

Comparative evaluation of standardized alcoholic, hydroalcoholic, and aqueous extracts of *Phyllanthus maderaspatensis* Linn. against galactosamine-induced hepatopathy in albino rats

U. K. Ilyas, Deepshikha P. Katare¹, Vidhu Aeri

Department of Pharmacognosy and Phytochemistry, Faculty of Pharmacy, Hamdard University, New Delhi, ¹Amity Institute of Pharmacy, Amity University, Noida, Uttar Pradesh, India

Submitted: 23-07-2014

Revised: 31-08-2014

Published: 12-03-2015

ABSTRACT

Background: *Phyllanthus maderaspatensis* species (Euphorbiaceae) has been used in folk medicine of many countries as a remedy against several pathological conditions including jaundice and hepatitis. This study is an attempt to evaluate hepatoprotective activity of *P. maderaspatensis* against galactosamine-induced toxicity and also investigation of polyphenols in each extract.

Materials and Methods: The extraction of *P. maderaspatensis* as per Ayurveda was simultaneously standardized and quantified for biochemical markers viz., polyphenols: Kaempferol, quercetin, catechin, rutin, and ellagic acid by high-performance thin layer chromatography. Hepatotoxicity was induced albino adult rats by intraperitoneal injection of galactosamine (400 mg/kg). The quantified aqueous, hydroalcoholic and alcoholic extract of *P. maderaspatensis* (200 and 400 mg/kg body weight/day) were compared for evaluation of hepatoprotective potential, which were assessed in terms of reduction in histological damage, change in serum enzymes such as aspartate amino transaminase, alanine amino transaminase and alkaline phosphatase and increase thiobarbituric acid reactive substances. **Results and Discussion:** The hydroalcoholic extract was found to contain comparatively high amount of kaempferol, quercetin, catechin, rutin, and ellagic acid which are responsible for hepatoprotection. Antioxidant parameters such as glutathione, catalase, and superoxide dismutase activity in liver tissues were restored toward the normalization more significantly by the hydroalcoholic extract when compared with other extracts. The biochemical observations were supplemented with histopathological examination. **Conclusion:** The hydroalcoholic extract standardized with respect to known biomarkers may be considered as a potent extract against hepatotoxicity.

Key words: Biochemical markers, galactosamine, hepatoprotection, high-performance thin layer chromatography, *Phyllanthus maderaspatensis*

INTRODUCTION

Hepatitis is an inflammation of the liver and characterized by the presence of inflammatory cells in the tissue of the organ. There are five main viruses; referred to as type A, B, C, D, and E. These five types are of greatest concern because of the burden of illness and death. The condition

can be self-limiting (healing on its own) or can progress to fibrosis (scarring) and cirrhosis. Hepatitis may occur with limited or no symptoms, but often leads to jaundice, anorexia (poor appetite), and malaise. Hepatitis is acute when it lasts <6 months and chronic when it persists longer. Hepatic trouble which includes parasites and viral infections, autoimmune diseases and intoxication with various xenobiotics such as alcohol, herbal medicine, drugs, chlorinated solvents, peroxidized fatty acids, fungal toxins, industrial pollutants, and radioactive isotopes. In particular, type A and C lead to chronic disease in hundreds of millions of people and together, are the most common cause of liver cirrhosis and cancer. In India, Medicinal

Address for correspondence:

Dr. Vidhu Aeri,
Department of Pharmacognosy and Phytochemistry,
Faculty of Pharmacy, Hamdard University, Hamdard Nagar,
New Delhi - 110 062, India
E-mail: vidhuaeri@yahoo.com

Access this article online

Website:

www.phcog.com

DOI:

10.4103/0973-1296.153079

Quick Response Code:



plants are significant sources of hepatoprotective drugs. According to one estimate, more than 700 mono and poly herbal preparation in the form of decoction, tincture, and tablets have been used in various liver disorders. The present investigation was carried out to evaluate the comparison of alcoholic, hydroalcoholic, and aqueous extracts of whole plant for hepatoprotective activity due to several bioactive molecules such as flavonoids, phenolic compounds, and tannins.^[1]

Galactosamine-induced experimental model system in rats is recognized to be much like viral hepatitis in humans from both morphological and functional points of view.^[2] Galactosamine has great liver specificity compared to other toxic group like paracetamol, acetaminophen, carbon tetrachloride, etc., because hepatocytes have high levels of galactokinase and galactose-1-uridylyltransferase and galactosamine does not affect other organs. Galactosamine induces hepatotoxicity with spotty hepatocytes necrosis and marked portal and parenchymal infiltration.^[3] Galactosamine also induces depletion of uridine diphosphate (UDP) by increasing the production of UDP-sugar derivatives, which causes inhibition of RNA and protein synthesis leading to cell membrane deterioration.^[4] In present investigation, aqueous, hydroalcoholic, and alcoholic crude extracts of plants in galactosamine-induced liver damage of rats showed the hepatoprotective activity.

Phyllanthus maderaspatensis species have been reported for treatment of diabetes, gall bladder, liver disease and jaundice in Indian traditional system.^[1,5] It is commonly known as Madras leaf-flower and also called as Nila-Nelli in Tamil, Hajarmani in Hindi, Nalla usirika in Telugu, Madaraas Nelli in Kannada and Bhuiavali in Marathi. It is widely distributed in most tropical and subtropical countries. In India, an alcoholic extract of whole plant is reported as an effective hepatoprotective agent, effective also in hepatitis B infection. The hexane fraction of the plant has been screened for its to protect rats from paracetamol, acetaminophen, carbon tetrachloride, and thioacetamide induced liver toxicity.^[6] The hexane fraction of *P. maderaspatensis* contains phyllanthin and hypophyllanthin were attributed for protection of hepatocytes against carbon tetrachloride and galactosamine-induced hepatotoxicity.^[7] However, these compounds are reported to be absent in *P. maderaspatensis*. It may be concluded that phyllanthin and hypophyllanthin may not responsible for hepatoprotective action.^[8] The present study was conducted to correlate hepatoprotective potential of *P. maderaspatensis* with phytochemical profile of its alcoholic, hydroalcoholic, and aqueous extracts and to subsequently in absence of phyllanthin and hypophyllanthin and search for polyphenols might to be responsible for its biological effect.

MATERIALS AND METHODS

Chemicals

Reference standards viz., rutin, catechin, ellagic acid, kaempferol, and quercetin were purchased from Natural Remedies Pvt. Ltd. (Bangalore, India). alanine amino transaminase (ALT), aspartate amino transaminase (AST) and alkaline phosphatase (ALP) kits were purchased from Span Diagnostics Ltd, Surat, India. Silymarin suspension (Silybon is manufactured by micro labs Ltd, Bangalore, India) and galactosamine was procured from Sclerotium rolfsii lectin, Mumbai India. All chemicals used were of analytical grade.

Animals

Adult Wistar rats (150–200 g) were obtained from the Central Animal House facility (Registration No. 173/CPLSEA/837) of Jamia Hamdard, New Delhi, India. Animals were maintained under standard laboratory conditions (12 h light/dark cycles, at 25°C ± 2°C and humidity 45–65%) and were fed with standard rodent diet and water *ad libitum*.

Collection of plant material

It was authenticated by a taxonomist in Tropical Botanical Garden and Research Institute (TBGRI), Trivandrum, India. The voucher specimen (voucher No: 65616) has been deposited in (TBGRI), Trivandrum for future reference.

Extraction of plant materials

Dried powdered plant materials were subjected to extraction by using 95% ethanol and hydro alcohol (50%) for 6 h at 37°C, respectively. The extraction procedure repeated thrice. The combined ethanolic and hydro-alcoholic extracts were pooled and evaporated to dryness under reduced pressure at 40°C with rotator evaporator. The percentage yields of the crude extracts were found to be 14.98% w/w for 95% ethanolic and 15.58% w/w for hydroalcoholic extracts. For the preparation of aqueous extract, dried plant material was boiled with 10 time of distilled water for 2 h at 60–70°C. The decoction was filtered and evaporated on the water bath at 50–60°C. The percentage yield was found to be 13.7% w/w.

Phytochemical analysis of extracts from *Phyllanthus maderaspatensis*

The crude extracts of *P. maderaspatensis* were dissolved in 10% distilled water and were fractionated thrice with hexane, chloroform, ethyl acetate. The fractions were subjected to preliminary phytochemical analysis for detecting the presence of alkaloid, flavonoids, phenolic and tannins, glycoside, steroids, and carbohydrate. The simultaneous quantitative analysis of extracts was performed using high-performance

thin layer chromatography (HPTLC). A mixture of toluene: Ethyl acetate: Formic acid: Methanol (3:3:0.8:0.2) was used as mobile phase. The amount of ellagic acid, rutin, kaempferol, quercetin, and catchin were determined using CAMAG densitometry (CAMAG model-3) and thin layer chromatography scanner equipped with CAMAG winCATS-4 software was manufactured by Camag, Muttenz, Switzerland.

Gas chromatography-mass spectrometry analysis of the hexane fraction

The chemical composition of hexane fraction was analyzed and 20 active compounds were characterized by gas chromatography-mass spectrometry (GC-MS) using Agilent 7890AGC system coupled with 5975C inert XL EI/CI Mass Selective Detector MS system equipped with a 30 m × 250 μm × 0.25 μm HP-5MS capillary column, and CTC CombiPAL injector operated in split mode (50:1) and the injection volume was 2.0 μL. The inlet temperature was kept at 270°C with helium flow rate at 1.0 ml/min. The temperature of the column was increased programmatically from 60 to 300°C at 5°C/min with total run time of 40 min at Scan mode.

Experimental design

Animals were randomly divided into nine groups ($n = 6$). Group I served as Vehicle control, which received normal saline for 14 days. Group II served as toxic control and also received normal saline (1 ml/kg, p.o) for 14 days, respectively. Groups III to VIII were prophylactically treated with different concentration (200 mg/kg and 400 mg/kg b.w. p.o) of aqueous, hydro-alcoholic and alcoholic extract of *P. maderaspatensis* in 0.1% carbon methyl cellulose for 14 days. Group IX received Silymarin (25 mg/kg, p.o) for 14 days. Hepatotoxicity was induced in Group II to IX on 15th day with 400 mg/kg, i.p of galactosamine.^[9]

Assessment of liver function

Biochemical estimation

After 24 h of galactosamine administration, blood was collected from retro-orbital plexus under light ether

anesthesia. Immediately, after blood withdrawal, all the groups were sacrificed. Liver samples were collected for histological and biochemical estimations. Serum was separated by centrifugation at 37°C and was used for various biochemical parameters. Liver samples were washed with chilled normal saline, weighed and 10% (w/v) liver homogenates were made in ice cold 0.15M KCl solution using motor driven Teflon pestle. The serum was further used for estimating the biochemical parameters viz., AST, ALT,^[10] ALP.^[11] The supernatant of the liver homogenate was subjected for the estimation of antioxidant enzyme like catalase (CAT),^[12] superoxide dismutase (SOD) by colorimetric method,^[13] glutathione was estimated by 5,5-dithiobis-2-nitrobenzoic acid method reported^[14] and thiobarbituric acid reactive substances (TBARS) was used as an index of lipid peroxidation and measured by the modified method.^[15]

Histological studies

Liver tissues were quickly removed and preserved in neutral buffered formalin. Liver sections for histological studies were prepared.^[16]

Statistical analysis

Results are expressed as mean ± standard error of the mean. Total variation present in a set of data was estimated by one-way analysis of variance followed by Dunnett's *post-hoc* test. $P < 0.01$ was considered as significant.

RESULTS

Phytochemical analysis

The presence of different phytoconstituents in alcoholic, hydroalcoholic, and aqueous extracts of *P. maderaspatensis* was detected indifferent solvents as part of preliminary phytochemical investigation [Table 1]. The extracts were quantified with regards to quercetin, kaempferol, ellagic acid, rutin, and catechin using HPTLC coupled with CAMAG densitometry as presented in Figure 1. The calibration curves for the markers were linear over the range of 100–1600 ng. The correlation coefficients were >0.992

Table 1: Qualitative analysis for phytoconstituents

Phytoconstituents	Alcoholic extract				Hydroalcoholic extract				Aqueous extract	
	P. Ether	Chloroform	n-butanol	Aqueous	P. Ether	Chloroform	n-butanol	Aqueous	n-butanol	Aqueous
Alkaloid	-	+++	-	-	-	+++	-	-	-	-
Glycoside	-	++	++	+	-	++	+++	++	++	+
Flavonoids	-	+	++	+	-	+	+++	+	+	+
Phenolic and tannins	+	-	+	++	+	-	++	+++	++	+++
Saponins	-	-	-	++	-	-	-	++	-	++
Steroid	+++	-	-	-	+++	++	-	-	-	-
Carbohydrate	-	-	+	+++	-	-	-	+++	+	+++

-ve: Absent; +ve: Positive; ++: More present; +++: High amount

for all standard curves. A mixture of toluene: Ethyl acetate: Formic acid (5:4:1) was used as mobile phase yielded a good resolution with reproducible peaks at $R_f = 0.06$, $R_f = 0.71$, $R_f = 0.36$, $R_f = 0.31$ and $R_f = 0.08$ for quercetin, kaempferol, ellagic acid, rutin, and catechin, respectively. The compounds were identified and quantified at 254. The results revealed that the alcoholic extract contains 0.035% w/w quercetin, 0.01468% w/w kaempferol and 0.0198% w/w rutin. The components identified in hydroalcoholic extract were 0.108% w/w quercetin, 0.172% w/w ellagic acid, 0.06% w/w kaempferol, 0.344% w/w rutin, 0.262% w/w catechin, and aqueous extract also contained 0.0013% w/w rutin.

Composition of the hexane fraction

Identification of chemical constituents was based on matching recorded mass spectra with those obtained from the library-Wiley/NIST. Overall, 26 components were characterized from hexane fraction, among which hexanaphthene (104.55%), bicyclo (2.2.1) hepta-2, 5-diene (0.87%), 1, 3, 5-cycloheptatriene (0.62%), hemimellitene (0.08%), hendecane (0.05%), n-eicosane (0.03%), isododecane (0.03%), heneicosane (0.07), fumaric acid (0.06%), isopropyl ester of luaric acid (0.07), 11-(1-ethylpropyl) heneicosane (0.05%), ([p-chlorobenzylidene] cyano) acetic acid (0.06%), methyl palmitate (0.16%), palmitic acid (0.25%), 2-acetyl-N-methylaniline (0.13%), ethyl palmitate (0.34%), silane (0.04%), ethyl linoleolate (0.17%), silicic acid (0.06%), linolenic acid (0.34%), ethyl 9,12,15-Octadecatrienoate ethyl linoleolate (0.42%), 1,2-bis (trimethylsilyl) benzene (0.09%), 1,1,3,3,5,5-hexamethyl-cyclohexasiloxane (0.07%), deca methyltetrasiloxane (0.03), 2-depentyl-cetate ester perhydro-hx-2-one (0.18%) and cyclotrisiloxane (0.41%).

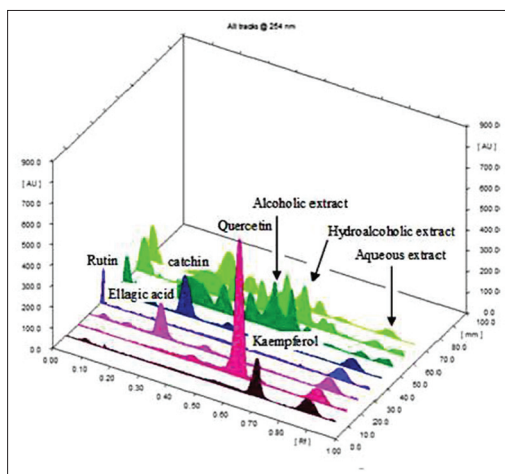


Figure 1: High-performance thin layer chromatography profile of alcoholic, hydroalcoholic, and aqueous extracts with different markers simultaneously. Eluent: Toluene: Ethyl acetate: Formic acid (5:4:1) (v/v). Detection was 254 nm

Effect of different extracts of *Phyllanthus maderaspatensis* on biochemical parameters in rats

The analyzed biochemical parameters, which included AST, ALT, ALP, and TBARS, were observed to be significantly increased in animals treated with galactosamine in contrast to normal control group ($P < 0.01$). There was also a significant decrease in tissue SOD, CAT and glutathione levels ($P > 0.05$). The hydroalcoholic extract was observed to be more effective compared to ethanolic and aqueous extract [Figure 2]. The effect of alcoholic, hydroalcoholic, aqueous extracts, and silymarin pretreatment on biochemical parameters of the rats intoxicated with galactosamine is summarized in Table 2.

Histopathological observation

Histology of the liver sections (H and E, $\times 400$) of control, standard silymarin, and hydroalcoholic extracts showing portal triad, the normal arrangement of hepatocytes with nuclei [Figure 3a]. Galactosamine over dosage caused

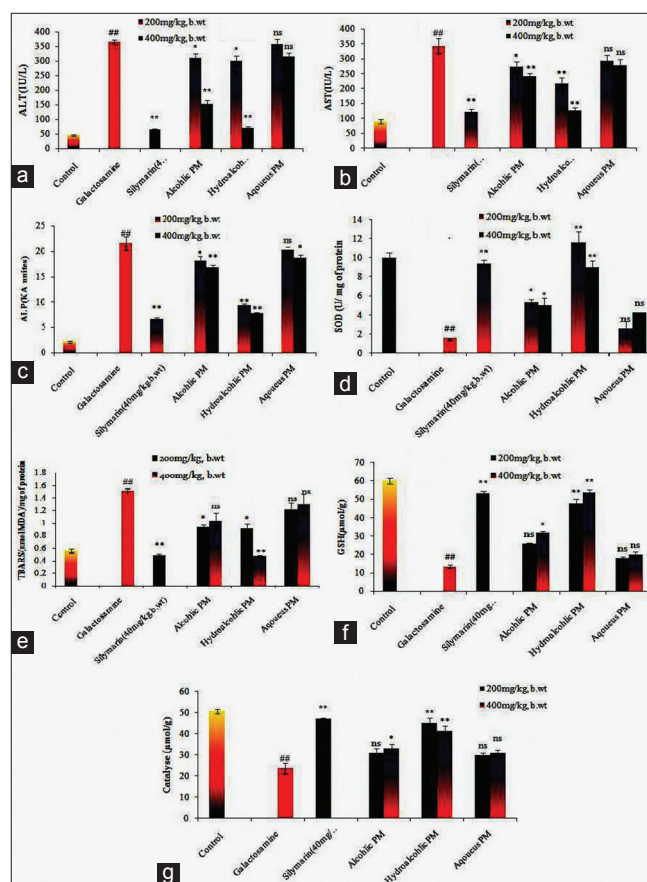


Figure 2: Effect of alcoholic, hydroalcoholic, aqueous, standard silymarin on alanine amino transaminase, aspartate amino transaminase, and alkaline phosphatase in the serum level representative Bar graph photo (a-c) tissue superoxide dismutase activity, glutathione level and catalase activity (d-g) and on thiobarbituric acid reactive substances formation (expressed as malondialdehyde) (e) of liver in galactosamine-induced oxidative stress (extracts were administered at two different doses, 200 and 400 mg/kg, body weight orally for 14 days) pretreatment of galactosamine administration intraperitoneally on the 15th day. Each value represents means \pm standard error of the mean, ns $P > 0.05$, ** $P < 0.01$, * $P < 0.05$

Table 2: Effect of three extracts and silymarin on serum and tissue biochemical parameters of the rat intoxicated with GalN

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA Unites)	Tissue SOD (U/mg of protein)	Tissue GSH (U/mg of protein)	Tissue catalyses (U/mg protein)	Tissue TBRS (nmolMDA/mg protein)
Control	44.46±2	89.87±6	2.1±0.6	9.99±0.5	58.88±1	50.63±0.9	0.553±0.3
GalN	365.69±7.8**	343.29±24**	21.5±1.3**	1.58±0.2**	13.58±1.1**	23.674±2.5**	1.5356±0.3**
Silymarin + GalN	66.12±2**	122.62±8.1**	6.7±0.2**	9.416±0.3**	56.38±1.2**	47.03±0.6**	0.4983±0.14**
Alcoholic extract (400 mg/kg) + GalN	153.756±12.5**	241.81±11.5**	16.79±0.4**	5.03±0.75*	22.956±0.6*	32.80±2.6*	1.06±0.2 ^{ns}
Alcoholic extract (200 mg/kg) + GalN	301.43±15.9*	273.66±17.5*	18.06±0.89*	5.36±0.3*	21.8±0.3 ^{ns}	30.84±2.3 ^{ns}	0.9333±0.04*
Hydroalcoholic extract (400 mg/kg) + GalN	70.66±3.6**	125.43±8.6**	7.73±0.2**	11.56±1.7**	53.62±1.4**	45.06±2.3**	0.48±0.01**
Hydroalcoholic extract (200 mg/kg) + GalN	316.03±11.4 ^{ns}	216.36±19.4**	9.31±0.4**	8.99±0.8**	46.253±2.3**	41.25±1.8**	0.9256±0.1*
Aqueous extract (400 mg/kg) + GalN	310.55±26 ^{ns}	278.40±19.5 ^{ns}	18.66±0.5*	4.28±0.7 ^{ns}	16.96±1.7 ^{ns}	31.17±2.4 ^{ns}	1.16±0.2 ^{ns}
Aqueous extract (200 mg/kg) + GalN	359.03±17 ^{ns}	292.12±21 ^{ns}	20.3±0.6 ^{ns}	2.59±0.5 ^{ns}	16.9±0.9 ^{ns}	29.84±1.1 ^{ns}	1.06±0.1 ^{ns}

When Groups III–IX was compared against Group II (GalN) using Dunnett's *post-hoc* test. Values are mean±SEM ($n=4$) $P>0.05$: Nonsignificant; * P : Significant; ** P : More significant. SEM: Standard error of the mean; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; SOD: Superoxide dismutase; GSH: Glutathione; TBRS: Thiobarbituric-acid-reactive substances; GalN: Galactosamine

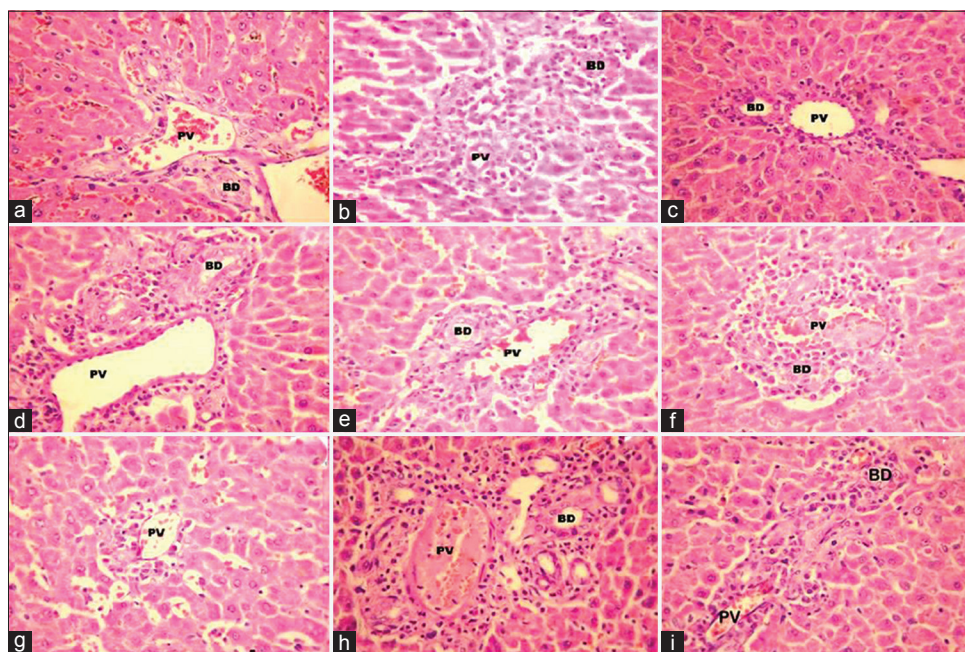


Figure 3: Liver section: (a) Control group showing normal hepatic architecture; (b) galactosamine caused necrotic liver cells and focal hemorrhages in the periportal area are seen; (c) standard silymarin plus galactosamine showing mild inflammatory cell infiltration; (d and e) alcoholic extracts plus galactosamine showing moderate inflammatory cell infiltration; (f and g) hydroalcoholic extracts plus galactosamine arrangement of cells in the liver lobule with only mild inflammatory cell infiltration; (h and i) aqueous extracts plus galactosamine-sever inflammatory cell infiltration

marked hepatic cells with severe toxicity characterized by inflammatory cell collection, scattered inflammation across liver parenchyma, necrotic liver cells, and focal hemorrhages in the periportal area [Figure 3b]. Standard silymarin [Figure 3c], alcoholic [Figure 3d and e], hydroalcoholic extracts [Figure 3f and g], and aqueous [Figure 3h and i] extracts was also evaluated for its hepatoprotective activity against galactosamine

induced hepatopathy in rats. Galactosamine + silymarin group showing a portal triad with mild inflammatory cell infiltration around the portal triad structures. PV = portal vein. Galactosamine + hydroalcoholic extracts appeared to be more significantly when compared with other two extracts. It may be due to a substantial amount of polyphenolic compounds.

DISCUSSION

Preliminary phytochemical analysis of fractionated alcoholic, hydroalcoholic and aqueous extract of *P. maderaspatensis* revealed the presence of flavonoids, phenolic compounds, alkaloids, glycosides, steroids, and tannins. In the previous published reports, n-hexane extract of *P. maderaspatensis* has been reported to exhibit significant hepatoprotection against carbon tetrachloride, thioacetamide, and acetaminophen-induced hepatotoxicity. The role of phyllanthin and hypophyllanthin has been co-related with hepatoprotective potential of *Phyllanthus* species.^[6] However, our examination is in contrast to earlier published reports on this plant. The GC coupled MS analysis of hexane extract led to the identification of 26 known compounds and revealed the absence of phyllanthin and hypophyllanthin. The significant hepatoprotective activity of alcoholic extract of *P. maderaspatensis* against tert-butyl hydroxide-induced cytotoxicity using HepG2 cell line indicated the absence of phyllanthin and hypophyllanthin.^[17] However, flavonoids,^[18] triterpenoids,^[19] polyphenolic compound,^[20] and saponins^[21] are known to possess a hepatoprotective activity in animals. The hepatoprotection can possibly be attributed to various flavonoid and polyphenolic moieties which are found to be present in hydroalcoholic extract of *P. maderaspatensis* in high amount. Such moieties include kaempferol, quercetin, rutin, epicatechin, and ellagic acid and were simultaneously quantified using HPTLC. Their concentration was found to be the highest in hydroalcoholic extract of this plant species. This along with potent free radical scavenging ability of hydroalcoholic extract provides evidence for the role of flavonoids as potential secondary metabolite responsible for hepatoprotection. Their action can be possibly mediated through prevention of liver injury by blocking the formation of lipid peroxides and inhibition of oxidative mechanism that lead to degeneration of liver hepatocytes.

The protection provided by hydroalcoholic extract has been evaluated against galactosamine-induced liver damage. Galactosamine is a hepatotoxic compound which induces necrosis in live tissues. The administration of hydroalcoholic extract to galactosamine-treated rats showed formation of normal hepatic cells and absence of necrosis. This observation bolsters the hepatoprotective potential of *P. maderaspatensis* which was found to be comparable to silymarin ($P < 0.01$) at dose level of 25 mg/kg, p.o.

REFERENCES

- Calixto JB, Santos AR, Cechinel Filho V, Yunes RA. A review of the plants of the genus *Phyllanthus*: Their chemistry, pharmacology, and therapeutic potential. *Med Res Rev* 1998;18:225-58.
- Keppler D, Lesch R, Reutter W, Decker K. Experimental hepatitis induced by D-galactosamine. *Exp Mol Pathol* 1968;9:279-90.
- Keppler D, Decker K. Studies on the mechanism of galactosamine-1-phosphate and its inhibition of UDP-glucose pyrophosphorylase. *Eur J Biochem* 1969;10:219-25.
- Decker K, Keppler D, Pausch J. The regulation of pyrimidine nucleotide level and its role in experimental hepatitis. *Adv Enzyme Regul* 1973;11:205-30.
- Dhiman RK, Chawla YK. Herbal medicines for liver diseases. *Dig Dis Sci* 2005;50:1807-12.
- Asha VV, Sheeba MS, Suresh V, Wills PJ. Hepatoprotection of *Phyllanthus maderaspatensis* against experimentally induced liver injury in rats. *Fitoterapia* 2007;78:134-41.
- Asha VV, Akhila S, Wills PJ, Subramoniam A. Further studies on the antihepatotoxic activity of *Phyllanthus maderaspatensis* Linn. *J Ethnopharmacol* 2004;92:67-70.
- Khatoon S, Rai V, Rawat AK, Mehrotra S. Comparative pharmacognostic studies of three *Phyllanthus* species. *J Ethnopharmacol* 2006;104:79-86.
- Najmi AK, Pillai KK, Pal SN, Aqil M. Free radical scavenging and hepatoprotective activity of jigrine against galactosamine induced hepatopathy in rats. *J Ethnopharmacol* 2005;97:521-5.
- Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *Am J Clin Pathol* 1957;28:56-63.
- Kind PR, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. *J Clin Pathol* 1954;7:322-6.
- Johansson LH, Borg LA. A spectrophotometric method for determination of catalase activity in small tissue samples. *Anal Biochem* 1988;174:331-6.
- Marklund S, Marklund G. Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974;47:469-74.
- Ellman GL. Tissue sulfhydryl groups. *Arch Biochem Biophys* 1959;82:70-7.
- Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351-8.
- Luna LG. *Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology*. 3rd ed. New York, Blakiston Division, McGraw-Hill;1968. p. 175-76.
- Sharma SK, Arogya SM, Bhaskarmurthy DH, Agarwal A, Velusami CC. Hepatoprotective activity of the *Phyllanthus* species on tert-butyl hydroperoxide (t-BH)-induced cytotoxicity in HepG2 cells. *Pharmacogn Mag* 2011;7:229-33.
- Gilani AH, Janbaz KH, Shah BH. Quercetin exhibits hepatoprotective activity in rats. *Biochem Soc Trans* 1997;25:S619.
- Gao J, Tang X, Dou H, Fan Y, Zhao X, Xu Q. Hepatoprotective activity of *Terminalia catappa* L. leaves and its two triterpenoids. *J Pharm Pharmacol* 2004;56:1449-55.
- Payá M, Ferrandiz ML, Sanz MJ, Alcaraz MJ. Effects of phenolic compounds on bromobenzene-mediated hepatotoxicity in mice. *Xenobiotica* 1993;23:327-33.
- Tran QL, Adnyana IK, Tezuka Y, Nagaoka T, Tran QK, Kadota S. Triterpene saponins from Vietnamese ginseng (*Panax vietnamensis*) and their hepatocytoprotective activity. *J Nat Prod* 2001;64:456-61.

Cite this article as: Ilyas UK, Katare DP, Aeri V. Comparative evaluation of standardized alcoholic, hydroalcoholic, and aqueous extracts of *Phyllanthus maderaspatensis* Linn. against galactosamine-induced hepatopathy in albino rats. *Phcog Mag* 2015;11:277-82.

Source of Support: NMPB, Govt. of India, **Conflict of Interest:** None declared.