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Original Article

Nephroprotective Activity of Methanolic Extract of *Lantana camara* and Squash *(Cucurbita pepo)* on Cisplatin-Induced Nephrotoxicity in Rats and Identification of Certain Chemical Constituents of *Lantana camara* by HPLC-ESI- MS

Heba Abdel-Hady[1*], Mortada Mohamed El-Sayed[1] , Afaf Ahmed Abdel-Hady[2], Maher Mahmoud Hashash[3], Ahmed Mohamed Abdel-Hady[4], Tarek Aboushousha[4], El-Sayed Saleh Abdel-Hameed[1], Ezzat El-Sayed Abdel-Lateef[1], Eman Abdalla Morsi[1]

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[1]Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, EGYPT.
[2]ClinicalChemistry Department, Theodor Bilharz Research Institute, Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, EGYPT.
[3]Department of Chemistry, Faculty of Science, Ain Shams University, , Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, EGYPT.
[4]Pathology Department, Theodor Bilharz Research Institute, , Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, EGYPT.

Correspondence

Heba Abdel Hady

Medicinal Chemistry Department, Theodor Bilharz Research Institute, Kornish El-Nile Street, Warrak El-Hadar, Imbaba, Giza, EGYPT.

Phone Number: 02-01000776485
Fax Number: 02-0235408125

E-mail: h_hady10@yahoo.com

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ABSTRACT

Introduction: Cisplatin is a highly effective chemotherapeutic agent; its clinical use is severely limited by serious side effects as nephrotoxicity. The aim of this study is to evaluate the nephroprotective activity of defatted methanolic extract of two Egyptian plants: *Lantana camara* and *Cucurbita pepo* and certain fractions derived from the defatted methanolic extract of *L. camara* on cisplatin-induced nephrotoxicity in rats. Also, identification of certain chemical constituents of *L. camara* by HPLC-ESI- MS. **Methods**: Nephrotoxicity was induced in rats by single dose of cisplatin. The effect of plants extract at doses 100-400 mg/kg.b.wt comparing with standard; ascorbic acid; was determined using serum urea, creatinine and some ions. Furthermore, the effect of these extracts on some renal antioxidant enzymes and histopathological examination of kidneys were examined. **Results**: The defatted methanolic extract and ethyl acetate fraction of *L. camara* showed the highest improvement of renal parameters. Also, HPLC-ESI-MS analysis of *L. camara* extracts exhibited bioactive phenolic compounds including phenyl ethanoid, flavonoids and phenolic acids. **Conclusion:** The phytochemical constituents of *L. camara* are responsible for their nephroprotective activity.
Key words: Antioxidant enzymes, Ascorbic acid, Creatinine, Flavonoids, Histological studies, Phenyl etanoid.

INTRODUCTION

The Kidneys play an important role in the maintenance of our endocrine, acid-base balance, blood pressure and erythropoiesis. The main functions of the kidney are formation of urine, maintenance of water and electrolyte balance as well as production of hormones and enzymes. Kidneys have some delicate tasks, especially when they have to deal with unwanted substances. Nephrotoxicity is renal dysfunction that arises as a direct result of exposure to external agents such as drugs and environmental chemicals.[1,2]

Cisplatin is extensively used for the treatment of several cancers. Unfortunately, it is conjoined with a brutal side effect as it induces nephrotoxicity. Nephrotoxicity induced by cisplatin is due to apoptosis, inflammatory mechanism and generation of reactive oxygen species (ROS).[3] Abnormal production of ROS may damage some macromolecules to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids; protein denaturation and DNA damage.[4,5] There is a continuous search for agents that provide nephroprotection against the renal impairment caused by drugs like cisplatin for which allopathy offers no remedial measures. Thus, it is obligated to turn towards alternative systems of medicine as natural products. So, plants which containing antioxidant properties are recommended as nephroprotective agents.[3,4,5]

Lantana camara L. (Family Verbenaceae) is distributed throughout many countries. It is an important medicinal plant, which exhibits anti-inflammatory, antihyperglycemic, antioxidant activity and hepatoprotective activities.[6,7,8] On the other hand, Several Squash plants (*Cucurbita pepo*) (Family Cucurbitaceae) have health benefits such as anti-diabetic, antifungal, antibacterial and anti-inflammation.[9,10] Therefore, the objective of this study is evaluating the nephroprotective effect of the defatted methanolic extract of the leaves of two plants grown in Egypt; *Lantana camara* and *Cucurbita pepo* (squash). Also,

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the identification of chemical constituents of *Lantana camara* by HPLC-ESI- MS was carried out.

MATERIALS AND METHODS

Plant materials

L. camara leaves were collected from Garden of Theodor Bilharz Research Institute, Giza, Egypt whereas, *Cucurbit pepo* leaves (Squash) were collected from local markets in Giza, Egypt. Both plants were identified by Asst.Prof. Rim Samir Hamdy Professor of Plant Taxonomy, Faculty of Science, CairoUniversity.[11] The voucher specimen of each plant was stored in Medicinal Chemistry Department, Theodor Bilharz Research Institute.

Chemicals

Cisplatin and Ascorbic acid were purchased from Sigma –Aldrich (USA), Lipid Peroxide (Malondialdehyde), Superoxide dismutase, Catalase and Glutathione peroxidase were purchased from Biodiagnostics, Egypt.

Extraction and fractionation

Leaves of each plant under investigation were dried and powdered by grinder. 500 g dry powders of each plant was separately extracted with 85% methanol (MeOH) then evaporated under vacuum using rotatory evaporator till dryness. The dried methanolic extract was defatted with petroleum ether. The aqueous defatted methanolic extract of the leaves of *L. camara* was fractionated using dichloromethane, ethyl acetate (EtOAc) and n-butanol (N-BuOH). Each fraction was concentrated and dried using rotatory evaporator then kept away from moisture.

Experimental animals

Male Wister albino rats, weighing 120-200 g were purchased from animal house of National Research Centre, Giza, Egypt. Animals were housed in polypropylene cages for one week under standard laboratory conditions (25±2˚C, 55±5% humidity and 12h light/ dark cycle). They were fed standard diet and water *ad libitum*. The experiment followed the ethical standards for care and use of laboratory animals.

Acute oral toxicity studies

Acute toxicity studies of the defatted methanolic extract of the leaves of each plant were performed according to.[12] The detailed experiments and results were reported in our previous study.[13]

Nephroprotective activity

Experimental design

Each group consists of 6 animals and divided as follows:

Group I (Normal control): was orally administered with 9% normal saline solution (1 ml/Kg .b. wt.) for 10 days.**Group 2 (Toxic control):** was injected intraperitoneally with cisplatin at a single dose of (10 mg/ Kg.b.wt.) on the 10th day. **Group 3 (standard group):** was orally administrated with ascorbic acid (200 mg/ kg.b.wt.).

Group 4, 5 and 6 (treated groups): were orally administrated with different concentration of the defatted methanolic extract of *L. camara* (100, 200 and 400 mg/kg.b.wt.). **Group 7,8 and 9 (treated groups):** were orally administrated with three concentrations of the defatted methanolic extract of Squash (*C. pepo*) (100,200 and 400 mg/kg.b.wt.). **Group 10, 11 and 12 (treated groups):** were orally administrated with different concentration of the ethyl acetate extract of *L. camara* leaves (100, 200 and 400 mg/kg. b. wt.). **Group 13, 14 and 15 (treated groups):** were orally administrated with different concentration of the butanolic extract of *L. camara* leaves (100, 200 and 400 mg/kg. b. wt.).

Ascorbic acid and all the extracts of above mentioned dose were administrated for 10 days before cisplatin. Whereas, the rats were recovered with free access to food and water *ad libitum* for five days after cisplatin injections (all experiment time 15 days).[14,15]

Blood collection

The animals were sacrificed by decapitation after 15 days and blood samples were collected from the tested animals in clean centrifuge tubes and centrifuged at 3000 rpm for 15 min. Serum parameters including creatinine, urea, magnesium, potassium and sodium were estimated. The biochemical estimations were done using a Biochemical-semi-auto analyser by standard procedures.

Calculate relative kidney weight

The kidneys and livers were isolated, weighed and relative weight of them was calculated from the ratio of organ weight to body weight.[16]

$$\text{Relative weight} = (\text{The organ wt.} / \text{Body wt.}) \times 100$$

Preparation of tissue sample

One kidney was homogenised in 5-10 ml/g tissue of cold phosphate buffer saline solution (PH 7.4) using an electronic homogenizer to prepare homogenate. The homogenate was centrifuged at 4000 rpm for 15 min at 4˚C then the supernatant was used for the estimation of lipid peroxidation as malondialdehyde (MDA),[17] superoxide dismutase (SOD),[18] catalase (CAT)[19] and Glutathione peroxidase (GPx).[20]

Histopathology studies

The other kidney was weighed and preserved in 10% neutral buffered formalin. A 5µ thickness of tissue sections was stained withhematoxylin and eosin stain (H and E stain) for routine histopathological examination.

HPLC-ESI-MS conditions

The samples (5 mg/mL) of *L. camara* extracts were injected to HPLC hyphenated with mass spectrometer.LC-ESI-MS analysis system consists of HPLC (Waters Alliance 2695) and mass spectrometry (Waters 3100). The mobile phases were prepared daily by filtering through 0.45 mm membrane disc filter and degassed by sonication before use. The mobile phase for gradient elution consists of two solvents: solvent A (0.1% formic acid (FA) in H_2O) and solvent B (0.1% FA in CH_3CN/MeOH (1: 1; v/v). The linear gradient profile was as follows: 95% A (5 min), 95-90% A (10 min), 90-50% A (55 min), 50-95% A (65 min), and 95% A (70 min). The injection volume was 10 μL. The flow rate (0.6 mL/min) was split 1: 1 before the MS interface. The negative ion mode parameters were as follows: source temperature 150°C, desolvation temperature 350˚C, cone gas flow 50 L/h, cone voltage 50 eV, capillary voltage 3 kV, and desolvation gas flow 600 L/h. Spectra were recorded in the ESI negative mode between m/m 50-1000. The peaks and spectra were processed using the Masslynx 4.1 software. The compounds were tentatively identified by comparing their retention time (*tR*) and mass spectrum with literature.

Statistical analysis

The results are reported as mean ± SD. Data were analyzed by using one-way analysis of variance (ANOVA) by SPSS software package. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Acute Toxicity

The purpose of preliminary acute toxicity studies is to determine the LD_{50} values to help us in evaluating the safe dose range at which the drug can be used without any harmful or lethal effect on the experimental

animals. In our study, results of each defatted methanolic extract of *L. camara* and *C. pepo* leaves revealed that there is no death or any clinical signs of toxicity in the tested rats. Therefore, the LD_{50} value of the defatted methanolic extract of each plant extract was found to be higher than 5000 mg/kg.[13]

Nephroprotective activity

Unfortunately, kidney diseases may be silent for a long time. Early detection is the key to preventing kidney diseases. The kidneys provide the final common pathway for the excretion of many drugs and their metabolites. Therefore, they are frequently subjected to high concentrations of potentially toxic substances.[21]

The present study is an effort to the evaluation of the potency of a famous natural antioxidant vitamin C.[22,23]

The body, relative kidney and liver weights

The results in (Table 1) exhibited that the initial body weights of all rats were almost the same. In the normal (control group), there is an obvious change in the body weights of rats between the initial and final body. The rat group treated with cisplatin (Toxic group) showed a significant decrease in their body weights as comparing with control group whereas, the relative weights of liver and kidney increased. These results suggested that the loss of weights of cisplatin-induced experimental animals may be due to the gastrointestinal toxicity of these animals and due to reducing of food ingestion by the animals.[24]

The administration of one dose of vitamin C (standard group) along with injection this group with of one dose of cisplatin on day 10 led to significant increase in the final body weights of the rats whereas decreased the relative kidney and liver compared with the toxic group. These results are in agreement with the previous results on other plant extracts.[25]

The groups treated with each dose 100, 200 and 400 mg /kg b.wt./day of the defatted methanolic extract of *L. camara* increased the final body weights of the rats whereas the relative kidney and liver were decreased comparing with the toxic group. The increasing of the final body rat weights is higher with a concentration of *L. camara* extract 400 mg / kg b.wt./day than concentration of 100 and 200 mg /kg b.wt./day. Also, the decreasing of the relative kidney and liver is associated with the high concentration of the plant extract 400 mg /kg b.wt./day as showed in (Table 1).

In the groups treated with the defatted methanolic extract of *C. pepo*, it was obvious that the increasing of the final body rat weights and the decreasing of the relative kidney and liver of rats are lesser than as in the defatted methanolic extract of *L. camara* as showed in (Table 1).

So, the defatted methanolic extract of *L. camara* had higher effect than the extract of *C. pepo*. Also the results appeared that the extract of each plant has the ability in protective of the rats against cisplatin-induced nephrotoxicity and improvement of the relative liver and kidney of the experimental rats. These results agree with previous studies on other plant extracts.[26,27]

Owing to the high nephroprotective activity of the defatted methanolic extract of *L. camara*, this extract was submitted to fractionation process using EtOAc and n-BuOH and the two fractions were evaluated as protective agents against cisplatin-induced nephrotoxicity for the experimental animals (Rats).Also, results in (Table 1) indicated the two fractions (EtOAc and n-BuOH) increase the final weight of rats and improve the relative kidneys of the rats as comparing with the toxic group. Also, EtOAc fraction showed higher effect than an n-BuOH fraction. Therefore this result means that the two fractions have nephroprotective effect against cisplatin- induced nephrotoxicity.

Kidney parameters

In the present study, the effect of oral doses 100, 200 and 400mg/kg b. wt./day of each defatted methanolic extract of *L. camara* and Squash (*C. Pepo*) on serum urea, creatinine, and certain ions in cisplatin-induced nephrotoxic rats were performed. The results in (Table 2) revealed that:

In a toxic group, it appears that there is the significant elevation of serum urea and creatinine values compared to the normal group. Also, the levels of sodium and potassium ions in the toxic group were significantly decreased compared to control group whereas, the magnesium ion was significantly increased. These results indicate that injury of the kidney of the rats occurred. [28]

Administration of the standard substance vitamin C (standard group) to rats which injected with one dose of cisplatin on day 10 led to significant reduction to the elevated levels of serum urea and creatinine as well as Mg ions whereas Na and K ions increased significantly comparing to the toxic group. The groups treated with different concentrations 100, 200 and 400 mg/kg/b.wt./day of each defatted methanolic extract of *L. camara* and *C. pepo* led to significant reduction of the levels of serum urea and creatinine as well as Mg ions values whereas Na and K significant increased compared to the toxic group. 400 mg/kg.b.wt./day of each plant extract gave good results. Also *L. camara* extract gave higher effect than *C. pepo* extract. The results mean that the two plant extracts have nephroprotective effect against cisplatin-induced nephrotoxicity and improve the kidney functions. These results are in agreement with a study on other plant extracts.[25]

Results in (Table 2) indicated the two fractions (EtOAc and n-BuOH) reduce the levels of serum urea and creatinine as well as Mg ions values whereas Na and K significant increased compared to the toxic group. 400 mg/kg b.wt./day of two fractions gave good results than 100 mg/kg b. wt./ day. Also, EtOAc fraction showed higher effect than an n-BuOH fraction. Therefore this result means that the two fractions have nephroprotective effect against cisplatin- induced nephrotoxicity.

Enzymatic antioxidants

It was reported that cisplatin increases the production of free oxygen radicals and decreases the antioxidants; this led to the disturbance of the oxidant/antioxidant balance.[29,30] The reactive oxygen radicals accumulated in tissues resulted in the production of hydroxyl radicals, superoxide anions, nitric oxide and hydrogen peroxide.[31] Thus the reactive oxygen species effect on the antioxidant defensemechanism through enhances lipid peroxidation process and led to the elevation of MDA levels[32] as well as reduce CAT, GPx and SOD levels. [33]

The results in a (Table 3) exhibited that: In (Toxic group), the antioxidant enzymes such as CAT, GPx and SOD significant decrease but MDA levels significant increase in the cisplatin group compared to the control. These observations reflected that the rat kidney is injured and this support that the nephrotoxicity induced by cisplatin in rats is occurred and partially related to the depletion of the renal antioxidant system. DeWoskin and Riviere[34] reported that this decreasing may either be due to loss of the certain mineral as copper and zinc, which are essential for the activity of the enzyme or due to ROS- induced inactivation of the enzyme. Moreover, the decreasing of CAT and GPx activities in the cisplatin group led to increasing the H_2O_2 concentration and enhanced the lipid peroxidation.[35] In the same time, the concentration of MDA as a result of lipid peroxidation increased in the toxic group.

Administration of vitamin C or the defatted methanolic extract of each plant *L. camara* and *C. pepo* prevents the lipid peroxidation by enhancing the renal SOD, CAT, and GPx activities. So, vitamin C or the defatted methanolic extract of each plant *L. camara* and *C. pepo* could significantly protect the cisplatin-induced renal damage. However, the nephro-

Table 1: Effect of graded oral doses 100-400 mg/ kg b. wt. / day of *L. camara* and Squash (*C. Pepo*) extracts on the average body weight and kidney and liver weights of cisplatin-induced nephrotoxic rats (g/animal).

Weight	Normal group	Toxic group	Standard group	C. pepo MeOH ext.			L. camara MeOH ext.			L. camara EtOAc. fraction			L. camara BuOH fraction		
				100	200	400	100	200	400	100	200	400	100	200	400
Initial body wt.	154±8.9	153.3±5.2	154.7±5.2	153.3±8.2	154.7±20.6	155±16.4	155.3±13.3	154±12.2	155±10.95	154.70±18.6	153.71±17.9	153.7±12.6	153.35±12.6	154.30±5.2	154.3±9.8
Final Body wt.	163.6±7.1	147.9±17.3#	152.8±13.4*	147.1±11.75	150.5±26.4	151.78±10.3	151.1±19.2	152.6±15.7*	153.5±20.7*	150.42±21.1	150.7±18.8*	151.5±11.1*	149.28±11.7	149.5±10.4	150.8±12.2*
Relative kidney wt.	0.77±0.9	0.97±0.5#	0.86±1.6*	0.93±0.6	0.92±0.87	0.91±1.26	0.90±1.04	0.91±1.4	0.89±0.46*	0.90±0.6	0.88±10.6*	0.87±0.96*	0.91±2.14	0.89±3.56*	0.88±1.2*
Relative liver wt.	3.40±12.5	3.80±3.64#	3.60±3*	3.70±4.9	3.67±5*	3.67±7.2*	3.65±3	3.63±4.5	3.62±3.2*	3.66±4.3	3.63±3.5	3.63±5.8*	3.69±5.2	3.67±4.3*	3.66±3.3*

Data represents as: mean±standered deviation, *: P<0.05 vs Toxic group, #: P<0.05 vs Normal group

Table 2: Effect of graded oral doses 100-400mg/ kg b. wt./ day of L. Camara and Squash (C. Pepo) extracts on serum urea, and creatinine, and certain ions in cisplatin-induced nephrotoxic rats.

Kidney parameters	Normal group	Toxic group	Standard group	C. pepo MeOH ext.			L. camara MeOH ext.			L. camara EtOAc. fraction			L. camara BuOH fraction		
				100	200	400	100	200	400	100	200	400	100	200	400
Urea (mg/l)	34.67±3.98	73±18.4#	52.60±11*	71.82±1.9	66.48±2.25	62.6±3.2	67.80±2.2	60.1±3.8*	55.97±3.6*	55.45±16.6*	54.60±2.9*	53.37±9.4*	57.18±2.6*	55.23±1.95*	54.12±2.7*
Creatinine (mg/dl)	0.46±0.025	0.72±0.06	0.53±0.04*	0.67±0.05*	0.66±0.04*	0.66±0.05*	0.63±0.07*	0.57±0.03*	0.55±0.03*	0.58±0.1*	0.55±0.05*	0.54±0.02*	0.59±0.05*	0.57±0.04*	0.55±0.03*
Na (mmol/)	137±3.58	118.67±2.7#	134.3±3.7*	122.7±3.6*	123.3±3.7*	126.2±6.5*	124.7±3.6*	126.7±4.2*	128.6±4.8*	127.7±4.9*	128.2±1*	132.8±1*	125.4±1.53*	126.3±0.58*	130.81±1*
K (mmol/l)	4.68±0.27	2.82±0.28#	4.40±0.14*	3.64±0.2*	3.66±0.3*	3.91±0.18*	3.73±0.23*	3.95±0.18*	3.97±0.23*	4.47±0.23*	4.44±0.18*	4.42±0.23*	4.49±0.74*	4.46±0.15*	4.44±0.2*
Mg (mmol/)	2.63±0.59	3.29±0.38#	2.72±0.38*	2.62±0.09*	2.63±0.12*	2.65±0.2*	2.67±0.2*	2.69±0.4*	2.70±0.38*	2.79±0.26	2.76±0.42*	2.74±0.67*	2.80±0.31*	2.78±0.25*	2.76±0.32*

Data represents as: mean±standered deviation, *: P<0.05 vs Toxic group, #: P<0.05 vs Normal group

Table 3: Effect of graded oral doses 100-400mg/ kg b. wt. / day of the defatted methanolic extract of *L. camara* and Squash (*C. Pepo*) on certain enzymatic antioxidants in normal and experimental rats in cisplatin-induced nephrotoxic rats

Enzymatic antioxidants	Normal group	Toxic group	Standard group	C. pepo MeOH ext.			L. camara MeOH ext.			L. camara EtOAc. fraction			L. camara BuOH fraction		
				100	200	400	100	200	400	100	200	400	100	200	400
CAT(nmol/)	49.89±12.9	30.27±8.5#	45.7±10.78*	31.2±4.68	34.7±4.6	35.2±10.4	36.8±9.7	38.28±2.2*	42.35±4.71*	39.88±20.6	40.52±22.6	43.08±2.56*	36.7±6.8	38.1±10.3	39.2±7.4*
GPx(U/gT)	59.1±20.78	32.4±15.76#	48±10.65*	32.68±12.61	36.08±9.4	38.38±13.4	41.67±5.44	42.1±11.05	45.50±13.7*	41.3±5.5	43.8±9.4*	46.22±12.6*	39.2±18.15	40.2±17.3	41.7±15.9*
SOD(nmol/g)	13.61±0.62	7.68±0.51#	11.44±1.09*	8.32±0.44	8.17±4.3	9.17±3.11	8.82±0.72*	9.37±2.34	9.79±0.63	9.9±0.7*	10.4±1.1*	10.89±1.52*	9.41±0.67*	9.82±1.6*	10.15±0.39*
MAD(nmol/)	35.08±5.94	70±22.28#	40.17±8.87*	52.28±6.8*	51.45±10.1*	49.31±21.5*	50.19±23.8*	47.17±2.4*	43.18±18.2*	48±32.8	47.1±14.95*	46.18±23.4*	52.11±4.75*	50.8±5.9*	48.25±10.4*

Data represents as: mean±standered deviation, *: P<0.05 vs Toxic group, #: P<0.05 vs Normal group

protective activity at (400 mg/kg. b. wt./ day) dose of each plant extract was higher than that at (100 mg/kg. b. wt./ day).

Results in (Table 3) revealed that both two fractions of *L. camara* (EtOAc and n-BuOH) showed a significant elevation in SOD, CAT, GPx enzymes while significantly decreased MDA levels. Although, the high dose (400 mg/kg.b.wt.) of the two fractions has a significant effect, but EtOAc fraction showed higher significant effect than an n-BuOH fraction.

Preliminary phytochemical screening of *L. camara* and *C. pepo* showed the presence of major antioxidant polyphenolic compounds in a chemical constituent of the two plants. These compounds protect the cisplatin-induced renal oxidative damage in rat and led to increasing of the renal antioxidant status, such as SOD, CAT, GPx activities by attack the free radicals (scavenges the free radicals) which generated during cisplatin-induced oxidative damage of renal parenchyma and improve the kidney functions.[24,36,37,38]

Cisplatin represents a class of antineoplastic drugs containing a heavy metal, platinum. It has been shown to cause nephrotoxicity in patients as well as in a variety of animal species.[26] A higher dose of cisplatin (10 mg/kg. b. wt.) was sufficient to induce nephrotoxicity in rats corresponds to that currently being used in clinical practice.[15,26] Although, the exact mechanism of cisplatin-induced nephrotoxicity is still not fully understood, however, cisplatin causes free radical production and lipid peroxidation in tubular cells. So, it has been suggested that these free radicals are responsible for the oxidative renal damage.[39,40] Therefore, the antioxidants especially natural antioxidants are a good nephroprotective agent against cisplatin-induced renal injury.[23,28,40,41]

Histopathological examination

Histopathological examination of the control group, toxic group, standard group and treated groups of kidney tissue of the experimental animals (Table 4) showed that: the control group exhibit normal renal cells as shown in (Figure 1). Cisplatin group (Toxic group) showed smaller glomeruli, interstitial oedema in tubular cells with mild hyaline casts in their lumens as in (Figure 2) comparing to control group. The standard group exhibits congested glomeruli as well as remarkable degeneration of the renal tubules with the presence of luminal hyaline casts and mild stromal interstitial mononuclear cellular infiltration as showed (Figure 3) compared to the toxic group. These results agreed with previous reports.[42,43]

The groups treated with concentrations 100 and 200 mg/kg. b. wt. of the defatted methanolic extract of *L. camara* showed average sized glomerulus with mild congestion as well as mild tubular degeneration and few luminal casts as shown in (Figure 4) compared to the toxic group. The group treated with concentration 400 mg/kg. b. wt. of the defatted methanolic extract of *L. camara* showed lesser glomerular congestion and milder degree of tubular degeneration as shown in (Figure 9) compared to the toxic group. The group treated with concentration 100 and 200 mg/kg. b. wt. of ethyl acetate fraction derived from the defatted methanolic extract of *L.* camara showed average sized glomeruli and mild tubular degeneration as shown in (Figure 5) compared to the toxic group. The group treated with concentration 400 mg/kg. b. wt. of ethyl acetate fraction derived from the defatted methanolic extract of *L. camara* showed normalisation of the histopathological features (Figure 7) Compared to the toxic group. The groups treated with concentrations 100 and 200 mg/kg. b. wt. of the butanolicfraction derived from the defatted methanolic extract of *L. camara* showed histopathological changes comparable to the cisplatin-treated group, except for the absence of stromal inflammation and mild tubular degeneration as showed in (Figure 6) compared to the toxic group (Figure 2).

The group treated with concentration 400 mg/kg. b. wt. of the butanolic-fraction derived from the defatted methanolic extract of *L. camara* has

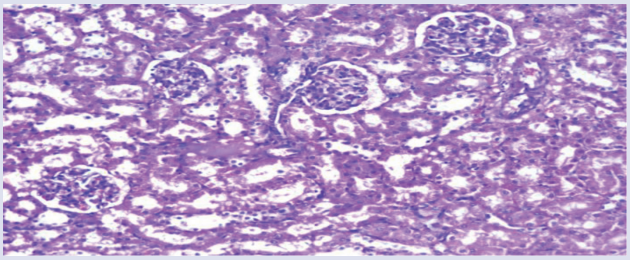

Figure 1: Section in kidney tissue of normal control rat, showing average sized glomeruli and proximal tubules with unremarkable pathological changes (Hematoxylin and eosin stain, X 200).

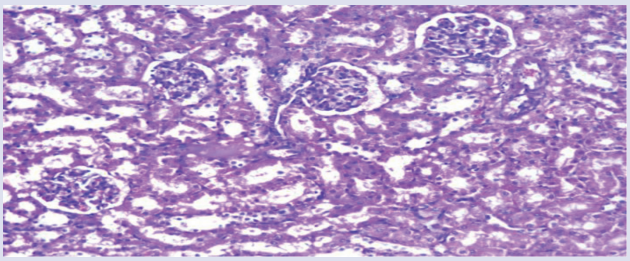

Figure 2: Section in kidney tissue of cisplatin-treated rat (toxic control), showing: (A) smaller glomeruli and degenerated proximal tubules.(B) degenerated distal tubules with many hyaline casts in their lumens (Hematoxylin and eosin stain, X 200).

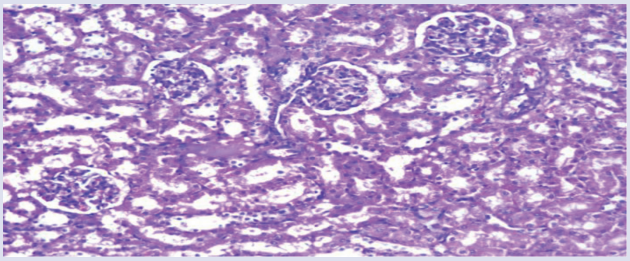

Figure 3: Section in kidney tissue of cisplatin-treated rat proceeded by vitamin C (200 mg/ kg b. wt. / day) (standard group), showing tubular degeneration and many luminal casts. (Hematoxylin and eosin stain, X400).

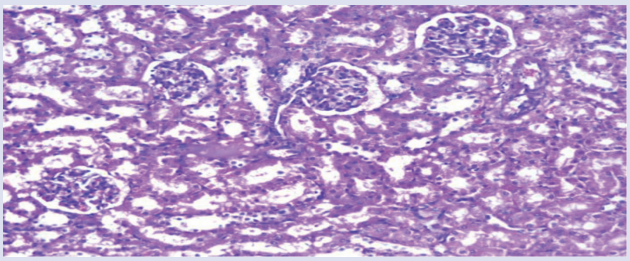

Figure 4: Section in kidney tissue of cisplatin -treated rat preceded by MeOH extract of *L. camara*(C) (100 mg/kgb.wt.) and (D) (200 mg/ kg b. wt. / day), showing average sized glomerulus with mild congestion as well as mild tubular degeneration and few luminal casts compared to the toxic group (Hematoxylin and eosin stain, X 400)

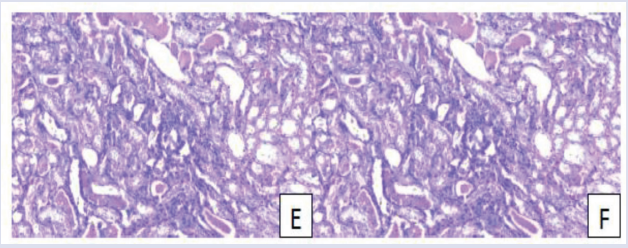

Figure 5: Section in kidney tissue of cisplatin -treated rat preceded by EtOAc fraction of L. *camara* (E) (100 mg/ kg b. wt. / day), showing average sized glomeruli and mild tubular degeneration (F) (200 mg/ kg b. wt. / day), showing moderate tubular degeneration and some luminal casts (Hematoxylin and eosin stain, X 200).

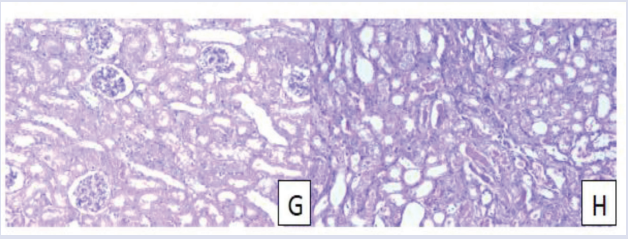

Figure 6: Section in kidney tissue of cisplatin -treated rat preceded by BuOH fraction of *L. camara*(G) (100mg/kg b.wt.) and (H) (200 mg/ kg b. wt. / day), showing moderate tubular degeneration and many luminal casts (Hematoxylin and eosin stain, X 200).

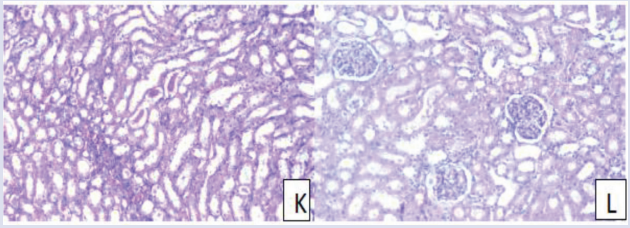

Figure 7: Section in kidney tissue of cisplatin -treated rat preceded by (K) EtOAc fraction of *L. camara* (400 mg/ kg b. wt. / day), showing average sized glomeruli and mild tubular degeneration, (L) BuOH fraction of *L. camara* (400 mg/ kg b. wt. / day), showing milder tubular degeneration and fewer luminal casts (Hematoxylin and eosin stain, X 200).

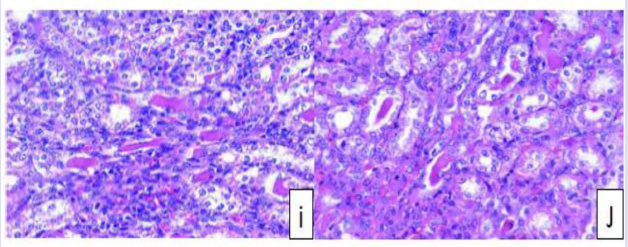

Figure 8: Section in kidney tissue of cisplatin -treated rat preceded by MeOH extract of *C. Pepo* (I) (100 mg/ kg b. wt. / day), showing tubular degeneration and many luminal casts, (J)(200 mg/ kg b. wt. / day), showing mild hydropic degeneration of distal tubules and fewer hyaline casts (Hematoxylin and eosin stain, X 400).

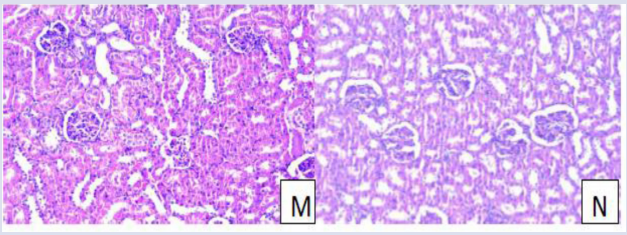

Figure 9: Section in kidney tissue of cisplatin -treated rat preceded by (M) MeOH extract of *L. camara* (400 mg/ kg b. wt. / day), showing milder degenerative changes of glomeruli and proximal tubules compared to the toxic group, (N) MeOH extract of *C. Pepo*(400 mg/ kg b. wt. / day), showing mild collapse of glomeruli and degeneration of proximal tubules (Hematoxylin and eosin stain, X 400).

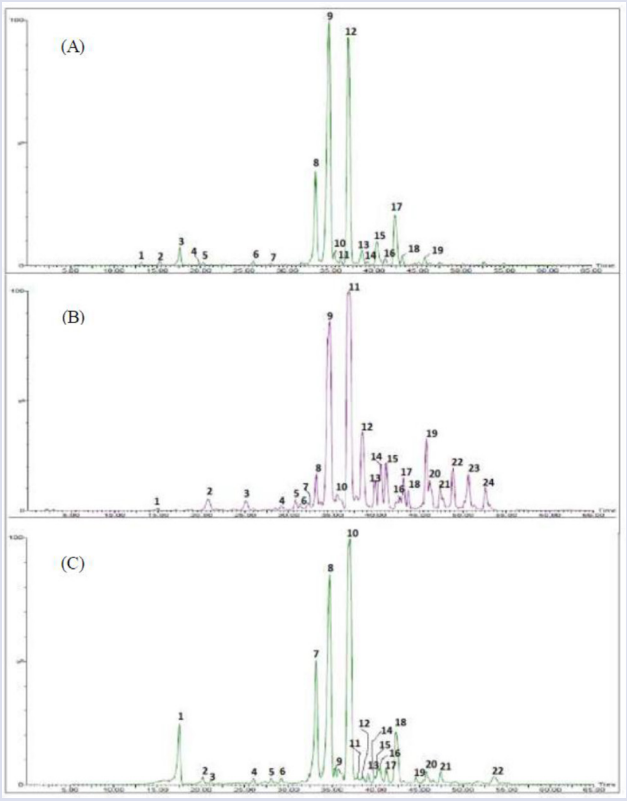

Figure 10: Total ion current (TIC) chromatogram of MeOH extract (A), EtOAc fraction (B) and BuOH fraction (C) of *L. camara.*

not showed glomerular congestion but mild interstitial infiltration by mononuclear inflammatory cells as showed in (Figure 7) Compared to the toxic group (Figure 2). These results indicated the nephroprotective activity of *L. camara* as reported in other previous studies.[44,45]

The groups treated with concentrations 100, 200 and 400 mg/kg. b. wt. of the defatted methanolic extract of *C. pepo* exhibited marked tubular degeneration and frequent luminal hyaline casts associated with glomerular congestion and stromal inflammation comparable to the group treated with cisplatin (Figure 8). However, the 400 mg/kg. b. wt. treated group showed milder stromal inflammation as shown in (Figure 9) compared to the toxic group (Figure 2).

The above results of histopathological examination of the rats exhibited that: The groups treated with the defatted methanolic extract of the two plants *L. camara* and *C. pepo* had a protective effect against degenerative kidney injury caused by cisplatin.

The defatted methanolic extract *L. camara* gave better protective property against the injury of kidney induced with by cisplatin in rats than *C. Pepo*. Also, it was obvious that the higher concentration of the ethyl acetate fraction derived from the defatted methanolic extract of *L. camara* has high protective effect against kidney injury in rats induced bycisplatin than the defatted methanolic extract and the butanolic fraction of *L. camara*.

The histopathological study of kidney against cisplatin-induced nephrotoxicity are in agreement with measurements of all parameters such as the changes in body weight, average kidney weights of rats and certain serum parameters as urea and creatinine as well as certain enzymatic antioxidants and ions of certain metals for toxic and treated groups.

HPLC-ESI-MS analysis of the defatted methanolic extract of *L. camara* as well as ethyl acetate and butanolic fractions

Owing to the high nephroprotective activity of the defatted methanolic extract of the leaves of *L. camara* as well as the ethyl acetate and butanolic fractions ,these extracts were submitted to HPLC ESI-MS analysis followed by identify of the major components of these extracts. ESI-MS full scan mode analyses were performed, in order to identify the deprotonated molecular ions $[M-H]^-$, followed by ESI-MS/MS product ion experiments in negative ionization mode using the deprotonated molecular ion as a precursor to studying the fragmentation of the compounds. The identification of each compound based on its main molecular ions and different fragments as well as on the reported data of standards fragmentation especially sugar moieties as 162 = hexose; 146 = deoxyhexose; 176 = glucuronic or galacturonic acid; 86 = malonyl residue.

The components of the defatted MeOH extract, EtOAc and BuOH fractions shown in (Figure 10) and (Tables 5, 6 and 7) respectively revealed that major components of these extracts are polyphenol groups such as phenolic acid derivatives, phenylethanoid glycosides, flavonoids and iridoids.

a-Phenolic acid derivatives

Peak 1 in EtOAc fraction (*tR*= 14.86 min) was identified as protocatechuic acid-*O*- rhamnosyl-hexoside with deprotonated molecule at *m/z* 461 $[M-H]^-$, other fragment ions at *m/z* 315 $[M-H-146]^-$, 153 $[M-H-146-162]^-$ and base peak at *m/z*109.[46] Peak 2 in EtOAc fraction (*tR*= 20.70 min) was identified as *p*-hydroxybenzoic acid, which represents $[M-H]^-$at *m/z* 137and base peak at *m/z* 93 $[M-H-44]^-$.[46] Peak 3 in EtOAc fraction (*tR*= 25.13 min) was identified as caffeic acid according to literature.[47] Peak 6 in EtOAc fraction (t_R= 31.39 min)exhibited a deprotonated molecular ion at m/z at 501 $[M–H]^-$, and other fragments at m/z 429, 267, these fragments are characteritic forFerulic acid derivative.[48,49] as shown in (Table 6).

b-Flavonoids

Peak 7 in MeOH extract (*tR*= 28.13 min) and peak 5 in BuOH fraction (*tR*= 28.05 min) as shown in (Table 5 and 7) were tentatively identified as apigenin 6, 8-di-*C*-glucoside (Vicenin-2), that had $[M-H]^-$ at *m/z* 593 and other fragment ions at *m/z* 473 $[M-H-120]^-$ and *m/z* 353 $[M-H-140]^-$ duo to liberation of two C-glucoside units.[50] Peak 11 in MeOH extract (*tR*= 36.15 min) as shown in Table (5) was tentatively identified as luteolin-*O*- dihexuronide-pentoside exhibited $[M-H]^-$ at *m/z* 769 and other fragments at *m/z* 593 $[M-H-176]^-$, *m/z* 417 $[M-H-(2\times176)]^-$ revealed that the loss of two hexuronide units, and *m/z* 285 $[M-H-(2\times176)-132]^-$ reflected

Table 4: Histopathological evaluation of kidney tissue in different groups:

Histopathological parameters	Normal group	Toxic group	Standard group	*C. Pepo* MeOH ext.			*L. Camara* MeOH ext.			L. Camara EtOAC Fr.			*L. Camara* BuOH Fr.		
				100 mg	200 mg	400 mg	100 mg	200 mg	400 mg	100 mg	200 mg	400 mg	100 mg	200 mg	400 mg
Glomeruli															
Number	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Congestion	-	++	++	++	++	++	+	+	+	-	-	-	+	+	-
Size	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
Tubules															
Degeneration	-	++	+++	+++	+++	+++	++	++	+	++	++	+	++	++	+
Casts	-	+	+	++	++	++	+	+	-	+	+	+	+	+	-
Stroma															
Inflammation	-	+	+	++	+	-	-	-	-	+	+	+	-	-	-
Fibrosis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Control showed normal glomerular and tubular histology whereas cisplatin treated rats shows glomerular capillary congestion and tubular degeneration as well as patchy mononuclear cell infiltrated mainly in the sub-capsular region.

Table 5: Tentative identification of polyphenol compounds in defattedmethanolic extract of *L. camara*by HPLC-ESI -MS.

Peak No.	t_R(min)	MW	$[M-H]^-$	MS fragments	Proposed compounds
1	13.10	390	389	345, 209, 121	Theveside
2	15.28	554	553	461, 315, 209, 165	Phenylethanoid derivative
3	17.61	462	461	315, 161, 135	Verbasoside
4	19.95	594	593	461, 179, 161	Verbasoside -pentoside
5	20.37	488	487	179 , 161, 135, 93	Cistanoside (F)
6	26.05	388	387	207	Tuberonic acid glucoside
7	28.13	594	593	473,383, 353, 179, 161	apigenin 6,8-di C-glucoside (vicenin 2)
8	33.23	756	755	593, 179, 161	Forsythoside B
9	34.90	624	623	461, 315, 161	Verbascoside
10	35.48	770	769	706, 623, 461, 161	Leonoside A
11	36.15	770	769	593, 417, 285, 161	Luteolin- di-O-hexuronide-pentoside.
12	37.07	624	623	461, 315, 161	Isoverbascoside
13	38.66	624	623	491, 315, 300, 161, 133	Isorhamntin-*O*- pentosyl- hexuronide.
14	39.24	594	593	461, 299 (100%), 179, 161	Hispidulin-*O*- pentosyl- hexoside
15	40.33	784	783	621, 461, 299 (100%), 285	Hispidulin-tri-*O*- hexoside
16	41.24	638	637	461,193, 175.161	Cistanoside C
17	42.58	476	475	299 (95%), 285	Hispidulin-7-*O*- hexuronide
18	43.25	652	651	475,193, 175	Martynoside
19	45.92	652	651	475, 193, 175	Iso-martynoside

Table 6: Tentative identification of polyphenol compounds in ethyl acetate fraction derived from the defatted methanolic extract of L. camara by HPLC-ESI -MS.

Peak No.	t_R(min)	MW	$[M-H]^-$	MS fragments	Proposed compounds
1	14.86	462	461	315, 153, 109 (100%)	Protocatechuic acid- *O*- rhamnosyl -hexoside
2	20.70	138	137	93 (100%), 65	*p*- Hydroxybenzoic acid
3	25.13	180	179	135 (100%),107	Caffeic acid
4	29.14	622	621	487, 179, 161	Isomer of suspensaside A
5	30.72	624	623	477, 461, 315	Forsythoside A
6	31.39	502	501	429, 267, 193 (100%), 161	Ferulic acid derivatives
7	32.31	638	637	475, 293, 179, 161, 135	Phenylethanoid derivative
8	33.14	756	755	593, 179, 161	Forsythoside B
9	34.65	624	623	461, 315, 161	Verbascoside
10	35.73	654	653	621 (100%), 179, 161	Suspensaside methyl ether
11	36.9	624	623	461, 315,161	Isoverbascoside
12	38.49	638	637	461, 315, 133	Cistanoside C
13	39.91	608	607	461, 179, 161, 145	Lipedoside A
14	40.58	784	783	621 (100%), 461, 161	Leucosceptoside B
15	41.24	638	637	461,193, 175.161	Isocistanoside C
16	42.75	778	577	461, 299, 179, 161	Phenylethanoid derivative
17	43.25	652	651	475, 193, 175	Martynoside
18	43.75	592	591	445, 193, 179, 163, 145	Osmanthuside B
19	45.84	652	651	475, 193,175	Isomartynoside
20	46.34	592	591	269, 179, 161	Apigenin-di-O- hexoside
21	47.42	624	623	489 (100%), 285, 161	Luteolin-O-pentosyl-acetylhexoside
22	48.93	592	591	445, 193, 179, 163, 145	Osmanthuside B isomer
23	50.68	570	569	299, 161	Hispidulin-di-O- pentoside
24	52.68	300	299	285 (100%), 137	Hispidulin

Table 7: Tentative identification of polyphenol compounds in butanolic fraction derived from the defatted methanolic extract of L. camara by HPLC-ESI -MS.

Peak No.	t_R(min)	MW	[M-H]$^-$	MS fragments	Proposed compounds
1	17.45	462	461	315, 297, 135	Verbasoside
2	20.29	488	487	461, 299, 179 (100%), 161, 93	Cistanoside F
3	21.07	608	607	461, 179, 161	Lipedoside A
4	26.05	388	387	207, 161	Tuberonic acid glucoside
5	28.05	594	593	473, 383, 353, 179, 161	apigenin 6,8-di C-glucoside (vicenin2)
6	29.22	622	621	487, 179, 161	Suspensaside A
7	33.14	756	755	593, 179, 161	Forsythoside B
8	34.56	624	623	461,315, 161	Verbascoside
9	35.32	770	769	623,706, 461, 179, 161	Leonoside A
10	36.99	624	623	461, 315, 161	Isoverbascoside
11	37.99	770	769	623,607, 461, 179, 161	Poliumoside
12	38.49	624	623	491, 315, 300, 161, 133	Isorhamntin-*O*- pentosyl- hexuronide.
13	39.16	594	593	461, 299 (100%), 179, 161	Hispidulin-*7-O*- pentosyl- hexoside
14	39.99	608	607	461, 179, 161	Lipedoside A
15	40.24	784	783	621, 461, 299 (100%), 284, 161	Hispidulin-tri-*O*- hexoside
16	40.58	622	621	461 (100%), 299, 283, 161	Hispidulin-di-*O*- hexoside
17	41.24	638	637	461,193, 161	Cistanoside C
18	42.33	476	475	299, 284	Hispidulin-7-O- hexuronide
19	44.58	680	679	619, 461, 179, 161	Cistanoside K
20	45.84	652	651	621, 487, 179, 175	Isomartynoside
21	47.42	624	623	461, 161	Isoverbascoside
22	53.60	828	827	623, 179, 161, 135	Acetyl echinacoside

the loss of pentose unit. Peak 21 in EtOAc fraction (*tR*= 47.42 min) as shown in (Table 6) showed [M-H] at *m/z* 623, base peaks at *m/z* 489 [M-H-134]$^-$ and *m/z* 285 [M-H-134-204]$^-$. This reflected the loss of pentose unit and acetyl hexoside unit. So, it was tentatively identified as Luteolin-*O*-pentosyl-acetyl hexoside. Peak 13 (*tR*= 39.91min) in MeOHas in (Table 5) and peak 12 in BuOH fraction (*tR*= 38.49 min) as in (Table 7) showed [M-H]$^-$*m/z* 623 and other fragments at *m/z* 491 [M-H-132]$^-$ and 315 [M-H-132-176]$^-$, this reflected that the liberation pentose and hexuronide units. So, this compound was identified as isorhamnetin-*O*- pentosyl- hexuronide. Peak 20 in EtOAc fraction (*tR*= 46.34 min) as in (Table 6) was tentatively identified as apigenin-di-*O*- hexosides that had [M-H]$^-$*m/z* 591 [M-H]$^-$ and another fragment at *m/z* 269 [M-H-322]$^-$, this indicated the loss of two hexoside units. Peak 14 in MeOH (*tR*= 39.24 min)as in (Table 5) and Peak 13 in BuOH fraction (*tR*= 39.16 min)as in (Table 7) showed [M-H]$^-$ at *m/z* 593 and other fragments at *m/z* 461 [M-H-132]$^-$ and 299[M-H-132-162]$^-$. Therefore, this compound was characterized as hispidulin-*O*- pentosyl- hexoside**.** Peak 15 in MeOH (*tR*= 40.33 min) as in (Table 5) and peak 15 in BuOH fraction (*tR*= 40.24 min) as in (Table 7) was identified as hispidulin-tri-*O*- hexosides as suggested from deprotonated molecular ion at at *m/z* 783[M-H]$^-$ and other fragments at *m/z* 621 [M-H-162]$^-$, 461 [M-H-(2×162)]$^-$ and 299 [M-H-(3 ×162)]$^-$. Peak 16 in BuOH fraction (*tR*= 40.58 min) as in (Table 7) was characterized as hispidulin-di-*O*-hexosides which have [M-H]$^-$ at *m/z* 621 and other fragments at *m/z* 461 [M-H-162]$^-$ and 299 [M-H-(2×162)]$^-$. Peak 17 in MeOH (*tR*= 42.58 min) as in (Table 5) and peak 18 in BuOH fraction (*tR*= 42.33 min) as in (Table 7) were identified as of hispidulin-7-*O*- hexuronoidedue to it had *m/z* 475 [M-H]$^-$ and base peak at *m/z* 299 (100%) [M-H-176 (hexuronoide)]$^-$. Peak 23 in EtOAc fraction (*tR*= 50.68 min) as in (Table 6) showed a deprotonated molecular ion at *m/z* 569 [M-H]$^-$ and another fragment at *m/z* 299 (100%) [M-H- 2x pentoside]$^-$ reflected to the liberation of two pentose units. Therefore, this compound was tentatively identified as Hispidulin-di-*O*-pentoside. Peak 24 in EtOAc fraction (*tR*= 52.68 min) showed *m/z* 569 [M-H]$^-$ and another fragment at *m/z* 299 (100%) [M-H- (2×132)]$^-$. So, this compound was identified as Hispidulin-di-*O*-pentoside.[51]

c-Phenylethanoids

Peak 3 in MeOH extract (*tR*= 17.61 min) as in (Table 5) and Peak 1 in BuOH fraction (*tR*= 17.45 min) as in (Table 7) exhibited *m/z* 461[M-H]$^-$ other fragment ions appeared at *m/z* 315 and 297 corresponding to the loss of rhamnose moiety followed by water elimination. Also, other fragments were detected at *m/z* 161 and 135. Therefore, this compound was identified as Decaffeoyl-verbascoside (verbasoside). Peak 4 in MeOH extract (*tR*= 19.95 min) as in (Table 5) exhibited *m/z* 461[M-H]$^-$ other fragment ions appeared at *m/z* 315 and 297 corresponding to the loss of rhamnose moiety followed by water elimination. Also, other fragments were detected at *m/z* 161 and 135. Therefore, this compound was identified as Decaffeoyl-verbascoside (verbasoside)[52] (Quirantes-Pine *et al.,* 2009). Peak 5 in MeOH extract (*tR*= 20.37 min) as in (Table 5) and Peak 2 in BuOH fraction (*tR*= 20.29 min) as in (Table 7) were identified as Cistanoside F due to it had [M-H]$^-$*m/z* 487. Other fragment ions were appeared at *m/z* 179 (100%) [M-H-caffeoyl-rhamnosyl]$^-$, *m/z* 161 [M-H- caffeoyl- rhamnosyl- H_2O]$^-$, and *m/z* 135 [caffeic acid-CO_2].[52] Peak (16) in MeOHextract (*tR*= 41.24 min), peak 15 in EtOAc fraction (*tR*= 41.24 min), and peak 17 in BuOH fraction (*tR*= 41.24 min) gave *m/z* 637 [M-H]$^-$, *m/z* 461, 193 and 161. Therefore, this compound was

tentatively identified as Cistanoside C according to literature.[53,54] Peak 8 in MeOHextract (*tR*= 33.23 min) as in (Table 5), Peak 8 in EtOAc fraction (*tR*= 33.14 min) as in (Table 6) and Peak 7 in BuOH fraction (*tR*= 33.14 min)as in (Table 7) were identified as Forsythoside B. It exhibited deprotonated molecular ion at *m/z* 755 $[M-H]^-$, other ions at *m/z* 593 [M-H- caffeoyl]$^-$ and *m/z* 461[M-H- caffeoyl- pentose]$^-$.[55,56] Peak 9 in MeOH extract (*tR*= 34.90 min)as in (Table 5), peak 9 in EtOAc fraction (*tR*= 34.65 min) as in (Table 6) and peak 8 in BuOH fraction (*tR*= 34.56 min) as in (Table 7) exhibited *m/z* 623 $[M-H]^-$ and other fragments at *m/z* 461 [M-H- 162]$^-$ and *m/z* 315 [M-H-162-146]$^-$ correspond to the liberation of caffeoyl moiety and, rhamnose unit. Therefore this compound was tentatively identified asVerbascoside.[57,58,59,60] Peak 12 in MeOH extract (*tR*= 37.07 min) as in (Table 5), peak11 in EtOAc fraction (*tR*= 36.90 min) as in (Table 6), and peaks 10 and 21 in BuOH fraction (*tR*= 36.99 and 47.42 min, respectively) as in (Table 7) were identified as iso-verbascoside according to literature.[52,57,60] Peak 18 in MeOH extract (*tR*= 43.25 min), and peak 17 in EtOAc fraction (*tR*= 43.25 min) were identified as martynoside, that gave *m/z* 651 $[M-H]^-$, and another fragment ion was detected at *m/z* 475 [M-H- 176 due to the loss of feruloyl moiety.[60,61] Peak 19 in MeOH extract (*tR*= 45.92 min) as in (Table 5), peak19 in EtOAc fraction (*tR*= 45.84 min) as in (Table 6) and peak 20 in BuOH fraction (*tR*= 45.84 min) as in (Table 7) exhibited deprotonated molecular ion at *m/z* 651 $[M-H]^-$ was identified as isomartynoside according to literature.[60] Peak 10 in MeOHextract (*tR*= 35.48 min) and peak 9 in BuOH fraction (*tR*= 35.32 min) gave molecular ion peak *m/z* 769 $[M-H]^-$and other fragments at *m/z* 623[M-H-146]$^-$, *m/z* 461[M-H-146- 162]$^-$ revealed to the loss of rhamnosyl unit and caffeoyl moiety respectively. Thus, this compound was identified as leonoside A.[62,63] Peak 11 in BuOH fraction (*tR*= 37.99 min) as in (Table 7) was identified as polumoside. it showed deprotonated molecule at *m/z* 769 $[M-H]^-$, other fragment ions at *m/z* 623 [M-H-146 (rhamnosyl unit)]$^-$ and *m/z* 607,461, 179 and 161.[64] Peak 4 in EtOAc fraction (*tR*= 29.14 min) as in (Table 6) and peak 6 in BuOH fraction (*tR*= 29.22 min) as in (Table 7) gave a molecular ion at *m/z* 621 $[M-H]^-$. Other fragment ions of this compound appeared at *m/z* 179 and 161. This fragmentation pathway is characteristic for suspensaside A.[52,65] Peak 10 in EtOAc fraction (*tR*= 35.73 min) as in (Table 6) was characterized as suspensaside methyl ether because it had a molecular ion at *m/z* 653 $[M-H]^-$ and another fragment at *m/z* 621 $[M–H- OCH_3]^-$.[66] Peak 13 in EtOAc fraction (*tR*= 39.91 min) as in (Table 6) and peaks 3 and 14 in BuOH fraction (*tR*= 21.07 and 39.99 min, respectively)as in (Table 7) were was tentatively identified as lipedoside A, it had a molecular ion at *m/z* 607 $[M-H]^-$, other fragment ions at *m/z* 461[M-H-146 (rhamnose unit)]$^-$ .The characteristic ions at *m/z* 163 and 145 suggested the presence of a coumaroyl moiety.[65] Peak 5 in EtOAc fraction (*tR*= 30.72 min) as in (Table 6) was identified as Forsythoside A according to literature, it had a deprotonated molecular ion at *m/z* 623. The fragmentation pattern of this compound gave fragment ions *m/z* 477 [M-H- rha]$^-$, m/z 461 [M–H-caffeoyl]$^-$ and 315 [461-rha]$^-$.[66]

Peak 12 in EtOAc fraction (*tR*= 38.49 min) as in (Table 6) showed a deprotonated molecular ion at *m/z* 637$[M–H]^-$. Other fragment ions were appeared at *m/z* 461[M-H-176 (feruloyl)]$^-$, 193 and 133.So, it was tentatively characterized as leucoceptoside A.[59,61,63] Peak 14 in EtOAc fraction (*tR*= 40.58 min) as in (Table 6) was tentatively identified as leucosceptoside B, which showed deprotonated molecular ion at *m/z* 783 $[M-H]^-$. High abundance fragment ions were observed at *m/z* 607 [M-H-176]$^-$ and *m/z* 461[M-H-176-146]$^-$ suggested the loss of feruloyl unit, and rhamnose unit.[65] Peaks 18 and 22 in EtOAc fraction (*tR*= 43.75 and 48.93 min, respectively) as in (Table 6) was identified as osmanthuside B due to it had $[M-H]^-$at *m/z* 591, other fragments at *m/z* 445, 163 and 145.[53,65] Peak 19 in BuOH fraction (*tR*= 44.58 min) as in (Table 7) gave a deprotonated molecular ion at *m/z* 679 $[M-H]^-$. Other fragment ions appeared at *m/z* 619, 461, 179 and 161. This fragmentation pattern is full agreement with Cistanoside K.[67,68] Peak 22 in BuOH fraction (*tR*= 53.60 min) as in (Table 7) was tentatively identified as acetyl echinacoside according to literature because it had a deprotonated molecular ion at *m/z* 827 $[M-H]^-$, and other fragment ions at *m/z* 723 [M-H-hexose- acetyl unit] and *m/z* 577 [M-H- pentose-acetyl-rhamnose].[65]

d-Iridoids

Peak 1 in MeOH extract (*tR*= 13.10 min) as in (Table 5) was tentatively identified as theveside with *m/z* 389 $[M-H]^-$. Other fragment ion appeared at *m/z* 345 [M-H-44] $^-$ correspond to the loss of CO_2 molecule, *m/z* 209 and 121. The fragment at m/z 121 corresponded to the elimination of the 3-oxopropanic acid.[52,69]

e-Other compound

Peak 6in MeOH extract (*tR*= 26.05 min) as in (Table 5) was identified as tuberonic acid -*O*- glucoside and demonstrated $[M-H]^-$ *m/z* 387 and other fragment ions at *m/z* 207 [M-H-Glc-OH]$^-$, which were consistent with the loss of glucoside moiety followed by successive dehydration and decarboxylation, respectively.[69]

CONCLUSION

Each defatted methanolic extract of the two plants under investigation is found to have nephroprotective effect on cisplatin-Induced nephrotoxicity in rats. Also, ethyl acetate and butanolic which derived from the methanolic extract of *L. camara* had high activity. The results showed that there is a positive correlation between the plants phytochemical constituents (phenolic acid derivatives, favonoids, phenylethanoids and Iridoids) and their nephroprotective activity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATION USED

ROS: Reactive Oxygen Species; **EtOAc:** Ethyle Acetate; **n-BuOH:** n-Butanol; **MDA:** Malondialdehyde; **SOD:** Superoxide dismatase; **CAT:** Catalase; **GPx:** Glutathione peroxidase.

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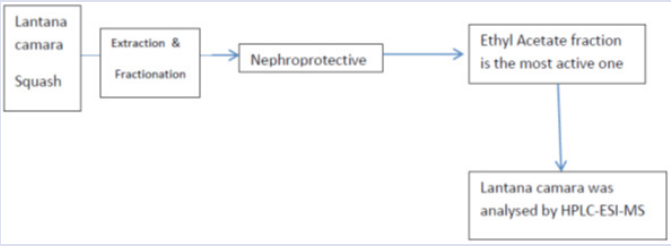
GRAPHICAL ABSTRACT



SUMMARY

- This study tested the nephroprotective activity of *Lantana camara* and Squash on cisplatin induced nephrotoxicity in rats.
- Both plants showed promising nephroprotective effect on kidney functions, antioxidant enzymes as well as histopathological studies on kidney tissue especially the ethyl acetate fraction of *Lantana camara*.
- HPLC-ESI-MS analysis of *Lantana camara* extracts demonstrate the presence of bioactive phenolic compounds including phenyl ethanoid, flavonoids and phenolic acids.

ABOUT AUTHORS

Heba Abdel-hady: Lecturer of Medicinal Chemistry, Meicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Mortada Mohamed El-Sayed: Professor of Medicinal Chemistry, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Afaf Ahmed Abdel-Hady: Professor of Clinical Chemistry, Clinical Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Maher Mahmoud Hashash: Professor of Organic Chemistry, Department of Chemistry, Faculty of Science, Ain Shams University, Cairo, Egypt.

Ahmed Mahamed Abdel-Hady: Professor of Pathology, Pathology Department, Theodor Bilharz Research Institute, Giza, Egypt.

Tarek Aboushousha: Professor of Pathology, Pathology Department, Theodor Bilharz Research Institute, Giza, Egypt.

Sayed Saleh Abdel-Hameed: Professor of Medicinal Chemistry, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Ezzat El-Sayed Abdel-Lateef: lecturer of Medicinal Chemistry, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

Eman Abdalla Morsi: Lecturer of Medicinal Chemistry, Medicinal Chemistry Department, Theodor Bilharz Research Institute, Giza, Egypt.

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