Hepatoprotective and Antioxidant Activity of Ethanol Extracts of *Dipterocarpus turbinatus* (Dipterocarpaceae) from Tripura

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Abstract

Ethanol extracts from the bark of *Dipterocarpus turbinatus* (EEDT) was evaluated for its hepatoprotective activity by paracetamol and carbon tetrachloride induced hepatotoxicity in rats. Hepatotoxicity was done by using carbon tetrachloride (2ml/kg) and paracetamol (3gm/kg) in rats. 200mg/kg and 400mg/kg of ethanol extracts of *D. turbinatus* (EEDT) was used as test drugs while silymarin (50mg/kg) used as a standard. Blood was collected by retro orbital pluxes and various biochemical parameters were evaluated related to liver disorder. Various parameters like SGOT, SGPT, ALP, cholesterol, serum bilirubin and levels significantly decreased with the dose dependence manner for EEDT as compared to paracetamol and CCl4 induced hepatotoxicity in rats. Total protein level was significantly increasing to test drug treated group as compared to the toxic control. Anti oxidant potency of drug was determined by various in-vitro methods and the drug extracts have shown positive results. Therefore, we conclude that it can be used for the treatment of hepatic damage where anti oxidant property is supported for its hepatoprotective activity.

Keywords: Anti oxidant, *Dipterocarpus turbinatus*, Hepatoprotective, Silymarin

1. Introduction

Liver is vital organ in human body that perform detoxification of exogenous xenobiotics, infection and regulate various biochemical enzymes for metabolisms thus help to fight against disease1. Liver disease is a worldwide problem which is caused by various agents like alcohol, drugs and chemicals2. In spite of phenomenal growth of modern medicine there are no synthetic drugs available for the treatment of hepatic disorder. However herbal formulation claimed to have beneficial effect on liver disorder3. Preliminary phyto chemical analysis of the extracts shows presence of phenol and flavonoids4 which is known to be hepatoprotective and anti oxidant activity. Free radicals like ROS and NOS due to the special characters can initiate the lipid peroxidation, break nucleic acid strands and oxidize virtually all molecules in the biological membrane resulting in the injury of liver. Thus anti oxidant effects drug can prevent liver’s oxidative damage5.

*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 and Andaman. *D. turbinatus* is used traditionally as anti diarrhoeal, astringent, wound healer, antiulcer, burns, tuberculoid leprosy\(^6\)-\(^8\). In Ayurveda, *Asanadi gana* was one of the ingredients out of 23 plants which were used in the diabetic treatment\(^9\). Tribal people of Tripura use *D. turbinatus* plant and plant derived product for various purposes. This drug possess anti oxidant activity\(^10\), aqueous extracts of the plant used for reducing gum and tooth ache\(^11\). It shows cyto-toxic activity\(^12\) and other species of the plants also shows anti cancer properities\(^13\). In this work we have done the hepatoprotective and anti oxidant activity of ethnolic extract of *D. turbinatus* bark by *in vivo* and *in vitro* method respectively.

Barks were procured from wild source from the forest of Tripura. The plant material was authentified by Prof P. Jayaraman, M.Sc., Ph.D., Director PARC., Chennai. With reference number (PARC/2012/1277). The specimen was preserved at the herbarium of the institute.

1.1 Chemical and Reagent
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 kits) were procured form 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 centrifuge 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, were used for various analyses.

Coarsely powdered dried bark of 500 g was extracted up to exhaustion with petroleum ether, chloroform, and ethanol (70%) using soxlet apparatus. Ethanol extracts (18% w/w) thus obtained was dried under controlled temperature and was selected for the study.

2. Experimental Animals
The animals (Wistar albino rats for hepatoprotective activity and albino Mice for acute toxicity) were kept at Standard environmental conditions for 7 days before the experiments. All the animals were maintained under strict hygienic conditions with normal feed and water. The entire animals were utilized for the studies and used according to the protocol approved by (No. IAE/ICESCOP/2016-07) Institutional Animal Ethics Committee. For each hepatoprotective activity 5 groups were made for both models (paracetamol and carbon tetrachloride) containing 6 animals. Group I serves as vehicle control, group II contains hepatotoxin, group III contains standard (Silymarin), group IV and V serve as drugs treated (200mg/kg & 400mg/kg of EEDT) group.

Acute toxicity for EEDT was determined by the method adapted by CPCSEA, Government of India, – acute toxic class method (OCED Guideline no. 423, Annexure–2d) in albino mice. The mortality was observed after oral administration of 2000mg/kg b.w of test sample\(^14\). Common side effects like mild diarrhea, weight loss and depression of treated groups of animals were recorded within the one week of observation\(^15\).

Group I (vehicle control) was given 2% acacia suspension in normal saline, group II received paracetamol (3gm/kg)\(^16\) as a single dose at 0hr followed by vehicle for 1hr, 24hr, 48hr. Group III received paracetamol (3gm/kg/p.o) as a single dose at 0hr followed by Silymarin (50mg/kg) for 1hr, 24hr, 48hr. Group IV and V received paracetamol 3gm/kg at 0hr followed by drug extracts 200mg/kg and 400mg/kg at 1hr, 24hr, 48hr. At 72\(^{nd}\) hr, blood was collected from animals and biochemical parameters were evaluated related to liver disorder to check the therapeutic effects of the drug extracts\(^17\). All the dosage has administrated by oral route.

2.1 Carbon Tetra Chloride Induced Hepatotoxicity
Group I serves as (–ve) control and were received 1 ml 2% acacia solution for 5 days and olive oil 2ml/kg on 2\(^{nd}\) and 3\(^{rd}\) day. Group II serves as (+ve) control, receiving acacia suspension for 5 days; Group III receiving standards Silymarin 50mg/kg for 5 days; Group (IV & V) receiving EEDT 200mg/kg & 400mg/kg for 5 days. CCl\(_4\) was administrated by IP route by mixing with olive oil (1:1) and all other treatment was given by oral route. On 2\(^{nd}\) and 3\(^{rd}\) day group II, III, IV & V received carbon tetra chloride at a dose 2ml/kg\(^18\) 30 minutes later.
of drugs treatment. Blood samples were collected on 6th day by retro orbital plexus route for evaluating various biochemical parameters.

Assessments of liver function were done by collecting blood from retro orbital puncture. Blood was centrifuged at 4000 rpm for 15 min and serum was collected. Various biochemical parameters like SGOT, SGPT, serum ALP, total cholesterol, total bilirubin and total protein were evaluated using biochemical kits.

The animals were sacrificed and liver were collected and washed with normal saline. Isolated liver were stored in 40% formalin solution and histopathology was done to evaluate the details change hepatic cell structure in each group microscopically for both the models.

### 2.2 Anti Oxidant Activity

#### 2.2.1 DPPH (2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

DPPH scavenging activity was measured according to the method of Sanjay Rai et al. with some modifications. 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 prepared in ethanol. 1 ml of this solution was added to 3 ml of all the extracts in water at different concentrations (1–10μg/ml). Thirty minutes later the absorbance was measured at 517 nm. Ascorbic acid is used as standard antioxidant. The results expressed as IC$_{50}$ or inhibitory concentration 50 value.

#### 2.3 Reducing Ability

Reducing power of EEDT was determined based on the ability of anti oxidant to form complex with potassium ferricyanide, TCA and FeCl$_3$. Add different concentration of sample(s) in 1 ml of distilled water in order to get 1μg, 2μg, 4μg, 8μg and 10μg/ml concentration. A test tube also makes as control (without any test sample). Add phosphate buffer (2.5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) to the sample. The mixture was incubated at 50°C for 20 min. Portions (2.5 ml) of trichloroacetic acid (10%) added to the mixture and centrifuged at 3000 rpm for 10 minutes. The upper layer of the mixture solution (2.5 ml) was mixed with distilled water (2.5 ml) and 0.1% ferric chloride (0.5 ml). The absorbance (OD) was measured at 700 nm by using Ascorbic as standard. The % reducing power was calculated by using conventional formula.

### 2.4 Superoxide Anion Scavenging Activity

The reaction mixture contains 100 μl riboflavin (20μg/ml) solution, 200 μl EDTA (12mM) solution, 200 μl Methanol and 100 μl NBT (0.1mg) solution in a test tube. The reaction mixture was diluted up to 3ml with phosphate buffer (50mM, pH 7.6). The absorbance of the mixture solution was measured at 560 nm using phosphate buffer as blank after illumination for 15 min. The OD was taken as control reading. Add different concentration (1 μg, 2 μg, 4 μg, 8 μg and 10 μg/ml) of samples in a test tube containing 100 μl Phosphate buffer. Add with 100 μl riboflavin, 200 μl EDTA, 200 μl ethanol and 100 μl NBT solution in respective test tubes. Then the reaction mixture was diluted up to 3 ml with phosphate buffer. The absorbance of the solution was measured after illumination for 5 min at 590nm. L-Ascorbic acid was used as a standard. Result was expressed as Inhibitory concentration 50 for the test drug.

Nitric oxide scavenging activity of extracts was determined by using Griess reagent (1% sulphonilamide, 2% phosphoric acid and 1% naphthyl ethylene diamine di hydrochloride). Reaction mixture containing 3 ml of sodium nitroprusside (10 M.mol) in phosphate buffer and test extracts in different concentrations (1μg, 2μg, 4μg, 8μg and 10μg/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 546nm using UV-visible spectrophotometer. Percentage inhibition was calculated and the activity was expressed as an inhibition concentration 50 (IC$_{50}$).

### 3. Statistical Analysis

Data were expressed as Mean values ± SEM. The differences between the groups were analyzed by one way ANOVA and compare with toxic group. A value *p* < 0.05 was considered as statistically significant.
3.1 Acute Toxicity Study (LD$_{50}$)

Acute toxicity study was carried out according to method of OECD 423 for EEDT. Mortality was not observed at 2000mg/kg in mice. Thus 2000mg/kg was considered as cut off dose. Thus 1/10$^{th}$ and 1/5$^{th}$ (200mg/kg and 400mg/kg) dose were taken as effective dose for all further in-vivo studies.

3.2 Effects of Ethanol Extracts of *D. turbinatus* on Various Biochemical Parameters Associated with Liver Toxicity

Animal treated with 200mg/kg and 400mg/kg P.O of ethanol extracts from *D. turbinatus* barks exhibit a significant (*p<0.05) reduction in various biochemical parameters. In paracetamol induced and carbon tetrachloride induced groups, there was an increase in the level of SGOT, SGPT, ALP, serum bilirubin and decrease in the level of total proteins. Treatment with the extracts 200mg/kg and 400mg/kg P.O take down to normal in the level of SGOT, SGPT, ALP, Cholesterol and serum bilirubin and increase the level of total proteins. The results were explained in Table 1 and Table 2. *D. turbinatus* bark extracts showed the degree of protection with the higher dose.

Histology of liver was done for all groups to find out change in liver architecture; like central vein, cytoplasm, nature of polygonal hepatocytes with their round nucleus and cytoplasm. Blood cell infiltration in hepatic vein, sinusoidal area and aggregation of Kuffer cells surrounding the central veins was also observed. Figure 1(a) and 2(a) shows the histopathology of the liver of untreated animals with normal hepatic vein, with less sinusoidal space polygonal hepatocytes without any ballooning. Figure 1(b) intoxicated with paracetamol shows damage in hepatic cell enlargement sinusoidal space and with infiltration of blood cell in hepatic vein, where as Figure 2(b) intoxicated with CCl$_4$ shows so much damage in the cellular system in liver cell. In both Figure 1(c) and 1(d) standard drug treated animal able to normalize the hepatic cell with less sinusoidal space, polygonal shape of kuffer cell, and hepatic vein with less blood cell in filtration. Figure 1(d) and 1(e) shows test drug treated with 200mg/kg and 400mg/kg, in paracetamol induced hepatotoxic animals liver, where we can see improvement of hepatocyte, sinusoidal space and hepatic vein as compared to the toxic (Paracetamol treated group) group. Figure 2(d) and 2(e) CCl$_4$ induced hepatotoxic animals liver treated with 200mg/kg and 400mg/kg EEDT with normalized liver cell.
Hepatoprotective and Antioxidant Activity of Ethanol Extracts of *Dipterocarpus turbinatus* (Dipterocarpaceae) from Tripura

**Table 1:** Hepatoprotective activity of ethanol extracts from barks of *D. turbinatus* against paracetamol induced hepatotoxicity

| Groups       | Treatment                          | Parameters                  |
|--------------|------------------------------------|----------------------------|
|              | SGOT (U/l) SGPT (U/l) APL (U/l)    | Cholesterol (mg/dl)        |
| Group I      | Normal control                     | 119.6 ± 5.431 63.61 ± 3.89 99.33 ± 5.18 108.5 ± 6.083 0.9783 ± 0.023 8.823 ± 0.445 |
| Group II     | Toxic (paracetamol)                | 588.5 ± 45.18 387.117 ± 11.76 270.9 ± 15.92 253.3 ± 11.65 2.490 ± 0.3002 3.692 ± 0.297 |
| Group III    | Standard (Paracetamol + Silymarin) | 121.7 ± 3.584*** 70.34 ± 3.22*** 92.61 ± 4.152** 107.8 ± 4.738*** 1.294 ± 0.1190*** 8.327 ± 0.7873*** |
| Group IV     | Test (EEDT 200mg/kg + Paracetamol) | 182.9 ± 5.377** 186.6 ± 12.20** 102.2 ± 5.560*** 169.9 ± 7.757** 0.5833 ± 0.0546* 7.252 ± 0.3618** |
| Group V      | Test (EEDT 400mg/kg + Paracetamol) | 165.3 ± 1.65*** 164.1 ± 6.432*** 79.81 ± 7.969*** 140.1 ± 6.10** 0.4567 ± 0.0445** 7.252 ± 0.3225** |

Values are the mean ± SEM six rats. Symbol represent statistically significance ***P<0.001., **P<0.01., *P<0.05 compared with toxic paracetamol group.

**Table 2:** Hepatoprotective activity of ethanol extracts from barks of *D. turbinatus* against Carbon tetra chloride induced hepato toxicity

| Groups       | Treatment                          | Parameters                  |
|--------------|------------------------------------|----------------------------|
|              | SGOT(U/l) SGPT(U/l) APL(U/l)       | Cholesterol (mg/dl)        |
| Group I      | Normal control                     | 140.9 ± 4.417 78.98 ± 4.296 55.45 ± 3.235 119.0 ± 5.235 0.2327 ± 0.0413 5.975 ± 0.2181 |
| Group II     | Toxic (CCl₄)                       | 453.3 ± 28.03 243.0 ± 12.71 189.1 ± 7.498 189.1 ± 7.498 0.2050 ± 0.217 9.239 ± 0.4360 |
| Group III    | Standard (+ Silymarin)             | 136.0 ± 13.15*** 156.3 ± 3.268*** 125.4 ± 2.474*** 125.4 ± 2.474*** 0.2733 ± 0.0272*** 5.377 ± 0.2397*** |
| Group IV     | Test (EEDT + CCl₄)                 | 288.0 ± 22.23ns 170.8 ± 7.912** 174.4 ± 6.817** 151.179 ± 9.372* 1.827 ± 0.1214*** 6.118 ± 0.389** |
| Group V      | Test (EEDT + CCl₄)                 | 155.3 ± 13.65*** 89.85 ± 4.813*** 131.2 ± 1.404*** 125.4 ± 3.003*** 1.192 ± 0.025*** 8.233 ± 0.543*** |

Values are the mean ± SEM six rats. Symbol represent statically significance ***P<0.001., **P<0.01., *P<0.05 compared with toxic paracetamol group, Where Ns represent not significant.

Fig. 1. (a) Normal liver section of rats (400X), (b) Liver section of rats (400X) intoxicated with Paracetamol (3gm/kg), (c) Liver section of rats (400X) intoxicated with Paracetamol (3gm/kg) and treated with Silymarin (50mg/kg), (d) Liver section of rats (400X) intoxicated with Paracetamol (3gm/kg) and treated with EEDT (200mg/kg), (e) Liver section of rats (400X) intoxicated with Paracetamol (3gm/kg) and treated with EEDT (400mg/kg).
Fig. 2. (a) Normal liver section (400X), (b) Liver section of rats (400X) intoxicated with carbon tetrachloride (2ml/kg). (c) Liver section of rats (400X) treated with Silymarin (50mg/kg) and intoxicated with Carbon tetrachloride (2ml/kg) (d) Liver section of rats (400X) treated with EEDT (200mg/kg) and intoxicated with Carbon tetrachloride (2ml/kg), (e) Liver section of rats (400X) treated with EEDT (400mg/kg) and intoxicated with Carbon tetrachloride (2ml/kg).

3.3 Anti Oxidant Activity

*D. turbinatus* bark extracts posses a significant anti oxidant activity. Table 3 illustrates the results of anti oxidant activity by various *in vitro* anti oxidant methods.

Preliminary phyto chemical analysis of the extracts shows presence of phenol and flavonoids which is known to be hepatoprotective and anti oxidant activity. Anti oxidant 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. Paracetamol is widely used as anti pyretic and analgesic. The metabolite of paracetamol N-acetyl–p-benzoquinonimine breaks sulphhydryl group of proteins and causes liver damage. CCl₄ has been widely used as an indicator of the liver protective activity of drug. CCl₄ gets metabolised in liver and release CCl₃ radicals, a free radical that binds to lipoproteins and leads to peroxidation of lipids of endoplasmic reticulum. CCl₄ causes liver injury that is characterized by centrilobular necrosis that is followed by hepatic fibrosis. In our study we performed paracetamol and carbon tetra chloride induced hepatotoxicity. In paracetamol induced hepatotoxicity, toxicant (Paracetamol) was given at 0 hr and followed by ethanol extract. In CCl₄ induced hepatotoxicity toxicant CCl₄ was administrated (at 2nd and 3rd day) along with ethanol extract from the bark.
of *D. turbinatus* in dose dependent manner for 0 to 5 days, once/day. The ethanolic extract from the barks of *D. turbinatus* able to decrease the elevated biochemical parameters like SGOT, SGPT, ALP, cholesterol, bilirubin level in both carbon tetra chloride and paracetamol induced hepatotoxicity in rats and also significantly increased the total protein in the blood of treated animals with test drugs and silymarin. These findings suggest that ethanolic extract of *D. turbinatus* is effective in bringing the functional improvement of liver. Protection in hepatocyte by EEDT at 200mg/kg and 400mg/kg also was confirmed by histopathological studies of the liver for various groups animal. Anti oxidant activity shows that the drug extract has beneficial anti oxidant property with significant IC$_{50}$ values with the reference standard of ascorbic acid.

Treatment with EEDT brought down the elevated levels of various biochemical parameters related to hepatic function and also restores the damage of liver cell. An overall result is concluded in these studies that the drug can be a therapeutic agent in liver damage in future. Hepatoprotective effect of EEDT may be due to its anti oxidant activity and ability to scavenging the free radicals which may be due to the presence of phenol and flavonoids in the extracts. However further studies are need to confirm involvement of cytochrome p450 enzyme inhibition to check the proper mechanism of liver protection from toxicity.

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### Table 3: Anti oxidant activity of ethanol extracts from the bark of *D. turbinatus*

| Scavenging methods                  | Positive control | EEDT          | Standard          |
|-------------------------------------|------------------|---------------|-------------------|
| DPPH scavenging activity            | Ascorbic acid    | 53.63 ± 1.282 | 10.63± 1.282      |
| Nitric oxide scavenging activity    | Ascorbic acid    | 47.01 ± 3.197 | 7.210 ±0.85       |
| Super oxide scavenging activity     | Ascorbic acid    | 45.72 ± 5.299 | 14.39 ±0.94       |
| Reducing power method               | Ascorbic acid    | 43.90 ± 2.866 | 15.26 ±1.994      |

Values are expressed as the mean SEM with p<0.01 compared to positive control group. Symbol represent *p<0.05., **p<0.01., ***p< 0.001.

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