO, Cairo, A.R.E).
DPPH: (Sigma[®] Chemical Company (EUA).

Apparatus:

- Spectrophotometer: Jeol EX-270 NMR spectrometer for ¹H NMR and ¹³C-NMR
- Ultraviolet visible recording spectrophotometer, UV-VIS Double Beam, UVD-3500. Lambomed Inc.
- Elexsys Bruker 500, operated at x-band frequency (Germany) for evaluation of antioxidant activity.

Isolation of Flavonoids:

500 g of air-dried powder of the leaves was successively extracted in a Soxhlet apparatus using petroleum ether, chloroform, ethyl acetate and ethanol 95%. Solvents were evaporated to dryness under vacuum at 40°C. The ethyl acetate extract was examined by paper chromatography (Whatmann No.1) using the solvent system *n*-butanol: acetic acid: water (3:1:1) (S₁) and 15% acetic acid (S₂). Chromatograms were visualized under UV before and after exposure to NH₃ and spraying with AlCl₃. R_f values and colors of the detected spots were recorded. Flavonoids were isolated by preparative paper chromatography (PPC) on Whatmann 3MM, using solvent system (S₁), and purified by repeated PPC using solvent system (S₂). Final purification was performed on Sephadex LH-20 column and elution with methanol.

Acid Hydrolysis of Glycosides:

Complete Acid Hydrolysis:

Five mg of each compound was dissolved in 5 mL methanol and an equal volume of 2N HCl aqueous solution was added. The mixture was refluxed on a boiling water bath for 3 hours. The methanol was evaporated and the aglycone was extracted with ether. The aqueous layer containing the sugar part was evaporated to dryness and the residue was dissolved in 10% isopropanol solution (Mabry *et al.*, 1970; Harborne *et al.*, 1973).

Identification of the Sugars:

The isopropanol solution containing the sugar moiety of each compound was applied on paper chromatogram along side with authentic sugars using the solvent system *n*-butanol: acetic acid: water (4: 1: 5 v/v) (S₃) and phenol: H₂O (80: 20 v/v) (S₄). After development they were visualized by spraying with aniline hydrogen phthalate reagent and heating at 110°C for 10 min (Harborne *et al.*, 1973).

Identification of Compounds:

The purified compounds were subjected to UV, ¹H-NMR and ¹³C-NMR spectral analysis. The chromatographic and UV data of these compounds were compared with published data (Mabry *et al.*, 1970 and Markham, 1982).

Investigation of Biological Activities:

Preparation of 80% Ethanol Extract:

500 gm. of the dried powdered leaves were refluxed in 80% ethanol for two hours. The extract was filtered and evaporated under reduced pressure. The residue was used for carrying out the biological activities.

Acute Toxicity Studies:

LD₅₀ of crude ethanol extract: was reported in a previous publication (El-Sawi and Sleem, 2009) following Miller and Tainter (1944) procedure and found to be (8.6 g/kg b.wt.).

Cytotoxic Activity of the Ethanol Extract of *S. surattensis* by SRB Assay:

Potential cytotoxicity of 80% ethanol extract of *S. surattensis* was tested using the method of Shehan *et al.*, (1990). Tumor cells were plated in 96-multiwell plate (Cellstar greiner bio-one, Germany) (10⁴ cells/well) for 24 h. before treatment with the extract to allow attachment of cells to the wall of the plate. Different concentrations of the extract (0, 1, 2.5, 5 and 10 µg/mL DMSO) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the extract for 48 hrs. at 37°C in atmosphere of 5% CO₂. After 48 h., cells were fixed, washed and stained with sulforhodamine B stain (Sigma). Excess stain was washed with acetic acid and attached stain was recovered with Tris EDTA buffer (Sigma). Color intensity was measured in an ELISA reader (Tecan Group Ltd., Sunrise, Germany). The relation between surviving fraction and extract concentration was plotted to get a survival curve of each tumor cell line after incubation with the extract. The potency of the extract was compared with reference Cisplatin (Glaxo).

Electron Spin Resonance (ESR) Measurements: (DPPH) Radical Scavenging Activity:

The potential antioxidant activity of the extract was assessed on the basis of scavenging activity of the stable DPPH free radical. Reaction mixtures contained test sample (0.5 mg/ml) dissolved in ethanol, and an equal volume of DPPH ethanolic solution (5 × 10⁻⁴ M) (Calliste *et al.*, 2001).

$$\text{The inhibition ratio} = \frac{\text{ref} - \text{extract}}{\text{ref}} \times 100$$

Determination of Hepatoprotective Activity:

Thirty adult male albino rats of the Sprague Dawley strain, weighing 130-150 g were used. They were kept on standard laboratory diet, and under the same hygienic conditions and normal diet. Induction of liver damage: induced according to method of Klassen and Plaa (1969) by intraperitoneal injection of 5 ml/kg of 25% carbon tetrachloride in liquid paraffin.

The rats were divided into three groups. The first group received a daily oral dose of 1 ml saline before and after liver damage (negative control). The second group received daily oral dose of 25 mg/kg b.wt. silymarin, as standard reference drug, before liver damage. Administration of the drug was continued after liver damage for another one month. The third group liver damaged rats pretreated with daily oral dose of 100 mg/kg of ethanol extract for 1 month. Administration of the extract was continued after liver damage for another one month. Followed by an overnight fast, whole blood was obtained from the retro orbital venous plexus through the eye canthus of anesthetized rats. Blood samples were collected at zero time and one month before liver damage and 72 hours and one month after liver damage. Serum was isolated by centrifugation. Serum ALT, AST (Thewfweld 1974) and ALP (Kind and King 1954) were measured.

Statistical Analysis of Data:

The data obtained were statistically analyzed using Student's "t" test (Sendecor and Cochran, 1971). Results with p < 0.01 were considered statistically significant.

RESULTS AND DISCUSSION

Identification of the Flavonoids in *S. surattensis* Leaf Extract:

Three compounds were isolated from the ethyl acetate extract of *S. surattensis* leaves:

Compound I:

Yellow amorphous powder. It had a yellow color under UV which did not change upon exposure to

ammonia vapors. $R_f = 0.63$ and 0.09 (systems S_1 and S_2 , respectively). On complete acid hydrolysis, no sugars could be detected using solvent systems S_3 and S_4 .

UV λ_{max} (nm) (MeOH): 255, 301sh, and 371, (NaOMe); 245, 327 (dec), (AlCl₃): 272, 305sh, 330, 454, (AlCl₃/HCl): 270, 297sh, 357, 425, (NaOAc): 275, 334, 387 (dec), (NaOAc/H₃BO₃): 262, 387.

¹H NMR (DMSO-*d*₆) δ 7.64 (1 H, d, $J = 8.5$ Hz, H-2), 7.49 (1H, q, $J = 8.5$ Hz, H-6), 6.85 (1H, d, $J = 8.5$ Hz, H-5), 6.37 (1H, d, $J = 2.5$ Hz, H-6) and 6.14 (1H, d, $J = 2.5$ Hz, H-8).

¹³C NMR (DMSO-*d*₆): δ 176.3 (C-4), 164.1 (C-7), 161.2 (C-5), 156.3 (C-9), 148.2 (C-4), 147.1(C-2), 145.0 (C-3), 135.9 (C-3), 121.7 (C-1), 121.0 (C-6), 116.1 (C-2), 115.6 (C-5), 103.7 (C-10), 98.5 (C-6) and 93.6 (C-8).

UV spectra revealed that decomposition occurred after 10 minutes upon addition of NaOMe or NaOAc indicating free 3,3',4' hydroxyl system. The presence of ortho-dihydroxyl group was confirmed by the bathochromic shift in band I (16 nm) in the presence of NaOAc/ H₃BO₃ relative to MeOH.

¹H NMR and ¹³C NMR showed characteristic signals of quercetin, while no signals of sugars could be detected.

Compound I was identified as the flavonol quercetin (Fig. 1).

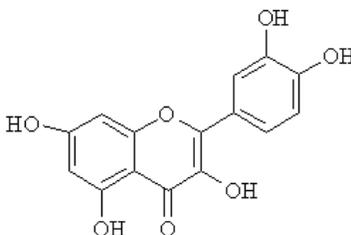


Fig. 1: Quercetin

Compound II:

Compound II was isolated as yellow amorphous powder which had purple color under UV light changed to yellow upon exposure to ammonia vapor or spraying with AlCl₃. The R_f values in solvent systems S_1 and S_2 were 0.40 and 0.62, respectively. It yielded two sugar moieties (glucose and rhamnose) upon complete acid hydrolysis in the aqueous phase (CoPC in solvent systems S_3 and S_4 , specific spray reagents) and quercetin in the organic phase (CoPC in solvent systems S_1 and S_2).

UV λ_{max} (nm): (MeOH): 257, 270sh, 299sh, 361, (NaOMe): 273, 324, 408, (AlCl₃): 271, 302sh, 428, (AlCl₃/ HCl): 267, 304, 363sh, 400, NaOAc): 272, 325, 398, (NaOAc / H₃BO₃): 263, 301, 384.

¹H-NMR (DMSO-*d*₆): δ (ppm)= 7.56 (2H, m, H-2', 6'), 6.85 (1H, d, $J = 8.5$ Hz, H-5'), 6.39 (1H, d, $J = 2.5$ Hz, H-8), 6.20 (1H, d, $J = 2.5$ Hz, H-6), 5.33 (1H, d, $J = 7.2$ Hz, H-1''), 4.37 (1H, s, H-1'''), 3 – 3.9 (sugar protons), 1.11 (3H, d, $J = 6$ Hz, H-6''').

¹³C NMR (DMSO-*d*₆): δ 177.9 (C-4), 164.8 (C-7), 161.6 (C-5), 156.9 (C-2), 156.6 (C-9), 148.8 (C-4'), 146.1 (C-3'), 133.8 (C-3), 121.3 (C-6'), 121.1 (C-1'), 116.9 (C-5'), 115.8 (C-2'), 104.5 (C-10), 101.5 (C-1'''), 101.3 (C-1''), 99.1 (C-6), 94.3 (C-8), 77.4 (C-3''), 75.9 (C-5''), 73.7 (C-2''), 72.3 (C-4''), 71.1 (C-4''), 70.9 (C-2''), 70.7 (C-3''), 70.4 (C-4''), 68.7 (C-5''), 67.1 (C-6''), 18.2 (C-6''').

The bathochromic and hypsochromic shifts were in a good agreement with quercetin 3-*O*-glycoside structure. Free 4'-OH group was clear from the bathochromic shift in Band I (47 nm) with the increase of its intensity upon addition of NaOMe. On addition of NaOAc, a bathochromic shift (16 nm) in Band II occurred due to free 7-OH. The bathochromic shift (67 nm) in Band I upon the addition of AlCl₃ indicated the substitution in position 3. The presence of ortho-dihydroxy groups in B ring was deduced from the hypsochromic shift in Band I in AlCl₃ / HCl spectrum (28 nm) relative to that in case of addition of AlCl₃ (Markham, 1982).

The presence of glucose and rhamnose was deduced from the ¹H-NMR and ¹³C NMR spectra. In the aliphatic region of ¹³C NMR 12 carbon resonances assigned for a rutinoid moiety among which the most downfield signals at δ 101.3 and 101.5 assigned for the two anomeric carbons C-1'' and C-1''', respectively. The downfield shift of C-6''' signal was due to rhamnoglucosylation. The anomeric proton signals at δ 5.33 (d, $J = 7.2$) and 4.37 (s) together with a doublet at δ 1.11 (3H, d, $J = 6$ Hz) accounted for a β -D-rutinoid moiety. Compound II was identified as quercetin-3-*O*- α -L-rhamnopyranosyl-(1'''-6''')-*O*- β -D-glucopyranoside (rutin, Fig. 2).

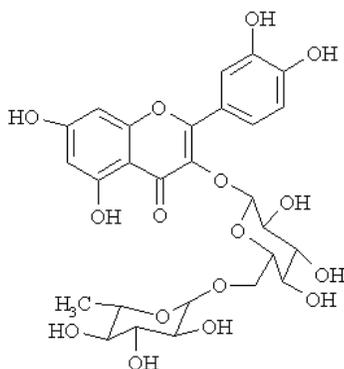


Fig. 2: Quercetin-3-O- α -L-rhamnopyranosyl-(1''-6'')-O- β -D-glucopyranoside (rutin)

Compound III:

Compound II obtained as yellow amorphous powder appeared deep purple under UV light, turning yellow when fumed with ammonia vapor or spraying with $AlCl_3$. The R_f values in solvent systems S_1 and S_2 were 0.33 and 0.63, respectively. Complete acid hydrolysis gave two sugar moieties (glucose and rhamnose) in the aqueous phase (CoPC in solvent systems S_3 and S_4 , specific spray reagents) while quercetin was detected in the organic phase (CoPC in solvent systems S_1 and S_2).

UV λ_{max} , (nm): (MeOH): 254, 266 (sh), 354; (NaOMe): 246, 276, 400 ($AlCl_3$): 273, 301 (sh), 339, 438; ($AlCl_3/HCl$): 273, 301 (sh), 363sh, 402; (NaOAc): 256, 294 (sh), 370, 414sh; (NaOAc/ H_3BO_3): 258, 294 (sh), 386.

1H NMR (DMSO- d_6) δ 7.73 (1 H, d, $J = 8.5$ Hz, H-2), 7.63 (1H, d, $J = 8.5$ Hz, H-6), 6.85 (1H, d, $J = 8.5$ Hz, H-5), 6.76 (1H, d, $J = 1.6$ Hz, H-8) and 6.48 (1H, d, $J = 1.6$ Hz, H-6), 5.63 (d, $J = 1.2$ Hz, H-1'') 5.36 (d, $J = 7.3$ Hz, H-1'), 4.1-3.30 (m, sugar protons), 1.26 (d, $J = 5.8$ Hz, H-1''').

^{13}C NMR (DMSO- d_6): δ 177.3 (C-4), 163.2 (C-7), 161.6 (C-5), 157.0 (C-2), 156.9 (C-9), 148.1 (C-4'), 146.5 (C-3'), 133.9 (C-3), 124.3 (C-1'), 121.0 (C-6'), 117.2 (C-5'), 115.1 (C-2'), 104.4 (C-10), 101.7 (C-1'''), 101.1 (C-1''), 99.6 (C-6), 94.8 (C-8), 76.4 (C-3''), 75.9 (C-5''), 71.8 (C-4'''), 71.0 (C-2''), 70.8 (C-3'''), 70.6 (C-2'''), 70.5 (C-4''), 68.7 (C-5'''), 60.1 (C-6''), 18.2 (C-6''').

UV spectroscopy of compound III revealed a bathochromic shift in band I (46 nm) with respect to MeOH with increase in intensity accounted for free 4'-OH. The bathochromic shift in band II in NaOAc is less than 5 which indicated occupied 7-OH. Occupied 3 or 7 hydroxyl group is clear from the shoulder at Band I in the presence of NaOAc. Bathochromic shift in Band I in NaOAc/ H_3BO_3 (34 nm) revealed ortho-dihydroxy group in ring B.

In ^{13}C NMR 12 carbon resonances assigned for glucose and rhamnose among which the most downfield signals at δ 101.1 and 101.7 assigned for the two anomeric carbons C-1'' and C-1''', respectively and a signal at δ 18.2 for C-6'''.

The 1H NMR spectrum of compound III showed the proton signals of quercetin. H-2' appeared as a doublet at δ 7.73 ($J = 8.5$ Hz) and H-6' as a doublet at δ 7.62 ($J = 8.5$ Hz) due to orthocoupling with H-5' which appeared at δ 6.82. The two aromatic protons of ring A appeared at δ 6.76 and 6.48 assigned to H-8 and H-6, respectively. The glucose moiety at C-3 was evidenced by the downfield location of anomeric proton at δ 5.36 as doublet with $J = 7.3$ Hz, while the anomeric proton of rhamnose appeared at δ 5.63 ($J = 1.2$ Hz). compound III was identified as quercetin 3-O-glucoside 7-O-rhamnoside (Fig. 3).

Evaluation of Cytotoxic Activity of Total Ethanol Extract of *S. surattensis*:

The ethanol extract of *S. surattensis* leaves (up to 10 mg/ml) show moderate activity against the five tested human tumor cell lines namely; cervix (Hela), lung (H460), breast (MCF7), brain (U251) and liver (HepG2). The most effective result obtained with Hela cell line in which the surviving fraction reached 63% at concentration 10 μ g/ml. Data of cytotoxic activities are represented in Table 1.

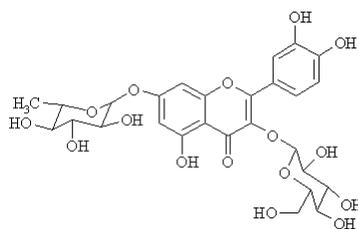


Fig. 3. Quercetin 3-O-glucoside 7-O-rhamnoside

Radical Scavenging Activity of *S. surattensis* Leaf Extract:

The radical scavenging activity of *S. surattensis* leaf extract was estimated by inhibiting the stable free radical of DPPH. The results were obtained by recording the double integration area of DPPH free radicals by ESR calculated after the addition of the extract. Table 2 and Fig. 4 illustrate a significant decrease in the concentration of DPPH radical due to scavenging ability of the alcoholic extract. Half mg/ml of the alcoholic extract resulted in 99% inhibition of the peak area of DPPH.

Table 1: Cytotoxic activity of total ethanol extract of *Senna surattensis* leaves.

Cell line	Conc. µg/mL	80% ethanol extract SF± MSE		Cisplatin SF± MSE	
1. H460	0.0	1.000	0.057	1.000	0.072
	1.0	1.023	0.043	0.796	0.020
	2.5	0.934	0.013	0.665	0.050
	5.0	0.837	0.026	0.484	0.026
	10.0	0.735	0.007	0.401	0.007
2. U251	0.0	1.041	0.134	1.000	0.068
	1.0	1.953	0.023	0.609	0.074
	2.5	0.886	0.044	0.564	0.045
	5.0	0.839	0.043	0.555	0.067
	10.0	0.776	0.051	0.494	0.051
3. Hela	0.0	1.000	0.112	1.000	0.075
	1.0	0.876	0.020	0.903	0.057
	2.5	0.652	0.033	0.424	0.210
	5.0	0.642	0.023	0.170	0.420
	10.0	0.632	0.018	0.059	0.024
4. MCF7	0.0	1.000	0.050	No significant effect	
	1.0	0.793	0.019		
	2.5	0.757	0.013		
	5.0	0.725	0.007		
	10.0	0.678	0.016		
5. HepG2	0.0	1.000	0.062	1.000	0.104
	1.0	1.097	0.021	0.601	0.043
	2.5	1.082	0.005	0.593	0.055
	5.0	1.036	0.018	0.524	0.040
	10.0	0.949	0.014	0.518	0.054

H460: lung, U251: brain, Hela: cervix, MCF7: breast, HepG2: liver, SF: survived fraction; MSE: mean standard error.

Table 2: Radical scavenging activities of 80% ethanol extract of leaves of *Senna surattensis*.

	Concentration mg/ml	Double integration area	% inhibition
DPPH*	0.5	2168028	0%
80% ethanol extract	0.5	14859	99%
Ascorbic acid	0.5	0	100%

Hepatoprotective Activity of *S. surattensis* Leaf Extract on Acute Hepatic Injury Induced by CCl_4 in Rats:

Acute liver injury was induced in the rats by intraperitoneal injection of CCl_4 into rats. Table 3 showed that CCl_4 significantly increased serum AST, ALT and ALP activities after 72 hours and 30 days compared with the control group (141.2 ± 6.3 and 158.1 ± 7.5 vs. 29.5 ± 0.8 u/L; 128.5 ± 5.9 and 142.3 ± 6.4 vs. 27.6 ± 0.4 u/L; 51.3 ± 1.7 and 49.8 ± 1.4 vs. 6.9 ± 0.2 , respectively). In comparison with the CCl_4 -treated group, the elevated levels of serum AST, ALT, ALP were significantly reduced ($P < 0.01$) in the animal groups pretreated with the extract or silymarin. After 72 hours of liver damage, the reduction of serum enzymes in animals pretreated with extract or silymarin was as follows: AST: 64 and 74%, respectively; ALT: 56 and 68%, respectively; ALP: 68 and 79%, respectively. The maximum inhibition in levels of AST, ALT and ALP recorded after 30 days in treated animals was as follows: AST: 76 and 84% for the extract and silymarin, respectively; ALT: 70 and 82%, respectively; ALP: 76%, and 87%, respectively. The relative

potencies of the extract with respect to silymarin in reducing serum AST, ALT and ALP after 30 days of administration were 90%, 85% and 87%, respectively.

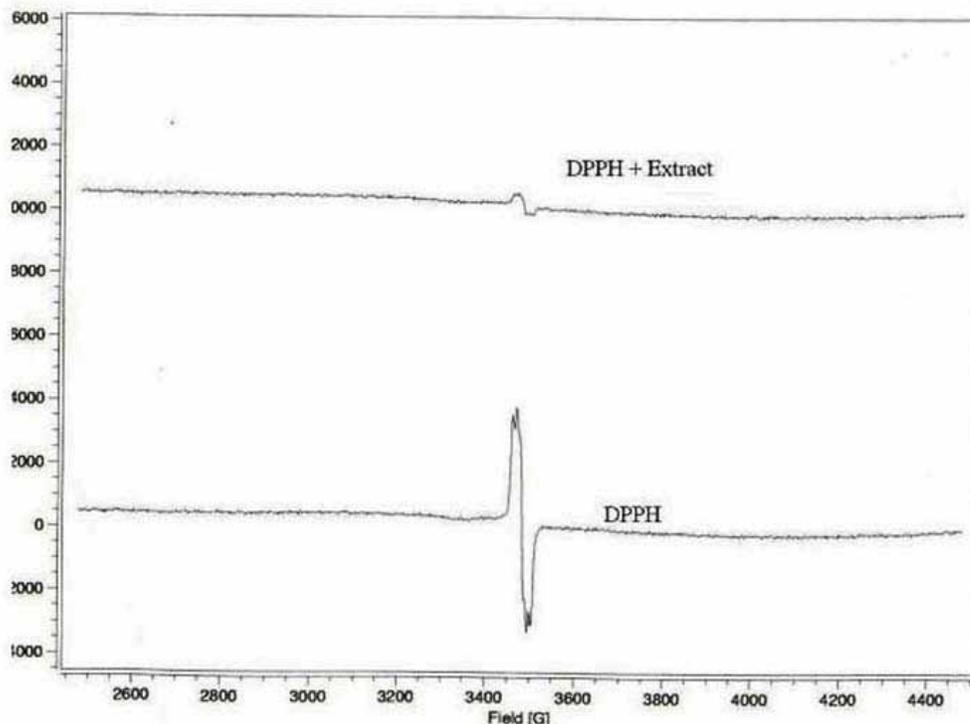


Fig. 4: DPPH radical scavenging assay of alcoholic extract of leaves of *Senna surattensis* using electron spin resonance spectroscopy

Table 3: Effect of alcoholic leaf extract of *Senna surattensis* and silymarin on acute hepatic injury induced by CCl₄ in rats.

Group	AST (u/L)				ALT (u/L)				ALP (u/L)			
	Before liver damage		After liver damage		Before liver damage		After liver damage		Before liver damage		After liver damage	
	zero	30d	72h	30d	zero	30d	72h	30d	zero	30d	72h	30d
Control												
1 ml saline	29.5±0.8	28.3±0.9	141.2±6.3	158.1±7.5	28.2±0.8	27.6±0.4	128.5±5.9	142.3±6.4	6.7±0.1	6.9±0.2	51.3±1.7	49.8±1.4
Alcohol	32.4±1.5	30.2±1.1	51.3*±1.4	36.9*±1.7	33.6±1.8	31.4±1.2	56.9*±2.4	43.2*±2.3	6.9±0.3	6.8±0.2	16.5*±1.3	11.2*±0.8
extract 100 mg/kg												
Silymarin	30.2±1.1	28.7±0.9	36.3*±0.8	24.5*±0.7	28.9±0.7	27.3±0.2	41.5*±1.6	26.3*±1.2	7.2±0.3	6.9±0.2	10.8*±0.7	6.5*±0.3
25 mg/kg												

Values are the mean ± S.E.M. of ten rats. Symbols represent statistical significance: *Significantly different from zero time at P < 0.01. •Significantly different from 72 hrs. time at P < 0.01.

The present investigation revealed that the given dose of CCl₄ (5 ml/kg of 25% CCl₄ in liquid paraffin) produced significant elevation in AST, ALT and ALP indicating all impaired liver function and these parameters have been reported to be sensitive indicator of liver injury (Zimmerman and Seeff, 1970). CCl₄ is a well-known hepatotoxic chemical (Yu *et al.*, 2002 and Marucci *et al.*, 2003). The main cause of acute liver injury by CCl₄ is free radicals of its metabolites. By the activation of liver cytochrome P-450, CCl₄ generates methyltrichloride radicals (CCl₃), which are highly unstable and immediately react with membrane components. They form covalent bonds with unsaturated fatty acids, or take a hydrogen atom from the unsaturated fatty acids of membrane lipids, resulting in the production of chloroform and lipid radicals. The lipid radicals react with molecular oxygen, which initiates peroxidative decomposition of phospholipids in the endoplasmic reticulum. The peroxidation process results in the release of soluble products that may affect cell membrane. Cell membrane integrity is broken and the enzymes (such as ALT, AST, etc.) in cell plasma leak out. Scavenging of free radicals is one of the major antioxidation mechanism to inhibit the chain reaction of lipid peroxidation.

The free radical-scavenging activity of the extract was evaluated by DPPH assay. DPPH is known to abstract labile hydrogen (Constantin *et al.*, 1990 and Matsubara *et al.*, 1991). Simultaneously, the

hepatoprotective effects of the extract of *S. surattensis* leaves in rats were compared with those treated with silymarin, which is an active constituent of the fruit of the milk thistle (*Silybum marianum*, Compositae). In this view, the reduction in levels of AST and ALT by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl₄. This effect is agreement with the commonly accepted view that serum levels of transaminases return to normal with the healing of hepatic parenchyma and regeneration of hepatocytes (Thabrew *et al.*, 1987). Alkaline phosphate is the prototype of these enzymes that reflects the pathological alteration in biliary flow (Ploa and Hewitt, 1989). Thus, administration of ethanolic extract of leaves revealed hepatoprotective activity of *S. surattensis* leaves against the toxic effect of CCl₄.

Phytochemical analysis of the ethyl acetate extract has shown the presence of the flavonoids quercetin, rutin and quercetin 3-*O*-glucoside-7-*O*-ramnoside which have been known for their antioxidant and hepatoprotective activities (Di Carlo *et al.*, 1999). The mechanism by which quercetin, a natural antioxidant, inhibit lipid peroxidation by blocking the enzyme xanthine oxidase (Cheng and Breen, 2000), Chelating iron (Da Silva *et al.*, 1998) and directly scavenging hydroxyl, peroxy and superoxide radicals (De Whalley *et al.*, 1990) reveals its antioxidant properties. Quercetin also protects antioxidative defense mechanism by increasing the absorption of Vitamin C (Vinson and Bose, 1998). Quercetin has been shown to inhibit structural damage to proteins (Salvi *et al.*, 2001) and the release and the protection of oxidative products generated by the respiratory burst in phagocytes (Zielinska *et al.*, 2000). Pretreatment of animals with rutin prevented the CCl₄-induced rise in serum level of transaminases and prolongation in pentobarbital-sleeping time, confirming a protective effect of rutin against CCl₄-induced damage to hepatocytes (Janbaz *et al.*, 2002).

Conclusion:

Investigation of the flavonoidal contents of *S. surattensis* leaves revealed that the leaves are rich in flavonols. Quercetin and its glycosides rutin and quercetin 3-*O*-glucoside 7-*O*-ramnoside were isolated for the first time from the leaves.

The extract exhibited moderate cytotoxic activity against the five human tumor cell lines tested. Hela cell line was the most sensitive one.

In vivo treatment with *S. surattensis* leaf extract decreases the CCl₄-induced elevation in biochemical parameters. These findings suggest that the extract was effective in bringing about functional improvement of hepatocytes. The possible mechanism of hepatoprotective activity of *S. surattensis* may be due to its free radical-scavenging and antioxidant activity, which may be due to the presence of flavonoids in the extract. The antioxidant activity of flavonoids can scavenge free radical and protect the cell membrane from destruction. Hence, the transaminases (ALT/AST) may not leak into the blood from the necrotic hepatocytes. This finding indicates the efficacy of *S. surattensis* leaf extract as a hepatoprotective.

REFERENCES

- Calliste, C.A., P. Trouillas, D.P. Allias, A. Simon and J.L. Duroux, 2001. Free Radical Scavenging Activities Measured by Electron Spin Resonance Spectroscopy and B16 Cell Antiproliferative Behaviours of Seven Plants. *Journal of Agriculture and Food Chemistry*, 49: 3321-3327.
- Cheng, L.E., K. Breen, 2000. On the ability of four flavonoids, baicilin, luteolin, naringenin, and quercetin to suppress the fenton reaction of the iron-ATP complex. *BioMetals*, 13: 77-83.
- Chopra, R.N., S.L. Nayar and I.C. Chopra, 1956. *Glossary of Indian medicinal plants* (first ed.), CSIR, New Delhi, India, pp: 54.
- Constantin, M., C. Bromont, R. Fickat and R. Massingham, 1990. Studies on the activity of Bepridil as a scavenger of free radicals. *Biochemical Pharmacology*, 40: 1615-1622.
- Da Silva, E.J., M.K. Diskala, N. Yamamoto, J.H. Moon and J. Tro, 1998. Quercetin metabolites inhibit copper ion induced lipid peroxidation in rat plasma. *FEBS Lett*, 430: 405-409.
- De Walley, C.Y., J.F. Rankin and S.M. Rankin, 1990. flavonoids inhibit the oxidative modification of low density lipoproteins. *Biochemical Pharmacology*, 39: 1743-1749.
- Di Carlo, N.M., A.A. Izzo and F. Capasso, 1999. Flavonoids: old and new aspects of a class of natural therapeutic drugs. *Life Sciences*, 65: 337.
- El-Sawi, S.A. and A.A. Sleem, 2009. Antihyperlipidemic, antihyperglycemic and chemical composition of *Senna surattensis* (Burm.f.) leaves. *Canadian Journal of Pure and Applied Sciences*, 3(2): 779-785.
- Hemlata, S. and S.B. Kalidhar, 1994. Chemical components of *Cassia glauca* Lam.. *Indian Journal of Pharmaceutical Sciences*, 56(1): 33-34.

- Janbaz, K.H., S.A. Saeed and A.H. Gilani, 2002. Protective effect of rutin on paracetamol- and CCl₄-induced hepatotoxicity in rodents. *Fitotrapia*, 73(7-8): 57-63.
- Khare, G.C., A.K. Dixit and P.C. Gupta, 1994. Phytochemical investigation of the *Cassia glauca* seeds. *National Academy of Sciences letters*, 17(1-2): 21-23.
- Kind, P.R. and E.G. King, 1954. Colorimetric Method for the Determination of Serum ALP. *Journal of Clinical Pathology*, 7: 322-324.
- Klassen, C.D. and G.L. Plaa, 1969. Comparison of the Biochemical Alteration Elicited in Liver of Rats Treated with CCl₄ and CHCl₃. *Toxicology and applied Pharmacology*, 18: 2019-2022.
- Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. *The Systematic Identification of Flavonoids*. Springer Verlag, Berlin.
- Markham, K.R., 1982. *Techniques of Flavonoid Identification*. Academic Press, London.
- Marucci, L., G. Alpini, S.S. Glaser, D. Alvaro, A. Benedetti, H. Francis, J.L. Phinizy, M. Marzioni, J. Mauldin, J. Venter, B. Baumann, L. Ugili and G. Le Sege, 2003. Taurocholate feeding prevents CCl₄-induced damage of large cholangiocytes through PI3-kinase-dependent mechanism. *American Journal of Physiology Gastrointestinal Liver Physiology*, 284: G290-G301.
- Matsubara, N., S. Fuchimoto, H. Iwagaki, Y. Nonaka, T. Kimura, H. Kashino, R. Edamatsu, M. Hiramatsu and K. Orita, 1991. The possible involvement of free radical scavenging properties in the action of cytokines. *Research Communications in Chemical Pathology and Pharmacology*, 71: 239-242.
- Miller, L.C. and M. I. Tainter, 1944. Estimation of LD₅₀ and its error by means of logarithmic probit graph paper. *Proceedings of Society of Experimental. Biobiology and Medicine*, 57: 261-264.
- Ney, D.M., J.B. Laska and E.F. Shinnick, 1988. Normal diet and Induction of hyperlipidemia. *Journal of nutrition*, 118: 1455.
- Paget, G. and E. Berne's, 1964. Toxicity Tests in Evaluation of Drug Activities "cited in the laboratory rat". Eds. Laurence, D.R. and Bacharach, A.L., Academic Press. London, pp: 135-160.
- Ploa, G.L. and W.R. Hewitt, 1989 In: A. Wallace Hyes, Editor, *Principle and Methods of Toxicology* vol. II, Raven Press, New York, pp: 399.
- Rai, K.N. and R.A. Roy, 1991. A new anthraquinone glycoside from the stem of *Cassia glauca*, Lam. *Journal of Bangladesh Academy of Sciences*, 15(2): 193-198.
- Rai, K.N., K. Kaushalendra and J. Singh, 1997. Chemical constituents from the pods of *Cassia glauca* Lam. *Asian Journal of Chemistry*, 9(1): 44-49.
- Salpekar, J. and S.A. Khan, 1996. Luteolin-7-O-β-D-glucopyranosyl-(1-4)-O-α-L-arabinopyranoside from *Cassia glauca*. *Ultra Scientist of Physical Sciences*, 8(2): 260-261.
- Salvi, A., P. Carrapt, J. Tillement and B. Testa, 2001. Structural damage to protein and influence of protein binding. *Biochemical Pharmacology*, 61: 1237-1245.
- Shehan, P., R. Storeng, D. Scudiero, A. Monks, J. Mc Mahon and D. Vistica, 1990. New coloremtric cytotoxicity assay for anti-cancer drug screening. *Journal of National Cancer Institute*, 82: 1107-1112.
- Snedecor, W.G. and G.W. Cochran, 1971. *Statistical methods*, 10th ed. Iowa State University Press, USA.
- Thabrew, M.I., P.D.T.M. Joice and W.A. Rajatissa, 1987. Comparative study of efficacy of *Paetta indica* and *Osbeckia octandra* in the treatment of liver dysfunction, *Planta Medica*, 53: 239-241.
- Thewfweld, W., 1974. Enzymatic Method for Determination of Serum AST and ALT. *Deutsch Med wochen*, 99: 343-347.
- Tiwari, H.P. and M. Misra, 1993. Phytochemical investigation of *Cassia glauca* bark, *Journal of Indian Chemical Society*, 70(7): 653.
- Vison, J.A. and P. Bose, 1998. Comparative bioavailability to humans of ascorbic acids alone or in citrus extract. *American Journal of Clinical Nutrition*, 48: 601-604.
- Yu, C., F. Wang, C.L. Jin, X. Wu, W.K. Chan, W.L. McKeehan, 2002. Increased carbon tetrachloride-induced liver injury and fibrosis in FGFR4- deficient mice. *American Journal of Pathology*, 161: 2003-2010.
- Zielinska, M., A. Kostrzewa and E. Lognatowicz, 2000. Antioxidative activity of flavonoids in stimulated human neutrophils. *Folia Histochemica et Cytobiologica*, 38: 25-30.
- Zimmerman, H.J. and L.B. Steef, 1970. *Enzymes in hepatic disease*, *Diagnostic Enzymology* (Lea and Febiger, Philadelphia), pp: 1.