View metadata, citation and similar papers at core.ac.uk

brought to you by CORE

provided by University of Groningen

University of Groningen

Hepatoprotective and Antioxidant Activities of Tribulus Terrestris

Harraz, Fathalla M; Ghazy, Nabila M; Hammoda, Hala M; Nafeaa, Abeer A.; Abdallah, Ingy I.

Published in:
Journal of Physiology and Pharmacology Advances

DOI:
10.5455/jppa.20151111080318

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2015

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):
Harraz, F. M., Ghazy, N. M., Hammoda, H. M., Nafeaa, A. A., & Abdallah, I. I. (2015). Hepatoprotective and Antioxidant Activities of Tribulus Terrestris. Journal of Physiology and Pharmacology Advances, 5(11), 787-794. https://doi.org/10.5455/jppa.20151111080318

Copyright
Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

Take-down policy
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): http://www.rug.nl/research/portal. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Download date: 12-11-2019

JOURNAL OF PHYSIOLOGY AND PHARMACOLOGY ADVANCES

Hepatoprotective and Antioxidant Activities of *Tribulus Terrestris*

Harraz F. M., Ghazy N. M., Hammoda H. M., Nafeaa A. A. and Abdallah I. I.

J Phys Pharm Adv 2015, 5(11): 787-794

DOI: 10.5455/jppa.20151111080318

Online version is available on: www.grjournals.com

Original Article

Hepatoprotective and Antioxidant Activities of *Tribulus Terrestris*

[1]Harraz F. M., [1]Ghazy N. M., [1]Hammoda H. M., [*2]Nafeaa A. A. and [1]Abdallah I. I.

[1] Department of Pharmacognosy, Faculty of Pharmacy, Alexandria University, Egypt.

[2] Department of Physiology, Faculty of Veterinary Medicine, Banha University, Egypt.

Abstract

Tribulus terrestris L. has been used in folk medicine throughout history. The present study examined the acute toxicity of the total ethanolic extract of *T. Terrestris* followed by investigation of the hepatoprotective activity of the total ethanolic extract and different fractions of the aerial parts of the plant compared to silymarin against carbon tetrachloride- induced hepatic damage in rats. In addition, *in vivo* antioxidant activity was examined and linked to the previous *in vitro* DPPH free radical scavenging activity investigation. This study established the plant's safety and the hepatoprotective effect of the total ethanolic extract of the aerial parts of the plant and its different fractions due to significant decrease in CCl_4- induced rise in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin in rats. Treatment with the ethyl acetate fraction significantly reduced oxidative stress in CCl_4- intoxicated rats, as evident by a decrease inmalondialdehyde (MDA) content associated with elevation of hepatic reduced glutathione (GSH) content and superoxide dismutase (SOD) activity. Hence, this hepatoprotective effect could be due to the antioxidant activity of the plant which is mainly imparted by the two major di-*p*-coumaroylquinic acid derivatives isolated from the ethyl acetate fraction.

Keywords: *Tribulus terrestris* L., carbon tetrachloride; hepatoprotective activity, antioxidant activity, silymarin, acute toxicity study.

[*] Corresponding author: Department of Physiology, Faculty of Veterinary Medicine, Banha University, Egypt.

Received on: 04 Sep 2015
Revised on: 14 Sep 2015
Accepted on: 11 Nov 2015
Online Published on: 30 Nov 2015

Introduction

Liver plays a vital role in transforming and clearing chemicals, so it is susceptible to the toxicity induced by these agents (Kashaw *et al.,* 2011). Studies indicate that, oxidative stress might be a key originating factor in the pathogenesis of liver diseases including drug- induced hepatic damage. Over production of free radicals is toxic to hepatocytes and triggers reactive oxygen species (ROS) mediated cascade causing hepatocyte death (Kaplowitz, 2000; Bissel *et al.,* 2001; Jaeschke *et al.,* 2002). Accordingly, the need for reliable hepatoprotective agents is crucial. Nature has afforded some plants with the ability to prevent and cure hepatic disturbances. Thus, medicinal plants especially those rich in antioxidants may serve as a vital source for the development of effective therapy to combat liver problems (Kashaw *et al.,* 2011).

Tribulus terrestris L. is a flowering plant native to warm temperate and tropical regions. It has been used in folk medicine throughout history for a variety of conditions such as hypertension, inflammation and sexual dysfunction (Vesilada *et al.,* 1995). In ethno- botanical claims, the fruits of *T. terrestris* have been used in China for liver and eye diseases (Li *et al.,* 1998), as well as, the hot water extract of the dried seeds which have been taken orally for liver diseases (Ross, 2005). Also, *T. terrestris* has been reported in Indian traditional medicine to be effective in treating jaundice (Pushpangadan and Atal, 1984).

The aim of the present study is to establish the ethno- botanical claims by evaluating the hepatoprotective activity of the total ethanolic extract and different fractions of the aerial parts of the plant against carbon tetrachloride- induced hepatic damage in rats. Also, we attempt to understand the cause of the hepatoprotective activity by evaluating the *in vivo* antioxidant activity and linking the results to the previously examined *in vitro* DPPH free radical scavenging activity (Hammoda, *et al.,* 2013) in order to determine the main compound(s) responsible for the antioxidant and hepatoprotective effects of the plant. Moreover, acute toxicity study was carried out to ensure the safety of the plant.

Materials and Methods

Chemicals and Reagents

Thiobarbituric acid (TBA) and 5, 5`-dithiobis-2-nitrobenzoic acid (DTNB) were bought from El Gomhoria Company, Egypt. Silymarin was obtained from Xiamen Forever Green Source Biochem. Tech. Company, China. Solvents used in this work; petroleum ether (40-60 °C), chloroform, ethyl acetate, *n*-butanol and ethanol were of analytical grade, purchased from Sigma-Aldrich (St. Louis, MO, USA).

Plant Material and Extraction

Tribulus terrestris L. was collected in August and September 2013, growing wildly on train railway and along the Mahmudiya Canal, Alexandria, Egypt. The plant material was identified by Prof. Dr. Salama Mohamed El-Dareer (Department of Botany and Microbiology, Faculty of Science, Alexandria University, Egypt). A voucher specimen (TT206) has been deposited in the Pharmacognosy Department, Faculty of Pharmacy, Alexandria University, Egypt.

Air dried powdered aerial parts (4 kg) of *T. terrestris* were extracted twice at room temperature (each for 7 days using 10 L 95% ethanol). The solvent was distilled off under reduced pressure to yield 400 g of total dry ethanolic extract. Three hundred and fifty gram of this dry extract was added to a mixture of water and ethyl alcohol (8:2). The hydroalcoholic phase (600 ml) was partitioned successively with chloroform, ethyl acetate and *n*-butanol to yield 18 g, 10 g and 15 g, respectively, which were separately dissolved in 0.3% sodium carboxymethylcellulose (CMC-Na) for oral administration to experimental animals.

Extraction yield (%) = (weight of the final dried extract x 100) / (weight of the original sample).

Extraction yield of ethanol = (400/4000) x 100 = 10%

Extraction yield of chloroform = (18/350) x 100 = 5%

Extraction yield of ethyl acetate = (10/350) x 100 = 3%

Extraction yield of ethanol = (15/350) x 100 = 4.5%

Animals

Male Wister rats weighing between 180 and 200 g were used in this study. Rats were kept at a controlled temperature of 23±2°C, relative humidity of 60-70% and a light regime of 12 hr light: 12 hr dark (lights on at 6:00 a.m.). Animals were quarantined and acclimatized for 7 days prior to the initiation of the study; animals were kept in groups of six and were given a well-balanced pellet diet ***and tap water ad libitum. The use of animals in our*** study is conformed to the guidelines and bioethics of the Egyptian Scientific Research Academy that coincide well with the U.S. Department of Agriculture through the Animal Welfare Act (7USC 2131) 1985 and Animal Welfare Standards incorporated in 9 CFR Part 3, 1991.

Acute Toxicity Study

Thirty-six male Wister rats were randomly assigned to six groups of six rats each. Five groups then received orally one of the following doses; 100, 200, 300, 500 or 800 mg/kg body weight of the total ethanolic extract dissolved in 0.3% sodium carboxymethylcellulose (CMC-Na). The control group (group 6) received 0.3% CMC-Na in distilled water (1ml/kg body weight, orally.). The groups were returned to their home cages, and provided with food and water *ad libitum.* Animals were observed for signs of toxicity and mortality for 72 hr(Litchfield and Wilcoxon, 1949).

Experimental Design for Hepatoprotective and in Vivo Antioxidant Study

Seventy-two male Wister rats were randomly assigned to twelve experimental groups (n=6). Group 1 (negative control) rats were given 0.3% CMC-Na in distilled water (1.0 ml/kg body weight) orally for 14 consecutive days. On days 11 and 12 the animals were injected with corn oil (1.0 ml/kg body weight, intraperitoneal- Jain, *et al.,* 2008). Group 2 (positive control) rats were given 0.3% CMC-Na in distilled water (1.0 ml/kg body weight) orally for 14 consecutive days. On days 11 and 12 the animals were injected with fresh mixture of equal volumes of carbon tetrachloride and corn oil (2.0 ml/kg body weight, intraperitoneal- Huang *et al.,* 2010). Groups 3 and 4 rats received silymarin dissolved in 0.3% CMC-Na at a dose of 200 mg/kg orally as a reference treatment for 14 consecutive days (Huang, *et al.,* 2010). Groups 5 and 6 rats received the total ethanolic extract of *T. terrestris* dissolved in 0.3% CMC-Na at a dose of 500 mg/kg orally for 14 consecutive days. Groups 7 and 8 rats received the chloroform fraction of *T. terrestris* dissolved in 0.3% CMC-Na at a dose of 250 mg/kg orally for 14 consecutive days. Groups 9 and 10 rats received the ethyl acetate fraction of *T. terrestris* dissolved in 0.3% CMC-Na at a dose of 250 mg/kg orally for 14 consecutive days. Groups 11 and 12 rats received the *n*-butanol fraction of *T. terrestris* dissolved in 0.3% CMC-Na at a dose of 250 mg/kg orally for 14 consecutive days. On days 11 and 12 the animals in groups 4, 6, 8, 10 and 12 were injected with fresh mixture of equal volumes of carbon tetrachloride and corn oil (2.0 ml/kg body, intraperitoneal). Twenty-four hours after the last animal group treatment (day 15), the rats were anaesthetized by the mixture (alcohol: chloroform: ether, 1: 2: 3). Blood samples were collected from each rat in all groups by cardiac puncture (using 20 gauge needle and 5 ml syringe) in heparinized tubes. The plasma was separated and stored at -20 ºC for screening of plasma liver markers.

Biochemical Analysis of Plasma

The plasma levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined according to the method described by Reitman and Frankel, (1957), total bilirubin by the method of Malloy and Evelyn (1937) and Tietz (1976), total protein by the method of Gornall *et al.,* (1949) and albumin by the method of Dumas *et al.,* (1997). Kits for determination of total proteins, albumin and transaminases activity were purchased from Bio Diagnostic Research office (Dokii, Giza, Egypt). The kit for determination of total bilirubin was bought from Diamond Diagnostics, El Gomhoria Company, Egypt.

Hepatoprotection Percentage

The hepatoprotection percentages of silymarin, total ethanolic extract of *T. terrestris* and its fractions (% H) were calculated for each biochemical parameter separately using the following equation (Wakchaure, *et al.,* 2011).

$$\% H = \{1-[(T-N)/(C-N)]\} \times 100$$

T = mean value of treatment group.
C = mean value of the positive control group.
N= mean value of the negative control group.

Moreover, total hepatoprotection percentage was compared to silymarin as follows: {(sum of % H of the biochemical parameters of each extract or fraction / sum of % H of the biochemical parameters of silymarin) × 100}.

Estimation of Hepatic Antioxidant Activity

After collection of blood samples, animals of groups 1, 2, 6 and 10 were sacrificed; livers were rapidly isolated and rinsed with ice cold saline. Tissue was then trimmed, washed in cold saline, blotted dry and immediately held on ice. Portions of the tissue were weighed (1.0g) and homogenized with 9 volumes of ice cold 0.05M phosphate buffer at pH 7.4, using electrical tissue homogenizer. Cell debris was removed by centrifugation at 12,000 r.p.m. (4°C) for 20 min. to collect supernatants for determination of malondialdehyde (MDA) concentration (Draper and Hadley, 1990), reduced glutathione (GSH) content (Chanarin, 1989) and superoxide dismutase (SOD) activity (Giannopolitis and Ries, 1977).

Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Differences between means in different groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's test and *P* value of 0.05 or less was considered significant using the statistical analysis system (SPSS, 1996).

Results

Acute Toxicity Study

Oral administration of the total ethanolic extract of *T. terrestris* in doses up to 800 mg/Kg body weight showed no signs of toxicity or mortality in rats.

Effects of Total Ethanolic Extract and Fractions on AST, ALT, Total Bilirubin, Albumin and Total Protein

The tested total ethanolic extract and different fractions of *T. terrestris* showed no effect on serum biochemical parameters in normal rats (Table 1). On the other hand, the CCl_4 intoxication significantly elevated the bilirubin content and transaminases (AST and ALT) activity as compared to the negative control group. Course treatment with *T. Terrestris* ethanolic extract and its fractions significantly decreased serum AST, ALT and bilirubin in rats with CCl_4- induced hepatotoxicity, moreover, the total ethanolic extract significantly elevated the level of serum albumin as shown in Table 1.

Table 1: Effects of silymarin, total ethanolic extract and different fractions of *T. terrestris* on liver function markers in normal and CCl_4 - intoxicated rats.

Group	Treatment	AST (U/ml)	ALT (U/ml)	Billirubin (mg/dL)	Albumin (g/L)	Total protein (g/L)
1	Negative control	53.00±1.90^b	25.66±2.32^b	0.59±0.01^b	36.87±0.69^b	57.15±1.05^b
2	Positive control (CCl_4)	203.20±10.07^a	178.80±18.44^a	1.10±0.06^a	34.56±0.47^b	57.49±1.06^b
3	Silymarin	57.67±2.38^b	30.00±1.73^b	0.65±0.04^b	36.27±1.35^b	55.47±1.42^b
4	Silymarin + CCl_4	53.80±3.50^b	39.60±3.14^b	0.67±0.05^b	36.57±0.58^b	55.73±1.49^b
5	Ethanolic extract	59.50±2.63^b	28.17±1.99^b	0.69±0.05^b	38.10±1.18^a	57.68±1.32^b
6	Ethanolic extract + CCl_4	58.17±2.99^b	34.17±2.07^b	0.69±0.05^b	38.19±0.38^a	55.20±0.82^b
7	Chloroform fraction	52.80±1.50^b	25.20±1.53^b	0.58±0.03^b	36.02±0.31^b	62.33±1.64^a
8	Chloroform fraction + CCl_4	56.17±2.81^b	33.67±2.50^b	0.55±0.02^b	35.31±1.30^b	55.57±2.35^b
9	Ethyl acetate fraction	55.33±2.14^b	26.17±1.96^b	0.62±0.03^b	35.06±1.48^b	55.31±0.60^b
10	Ethyl acetate fraction + CCl_4	65.20±3.79^b	30.20±1.53^b	0.75±0.03^b	36.95±0.66^b	53.60±0.84^c
11	*n*-Butanol fraction	48.33±1.98^b	26.00±0.93^b	0.70±0.05^b	39.29±1.03^a	53.78±0.91^c

12	*n*-Butanol fraction + CCl_4	62.80±2.82[b]	30.40±2.25[b]	0.80±0.05[ab]	35.32±0.67[b]	58.53±1.85[b]

Values are presented as means± S.D., n=6 in each group.
[a] Significantly different compared to the negative control group ($P<0.05$).
[b] Significantly different compared to the positive control group ($P<0.05$).

Hepatoprotection Percentage

Hepatoprotection percentage (% H) indicated that the total ethanolic extract of *T. terrestris* at a dose of 500 mg/kg has the highest hepatoprotective effect (99.07%) compared to silymarin followed closely by the ethyl acetate fraction at a dose of 250 mg/kg (97.90%) as shown in Table 2.

Table 2: Hepatoprotection percentages of the total ethanolic extract and different fractions of *T. terrestris* compared to silymarin following CCl_4 - induced hepatotoxicity in rats.

	Silymarin	Ethanolic extract	Chloroform fraction	Ethyl acetate fraction	*n*-Butanol fraction
AST	99.47	96.56	91.90	91.88	93.48
ALT	90.90	95.24	94.77	97.04	96.90
Bilirubin	84.31	80.39	92.16	68.62	58.82
Albumin	87.01	86.15	32.50	96.54	32.90
Total % H	361.69	358.34	311.33	354.08	282.10
% H compared to silymarin	100	99.07	86.08	97.90	77.99

Effects of total ethanolic extract and Ethyl Acetate Fraction on MDA, GSH and SOD

CCl_4 administration increased MDA concentration and decreased GSH content and SOD activity markedly compared with the negative control rats, suggesting that lipid peroxidation was a result of CCl_4 poisoning. Treatment with the total ethanolic extract and ethyl acetate fraction of *T. terrestris* decreased MDA concentration and restored SOD activity and GSH content effectively relative to CCl_4 treated rats as shown in Table 3. The ethyl acetate fraction significantly decreased MDA concentration to 0.43±0.039 nmol/mg nearly restoring it to its original concentration in the negative control group (0.36±0.031 nmol/mg), while the total ethanolic extract was less effective (0.55±0.027 nmol/mg). Both the total ethanolic extract and the ethyl acetate fraction restored the concentration of GSH content and even elevated its concentration compared to the negative control group. Also, both fractions partially improved SOD activity but could not restore it to its original state.

Table 3: Effects of the total ethanolic extract and ethyl acetate fraction of *T. terrestris* on MDA, GSH and SOD following CCl_4 - induced hepatotoxicity in rats.

Group	Treatment	MDA (nmol/mg)	SOD (U/mg)	GSH (µMol/mg)
1	Negative control	0.360±0.031 [b]	0.057±0.005 [a]	3.770±0.230 [c]
2	Positive control (CCl_4)	0.770± 0.065[a]	0.042±0.004[b]	1.880±0.080[c]
6	Ethanolic extract + CCl_4	0.550±0.027[b]	0.051±0.019[ab]	6.650±0.170[b,b]
10	Ethyl acetate fraction + CCl_4	0.430±0.039[b]	0.048±0.035 [ab]	9.430±0.190[a,b]

Values are presented as means± S.D., n=6 in each group.
[a] Significantly different compared to the negative control group ($P<0.05$).
[b] Significantly different compared to the positive control group ($P<0.05$).

Discussion

In the present experiment, acute toxicity study proved the safety of the plant extract in doses up to 800 mg/kg. The total ethanolic extract and different fractions of the aerial parts of *T. terrestris* were evaluated for the hepatoprotective activity using CCl_4 induced hepatotoxicity model in rats to find out the therapeutically more efficacious extract or fraction. In addition, an attempt was made to find out the correlation between antioxidant and

hepatoprotective activities in order to determine the secondary metabolites responsible for these activities. The first part of the study was designed to evaluate the competency of the total ethanolic extract and different fractions of the aerial parts of *T. terrestris* in preventing CCl_4- induced hepatic damage. Liver damage induced by CCl_4 is a commonly used model for the screening of hepatoprotective drugs. The rise in serum levels of AST and ALT has been attributed to the damaged structural integrity of the liver, because they are cytoplasmic in location and released into the circulation subsequent to cellular damage. The rise in the level of serum bilirubin is the most sensitive and confirms the intensity of jaundice (Krishna Mohan *et al.,* 2007; Atta *et al.,* 2010).

Hence, treatment of rats in this study with hepatotoxic CCl_4 resulted in severe damage of hepatocytes as indicated by the significant increase of serum transaminases (AST and ALT). Severe jaundice and hepatic cell injury were indicated by increased level of serum bilirubin.

Pretreatment of rats with the total ethanolic extract of the aerial parts of *T. terrestris* at a dose of 500 mg/kg induced a statistically significant decrease in the levels of AST, ALT and bilirubin, as well as, a significant increase in the level of serum albumin where stimulation of protein synthesis has been reported as a contributory hepatoprotective mechanism as it accelerates the regeneration process and the production of liver cells (Krishna Mohan *et al.,* 2007;Atta *et al.,* 2010).

The hepatoprotective activities of the different fractions of the ethanolic extract at a dose of 250 mg/Kg were also studied. The results revealed that the chloroform, ethyl acetate and *n*-butanol fractions induced a statistically significant decrease in the level of serum liver biochemical parameters to different extents.

The hepatoprotection percentage of the total ethanolic extract and different fractions was calculated compared to silymarin as shown in Table 2, aiming to identify which sample has the highest hepatoprotective effect. The results revealed that the total ethanolic extract, chloroform, ethyl acetate and *n*-butanol fractions caused 99.07, 86.08, 97.90 and 77.99 % protection, respectively. The total ethanolic extract at a dose of 500 mg/kg showed the highest hepatoprotective activity, closely followed by the ethyl acetate fraction at a dose of 250 mg/kg.

It has been reported in literature that one of the main causes of CCl_4- induced liver injury is lipid peroxidation induced and accelerated by oxidative stress due to free radical derivatives of CCl_4. CCl_4 is metabolically activated by cytochrome P450 dependent monooxygenase leading to the production of trichloromethyl radical ($CCl^{\cdot}_3$). The $CCl^{\cdot}_3$ radicals attack polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides contributing to hepatic damage. The lipid peroxidation is accelerated when free radicals are formed as the results of losing a hydrogen atom from the double bond in the structure of unsaturated fatty acids. Scavenging of free radicals is one of the major antioxidation mechanisms known to inhibit the chain reaction of lipid peroxidation (Ashok Shenoy *et al.,* 2001; Jain *et al.,* 2008; Huang *et al.,* 2010; Desai *et al.,* 2012).

Accordingly, the second part of the study was carried out as an attempt to elucidate the probable cause of the hepatoprotective activity and the role of the plant in reducing liver damage caused by oxidative stress. The *in vivo* antioxidant activity was investigated where the total ethanolic extract and ethyl acetate fraction were chosen as good candidates for this part of the study owing to their highest hepatoprotective activity. The *in vivo* antioxidant activity was assessed according to the effect of the candidates on superoxide dismutase (SOD) enzyme activity, malondialdehyde (MDA) and reduced glutathione (GSH) concentrations.

SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady state level of O_2^-. CCl_4 administration significantly reduced SOD activity compared to the negative control group. This reduction may be due to enhanced reactive oxygen radicals (superoxide and hydrogen peroxide) generation, which in turn overwhelmed the activity of the enzyme (Akinloye and Olaniyi, 2011). The first evidence of the antioxidant activity of the total ethanolic extract and ethyl acetate fraction was the partial improvement of SOD activity.

GSH, a pervasive tripeptide, is a major thiol in living organisms. It is present at a large concentration in the liver and plays a major role in

the elimination and detoxification of many exogenous toxicants. Also, it has a central role in the non-enzymatic antioxidant defense system where GSH metabolism is important in quenching the reactive intermediates and radical species generated during oxidative stress so coordinating the body's antioxidant defense processes. Thus, disruption of GSH status of a biological system can lead to serious consequences (Lee *et al.,* 2000;Mohammad *et al.,* 2011). In this study, reduction of GSH stores of liver tissue in CCl_4 intoxicated rats suggested that oxidative stress due to free radical damage is one of the possible mechanisms in the pathophysiology of CCl_4-induced hepatopathy. On administration of the total ethanolic extract and ethyl acetate fraction of the plant, GSH levels increased markedly. The ethyl acetate fraction showed higher increase in GSH content (9.43±0.19 µMol/mg) compared to the total ethanolic extract (6.650±0.17 µMol/mg).

MDA is the last product of lipid breakdown caused by oxidative stress. The increase in MDA levels in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals (Ashok Shenoy *et al.,* 2001;Wu *et al.,* 2007). CCl_4 intoxication increased the level of MDA compared to the negative control group. Pretreatment with the total ethanolic extract and the ethyl acetate fraction significantly reversed this increase. Hence, it is possible that the hepatoprotection of *T. terrestris* is due to its antioxidant effect. Moreover, it is apparent that the ethyl acetate fraction showed more potent effect than the total ethanolic extract.

Results of the *in vivo* antioxidant investigation were correlated to the former study of *in vitro* DPPH free radical scavenging activity, where both revealed that the ethyl acetate fraction possess the highest antioxidant effect. These findings prompted the phytochemical investigation of the most active fraction (ethyl acetate) leading to the isolation of two major phenolic compounds (4,5-di-*p-cis*-coumaroylquinic acid and 4,5-di-*p-trans*-coumaroylquinic acid) showing IC_{50} very close to that of ascorbic acid proving their responsibility for the antioxidant effect (Hammoda *et al.,* 2013).

In conclusion, the data of this study provided convincing evidence that the ethyl acetate fraction of the aerial parts of *T. terrestris* has a significant hepatoprotective effect on acute liver injuries induced by CCl_4. It can be postulated that this hepatoprotective effect may be due to its antioxidant activity proved by the *in vivo* and *in vitro* studies. Two major phenolic compounds (4, 5-di-*p-cis*-coumaroylquinic acid and 4, 5-di-*p-trans*-coumaroylquinic acid) isolated from the bioactive ethyl acetate fraction can be identified as the secondary metabolites responsible for the antioxidant effect and consequently the hepatoprotective activity of the plant.

References

Akinloye OA, Olaniyi MO (2011). Hepatoprotective effect of Cajanus cajan on tissue defense system in D-galactosamine-induced hepatitis in rats. Turk. J. Biochem., 36: 237-41.

Ashok Shenoy K, Somayaji SN, Bairy KL (2001). Hepatoprotective effects of Ginkgo biloba against carbon tetrachloride induced hepatic injury in rats. Indian J. Pharmacol., 33: 260-6.

Atta AH, Elkoly TA, Mouneir SM, Kamel G, Alwabel NA, Zaher S (2010). Hepatoprotective effect of methanol extracts of Zingiber officinale and Cichorium intybus. Indian J. Pharm. Sci., 72: 564-70.

Bissell DM, Gores GJ, Laskin DL, Hoofnagle JH (2001). Drug-induced liver injury: mechanisms and test systems. Hepatol., 33: 1009-13.

Chanarin I (1989). Laboratory Haematology: An Account of Laboratory Techniques. Churchill Living stone, New York.

Desai SN, Patel DK, Devkar RV, Patel PV, Ramachandran AV (2012). Hepatoprotective potential of polyphenol rich extract of Murraya koenigii L.: an in vivo study. Food Chem. Toxicol., 50: 310-4.

Draper HH, Hadley M (1990). Malondialdehyde determination as index of lipid peroxidation. Meth. Enzymol., 186: 421-31.

Dumas BT, Watson WA, Biggs HG (1997). Albumin standards and the measurement of serum albumin with bromocresol green. Clin. Chim. Acta., 258: 21-30.

Giannopolitis CN, Ries SK (1977). Superoxide dismutases: I. Occurrence in higher plants. Plant Physiol., 59: 309-14.

Gornall AG, Bardawill CJ, David MM (1949). Determination of serum proteins by means of the biuret reaction. J. Biol. Chem., 177: 751-66.

Hammoda HM, Ghazy NM, Harraz FM, Radwan MM, ElSohly MA, Abdallah II (2013). Chemical constituents from Tribulus terrestris and screening of their antioxidant activity. Phytochem., 92: 153-9.

Huang B, Ban XQ, He JS, Tong J, Tian J, Wang YW (2010). Hepatoprotective and antioxidant activity of ethanolic extracts of edible lotus (Nelumbo nucifera Gaertn.) leaves. Food Chem., 120: 873-8.

Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ (2002). Mechanisms of hepatotoxicity. Toxicol Sci., 65: 166-76.

Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB (2008). Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioicaRoxb. leaves. J. Ethnopharmacol., 115: 61-6.

Kaplowitz N (2000). Mechanisms of liver cell injury. J. Hepatol., 32: 39-47.

Kashaw V, Nema AK, Agarwal A (2011). Hepatoprotective prospective of herbal drugs and their vesicular carriers-A review. IJRPBS., 2: 360-74.

Krishna Mohan G, Pallavi E, Ravi Kumar B, Ramesh M, Venkatesh S (2007). Hepatoprotective activity of Ficus carica Linn. leaf extract against carbon tetrachloride-induced hepatotoxicity in rats. DARU., 15: 162-6.

Lee MK, Yeo H, Kim J, kim YC (2000). Protection of rat hepatocytes exposed to CCl_4 in-vitro by cynandione A, a biacetophenone from Cynanchum wilfordii. J. Pharm. Pharmacol., 52: 341-5.

Li JX, Shi Q, Xiong QB, Prasain JK, Tezuka Y, Hareyama T, *et al.,* Tribulusamide A and B (1998). New hepatoprotective lignanamides from the fruits of Tribulus terrestris: indications of cytoprotective activity in murine hepatocyte culture. Planta Med., 64: 628-31.

Litchfield JT , Wilcoxon FA (1949). Simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.

Malloy HT, Evelyn KA (1937). The determination of bilirubin with the photoelectric colorimeter. J. Biol. Chem., 119: 481-90.

Mohammad R, Daryoush M, Ali R, Yousef D, Mehrdad N (2011). Attenuation of oxidative stress of hepatic tissue by ethanolic extract of saffron (dried stigmas of Crocus sativus L.) in streptozotocin (STZ)-induced diabetic rats. Afr. J. Pharm. Pharacol., 5: 2166-73.

Naik SR, Panda VS (2008). Hepatoprotective effect of Ginkgoselect Phytosome® in rifampicin induced liver injury in rats: Evidence of antioxidant activity. Fitoterapia. 79: 439-45.

Pushpangadan P, Atal CK (1984). Ethno-medico-botanical investigations in Kerala.I. Some primitive tribals of Western Ghats and their herbal medicine. J. Ethnopharmacol., 11: 59-77.

Reitman S, Frankel S (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.

Ross IA (2005). Medicinal Plants of the World: Chemical Constituents, Traditional and Modern Uses. Totowa New Jersey: Human Press.

Tietz NW (1976). Fundamentals of clinical chemistry. 2nd Ed. Philadelphia: Saunders WB, Co.

Wakchaure D, Jain D, Singhai AK, Somani R (2011). Hepatoprotective activity of Symplocos racemosa bark on carbon tetrachloride-induced hepatic damage in rats. J. Ayurveda Integr. Med., 2: 137-43.

Wu ZM, Wen T, Tan YF, Liu Y, Ren F, Wu H (2007). Effects of salvianolic acid A on oxidative stress and liver injury induced by carbon tetrachloride in rats. Basic Clin. Pharmacol. Toxicol., 100: 115-20.

Yesilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, (1995). Traditional medicine in Turkey.V. Folk medicine in the Inner Taurus Mountains. J. Ethnopharmacol., 46: 133-52.