Journal of Materials and Environmental Sciences
ISSN : 2028-2508
CODEN : JMESCN

Copyright © 2018,
University of Mohammed Premier
Oujda Morocco

J. Mater. Environ. Sci., 2018, Volume 9, Issue 6, Page 1908-1914

https://doi.org/10.26872/jmes.2018.9.6.210

http://www.jmaterenvironsci.com

Anti-inflammatory, Antinociceptive and Nephroprotective activities of *Tilia cordata* and Isolation of Bioactive Compounds

G. Fawzy[1*] , K. Younes [2], E. Waked[3], H. Mahmoud[4]

[1]*Pharmacognosy Department, National Research Centre, Elbehoos Street, Dokki 12622, Giza, Egypt.*
[2]*Internal Medicine Department, Medical Division, National Research Centre, Giza Egypt*
[3] *Nephrology Department, Theodor Bilharz Research Institute, Giza Egypt*
[4] *Food Industry, Faculty of Agriculture, Cairo University, Cairo, Egypt*

Received 05 Jun 2017,
Revised 04 Nov 2017,
Accepted 14 Nov 2017

Keywords

- Anti-inflammatory Antinociceptive
- Bioactive Nephroprotective
- *Tilia cordata*

Gehan Fawzy Abdel Raoof,
ahmedkhaled_1@hotmail.com
gehankandeel9@yahoo.com
Tel.: +201223417848

Abstract

Many studies aim to manage many diseases by discovering new drugs from medicinal plants. This study was designed to evaluate anti-inflammatory, antinociceptive and nephroprotective activities of 70% methanol extract of *Tilia cordata* aerial parts and to isolate and identify its phytoconstituents. Anti-inflammatory activity was evaluated by using the carrageenan-induced rat paw edema method, antinociceptive activity was evaluated by using the writhing test in mice, nephroprotective activity against the toxic effect of cadmium chloride ($CdCl_2$) on the renal function was determined by measuring creatinine and uric acid levels. Moreover, the study showed the isolation and identification of the bioactive compounds using chromatographic and spectrophotometric methods .Six phenolic compounds were isolated which were identified as; kaempferol, quercetin , vitexin , kaempferol 3-O-α-rhamnoside , quercetin 3-O-β-galactoside and kaempferol 3-O-rutinoside. The extract of *Tilia cordata* showed a powerful anti-inflammatory, antinociceptive and nephroprotective activities. This research aims to enter the field of drug discovery by using the methanol extract of *Tilia cordata* aerial parts in the treatment of inflammation, pain and nephrotoxicity

1.Introduction

Many studies aim to manage many diseases by discovering new drugs from medicinal plants. Many parts of medicinal plants were used for extraction and isolation of bioactive compounds which have many medicinal activities. Previous studies showed that medicinal plants are playing an important role against many diseases [1–3]. The phenolic compounds which are found in the medicinal plants are responsible for these activities [4]. More studies are nedded for evaluation of the safety and the modes of action of the bioactive compounds of the medicinal plants which are used in folk medicine.

Tilia cordata (Tiliaceae family) is a deciduous tree, in folk medicine *Tilia cordata* flowers are widely used for the treatment of fever and anxiety. It contains tannins ,volatile oils and flavonoids [5].The recent study showed that the extract of the flower of *Tilia cordata* had a potent antioxidant activity [6]. Phytochemical screening of the aerial parts of *Tilia* cordata showed the presence of carbohydrates, saponins, tannins, flavonoids, triterpenes and coumarins. In addition, the extract showed anti-tyrosinase and antioxidant activities [7]. Phytoconstituents and other biological activities of the leaves and stem (aerial parts) of *Tilia cordata* haven't been described yet in previous studies.

This study is aiming to evaluate anti-inflammatory, antinociceptive and nephroprotective activities of 70% methanol extract of *Tilia cordata* aerial parts and to isolate and identify its phytoconstituents .

2. Materials and Methods

2.1. *General experimental procedures*

UV/VIS: Shimadzu UV-visible recording spectrophotometer model-UV 240 (NRC,Egypt). ^{1}H-NMR and ^{13}C-NMR (Varian Unity Inova). MS (Finnigan MAT SSQ 7000, 70 ev). (Silica gel (0.063– 0.200 mm for column chromatography) and Sephadex LH-20 (Pharmacia Fine Chemicals).Thin layer chromatography (TLC) F_{254}

plates. Solvent mixtures; S_1:BAW (n-butanol/acetic acid/ water) 4:1:5, (v/v/v) upper phase, S_2: 15% acetic acid (Water/glacial acetic acid, 85:15,(v/v)) and S_3: (30:70 methanol:chloroform). Paper Chromatography (PC) Whatman No.1, (Whatman Led. Maid Stone, Kent, England), sheets for qualitative detection of flavonoids and sugars.

2.2. *Plant identification and collection*

Tilia cordata aerial parts were collected from the Agricultural Research Centre, Giza, Egypt, in March 2016. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National research centre (NRC) and by Mrs. Tereeza Labib consultant of plant taxonomy at the ministry of agriculture and director of Orman botanical garden, Giza, Egypt.

2.3. *Preparation of the extract*

Air-dried powder of *Tilia cordata* aerial parts (900 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 (44g) of the crude extract.

2.4. Isolation of the bioactive compounds from methanol (70%) extract of Tilia cordata aerial parts

Methanolic extract (40 g) was defatted with n-hexane and the defatted extract (33 g) was subjected to silica gel column chromatography eluting with dichloromethane, ethyl acetate and methanol gradually. One hundred and forty fractions of 100 ml conical flask were collected. The fractions that showed similar PC in S_1 and S_2 were combined to give three main fractions (I, II, and III). Fraction I (4.85 g) was subjected to sub-column of silica gel eluted with dichloromethane and ethyl acetate. Elution of (dichloromethane:ethyl acetate (90:10, v/v)) gave compound 1, further elution with (dichloromethane:ethyl acetate (80:20,v/v)) gave compound 2 and further elution with (ethyl acetate: dichloromethane (60:40, v/v)) yielded compound 3. Fraction II (3.65 g) was subjected to sub-column of silica gel eluted with dichloromethane and methanol. Elution of (dichloromethane: methanol (85:15,v/v)) yielded compound 4,and further elution with (dichloromethane:methanol (80:20, v/v)) gave compound 5.Fraction III (6.35 g) was subjected to sub-column of silica gel eluted with dichloromethane and methanol. Elution of (dichloromethane:methanol (75:25, v/v)) yielded compound 6. All the isolated compounds were purified on Sephadex LH-20 column using different systems of methanol and mixture of methanol and distilled water.

2.5. *General method for acid hydrolysis of flavonoid glycosides*

Five milligrams of each flavonoid glycoside in 5 ml 10% HCl were heated for 5 h. The aglycones were extracted with ethyl acetate and identified by co-TLC with authentic standards. The sugars in the aqueous layer were identified by co-PC with authentic markers on Whatman No. 1 sheets in the solvent system: S_1

2.6. Animals

Adult albino rats (120–150 g) were used for the evaluation of the anti-inflammatory and renal function protective activities. Adult Swiss albino mice (18–25 g) of either sex were used for the evaluation of the antinociceptive activity. Animals were obtained from the animal-breeding unit of the National Research Centre, Giza, Egypt. Animals were housed under standardized conditions of light and temperature (room temperature 23 ± 2°C, relative humidity 55 ± 5%, 12 h light/dark cycle) and received standard rat chow and tap water . This study was performed according to the guidelines of the ethical committee of the National Research Centre for experimental animal use.

2.7. *Determination of anti-inflammatory activity*

The anti-inflammatory activity was evaluated using the carrageenan-induced rat paw edema according to the method described by Winter *et al*. [8]. Four groups of rats (six rats in each) were prepared; group 1: is the control group that received distilled water (10 ml/kg b.wt), group 2: received 10 mg/kg indomethacin ,groups 3 and 4: received 150 and 300 mg/kg of 70% methanol extract of *Tilia cordata* aerial parts, respectively. All groups of adult male albino rats were fasted with free access of water for 12 h prior to the test. The rats were orally dosed with 150 and 300mg/kg of 70% methanol extract and indomethacin (Sigma-Aldrich, St. Louis, MO) (10mg/kg, p.o.) 2 h before carrageenan challenge. Rat paw edema was induced by sub-planter injection of 0.1ml of 1% suspension of carrageenan (SigmaAldrich, St. Louis, MO) in saline into the planter tissue of one hind paw. An equal volume of saline was injected into the other hind paw and served as control. The thickness of the rat hind paws was measured by Vernier Caliper (SMEC, Shanghai, China) 1, 2, and 3 h after carrageenan

challenge. The percentage swelling of the paw was calculated by measuring the difference between the thicknesses of the two paws. The percentage inhibition of the two concentrations of the test extract as well as indomethacin, employed as reference, was calculated relative to carrageenan.

2.8. *Antinociceptive activity*

This activity was determined by performing the writhing test [9]. Mice were divided into four groups; Group I served as positive control and received the vehicle. Group II was injected with the reference drug (acetylsalicylic acid 100mg/kg, p.o.), while groups III and IV were treated with two different extract concentrations (150 and 300 mg/kg, p.o. of the plant under investigation). The animals were orally administrated with the vehicle, reference drug or the extract 1 h before the intraperitoneal injection of freshly prepared acetic acid (2% (w/v) in saline), 10ml/kg body weight. The mice were individually separated and observed for 30 min starting from 5 min post acetic acid injection. The observation included counting the number of writhes produced by the mice which is a response consisting of an abdominal wall and pelvic rotations followed by hind limb extension.

2.9. Nephroprotective activity

Mice were assigned into four groups; animals of group 1 (control) received the vehicle (10 mg/kg body weight/day) for 5 days. Animals of group II received the vehicle for 5 days. Animals of group III received lower dose 150 mg/kg body weight/day of the 70% methanol extract suspended in the vehicle (10 ml/kg) for 5 days. Animals of group IV received the higher dose (300 mg/kg body weight/day) of 70% methanol extract suspended in the vehicle (10 ml/kg) for 5 days. A single dose of cadmium chloride (20 mg/kg body weight) which was obtained from Merck (Darmstadt, Germany) [10], was administered subcutaneously in the neck region in a volume of 1 ml/kg, on fourth day to all the animals except of group 1. Group 1 received normal saline only. On the sixth day, blood samples were withdrawn from retro-orbital venous plexus and serum was separated for the estimation for urea and creatinine concentrations. The drug solutions or vehicle were administered orally by gastric intubation using syringe to assess the experiments. At the end of experimental period, mice were anaesthetized with ether according to the method described by Cocchetto and Bjornsson [11]. 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.

2.9.1.*Uric acid assay*

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 [12].

2.9.2.*Creatinine assay*

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 [13]. In the assay, creatinine is converted to creatine by creatininase, it is then converted to sarcosine, which is specifically oxidized to produce a product which reacts with a probe to generate red color (λmax = 075 nm).

2.10.Statistical analysis

Results were expressed as means ± standard deviation (SD). Statistical analysis of the obtained data was performed using one-way analysis of variance (ANOVA) followed by a Student–Newman–Keuls post hoc comparison. A result was considered statistically significant where $p<0.05$.

3.Results and discussion

Different spectroscopic methods were used in elucidation of the chemical structures of the compounds that were isolated from 70% methanol extract of *Tilia cordata* aerial parts.

3.1. Structure elucidation of the isolated compounds

Compound (1): 6 mg, yellow powder. (+) ESI-MS: m/z 287 $[M + H]^+$. ^{1}H-NMR (DMSO-d6): δ 6.19 (1H, d, J = 2 Hz, H-6), δ 6.47 (1H, d, J = 2 Hz, H-8), δ 6.96(2H,d,J=8Hz,H-3', 5'), δ 8.12 (2H,d,J=8Hz,H-2',6').

Compound (2): 5 mg, yellow powder, EIMS: m/z 302. UV λmax (MeOH): 263, 374; (NaOAc): 269, 410; (NaOAc/H3BO3): 257, 385. (AlCl3): 272, 447; (AlCl3/HCl): 263, 415 ,(NaOMe): 272, 410.

Compound (3): 10 mg, yellow powder. (−) ESI-MS: m/z 431 $[M-H]^-$, ^{1}H-NMR (DMSO-d6): δ 6.23 (1H, s, H-3), δ 6.71 (1H, s, H-6), δ 6.84 (2H, d, J = 8.9 Hz, H-3', 5'), δ 7.91 (2H, d, J = 8.9 Hz, H-2', 6'), δ 13.10 (1H, s, 5-OH), 4.59 (1H, d, J = 10 Hz, H-1''), 3.1–3.8 (other sugar protons, H-2''-6''). UV λmax (MeOH): 269,341; (NaOAc): 278,371; (NaOAc/H3BO3): 270, 346. ;(AlCl3):277,303sh, 350; (AlCl3/HCl): 270,350; (NaOMe):279,391.

Compound (4): 15 mg, yellow powder. ^{1}H-NMR (CD3OD): δ ppm 0.93 (CH3, d, J = 6.2 Hz). 5.40 (1H,d,J=2.4 Hz,H-1''),6.23 (1H,d,J=2.2 Hz,H-6).6.42 (1H, d, J = 2.2 Hz, H-8),6.90 (2H, d, J = 8.2 Hz, H-3', 5').7.70 (2H, d, J = 8.2 Hz, H-2', 6).13C-NMR (CD3OD): δ ppm 101.11 (C-1''), 102.74 (C-5'), 71.27 (C-4''), 71.33 (C-2''), 71.46 (C-3''), 72.13 (C-5''), 93.90 (C-6), 94.13 (C-8),107.62 (C-10),105.19 (C-1'),119.23 (C-3'), 115.77 (C-2'), 124.88 (C-6'), 133.22 (C-3),138.45 (C9), 159.99 (C-2), 158.55 (C-4'), 162.83 (C-5), 165.99 (C-7), 177.99 (C-4), 18.91 (CH3-rhamnosyl).

Compound (5): 13 mg, as needles with yellow colour. ^{1}HNMR(CD3OD): δ ppm 7.79 (1H, d,J=2Hz,H-2'), 7.66 (1H,dd,J = 2,7.7 Hz,H-6'),6.86 (1H,d,J = 8.2 Hz,H-5'),6.32 (1H, d, J = 2 Hz, H-8), 6.13 (1H, d, J = 2 Hz, H-6), 5.07(1H,d,J=7.8Hz,H-1''). UV λmax (MeOH): 259, 349; (NaOAc): 278, 390; (NaOAc/H3BO3):269 ,374, (AlCl3): 279, 429; (AlCl3/HCl): 278, 410; (NaOMe): 275, 406.

Compound(6): 19 mg. yellow amorphous powder , ^{1}H NMR (DMSO-d6) δ ppm 6.41 (1H, s, H-8), 6.23 (1H, s, H-6), 6.83 (2H, d, J = 8.5 Hz, H-3′, 5′), 7.93 (2H, d, J = 8.5 Hz, H-2′, 6′), 1.11(3H, d, J = 6.5 Hz, -CH3-rhamnosyl), 4.38 (1H, s, H-1‴),5.43 (1H, d, J = 7.2 Hz, H-1″), 13C-NMR (DMSO-d6): δ ppm 17.71 (C-6‴), 68.23 (C-5‴), 72.73 (C-4‴), 70.61 (C-3‴), 70.33 (C-2‴), 100.83 (C-1‴), 66.91 (C-6″), 75.71 (C-5″), 69.73 (C4″), 76.44 (C-3″), 74.25 (C-2″), 101.47 (C-1″), 159.97 (C-4′) , 115.33 (C-3′, 5′), 131.31 (C-2′, 6′), 120.69 (C-1′), 103.69 (C-10), 156.69 (C-9), 93.77 (C-8), 164.39 (C-7), 98.39 (C-6), 161.78 (C-5), 177.33 (C-4), 133.23 (C-3), 156.33 (C-2), UV λmax (nm): MeOH 270, 351; (NaOAc) 278, 389; (NaOAc/H3BO3) 269, 358. (AlCl3): 277, 305, 359, 393; (AlCl3/HCl) 277, 307, 349, 389sh; (NaOMe) 279, 397. Acidic hydrolysis showed that sugar moieties were glucose and rhmanose, while aglycone was kaempferol.

3.2. Identification of the isolated compounds

Compound 1: under UV light gave a yellow colour that changed to yellowish green fluorescent on exposure to ammonia or using AlCl3 reagent. According to its spectral data and comparison with the literature [14]. Compound 1 was identified as kaempferol

Compounds 2: based on its spectral data , using the authentic sample and comparison with the literature [15]. Compound 2 was identified as Quercetin

Compounds 3: by UV light gave deep purple colour and by using ammonia and AlCl3 it changed to yellow. By using the authentic sample and comparing its spectral data with the literature [16] , compound 3 could be identified as vitexin

Compound 4: by using UV light, it gave deep purple colour which changed into a yellow fluorescent colour with ammonia and AlCl3 reagent, acidic hydrolysis showed that aglycone was kaempferol and sugar moiety was rhamnose. Its spectral data were identical with the published data [17] . So compound 4 could be identified as kaempferol 3-O-α-rhamnoside

Compound 5: it gave deep purple colour under UV light, and gave a yellow fluorescent colour with ammonia and AlCl3 reagent. Quercetin and galactose were produced by acid hydrolysis as an aglycone and a sugar moieties, respectively. By comparing its spectral data with the published data [18] , compound 5 was identified as quercetin 3-O-β-galactoside

Compound 6 : yellow amorphous powder, kaempferol was produced as an aglycone by acidic hydrolysis, while sugar moieties were glucose and rhmanose. According to its spectral data and by comparison with the literature [19], compound 6 could be identified as Kaempferol 3-O-rutinoside.

Chemical structures of the isolated compounds were illustrated in (Figure 1).

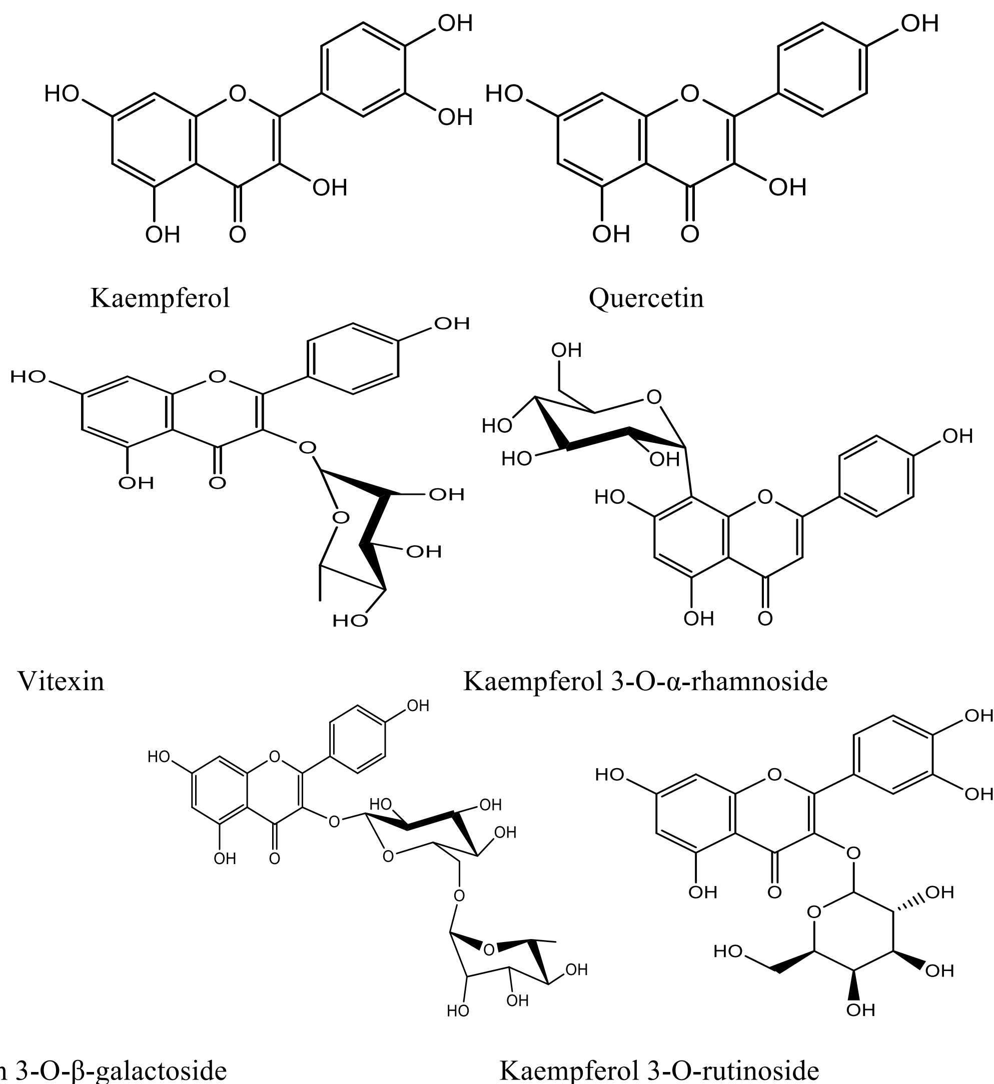

Figure 1. Chemical structures of the compounds isolated from 70% methanol extract of *Tilia cordata* aerial parts

3.3. Anti-inflammatory activity

The carrageenan-induced rat paw edema test was used to evaluate the anti-inflammatory activity of 70% methanol extract of *Tilia cordata* aerial parts. The results were summarized in table 1, from these results we found that the swelling that was caused by carrageenan was significantly decreased by using Indomethacin (the reference drug), comparing with the carrageenan group. After 1 , 2 and 3 h of carrageenan injection for rats treated with 70% methanol extract (150 mg/kg) and after 1 and 2 h for rats treated with the higher dose (300 mg/kg), the results showed a significant reduction in swelling that was produced by carrageenan, but these percentages were significantly increased than that was produced by indomethacin . 70% methanol extract (300mg/kg) induced the same decrease in the percentage of swelling as that produced by indomethacin after 3 h of carrageenan injection. 70% extract (150 mg/kg) induced the inhibition of the inflammation with percentage 65, 67 and 87% at 1, 2 and 3 h , respectively, while 70% methanol extract (300 mg/kg) induced the inhibition of the inflammation with percentage 69,77 and 97% at 1,2 and 3 h , respectively.

Table 1: Anti-inflammatory effect of 70% methanol extract of *Tilia cordata* aerial parts

Groups	%Swelling			% Inhibition		
	1h	2h	3h	1h	2h	3h
Carrageenan	39	59	88	-	-	-
Indomethacin	11[a]	8[a]	6[a]	83	87	95
Extract (150 mg/kg)	15[ab]	13[ab]	11[ab]	65	67	87
Extract (300 mg/kg)	13[ab]	11[ab]	6[ab]	69	77	97

One-way ANOVA test, significant at P-value <0.05, [b]significantly different from indomethacin ,[a]significantly different from carrageenan .

3.4. Antinociceptic activity

Acetic acid affects on peritoneal receptors which causes inflammatory pain via release of special mediators; as cytokines prostaglandins F2,E2 and bradykinin which cause writhing response that was detected by twisting of the pelvis and trunk, arching of back, contraction of the abdomen and finally, the elongation of limbs [20]. Table 2 showed that the number of writhing was significantly decreased by the two doses of the plant extract (150 and 300 mg/kg) and acetylsalicylic acid (100mg/kg) by 72, 89 and 88% ,respectively, compared with the effect of acetic acid only. The results showed that the higher dose of the plant extract (300 mg/kg) showed the same effect that produced by acetylsalicylic acid. The anti-inflammatory and antinociceptive activities of the 70% methanol extract of *Tilia cordata* may be due to the presence of phenolic contents such as flavonoids that reported to have anti-inflammatory and antinociceptive activities [21- 26].

Table 2:Antinociceptive activity 0f 70% methanol extract of *Tilia cordata* aerial parts

Groups	Number of writhing	% Inhibtion
Acetic acid	59.90±1.03[b]	-
Acetylsalicylic acid	18.80±1.02[a]	88%
Extract (150 mg/kg)	36.60±1.77[ab]	72%
Extract (300 mg/kg)	18.20±1.01[ab]	89%

One-way ANOVA test, significant at P-value <0.05,[a]significantly different from acetic acid , [b]significantly different from acetylsalicylic acid

3.5. Nephroprotective activity

Table 3 showed that serum creatinine and blood urea levels were significantly increased as a result of nephrotoxicity which caused by cadmium chloride compared with control group. Both doses of plant extract (150 mg and 300 mg/kg body weight) significantly decreased the serum creatinine and blood urea compared with cadmium chloride treatment group. The results showed that the 70% methanol extract of *Tilia cordata* aerial parts had the protective effect against nephrotoxicity that was caused by cadmium chloride in rats, this activity may be due to the powerful antioxidant activity, that was reported for the plant [6,7].

Table 3: Nephroprotective activity of 70% methanol extract of *Tilia cordata* aerial parts against the toxicity of cadmium chloride

Groups	Creatinine (mg/dl)	Urea (mg/dl)
Control	0.159±0.01	12.9±0.9
Cadmium chloride	0.405±0.011[a]	39.44±1.9[a]
Extract (150 mg/kg)	0.179±0.013[b]	16.04±1.4[b]
Extract (300 mg/kg)	0.168±0.012[b]	13.98±1.8[b]

One-way ANOVA test, significant at P-value <0.05; [b] compared to cadmium chloride treatment group, [a] compared to control treatment group.

Conclusion

This study showed that 70% methanol extract of *Tilia cordata* aerial parts decreased the inflammation that was produced by carrageenan, the pain induced by acetic acid and nephrotoxicity due to administration of cadmium chloride . These results showed that *Tilia cordata* aerial parts has anti-inflammatory, antinociceptive and nephroprotective activities, which could play an important role in controlling pain, inflammation and nephrotoxicity.

Conflicts of interest - There are no conflicts of interest.

Acknowledgment - We acknowledge to National Research Centre, Theodor Bilharz Research Institute and Faculty of Agriculture, Cairo University for using laboratory instruments in doing research.

References

1. M.V. Eberhardt, C.Y. Lee, R.H. Liu, Antioxidant activity of fresh apples. *Nature*., 405(2000) 903–904.
2. L.A. Bazzano, J. He, L.G. Ogden, C.M. Loria, S. Vupputuri, L. Myers, P.K. Whelton, Fruit and vegetable intake and risk of cardiovascular disease in US adults: The first national health and nutrition examination survey epidemiologic follow-up study. *Am. J. Clin. Nutr.*, 76(2002) 93–99.
3. D. Del Rio, A. Rodriguez-Mateos, J.P.E.Spencer, M. Tognolini, G. Borges, A. Crozier, Dietary (poly) phenolics in human health: Structures, bioavailability, and evidence of protective effects against chronic diseases. *Antioxid. Redox Signal.*, 18(2013) 1818–1892.

4. A. Crozier, I. Jaganath, E.M.Clifford, R. Miri, M.Tavakkoli, S. Pourali , L. Saso, F. Borges, O. Firuzi, Dietary phenolics: Chemistry, bioavailability and effects on health. *Nat. Prod. Rep.*, 26(2009) 1001–1043.
5. P. Bradley, *ed. British Herbal Compendium*., 1(1992) 142–144.
6. A.F. Vinha, V.P.B. Sérgio, C. Ana and M. Marisa, Comparison Between the Phytochemical and Antioxidant Properties of Plants Used in Plant Infusions for Medicinal Purposes. *J. Agric. Sci.,* 5(11) (2013)11-19.
7. K. Rashed, R. Medda, D. Spano, F. Pintus, Evaluation of antioxidant, anti-tyrosinase potentials and phytochemical composition of four Egyptian plants**.** *Int. Food .Res. J.*,23(1) (2016) 316-321
8. C.A. Winter, E.A. Risley, G.W. Nuss, Anti-inflammatory and antipyretic activities of indomethacin, 1-(p-chlorobenzoyl)-5-methoxy-2-methyl-indoie-3-acetic acid. *Pharm. Exp. Therapy*., 141 (1963) 369–76.
9. M. Hernandez-Perez, R.M. Rabanal, M.C. De la Torre,B.Rodriquez, Analgesic, anti-inflammatory, antipyretic and haematological effects of aethiopinone, an o-naphthoquinone diterpenoid from *Salvia aethiopis* roots and two hemisynthetic derivatives*. Planta Med*., 61(1995) 505–9.
10. D. Jonker, M.A. Jones, P.J. Van Bladeren, R.A. Woutersen, H.P. Til, V.J. Feron, Acute (24 hr) toxicity of a combination of four nephrotoxicants in rats compared with the toxicity of the individual compounds. *Food. Chem. Toxicol*., 31(1993)45-52.
11. D.M. Cocchetto, T.D. Bjornsson, Methods for vascular access and collection of body fluids from the laboratory rat*. J. Pharm .Sci*.,72(1983) 465- 492.
12. D. Barham, P. Trinder, An improved colour reagent for the determination of blood glucose by the oxidase system. *Analyst*., 97 (1972) 142-145.
13. H. Bartles, M. Bohmer, C. Heirli, Serum creatinine determination without protein precipitation. *Clin. Chem. Acta.,* 37 (1972) 193-197.
14. A. Gangwal, S. K. Parmar, N. R. Sheth, Triterpenoid, flavonoids and sterols from Lagenaria siceraria fruits. *Der Pharm. Lett.*, 2(1) (2010) 307–317.
15. L.O.A. Manguro, I. Ugi, and P. Lemmen, Flavonol Glycosides from the Leaves of Embelia keniensis*. J. Chin. Chem. Soc.*, 52(2005) 201-208.
16. L.Yun-Lian , K.Yueh-Hsiung, S.Ming-Shi , C. Chien-Chih , O. Jun-Chih, Flavonoid glycosides from Terminalia catappa L. *J. Chin. Chem. Soc.*, 47 (2000) 253–256.
17. O. Lawrence, M. Arot, U. Ivar, L. Peter, Flavonol Glycosides from the Leaves of Embelia keniensis. *J. Chin. Chem. Soc.*, 52 (2005) 201–208.
18. Z. Guvenalp and L.O. Demirezer, Flavonol Glycosides from Asperula arvensis L., *Turk. J. Chem*., 29 (2005) 163-169.
19. T.N. Bao, S.L. Peng and Z.Z. Zhou, Chemical Constituents of Buckwheat. *Nat. Prod. Res. Dev.*, 15(2003) 24-26.
20. Y. Ikeda, A. Ueno, H. Naraba, S.Oh-ishi *,* Involvement of vanilloid receptor VR1 and prostanoids in the acid-induced writhing responses of mice. *Life Sci*., 69 (2001) 2911–19.
21. M.M. Ahmad, S. Quresh, A. Shah, N. S. Qazi, R. M. Rao , M. Albakiri, Antiinflammatory activity of Caralluma tuberculata alcoholic extract. *Fitoterapia*., 46(1983) 357-360.
22. C. La Casa, I. Villegas, C. Alarcon de la Lastra,V. Motilva, M.J. Martin, Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions. *J. Ethnopharmacol*., 71(2000) 45-53.
23. Z.V. Danieli, S.O.Vinícius ,S.A. Jucicléia, C.P. Ana, A.L.Claudia ,R.M. Iriani, A.L.Cândida , J.S.Eliana , Anti-inflammatory, and antinociceptive effects of Campomanesia adamantium microencapsulated pulp. *Rev. bras. Farmacogn.*, 27(2) (2017) 220-227.
24. L.L. Nwidu, B. Airhihen, A. Ahmadu, Anti-Inflammatory and Anti-Nociceptive Activities of Stem-Bark Extracts and Fractions of *Carpolobia Lutea* (Polygalaceae). *J. Basic Clin. Pharma*.,8 (2017) 25-32.
25. H. Bendaif, A. Melhaoui , A. Bouyanzer , B. Hammouti , Y. El Ouadi,The study of the aqueous extract of leaves of Pancratium Foetidum Pom as: Characterization of polyphenols, flavonoids, antioxidant activities and Ecofriendly corrosion inhibitor. *J. Mater. Environ. Sci*., 8(12) (2017) 4475-4486.
26. M.M. El-Sherei, A.Y. Ragheb, S.A. Mosharrafa, M.M. Marzouk, M.E.S. Kassem, N.A.M. Saleh, Pterygota alata, (Roxb.) R. Br.: Chemical constituents, Anti-hyperglycemic Effect and Anti-oxidative Stress in Alloxan-induced Diabetic rats. *J. Mater. Environ. Sci.*, 9(1) (2018) 245-255.

(2018) ; http://www.jmaterenvironsci.com