

Mitigation of Alpha-Cypermethrin-Induced Hepatotoxicity in Rats by *Tribulus terrestris* Rich in Antioxidant Compounds

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Abstract

Tribulus terrestris is used traditionally as a medicinal herb to improve the sexual functions. However, its hepatoprotective effects against alpha-cypermethrin (an insecticide) hepatotoxic effects are still not fully elucidated. The present study investigates for the first time the hepatoprotective effect of the *T. terrestris* extract against alpha-cypermethrin induced liver toxicity in addition to its phytochemical composition and *in vitro* antioxidant activity. Several phenolic and flavonoids' compounds (ellagic and ferulic acid, hesperidin and quercetin) were screened in the *T. terrestris* extract by HPLC. The *T. terrestris* extract exhibited *in vitro* antioxidant activity using DPPH[•], ABTS^{•+}, and reducing power assays. The remarkable effects of the *T. terrestris* extract in the attenuation of hepatotoxicity induced by α -cypermethrin in rats are investigated for the first time in the current study. The administration of the *T. terrestris* extract decreased liver enzymes; alanine amino transferase (ALT), and aspartate amino transferase (AST). Also, it increased antioxidant; glutathione (GSH) and paraoxnase-1 (POX-1) enzyme, and decreased oxidant; malondialdehyde (MDA) and nitric oxide (NO). Furthermore, it decreased liver inflammatory markers; tumor necrosis factor alpha (TNF - α), adiponectin and lipocalin. The hepatoprotective effect of *T. terrestris* extract could be attributed to its ability to hunt free radicals and induce the antioxidant enzymes expression in addition to the down-regulation of pro-inflammatory markers in liver injuries.

Keywords: α -Cypermethrin, Hepatotoxicity, HPLC, Polyphenolic compounds, *Tribulus terrestris*.

1. Introduction

Pesticides are toxic chemicals, which contaminate the entire environment including air, soil, water, and have been detected in human and animal tissue samples all over the world (Carvalho, 2017). Prolonged exposure to toxic pesticides has shown harmful effect to the skin, eyes, liver, the gastrointestinal tract, kidneys, the reproductive system, nervous system, cardiovascular system, blood and the endocrine system (IPCS, 2010).

Liver is the main target organ for drugs, xenobiotics, and other toxic chemicals because of the liver's complex anatomical texture, different metabolic functions, and direct association with the gastrointestinal tract. Pesticides including cypermethrin are aggressive chemicals that cause liver damage including hepatic fibrosis, cirrhosis, steatosis and inflammation (Cataudella *et al.*, 2012).

Alpha Cypermethrin (α -CYP), a synthetic type II pyrethroid insecticide, is used extensively in different countries for pest control, because of its high influence against a wide range of insects and because of its low toxicity to mammals (Abdou *et al.*, 2012). Despite the low toxicity of α -CYP, its accumulation in various food chains

and persistence in different mammalian tissues including the liver, kidney, and the brain are the reasons behind its higher toxic levels (Yavasoglu *et al.*, 2006; Singh *et al.*, 2012). It induces mitochondrial dysfunction and oxidative stress especially lipid peroxidation in animal models, resulting in both an elevation of oxidative stress markers and a reduction in antioxidant activities (El-Demerdash, 2011). Moreover, Yavasoglu *et al.* (2006) reported that α -CYP treatment increased the apoptotic index in the liver of rats, and it might cause hazardous effects in different levels to non-target organisms.

Several studies have reported that the pretreatment with different plant extracts as a source for natural antioxidants can alleviate the side effects and toxicity of cypermethrin in rats (Sushma and Devasena, 2010; Abdou *et al.*, 2012).

Tribulus terrestris L. is an annual flowering creeping plant that belongs to the family Zygophyllaceae, which is native to the Mediterranean region. However, it grows widely in the warm regions of Africa, Asia, America, Europe, and Australia (Qureshi *et al.*, 2014). It is known by different Arabic names: Ders El-Agouz, Hasak, Qutiba and Al-Gutub (Al-Ali *et al.*, 2003), with some common names such as caltrop, goat head, puncture vine, bull's head, devil's thorn, and ground burr nut (Kostova and

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Dinchev, 2005). In Egypt, China, India, and other countries, it is used traditionally as a medicinal herb to improve sexual functions in both sexes (Kamenov *et al.*, 2017). Also, anticancer (Kim *et al.*, 2011), antibacterial (Soleimanpour *et al.*, 2015), antifungal (Zhang *et al.*, 2005), hypoglycemic (El-Shaibany *et al.*, 2015) effects and antioxidant activities (Hammoda *et al.*, 2013) of different extracts of *T. terrestris* have been reported. The phytochemical investigation of *T. terrestris* revealed that it contains different active compounds including saponins (Kostova and Dinchev, 2005; Hammoda *et al.*, 2013), alkaloids (Wu *et al.*, 1999), flavonoids and sterols (Ivanova *et al.*, 2011).

To the authors' knowledge, there are no reports on the hepatoprotective effect of *T. terrestris* especially against α -cypermethrin, an insecticide used widely in Egypt. Because of the previously reported hepatic free radical-mediated tissue damage and dysfunction effect of alpha-cypermethrin in rats, these findings intrigued authors to investigate the phytochemical composition of *T. terrestris* extract. In addition, its hepatoprotective effect against alpha-cypermethrin-induced liver injury in rats in the context of its antioxidant and anti-inflammatory properties were also assessed.

2. Materials and Methods

2.1. Materials

2.1.1. Chemicals

Alpha-cypermethrin (10 % EC) was purchased from KZ pesticides company, Egypt. ABTS⁺ (2, 2'-azino-bis (3-ethyl benzothiazoline - 6 - sulfonic acid)), Folin-Ciocalteu reagents, Gallic acid, quercetin, DPPH^{*} (2, 2-diphenyl-1-picrylhydrazyl), BHT: Butyl Hydroxy toluene and, potassium ferricyanide, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were directly purchased from (Sigma Chemical Co., St. Louis, MO, USA).

2.1.2. Plant Samples

Tribulus terrestris aerial parts were collected from National Research Centre farm in Giza, Egypt. They were air-dried and then milled by a mixer grinder to a fine powder to be used to prepare the extracts.

2.1.3. Animals

Forty male three-month old albino rats (weighting 150-170 g) were obtained from the breeding unit of the Toxicology and Forensic Medicine Department of the Faculty of Veterinary Medicine at Cairo University. The animals were housed in plastic cages, and were fed a standard laboratory diet and water *ad libitum*. All animal experiments were carried out in accordance with the guide of the care and use of laboratory animals published by the National Institute of Health (NIH Publication No.85-23, revised 1996), and revised by the animals' experiments local ethics committee at Cairo University, Egypt.

2.2. Methods

2.2.1. *Tribulus terrestris* Extraction

One kg of dried powder of *T. terrestris* was extracted with three liters of the solvent mixture of methanol:

acetone: H₂O (2:2:1, respectively), under shaking at 23±1 °C on an orbital shaker (Heidolph Unimax 2010, Germany) for forty-eight hours. The extract was filtered using Whatman No.1 filter paper. The plant residues were re-extracted twice with the same solvent mixture. The pooled filtrates were concentrated under vacuum at 40°C to dryness. The dried crude extract was re-dissolved in methanol for further phytochemical and antioxidant analysis, and was dissolved in distilled water for biological experiment. Unless noted otherwise, all extraction and subsequent characterization experiments were done using three replicates.

2.2.2. Phytochemical Analysis

2.2.2.1. Total Phenolic Content

The total phenolic (TP) content was determined by Folin Ciocalteu reagent assay as described by Singleton and Rossi (1965). A sample aliquot (1 mL) of the *T. terrestris* extract was added to a 25 mL volumetric flask, containing 9 mL of distilled water. One milliliter of Folin Ciocalteu phenol reagent was added to the mixture and shaken. After five minutes 10 mL of 7 % Na₂CO₃ solution were added to the mixture. The solution was diluted to 25 mL with distilled water and mixed using a magnetic stirrer. After incubation for ninety minutes at room temperature, the absorbance was measured at 750 nm with a spectrophotometer (Unicum UV 300, England) against prepared reagent as blank. The total phenolic content was expressed as mg Gallic acid equivalents (GAE)/g dry weight.

2.2.2.2. Total Flavonoid Content

The aluminum chloride method was used for the determination of the total flavonoid (TF) content (Zhishen *et al.*, 1999). One mL of the *T. terrestris* extract was added to a 10 mL volumetric flask, containing 4 mL of distilled water. To the flask, 0.3 mL 5 % NaNO₂ was added followed by the addition of 0.3 mL 10 % AlCl₃ after five minutes. After six minutes, 2 mL 1M NaOH were added, and the total volume was made up to 10 mL with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm using a spectrophotometer (Unicum UV 300, England). The total flavonoid content was expressed as mg quercetin equivalents (QE)/ g dry weight.

2.2.2.3. Identification of Phenolic Acids and Flavonoid Compounds

The dried crude extract of *T. terrestris* (10 mg) was dissolved in 2 mL methanol HPLC spectral grade by vortex mixing for fifteen minutes. The extract was filtrated through a 0.2µm Millipore membrane filter, and was set up to a known volume (2 mL). The phenolic and flavonoid compounds were identified by HPLC (Agilent Technologies 1100 series, Germany), equipped with a quaternary pump (G131A motendel). Separation was achieved on ODS reversed phase column (C18, 25×0.46 cm i.d. 5 µm, Netherlands). The injection volume (35 µL) was carried out with an auto sampling injector. The column temperature was maintained at 35°C. Gradient phenolic compounds' separation was carried out with an aqueous formic acid solution 0.1 % (A) and methanol (B) as a mobile phase at a flow rate of 0.3 mL/min following

the method of Goupy *et al.* (1999). In addition, the flavonoid compounds' separation was carried out with 50 mM H₃PO₄, pH 2.5 (solution A) and acetonitrile (solution B) as a mobile phase at a flow rate of 0.7 mL/min as described by Mattila *et al.* (2000). Elutes were monitored using a UV detector set at 280 nm for the phenolic acids, and at 330 nm for flavonoids. Chromatographic peaks were identified by comparing the retention times with the respective retention times of known standard reference material. Phenolic acids and flavonoid compounds' concentration were calculated by comparing its peak areas with the peak areas of used standards (with known concentration) based on the data analysis of Hewlett Packard software. Phenolic acids and flavonoid compounds were expressed as mg/g dry extract.

2.2.3. Antioxidant Activity

2.2.3.1. DPPH[•] Radical Scavenging Assay

The DPPH[•] (2, diphenyl-1-picryl hydrazyl) radical scavenging activity of the *T. terrestris* extract was determined by the method of Chu *et al.* (2000). The DPPH[•] (0.1 mM) in methyl alcohol was prepared and 0.5 mL of this solution was added to 1 mL of the *T. terrestris* extract at different concentrations (25, 50, 75, 100 µg/mL). The mixture was shaken vigorously, and was allowed to stand at room temperature in the dark for thirty minutes. Butyl hydroxytoluene (BHT, Sigma Aldrich, St. Louis, MO, USA) was used as positive control, whereas the negative control contained the entire reaction reagent minus the extract. Then the absorbance was measured at 515 nm against blank. The capacity to scavenge the DPPH[•] radical was calculated using the following equation:

$$\text{DPPH}^{\bullet} \text{ scavenging effect (Inhibition \%)} = ((A_c - A_s) / A_c) \times 100$$

Where A_c was the absorbance of the control reaction and A_s was the absorbance in the presence of the plant extracts. The results were expressed as IC₅₀ (the concentration (mg/mL) of the plant extract that scavenge 50 % of DPPH[•] radical).

2.2.3.2. Reducing Power

The reducing power was assayed as described by Kuda *et al.* (2005). One mL of the *T. terrestris* extract at different concentrations (25, 50, 75, 100 µg/mL) was mixed with 2.5 mL of phosphate buffer (50 mM, pH 7.0) and 2.5 mL of 1 % potassium ferricyanide. The mixture was then incubated at 50°C for twenty minutes. After the addition of 2.5 mL of trichloroacetic acid (10 %) to the mixture, centrifugation at 3000 rpm for ten minutes was performed. Finally, 1.25 mL from the supernatant was mixed with 1.25 mL of distilled water and 0.25 mL FeCl₃ solution (0.1 %, w/v). The absorbance was measured spectrophotometrically at 700 nm. BHT was used as positive control. The results were expressed as EC₅₀ (the concentration (mg/mL) of the plant extract that provided the reading of 0.5 absorbance at 700 nm).

2.2.3.3. ABTS^{•+} Antioxidant Assay

ABTS^{•+} assay was carried out according to Arnao *et al.* (2001). Briefly, ABTS^{•+} was dissolved in double distilled water to 7.4 mM concentration, and potassium persulphate was added to a concentration of 2.6 mM. The working solution was then prepared by mixing the two stock

solutions in equal quantities. They were allowed to react for 12-16 hours at room temperature in the dark. The solution was then diluted by mixing 1 mL of the ABTS^{•+} solution with 60 mL of methanol to obtain an absorbance of 1.1 ± 0.02 at 734 nm using the spectrophotometer. The *T. terrestris* extracts (150 µL) at different concentration (25, 50, 75, 100 µg/mL) were allowed to react with 2850 µL of the freshly prepared ABTS^{•+} solution for two hours in the dark at room temperature. Then the absorbance was recorded at 734 nm. Trolox was used as a positive control. ABTS^{•+} scavenging activity (%) was calculated using the equation:

$$\text{ABTS}^{\bullet+} \text{ scavenging \%} = ((A_0 - A_1) / A_0) \times 100$$

Where A₀ is the ABTS^{•+} absorbance of the control (the entire reaction reagent minus the sample), and A₁ is the ABTS^{•+} absorbance in the presence of the sample. The results were expressed as IC₅₀ (the concentration (mg/mL) of the plant extract that scavenge 50 % of ABTS^{•+} radical).

2.2.4. Biological Analysis

2.2.4.1. Estimation of Median Lethal Dose 50 (LD₅₀)

Twenty mature male rats were divided into four groups (five rats per group) then they were orally administered alpha-cypermethrin in four doses (14, 11.666, 9.722, and 8.101 mg/Kg BW); each group was administered only one dose one time and was monitored for fourteen days. Mortality was assessed and counted in the four groups. The median lethal dose LD₅₀ was determined according to the method of Weil (1952). The LD₅₀ dose was calculated as 10.65 mg/Kg BW by the end of fourteen days.

2.2.4.2. Experimental Design

Forty mature rats were randomly assigned into four equal groups group1 (Control), rats were kept as control group; group 2 (TT), rats were orally administered (100 mg of *T. terrestris* extract/kg BW); groups 3 (CYP), rats were orally administered 1/20 LD₅₀ of alpha-cypermethrin (0.533 mg/kg, BW); and group 4 (TT+CYP), rats were orally administered alpha-cypermethrin at a dose level of 0.533 mg/kg BW along with 100 mg of the *T. terrestris* extract /kg BW. Gastric intubation was used for the administration of both alpha-cypermethrin and the *T. terrestris* extract for sixty-five days.

2.2.4.3. Blood and Tissue Sampling

After the experimental period, blood was withdrawn from the retro-orbital venous plexus of the eye using capillary tubes. It was collected in dry clean tubes, and was centrifuged at 2,000 rpm for ten minutes using cooling centrifuge (Laborzentrifugen, 2k15, Sigma, Germany). Serum was separated and stored at -80°C for biochemical parameters estimations.

The rats were sacrificed by decapitation. Their livers were removed quickly and perfused with pH 7.4 iced phosphate-buffered saline (PBS) to remove blood cells, then blotted on filter paper and frozen at -80°C for biochemical parameters estimation.

Part of the frozen tissues were cut into small pieces and homogenized in 5 mL of cold buffer (0.5 g of Na₂HPO₄ and 0.7 g of NaH₂PO₄ in 500 mL deionized water) (pH 7.4) per gram tissue. After that, they were centrifuged at 4,000 rpm for fifteen minutes at 4°C using a cooling centrifuge, and the supernatant was removed for the

estimation of different biochemical parameters (Hussein *et al.*, 2011).

2.2.4.4. Biochemical Analysis

Serum alanine amino transferase (ALT) and aspartate amino transferase (AST) were determined using commercial kit purchased from Bio Med Diagnostics (Egypt) based on the method of Reitman and Frankel (1957). Liver homogenates were used for the estimation of MDA, GSH and NO levels according to the methods of Watanabe *et al.* (2001), Ellman (1959) and Moshage *et al.* (1995), respectively.

2.2.4.5. Determination of Serum Paraoxonase Activity

The aryl esterase activity of paraoxonase (POX-1) was measured spectrophotometrically in supernatants using phenyl acetate as a substrate. In this assay, aryl esterase/paraoxonase catalyzes the cleavage of phenyl acetate, resulting in phenol formation. The rate of phenol formation is measured by monitoring the increase in absorbance at 270 nm at 25 °C. The working reagent consisted of 20 mM of Tris/HCl buffer, pH 8.0, containing 1 mM of CaCl₂ and 4 mM of phenyl acetate, as the substrate. The samples diluted in a ratio of 1:3 in buffer were added, and the change in absorbance was recorded following a twenty- second lag time. Absorbance at 270 nm was taken every fifteen seconds for 120 seconds using a UV spectrophotometer (Hussein *et al.*, 2013).

2.2.4.6. Determination of Serum Adiponectin

Serum adiponectin was assayed by an enzyme-linked immunosorbent assay (ELISA) according to Watanabe *et al.* (2006); the kit was supplied by Orgenium Laboratories, Finland.

2.2.4.7. Determination of Serum Tumor Necrosis Factor Alpha (TNF - α)

TNF- α was determined by an enzyme amplified sensitivity immunoassay (EASIA) according to Aukrust *et al.* (1994); the kit was purchased from Biosource, Belgium.

2.2.4.8. Determination of Serum Lipocalin-2

Lipocalin-2 was estimated by ELISA using DRG rats Lipocalin-2 diagnostic kit provided by (DRG International, Inc., USA) according to the manufacturer's instructions.

2.2.5. Statistical Analysis

The data were statistically analyzed using SPSS statistical package, (release 16) for windows. Data were expressed as mean \pm SE. Differences between two groups were analyzed by student T test. Multiple comparisons were performed by one-way ANOVA tests.

3. Results

3.1. Total Phenolics and Total Flavonoids

The *T. terrestris* extract showed total phenolic and total flavonoid content of 14.48 \pm 0.16 mg GAE/g dry weight and of 3.39 \pm 0.08 mg quercetin equivalent/g dry weight, respectively (Table 1).

Table 1. Total phenolic (TP), total flavonoid (TF) and antioxidant activity (DPPH[•] scavenging, ABTS^{•+} scavenging and reducing powers) of *T. terrestris*.

Sample	TP (mg GAE/g)	TF (mg QE/g)	DPPH [•] IC ₅₀ (μ g/mL)	ABTS ^{•+} IC ₅₀ (μ g/mL)	Reducing power EC ₅₀ (μ g/mL)
<i>T. terrestris</i>	14.48 \pm 0.16	3.39 \pm 0.08	29.55 \pm 0.49	143.30 \pm 8.10	115.98 \pm 4.81
BHT	-	-	9.78 \pm 0.04	-	11.20 \pm 0.33
Trolox	-	-	-	16.38 \pm 0.43	-

Each assay was carried out in triplicate. Data expressed as mean value \pm SD. GAE, gallic acid equivalents. QE, quercetin equivalent. BHT, butylated hydroxytoluene.

3.2. HPLC Profile of Polyphenolic Compounds

The total content of phenolic and flavonoids has been reported previously in *T. terrestris*; however, the characterization of different phenolic and flavonoid compounds is still limited. HPLC screening of the *T. terrestris* extract in the present study revealed the presence of 45 polyphenolic compounds identified as twenty-three phenolic compounds and twenty-two flavonoid compounds (Table 2). In regard to the phenolic compounds, e-vanillic, oleuropen, ellagic, pyrogallol, and vanillic were the leading compounds detected in the highest concentration (28.82, 10.07, 3.12, 2.08, and 1.88 mg/g extract, respectively) of the *T. terrestris* extract in addition to other several phenolic acids, as presented in Table 2.

The major flavonoid compounds (Table 2) detected in the *T. terrestris* extract were hesperidin (73.96 mg/g extract), luteolin 6-arbinose 8-glucose (52.09 mg/g extract), kampferol 3, 2-p-comaroylglucose (19.35 mg/g extract), acacetin (6.65 mg/g extract), quercetrin (4.85 mg/g extract), and apigenin 6-glucose 8-rhamnose (2.10 mg/g extract) in addition to other several flavonoids listed in Table 2.

Table 2. HPLC profile of polyphenolic compounds of *T. terrestris*.

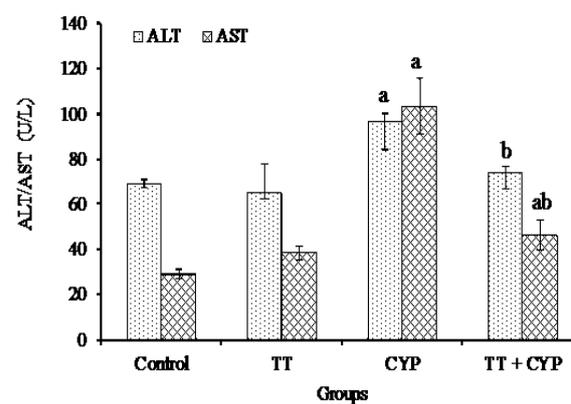
No.	Phenolic compounds	Concentration (mg/g, extract)	Flavonoid compounds	Concentration (mg/g, extract)
1	Pyrogallol	2.08	Luteolin 6-arbinose 8-glucose	52.09
2	Gallic acid	0.16	Luteolin 6-glucose 8-arbinose	1.31
3	4-Amino-Benzoic acid	0.74	Apigenin 6-arbinose 8-glucose	0.91
4	Protocatechuic	0.68	Apigenin 6-rhamnose 8-glucose	1.35
5	Catechein	0.49	Apigenin 6-glucose 8-rhamnose	2.10
6	Catechol	1.26	Luteolin 7-glucose	0.50
7	Epiucatachin	0.66	Narengin	1.08
8	<i>P</i> -OH-benzoic	0.93	Hesperidin	73.96
9	Caffeine	0.69	Rutin	1.08
10	Chlorogenic acid	0.46	Apigenin 7-O-neohespiroside	1.09
11	Vanillic acid	1.88	Kampferol 3,7-dirhamoside	1.21
12	Caffeic acid	0.73	Quercetrin	4.85
13	<i>p</i> - Coumaric acid	0.25	Rosmarinic	0.22
14	Ferulic acid	0.92	Quercetin	1.75
15	<i>e</i> -Vanillic acid	28.82	Naringenin	1.89
16	Resveratrol	0.55	Kampferol 3,2- <i>p</i> -comaroyl glucose	19.35
17	Oleuropen	10.07	Hespirtin	1.04
18	α - Coumaric acid	0.96	Kampferol	0.25
19	Ellagic acid	3.12	Rhamenetin	0.21
20	3,4,5-Methoxy-Cinnamic acid	1.02	Apigenin	0.18
21	Coumarin	0.37	Apigenin-7-glucose	0.18
22	Cinnamic acid	0.98	Acacetin	6.65
23	Salycilic acid	0.38	-	-

3.3. *In vitro* Antioxidant Activity

Due to the diversity of the antioxidant components in the plant extracts and their mechanism of action, three different assays (DPPH[•], ABTS^{•+} and reducing power) were used in the present study to assess the *in vitro* antioxidant activity of the *T. terrestris* extract. The DPPH[•] and ABTS^{•+} radical scavenging activity of the *T. terrestris* extract were compared to BHT and Trolox, respectively, and were expressed as IC₅₀ (concentration of the extract where the absorbance of DPPH[•] or ABTS^{•+} were reduced to 50 % in comparison to absorbance of blank). As presented in Table1, the *T. terrestris* extract possessed DPPH[•] activity (IC₅₀ 29.55 ± 0.49 µg/mL) and ABTS^{•+} activity (IC₅₀ 143.30 ± 8.10 µg/mL), compared to BHT and Trolox IC₅₀ values (9.78 ± 0.04 and 16.38±0.43µg/mL), respectively. In regard to the reducing power activity, the effective concentration of *T. terrestris* extract that provides the reading 0.5 absorbance at 700 nm (EC₅₀) was found to be 115.98 ± 4.81 µg/mL compared to BHT (11.20 ± 0.33 µg/mL) as presented in Table 1.

3.4. Liver Enzymes

It was observed that treating rats with alpha-cypermethrin (CYP) significantly ($p \leq 0.05$) increased the ALT and AST activity compared to the control group. Whereas, pretreatment with the *T. terrestris* extract (TT) significantly decreased these values in the treated group compared to CYP (Figure 1).

**Figure 1.** Serum ALT and AST levels in different studied groups.

Data presented as mean ± SE, Number of rats per group n = 10.

^aSignificant difference at $P \leq 0.05$ compared to the control group.

^bSignificant difference at $P \leq 0.05$ compared to the CYP group.

3.5. Oxidant /Antioxidant Parameters

MDA and NO levels were significantly ($p \leq 0.05$) increased in the CYP group when compared to the control group. Whereas, the co-administration of TT significantly alleviated MDA and NO values in the treated group compared to CYP group (Table 3). A significant ($p \leq 0.05$) reduction in POX-1 and GSH was observed in the CYP group compared to the control group. However, the co-administration of TT significantly ($p \leq 0.05$) improved these values in the treated group, making it close to that of the control group (Table 3).

Table 3. Oxidant and antioxidant parameters in different studied groups.

Groups	NO ($\mu\text{mol/g}$) tissue	POX (IU/g) tissue	GSH ($\mu\text{g/g}$) tissue	MDA (nmol/g) tissue
Control	4.8 \pm 0.3	34.0 \pm 1.8	28.6 \pm 0.6	119.0 \pm 7.8
TT	4.6 \pm 0.4	34.6 \pm 1.4	29.3 \pm 0.3	127.3 \pm 3.9
CYP	17.5 \pm 0.8 ^a	16.5 \pm 0.3 ^a	17.0 \pm 0.8 ^a	248.6 \pm 14.2 ^a
TT + CYP	10.7 \pm 0.7 ^{ab}	25.2 \pm 2.9 ^{ab}	23.3 \pm 0.6 ^{ab}	191.0 \pm 5.1 ^{ab}

Data presented as mean \pm SE, Number of rats per group n = 10.
^a:Significant difference at $P \leq 0.05$ compared to the control group. ^b:Significant difference at $P \leq 0.05$ compared to the CYP group.

3.6. Inflammatory Markers

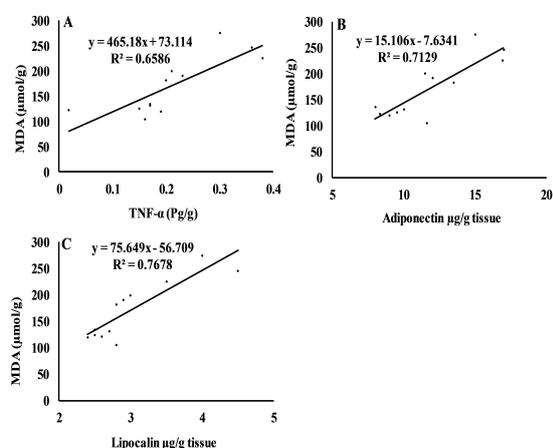
The liver inflammation markers (TNF- α , lipocalin, and adiponectin) increased in the CYP group compared to the control group; however, these levels were modulated in the treated group (TT+CYP) to be partially similar to the control compared to the CYP group (Table 4).

Table 4. TNF- α , lipocalin and adiponectin levels in different studied groups.

Groups	TNF- α (Pg/g) tissue	Lipocalin ($\mu\text{g/g}$) tissue	Adiponectin (ng/g) tissue
Control	0.17 \pm 0.008	2.63 \pm 0.1	10.2 \pm 0.7
TT	0.11 \pm 0.04	2.5 \pm 0.03	8.6 \pm 0.4
CYP	0.34 \pm 0.02 ^a	4.0 \pm 0.2 ^a	16.3 \pm 0.6 ^a
TT + CYP	0.21 \pm 0.008 ^b	2.9 \pm 0.05 ^b	12.3 \pm 0.6 ^b

Data presented as mean \pm SE, Number of rats per group n = 10.
^a Significant difference at $P \leq 0.05$ compared to the control group. ^b Significant difference at $P \leq 0.05$ compared to the CYP group.

Person's correlation coefficients were calculated between MDA level and inflammatory markers TNF- α , adiponectin and lipocalin in all groups. There were highly significant positive correlations between the MDA level and the inflammatory markers TNF- α , adiponectin and lipocalin in all groups ($p < 0.00$) (Figure 2).

**Figure 2.** Correlation coefficient between A) MDA and TNF α ; B) MDA and adiponectin, and C) MDA and lipocalin.

4. Discussion

Liver diseases represent a critical health problem around the world. In addition to the toxic chemicals such as aflatoxins, high doses of paracetamol and chlorinate hydrocarbons, viral infection; apoptosis, oxidative stress, and inflammation are the main processes that cause the initiation and progression of hepatic disorders (El-Khayat *et al.*, 2013; Miltonprabu *et al.*, 2017). Different herbal medications are used in traditional therapy for their preventive and curative properties against hepatic diseases. Most of these herbal medications have a free radical scavenging effect, and others have variable antioxidant properties (Hussein *et al.*, 2016). In recent years, food and pharmaceutical supplements using new bioactive herbal medications have gained abundant attention for the improvement of hepatic dysfunction (Lin *et al.*, 2014).

The phytochemical analysis of the *T. terrestris* extract in the current study showed an adequate amount of both total phenolic and total flavonoids, in addition to several phenolic and flavonoid compounds which were further screened by HPLC. These results are in accord with previous studies, which reported several phenolic, flavonoids and flavonoid glycosides in *T. terrestris* from different regions (Ivanova *et al.*, 2011; Hammada *et al.*, 2013).

The *T. terrestris* extract showed antioxidant activity with different mechanisms including free radical scavenging activity in DPPH $^{\bullet}$ and ABTS $^{2+}$ assays, besides the capability to reduce Fe $^{3+}$ to Fe $^{2+}$ in reducing power assay. In this regard, Hammada *et al.* (2013) reported the antioxidant activities of the *T. terrestris* extract using DPPH $^{\bullet}$ assays, however, Zheleva-Dimitrova *et al.* (2012) evaluated the antioxidant activity of *T. terrestris* containing different commercial herbal preparations from Bulgaria using DPPH $^{\bullet}$, ABTS $^{2+}$ and ferric-reducing antioxidant power. As total polyphenols (a wide class of compounds including phenolic acids, flavonoids, flavonols and anthocyanins) can scavenge free radicals *in vitro* and *in vivo*, the observed antioxidant activities of the *T. terrestris* extract, rich in phenolic and flavonoid compounds in the present study might be attributed to the components of polyphenolic compounds. Sasipriya and Siddhuraju (2015) reported that the antioxidant properties of phenolic compounds can be generally attributed to their redox properties, through different possible mechanisms, such as quenching singlet and triplet oxygen, absorbing and neutralizing free radicals, the transition of metal chelating activity, and/or decomposing peroxides.

The liver is an important organ. Its function is mainly involved in the decomposition and detoxification of toxic compounds, including xenobiotics (pesticides). Subsequently, the permanent alteration in its function causes hepatotoxicity and health implications. Fluctuation of liver enzymes such as ALT and AST are one of symptoms of hepatic damage and liver dysfunction (Rjeibi *et al.*, 2016). The administration of CYP to rats in the present study significantly increased the ALT and AST levels. These results are in accord with the results of Abdou *et al.* (2012). The increase in ALT and AST could be due to the accumulation of CYP in liver tissues, thereby leading to damage in cell membrane; consequently releasing the enzymes in the blood stream. On the other

hand, the pretreatment of rats with TT (TT + CYP group) significantly decreased the levels of ALT and AST enzymes. These results suggest the capability of the TT extract and its polyphenolic bioactive components in the alleviation of liver toxicity induced by CYP through the maintenance of liver cellular membranes' integrity.

CYP increases hepatic lipid peroxidation in rats, and reduces the antioxidative cellular reserves (both the enzymatic and non-enzymatic) leading to a condition of oxidative stress and causing free radical-mediated tissue damage and dysfunction (Sushma and Devasena, 2010).

The current results demonstrated that oxidative stress caused by CYP administration was marked by high oxidative parameters (MDA and NO) and low antioxidant defense parameters (POX-1 and GSH). These findings were in agreement with previous studies that showed an elevation of serum MDA and NO as well as a decrease in the antioxidant enzymes with the CYP exposure (Wang *et al.*, 2009; Hocine *et al.*, 2016).

Pyrethroids-induced oxidative damage may be attributed to their lipophilicity, thus, they can easily penetrate the cell membrane causing membrane lipid peroxidation (Prasanthi and Rajini, 2005); this mechanism could explain the high levels of liver MDA in the current results. The elevation of liver NO in this study was also explained by Wang *et al.* (2009) who indicated that the CYP administration increased inducible NOS (iNOS) and total NOS (T-NOS) concentrations raising the NO level in rats; this oxidative state leads to different organ damages with both biochemical and physiological alterations.

The significant decreases in POX-1 and GSH levels in the current study may be caused by either the inhibition of GSH synthesis or augmented consumption of GSH and POX-1 for scavenging of excess free radicals which cause tissue damage (Raina *et al.*, 2009).

In contrast, the oral administration of the TT extract with CYP in (TT+CYP group) revealed significant increase in liver GSH and POX-1 together with a significant reduction in the liver MDA and NO compared to the CYP group. These results agree with former results of Amin *et al.* (2006) who found a significant increase in liver GSH and a reduction of MDA in diabetic rats treated with TT extract. Also, Lakshmi *et al.* (2012) pointed out that the *T. terrestris* extract administration relieved cadmium toxicity in rats through the restoration of antioxidant, peroxidation and functional indicators in the liver and kidney tissues.

The alleviation effect of the TT extract in the current study could be attributed to the rich presence of flavonoid compounds that have a potential antioxidant activity, such as kaempferol, which causes singlet oxygen scavenging, iron chelation, NADPH oxidase inhibition, and lipid peroxidation chain reaction termination (Fatima *et al.*, 2015).

Quercetin is the most important flavonol generally detected in several natural resources as well as in the *T. terrestris* extract (as observed in Table 2). The Quercetin dose not only act as an antioxidant agent through direct donation of hydrogen atoms and quenching of ROS, but it also induces the intracellular anti-oxidative defense system by the direct interaction with the intracellular signaling cascades related to the antioxidant function (Williams *et al.*, 2004). Similarly, Huang *et al.* (2013) revealed that

apigenin, luteolin, and chrysin are all able to attenuate hepatic oxidative stress induced by *tert*-butyl hydroperoxide (tBHP) by means of regulating the gene transcription of hemeoxygenase 1 (HO-1) and glutamate cysteine ligase catalytic (GCLC) and modifier subunit (GCLM) through the activation of extracellular signaling pathways of signal-regulated protein kinase 2 (ERK2), nuclear factor erythroid 2-related factor 2 (Nrf2) nuclear translocation, and nuclear Nrf2-antioxidant responsive element(ARE) in rat primary hepatocytes.

Hepatic oxidative stress stimulates the proliferation of different inflammatory cells which can increase liver deterioration by releasing many cytokines inflammatory markers (Xin *et al.*, 2015). Lipocalin as an inflammatory marker is induced in damaged hepatocytes in deleterious conditions including infection, intoxication, and inflammation in addition to other conditions of oxidative stress (Borkham-Kamphorst *et al.*, 2013). Adiponectin is a surrogate marker for inflammation through the release of proinflammatory cytokines with the special participation of TNF- α (Pennathur and Heinecke, 2007). In the present study, rats intoxicated by CYP showed a significant increase in the hepatic inflammatory markers, namely TNF- α , lipocalin, and adiponectin.

The results of the present study agree with the findings of Salman *et al.* (2010) who reported high plasma adiponectin in chronic liver disease due to the imbalance between its production by adipocytes and metabolism in the liver as a result of the suppression of hepatic catabolism. Moustafa and Hussein (2016) demonstrated that the insecticide pyrethroid increases TNF- α inflammatory gene expression in rats. Also, Raszewski *et al.* (2015) reported that CYP is involved in the production of cytotoxic T cells and activated NK cells, which consequently elevated the TNF- α level. All these studies emphasize the stimulation of liver injury following the CYP administration.

In the current study, the administration of the TT extract in (TT+CYP) group modulated these markers to be partially similar to that of the control group. The effect of the protecting potential of the TT extract through the modulation of TNF- α , lipocalin, and adiponectin levels in rats might be attributed to the bioactive chemical contents of the TT extract specifically the diversity of polyphenolics presented in Table 2. Several studies indicated the involvement of different polyphenolics isolated from natural resources in down-regulation of pro-inflammatory markers including TNF- α , cyclooxygenase-2 (COX-2), iNOS, C-reactive protein (CRP), interleukin-1 β (IL-1 β), and adiponectin in different experiments concerning liver toxicity (Chtourou *et al.*, 2015).

5. Conclusions

Results of the present study revealed the adequate amount of total phenolic and total flavonoids and several other compounds of phenolic and flavonoids screened for the first time by HPLC in *T. terrestris* growing in Egypt. The *T. terrestris* extract was found to exhibit a potent *in vitro* antioxidant activity using DPPH, ABTS, and reducing power assays. The significant effect of *T. terrestris* extract in the attenuation of hepatotoxicity induced by α -cypermethrin in rats was also investigated

for the first time. The administration of *T. terrestris* extract decreased liver enzymes (ALT and AST), increased liver GSH and POX-1, decreased liver MDA and NO, and decreased hepatic inflammatory markers (TNF- α , lipocalin, and adiponectin). The significant hepatoprotective effect of the *T. terrestris* extract may be attributed to its ability to hunt formed free radicals and induce the antioxidant enzymes expression in addition to the down-regulation of the pro-inflammatory markers that are known to be released following the injury of liver tissues. In summary, these results emphasize the therapeutic potential of the Egyptian *T. terrestris* and suggest its use as a natural hepatoprotective source and health-promoting product.

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