Natural Product Sciences
12(1) : 8-13 (2006)

Protective Effect of *Pterocarpus santalinus* on Galactosamine Induced Liver Damage

S. P. Dhanabal*, G. Syamala[1], K. Elango, and B. Suresh

Department of Pharmacognosy and Pharmacology, JSS College of Pharmacy, Rocklands, OOtacamund-643001
[1]PSG College of Pharmacy, Coimbatore-641004 Tamilnadu, India

Abstract – The present study was carried out to investigate the hepatoprotective effect of the extract of *Pterocarpus santalinus* Linn on acute hepatotoxicity induced in Wistar albino rats by a single dose of Galactosamine (400 mg/kg). Suspensions of methanolic extract of heartwood of *P. santalinus* (200 and 400 mg/kg) in 0.3% Carboxy Methyl Cellulose (CMC) were administered p.o. to experimental animals and hepatoprotective activity was monitored by estimating aspartate amino transferase (ASAT, GOT), alanine amino transferase (ALAT, GPT), alkaline phosphatase (ALP), total bilirubin (TB), lactate dehydrogenase (LDH), total cholesterol (TC), triglycerides (TGL), albumin, total protein (TP) levels. The methanolic extract significantly reduced the elevation of serum transaminases and alterations of biochemical parameters induced by hepatotoxin, and alleviated the degree of liver damage. The results were supported by histopathological studies of liver samples showing regeneration of hepatocytes in treated animals. Silymarin (25 mg/kg), a known hepatoprotective drug was used for comparison. Based on the results obtained, it can be concluded that *P. santalinus* exerts hepatoprotective activity and may serve as a useful adjuvant in several clinical conditions associated with liver damage.
Keywords – *Pterocarpus santalinus* Linn., galactosamine, silymarin, hepatoprotective effect, histopathology

Introduction

Herbs are known to play a vital role in the management of various liver disorders. Ayurveda, the ancient system of Indian Medicine identified liver diseases quite early and recommended a number of herbal remedies. Ayurvedic and other traditional medical practitioners from different countries have claimed for centuries that extracts of plants can be effectively used for the alleviation of different types of liver diseases (Ding, 1987; Attygalle, 1952) but most claims are anecdotal and very few have received adequate medical and scientific evaluation until the 1970s. Investigations carried out since then have provided experimental evidence confirming that many of these plants do have hepatoprotective properties (Bertelli, 1975).

Pterocarpus santalinus Linn (Fabaceae) (PS) commonly known as "Red sanders" is a small to medium sized tree, attaining 7.5 m, with extremely hard, dark purple heartwood with bitter flavor. In indigenous system of medicine, the wood has been reported to possess various medicinal properties viz., cooling, antipyretic, anthelmintic, tonic, aphrodisiac, useful in vomiting, thirst, eye diseases, mental aberrations, ulcers, diaphoretic, paste applied to inflammations and to forehead in headache and the seeds stop haemorrhage of the urethra; useful in dysentery. The wood, rubbed up with water, is advantageously employed as a wash in superficial excoriation of the genital organs. The wood in combination with other drugs is prescribed for the treatment of snake-bite and scorpion-sting (Warrier *et al.*, 1995).

Heartwood contains pterocarpol, santalin A, B, pterocarptriol, ispterocarpolone (Yoganarasimhan, 2000) and also the presence of Auron glycosides have been reported (Krishnaveni and Srinivas Rao, 2000). Decoction of the heartwood showed CNS depression, anti-inflammatory activity in formalin (3%) induced hind paw edema in rats and effect of bark extract on blood glucose level in experimental animals have also been reported (Varma and Vijayamma, 1991). The methanolic and aqueous extracts of heartwood of another species *Pterocarpus marsupium* showed antihepatotoxicity in CCl_4 induced hepatoxicity and reduced elevated levels of SGOT, SGPT and alkaline phosphatase (Anupa Rane and Nirmala D.Gramarc, 1998). Himoliv, a polyherbal Ayurvedic formulation containing PS as one of the ingredient has been reported to possess hepatoprotective activity (Battacharya *et al.*, 2003).

*Author for correspondence
Fax: +0423 –2442937; E-mail: dhanskanaksahil@rediffmail.com

Hence, the hepatoprotective effect of this species has not been determined; the present study was intended to investigate the *in vivo* anti-hepatotoxic effects of the heartwood of PS.

Experimental

All chemicals used were of analytical grade, obtained from SD Fine Chemicals, Mumbai. Ecoline diagnostic kits were purchased from E. Merck, India. Galactosamine Hydrochloride (D-GalN) was obtained from Sigma Chemical Co., USA and Silymarin (Muneesh Pharmaceuticals, Pondicherry) a marketed formulation, was used as a reference standard. Autoanalyzer (Microlab 200), Vital Scientific, The Netherlands was used for the estimation of all biochemical parameters.

Plant material – The wood part of PS was collected from the fields nearby Coimbatore, Tamilnadu State in the month of June 2003 and authenticated by comparing with voucher specimen preserved at Survey of Medicinal Plants and Collection Unit, Ootacamund. A voucher specimen of the species has been deposited at the Dept. of Pharmacognosy, JSSCP, Ootacamund (No.JSSCP/PCOG/203/2004).

Preparation of crude extract – The heartwood of PS (320 g) was subjected to hot continuous extraction in a Soxhlet extractor using methanol (2.0 L) for 20 - 24 h. The methanol extract (ME) was then concentrated to dryness under reduced pressure and controlled temperature to yield a dark brownish semisolid mass (16.00% w/w), which was preserved in refrigerated condition. The ME and the standard powder were suspended in sodium carboxy methyl cellulose (CMC) (0.3%) in distilled water separately and used for *in vivo* investigations.

Animals – Healthy adult albino rats of Wistar strain weighing 180 - 220 g and Swiss albino rats procured from JSSCP animal house, Ootacamund were housed in large spacious hygienic polypropylene cages and fed with rat pellet feed supplied by Hindustan Lever Ltd., Bangalore, India and water *ad libitum* (aquaguard filter water). The study was cleared by the IAEC (Regn. No: JSSCP/IAEC/PH/COGNOSY/M.PHARM/02/2004-05).

Toxicity studies – The ME was administered orally (200 - 4000 mg/kg) to different groups of mice. There was no lethality in any of the groups. Mice, which received extract in doses above 3000 mg/kg exhibited ptosis (dropping of upper eyelids) and were found lethargic. One tenth of the maximum dose of the extract tested for acute toxicity was selected as maximum dose for evaluation of antihepatotoxic activity i.e., 400 mg/kg, p.o. (Hand, 1991).

Assessment of in vivo hepatoprotective effect

Induction of hepatotoxicity – Experimental hepatotoxicity in Wistar albino rats was induced by i.p. administration of GalN at 400 mg/kg b.w. in sterile water (Ferencikova *et al.*, 2003).

Grouping of animals – The investigation was carried out in five groups with six animals in each group and carried out in the following regimen.

Group I : Served as solvent control, which received 0.3% CMC (1 ml/kg b.w.) orally for 15 days.

Group II : Received 0.3% CMC (1 ml/kg b.w.) for 14 days orally and hepatotoxicant GalN as a single dose of 400 mg/kg b.w. i.p. on 14th day.

Group III : Received silymarin (25 mg/kg b.w.) for 14 days orally and hepatotoxicant on 14th day i.p.

Group IV : Received ME of PS (200 mg/kg b.w.) for 14 days orally and hepatotoxicant on 14th day i.p.

Group V : Received ME of PS (400 mg/kg b.w. for 14 days orally and hepatotoxicant on 14th day i.p.

Silymarin, a known hepatoprotective agent, was used as reference standard for comparison at a dose of 25 mg/kg body weight in 0.3% CMC (Detlef *et al.*, 1999).

Assessment of liver function – After 14 days of treatment and intoxication, blood was collected and serum separated and *used* for the estimation of biochemical parameters viz., ASAT (GOT), ALAT (GPT), alkaline phosphatase (ALP), total bilirubin (TB), total protein (TP), lactate dehydrogenase (LDH), triglycerides (TGL) total cholesterol (TC) and albumin using E-coline diagnostic kits (Yoshinobu *et al.*, 1983) to determine the functional state of the liver.

Histological investigation of the liver slices was processed for paraffin embedding for 48 h in 10% formalin, following the standard microtechnique (Galigher and Koyloff, 1971). Five micron sections of the livers stained with haematoxylin and eosin were examined for histopathological changes under a light microscope.

Statistical analysis – Results of biochemical parameters were reported as Mean ± S.D. for determination of significant intergroup differences. Each parameter was analyzed separately and a one way analysis of variance (ANOVA) was carried out (Alfanso *et al.*, 1995) followed by Dunnett's "t" test for individual comparisons. The results were judged significant if $P < 0.05$.

Results

The effect of ME of PS on GalN intoxicated rats is recorded in Table 1. Rats intoxicated with GalN significantly altered the biochemical parameters when compared

Table 1. Effect of methanol extract of *Pterocarpus santalinus* on different biochemical parameters in the serum of rats (n = 6)

Groups	TB (mg/dl)	ASAT (U/L)	ALAT (U/L)	ALP (U/L)	TGL (mg/dl)	TC (g/dl)	Albumin (g/dl)	TP (g/dL)	LDH (U/L)
solvent control	0.3 ± 0.2	65.58 ± 0.62	35.63 ± 0.76	435.57 ± 3.26	71.31 ± 1.11	55.03 ± 1.38	4.92 ± 0.03	7.08 ± 0.08	423.65 ± 2.00
heptato toxicant (D-GalN)	2.17 ± 0.09	136.22 ± 1.85^a	73.47 ± 1.69^a	903.21 ± 6.09^a	26.36 ± 0.36^a	170.47 ± 2.42^a	3.61 ± 0.04^a	4.03 ± 0.03^a	733.97 ± 3.26^a
innovator (silymarin 25 mg/kg)	0.45 ± 0.02^b	80.03 ± 1.18^b	46.64 ± 1.97^b	494.07 ± 9.32^b	59.85 ± 0.69^b	76.13 ± 1.01^b	4.67 ± 0.05^b	6.49 ± 0.12^b	502.9 ± 2.84^b
PS - ME (200 mg/kg)	1.99 ± 0.08^{ns}	134.47 ± 0.84^{ns}	66.97 ± 0.48^c	872.83 ± 0.95^c	26.25 ± 0.52^{ns}	163.83 ± 1.01^c	3.44 ± 0.01^d	4.17 ± 0.01^{ns}	716.08 ± 09^b
PS - ME (400 mg/kg)	1.72 ± 0.03^b	126.21 ± 0.61^b	61.71 ± 0.4^b	849.33 ± 1.96^b	33.40 ± 0.8^b	155.53 ± 0.99^b	3.55 ± 0.04^{ns}	4.39 ± 0.02^c	704 ± 1.07^b

TB - total bilirubin, ASAT - aspartate aminotransferase, ALAT - alanine aminotransferase, ALP - alkaline phosphatatse, TGL - triglycerides, TC - total cholesterol, TP - total protein, LDH - lactate dehydrogenase
$^aP < 0.001$ when compared to solvent control group; $^bP < 0.001$, $^cP < 0.01$ and $^dP < 0.05$ when compared to hepatotoxicant (GalN) group.
ns, non-significant from control group.

Table 2. Findings of histopathology study

microscopic findings	CMC solvent only	GalN only	GalN + silymarin	GalN + methanol extract	
				200 mg	400 mg
a) architecture	normal	lobular disarray	near normal	normal	normal
b) hepatocytes (I) balloon degeneration	-	+++	+	–	–
(II) foamy degeneration	-	-	-	+	+
(III) focal necrosis	-	+++	+	+	+
(IV) zone 1 necrosis	-	+ (focal)	+ (focal)	–	–
(V) council man bodies	-	++ (random)	+ (random)	–	–
(VI) regeneration	-	-	+	–	+
c) portal tract nature of infiltrate	lymphocytes	lymphocytes predominant, few plasma cells, polymorphs and eosinophils	lymphocytes	lymphocytes	lymphocytes
bile duct hyperplasia	–	+ (focal)	+ (focal)	–	–

+, slight; ++, marked (below 50%); +++, very intense (overall).

with the normal control rats ($P < 0.001$). Treatment with ME at 200 and 400 mg/kg b.w. showed significant decrease in ASAT, ALAT, ALP, TB, LDH, TC levels ($P < 0.001$, $P < 0.01$, and $P < 0.05$) and a significant elevation in the TP ($P < 0.01$), TGL ($P < 0.001$), and albumin levels ($P < 0.05$) in serum when compared with GalN treated rats. Standard drug, silymarin (25 mg/kg) also exhibited similar results significantly ($P < 0.001$).

Findings of liver histopathology – The observations of comparative histopathological studies of liver from different groups are given in Table 2.

Control rats treated with CMC (Solvent) (Fig. 1) – Sections show structure of liver whose architecture is well preserved. The hepatocytes in all the three zones (Zones

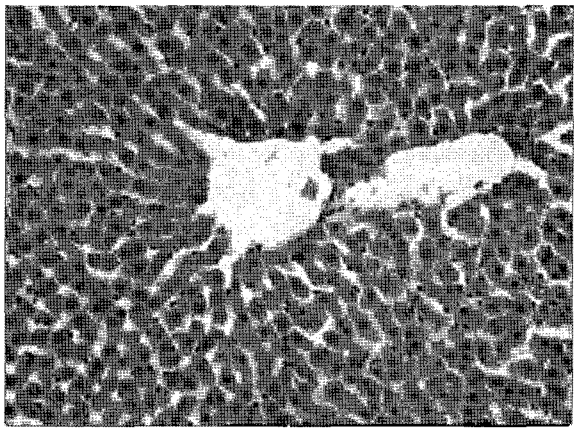

Fig. 1. Control rats treated with CMC Hepatocytes are normal. No liver cell injury/necrosis.

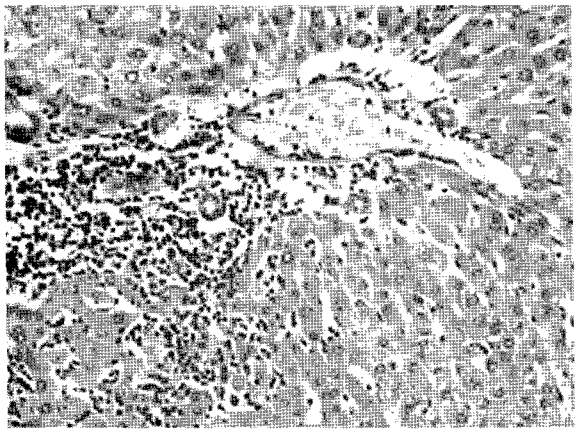

Fig. 2. Rats treated with GalN hepatocytes show ballooning degeneration as well as focal necrosis within lobules. Randomly scattered councilman bodies are seen.

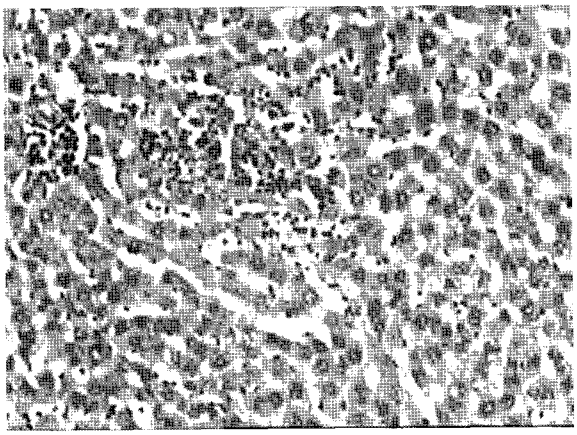

Fig. 4. Rats treated with GalN and ME at 200 mg/kg. Mild foamy degeneration of hepatocytes. No councilman bodies or zonal necrosis.

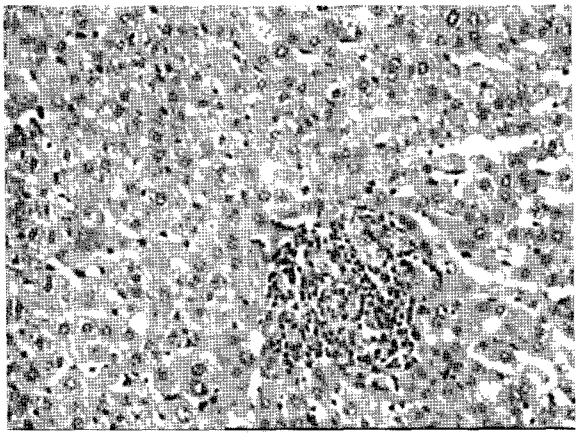

Fig. 3. Rats treated with GalN and silymarin hepatocytes show two to three nuclei suggestive of regenerative activity.

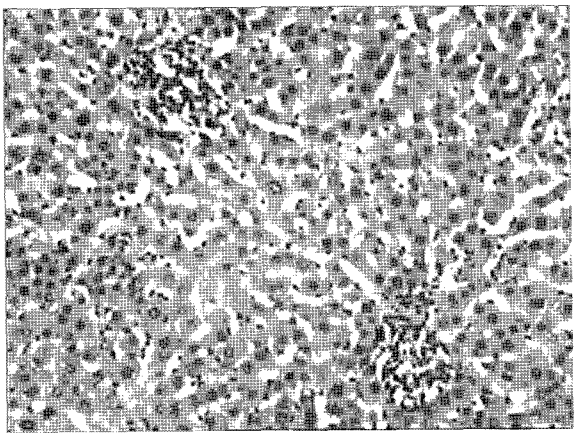

Fig. 5. Rats treated with GalN and ME at 400 mg/kg, respectively. Mild foamy degeneration of hepatocytes. No councilman bodies or zonal necrosis.

1, 2 and 3) are normal. The sinusoids show a fenestrated endothelial cell lining. The central veins appear normal and the hepatocytes, appear to be arranged radiating away from it. The portal tracts composed of portal vein radical hepatic arteriole and bile duct radical are seen. No liver cell injury/necrosis is seen.

Rats treated with GalN (Fig. 2) – Sections of liver tissue displays mild lobular disarray with inflammatory cells in the sinusoids of the lobules. The hepatocytes show ballooning degeneration as well as focal necrosis within lobules. One focus shows necrosis of zone 1 hepatocytes. Randomly scattered councilman bodies suggestive of apoptosis are seen. These bodies represent isolated hepatocytes with shrunken and intensely eosinophilic cytoplasm. Most of the portal tracts show intense periportal inflammation. The infiltrate is composed predominantly of lymphocytes, along with occasional plasma cells, neutrophils and eosinophils. Some of the portal tracts show bile duct hyperplasia.

Rats treated with GalN and silymarin (Fig. 3) – Sections show findings similar to those described above, albeit of less intensity. However one finding which is conspicuous in some areas is thickening of hepatocyte cords. The hepatocytes in these areas show two to three nuclei suggestive of regenerative activity. Thus, Silymarin has produced only minimal reversal of effects of GalN but there is regenerative activity in addition, which may eventually lead to restoration of normal architecture and function.

Rats treated with GalN and ME at 200 and 400 mg/kg respectively (Fig. 4 and 5) – Analysis reveals that there is mild foamy degeneration of hepatocytes and focal

necrosis of hepatocytes in both the doses. However there are no councilman bodies or zonal hepatocyte necrosis. There is no significant difference in microscopic findings in both the doses.

Discussion

Liver injuries could be induced by various hepatotoxins, such as β-galactosamine in rodents. This animal model is frequently used in prospective studies of hepatoprotective agents because of the experimental ease and its mechanism of actions are well documented (Dumont *et al.*, 1987). After a single injection with GalN the uracil nucleotides are considerably decreased in the liver. This causes a rapid inhibition of hepatic synthesis of both RNA and protein and provokes inflammatory reactions, resulting in a histological picture closely resembling viral hepatitis (Decker and Keppler, 1974).

Hepatocellular necrosis leads to very high levels of GOT and GPT released from liver in the blood. Among the two, GPT is a better index of liver injury, as liver GPT activity represents 90% of total enzyme present in the body (Achiliya *et al.*, 2003). ALP activities on the other hand are related to the functioning of hepatocytes, increase in its activity is due to increased synthesis in presence of increased biliary pressure (Moss and Butterworth., 1974).

Reduction in the levels of GOT and GPT towards the respective normal value is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by GalN. Suppression of increased activity with concurrent depletion of raised bilirubin level suggests the stability of the biliary dysfunction in rat liver during hepatic injury with GalN (Mukherjee, 2002).

In GalN induced acute hepatotoxic model, pretreatment with methanolic extract of PS offered hepatoprotection as evidenced by the inhibition in the rise in ASAT (SGOT), ALAT (SGPT), ALP, total bilirubin and total cholesterol levels. The decreased level of serum bilirubin indicates the effectiveness of the normal functional conditions of the liver. A similar kind of effect with the extracts of *Boussingaultia gracilis* as well as root extract of *B. chinense* (Chun-Ching Lin *et al.*, 1994) and extracts of *Cyperus rotundus* Linn (Suresh Kumar and Mishra., 2004) and extracts of *Curcuma longa* (Deshpande *et al.*, 2003) have been reported.

Also the absence of necrotic lesions in liver samples from the extract treated group, suggested that its hepatoprotective action might be due to its membrane stabilizing effect of hepatic cells. These findings could be correlated with an earlier study (Sree Ramamurthy and Srinivasan, 1993) where it was reported that pretreatment of rats with *Tephrosia purpurea* offered hepatoprotection, which may be due to membrane stabilizing effect on hepatic cells.

Silymarin, a standardized extract from the milk thistle *Silybum marianum* (Compositae) afforded good hepatoprotective activity by reversing the hepatotoxin induced alterations of biochemical parameters and has so far been the most thoroughly investigated of all the plant substances known to possess antihepatotoxic activity (Ira Thabrew and Robin D. Hughes, 1996).

The phytochemical investigation showed that the ME contains alkaloids, flavonoids and triterpenoids. Several steroidal alkaloids isolated from *Solanum* species inhibited hepatotoxicity in mice (Gan *et al.*,1993). Capsimine and isocapsicastrine, two steroidal alkaloids isolated from *Solanum capsicastrum* Linn exhibited strong hepatoprotective effects (Chun and Kim, 1989). The total alkaloidal fraction of methanolic extract of leaves of *Solanum pseudocapsicum* exhibited hepatoprotective activity *in vivo and in vitro* (Vijayan *et al.*, 2003). Flavonoids are well known for hepatoprotective and antioxidant activities. Nirocil, rich in flavonoids is comparable to silymarin in protecting liver (Venkatesan *et al.*, 2003). The triterpenoid daturalone isolated from *Solanum arundo* Juss. (Solanaceae) showed potent hepatoprotective effect in both acute and chronic liver damage induced in rats (Grace and Salen, 1996). Hence the hepatoprotective activity of PS may be correlated to the presence of alkaloid, triterpenoid and flavonoidal constituents.

Based on the results obtained, it can be concluded that *P. santalinus* exerts hepatoprotective activity and may serve as a useful adjuvant in several clinical conditions associated with liver damage.

Acknowledgements

Authors wish to thank His Holiness Jagadguru Sri Sri Shivarathreeshwara Deshikendra Mahaswamigalavaru of Sri Suttur Mutt, JSS Mahavidyapeetha, Mysore, Karnataka for providing necessary facilities. We greatly acknowledge Dr. K. Elango, HOD, Mr. M. N. Satish Kumar, Asst Professor and Mr. E. P. Kumar Asst Professor, Department of Pharmacology for their generous help.

References

Achiliya, G.S., Kotgale, S.G., Wadodkar, A.K., and Dorle, A.K., Hepatoprotective activity of Panchagavya gritha in carbon tetra chloride induced hepatotoxicity in rats. *Ind. J. Pharmacol.* **35**,

311-315 (2003).

Alfanso, R., Gennaro. and Remington., *The Science and Practice of Pharmacy* ed. 19, Vol I, Mack Publishing Co, Easton PA, 1995, pp. 111.

Anupa, Rane and Nirmala D. Gramarc., Hepatoprotective activity of *Pterocarpus marsupium* and *Butea koen-ex-Roxb. Ind. J. Pharm. Sci.* **5**, 182-184 (1998).

Attygalle., *Sinhalese Materia Medica* 2nd ed; M.D.Gunasena & Co Ltd., Colombo, Srilanka, 1952.

Battacharya, D., Mukherji, R., Pandit, S., Das, N., and Sur, T.K., Prevention of carbon tetra chloride induced hepatotoxicity in rats by Himoliv, a polyherbal formulation. *Ind. J. Pharmacol.* **35**, 183-185 (2003).

Bertelli, A., *New trends in therapy of liver diseases*, Karger Basel, 1975.

Chun, N.L. and Kim, H.G., Antihepatotoxic principles of *Solanum capsicastrum. Planta. Med.* **55**, 48-50 (1989).

Chun, C.L., Tuan, C.S., and Yen, M.H., The anti inflammatory and liver protective effects of *Boussingaultia gracils* var. *Pseudobasseloides* extract in rats. *Phytother. Res.* **8**, 201-207 (1994).

Decker, K. and Keppler, D., Galactosamine hepatitis. Key role of the nucleotide deficiency period in the pathogenesis of cell injury and cell death. *Rev. Physiol. Biochem. Pharmacol.* **71**, 78-105 (1974).

Deshpande, U.R., Joseph, L.J., and Samuel, A.M., Hepatobiliary clearance of labeled Mebrofenin in normal and D-GalN Hcl induced hepatitis rats and the protective effect of turmeric extract. *Ind. J. Pharmacol.* **47**, 332-336 (2003).

Detlef Schuppan, Ji-Dong Jia, Benno Brinkhaus, and Eckhart G Hahn, Herbal products for liver diseases. A therapeutic challenge for the New Millenium. *Hepato.* **30**, 1099-1104, (1999).

Ding, G.S., Traditional Chinese Medicine and Modern Pharmacy. *Int. Pharm. J.* **1**, 11-15 (1987).

Dumont, J.M. Maignan, M.F., and Perrissoud, D., Protective effect of malotiate against galactosamine intoxication in the rat. *Arch. Int. Pharmacodyn. Ther.* **289**, 269-310 (1987).

Ferencikova, R., Cervinkova, Z., and Drahota, Z., Hepatotoxic effect of D-Galactosamine and protective role of lipid emulsion. *Physiol. Res.* **52**, 73-78 (2003).

Galigher, A.E. and Koyloff, E.N., *Essential of Practical Microtechnique*, 2nd Ed, Lea and Febiger, Philadelphia, 1971, pp. 77.

Gan, K.H., Lin, C.N., and Won, S.J., Cytotoxic principles and their derivatives of Formosan *Solanum* plants. *J. Nat. Prod.* **56**, 15-21 (1993).

Grace, M.H. and Salen, M.M., Hepatoprotective effects of Daturolone isolated from *Solanum arundo. Pharmazie.* **51**, 593-595 (1996).

Handa, S.S., Management of hepatic ailments. *Pharma Times.* **23**, 13 (1991).

Ira Thabrew, M and Robin D. Hughes, Phytogenic agents in the therapy of liver disease. *Phytother. Res.* **10**, 461-467 (1996).

Krishnaveni, K.S. and Srinivas Rao, J.V., Aurone glycosides from *Pterocarpus santalinus* Linn. *Chem. Pharm. Bull.* **48**, 1373-1374 (2000).

Moss, D.W. and Butterworth, D.J., *Enzymology and Medicine*, Pitman Medical, London, 1974, pp. 139.

Mukherjee, P.K., *Quality Control of Herbal Drugs* 1st edn, Business Horizons Pharmaceutical Publications, New Delhi, 2002, pp. 531.

Sree Ramamurthy, M. and Srinivasan, M., Hepatoprotective effect of *Tephrosia purpurea* in experimental animals. *Ind. J. Pharm.* **25**, 34-36 (1993).

Suresh Kumar, S.V. and Mishra, S.H., Protective effect of rhizome extract of *Cyperus rotundus* Linn. against thioacetamide induced hepatotoxicity in rats. *Ind. Drugs.* **41**, 165-169 (2004).

Varma, R.R. and Vijayamma, N., Pharmacological studies on Rakthachandana. *J. Res. Ayur. Sidd.* **12**, 190-199 (1991).

Venkatesan, P., Satyan, K.S., Sudhir Kumar, M., and Prakash A., Protective effect of aqueous extract of *Phyllanthus amarus* Linn., Phyllanthin and Nirocil against carbon tetra chloride induced liver and brain toxicity. *Indian. J. Pharm. Sci.* **65**, 309-312 (2003).

Vijayan, P., Prasanth, H.C., Preethi Vijayaraj, Dhanaraj, S.A., and Shrishailappa Badami., Hepatoprotective effect of the total alkaloid fraction of *Solanum pseudocapsicum* leaves. *Pharmac. Biol.* **41**, 443-448 (2003).

Yoganarasimhan, S.N., *Medicinal Plants of India (Tamil Nadu)*, Vol. II, 2000, pp. 449.

Yoshinobu, K., Masahiro, T., and Hiroshi, H., Assay method for Antihepatotoxic activity using carbon tetrachloride induced cytotoxicity in primary cultured hepatocytes. *Planta. Med.* **49**, 222-225 (1983).

Warrier, P.K., Nambiar, V.P.K., and Raman Kutty, C., *Indian Medicinal Plants*, Orient Longman Ltd., Vol. IV, 1995, pp. 384-387.

(Accepted January 5, 2006)