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Hemidesmus indicus Linn. extract protects multiple organ injury due to its pharmacologically suitable physicochemical properties

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Hemidesmus indicus Linn. is used in the *Ayurveda* system of medicine in India. Co-exposure to D-galactosamine and lipopolysaccharide is employed in experiments to mimics human viral hepatitis in rats but also damages other organs as D-galactosamine depletes uridine triphosphate pool and inhibits protein synthesis, whereas lipopolysaccharide induces inflammation. We investigated physicochemical properties of extract of *Hemidesmus indicus* root and its protective potential against multiple organ injury in rats. Doses of *Hemidesmus indicus* (50, 100 and 200 mg/ kg) were orally administered to different groups of rats before co-exposure to D-galactosamine (300 mg/kg) and lipopolysaccharide (50 µg/kg) intraperitoneally. Hematology, serology, and tissue biochemistry were performed. Aspartate aminotransferase, alanine aminotransferase, bilirubin, cholesterol, urea, uric acid, and creatinine were increased in serum; aniline hydroxylase, lipid peroxidation and cholesterol level in liver, kidney, brain, testis, and spleen tissues were increased. Serum albumin, glucose, and red blood cells; superoxide dismutase, catalase, and glycogen in tissues were decreased after co-exposure to D-galactosamine and lipopolysaccharide. Physicochemical characterization indicated particle size of extract about 43 nm, presence of caffeic acid, quercetin, naringenin and trans-cinnamic acid, and presence of carboxylic acid, aromatic compounds, alkenyl and alkyl groups, alkenes and atmospheric CO_2 in *Hemidesmus indicus* root extract. It was concluded that prophylactic treatment of root extract of *Hemidesmus indicus* root extract protects multiple organs from injury in dose dependent manner by maintaining diagnostic indices towards control due to its excellent antioxidant potential.

Keywords: Antioxidant potential, D-galactosamine, *Hemidesmus indicus*, Lipopolysaccharide, Physicochemical characterization

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Use of traditional remedies predates the application of modern scientific methods of healthcare. Development of various medications against a plethora of diseases has been greatly aided by plant-based natural compounds in modern medicine[1] as several plant-based medicines are safer than synthetic medicaments[2]. Traditional knowledge-based use of herbal medicines and products is expanding around the world as an alternative to conventional medicines in addition to food supplements, low-calorie diets, and preparations to replace caffeinated beverages[3]. *Hemidesmus indicus* (Linn.) (HI), commonly known as *Anantamul* (endless root) in Hindi language, is utilized in drug formulations in the *Ayurveda* medicine in India. It belongs to family of Asclepiadaceae, which is derived from the word "Asklepios" means God of Medicines[4]. The name *Hemidesmus* comes from Latin term *hemidesmos*, which means half link and *indicus* represents India[5]. The plant comes in two colours: black known as *Krishna Sariva*, and white known as *Sariva*[6,7]. Various biological activities of HI, especially of its roots, remains the basis of its wide use in traditional medicine in different parts of Indian subcontinent. *Ayurveda* based literature explains healing properties of HI in the distich as written in books "*Dhanvantari nighattu*", "*Kaiyaadevnighattu*" and "*Bhavprakash*"[8]. It has been used as antiallergic, demulcent, diaphoretic, diuretic and hepatoprotective[9]. It helps to increase sperm count, boosts up digestive fire, removes toxic substances from body as an antidote and helpful to treat fever, diarrhoea, and blood disorders. Extract from leaves, stem and root of HI of Chhattisgarh state origin contains alkaloids, flavonoids, tannins, saponins, terpenoids, carbohydrate, glycosides, proteins,

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flavonone, flavone, flavonol and polyphenolic contents; and hydrogen peroxide scavenging activity[10]. Fast-growing countries, including India face challenges associated with number of diseases and organ injuries that need more and more plants to be evaluated for medicinal purposes. Considering widespread traditional use of HI, it was felt to assess its physicochemical properties, which remain responsible against various diseases and documentation of its medicinal potential with scientific evidences.

Lipopolysaccharide (LPS) is the major endotoxin, found in gram-negative bacteria's cell wall and plays significant role in endotoxic damage[11]. Hepatocytes with low uridine nucleotides may selectively metabolise D-galactosamine (D-GalN), which hampers synthesis of macromolecules in liver. The LPS primarily targets liver and enhances lethal outcome of D-GalN. Because of this, acute co-exposure to D-GalN and LPS is frequently used as experimental model to induce acute liver injury, whereas their long-term exposure at lower doses induce chronic inflammatory responses that resemble hepatic fibrosis[12,13].

Co-exposure to D-GalN and LPS generate reactive oxygen species (ROS) that elevate oxidative stress, which subsequently cause inflammation and dysfunctions in multiple organs[14,15]. Thus, present investigation aimed to validate pharmacologically suitable physico-chemical properties of ethanolic extract of roots of HI responsible for its protective potential against acute injury in liver, kidney, brain, testis, and spleen in rats.

Materials and Methods

Maintenance of animals and procurement chemicals

Wistar male rats (150±10 g body weight) were transported from "M/S Chakraborty Enterprise Laboratory Animal Supplier (CPCSEA Reg No: 1443/PO/Br/11/CPCSEA)"to institutional animal facility where they were maintained under standard husbandry conditions with access to pelleted animal feed and water *ad libitum*. Chemicals and diagnostic kits used in study were of pure and analytical grade. The LPS (Lot no114M4009V) was procured from Sigma Aldrich Co Ltd., USA and D-GalN (Batch no 3647503) from SRL Pvt. Ltd, which were used to induce injury in liver, kidney, brain, testis and spleen[16].

Physicochemical characterisation of plant extracts

Hydroethanolic extract of HI roots was prepared[17] for physicochemical characterisation through X-Ray diffraction (XRD) analysis, particle size analysis (PSA), high performance liquid chromatography (HPLC) and Fourier transform infrared spectroscopy (FTIR). Rigaku's Mini Flex 600 diffractometer was used for XRD analysis using Cu-Kα. The instrument was run at 40 kV voltage with15 mA of current, scan speed of 10 degrees/ min and scan angle range of 2° to 90°. Intensity against 2θ was used to produce dendrogram. Particle size analyser (Shimadzu's SALD- 2300) was employed for particle size analysis of HI root extract. Sample was prepared in 50ml distilled water and sonicated for 20 min. Pump speed of 5 cm^3/min was used for analysis of the sample by scattering of laser light. Reverse phase HPLC analysis was carried out with diode array detector. Mobile phase contained 0.5% sulpho-salicylic acid dissolved in mixture of HPLC grade ethanol and distilled water (1:1). About 20 µL of HI extract was dissolved in HPLC grade absolute ethanol and injected into column with flow rate of 0.5 mL per min. Retention time peak was obtained in the form of a chromatogram at λ 281 nm. Presence of various functional groups in HI root extract was determined with FTIR analysis (Perkin Elmer Modal no. Spectrum Two Serial no. 105627 FT-IR Spectrum). The sample was scanned from 4000 cm^{-1} to 450 cm^{-1} to record transmittance.

Experimental design

Thirty rats were assigned into five groups. Group 1 was administered with vehicle at 10:00 am, 06:00 pm and 07:00 pm daily from day 1 to 5 and served as control. Group 2 was administered with vehicle at 10:00 am daily from day 1 to 5 and served as experimental control. Groups 3, 4 and 5 were orally administered HI root extract at 50, 100 and 200 mg/ kg doses at 10:00 am, respectively daily for 1 to 5 days. On day 6, D-GalN and LPS were administered intraperitoneally to animals of group 2 to 5. The D-GalN was administered at a dose of 300 mg/ kg at 06:00 pm, whereas LPS was administered at a dose of 50 µg/ kg at 07:00 pm. On the day 7, all the animals were euthanized at 10:00 am, blood was drawn to isolate serum with routine method, and tissues from liver, kidney, brain, testis, and spleen were surgically dissected, blotted and stored at -20°C for further analysis.

Hematological and serological study

Blood from all animals were collected in heparinized tubes to determine red blood cell (RBC)

count using blood analyser (Analytica HEMA 2062+). Blood was also collected in glass vials and allowed to clot at room temperature, centrifuged at 500Xg for 10 min, serum was isolated and kept in refrigerator at -20°C. Markers for liver functions, including aspartate aminotransferase (AST), alanine aminotransferase (ALT), total bilirubin, glucose, and albumin; kidney functions, including urea, uric acid, and creatinine were determined by kit method (Meril India Pvt Ltd, Gujarat, India).

Preparation of microsomes and assessment of CYP2E1 activity

Liver tissues, perfused with normal saline, were homogenised in tris-hydrochloride buffer (Tris/HCl) (10mM), and centrifuged at 5600Xg for 20 min to separate microsomes[18]. After removing fat layer and adding calcium chloride ($CaCl_2$) (40 mM), sample mixture was again centrifuged at 5600Xg for 20 min. The pellet was collected and mixed with potassium chloride (KCl) solution (150 mM) and centrifuged again at 5600Xg for 20 min. Remaining pellet was then suspended in KCl solution (10 mM) and stored at -70°C to determine CYP2E1 activity; lipid peroxidation[19] and protein content[20] in microsomes. Aniline hydroxylase (AH)[21] was determined to assess CYP2E1 activity.

Assessment of oxidative stress

Lipid peroxidation (LPO) was determined to assess oxidative stress. Every tissue was were homogenised separately in 0.15 M KCl solution, and thiobarbituric acid reactive substances (TBARS) were determined spectrophotometrically at λ 535 nm[19].

Assessment of antioxidant status

Tissue were homogenised in sucrose solution (1%) to assess reduced glutathione (GSH) with Ellaman's reagent as chromogenic reagent, spectrophotometrically at λ 412 nm[22]. Homogenates were prepared in normal saline for superoxide dismutase (SOD) and catalase, which were determined respectively at λ 480 nm[23] and at λ 240 nm[24].

Assessment of protein content

Tissues were homogenised in hypotonic solution to determine total proteins with Folin-Ciocalteu reagent, spectrophotometrically at λ 625 nm[20].

Assessment of cholesterol level and glycogen

Homogenates of liver and kidney were prepared in hypotonic to determine cholesterol[25] spectrophotometrically at λ 560 nm with ferric chloride ($FeCl_3$) colouring reagent. Liver and kidney tissues were digested with 30% potassium hydroxide (KOH) solution for assessment of glycogen with Anthrone reagent and absorbance was recorded at λ 620 nm against blank[26].

Statistical analysis

The results were subjected to one way analysis of variance (ANOVA) followed by students t-test at significance level of 5%. All data are expressed as mean ± standard error[27].

Results

Physicochemical characterisation of plant extract

Results from XRD showed that HI root extract was amorphous in nature. The peak obtained at 2θ (degree) was at 29.309, 31.615, 79.63 and 86.50. 2θ angle, and at 31.615° showed maximum intensity (cps) of 14854 (Fig. 1 a) indicating its translational symmetry. The particle size of HI extract obtained by PSA was between 0.031 µm and 0.063 µm. Mean particle size obtained was 0.043 µm or 43 nm (Fig. 1 b). The HPLC peak analysis showed the presence of caffeic acid (3.708), quercetin (6.304), naringenin (6.927) and trans-cinnamic acid (7.327) (Fig. 1 c). The FTIR analysis showed the presence of carboxylic acid, aromatic compounds, alkene group, alkyl group, alkene group and atmospheric CO_2 (Fig. 1 d) as corresponding peaks are given in Table 1.

Hematological and serological analysis

Co-exposure to D-GalN and LPS reduced RBCs count significantly and administration of HI root extract significantly increased RBCs count in dose dependent manner. Liver function markers, AST, ALT, bilirubin and cholesterol level were increased and albumin and glucose level were decreased significantly after co-exposure to D-GalN and LPS, which implicated acute liver injury. Kidney function markers, urea, uric acid and creatinine were increased significantly as compared to control after co-exposure to D-GalN and LPS. Pre-treatment of HI root extract

Table 1 — FTIR peaks of *Hemidesmus indicus* root extract

Sl. No.	Peak (λ)	Compound
1	3260.45	Carboxylic acid
2	2928.13	Aromatic compound
3	1606.42	Alkene
4	1519.94	Aromatic compound
5	1440.77	Weak carboxylic acid
6	1371.98	Alkyls
7	1281.95	Acetate
8	1036.00	Atmospheric CO_2
9	967.20	Alkanes

offered dose dependent protection by reversing serological indices. Treatment with HI root extract at 50 mg/ kg dose could not reverse AST, albumin, glucose, and urea significantly, whereas 100 and 200 mg/ kg doses significantly restored all the parameters towards their respective control indicating protection of organs. Serum ALT, bilirubin, cholesterol, uric acid and creatinine showed significant reversal at all the three doses of HI root extract significantly. More than 70% protection was noted after administration of 200 mg/ kg dose of HI root extract (Table 2).

Effect of HI root extract on CYP2E1 activity, LPO and protein contents in microsomal fraction of liver

Co-exposure to D-GalN and LPS significantly reduced concentration of AH, LPO and protein content in microsomal fraction of liver (Fig. 2 a-c, respectively). The HI root extract significantly

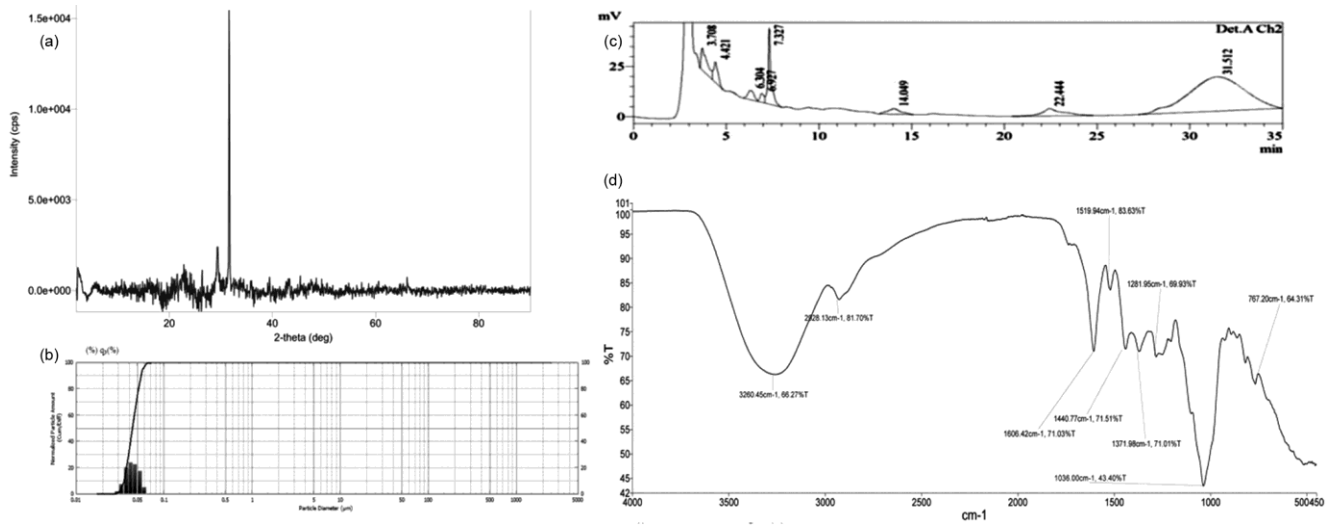


Fig. 1 — (a) X-Ray powder diffraction analysis of HI root extract, (b) Particle size analysis of HI root extract, (c) High performance liquid chromatography analysis of HI root extract, (d) Fourier transform infrared analysis of HI root extract

Table 2 — Protective role of *Hemidesmus indicus* against D-GalN + LPS induced deviations of liver and kidney function markers

Parameters	Groups					
	Control	D-GalN + LPS	HI 50 + D-GalN + LPS	HI 100 + D-GalN + LPS	HI 200 + D-GalN + LPS	ANOVA
AST (IU/L)	65.3 ± 3.61	227 ± 12.5$^{\theta}$	209 ± 11.5 11%	167 ± 9.23$^{\varphi}$ 37%	111 ± 6.14$^{\varphi}$ 72%	64.0$^{¥}$
ALT (IU/L)	36.0 ± 1.99	171 ± 9.45$^{\theta}$	136 ± 7.52$^{\varphi}$ 26%	98.9 ± 5.47$^{\varphi}$ 53%	80.1 ± 4.42$^{\varphi}$ 67%	80.8$^{¥}$
Bilirubin (mg/dL)	0.39 ± 0.02	1.03 ± 0.06$^{\theta}$	0.65 ± 0.04$^{\varphi}$ 59%	0.62 ± 0.03$^{\varphi}$ 64%	0.55 ± 0.03$^{\varphi}$ 75%	47.1$^{¥}$
Albumin (g/dL)	4.83 ± 0.27	2.83 ± 0.16$^{\theta}$	3.45 ± 0.19 31%	3.74 ± 0.21$^{\varphi}$ 46%	3.95 ± 0.22$^{\varphi}$ 56%	14.4$^{¥}$
Cholesterol (mg/ dL)	14.7± 0.81	45.2 ± 2.50$^{\theta}$	36.5 ± 2.01$^{\varphi}$ 28%	33.6 ± 1.86$^{\varphi}$ 38%	21.8 ± 1.21$^{\varphi}$ 76%	55.4$^{¥}$
Glucose (mg/dL)	120 ± 6.63	83.0 ± 4.59$^{\theta}$	96.0 ± 5.31 35%	103 ± 5.70$^{\varphi}$ 54%	113 ± 6.25$^{\varphi}$ 81%	7.63$^{¥}$
Urea (mg/dL)	22.3 ± 1.23	69.6 ± 3.85$^{\theta}$	64.9 ± 3.59 10%	57.8 ± 3.19 25%	56.7 ± 3.13 27%	42.3$^{¥}$
Uric acid (mg/dL)	1.39 ± 0.08	5.01 ± 0.27$^{\theta}$	3.67 ± 0.20$^{\varphi}$ 37%	3.32 ± 0.18$^{\varphi}$ 47%	2.08 ± 0.12$^{\varphi}$ 81%	70.2$^{¥}$
Creatinine (mg/dL)	0.57 ± 0.03	1.98 ± 0.11$^{\theta}$	1.13 ± 0.06$^{\varphi}$ 60%	0.99 ± 0.05$^{\varphi}$ 70%	0.82 ± 0.04$^{\varphi}$ 82%	78.4$^{¥}$
RBC (10^6/mm^3)	7.56 ± 0.42	4.99 ± 0.08$^{\theta}$	7.28 ± 0.40$^{\varphi}$ 89%	7.35 ± 0.41$^{\varphi}$ 92%	7.46 ± 0.41$^{\varphi}$ 96%	9.51$^{¥}$

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide; AST: aspartate aminotransferase; ALT: alanine aminotransferase; RBC: red blood cell count

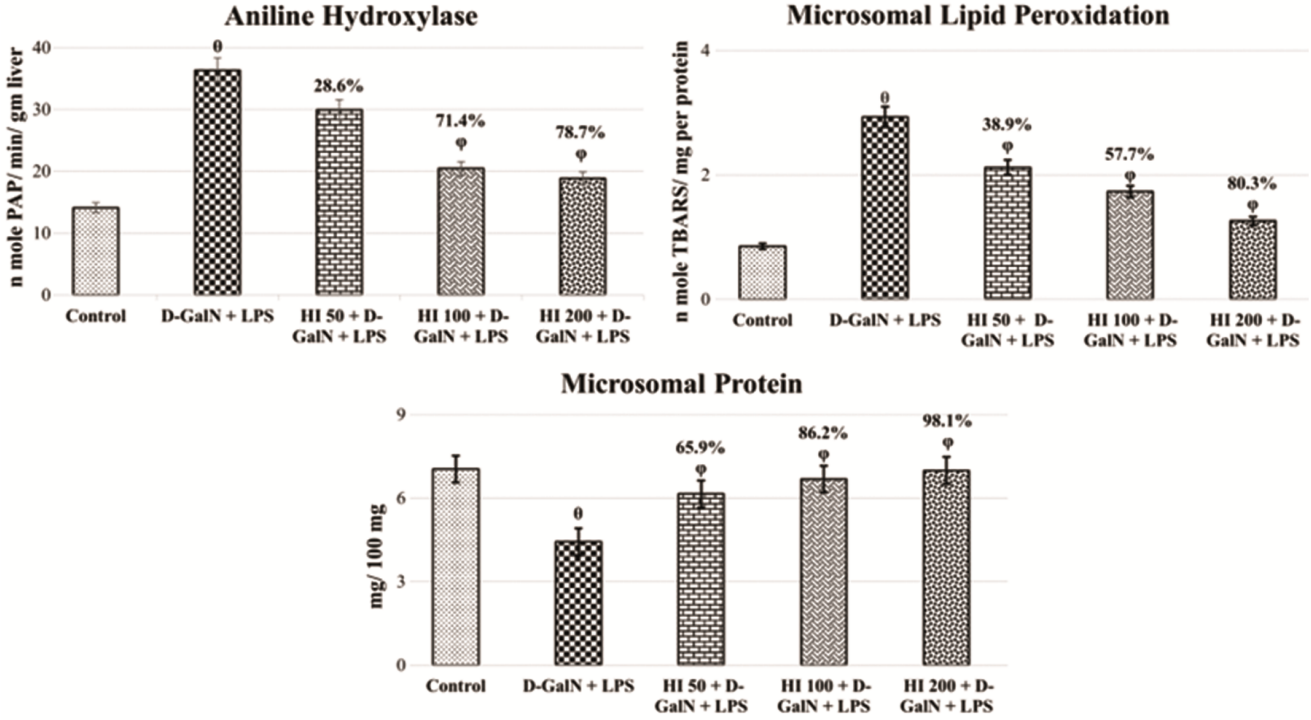


Fig. 2 — (a) Activity of microsomal aniline hydroxylase, (b) Assessment of microsomal lipid peroxidation, (c) Determination of microsomal protein content

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Aniline hydroxylase	Microsomal lipid peroxidation	Microsomal protein
F variance	49.9$^{¥}$	68.7$^{¥}$	11.5$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.

recovered AH activity at 100 and 200 mg/kg doses, whereas all the three doses significantly inhibited LPO and increased protein content in liver microsomes. Approximately, 80% protection was observed at 200 mg/ kg dose showing maximum protective efficacy on liver microsomal fraction.

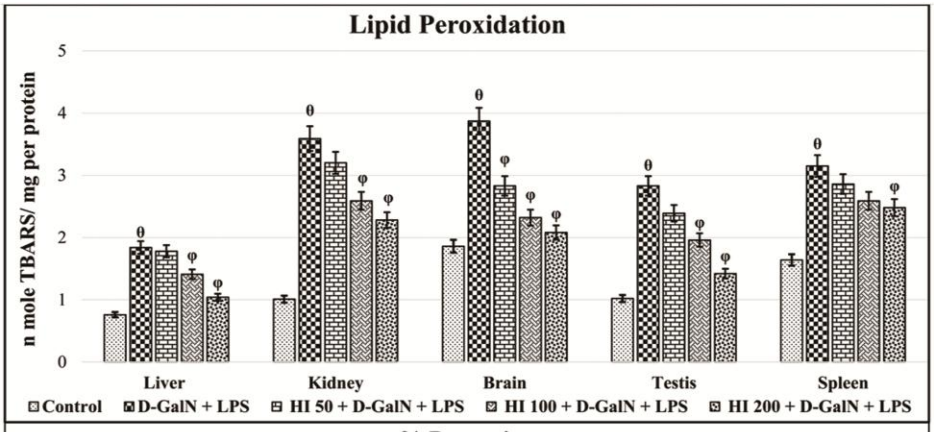
Effect of HI root extract on oxidative stress in liver, kidney, brain, testis, and spleen

Lipid peroxidation was significantly increased in liver, kidney, brain, testis, and spleen after co-exposure to D-GalN and LPS Figure 3. Pre-treatment with HI root extract inhibited LPO across all the tissues in dose dependent manner. In liver, kidney, and testis, 100 and 200 mg/ kg doses of HI root extract showed significant decrease in LPO; in brain all the doses showed significant recovery, whereas only 200 mg/ kg dose of HI extract significantly reduced TBARS in spleen. More than 70% protection was observed in liver, brain and testis and about 50% protection was seen in kidney and spleen tissues at 200 mg/ kg dose of HI root extract.

Effect of HI root extract on antioxidant status in liver, kidney, brain, testis, and spleen

Co-exposure to D-GalN and LPS significantly lowered GSH in liver, kidney, brain, testis, and spleen Figure 4. Pre-treatment of HI root extract significantly recovered GSH in liver and kidney at 50 and 100 mg/ kg doses, whereas all the three doses significantly recovered GSH in brain, testis, and spleen. The HI root extract at 200 mg/ kg dose offered maximum protection with more than 80% protection in kidney, brain, and testis and more than 60% protection in liver and spleen.

Activity of SOD and catalase were significantly diminished after co-exposure to D-GalN and LPS in all tissues Figure 5 and Figure 6, respectively. Tissues showed significant recovery in SOD activity at all three doses of HI except for liver and testis of 50 mg/kg dose. Liver, brain, and spleen revealed significant increase in catalase activity at all the three doses of HI extract. Kidney and testis significantly recovered catalase only at the highest dose. Maximum protection of about 60% was seen with pre-treatment of 200 mg/ kg dose of HI root extract in both SOD and catalase.



% Protection

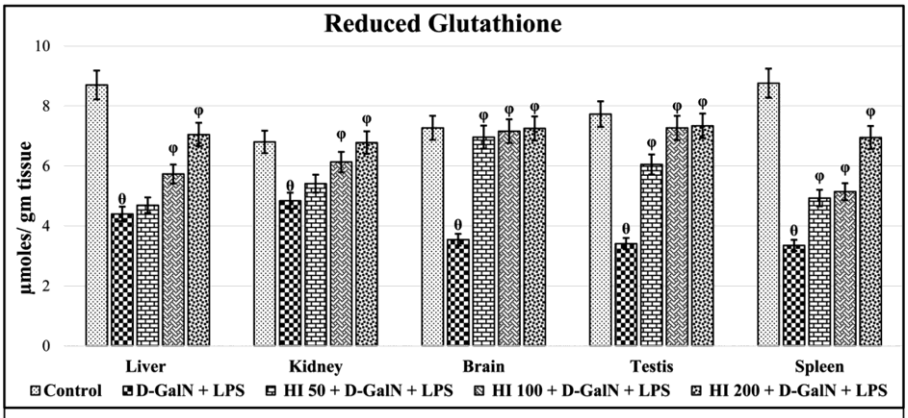
Groups	Liver	Kidney	Brain	Testis	Spleen
HI 50 +D-GalN + LPS	5.56 %	15.1 %	51.7 %	24.3 %	19.2 %
HI 100 +D-GalN + LPS	39.8 %	38.7 %	77.1 %	48.1 %	37.1 %
HI 200 +D-GalN + LPS	74.1 %	50.8 %	89.0 %	77.9 %	44.4 %

Fig. 3 — Effect of HI root extract on lipid peroxidation

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver	Kidney	Brain	Testis	Spleen
F variance	41.9$^{¥}$	53.8$^{¥}$	34.8$^{¥}$	50.2$^{¥}$	18.8$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.



% Protection

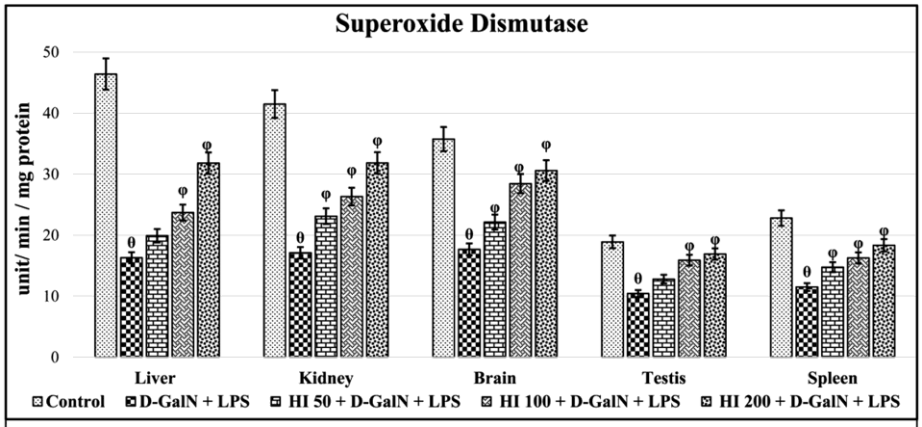
Groups	Liver	Kidney	Brain	Testis	Spleen
HI 50 +D-GalN + LPS	6.74 %	29.1 %	91.7 %	61.1 %	29.2 %
HI 100 +D-GalN + LPS	30.9 %	65.8 %	97.0 %	89.3 %	33.1 %
HI 200 +D-GalN + LPS	61.7 %	98.9 %	99.5 %	90.9 %	66.5 %

Fig. 4 — Effect of HI extract on reduced glutathione

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver	Kidney	Brain	Testis	Spleen
F variance	31.2$^{¥}$	7.95$^{¥}$	23.8$^{¥}$	28.5$^{¥}$	18.8$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.



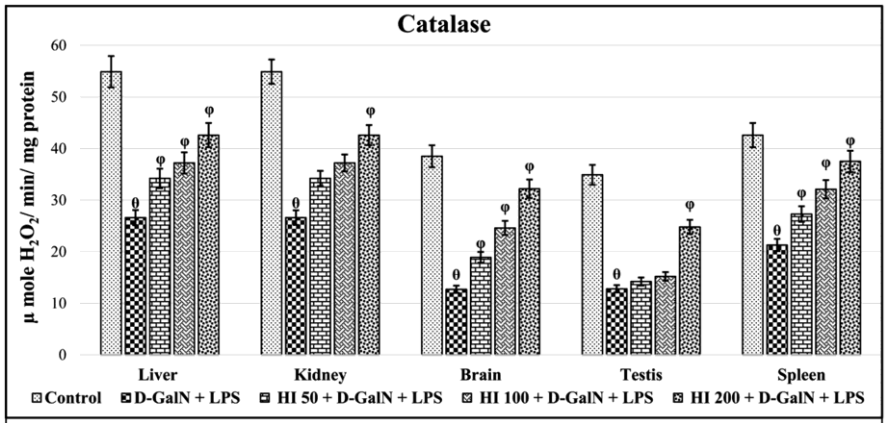
% Protection					
Groups	**Liver**	**Kidney**	**Brain**	**Testis**	**Spleen**
HI 50 +D-GalN + LPS	**11.9 %**	**24.6 %**	**24.7 %**	**28.1 %**	**28.8 %**
HI 100 +D-GalN + LPS	**24.6 %**	**37.8 %**	**37.8 %**	**64.6 %**	**42.2 %**
HI 200 +D-GalN + LPS	**51.5 %**	**60.4 %**	**60.4 %**	**76.4 %**	**60.4 %**

Fig. 5 — Effect of HI root extract on superoxide dismutase

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver	Kidney	Brain	Testis	Spleen
F variance	64.1$^{¥}$	39.5$^{¥}$	25.9$^{¥}$	19.1$^{¥}$	23.6$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.



% Protection					
Groups	**Liver**	**Kidney**	**Brain**	**Testis**	**Spleen**
HI 50 +D-GalN + LPS	**26.9 %**	**5.30 %**	**24.0 %**	**6.30 %**	**28.2 %**
HI 100 +D-GalN + LPS	**37.5 %**	**23.5 %**	**46.1 %**	**10.9 %**	**50.7 %**
HI 200 +D-GalN + LPS	**56.5 %**	**55.9 %**	**75.6 %**	**54.3 %**	**76.1 %**

Fig. 6 — Effect of HI root extract on catalase

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50, 100, 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver	Kidney	Brain	Testis	Spleen
F variance	27.0$^{¥}$	18.5$^{¥}$	56.9$^{¥}$	71.3$^{¥}$	25.1$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.

Effect of HI root extract on total protein content in liver, kidney, brain, testis, and spleen

Co-exposure to D-GalN and LPS significantly reduced total protein content in all the tissues as compared to control Figure 7. Pre-treatment of HI at all doses significantly recovered protein content in testis and spleen, whereas 100 and 200 mg/ kg doses showed significant increment in protein content in liver, kidney and brain. About 60% protection was offered by 200 mg/ kg dose.

Effect of HI root extract on cholesterol and glycogen level in liver and kidney

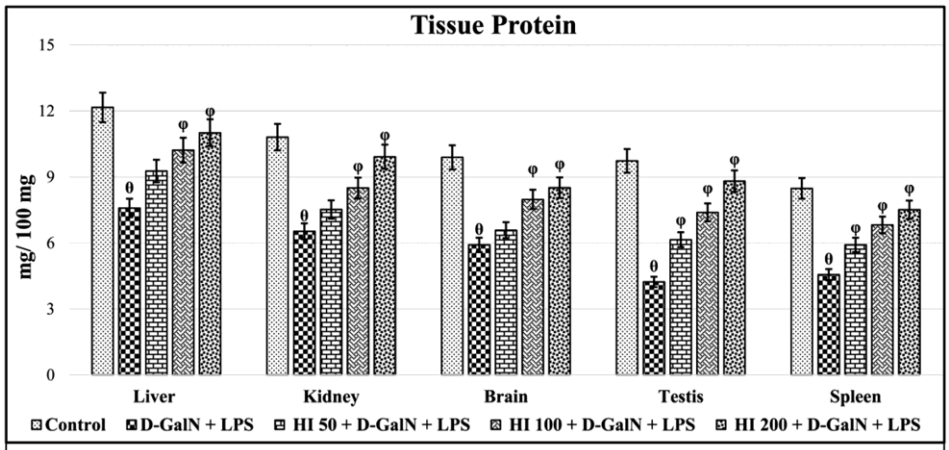
Cholesterol was significantly increased following co-exposure to D-GalN and LPS indicating disturbances in lipid metabolism in liver and kidney (Fig. 8 a). Except 50 mg/ kg dose in kidney, all other doses of HI root extract significantly reduced cholesterol level both in liver and kidney in dose dependent manner. Co-exposure to D-GalN and LPS reduced glycogen level in liver and kidney (Fig. 8 b,c, respectively). Only 200 mg/ kg dose of HI root extract significantly raised glycogen in both tissues as compared to control. Pre-treatment with 200 mg/ kg dose offered about 70% protection in cholesterol and glycogen in liver and kidney.

Discussion

Co-exposure to D-GalN and LPS produce severe damage in liver that mimics viral hepatitis[28]. Beyond damage to liver, this combination also elicit injury to multiple organs[29]. Through this investigation, prophylactic potential of *Hemidesmus indicus* root extract was documented against multiple organ injury.

Molecules with less than 100 nm size are readily absorbed by cells in the body[30]. Smaller size of the HI root extract[17] as determined by PSA facilitated movement through membrane and amplified its efficacy as antioxidant. The FTIR analysis revealed the presence of alkanes, alkenes, carboxylic acid group, acetate and cyclic compounds as moiety of secondary plant metabolites[31] responsible for diverse medicinal properties of HI root extract.

Liver damage due to D-GalN and LPS was characterised by disruptions in metabolism of liver cells, which altered serum enzyme activity in a distinctive way. Increased in AST and ALT after D-GalN and LPS administration suggested damage to liver cells[32]. Albumin and bilirubin are markers of synthesis activity of liver cells. Co-exposure to model toxicants reduced level of albumin, suggested



% Protection

Groups	Liver	Kidney	Brain	Testis	Spleen
HI 50 +D-GalN + LPS	36.8 %	23.1 %	16.8 %	34.9 %	34.4 %
HI 100 +D-GalN + LPS	57.5 %	46.0 %	52.0 %	57.5 %	57.7 %
HI 200 +D-GalN + LPS	74.8 %	79.2 %	65.3 %	83.3 %	75.3 %

Fig. 7 — Dose dependent protective effect of HI root extract in protein content

Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver	Kidney	Brain	Testis	Spleen
F variance	11.4$^{¥}$	15.3$^{¥}$	15.7$^{¥}$	32.9$^{¥}$	19.2$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.

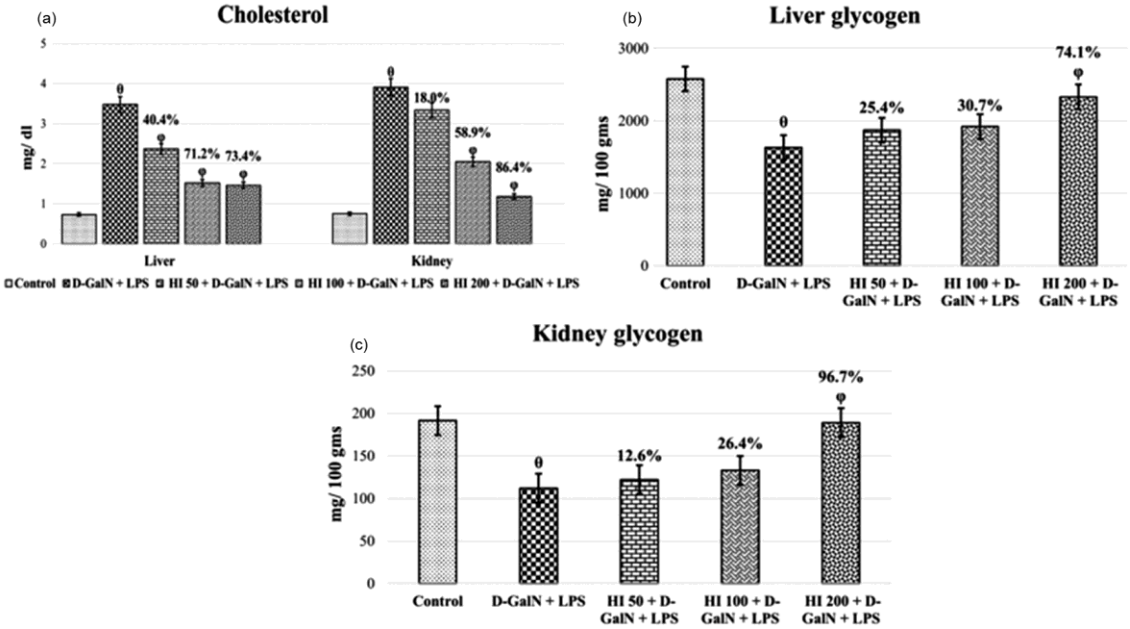


Fig. 8 — (a) Effect of HI root extract on level of total cholesterol, (b) Effect of HI root extract of different doses against D-GalN + LPS induced toxicity in liver glycogen, (c) Effect of HI root extract different doses against D-GalN + LPS induced toxicity in kidney glycogen Data are presented as mean ± SE (n = 6); $^{\theta}$ Control versus D-GalN + LPS; $^{\varphi}$ D-GalN + LPS versus HI (50/ 100/ 200 mg/ kg) + D-GalN + LPS for students t-test at $p \leq 0.05$; $^{¥}$ Represents significant value of ANOVA at 5%.

ANOVA	Liver total cholesterol	Kidney total cholesterol	Liver glycogen	Kidney glycogen
F variance	95.6$^{¥}$	111$^{¥}$	12.9$^{¥}$	24.1$^{¥}$

HI 50: *Hemidesmus indicus* at 50 mg/ kg; HI 100: *Hemidesmus indicus* at 100 mg/ kg; HI 200: *Hemidesmus indicus* at 200 mg/ kg; D-GalN: D-Galactosamine; LPS: lipopolysaccharide.

impairment in synthesis activity of liver. Increased bilirubin after co-exposure to model toxicants indicated hepatobiliary obstruction similar to alcohol toxicity[33]. Co-exposure to D-GalN and LPS declined RBCs count with simultaneously increased bilirubin. It may be postulated that D-GalN, LPS and their metabolites could induce damage to RBCs that declined RBCs count. On the other hand, damaged RBCs released hemoglobin that metabolized into bilirubin and increased bilirubin in circulation. Low glucose level could be due to increased glycolysis and decreased gluconeogenesis after co-exposure to D-GalN and LPS. Additionally, hepatic and renal glycogen stores were significantly depleted following D-GalN and LPS co-exposure, likely due to accelerated glycogenolysis to meet energy demands and a reduction in glycogenesis, potentially linked to the preferential production of UTP hexosamines over UTP hexoses[29]. Increased tissue cholesterol could be due to breakdown of membrane and disruption in lipid metabolism. Pre-treatment with HI root extract at different doses attenuated D-GalN and LPS induced damage as evident by stabilisation of AST, ALT, albumin, and bilirubin in serum. Administration of HI root extract prevented membrane from fragility and stabilised them in various tissues; thus reduced leakage of marker enzymes into circulation. Increased bilirubin suggested potential of HI root extract being able to improve biliary functions. Sustained glucose and cholesterol level in serum implicated restoration of gluconeogenesis, which supported hyperglycaemic and antihyperlipidemic properties of HI root extract. Elevated level of serum urea and uric acid in experimental control group suggested excretory impairment and heavy nitrogenous metabolism. Creatinine was more rapidly removed through kidney as compared to urea and uric acid. Higher concentration of serum creatinine level could be due to lower glomerular filtration and higher conversion of creatinine phosphate to creatinine for energy requirements[17]. Reversal in these parameters after administration of HI extract suggested its antioxidant potential and ability to protect kidneys from untoward effects.

Lipid peroxidation is oxidative degradation of polyunsaturated fatty acids triggered by free radicals. Lipid peroxidation in liver, kidney, brain, testis, spleen and microsomes was increased as a result of co-exposure to D-GalN and LPS. Increased TBARS

denoted increased lipid peroxidation that caused tissue damage and break down of antioxidant defence system. Cytotoxic effect of D-GalN has been influenced by peroxidation of endogenous lipids[34]. Lower production of TBARS due to pre-treatment with HI root extract indicated its stabilising effect on cell membrane of studied organs. Oxidative stress causes lipids, proteins, and DNA damage, which hampers cellular growth and cause senescence or death. Moreover, ROS play a crucial role in signal transmission and act as damage mediators during cellular processes like apoptosis and necrosis[35]. The GSH, an essential endogenous antioxidant plays a crucial role in protective mechanisms. The GSH acts as a preventive measure for cellular lipid peroxidation[36,37]. Exposure to D-GalN and LPS induced hepatitis could be linked to low GSH concentration in organs. The lipid peroxidation and calcium ion (Ca^{2+}) disturbance caused by hazardous substances are both intimately related decreased cellular GSH. Amounts of ROS and GSH were found to be reversed after pre-treatment with HI root extract. The SOD and catalase convert superoxide radicals to hydrogen peroxide and H_2O_2 to water respectively; thus, prevent cell from oxidative damage. Co-exposure to D-GalN and LPS increased oxidative stress by decreasing activity of these two enzymes[35,38]. Pre-treated group with HI root extract showed antioxidant potential by increasing SOD and catalase.

The hepatoprotective, neuroprotective, nephroprotective, anti-ulcer activity, anti-cancer activity of HI root extract could be due to the presence of multiple pharmacologically active compounds, including 2-hydroxy-4-methoxybenzoic acid, isovanillin, vanillin, gallic acid in more or less amounts[39]. The HPLC analysis of this study also revealed the presence of essential phytochemicals, including quercetin, caffeic acid, naringenin and trans-cinnamic acid, which could exhibit prophylactic and curative potential against multiple organ injury. The above-mentioned phytochemicals inhibited hepatic stellate cell proliferation probably by triggering cell cycle arrest in G1 phase and lowering oxidative stress[40]. In another investigation, these active metabolites dramatically decreased ALT and AST while increased SOD in rats under co-exposure to D-GalN and LPS[13]. This study reported for the first time that *Hemidesmus indicus* not only has a hepatoprotective impact but also has protective potential on kidney, brain, testis, and spleen. Early phytochemistry reported occurrence of flavonoids and phenolic compounds in *Hemidesmus indicus*, which are linked to antioxidant and hepatoprotective properties[17]. These active metabolites reduced oxidative stress and boosted the activity of enzymatic and non-enzymatic antioxidants.

Conclusion

Hemidesmus indicus root extract exerted direct antioxidant effects that hampered overproduction of ROS, which subsequently helped in reducing acute injury in liver, kidney, brain, testis and spleen. Pharmacologically suitable physicochemical properties on nano scale and functional group diversity in HI root extract contributed in maintaining redox homeostasis; thus, *Hemidesmus indicus* elucidated its protective potential against multiple organ injury.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

MB: conceptualisation, research design, data curation and analysis; AAS: manuscript drafting, editing; PB: animal experimentation; SS: physiochemical characterisation; AM: formal analysis; SKN: conceptualization, experimentation, formal analysis, manuscript editing, revising and finalisation.

Ethics Approval

Experimental design was approved by IAEC of Guru Ghasidas Vishwavidyalaya, Bilaspur (Ref: 271/B/ IAEC/ Pharmacy).

Data Availability

The authors confirm that the data supporting the findings of this study are available within the article. The data may also be provided by the corresponding author upon reasonable request.

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