

Research Article**Hepatoprotective activity of the ethanolic extract of *Dipterocarpus turbinatus* leaves in Paracetamol induced hepatotoxicity in rats****Diptanu Biswas^{1,2*}, G. Nirmala², P. Anjaneyulu², B. Praveen Kumar², K. Swathi², Suresh Kumar S. V.²**¹Department of Pharmacognosy, Eminent College of Pharmaceutical Technology, Barasat, Kolkata-700126, India²Department of Pharmacognosy, Creative Educational Society's College of Pharmacy, NH-7, Chinnatekur, Kurnool, Andhra Pradesh-518218, India

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Abstract

Objective: *Dipterocarpus turbinatus* is a large woody plant widely grown in Northeastern state, Tripura. Ethanol extract of *D. turbinatus* leaves was evaluated for its antioxidant and hepatoprotective activity. **Materials and Methods:** The preliminary phytochemical study was carried out to find out the presence of various phytoconstituents in different extracts obtained by cold maceration. Antioxidant activity was done by Nitric oxide scavenging and DPPH* scavenging. Paracetamol (3gm/kg) was used to induce hepatotoxicity in rats and 200mg/kg & 400mg/kg of ethanol extract of leaves of *D. turbinatus* (EEDT) was used for the study, while silymarin (50mg/kg) was used as a standard. Blood was collected by retro-orbital puncture and various biochemical parameters were determined by photometric analysis. **Results:** Ethanolic extract showed different bioactive constituents like flavonoid, phenolic, alkaloid, tannin. Plant extract was exhibited antioxidant properties. Tested extracts showed a significant reduction in the elevated level of various biochemical parameters like SGOT, SGPT, ALP, total bilirubin & increase the level of protein level with the dose-dependent manner. Histopathological studies of the liver of the tested animal showed there is an improvement in the architecture of the hepatic cell with plant extract treatments. **Conclusion:** The results suggest that ethanolic extract of *D. turbinatus* leaves in different dose shows significant hepatoprotective activity against paracetamol-induced hepatotoxicity in rats.

Keywords: Antioxidant, *Dipterocarpus turbinatus*, hepatoprotective, Paracetamol

Introduction

Since the ancient age, nature has shown its own benefit on mankind as it provides essential elements for human need. Importance of plant in health is directly or indirectly showing its medicinal benefit to the society (Newman and Cragg, 2007). The liver is the chemical factory which regulates synthesis, store and secrets of important macromolecules in the body (Walker and Whittlesea, 2012). Liver diseases are a global problem and the synthetic drugs are available for the treatments of liver disorder are believed to produce serious adverse effects on the

biological system (Ramchadrasetty et al., 2007). A herbal drug plays a major role in the management of various liver functions and their formulations are used for the treatment of a liver disorder in ethnomedicinal practice and traditional system of medicine in India (Harikumar et al., 2013). *Dipterocarpus turbinatus* is a large woody plant height of 100-120 ft. and a girth of 8-15 ft. It is found in the tropical forests of Tripura, Assam & Andaman (Chatterjee and Prakash, 1995). Indigenous people of Tripura use it for various purposes (Sharma et al., 2014). Stem leaf and fruits of *D. turbinatus* possess antioxidant property (Rajendra et al., 2009). Thin bark of *D. turbinatus* is boiled in water for the treatment of gum and toothache by Bangladeshi Mro community in Chittagong hilly area (Haque et al., 2012). *D. turbinatus* shows cytotoxic against breast cancer cell line (MCF -7 and MDAMB-231 (Aker et al., 2014). Five new tri-terpenes, 3-oxo-20-hydroxy-30 α -methyl,17(29)- α -epoxy-28-norlupane (1); 3-oxo-20 hydroxy-30 β -methyl-

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17(29) α -epoxy-28-norlupane (2); 3, 20-dooxo-28, 29-norlupane17 α -ol (3); 27-dimethyl-20(s) dammer-23-ene-20-ol-3, 25 dione (4); 3 epi-ceropic acid (5); together with 13 known compound including diterpane, sesquiterpenes and triterpenes were isolated from the stem of *D. obtusifolius* and were tested for cytotoxic activity (Khiev et al., 2012). A cytotoxic compound Diptindonein E was isolated from the acetone extracts of the bark of *D. hasseltii* (Muhtadi et al., 2009). Ethanolic extract of the *D. turbinatus* bark showed antioxidant and hepatoprotective activity (Biswas et al., 2016). Our present study was carried out for the determination of antioxidant and hepatoprotective activity of the ethanolic extract of *D. turbinatus* leaves.

Materials and Methods

Plant materials

Plant material was collected from the forest of the Gomuti Dist. of Tripura and was identified and authenticated by Prof. P. Jayaraman, M.Sc., Ph.D; Director Plant anatomy research Centre, Tambaram, Chennai. Voucher specimens (No. PARC/2012/1277) were deposited for further reference. Plant materials were dried under shade and made coarse powder by pulverized in our college laboratory.

Chemicals and reagents

Paracetamol and silymarin were procured from the Micro Lab Ltd, India. Standard biochemical kits [Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Serum Alkaline Phosphate (ALP), Bilirubin, Protein] were procured from Agappe diagnostics Ltd, Kerala, India. DPPH was procured from Hi Media Mumbai. All other reagents for the study were procured from SD Fine Lab, Mumbai, India. Remi research centrifuged was used for centrifugation purpose. Mispa excel semi-autoanalyzer made of Agappe diagnostic Ltd, Kerala, India and double beam UV-Visible spectrophotometer of Lab India, was used for various analysis.

Physicochemical studies (Kokate et al., 2012)

Determination of Ash values

The ash values of plant material were determined for measuring total ash, acid insoluble ash and water-soluble ash as per the procedure mention in standard guidelines (WHO guide Line 2003).

Determination of extractive values

Extractive values for the leaves powder of *D. turbinatus* was determined by cold maceration process as a standard guideline.

Extraction of plant material

About 500g pulverized powder of the leaf is extracted successively with petroleum ether, chloroform and ethanol (70%) by cold maceration. The extracts were dried in a vacuum evaporator, till the solid mass obtained and kept in desiccators to

for complete drying.

Animals for the study

For the study adult Wistar albino rats (either sex) weighing between 180 to 220 g were selected. The animals were housed in polypropylene cages and watered. Animals were fed with the standard rodent pellet diet. The entire animals were utilized for studies according to the protocol approved by the Institutional Animal Ethics Committee (approval ID No. IAEC/CESCOP/2015-02).

Preliminary phytochemical studies

Preliminary phytochemical studies for various extract were done to determine the presence of various phytoconstituents. Preliminary phytochemical studies were done by standard procedure.

Toxicity study

Acute toxicity was performed for ethanolic extract of *D. turbinatus* by the method adopted by CPCSEA, Government of India – acute toxic class method (OCED Guideline no. 423, Annexure-2d) in female albino rats. The mortality was observed after oral administration of up to 2000mg/kg b.w. of the test sample (Veeraraghavan, 2001). Common side effects like mild diarrhoea, weight loss and depression of treated groups of animals were recorded within the one week of observation (Silva et al., 2011).

Antioxidant activity

Nitric Oxide scavenging activity

Nitric oxide scavenging activity of extracts was determined by using *Griess reagent* (1% sulphonilamide, 2% phosphoric acid & 1% naphthyl ethylene diamine di hydrochloride). Reaction mixture containing 3 ml of sodium nitroprusside (10 mmol) in phosphate buffer and test extracts in different concentrations (1g, 2g, 4g, 8g and 10g/mL) were incubated at 25°C for 150 minutes. Control was prepared by omitting the sample. After incubation, 0.5 ml of *Griess* reagent was added and the absorbance was measured at 546 nm using UV-visible spectrophotometer²⁴. Percentage inhibition was being calculated and the activity was expressed as an inhibition concentration 50 (IC₅₀) (Biswas et al., 2013).

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity

DPPH scavenging activity was measured according to the method of Sanjay Rai et al., with little modification. The free radical-scavenging activities of all samples were estimated in terms of hydrogen donating or radical scavenging ability. A Solution of 0.1 mM DPPH was being prepared in ethanol, 1 ml. of this solution was being added to 3 mL of all the

extracts in water at different concentrations (1g, 2g, 4g, 8g and 10g/mL). Thirty minutes later, the absorbance was to be measured at 517nm. Ascorbic acid was used as a standard antioxidant. The results expressed as IC_{50} or inhibitory concentration 50 values (Sanjay et al., 2006).

Paracetamol-induced hepatotoxicity in rats

Group I (vehicle control) was given 2% acacia suspension in normal saline, group II (toxic) received paracetamol (3gm/kg) (Singh and Handa, 1995) as a single dose at 0hr followed by vehicle for 1hr, 24hr, 48hr. Group III (standard) received paracetamol (3gm/kg/p.o.) as a single dose at 0hr followed by silymarin (50mg/kg) for 1hr, 24hr, 48hr. Group IV & V (test) received paracetamol 3gm/kg at 0hr followed by drug extracts 200mg/kg & 400mg/kg at 1hr, 24 hr, 48hr. At 72nd hr, blood was collected from animals and biochemical parameters were evaluated related to liver disorder to check the therapeutic effects of the drug extracts. All the dosage has administrated by the oral route.

Assessments of liver function were carried out by collecting blood from retro orbital puncture. Blood was centrifuged at 4000 rpm for 15min and collects the serum. Various biochemical parameters like SGOT, SGPT, serum ALP, total bilirubin and total protein were evaluated using biochemical kits (Bagban et al., 2012).

Histopathology

The animals were sacrificed and livers were collected and washed with normal saline. Isolated livers were stored in formalin solution and histopathology was done to evaluate the details change hepatic cell structure (Kumar et al., 2006). Samples were then embedded in paraffin wax with automatic tissue processor and five-micron sections were prepared by using rotary microtome. This thin section was stained with hematoxylin & eosin and mounted on a glass slide (William et al., 2003). Degrees of liver damaged were estimated under a light microscope (Magnus lab photo microscope) in 450 X.

Statistical analysis

The Mean values \pm standard error of the mean (SEM) were calculated for each parameter. A value of $*p < 0.05$ was considered statistically significant.

Results

Physicochemical evaluation

D. turbinatus leaves powder was subjected to various physicochemical studies. The loss on drying for the leaves powder was found to be 11.081 ± 0.024 . Ash values were determined in percentage for leaves powder of *D. turbinatus* and were found to be, total ash 3.158 ± 0.276 , acid insoluble ash 0.063 ± 0.7398 & water soluble ash 0.970 ± 0.027 for *D. turbinatus*. Extractive values for different solvent were done, where the value obtained for the leaves powder of *D. turbinatus* like this, petroleum ether soluble extractive 4.66 ± 0.134 ; chloroform extractive 8.840 ± 0.426 ethanol soluble extractive 20.834 ± 0.54 and water-soluble extractive 12.23 ± 0.94 .

Preliminary phytochemical analysis

The preliminary phytochemical test was done to find out the presence of various phytoconstituents present in the different extract obtained by extractive values. The result for the preliminary phytochemical studies is represented in the table 1.

Toxicity study

Acute toxicity study was carried out according to the method of OECD 423 for ethanolic extract of *D. turbinatus* leaves. Mortality was not observed at 2000 mg/kg in rats. Thus 2000mg/kg was considered as cut off dose and 1/10th and 1/5th (200mg/kg and 400mg/kg) dose were selected for evaluation of the hepatoprotective activity.

Antioxidant activity

Antioxidant activity for ethanolic extract of *D. turbinatus*

Table 1. Phytochemical analysis for different extract of the leaves of *D. turbinatus*

Tests	Petroleum ether extract	Chloroform extract	Ethanol extract	Water extract
Alkaloids	-	-	-	-
Steroid	-	+	+	-
Flavonoids	-	-	+	+
Tannins	-	-	+	+
Carbohydrate	-	-	+	+
Glycoside	-	-	+	+
Terpenoids	+	+	+	-
Saponin	-	-	+	+
Protein	-	-	-	+

leaves was done by *in vitro* methods. The results for the *in-vitro* antioxidant study for the extract are shown in the table 2.

Effects of ethanol extracts of *D.turbinatus* on various biochemical parameters associated with liver

Animal treated with 200mg/kg & 400mg/kg p.o of ethanol extracts from *D. turbinatus* leaves exhibit a significant (*p<0.05) reduction in various biochemical parameters. In paracetamol induced, there is an increase in the level of SGOT, SGPT, ALP, serum bilirubin and decrease the level of total proteins. Treatment with the extracts at a dose level of 200mg/kg & 400mg/kg P.o restore levels of SGOT, SGPT, ALP, Serum bilirubin and total proteins which are disturbed by paracetamol. The results were represented in table 3.

Histopathology

Histology of the liver was done for all groups to found the out change in liver architecture; like central vein, cytoplasm, nature of hepatocytes with their round nucleus & cytoplasm. Blood cell infiltration in hepatic vein, sinusoidal area and aggregation of Kuffer cells surrounding the central veins was also observed. Figure (A) Shows the histopathology of the liver of untreated animals with the normal hepatic vein, with less sinusoidal space polygonal hepatocytes without any ballooning. Figure (B) intoxicated with paracetamol shows damage in the hepatic cell enlargement sinusoidal space and with infiltration of blood cell in hepatic vein. Figure (C) Treatment with silymarin able to

normalize the hepatic cell, with less sinusoidal spaces, hepatic vein with fewer blood cells infiltration. Figure (D) Liver treated with 200mg/kg plant extract, we can see improvement of hepatocytes, sinusoidal space and hepatic vein as compared to the toxic (Paracetamol treated group) group. Figure (E) Histopathology of liver with the treatment of EEDT 400mg/kg also showed improvement of the architecture of the liver cells.

Discussion

Preliminary phytochemical studies are the 1st steps on the way of establishing and identifying a new plant material from the natural source. Physicochemical parameters like ash values and extractive values were determined to find out the ash and different solvent soluble extract of the leaves of *D.turbinatus*. Preliminary phytochemical studies were done to identifying phytochemical constituents present in the different extract (Harbone, 2005). It shows phytoconstituents like flavonoid, phenolic, constituents are present in the ethanolic extract of *D.turbinatus*, which are known to be an antioxidant and hepatoprotective (Zhou, et al., 2008). *In-vitro* antioxidant activity shows that the plant extracts have the antioxidant property with significant IC₅₀ values with the reference standard of ascorbic acid.

Paracetamol is widely used as antipyretic and analgesic and its hepatotoxicity of is attributed by the formation of a highly reactive metabolite of paracetamaol, N-acetyl-P-benzoquinoneimine (NAPQI) by the hepatic cytochrome P-450 (Hartmut et al., 2008). Paracetamol 3gm/kg as a single dose was given at 0 hr and followed by treated ethanol extract of *D.turbinatus* leaves at a dose of 200mg/kg, & 400mg/kg, where silymarin (50mg/kg) was administrated as standard. The ethanolic extract of the leaves of *D. turbinatus* was able to decrease the elevated biochemical parameters like SGOT, SGPT, ALP, bilirubin level paracetamol induced hepatotoxicity in rats and also significantly increased the total protein in the blood of treated animals with test drugs

Table 2. Antioxidant activity for the ethanol extract of the *D. turbinatus* leaves (N= 3)

Scavenging method	Standard	IC ₅₀	
		EEDT	Standard
Nitric oxide scavenging activity	Ascorbic acid	41.44 ± 0.5294	10.63 ± 1.282
DPPH radical activity	Ascorbic acid	52.18 ± 3.301	7.210 ± 0.85

Table 3. Effects of ethanol extracts of *D.turbinatus* leaves on biochemical parameters in Paracetamol intoxicated rats.

Groups	Treatments	Biochemical Parameters				
		SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Bilirubin (mg/dl)	Total Protein (g/dl)
Group I	Normal control	99.26 ± 5.466	65.31±5.809	61.45 ± 3.272	0.4367 ± 0.04529	4.579 ± 0.448
Group II	CCl ₄	400.0 ± 14.59	233.00± 7.725	189.1±2.842	2.365 ± 0.153	8.983 ± 0.4571
Group III	Silymarin + CCl ₄	122.7 ± 11.24***	59.65 ± 5.790***	78.00 ± 4.009***	0.5717 ± 0.1156***	4.710 ± 0.2143***
Group IV	EEDT(200mg/kg) + CCl ₄	252.9 ± 12.39***	181.9 ± 6.11***	130.5 ± 11.42***	1.2070 ± 0.1520*	7.169 ± 0.3947**
Group V	EEDT(400mg/kg) + CCl ₄	197.5 ± 17.50***	157.4 ± 8.288***	111.5±5.172***	0.883 ± 0.07504**	6.424 ± 0.2893**

Values are the mean ± SEM six rats. Symbol represent statically significance *P<0.05 compared with toxicant.

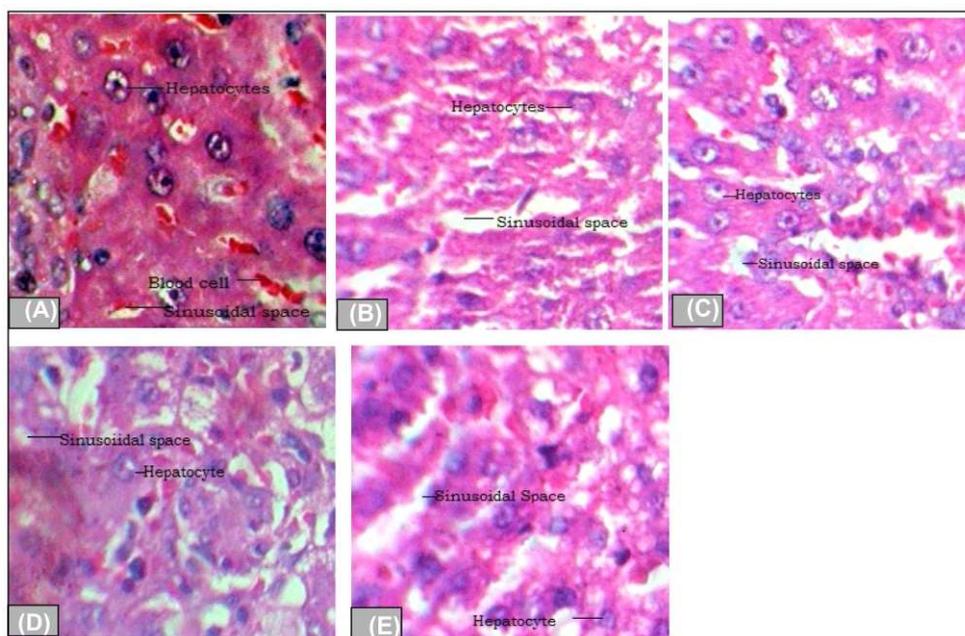


Figure 1. (A) Normal rat liver; (B) Rat intoxicated with paracetamol; (C) Rat treated with silymarin and intoxicated with paracetamol; (D) Rat treated with EEDT (200mg/kg) and intoxicated with paracetamol; (E) Rat treated with EEDT (400mg/kg) and intoxicated with paracetamol. (450X) haematoxylin –eosin stain

and silymarin. These findings suggest that ethanolic extract of *D. turbinatus* is effective in bringing the functional improvement of liver. Protection in hepatocytes by EEDT at 200mg/kg & 400mg/kg also was confirmed by histopathological studies of the liver for various groups animal. Antioxidant capacity of the plant extracts may be helpful in the regeneration of hepatic cells from their oxidative damage and protect the liver cell from toxicants (Jain et al., 2008).

Present study shows that, ethanol extract from the leaves of *D. turbinatus* possessed potent hepatoprotective in paracetamol induced hepatotoxic rats. Preliminary phytochemical test indicates the presence of flavonoids and phenolic contents and also *in-vitro* antioxidant studies shows the extract having antioxidant capacity. Silymarin and plant extracts decreased the elevated enzyme levels in tested groups, indicating the protection of structural integrity of hepatic cell & regeneration of damaged liver cells. Treatment with *D. turbinatus* extracts significantly reverses these changes. Hepatoprotective activity of ethanolic extract of leaves of *D. turbinatus* may be due to its phytoconstituents. Antioxidant properties may help in the protection of liver in paracetamol induced hepatotoxicity. to use this plant as safe prophylactic agent for liver disorder, more studies should be carried out to know all the active components and their mechanism of actions another types of experimental animals for a long period in order to judgment if this plant could be used as safe agents or not.

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Conflicts of interest

No conflict

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