

Antioxidant and hepatoprotective effects of aqueous and ethanol extracts of *Dendrophthoe falcata* Linn leaves

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Abstract

Dendrophthoe falcata L. is an important parasitic plant extensively used as traditional medicine against different diseases. Therefore present study was designed to investigate the antioxidant and hepatoprotective effects of aqueous and ethanol extracts of leaves of the plant. To investigate antioxidant potentials, we have performed qualitative phytochemical screening, quantitative determination of total phenol, flavonoids and antioxidants levels along with free radical (DPPH) scavenging and reducing capacities (Fe^{3+}) of the extracts. The hepatoprotective effect was examined on Long Evans rat model. Liver damage was induced by intraperitoneal administration of 25% carbon tetrachloride (CCl_4) in olive oil (1ml/kg, b.w, i.p) and the extent of damage was measured by assessing biochemical parameters. 30 rats were divided into five groups of six animals in each group. Group I (control) was given olive oil and DMSO, while group II- V were injected CCl_4 intraperitoneally. Animals of group II received only CCl_4 . Rats of group III, IV and V received silymarin (50mg/kg p. o.), aqueous extract (200mg/kg p.o.) of *D. falcata* leaves (AEDFL) and ethanol extract (200mg/kg p.o.) of *D. falcata* leaves (EEDFL) respectively along with intraperitoneal administration of CCl_4 for consecutive 7 days. At the end of the experiment (7days), serum and liver samples were collected for biochemical and histopathological analysis. The activities of liver markers: aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), total protein (TP) and bilirubin were measured in serum of each animal. Hepatotoxicity induced by CCl_4 was evidenced by significantly elevated levels of the markers. Co-administration of silymarin and the extracts (AEDFL and EEDFL) significantly ($P < 0.001$) prevented all the changes observed with CCl_4 -treated rats. The phytochemical analysis and antioxidant measurement revealed the presence of important phytochemicals like alkaloids, tannins, resins, phenols, flavonoids etc. and significant antioxidant effects of the extracts.

Key words: *Dendrophthoe falcata*, antioxidants, Hepatotoxicity, carbon tetrachloride

Introduction

Liver is the largest and pivotal gland the main functions of which are metabolism, detoxification, excretion and generation of a variety of coagulation factors (1, 2). On the other hand, it is also one of the most vulnerable organ and prone to be impaired by toxins, drugs and microorganisms (3,4). Millions of people are being affected by liver disease throughout the world (5) where drug induced hepatic injury is a major concern (6). It has been reported that the oxidative stress induced by free radicals is a major cause of liver disorder such as swelling, degeneration, necrosis, and apoptosis of hepatic cells (7, 8, 9). Free radicals induce an oxidative state that causes cellular membrane injury with the subsequent alteration in metabolic processes. Reactive oxygen species (ROS) plays a potential role in pathogenesis of diverse degenerative diseases of human and have been implicated in atherosclerosis, liver disorders, lung and kidney damage, aging and diabetes mellitus (10). In case of liver disorders the ability of natural antioxidant system is impaired. Free radicals are generated in cells by environmental factors such as ultraviolet radiation, pollutants, x-rays, or by normal metabolic process in mitochondria. The intracellular concentration of ROS depends on both their production by endogenous or exogenous factors and removal by various endogenous antioxidants including both enzymatic and non enzymatic components (11, 12). Carbon tetrachloride (CCl_4) is an extremely toxic chemical to the liver cells. It is a widely used model to evaluate hepatotoxic potential of experimental compounds. Histopathological sectioning of the liver tissues indicates the state of cell such CCl_4 induced fibrosis, cirrhosis and hepatocarcinoma (10, 13, 14). The toxic effect of CCl_4 is attributed by trichloromethyl radical (free radical) produced during oxidative stress (15). Management of liver diseases is still a challenge to the modern scientific community (16). Because the treatment options for common liver disease such as cirrhosis, fatty liver and chronic hepatitis are now still problematic. There are few conventional drugs available in market such as interferons, colchicines, penicillamine and corticosteroids that are inconsistent for proper treatment (17). These drugs have profound side

effects, and cannot offer hepato protection by stimulating liver function or regenerating hepatic cells (18). Inhibition of free radicals is very important in terms of liver pathology. Natural products from the plant kingdom are being investigated as a source of anti-oxidants, and all types of antioxidants are capable to resist oxidative stress (11, 12, 19). These traditional products may have the hepatoprotective potential, and therefore can be effectively used to treat acute and chronic liver diseases (20, 21, 22).

Dendrophthoe falcata belongs to the family Loranthaceae, commonly known as 'Porgassa in Bangla', and 'Banda' in Hindi (23). It is also familiar as "Bandaaka, Vrksaadani, Vrksruuhaa" in the Indian Ayurvedic System of Medicine (24). *D. falcata* is an evergreen perennial climbing woody hemiparasitic plant with bark smooth grey, leaves opposite unequal, thick 1.6 - 25.4 cm long, flowers single, orange-red or scarlet softly pubescent, berries soft ovoid-oblong, 1.3cm diameter (25,26). It is found in Bangladesh and also widely distributed in Australia, India, China, Malaysia, Myanmar, Srilanka, and Thailand (25, 26, 27, 28).

The entire plant is used extensively in traditional system of medicine as cooling, bitter, aphrodisiac, astringent, narcotic, diuretic, and is useful in pulmonary tuberculosis, asthma, menstrual disorders, swellings, wounds, ulcers, renal and vesical calculi (24, 26, 29). Leaf paste is used in skin diseases where it is applied on boils, setting dislocated bones and extracting pus (28, 23). The decoction of whole plant is used to treat joint pains and leaf juice is used for relief from chest pain (30, 31). *D. falcata* is reported to have cytotoxic, immunomodulatory activities and wound healing potentials. In the traditional system of medicine, *D. falcata* is recommended for the treatment of epilepsy (26, 32).

To find out active constituents a number of enzymes are separated from the leaves of *D. falcata* such as L-Threonine dehydratase, hexokinase, Glucan phosphatase (33). It has also been reported by the isolation and identification of several possible active chemical constituents such as β -amyryn acetate, β -sitostriol, stigmasterol, oleanolic acid (31), kaempferol, quercetin (27), quercetin-3-O-rhamnoside, rutin, myricetin and their glycosides, (+)-catechin, leucocyanidin, some, kaempferol-3-O- α -L-rhamnopyranoside and will

quercetin-3-O- α -L-rhamnopyranoside etc (26, 34). It also contains tannins comprising of gallic acid, chebulinic acid (28) ellagic acid (34), quercetin and (+) – catechin(28, 26). Three cardiac glycosides such as strosposide, odoroside F and neritaloside were isolated from the leaves of *D. falcata* (26). Pentacyclic triterpenes: 3β -acetoxy- 1β -(2-hydroxy-2-propoxy)- 11α -hydroxy-olean-12-ene (35), kaempferol-3-O- α -L-rhamnopyranoside, quercetin-3-O- α -L-rhamnopyranoside were also reported to have in the plant (26, 36).

The study was undertaken to evaluate antioxidant and hepatoprotective activity of aqueous, ethanol extract of *D. falcata* leaves in Long Evans rat which will unveil the rationality of use of the plant as traditional medicines.

Therefore, the great efforts have been invested to exploit the perfect antioxidant drug protecting liver from damage

Methods

Plant materials:

For the investigation, *Dendrophthoe falcata* L. leaves, mistletoe of *Swietenia fabrilis* tree were collected from Joypurhat, Bangladesh in September, 2012 and identified by experts of the Bangladesh National Herbarium, Dhaka, where a voucher specimen has also been retained with accession no. 39432. The collected plant parts were cleaned, dried for one week and pulverized into a coarse powder using a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark, and dry place until further analysis.

Extract preparation

Approximately 800 g of powdered material was placed in a clean, flat-bottomed glass container and soaked in ethanol and similarly 500g of the powder was soaked in distilled water. Both the containers with its contents was sealed and kept for 5 days. Then extraction was carried out using ultrasonic sound bath accompanied by sonication (40 minutes). The entire mixture then underwent a coarse filtration by a piece of clean, white cotton material. The extract then was filtered through Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and dried by electric oven at 45°C temperature and continued up to obtain ethanol (12g) and aqueous (16g) extracts. The gummy extracts were stored in an air tight container.

Drugs and chemicals

Silymarin, Diagnostic kits for serum: alanine aminotransferase (ALT) and aspartate amino transferase (AST), alkaline phosphatase (ALP), total proteins (TP) and bilirubin were purchased from Human, Germany. DPPH, quercetin, gallic acid, ascorbic acid were purchased from Sigma Aldrich, USA. Other chemicals and solvents were of highest analytical grade commercially available.

Animals

Long Evans rat of either sex weighing approximately 100-130 g were used for this experiment. The rats were purchased from the animal research branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). After their purchase, the rates were kept in standard environmental conditions (24.0 \pm 0°C & 55-65% relative humidity and 12 h light/dark cycle) for one week to acclimate and fed ICDDR, B formulated rodent food and water *ad libitum*. The experimental procedures involving animals were conducted in accordance with the guidelines of Southeast University, Dhaka, Bangladesh. The study protocol was approved by Institutional Animal, Medical Ethics, Biosafety and Biosecurity Committee of the University. The set of rules followed for animal experiment were approved by the institutional animal ethical committee (37).

Acute toxicity testing

Sixty six rats were divided in eleven groups of six animals. Two extracts (AEDFL and EEDFL) were administered orally at doses of 200, 400, 800, 1600 and 3200 mg/kg body weight to the animal groups (one dose per group). The control group received normal saline (mg/kg). General signs of weakness and symptoms of toxicity, food and water intake and mortality were recorded for a period of 48 hours and then for a period of 14 days.

Phytochemical screening

Qualitative tests of the AEDFL and EEDFL for the presence of alkaloids, tannins, resins, saponins, flavonoids, steroids and terpenoids were carried out.

Test for alkaloids

0.4 g of AEDFL and EEDFL were stirred with 8 ml of 1% HCl in two separate test tubes. The mixtures were warmed and filtered. 2 ml of filtrate two different samples were treated separately with (a)

with few drops of potassium mercuric iodide (Mayer's reagent) and (b) potassium bismuth (Dragendorff's reagent). Turbidity or precipitation with either of these reagents was taken as evidence for existence of alkaloids (38).

Test for saponins

The ability of saponins to produce emulsion with oil was used for the screening test. 20 mg of the extracts (AEDFL and EEDFL) were boiled in 20 ml of distilled water in a water bath for five min and filtered. 10 ml of the filtrate was mixed with 5 ml of distilled water and shaken vigorously for froth formation. 3 drops of olive oil were mixed with froth, shaken vigorously and observed for emulsion development (38).

Test for terpenoids

Presence of terpenoids in the extracts was carried out by taking 5 ml (1 mg/ml) of AEDFL and EEDFL in test tubes. Then 2 ml of chloroform, followed by 3 ml of concentrated H_2SO_4 were added in the test tubes. A reddish brown coloration of the interface confirmed the presence of terpenoids (38).

Test for flavonoids

To perform the test 50mg of AEDFL and EEDFL were suspended in 100ml of distilled water to get the filtrate. 5 ml of dilute ammonia solution was added to 10 ml of filtrate followed by few drops of concentrated H_2SO_4 . Presence of flavonoids was confirmed by yellow coloration (39).

Test for tannins

50 mg of AEDFL and EEDFL were boiled in 20 ml of distilled water then filtered. A few drops of 0.1% $FeCl_3$ was added in filtrate and observed for color change. Appearance of brownish green or a blue-black coloration indicated the presence of tannins (39).

Test for Steroids

One ml of the extract was dissolved in 10 ml of chloroform and equal volume of concentrated sulphuric acid was added by sides of the test tube. The upper layer turns red and sulphuric acid layer showed yellow with green fluorescence. This indicated the presence of steroids (38).

Test for reducing sugar

Both the extracts (AEDFL and EEDFL) were dissolved individually in 5 ml distilled water and filtered. The filtrates were used to test for the presence of carbohydrates. Filtrates were treated with Benedict's reagent and heated on a water bath. Formation of an orange red precipitate

indicates the presence of reducing sugars (40).

Test for Resin

Acetone-water Test: Extracts (AEDFL and EEDFL) were treated with acetone. Small amount of water was added and shaken. Appearance of turbidity indicates the presence of resins (40).

Determination of phytoconstituents

Determination of total phenols

Total phenol contents were determined using Folin-Ciocalteu reagent as described by Yang et al. (41) with slight modifications. Total phenolic assay was conducted by mixing 2.7 mL of deionised water, 0.01 ml (200 μ g/ml) of extracts, 0.3 ml 20% Na_2CO_3 and 0.10 ml Folin-Ciocalteu reagent. Absorbance of mixture was measured at 725 nm. A standard curve was prepared with gallic acid ($r_2=0.945$) and final results were given as mg/g gallic acid equivalent.

Determination of total flavonoids

1 ml of plant extract in methanol (200 mg/ml) was mixed with 1 ml aluminium trichloride in ethanol (20 mg/ml) and a drop of acetic acid, and then diluted with ethanol to 25 ml. The absorption at 415nm was read after 40 min. Blank samples were prepared using all the reagents with equal volume used in the sample except extract. The total flavonoid content was determined using a standard curve ($r_2=0.902$) of quercetin (12.5-200 mg/ml) where quercetin was used as standard sample. Total flavonoid content was expressed as mg/g of quercetin equivalent (42).

Antioxidant ability assays

Determination of total antioxidants

The total antioxidant activity of extracts was evaluated by phosphomolybdenum complex according to the method of Prieto et al. (43). 0.3 ml extracts (200 μ g/ml) was combined with 3ml of reagent solution (0.6M sulfuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min in water bath. Then the absorbance of the solution was measured at 695nm using a spectrophotometer against blank (methanol) after cooling to room temperature. Ascorbic acid have been used as standard antioxidant ($r_2=0.964$) and total antioxidant capacities of the extracts were expressed as mg/g equivalents of ascorbic acid.

DPPH • radical scavenging activity:

The DPPH free radical scavenging activity of leaf extracts of *D.falcata* (AEDFL and EEDFL) were measured in term of hydrogen donating or radical scavenging ability using the stable radical DPPH (44). Briefly, 0.004% w/v of DPPH radical solution was prepared in methanol and then 900 µL of this solution was mixed with 100 µl of extract solution (12.5–200 µg/ml) and kept in a dark place for thirty minutes. Then absorbance was measured at 517 nm where methanol (98%), DPPH solution and ascorbic acid were used as blank, control and standard antioxidant respectively. Scavenging capacity of DPPH radicals (% Inhibition) was measured by the following formula and finally calculated 50% inhibition concentration (IC₅₀) using software.

$$\text{Inhibition (\%)} = (A_0 - A_s) / A_0 \times 100$$

Where A₀=Absorbance of control group, A_s=Absorbance of sample

Ferric-reducing power assay

The Fe³⁺reducing power of the extracts were determined by the method of Oyaizu (45) with slight modifications. Different concentrations of the extracts and standard ascorbic acid (12.5, 25, 50, 100, 200 µg/ml) were prepared. 1ml of both the extracts and standard ascorbic acid of all concentrations were taken in separate test tubes and were mixed with 2.5 ml of phosphate buffer solution (0.2 M, pH 6.6). 2.5 mL of potassium ferricyanide (1%) was added in each test tube, and incubated at 50°C for 30 min. Then, 2.5 mL of trichloroacetic acid (10%) was added to the mixture which was then centrifuged at 4000 rpm for 10 min. Finally, 2.5 mL of supernatant was mixed with 2.5 mL of distilled water and 0.1 mL of FeCl₃ (0.1%) solution followed by incubation at 35°C for 10 minutes. The absorbance was measured at 700 nm and the reducing powers of the extracts were compared with the standard ascorbic acid.

Hepatoprotective studies

Hepatoprotective activity of extracts was evaluated by CCl₄ according to the method of Gerhard Vogel (46).

Briefly, thirty animals of both sexes were divided into 5 groups of six animals in each group and subjected to the following experiment. Group-I served as the control and received 2% gum acacia (1ml/kg p.o) daily for 7days. Group-II served as the toxic group (CCl₄ control) and received 25% CCl₄ in olive oil (1ml/kg.i.p) daily for 7 days. Group-III served as positive control and received the standard drug silymarin (50mg/kg p.o.) for 7 days. Group-IV and V were treated with AEDFL and EEDFL at 200 mg /kg.p.o for 7 days. On the 7th day animals of each group were sacrificed after anaesthesia carried out by chloroform. Blood sample were collected from anticubital vein and serum was separated by centrifugation for estimation of hepatic enzymes. Then the liver markers such as ALT, AST, ALP, TP and bilirubin were estimated from the serum by blood chemistry analyzer Olympus AU-400.

Histopathological examination

Liver of each animal were separated and washed with normal saline (0.9%) solution and preserved with 10% formalin for histopathological examination. Sections (4-5mm thick) were prepared from each liver and stained with Hemotoxylin and Eosin dye for photomicroscopic observation. The microscopic slides of the liver cells were photographed at a magnification of x100.

Statistical analysis

All the data are expressed as mean ± S.E.M. (n = 6 rats per group). Statistical significance (p) calculated by ANOVA done in SPSS, Version 15.0, Followed by Dunnett 's Test. P³<0.001 were considered to be statistically significant. All the graphs are prepared using Graph Pad Prism software.

Results

Phytochemical screening

Various Qualitative Phytochemical tests were performed on AEDFL and EEDFL. The Phytochemical screening demonstrated the presence of saponins, tannins, flavonoids, alkaloids, reducing sugars, terpenoids and steroids. (Table 1)

Total phenol, total flavonoid and total antioxidant contents

The total phenol, total flavonoid and total antioxidant contents of AEDFL and EEDFL were expressed as Gallic acid, Quercetin and Ascorbic acid equivalents (mg/g) respectively. (Table 2).

In vitro antioxidant activity

Antioxidant capacity of AEDFL and EEDFL was examined using following assays.

DPPH free radical scavenging activity

The antioxidant activity of AEDFL and EEDFL were assessed by the DPPH free radical scavenging assay. The leaves extracts exhibited significant DPPH free radical scavenging effects compared to standard ascorbic acid. IC_{50} (50% inhibition concentration) value of ascorbic acid was 12.55 ± 2.35 $\mu\text{g/ml}$ where as aqueous and ethanol extracts showed 19.88 ± 2.98 $\mu\text{g/ml}$ and 33.60 ± 2.15 $\mu\text{g/ml}$ respectively (Figure 1).

Reducing power capacity

The reducing power of a compound is related to its electron transfer ability and may therefore; serve as an indicator of its potential antioxidant activity. We found the dose response curves of the reducing powers of both the extracts of *D.falcata* leaves (12.5 - 200 $\mu\text{g/ml}$) and compared with standard ascorbic acid. It was found that the reducing capacity of each sample increased with the increase of concentration. Between the two extracts the aqueous portion showed more reducing activity (Figure 2).

Hepatoprotective studies

Specific liver enzymes, total protein and bilirubin

The plasma levels of specific liver enzymes and protein profile were assessed to determine the liver function of each rat. The liver damage, induced by hepatotoxic compound CCl_4 , significantly ($P < 0.001$) elevated the plasma level of specific liver enzymes (ALT, AST, ALP and bilirubin) and lowered total protein (TP) levels in the hepatotoxic rats compared with the normal group. However co-treatment with AEDFL and EEDFL (200 mg/kg bw) reduced the elevated levels of liver markers such as ALT, AST, ALP and bilirubin, as well as increased protein level against the CCl_4 group. Silymarin (50 mg/kg bw) prevented the alterations in the activity level of ALT, AST, ALP and bilirubin induced with CCl_4 . These data demonstrated that the effects of toxicity induced by CCl_4 on the liver function could

be effectively counter balanced by the treatment of *D.falcata* leaf extracts (AEDFL and EEDFL). (Table 3).

Histopathological evaluation

The microscopic assessment (H&E staining) of liver sections in the experimental Groups 1-5 are shown in Figures 3. Figure 3(a) shows that there was no pathological abnormality observed in the liver of normal rat and thus showing the absence of vascular or necrosis changes. Figure 3(b) shows that CCl_4 induced severe necrosis changes and substantial changes in liver section such as ballooning, microvesicular steatosis, fatty degeneration, increase in sinusoidal space (SS) dilation and central vein, in CCl_4 treated group as compared to normal group. On the other hand, livers of rats in all treatment groups (AEDFL and EEDFL and silymarin) showed noticeable recovery from CCl_4 -induced liver damages with less microvesicular steatosis and hepatocytes necrosis features compared to CCl_4 control group (Figure 3c, 3d and 3e) (Figure 3).

Discussion

Modern drugs, hazardous chemicals and pollutants are the main factors of liver damage (47). So it is very much important to find out safe and effective therapeutic options to minimize the situation. CCl_4 induced- hepatotoxicity is one of the most important methods for conducting the hepatoprotective study in animal model (48). Medicinal plants are sources of diverse nutrients, many of which display antioxidant properties that are capable of shielding the body against both cellular oxidative stress and harmful pathogens (49); hence these are considered an alternative therapeutic approach in folk medicine (50). In the present research, AEDFL and EEDFL were examined as a promising therapy for treating liver damage induced by CCl_4 in rat model. The protocol induced cirrhosis with similar pathology and etiology pattern to the human liver cirrhosis with the same biochemical values for typical human cirrhosis markers (51). The CCl_4 , after administrations to rats, is biotransformed in liver by cytochrome P450 (CYP2E1) of endoplasmic reticulum to the trichloromethyl free radical (NCCl_3) and then further converted to a peroxy radical ($\text{CCl}_3\text{O}_2\text{N}$) (52, 53). radicals lead to auto-oxidation (cascade of reactions) of cellular lipids

and proteins and , thereby not only change the structures of endoplasmic reticulum and other membrane but also generates endogenous toxicants that can readily react with adjacent molecules like membrane proteins or diffuse to more distant molecules like DNA, which may lead to more hepatic complications and functional anomalies such as liver cell necrosis, apoptosis, fibrosis, or cirrhosis (48, 54, 55 ,56) and significantly lower activities of antioxidant enzymes such as Catalase (CAT), peroxidase (POD), superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), glutathione reductase (GSR), glutathione-S-transferase (GST), and quinone reductase (QR) (56). As a consequence, the cell membranes of hepatocytes become more permeable, and enzymes such as ALT and AST, ALP, generated by cell membrane and mitochondrial damages in liver cells, (57) can easily leak out into the blood circulation. So, amounts of these enzymes are increased in the plasma compared with normal subjects (1). A high concentration of bilirubin in serum is an indication for increased erythrocyte degeneration rate. The liver is known to play a significant role in the serum protein synthesis. So reduced level of TP is the consequence of defective biosynthesis of protein in liver (48). These changes of markers level reflect the degree of hepatocyte damage and necrosis (53) and vice versa. So, the extent of liver damage can be effectively assessed by estimating the activities of the liver markers: ALT, AST, ALP, TP and bilirubin.

Antioxidants are the prominent bioactive compounds that can effectively protect cells of liver or other organs from damage by opposing the activities of the free radicals (NCCl_3 , $\text{CCl}_3\text{O}_2\text{N}$) (58). The coordinate action of antioxidant system is very critical for the detoxification of free radicals. SOD reduces the concentration of highly reactive superoxide radical by converting it to H_2O_2 whereas CAT and GSH-Px decomposes H_2O_2 and protect the tissues from highly reactive hydroxyl radicals. Studies have reported that CCl_4 reduces the activities of antioxidant enzymes and causes hepatopathy (59, 60).

Phytochemical screening of AEDFL and EEDFL revealed the presence of alkaloids, tannins, resins, saponins, flavonoids, terpenoids, and steroids that are biologically active compounds.

Significant amount of total antioxidants (329.79 ± 8.12 , 297.97 ± 7.73), total phenols (178.72 ± 3.49 , 121.79 ± 4.39) and total flavonoids (153.80 ± 6.39 , 132.50 ± 7.38) were also presents in the AEDFL and EEDFL respectively. These phytoconstituents have been reported to exert major antioxidant activities by scavenging of free radicals that cause lipid peroxidation (9, 55). These antioxidant effects of AEDFL and EEDFL were assessed by DPPH radical scavenging and reducing power methods. DPPH radical, rapid and sensitive free radical is considered to be a model of antioxidants actions of scavenging activity of free radicals by donating hydrogen atom (58). Median inhibition concentration (IC_{50}) of AEDFL and EEDFL were $19.88 \pm 2.98 \mu\text{g/ml}$ and $33.60 \pm 2.15 \mu\text{g/ml}$ respectively. Furthermore, reducing power of an antioxidant depicts the neutralizing of oxidants by donating electrons (58). AEDFL has showed more reducing ability than the EEDFL.

Co-treatment of AEDFL and EEDFL prevented the toxic effects of CCl_4 by restoring the activities of antioxidant enzymes towards the level of control animals. AEDFL and EEDFL which have significant antioxidant activities ameliorate the liver injuries by scavenging of free radicals, or by preventing cytochrom P450 enzyme which is further confirmed by the reduced amount of histopathological injury (55). A number of studies have revealed that GSH conjugates play a major role in eliminating the CCl_4 -induced toxic metabolites which are the main cause of liver injuries. Repeat administration of CCl_4 depletes GSH level (61). The maintenance of sufficient glutathione level is important for the prevention of CCl_4 -induced damages (62). So alternatively, the mechanism of hepatoprotection of AEDFL and EEDFL against the CCl_4 toxicity might be due to restoration of GSH concentration in the liver of experimental animal (63). Between the two extracts AEDFL showed better protection as it poses more antioxidant activity than EEDFL.

These *in vitro* and *in vivo* assays indicate that the plant extracts (AEDFL and EEDFL) are significant source of natural antioxidants which might prevent the progression of oxidative stress on various organs especially on hepatic tissues from its damage by free radicals. However, the specific compounds of the extracts which are responsible for the protection by antioxidant activity are still unclear.

Therefore, further studies are necessary to isolate and identify the antioxidant compounds and to clarify the actual mode of protection of hepatotoxicity.

Acknowledgments

The authors are grateful to Pharmacy department of Southeast University, Dhaka, Bangladesh for providing all required facilities to conduct the research in their lab. The authors are also grateful to authority of Arms forces Medical College Hospital, Dhaka, Bangladesh for providing histological examination of rat liver in their lab. The authors are also thankful to animal division of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B) for timely providing sufficient experimental rats.

Conflict of interest: Authors have no conflict of interest.

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| Sample | Alkaloid | Tannin | Resin | Saponin | Flavonoids | Steroid | Reducing sugar | Terpinoids |
|--------|----------|--------|-------|---------|------------|---------|----------------|------------|
| AEDFL | ++ | ++ | ++ | +++ | ++ | + | + | + |
| EEDFL | ++ | + | + | ++ | + | + | + | + |

+ = slightly present, ++ = moderately present, +++ = extensively present

Table 1: Phytochemical test for aqueous and ethanol extracts of *Dendrophthoe falcata* leaves (AEDFL and EEDFL).

| Type of extract | Total phenol ^a | Total flavanoid ^b | Total antioxidant ^c |
|-----------------|---------------------------|------------------------------|--------------------------------|
| AEDFL | 178.72 ± 3.49 | 153.80 ± 6.39 | 329.79 ± 8.12 |
| EEDFL | 121.79 ± 4.39 | 132.50 ± 7.38 | 297.97 ± 7.73 |

a: Gallic acid; b: Quercetin; c: Ascorbic acid equivalents mg/g dw plant material respectively. Each value is expressed as a mean ± standard deviation (n = 3).

Table 2: Quantitative estimation of phytochemicals and antioxidant activities of aqueous and ethanol extracts of *D.falcata* leaves (AEDFL and EEDFL).

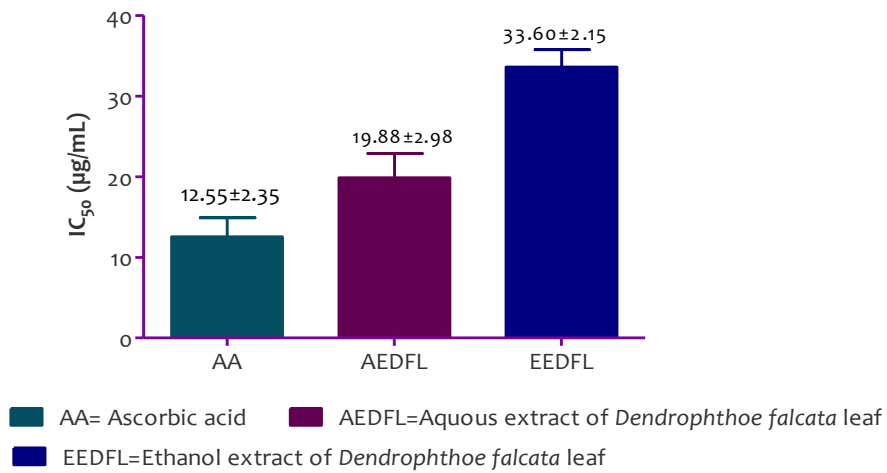


Figure-1: Determination of IC₅₀ of AEDFL and EEDFL. Data expressed as mean ± SEM (n=3) for all tested

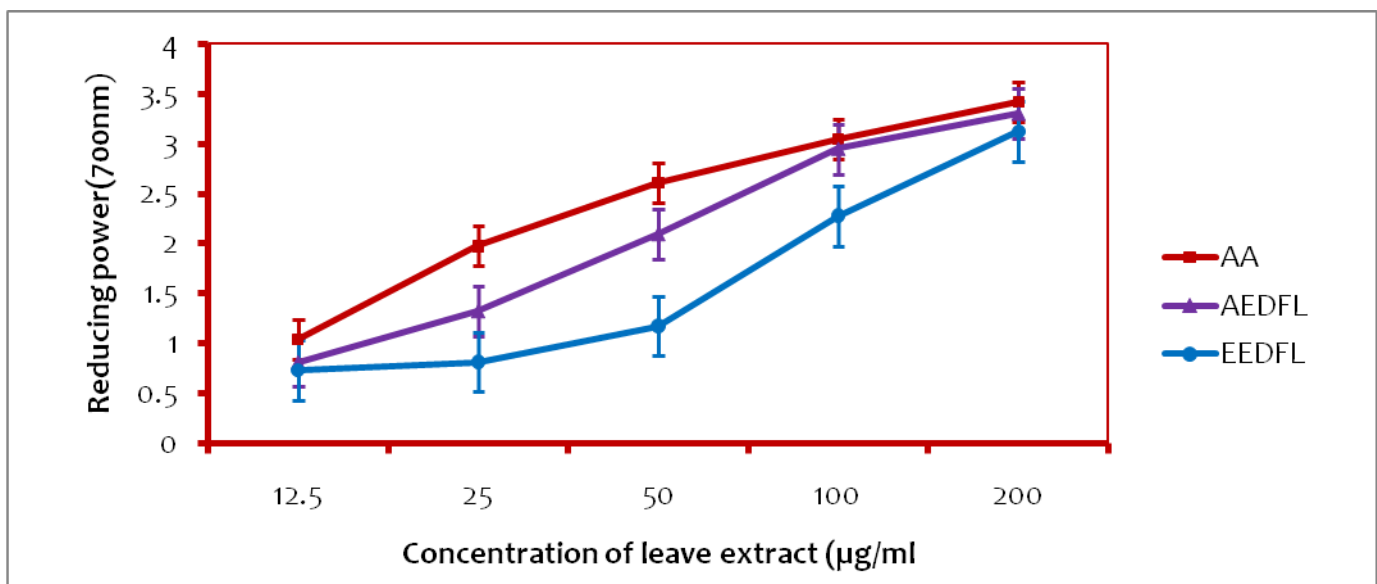


Figure 2: Reducing power of the aqueous and ethanol extract of *D.falcata* leaves (AEDFL and EEDFL) and ascorbic acid. n = 3. Error bars indicate standard error of mean.

| Group | Treatment | ALT (U/ml) | AST (U/ml) | ALP (U/ml) | TP (g/dl) | Bilirubin (mg/dl) |
|-------|-----------------------------------|--------------------------|--------------------------|-------------------------|------------------------|------------------------|
| I | Olive oil | 58.84±4.51 | 40.22±3.1 | 32.22±4.57 | 8.66±0.36 | 0.77±0.3 |
| II | CCl ₄ | 113.22±3.57 ^a | 107.22±4.01 ^a | 88.54±3.03 ^a | 2.38±0.25 ^a | 4.36±1.50 ^a |
| III | CCl ₄ +50 mg silymarin | 61.44±4.81 ^b | 47.20±4.11 ^b | 39.62±4.56 ^b | 7.16±0.76 ^b | 0.97±0.1 ^b |
| IV | CCl ₄ +200 mg AEDFL | 67.23±3.54 ^b | 56.14±2.52 ^b | 49.00±5.46 ^b | 6.72±0.52 ^b | 1.15±0.86 ^b |
| V | CCl ₄ +200 mg EEDFL | 89.01±3.57 ^b | 78.35±2.88 ^b | 66.51±4.51 ^b | 5.12±0.22 ^b | 1.02±0.97 ^b |

Data are Mean ± SEM, P^a<0.001 indicates significant change of liver markers compared with normal (I) group, P^b<0.001 is considered as significant level compared with control (II). ANOVA followed by Dunnet's T test is done in SPSS version 15.0.

Table 3: Effects of AEDFL and EEDFL on liver function tests in rat.

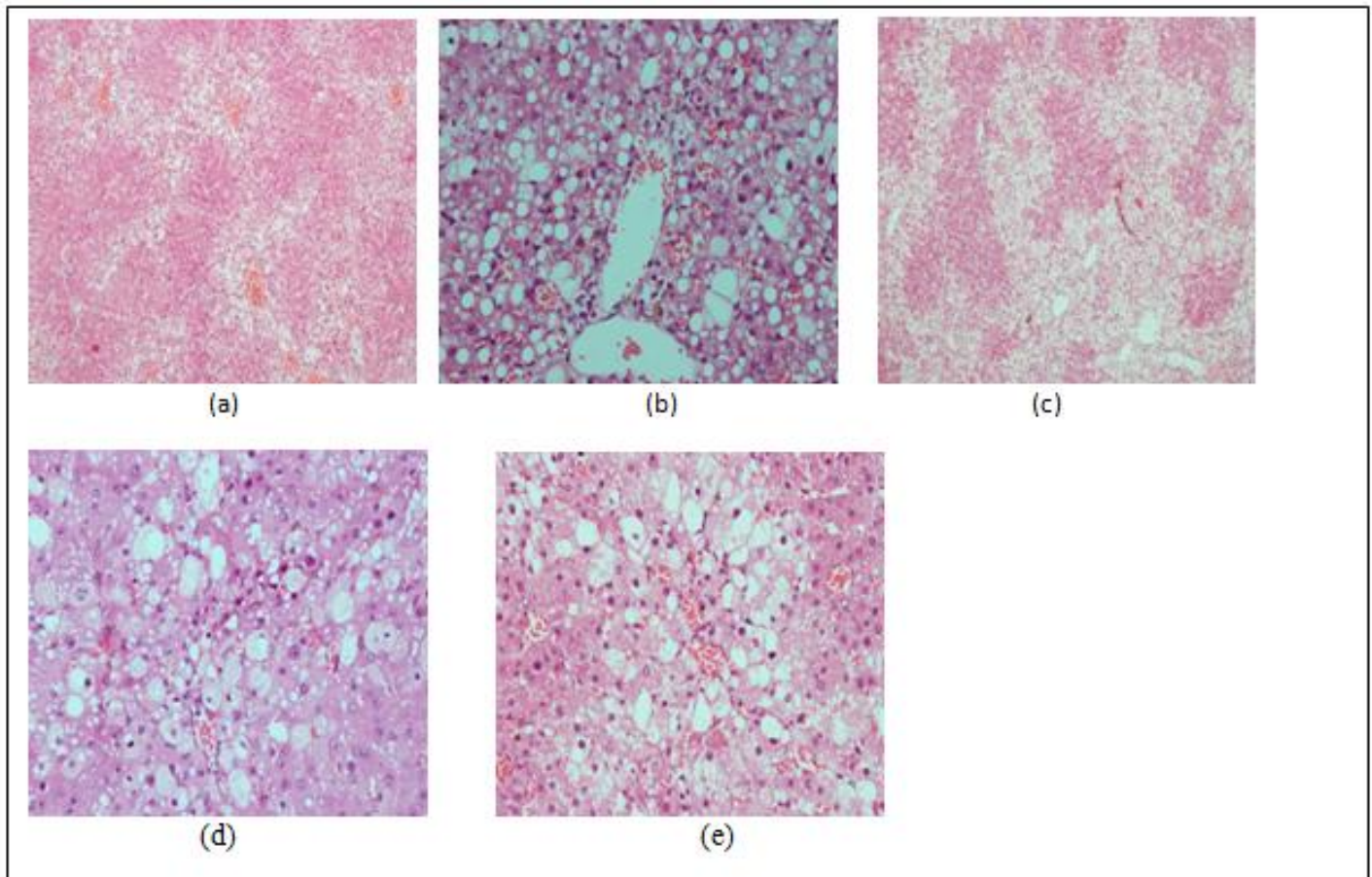


Figure 3: Micrograph of liver of rats (H & E stain). (a) Representative section of liver from the control group showing the normal histology. (b) CCl₄ (1ml/kg bw, 25% in olive oil) induced hydropic necrosis, lymphocytes infiltration, and ballooning of hepatocytes. (c) CCl₄+silymarin (50 mg/kg bw) repairing of hepatocytes. (d) CCl₄+AEDFL (200 mg/kg bw) repairing of hepatocytes (e) CCl₄+EEDFL (200 mg/kg bw) repairing of hepatocytes.