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ORIGINAL ARTICLE

Evaluation of the hepatroprotective and nephroprotective activities of *Scrophularia hypericifolia* growing in Saudi Arabia

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Abstract The hepatroprotective and nephroprotective effects of the ethanol extract of the aerial parts of *Scrophularia hypericifolia* growing in Saudi Arabia were evaluated at 250 and 500 mg kg^{-1} doses using Wistar albino rats as experimental animal model. Toxic doses of paracetamol were used to induce liver and kidney toxicities, while the standard drug silymarin was used as reference. The biochemical parameters such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT) and total bilirubin were estimated as reflections of the liver condition. Kidney condition was investigated through measurement of serum urea, serum creatinine, sodium and potassium levels. Liver and kidney samples of rats treated with 500 mg kg^{-1} of the extract were subjected to the histopathological study. The ethanol extract of the aerial parts of *S. hypericifolia* showed dose dependent moderate level of protection against paracetamol induced hepatrotoxicity and nephrotoxicity as indicated from the obtained results. The reduction of the sodium and potassium levels by the higher dose of the extract exceeded that obtained by silymarin.

1. Introduction

Liver and kidney diseases represent a major global health problem (Baranisrinivasan et al., 2009; Couser et al., 2011). Toxic chemicals, xenobiotics, alcohol consumption, malnutrition, and medications, cause liver and renal damage due to exposure to high quantities of free radicals leading to oxidative stress (Marina, 2006; Dianzani et al., 1991; Abubaker et al., 2012). Several hepatoprotective compounds were discovered from plants including the iridoid glycosides picroside I and kutkoside (Ram, 2001; Ansari et al., 1988). Some Chinese traditional plants such as *Astragalus membranaceus*, *Ligusticum wallichii*, *Tripterygium wilfordii* and *Rheum officinale* (Rhubarb), have a beneficial role in slowing the progression of chronic kidney diseases (Peng et al., 2005).

The genus *Scrophularia* is represented by over 300 species worldwide (Jiangsu New Medical College, 1977). In Saudi Arabia, about 5 species were described (Collenette, 1999).

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Genus *Scrophularia* is rich in iridoid glycosides, phenylpropanoids and flavonoids (Li et al., 1999; Yamamoto et al., 1993; Boros and Stermitz, 1990; Miyase and Mimatsu, 1999). Traditionally plants belonging to the genus are used for the treatment of wounds (Grieve, 1992), various inflammatory conditions, fever, constipation, swelling (Jiangsu New Medical College, 1977; Duck and Ayensu, 1985) and as remedy for kidney diseases (Perry and Metzger, 1980). Iridoids from Scrophularia species exert hapatoprotective activity (Garg et al., 1994).

2. Experimental

2.1. Plant materials

Plants of *Scrophularia hypericifolia* Wydler were collected in March, 2010 from the Al-Qassim province, Saudi Arabia. The plants were identified by Dr. Mohammad Atiqur Rahman, taxonomist of the Medicinal, Aromatic and Poisonous Plants Research Center (MAPPRC), College of Pharmacy, King Saud University, Riyadh, Saudi Arabia. A voucher specimen (# 13274) was deposited at the herbarium of this center.

2.2. Extraction

Air dried powdered aerial parts (500 g) were exhaustively extracted with 90% ethanol by percolation at room temperature. The ethanol extract was filtered and the solvent was distilled off under reduced pressure at 45 °C to give 15 g residue.

2.3. Animals and chemicals

Wistar albino rats (150–200 g) of both sexes obtained from the Experimental Animal Care Center, College of Pharmacy, King Saud University, Riyadh, were used. The animals were housed under constant temperature (22 ± 2 °C), humidity (55%) and light/dark conditions (12/12 h). They were provided with Purina chow and access to drinking water *ad libitum*.

All solvents used were of analytical grade. Silymarin and paracetamol were obtained from Sigma Aldrich (St. Louis, USA).

2.4. Hepatoprotective and nephroprotective activity

Animals were divided into 4 groups, of five animals each. Group I was used as the control group, Groups II, III and IV received 500 mg of paracetamol per kg body weight intraperitoneally for 3 days. Group II received only paracetamol. Group III was administered silymarin at a dose of 10 mg/kg p.o. Group IV was divided into two subgroups ($n = 5$) treated with the ethanol extract at 500 and 250 mg/kg. Drug treatment was started 5 days prior to paracetamol administration and continued till day 6. After 24 h of the third paracetamol dose administration, the animals were sacrificed under ether anesthesia. Blood samples were collected by cardiac puncture and the serum was separated for determining the different biochemical parameters. The livers and kidneys were immediately removed; small pieces were fixed in 10% formalin and kept for histopathological assessment.

2.5. Determination of biochemical parameters

Five biochemical parameters; AST, ALT, ALP, GGT and total bilirubin were estimated as reported by Edwards and Bouchier (1991). The enzyme activities were measured using diagnostic strips (Reflotron®, ROCHE) and were read on a Reflotron® Plus instrument (ROCHE).

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2.7. Determination of non-protein sulfhydryl groups (NP-SH)

The liver or kidney was cooled in a beaker immersed in an ice bath. The tissues were homogenized in 0.02 M ethylenediaminetetraacetic acid (EDTA) in a Potter–Elvehjem type C homogenizer. Homogenate equivalent to 100 mg tissues were used for the measurements. Non-protein sulfhydryl groups (NP-SH) were quantified by mixing homogenated with 4 ml of distilled water and 1 ml of 50% trichloroacetic acid (TCA) in 15 ml test tubes. The tubes were shaken intermittently for 10–15 min and centrifuged for 15 min at approximately 3000 rpm to precipitate the protein. 2 ml of the supernatant was mixed with 4 ml of 0.4 M Tris buffer, pH 8.9 and 0.1 ml of 0.01 M DTNB [5,5′-dithio-bis-(2-nitrobenzoic acid)] was added and the sample was shaken. The absorbance was measured spectrophotometrically within 5 min of addition of DTNB at 412 nm against a reagent blank with no homogenate (Sedlak and Lindsay, 1968).

2.8. Statistical analysis

For each set of experiments where two or more than two groups were compared, an analysis of variance (ANOVA) test was used to determine the significance of the differences. Differences between the control and paracetamol-treated group were compared for significance using Student's *t*-test for non-paired samples (Woolson and Clarke, 2002). All the values shown are the mean ± S.E.

2.9. Histopathological study

The liver was immediately removed; fixed in 10% formalin, dehydrated with ethanol xylene mixtures and fixed with paraffin wax. Thin sections (3 μm) were stained in Mayer's hematoxylin solution followed by eosin-phloxine solution. Details of the experimental procedures were described by Alqasoumi et al. (2009).

Table 1 Effect of *S. hypericifolia* (SH) on the liver serum biochemical parameters ($n = 5$).

Treatment	ALT (u/l)		AST (u/l)		GGT (u/l)		ALP (u/l)		Bilirubin (mg/dl)	
	(Mean ± S.E.)	% Change	(Mean ± S.E.)	% Change	(Mean ± S.E.)	% Change	(Mean ± S.E.)	% Change	(Mean ± S.E.)	% Change
Normal	86.4 ± 4.25		37.35 ± 2.88		3.46 ± 0.20		360.83 ± 9.44		0.54 ± 0.01	
Paracetamol 500 mg	292.83 ± 9.00[c]		274.16 ± 6.03[c]		13.48 ± 0.31[c]		633.33 ± 13.35[c]		2.86 ± 0.08[c]	
Silymarin 10 mg	152.16 ± 6.24[c]	48.03	102.1 ± 3.78[c]	62.75	4.93 ± 0.21[c]	63.41	434.5 ± 17.40[c]	31.39	1.20 ± 0.10[c]	57.98
SH 250 mg	196.33 ± 9.13[c]	21.52	184.83 ± 8.51[c]	32.58	10.81 ± 0.33[c]	19.77	505.83 ± 13.59[c]	20.13	1.89 ± 0.06[c]	25.36
Normal	80.03 ± 4.37		39.00 ± 2.87		4.29 ± 0.32		276.5 ± 10.09		0.56 ± 0.05	
Paracetamol 500 mg	245.5 ± 12.03[c]		218.66 ± 11.40[c]		15.46 ± 0.89[c]		532.5 ± 19.27[c]		3.21 ± 0.1[c]	
Silymarin 10 mg	133.83 ± 6.27[c]	45.48	115.26 ± 7.08[c]	47.28	6.34 ± 0.24[c]	58.96	331.00 ± 17.67[c]	37.84	1.05 ± 0.04[c]	67.32
SH 500 mg	192.66 ± 5.68[b]	32.95	165.00 ± 14.35[a]	24.54	11.31 ± 0.53[b]	26.83	376.66 ± 10.80[c]	26.26	2.39 ± 0.12[c]	33.74

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

3. Results and discussion

3.1. Hepatoprotective activity

Although therapeutic doses of paracetamol are safe, toxic doses can produce fatal hepatic necrosis in man, rats and mice (Mitchell et al., 1973; Ray et al., 1996). When administered in the regular therapeutic doses only 5% of the dose is converted into N-acetyl-p-benzoquineimine (NAPQI) (Eriksson et al., 1992). Toxic doses of paracetamol lead to saturation of the sulfation and glucoronidation routes consequently, higher percentage of the molecules is oxidized to highly reactive NAPQI. Semiquinone radicals, obtained by one electron reduction of NAPQI, rapidly conjugated with glutathione (GSH) leading to depletion of liver GSH pool (Remirez et al., 1995). Due to GSH depletion, NAPQI covalently binds to vital proteins, the lipid bilayer of hepatocyte membranes and increases the lipid peroxidation. The result is hepatocellular death and centrilobular liver necrosis (McConnachie et al., 2007). The hepatocytes transport function gets disturbed resulting in leakage of plasma membrane, thus causing an increase in serum enzyme levels (Zimmerman and Seeff, 1970).

Biochemical parameters such as AST, ALT, GGT, ALP and total bilirubin were taken as a measure to reflect the liver condition. Treatment of rats with paracetamol resulted in dramatic increase in the levels of AST, ALT, GGT, ALP and bilirubin (Table 1) (Edwards and Bouchier, 1991). The use of silymarin, significantly ($P < 0.001$) decreased the raised levels of AST, ALT, GGT, ALP and bilirubin induced by paracetamol indicating a good recovery from the hepatotoxic agent. The use of *S. hypericifolia* (SH) ethanol extract showed a dose dependent protection against paracetamol toxicity. Treatment with 250 mg kg^{-1} doses of SH resulted in reduction in AST, ALT, ALP and bilirubin by 21.52%, 32.58%, 20.13% and 25.36%, respectively. The level of GGT was slightly improved (19.77%) as compared with silymarin (63.41%). All the results were highly significant ($P < 0.001$) (Table 1). Higher doses of SH (500 mg kg^{-1}) resulted in more reduction in the levels of the measured biochemical parameters (32.95%, 24.54%, 26.83%, 26.26% and 33.745%). The obtained results were significant ($P < 0.05$, $P < 0.01$) (Table 1).

One of the most important liver functions is protein synthesis. Liver damage causes disruption and disassociation of polyribosomes on endoplasmic reticulum and thereby reducing the biosynthesis of protein. Restoring the normal levels of protein is an important parameter for liver recovery (Navarro and Senior, 2006). Paracetamol treatment resulted in sever decrease in liver protein level from 135.25 ± 13.23 to 22.24 ± 2.72 g/l. The level of protein increased with silymarin treatment to 90.57 ± 13.58 g/l, while treatment with 500 mg kg^{-1} of SH significantly improved the level to reach 61.78 ± 9.58 g/l (Table 3).

The ability of glutathione *S*-transferases (GSTs) to catalyze the conjugation of reduced glutathione – via a sulfhydryl group – to electrophilic centers on a wide variety of substrates enables the detoxification of endogenous compounds such as peroxidized lipids, and the breakdown of xenobiotics (Douglas, 1987). GSTs may also bind toxins and serve as transport proteins (Leaver and George, 1998). Treatment of animals with hepatotoxic agents leads to depletion of GSH and reduction in the non-protein sulfhydryl moiety (NP-SH) (Naaz et al., 2007; Abdel-Kader et al., 2010). The group

Table 2 Effect of *S. hypericifolia* (SH) on the kidney biochemical parameters ($n = 5$).

Treatment	Urea (mg/dl)		Creatinine (mg/dl)		Sodium (mmol/l)		Potassium (mmol/l)	
	(Mean ±)	% Change	(Mean ±)	% change	(Mean ±)	% Change	(Mean ±)	% Change
Normal	49.86 ± 1.69		3.3 ± 0.22		73.45 ± 2.01		6.40 ± 0.25	
Paracetamol 500 mg	150.5 ± 4.37[a]		13.11 ± 0.51[a]		182.00 ± 4.77[a]		14.13 ± 0.44[a]	
Silymarin 10 mg	94.48 ± 4.93[a]	37.22	4.23 ± 0.27[a]	67.72	100.85 ± 3.22[a]	44.58	8.46 ± 0.43[a]	52.40
SH 250 mg	119.33 ± 4.09[a]	20.70	8.58 ± 0.38[a]	34.56	112.26 ± 3.97[a]	38.31	8.23 ± 0.27[a]	48.16
Normal	47.43 ± 10.32		2.95 ± 0.17		82.38 ± 4.09		5.33 ± 0.28	
Paracetamol 500 mg	152.00 ± 3.70[a]		10.95 ± 0.67[a]		167.16 ± 8.05[a]		11.80 ± 0.90[a]	
Silymarin 10 mg	98.45 ± 3.97[a]	35.23	5.26 ± 0.29[a]	51.90	114.16 ± 4.26[a]	31.70	5.61 ± 0.23[a]	40.09
SH 500 mg	113.00 ± 3.19[a]	25.65	6.01 ± 0.21[a]	45.05	110.18 ± 3.00[a]	34.08	6.11 ± 0.21[a]	41.74

[a] $P < 0.001$.

treated with paracetamol showed reduction in the NP-SH from 8.29 ± 0.64 to 5.77 ± 0.27 nmol/g. The recovery of the NP-SH levels after treatment with 500 mg kg^{-1} of SH (7.21 ± 0.53 nmol/g) was closer to that observed in the silymarin treated group (7.73 ± 0.47 nmol/g).

3.2. Nephroprotective activity

Acute over doses of paracetamol may lead to death due to renal failure (Abraham, 2005). Paracetamol renal damage is mediated by its deacetylation into *p*-amino phenol excreted in urine (Mugford and Tarlolf, 1997). Glutathione conjugates formed in the liver are involved in paracetamol induced renal toxicity (Li et al., 2003). The kidney functions include removal of nitrogenous metabolic waste products such as urea, creatinine and uric acid as well as regulation of ions in plasma (Pocock and Richards, 2006). Elevations of serum electrolytes, urea and creatinine are reliable parameters for investigating drug-induced nephrotoxicity in animals and man (Adelman et al., 1981). The group treated with paracetamol showed tremendous rise in the levels of serum urea, serum creatinine, sodium and potassium (Table 2). Treatment with silymarin at 10 mg kg^{-1} dose resulted in highly significant reduction in the elevated levels of the measured parameters. SH extract showed dose dependent nephroprotective effect against the paracetamol toxicity. The higher dose of SH (500 mg kg^{-1}) showed highly significant ($P < 0.001$) 25.65% and 45.05% decrease in the levels of serum urea and creatinine, respectively. This decrease is slightly less than that observed in the silymarin treated group (35.23% and 51.90%). On the other hand, reduction of the sodium and potassium levels in the group treated with 500 mg kg^{-1} SH (34.08% and 41.74% respectively) exceeded that measured in the silymarin treated group (31.70% and 40.09%, respectively).

Paracetamol treatment resulted in sever decrease in kidney protein level from 71.19 ± 7.30 to 16.86 ± 2.05 g/l. The level of protein increased with silymarin treatment to 50.55 ± 5.28 g/l, while treatment with 500 mg kg^{-1} of SH significantly ($P < 0.05$) improved the level to reach 29.86 ± 4.36 g/l (Table 3). The group treated with paracetamol showed reduction in the kidney NP-SH from 4.63 ± 0.28 to 1.91 ± 0.33 nmol/g. The recovery of the NP-SH levels by 500 mg kg^{-1} of SH (3.96 ± 0.42 nmol/g) was closer to that detected in the silymarin treated group (4.18 ± 0.49 nmol/g).

3.3. Histopathological study

The histological appearance of the hepatocyte reflects their conditions (Prophet et al., 1994). Liver cells of rats treated with 500 mg kg^{-1} paracetamol (Fig. 1B) showed great damage represented by extensive focal necrosis, lymphocytic infiltrate, extensive hydropic swelling with rosette formation, lymphocytic exudates and dilated congested vessels in portal tracts. Liver cells treated with 10 mg kg^{-1} of the standard drug Sily (Fig. 1C) prior to paracetamol administration showed improvement in the liver cells histopathology with granular cytoplasm, mild congestion in central veins, mild portal tract infiltration and few focal necrosis. Treatment with SH 500 mg kg^{-1} (Fig. 1D) resulted in improvement in hepatocytes histological appearance comparable with that resulted from

Table 3 Effect of *S. hypericifolia* (SH) on the liver and kidney protein and NP-SH ($n = 5$, (mean ± S.E).

Treatment	Liver		Kidney	
	Protein (g/l)	NP-SH (nmol/g)	Protein (g/l)	NP-SH (nmol/g)
Normal	135.25 ± 13.23	8.29 ± 0.64	71.19 ± 7.30	4.63 ± 0.28
Paracetamol 500 mg	22.24 ± 2.72[c]	5.77 ± 0.27[b]	16.86 ± 2.05[c]	1.91 ± 0.33[c]
Silymarin 10 mg	90.57 ± 13.58[c]	7.73 ± 0.47[b]	50.55 ± 5.28[c]	4.18 ± 0.49[b]
SH 250 mg	34.71 ± 2.79[a]	6.86 ± 0.57	24.60 ± 3.10[a]	2.56 ± 0.37
SH 500 mg	61.78 ± 9.58[b]	7.21 ± 0.53[a]	29.86 ± 4.36[a]	3.96 ± 0.42[b]

[a] $P < 0.05$.
[b] $P < 0.01$.
[c] $P < 0.001$.

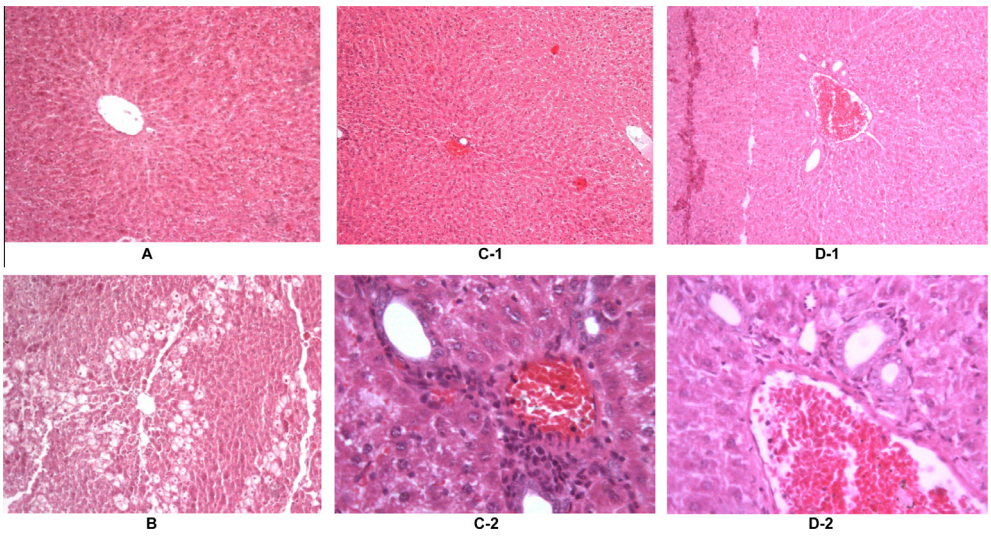

Figure 1 Histopathological study of liver cells; (A) normal cells; (B) liver cells of rats treated with Paracetamol; (C) liver cells of rats treated with Paracetamol and Silymarin; (D) liver cells of rats treated with Paracetamol and 500 mg kg^{-1} of SH.

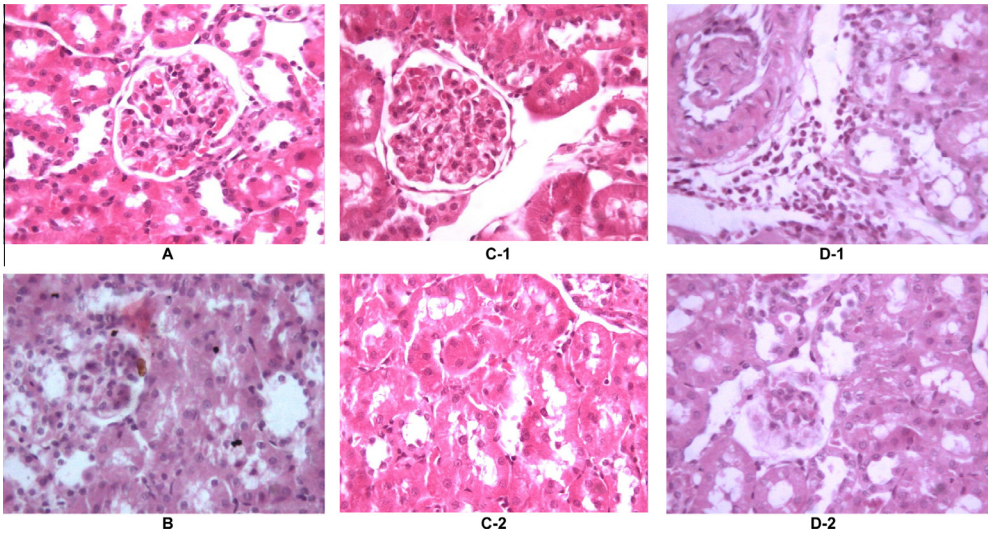

Figure 2 Histopathological study of kidney cells; (A) normal cells; (B) kidney cells of rats treated with Paracetamol; (C) kidney cells of rats treated with Paracetamol and Silymarin; (D) kidney cells of rats treated with Paracetamol and 500 mg kg^{-1} of SH.

treatment with silymarin. Congestion in portal tracts was less than that observed with the silymarin group along with mild sinusoidal dilation and slightly swollen cells.

Paracetamol treated rats showed sever damage in the kidney cells (Fig. 2B) appeared as variable size and atrophic cellular glomeruli, marked cloudy swelling in tubules and narrow lumens. The protective standard drug Silymarin at 10 mg kg^{-1} (Fig. 2c) helped in decreasing the cellular damage induced by paracetamol. Cellular appearance showed mostly nearly normal glomeruli with few variable size atrophic glomeruli, mild tubular degeneration, necrosis and cloudy swelling. Kidneys of animal treated with 500 mg kg^{-1} (Fig. 2D) showed marked neutrophilic exudate around blood vessels and in between tubules, neutrophilic infiltration and some atrophic glomeruli. Although the cells conditions are better than the paracetamol group, the protection was less than that observed with silymarin.

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References

Abdel-Kader, M.S., Alqasoumi, S.I., Hefnawy, M.M., AlSheikh, A.M., 2010. Hepatoprotective effect and safety studies of *Cleome droserifolia*. Alex. J. Pharm. Sci. 24 (1), 13–21.

Abraham, P., 2005. Oxidative stress in paracetamol-induced pathogenesis: (I) renal damage. Indian J. Biochem. Biophys. 42, 59–62.
Abubaker, S., Shanmukha, I., Rajendra, S.V., Ramachandra, S.S., 2012. Protective effect of *Spathodea campanulata* bark against paracetamol-induced nephrotoxicity in rats. Int. J. PharmTech Res. 4 (1), 398–403.
Adelman, R.D., Spangler, W.L., Beasom, F., Ishizaki, G., Conzelman, G.M., 1981. Frusemide enhancement of neltimicin nephrotoxicity in dogs. J. Antimicrob. Chemother. 7, 431–435.
Alqasoumi, S.I., Al-Dosari, M.S., AlSheikh, A.M., Abdel-Kader, M.S., 2009. Evaluation of the hepatoprotective effect of *Fumaria parviflora* and *Momordica balsamina* from Saudi folk medicine against experimentally induced liver injury in rats. Res. J. Med. Plants 3 (1), 9–15.
Ansari, R.A., Aswal, B.S., Chander, R., 1988. Hepatoprotective activity of kutkin, the iridoid glycoside mixture of *Picrorhiza kurroa*. Indian J. Med. Res. 87, 401404.
Baranisrinivasan, P., Elumalai, E.K., Sivakumar, C., Therasa, S.V., David, E., 2009. Hepatoprotective effect of *Enicostemma littorale* blume and *Eclipta alba* during ethanol induced oxidative stress in albino rats. Int. J. Pharm. 5 (4), 268–272.
Boros, C.A., Stermitz, F.R., 1990. Iridoids. An updated review. J. Nat. Prod. 53, 1055–1147.
Collenette, S., 1999. Wild Flowers of Saudi Arabia. East Anglian Engraving Co. Ltd., Norwich.
Couser, W.G., Remuzzi, G., Mendis, S., Tonelli, M., 2011. The contribution of chronic kidney disease to the global burden of major noncommunicable diseases. Kidney Int. 80 (12), 1258–1270.
Dianzani, M.U., Muzia, G., Biocca, M.E., Canuto, R.A., 1991. Lipid peroxidation in fatty liver induced by caffeine in rats. Int. J. Tissue React. 13 (1), 79–85.
Douglas, K.T., 1987. Mechanism of action of glutathione-dependent enzymes. Adv. Enzymol. Relat. Areas Mol. Biol. 59, 103–167.
Duck, J.A., Ayensu, E.S., 1985. Medicinal Plants of China. Reference Publications, Inc., Algonac, MI, p. 599.
Edwards, C.R.W., Bouchier, I.A.D., 1991. Davidson's Principles and Practice Medicine. Churchill Livingstone Press, UK.
Eriksson, L., Broome, U., Kahn, M., Lindholm, M., 1992. Hepatotoxicity due to repeated intake of low doses of paracetamol. J. Intern. Med. 231 (5), 567–570.
Garg, H.S., Bhandari, S.P.S., Tripathi, S.C., Patnaik, G.K., Puri, A., Saxena, R., Saxena, R.P., 1994. Antihepatotoxic and immunostimulant properties of iridoid glycosides of *Scrophularia koelzii*. Phytother. Res. 8 (4), 224–228.
Grieve, M., 1992. In: Leyel, C.F. (Ed.), A Modern Herbal. Tiger Books International, London, p. 313.
Henry, R.J., Cannon, D.C., Winkelman, J.W., 1974. Clinical Chemistry, Principles and Techniques. Harper Row, New York.
Jiangsu New Medical College, 1977. Dictionary of Chinese Traditional Medicines. Shanghai Scientific and Technical Publishers, Shanghai.
Leaver, M.J., George, S.G., 1998. A piscine glutathione S-transferase which efficiently conjugates the end-products of lipid peroxidation. Mar. Environ. Res. 46 (1–5), 71–74.
Li, Y., Jiang, S., Gao, W., Zhu, D., 1999. Iridoid glycosides from *Scrophularia ningpoensis*. Phytochemistry 50, 101–104.
Li, C., Liu, J., Saavedra, J.E., Keefer, L.K., Waalkes, M.P., 2003. The nitric oxide donor, V-PYRRO/NO, protects against acetaminophen-induced nephrotoxicity in mice. Toxicology 189 (3), 173–180.
Marina, N., 2006. Hepatotoxicity of antiretrovirals: incidence, mechanisms and management. J. Hepatol. 44 (1), S132–S139.
McConnachie, L.A., Mohar, I., Hudson, F.N., Ware, C.B., Ladiges, W.C., Fernandez, C., Chatterton-Kirchmeier, S., White, C.C., Pierce, R.H., Kavanagh, T.J., 2007. Glutamate cysteine ligase modifier subunit deficiency and gender as determinants of acetaminophen-induced hepatotoxicity in mice. Toxicol. Sci. 99 (2), 628–636.
Mitchell, J.R., Jollow, D.J., Potter, W.Z., Gillete, J.R., Brrodle, B.N., 1973. Acetaminophen induced hepatic necrosis. I. Role of drug metabolism. J. Pharmacol. Exp. Ther. 187 (1), 185–194.
Miyase, T., Mimatsu, A., 1999. Acylated iridoid and phenylethanoid glycosides from the aerial parts of *Scrophularia nodosa*. J. Nat. Prod. 62 (8), 1079–1084.
Mugford, C.A., Tarlolf, J.B., 1997. The contribution of oxidation and deacetylation to acetaminophen nephrotoxicity in female Sprague-Dawley rats. Toxicol. Lett. 93 (1), 15–22.
Naaz, F., Javed, S., Abdin, M.Z., 2007. Hepatoprotective effect of ethanolic extract of *Phyllanthus amarus* Schum. et Thonn. on aflatoxin B1-induced liver damage in mice. J. Ethnopharmacol. 113 (3), 503–509.
Navarro, V.J., Senior, J.R., 2006. Drug-related hepatotoxicity. N. Engl. J. Med. 35 (4), 731–739.
Peng, A., Gu, Y., Lin, S.Y., 2005. Herbal treatment for renal diseases. Annals Academy of Medicine Singapore 34 (1), 44–51.
Perry, L.M., Metzger, J., 1980. Medicinal Plants of Southeast Asia. MIT Press, Cambridge, MA and London, UK, p. 385.
Pocock, G., Richards, C.D., 2006. Human Physiology. The basis of Medicine, 3rd ed. Oxford University Press.
Prophet, E.P., Mills, B., Arrington, J.B., Sobin, L.H., 1994. Laboratory Methods in Histology, 2nd ed. American Registry of Pathology, Washington, DC.
Ram, V.J., 2001. Herbal preparations as a source of hepatoprotective agents. Drug News Perspect. 14 (6), 353–363.
Ray, S.D., Mumaw, V.R., Raje, R.R., Fariss, M.W., 1996. Protection of acetaminophen induced hepatocellular apoptosis and necrosis by cholesteryl hemisuccinole pretreatment. J. Pharmacol. Exp. Ther. 279, 1470–1483.
Remirez, D., Commandeur, J.N.M., Ed Groot, E., Vermeulen, N.P.E., 1995. Mechanism of protection of *Lobenzarti* against paracetamol-induced toxicity in rat hepatocytes. Eur. J. Pharmacol. Environ. Toxicol. Pharmacol. 293 (4), 301–308.
Sedlak, J., Lindsay, R.H., 1968. Estimation of total, protein-bound, and nonprotein sulfhydryl groups in tissue with Ellman's reagent. Anal. Biochem. 25, 192205.
Varley, H., Alan, H.G., 1984. Tests in renal disease. In: Practical Clinical Biochemistry, vol. 1123. William Heinemann Medical Book Ltd., London.
Woolson, R.F., Clarke, W.R., 2002. Statistical Methods for the Analysis of Biochemical Data, 2nd ed. John Wiley and Sons, Inc., New York.
Yamamoto, A., Miyase, T., Ueno, A., Maeda, T., 1993. Scrophulasaponins II-IV, new saikosaponin homologs from *Scrophularia kakudensis* FRANCH. Chem. Pharm. Bull. 41 (10), 1780–1783.
Zimmerman, H.J., Seeff, L.B., 1970. Enzymes in hepatic disease. In: Goodly, E.I. (Ed.), Diagnostic Enzymology, 1, Lea and Febiger, Philadelphia, USA.