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-ethylbenzthiazoline-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). In folk medicine, fruits, barks and leaves are commonly used for diarrhea and fever, roots and barks are used in the treatment of diabetes, stems are in viral fever while, leaves in burns, intestinal worms, and liver disorders. Different new and known bioactive constituents such as 3β-(Z)-coumaroyllupeol and cyclohexylideneaconitines were found in hypocotyls, dammarane triterpenes (bruguierins) in flowers while, brugnins, and bruguierols in stems. Other derivatives such as amyrins, lupeols, ursolic acid, taraxerol, gymnorphol, 2,2'-Azino-bis-3-ethyl benzthiazoline-6-sulphonic acid = 4.5:3:0.2) for 30 min and scanned at 280 nm using Camag TLC Scanner 3 (Camag, Linomat 5, USA).

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)
in a mobile solvent system (toluene:ethyl acetate:formic acid = 4.5:3:0.2) for 30 min and scanned at 280 nm using Camag TLC Scanner 3 (Camag, Linomat 5, USA).

In vitro antioxidant study
The reducing power, DPPH radical scavenging activity, superoxide anion scavenging action, nitric oxide radical formation, hydroxyl radical scavenging by Fenton reaction, and ABTS cation decolorization were conducted in a mobile solvent system (toluene:ethyl acetate:formic acid = 4.5:3:0.2) for 30 min and scanned at 280 nm using Camag TLC Scanner 3 (Camag, Linomat 5, USA).

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.

Hepatoprotective activity in rats
Two graded dose of BR (125 mg/kg and 250 mg/kg, p.o) was evaluated on GalN induced hepatic tissue injury (resembles to human viral hepatitis) in laboratory animals and also underlying mechanisms in details.

Standardization of extracts
BR was primarily evaluated by standard methods for qualitative analysis of bioactive groups. The total phenolic acids in BR was done according to Folin-Cioicalteau method. Aluminum chloride method was employed to determine the flavonoids present in BR. 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) 20 cm × 20 cm) from 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 using Camag TLC Scanner 3 (Camag, Linomat 5, USA).

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.

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. 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).
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, triterpenoids, 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 (0.311 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 (IC50) 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. The plant derived antioxidants; particularly polyphenols could be correlated with oxidative stress defense and hepatoprotection. Furthermore, mangrove is rich source of novel compounds with powerful antioxidant properties. 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

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

| Serum liver function test (mean ± SEM) | ALT (IU/L) | AST (IU/L) | AKP (IU/L) | Total protein (mg/dL) |
|----------------------------------------|-----------|-----------|-----------|---------------------|
| Normal control                         | 36 ± 1.06 | 40.3 ± 1.52| 67 ± 1.86 | 7.41 ± 0.01         |
| GalN control                           | 141 ± 2.86*** | 136.8 ± 2.84*** | 182.5 ± 2.86*** | 3.18 ± 0.12***     |
| GalN + BR (125)                        | 76.8 ± 2.75*** | 79.3 ± 2.49*** | 121 ± 3.19*** | 4.46 ± 0.12**       |
| GalN + BR (250)                        | 68.8 ± 2.27*** | 69.1 ± 1.66*** | 108.3 ± 3.43*** | 5.01 ± 0.11**       |
| GalN + SLY (25)                        | 58.1 ± 2.14*** | 80.8 ± 1.92*** | 91.1 ± 2.58*** | 5.46 ± 0.12***     |

*When compared with normal control, **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
their antioxidant and hepatoprotective actions.\textsuperscript{[29,40]}

\textit{In vitro} antioxidant studies show BR has strong reducing abilities and DPPH radical scavenging properties and also has capacities to inhibit generations of ABTS\textsuperscript{+} radicals and hydroxyl radicals. It is believed that hydroxyl radicals hamper normal liver functions by damaging and breaking DNA strands in hepatic tissues.\textsuperscript{[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.\textsuperscript{[42]}

Hepatotoxic, 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.\textsuperscript{[43,44]} These changes resulted in leakage of aminotransferases and DNA peroxides in the liver tissues.\textsuperscript{[41]} 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.\textsuperscript{[37]} Additionally, reduced GSH also functions as free radical scavenger and is important in the repair of free radical-induced biological damages.\textsuperscript{[33]} Decreased GSH and catalase levels in GalN induced hepatitis have been considered to be an indicator of oxidative stress.\textsuperscript{[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 polyphenols present in \textit{B. gymnorrhiza} L. leaves produce an antioxidant effect and have the potential to treat hepatic injuries.

### Conclusion

It may, therefore, suggest that \textit{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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