

Phytochemical Study and Protective effect of *Diospyros lotus* Against Carbon Tetrachloride-Induced Hepato and Nephro-Toxicity in Rats

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Abstract: The present work was carried out to study the protective effect of 70% methanolic extract of *Diospyros lotus* L. aerial parts against hepato and nephro-toxicity that was induced by carbon tetrachloride. In addition, phytochemical screening, total phenolic, total flavonoid contents and proximate analysis of the aerial parts were determined. Methanol (70%) extracts of the aerial parts of *Diospyros lotus* L. was prepared. Two main groups of rats were assigned which were normal and damaged liver groups that administered CCl₄ (carbon tetra chloride). Each group classified into 4 different groups normal control: was received distilled water, positive control: was administered silymarin (50 mg/kg), and treated groups: were administered *Diospyros lotus* tested extract at two dose levels (150 and 300 mg/kg) for 15 days. Serum alanine and aspartate aminotransferase activities were determined to evaluate hepatoprotective effect, while uric acid and creatinine levels were measured to study renal function protective effects. The results showed that the alterations induced by carbon tetrachloride were significantly restored by methanol extract of *Diospyros lotus* (150 and 300 mg/kg) in dose dependant manner. Alkaloids, carbohydrates, tannins, flavonoids, saponins and unsaturated sterols and/or triterpenes were detected as the main active constituents of the methanol extract. It can be concluded that the 70% methanol extract of *Diospyros lotus* L. aerial parts has a significant protective effect against acute hepato-toxicity and nephro-toxicity induced by CCl₄ in rats so it can be used as a safe hepato-nephro protective drug.

Keywords: Carbon Tetrachloride, *Diospyros Lotus*, Hepatoprotective, Nephro-Toxicity

1. Introduction

Liver is one of the most important organs in the human body which responsible for secretion of bile and storage of vitamins, detoxification as well as fat, protein and carbohydrate metabolism [1]. Hepatitis and Jaundice are two major hepatic disorders that considered the major worldwide health problems, which may lead to death [2]. Unfortunately, serious side effects were induced by synthetic

drugs that used in the treatment of liver diseases, so natural products can be used instead of synthetic drugs [3]. For decades, natural products are playing an important role in health care [4]. Drug induced liver toxicity is major health problem that challenges not only the pharmaceutical industry but also healthcare professionals [5]. The activity of hepatoprotective agents can be evaluated by the inhibition of free radical generation [6].

Genus *Diospyros* is widely distributed in tropical and

subtropical area, all members of the genus are trees [7]. *Diospyros* species are known for their medicinal uses in folk medicine. All parts of these plants have been used for medicinal purposes e.g. the bark is bitter, febrifuge and astringent, the seeds are sedative, the fruits are astringent, cure biliousness and carminative and the leaves are used for lumbago [8]. In Japan, Japanese persimmon *D. kaki* (Leaf extract) in combination with jasmine is used for making antitobacco smoking candies. The other research stated that triterpenoids that have been isolated from *Diospyros* species showed anti-inflammatory activity [9]. *Diospyros lotus* tree (family Ebenaceae) grows up to 9 m in height and around 6 m in width. In traditional medicine, *D. lotus* fruits were used as a febrifuge, nutritive, laxative, sedative, astringent, antitussive, antitumor, antidiabetic and as antiseptic [10-12]. In addition, *D. lotus* fruits are used to treat hypertension, dry coughs and diarrhea [13]. Previous phytochemical studies of *D. lotus* revealed the presence of naphthoquinones, terpenes, non volatile acids and some fatty acids [14-16].

To our knowledge, there is only one report dealing with the aerial parts of *D. lotus* which stated that the aerial parts of *D. lotus* showed antioxidant effect [17]. Hence the current study aims to investigate the chemical constituents of this part and evaluate its protective effect against carbon tetrachloride-induced hepato and nephro-toxicity in rats.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *Diospyros lotus* L. were collected from the Agricultural Research Centre, Giza, Egypt in April 2016 during flowering and identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen No. 2457 is deposited in the herbarium of Agricultural Research Centre, Giza, Egypt.

2.2. Preparation of the Plants Extracts

Air-dried powder of *Diospyros lotus* L. aerial parts (1000 g) was extracted with methanol/distilled water (70:30, v/v) several times at room temperature until exhaustion by maceration method. The extract was concentrated under reduced pressure to give 58 g of the crude extract.

2.3. Phytochemical Screening

Chemical tests were carried out on the methanolic extracts using standard procedure to identify the constituents as described by [18, 19].

2.4. Total Phenolic Assay

The total phenolic content (TP) was determined applying the Folin-Ciocalteu colorimetric method using gallic acid as a standard [20], and expressed as milligrams of gallic acid

equivalents (GAE)/g of the dry plant materials.

2.5. Total Flavonoid Assay

Total flavonoid content (TFC) was measured using an aluminum chloride colorimetric assay [21]. A calibration curve was established using quercetin as a standard. TFC was expressed as mg quercetin equivalent (QE)/g of the dry plant materials.

2.6. Proximate Analysis

Percentages of moisture content, total ash, water soluble ash, acid-insoluble ash, and crude fiber values were assessed according to the official methods [22].

2.7. Biological Evaluation

2.7.1. Animal

Sprague Dawley rats of both sexes weighing 130-150 g were used throughout the experiments. Animals were housed under standard environmental conditions ($23 \pm 1^\circ\text{C}$, $55 \pm 5\%$ humidity, 12:12 hr light-dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Animal care and the experimental protocols were approved by Institutional Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain.

2.7.2. Acute Toxicity Study

The LD₅₀ of methanol (70%) extract of the aerial parts of *Diospyros lotus* was determined using rats. Male and female albino rats (10 rats in each group) were orally administered the tested extract at doses ranging from 1.0 to 5.0 g/kg. LD₅₀ was calculated using Karber method [23]. Two dose levels of *Diospyros lotus* were chosen (150 and 300 mg/kg) to determine the most effective dose at exerting physiological activity.

2.7.3. Animal Grouping and Experimental Design

Rats were assigned into two main groups: normal and damaged liver groups that administered CCl₄ (carbon tetrachloride). Each group classified into 4 different groups (6 rats in each): group 1: was control received distilled water, group 2 was administered silymarin (50 mg/kg), and groups 3&4 were administered *Diospyros lotus* tested extract at two dose levels (150 and 300 mg/kg) for 15 days. The drug solutions or vehicle were administered orally by gastric intubation using syringe to assess the experiments. At the end of experimental period, rats were anaesthetized with ether according to the method described by Cocchetto and Bjornsson [24]. Blood samples were collected from orbital venous plexus in nonheparinized tubes, centrifuged at 3000 rpm for 15 min, and blood sera were collected and stored at 20°C before they were analyzed.

2.7.4. Biochemical Analysis

Determination of serum alanine aminotransferase (ALT) activity Serum alanine aminotransferase (ALT) activity was assessed colorimetrically using a test reagent kit according to

the method described by Reitman and Frankel [25]. Determination of serum aspartate aminotransferase (AST) activity Serum AST activity was assessed colorimetrically using a test reagent kit according to the method described by Reitman and Frankel [25]. Serum uric acid is the end product of purine metabolism, and is cleared through the kidney by glomerular filtration. Kit provides a convenient means for detecting uric acid in biological samples such as serum and urine. Pretreatment of samples are not required. Uric acid level can be measured using colorimetric method (at 570 nm) according to the method of Barham and Trinder [26]. Creatinine is a breakdown product of creatine phosphate. Creatinine is produced and excreted at a constant rate, and blood creatinine is used to determine glomerular filtration rate. Creatinine is measured in biological fluids (serum and urine) according to the method of Bartles and Bohmer [27].

2.7.5. Statistical Analysis

Data were analyzed using one-way ANOVA. P-value <0.05 was considered statistically significant.

3. Results and Discussion

3.1. Phytochemical Analysis

Table 1 showed that flavonoids, tannins, carbohydrates and/or glycosides as well as sterols and/or triterpenes were present in the aerial parts of *Diospyros lotus L.*, while coumarins, saponins and alkaloids and/or nitrogenous bases were absent.

Table 1. Results of phytochemical screening of the aerial parts of *Diospyros lotus L.*

Constituents	The aerial parts of <i>Diospyros lotus L.</i>
1. Carbohydrates and/or Glycosides	+ ve
2. Tannins	++ ve
3. Alkaloids and/or nitrogenous bases	-ve
4. Flavonoids	++ ve
5. Sterols and/or triterpenes	+ ve
6. Saponins	-ve
7. Coumarins	-ve

+ ve: the presence of the constituents, - ve: the absence of the constituents, ±ve: the presence of the constituents in minute amounts

3.2. Total Phenolic and Flavonoids Contents

The flavonoids play a vital role in protection of human

Table 3. Effect of 70% methanol extract of *Diospyros lotus L.* aerial parts on kidney function and liver enzymes in serum of normal rats.

Groups	AST	ALT	Uric acid	Creatinine
Control	44 ± 1.13	28.1 ± 1.24	1.30 ± 0.06	0.45 ± 0.07
Silymarin 50	38 ± 2.86*	23.2 ± 1.77*	1.28 ± 0.08	0.40 ± 0.03*
<i>D. lotus</i> extract 150	39.9 ± 1.21*	25.1 ± 1.33*	1.23 ± 0.09	0.33 ± 0.06*#
<i>D. lotus</i> extract 300	36.7 ± 2.33*#	22.8 ± 1.24*#	1.19 ± 0.07*#	0.36 ± 0.09*#

One-way ANOVA, significant at P-value ≤0.05; AST: aspartate aminotransferase, ALT: alanine aminotransferase.; # Compared to silymarin treatment group; * Significantly different from control group

from many diseases [28].

The result showed that total phenolic content of the aerial parts of *Diospyros lotus L.* was 20.5 mg gallic acid equivalent / g of the dry plant materials, while the total flavonoid was 17.1 mg quercetin equivalent (QE)/g of the dry plant materials.

3.3. Proximate Analysis

Table 2 showed the percentages of moisture content, crude fiber total ash, water soluble ash and acid-insoluble ash of the aerial parts of *Diospyros lotus L.*. From these results, it could be concluded that, these constants could be used as criteria for the purity of the in the aerial parts of *Diospyros lotus L.*

Table 2. Percentages of certain pharmacopoeial constants of the aerial parts of *Diospyros lotus L.*

Pharmacopoeial constants	The aerial parts of <i>Diospyros lotus L.</i>
% Moisture	7.23
% Crude fibre	8.62
% Total Ash	2.31
% Water-soluble ash	1.14
% Acid-insoluble ash	1.15

3.4. Acute Toxicity Study

No symptoms of mortality or morbidity were reported by oral administration of 70% methanol extract of *Diospyros lotus L.* aerial parts in doses up to 5 g/kg. This result stated that the tested plant is safe for use.

3.5. Hepato-nephro Protective Effects

Liver function was estimated by measuring the activity of ALT and AST. These enzymes are normally located in the cytosol of hepatocytes. The type and extent of hepatocellular damage can be detected when these enzymes are released in the plasma and increased their activity [1].

Liver enzymes significantly decreased in groups treated with 70% methanol extract of *Diospyros lotus L.* aerial parts (at both doses) comparing with control value and silymarin groups in dose dependant manner. As well as, ALT and AST significantly decreased in groups treated with silymarin (Table 3).

Creatinine serum concentration and uric acid level were decreased significantly in groups treated with the two doses of *Diospyros lotus* extract comparing with the control or silymarin groups. (Table 3).

Acute pathological changes in the liver of experimental

animals were induced by carbon tetrachloride administration

which were detected by the marked increase in serum ALT and AST activities. So liver enzymes values significantly elevated in CCL₄-toxicated rats than the other CCL₄toxicated treated groups. The result showed that the two dose levels of *Diospyros lotus* L. aerial parts extract resulted significant reduction in the elevated ALT and AST levels caused by CCL₄ in dose dependant manner as compared to control and silymarin treated group (Table 4). CCL₄ intoxication resulted in significant increasing inserum uric acid and creatinine

levels. Treated groups with the two dose levels of *Diospyros lotus* extract significantly reduced creatinine value, but still lower than the reduction due to silymarin treatment (Table 4). The uric acid level was significantly elevated by CCL₄-treatment, however it was significantly decreased by pretreating with silymarin. The uric acid value wassignificantly reduced in groups treated withthe two dose levels of *Diospyros lotus* extract in a dose dependent manner compared with CCL₄ or silymarin.

Table 4. Effect of 70% methanol extract of *Diospyros lotus* L. aerial parts on kidney function and liver enzymes and in serum of hepatic damage rats.

Groups	AST	ALT	Uric acid	Creatinine
CCL ₄	90.4 ± 2.2	48.3 ± 1.31	2.62 ± 0.03	1.34 ± 0.15
Silymarin 50+CCL ₄	69.3 ± 1.78*	27.5 ± 1.22*	1.94 ± 0.06*	0.78±0.16*
<i>D. lotus</i> extract 150+CCL ₄	67.3 ± 0.55*	39.3 ± 1.34*#	1.85 ± 0.06*#	0.94 ± 0.14*#
<i>D. lotus</i> extract 300+CCL ₄	63.8 ± 0.35*#	35.9 ± 0.65*#	1.79 ± 0.07*#	0.89 ± 0.12*#

One-way ANOVA test, significant at P-value ≤0.05: CCL₄: carbon tetra chloride, AST: aspartate aminotransferase; ALT: alanine aminotransferase,* compared to CCL₄- treatment group; # compared to Silymarin treatment group.

So we conclude that all treatment groups had hepatoprotective and renal function protective effects in both normal and hepatotoxic groups. This biological activity may be due to its free radical scavenging effect as the plant is reported to have strong antioxidant activity (17). Moreover, preliminary phytochemical study revealed the presence of carbohydrates and/or glycosides, tannins, flavonoids, and unsaturated sterols and/or triterpenes as major active constituents. Furthermore, phenolics contents of some medicinal plants play a vital role in liver homogenates [28]. Therefore the protective effect of plant extract against CCl₄ hepatotoxicity may be attributed to the presence of these biologically active constituents, which also play an important role in its antioxidant activity.

4. Conclusion

This study aims to study the protective effect of 70% methanolic extract of *Diospyros lotus* L. aerial parts against carbon tetrachloride-induced hepato and nephro-toxicity in rats. The results showed that the extract of *Diospyros lotus* L. aerial parts has a significant protective effect against acute hepato-toxicity and nephro-toxicity induced by CCl₄ in rats, this effect may be due to its free radical scavenging effect. Therefore, this study aims to use the methanolic extract of the aerial part of the plant as a safe hepato-nephro protective drug.

Conflicts of Interest

The authors declare that they have no competing interests.

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