Original Article

Antioxidant and hepatoprotective properties of Indian Sunderban mangrove *Bruguiera gymnorrhiza* L. leave

Abstract

Background: *Bruguiera gymnorrhiza* L. (family *Rhizophoraceae*) is a true mangrove habitat in Indian Sunderban and traditionally uses for liver disorders.

Objectives: The aim was to evaluate antioxidant and hepatoprotective actions of leave extract of *B. gymnorrhiza* L.

Materials and Methods: Hydro-methanolic extract of mangrove leaves (BR) was standardized using spectrophotometric and high-performance thin layer chromatography methods. Radical scavenging activities were assessed in different *in vitro* methods, like 1,1-diphenyl-2-picrylhydrazyl, 2,2'-azino-bis-3-ethyl benzthiazoline-6-sulphonic acid+, superoxides, nitric oxides and hydroxyl radicals. Hepatoprotective efficacy of BR (125 mg/kg and 250 mg/kg, p.o) was measured in D-galactosamine (GalN) induced (200 mg/kg, i.p) hepatitis in Wistar rats. Silymarin (25 mg/kg, p.o) was used as known hepatoprotective agent.

Results: Polyphenols such as gallic acid, quercetin, and coumarin obtained from BR exhibited powerful antioxidant properties. Moreover, it produced dose-dependent protection against GalN induced hepatitis in rats. It significantly reduced GalN induced elevation of enzymes (alanine transaminase, aspartate aminotransferase, and alkaline phosphatase) in serum and resist oxidative stress marked by lipid peroxides, glutathione, and catalase in hepatic parenchyma.

Conclusions: Polyphenols rich *B. gymnorrhiza* L. leaves ameliorate hepatic tissue injury through its antioxidant effects.

Key words:
Antioxidant, Bruguiera gymnorrhiza, D-galactosamine, liver, mangrove

Introduction

The mangrove flora is a diverse group of salt-tolerant plants growing in tropical and subtropical intertidal estuarine zones and usually categorized into two subgroups, true mangrove, and semi-mangrove according to their living environment.[1] True mangrove is confined to the typical intertidal mangrove habitats where the seawater salinity is usually 17–36%, however, semi-mangrove grow on the landward fringe of the mangrove habitats or in the terrestrial marginal zones that are subjected to irregularly high tides.[2] *Bruguiera gymnorrhiza* L. (family *Rhizophoraceae*) is a true mangrove widely distributed in the southern tropical Indian Ocean to tropical Australia.[3] It is the most abundant species in Indian Sunderban.[4] The higher degree of polymorphism might be attributed toward the comfortable growth of *B. gymnorrhiza* all along Sunderban.[5] It is a salt-tolerant large evergreen tree with elliptic-oblong thick leaves, rough reddish brown bark, short prop-roots, creamy white flowers, ovoid or turbinate single seed berries and viviparous and locally known as *Kakra* (Hindi),

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Kankra (Bengali), *Banduri* (Oriya), *Thuddu ponna* (Telegu) or *Sigapukokandam* (Tamil).[6-8] In folk medicine fruits, barks and leaves are commonly used for diarrhea and fever,[6,9] roots and barks are used in the treatment of diabetes,[10] stems are in viral fever[11] while, leaves in burns, intestinal worms, and liver disorders.[6,12-15] Different new and known bioactive constituents such as 3-β-(Z)-coumaroyllupeol and cyclohexylideneacetonitriles were found in hypocotyls,[14,16] dammarane triterpenes (bruguierins) in flowers[17] while, brugunins, and bruguierols in stems.[18] Other derivatives such as amyrins, lupeols, ursolic acid, taraxerol, gymnorhizol, ellagic acid, tannins have been isolated from different parts of this palnt.[2,19-22] Recent studies hypothesized that some of the compounds present in *B. gymnorrhiza* have potential inhibitory actions on hepatitis B virus[14] and also in HepG2 cells activations,[17] though no experimental evidence is still persist. Hence, this study was undertaken to find out the chemical nature and antioxidant properties of *B. gymnorrhiza* leaves and its role on D-galactosamine (GalN) induced hepatic tissue injury (resembles to human viral hepatitis) in laboratory animals and also underlying mechanisms in details.

Materials and Methods

Drug and chemicals

Ascorbic acid, bovine serum albumin, catalase, coumarine, 2,2'-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid (ABTS), 2-deoxyribose, GalN, 1,1-diphenyl-2-picrylhydrazyl (DPPH), gallic acid, glutathione(GSH), malonaldehyde, nitro blue tetrazolium, quercetin, thiobarbituric acid etc., were obtained from Sigma-Aldrich (St. Louis, MO, USA) and precoated silica gel plates (Merck, $60F_{254}$, 20 cm × 20 cm) from Germany. Other chemicals and solvents were AR grade and procured from Merck, India. Biological commercial kits were procured from Cogent (Span Diagnostics Ltd., Surat, India).

Plant material

The leaves of *B. gymnorrhiza* L. (family *Rhizophoraceae*) was collected from the mangrove forest area of Indian Sunderban, West Bengal, during the postmonsoon period (September to November) and identified by Botanical Survey of India, Howrah. Forest department gave the permission of collection of leaves and the specimen was collected under their supervision. A voucher specimen (DST/537/5G-4/09-04) was deposited in the departmental herbarium. The shed dried powdered leaves were extracted with hydro-methanol (20–80%) in Soxhlet apparatus. Then, it was concentrated and dried (BR) and percentage yield was recorded.

Animals

Wistar male rats (150–160 g) were used in this study. Principles of laboratory animal care guidelines were strictly followed.[23] The animals were maintained under 12:12 h dark: Light cycle and controlled temperature (25 ± 3°C) and fed with food (Provimi, Bangalore, India) and water *ad libitum*. The experiment was conducted in accordance with the guidelines of Institutional Animal Ethical Committee following due approval (544/c/PO/02/CPCSEA).

Standardization of extracts

BR was primarily evaluated by standard methods for qualitative analysis of bioactive groups.[24] The total phenolic acids in BR was done according to Folin-Ciocalteu method.[4] Aluminum chloride method was employed to determining the flavonoids present in BR.[4] Finally, the densitometric high-performance thin-layer chromatography (HPTLC) fingerprint of BR was carried out. Gallic acid (3,4,5-trihydroxybenzoic acid), quercetin (5,7,3',4'-tetrahydroxy flavonol), and coumarin (1,2-benzopyrone) were used as biomarkers. In brief, BR was diluted into 1 mg/ml in methanol and spotted in the form of bands with on a precoated silica gel plates (Merck, $60F_{254}$, 20 cm × 20 cm) by Camag Linomat 5. The plates were developed in a mobile solvent system (toluene:ethyl acetate:formic acid = 4.5:3:0.2) for 30 min and scanned at 280 nm suing Camag TLC Scanner 3 (Camag, Linomat 5, USA).[25]

In vitro antioxidant study

The reducing power,[4] DPPH radical scavenging activity,[26] superoxide anion scavenging action,[27] nitric oxide radical formation,[25] hydroxyl radical scavenging by Fenton reaction,[28] and ABTS cation decolorization[29] of BR was done spectrophotometrically following *in vitro* standard procedures.

In vivo pharmacological study

Acute toxicity

BR was examined for acute oral toxicity for determining the 50% lethal dose following the guideline no. 423 of OECD.[30]

Hepatoprotective activity in rats

Two graded dose of BR (125 mg/kg and 250 mg/kg, p.o) was evaluated on GalN induced hepatic toxicity in rats.[31] The dose has been selected on the basis of 5 point pilot experiment. Silymarin was used as known hepatoprotective agent. Healthy male Wistar rats (150–160 g) were randomized into five following groups (n = 6/group): Group I – normal control (2 ml/kg normal saline); Group II – treated control (2 ml/kg normal saline); Group III – BR treated (125 mg/kg); Group IV – BR treated (250 mg/kg) and Group V – Silymarin (25 mg/kg). The test drug was given orally for 7 consecutive days. On day 5, GalN was administered (200 mg/kg, i.p) to all rats, except normal control. The other treatments were continued as before. After 48 h of hepatotoxin injection, all animals were anesthetized by sodium pentobarbitone (40 mg/kg, i.p) and their blood was collected directly from the heart. Serum alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, and total protein were estimated using commercial kits.[32] Liver tissues were dissected out, homogenized in cold phosphate buffer (0.1M, pH 7.2) for estimating GSH,[33] catalase,[34] lipid peroxides,[35] and protein.[36]

Statistical analysis

Data have been summarized by routine descriptive statistics. All statistical analysis were performed using the SPSS software, version 17.0 (IBM, USA), differences between and within groups have been assessed for statistical significance by standard parametric and nonparametric tests, as appropriate. $P < 0.05$ was taken as level of statistical significance in all tests.

Results

Standardization of hydro-methanolic extract of *Bruguiera gymnorrhiza* leaves

The extractive of BR yields 14.82%. In phytochemical group analysis, BR denotes phenolics, flavonoids, triterpinoids, sterols, and tannins. Further, it shows [Table 1] enrich in phenolics (2.34 µg gallic acid equivalent/mg extract) and flavonoids (5.20 µg quercetin equivalent/mg extract). HPTLC densitometric fingerprint confirms gallic acid (5.90 µg/mg extract), quercetin (8.812 µg/mg extract) and coumarin (14.461 µg/mg extract) in BR [Figure 1].

In vitro antioxidant study

BR exhibits [Table 1] powerful reducing abilities (17.93 µg/ml), DPPH radical scavenging (0.355 µg/ml), nitric oxide radical inhibitions (0.305 µg/ml), hydroxyl radical scavenging action (0.311 µg/ml), superoxide radical inhibitions (0.356 µg/ml), and ABTS cation diminutions (0.056 µg/ml).

Table 1: Physicochemical natures and antioxidant properties of *Bruguiera gymnorrhiza* leaves

	Mean±SEM
Extractive value (%)	14.82±0.047
Quantitative assay	
Phenolics (µg GAE/mg extract)	2.34±0.039
Flavonoids (µg QE/mg extract)	5.20±0.115
HPTLC densitometric quantification (µg/mg)	
Gallic acid	25.90±0.016
Quercetin	8.81±0.051
Coumarin	19.46±0.202
Radical scavenging (IC_{50}) µg/ml	
Reducing power	17.93±0.161
DPPH radical scavenging	0.355±0.005
NO radical scavenging	0.305±0.004
SO radical scavenging	0.356±0.007
HO radical scavenging	0.311±0.004
ABTS radical scavenging	0.056±0.0003

n=3 or triplicate; hydro-methanolic extract. GAE: Gallic acid equivalent, QE: Quercetin equivalent, HPTLC: High-performance thin-layer chromatography, SEM: Standard error of mean, DPPH: 2,2-diphenyl-1-picrylhydrazyl

In vivo pharmacological study

The no adverse of BR shows 2.0 g/kg, p.o (limit test) in rats. Three serum liver marker enzymes, ALT, AST, alkaline phosphatise elevates, and protein levels significantly ($P < 0.001$) reduces within 48 h of GalN injection in rats, while pretreatment of BR shows significant ($P < 0.001$) and dose dependent protections on these parameters similar to Silymarin, a known hepatoprotective agent [Table 2]. Furthermore, GalN enhances lipid peroxides and diminishes GSH and catalase in hepatic tissues, which reverses significantly ($P < 0.001$) in pretreatments with BR and Silymarin [Table 3].

Discussion

Hepatic damage involves in most cases of oxidative stresses and is characterized by a progressive evolution from steatosis to chronic hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma.[37] The plant derived antioxidants; particularly polyphenols could be correlated with oxidative stress defense and hepatoprotection.[38] Furthermore, mangrove is rich source of novel compounds with powerful antioxidant properties.[11,25-26] In this study, leaves of *B. gymnorrhiza* L. (BR) report to have rich source in gallic acid, quercetin, and coumarin and there are considerable data to support

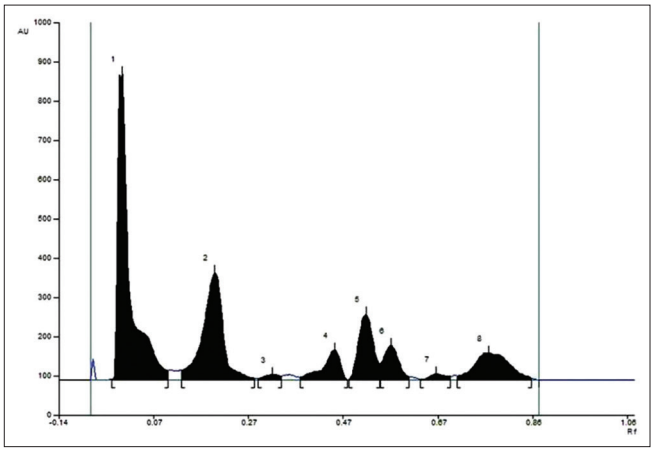

Figure 1: High-performance thin layer chromatography chromatogram of hydro-methanolic extract of *Bruguiera gymnorrhiza* L. leaves peak 2 = gallic acid, pick 4 = quercetin, pick 5 = coumarine

Table 2: Effect of *Bruguiera gymnorrhiza* leaves on liver functions in D-galactosamine hepatic injury in rats

	Serum liver function test (mean±SEM)			
	ALT	AST	AKP	Total protein
Normal control	36±1.06	40.3±1.52	67±1.86	7.41±0.10
GalN control	141±2.98^{a,***}	136.8±2.84^{a,***}	182.5±2.66^{a,***}	3.18±0.12^{a,***}
GalN + BR (125)	76.6±2.75^{b,***}	79.3±2.49^{b,***}	121±3.19^{b,***}	4.46±0.12^{b,**}
GalN + BR (250)	68.8±2.27^{b,***}	69.1±1.66^{b,***}	108.8±3.43^{b,***}	5.01±0.11^{b,***}
GalN + SLY (25)	58.1±2.41^{b,***}	60.8±1.92^{b,***}	91.1±2.58^{b,***}	5.46±0.12^{b,***}

^{a}When compared with normal control, ^{b}When compared with GalN control, $^{**}P<0.01$ and $^{***}P<0.001$. GalN: D-galactosamine (200 mg/kg, i.p.), BR: Hydro-methanolic extract of *B. gymnorrhiza* leaves (125 mg/kg and 250 mg/kg, p.o.), SLY: Silymarine (25 mg/kg, p.o.), *n*=6; All groups compared using statistical software (SPSS version 17). SEM: Standard error of mean, ALT: Alanine transaminase, AST: Aspartate aminotransferase, AKP: Alkaline phosphatase

Table 3: Effect of *Bruguiera gymnorrhiza* on liver tissues in D-galactosamine hepatic injury in rats

	Liver tissues (mean±SEM)		
	Lipid peroxides (nM MDA/mg protein)	Glutathione (nM/mg protein)	Catalase (μM of H_2O_2 decomposed/min/mg protein)
Normal control	1.63±0.03	4.31±0.042	1.26±0.03
GalN control	5.47±0.05[a,***]	1.14±0.017[a,***]	0.45±0.26[a,***]
GalN + BR (125)	4.42±0.06[b,***]	2.22±0.038[b,***]	0.62±0.02[b,**]
GalN + BR (250)	4.04±0.06[b,***]	2.35±0.027[b,***]	0.76±0.01[b,***]
GalN + SLY (25)	3.50±0.03[b,***]	2.85±0.045[b,***]	0.99±0.03[b,***]

[a]When compared with normal control, [b]When compared with GalN control, **P<0.01 and ***P<0.001. GalN: D-galactosamine (200 mg/kg, i.p.), BR: Hydro-methanolic extract of *Bruguiera gymnorrhiza* leaves (125 mg/kg and 250 mg/kg, p.o.), SLY: Silymarine (25 mg/kg, p.o.), n=6, All groups compared using statistical software (SPSS version 17), SEM: Standard error of mean

their antioxidant and hepatoprotective actions.[39,40] *In vitro* antioxidant studies show BR has strong reducing abilities and DPPH radical scavenging properties and also has capacities to inhibit generations of $ABTS^+$ radicals and hydroxyl radicals. It is believes that hydroxyl radicals hamper normal liver functions by damaging and breaking DNA strands in hepatic tissues.[41] Moreover, BR has nitric oxide and superoxide radicals scavenging also abilities and thereby it has strong antioxidant properties. Earlier, it was reported that the stems and the roots of this mangrove has potent antioxidant role on polycyclic aromatic hydrocarbon treated tissues.[15]

Hepatotoxin, GalN is mainly concerns either with insufficiency of uridine diphosphate (UDP)-glucose or UDP-galactose that leads to inhibition of energy metabolism on hepatocytes or damaging mitochondrial membrane to release cytochrome C from hepatocytes through oxidative stress.[42,43] These changes resulted in leakage of aminotransferases and alkaline phosphatase enzymes from hepatocytes into serum and considers as sensitive indicators for hepatic injuries. The rises of AST, ALT and alkaline phosphatase in serum are significantly attenuates during BR treatments suggests its stabilizing/protecting role on hepatocyte cell membrane. Further, BR treatment reduces the level of lipid peroxides, most likely due to its strong antioxidant properties. Any reduction in tissue catalase enzyme activity may result in a number of deleterious effects due to the accumulation of hydrogen peroxides in the liver tissues.[37] Additionally, reduced GSH also functions as free radical scavenger and is important in the repair of free radical-induced biological damages.[32] Decreased GSH and catalase levels in GalN induced hepatitis have been considered to be an indicator of oxidative stress.[43] BR resulted in dose dependent and significant restoration of both catalase and GSH in rat liver tissues suggests its potential oxidants defenses. Thus, this study shows that polyphonols present in *B. gymnorrhiza* L. leaves produce an antioxidant effect and have the potential to treat hepatic injuries.

Conclusion

It may, therefore, suggest that *B. gymnorrhiza* L. leaves exert a stabilizing effect on hepatocyte cell membrane and promote repair of injure hepatic tissues through its radical scavenging pathways, however, in due course, this study may lead to search more effective hepatoprotective compound(s) from this mangrove for harmonizing therapeutic approaches to hepatic disorders.

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Conflicts of interest
There are no conflicts of interest.

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