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Ameliorative Effects of *Urtica dioica* Aqueous Extract against Hepatotoxicity and Nephrotoxicity Induced by Insecticide Mixture in Adult Male Rats

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ABSTRACT

The present study was conducted to investigate the protective effects of *Urtica dioica* (*U. dioica*) against hepatotoxicity and nephrotoxicity induced by insecticide mixture deltamethrin (DLM) and chlorpyrifos (CPF). The rats were divided into five experimental groups: the control group, the group treated with deltamethrin and chlorpyrifos insecticide mixture, the group received a mixture of vitamins CE and insecticide mixture (deltamethrin and chlorpyrifos), the group received an aqueous extract of *U. dioica* and the group treated with the aqueous extract of *U. dioica* and simultaneously receive the mixture of deltamethrin and chlorpyrifos. *U. dioica* was found to contain large amounts of polyphenols (712.37µg/g GAE/mg of a dry plant). DLM/CPF induced a disturbance of red blood cells (RBC), hemoglobin (HB), and hematocrit (Ht), an increase in aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), lactate dehydrogenase (LDH), Alkaline phosphatase (ALP), creatinine, urea and uric acid levels, a significant increase in thiobarbituric acid reactive substances (TBARS), conjugated diene (CD), carbonyl protein (CP) and advanced oxidation of protein products (AOPP) levels. Our results revealed that *U.dioica* has a therapeutic effect on some health problem and has an important antioxidant and antiradical activity.

Keywords: *Antioxidant, U. Dioica, Deltamethrine, Chlorpyrifos, Oxidative stress, Toxicity.*

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INTRODUCTION

Pesticides are lipophilic compounds that involve low toxicity to mammals and neurotoxic effects [1-5]. Their use in agriculture is a major source of contamination that causes health problems. Exposure to different classes of pesticides such as organochlorines (OCs), organophosphorus (OP), carbamates and pyrethroids is responsible for neurological, carcinogenic, dermatological, respiratory, gastric, reproductive, and congenital problems [6]. Several studies have shown that pyrethroids caused alterations in hematologic, biochemical, and reproductive parameters [7]. In this context, we noted that DLM and CPF are two insecticides that belong to the different classes of pyrethroid (PYR) and organophosphorus (OP) respectively, their harmful effects are well studied [8]. Several studies have shown that DLM, a pyrethroid type II, that is widely used in agriculture and also in different domestic areas. Recent studies have indicated immunotoxic and genotoxic effects of DLM in mammalian species [9]. On another hand, CPF is responsible for oxidative stress, inflammation, and apoptosis [10]. The adverse effects of CPF led to a reduction in its use in the United States in 2002 [8]. The use of medicinal plants in folk

medicine is based on empirical knowledge for centuries by different populations. Their potential antioxidants are a strategy to prevent oxidative damage in healths [11]. In our case, *U. dioica* is defined as a medicinal plant. Several works have shown the beneficial effects of this plant in the treatment of nasal and menstrual hemorrhages, anemia, nephritis, hematuria, and so for the purification of blood. It has therapeutic properties on diabetes, atherosclerosis, cardiovascular diseases, and prostate cancer [12]. The use of this plant is used in traditional medicine to treat allergy, renal limestones, burns, internal bleeding, and diabetes [13]. This plant is also used to treat stomachaches in traditional medicine in Turkey and Iran. Also, it is used to treat rheumatic pain colds, cough [14], hypertension, allergic rhinitis, and cardiovascular [15]. It can lower the blood pressure by its diuretic action, reduces the tension at the vascular level [16]. The use of the aqueous extract of our plant showed a hypoglycemic effect, as well as the application of the hydroalcoholic extract of the leaves on the rats, shows an improvement of the secretion of insulin in the blood [17]. Therefore, the present study was conducted to find the protective effects of *U. dioica* extract against DLM and CPF induced hepatotoxicity, nephrotoxicity, and oxidative stress in rats.

MATERIALS AND METHODS

Plant Preparation of *U. dioica*

The leaves of the fresh plant *U.dioica* were dried at room temperature for a week in the laboratory. 10g of *U. dioica* leaves powder were boiled with 100 ml of distilled water for 20 min with occasional stirring. The decoction preparation was filtered and the extracts were then lyophilized for 48hours. The obtained lyophilic sate was stored at +4°C until its use.

Determination of total poly-phenolic content

Total phenolics content was determined using the Folin-Ciocalteu method of Wolfe et al. [18] adapted to a microscale, 10 µl diluted extract solution was shaken for 5 min with 50µl Folin-Ciocalteu reagent. Then, 150 µl of 20% Na_2CO_3 was added and the mixture was shaken once again for 1 min. Finally, the solution was brought up to 790µl by adding distilled water. After 90min, the absorbance at 760 nm was evaluated using a spectrophotometer; gallic acid was used as a standard for the calibration curve. The phenolic content was expressed as mg gallic acid equivalent/gram of dry extract using the linear equation based on the calibration curve.

Fer reducing antioxidant power (FRAP)

Reducing power is a significant indicator of the potential for antioxidant activity [19]. A volume of 0.5 ml of different concentrations of the extracts and the standard antioxidant (ascorbic acid) are mixed with 1ml of a 1% solution of potassium ferricyanide [$K_3Fe(CN)_6$] and 1ml of phosphate buffer (0.2 M, pH 6.6). The mixture is incubated at 50°C for 20 min, 1 ml of a trichloroacetic acid (TCA) solution (10%) is added to stop the reaction. Centrifugation for 10 min at 3000 rpm. After 1.5 ml of the supernatant is mixed with 1.5 ml of distilled water and 0.5 ml of iron chloride $FeCl_3$ (0.1%). Incubation for 10 minutes, the absorbance is measured at 700 nm. Ascorbic acid is used as a standard.

Scavenger power of 2, 2-diphenyl-1-picrylhydrazyle (DPPH)

The chemical compound 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was one of the first free radicals used to study the antioxidant structure-activity relationship of phenolic compounds [20]. In the presence of free radical scavengers, DPPH (2, 2 Diphenyl 1 picryl hydroxyl) violet color is reduced to 2, 2 diphenyl 1 picryl hydrazine yellow. A volume of 0.5 ml of different concentrations of the extracts and the standard antioxidant (ascorbic acid) is mixed with0.5ml of DPPH dissolved in methanol. After incubation 30 minutes in the dark, reading the absorbance is carried out at 517 nm. The anti-radical activity was evaluated concerning solution 100% which contains absolute methanol and DPPH solution.

Animals and experimental design

Adult male albino *Wistar* rats were obtained from the central pharmacy of Tunisia (SIPHAT, Tunisia). The animals were handled under standard laboratory conditions of a 12h light/dark cycle in a temperature and humidity controlled room. Food and water were available ad libitum. Our Institutional Animal Care and Use Committee approved the protocols for the animal study, and the animals were cared for following the institutional ethical guidelines.

After two weeks of acclimatization, the rats were divided into the following groups:

1. group 1 control rats receiving distilled water and a standard diet ad libitum.
2. group2 (DLM/CPF): the rats received a mixture of DLM/CPF by gavage (1mg/kg/day of DLM +5mg/kg/day of CPF respectively) [21] for 20 days.
3. group 3 (Vit CE+DLM/CPF): the rats received a mixture of vitamins CE (the dose of each vitamin is 100 mg/kg /day) [22] during 20 days of treatment and simultaneously received the mixture of DLM/CPF during the last 10 days by gavage.
4. group 4 (*U. dioica*): rats received an aqueous extract of *U. dioica* (200mg/Kg/day)by gavage for 20 days.
5. group5 (*U. dioica*+DLM/CPF): the rats received the aqueous extract of *U. dioica* (200mg/Kg/day) and simultaneously receive the mixture of DLM/CPF(1mg/kg/day of DLM +5 mg/kg/day of CPF, respectively)during the last 10 days by gavage.

Body weight was monitored throughout the treatment; the animals were killed on day 20 by decapitation. Blood samples were collected; allowed to clot at room temperature and serum was separated by centrifuging at 2700 g for 15 min for various biochemical parameters. The liver and kidney were quickly excised, minced with ice-cold saline blotted on filter paper. Homogenates were centrifuged at 10000g for 15 min at +4°C (Ultra Turrax T25, Germany) (1:2, w/v in 50 Mm/l phosphate buffer (pH= 7.4)). The supernatant and serum were frozen at 30°C in aliquots until analysis.

- **Hematology parameters**

Red blood cell count (RBC), Hemoglobin (Hb), hematocrit (Ht), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH),mean corpuscular hemoglobin concentration (MCHC), and White blood cell count (WBC) were measured with an automate Sysmex Kx-21N (CHU Habib Bourguiba Sfax).

- **Serum parameters**

Aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine, urea, and uric acid were determined using standardcommercial kits (France) (Ref: 80127; 92027; 80107; 80221; 020141, respectively) which were measured with an automate in CHU Hedi Chaker of Sfax, Tunisia. The activity of serum enzymes was expressed as U/L (ALAT and ASAT), creatinine (mmol/L), urea (mmol/L), anduric acid (µmol/L).

- **Protein quantification**

The liver and kidney protein contents were measured byusing bovine serum albumin as standard, according to themethod of Lowry et al. [23].

- **Advanced oxidation of protein products (AOPP) levels**

AOPP was determined according to the method of Kayali et al. [24]. Briefly, 0.4 mlof thesupernatant of tissue homogenate was treated with 0.8 ml TBS (0.1 mol/L; pH 7.4). After 2 min, 0.1 ml of 1.16 mol/L potassium iodide was added to the tube, followed by 0.2 ml of acetic acid. The absorbance of the reaction mixture was immediately recorded at 340 nm. The concentration of AOPP for each sample was calculated using the extinction coefficient of 261 $cm^{-1}mM^{-1}$ and the results were expressed as µmoles per milligram of protein.

- **Determination of carbonyl protein content**

Carbonyl protein was carried out according to the method described previously [25]. For determination of protein carbonyl content in the samples, 1ml of 10 mM 2, 4-dinitrophenylhydrazine (DNPH) in 2M HCl was added to the samples (1mg). Samples were incubated for 30 min at RT. Then, 1 ml of cold TCA (10%, w/v) was added to the mixture and centrifuged at 3000g for 10 min. The protein pellet was washed three times with 2 ml of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 ml of guanidine hydrochloride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH (e = 2.2 104 cm1M1). The data were expressed as nmol/mg protein.

- **Thiobarbituric acid-reactive substance (TBARS) measurements**

Lipid peroxidation was estimated by measuring TBARS and expressed in terms of malondialde-

hyde (MDA), which is the end product of lipid peroxidation, according to Yagi [26]. In brief, 125µl of supernatants were homogenized by sonication with 50µl of TBS, 125µl of TCA-BHT to precipitate proteins and centrifuged (1000×*g*, 10 min, 4°C), 200µl of obtained supernatant was mixed with 40µl of HCl (0.6M) and 160µl of TBA dissolved in Tris and the mixture was heated at 80°C for 10 min. The absorbance of the resultant supernatant was read at 530 nm. The amount of TBARS was calculated by using an extinction coefficient of 156×10^5 mM-1 cm-1.

- **Assay of conjugated dienes**

This assay consists of taking 25µl of sample and adding 3 ml of chloroform/methanol (2v/v) after centrifugation at 3000tours/min for 5 min on prelate 2ml extract obtained and left overnight in the drying oven at 45°C, adding 2 ml of methanol and reading the optique density (OD) at 190 nm in the quartz cuvet [27].

Histopathological study

The liver and kidney were collected and fixed in 10% formalin, dehydrated in graduated ethanol, cleared in xylene, and embedded in paraffin. Sections of 5µm thick were prepared and then stained with hematoxylin and eosin (H&E) dye and examined for histopathological changes under the microscope.

Statistical analysis

The results obtained have been expressed as mean values±standard error of the mean (SEM). Statistical significance was assessed using a one-way analysis of variance (ANOVA) followed by Tukey post hoc test. Values of $p<0.05$ were considered significant.

RESULTS

Total polyphenolics contents and scavenging capacity of U. dioica

*U. dioica*extract was found to contain 712.375 µg GAE/mg of total polyphenolics content dry plant extracts.

In the present study, the potential antioxidant activity of *U.dioica* was evaluated by FRAP reduction power and antiradical activity by using the DPPH free radical assay (IC_{50}=0.09 ± 1.3; 0.07±0.005) compared to the reference ascorbic acid (IC_{50}=0.07 mg/ml; 0.02±0.004mg/ml respectively) (Table 1).

Growth curve analysis of control and treated rats

Growth assessment is the single most useful tool for defining health and nutritional status.Our results revealed that *U.dioica* has a protective effect when in the DLM/CPF rats group and this is comparedto rats receiving only DLM/CPF mixture, both the mixture of vitamins CE also denotes a restoration of body growth and this in comparison with the group of rats who receiving only DLM/CPF (Fig. 1A).

The absolute weight of the rats at the end of the treatment revealed a significant decrease in the absolute bodyweight of the DLM/CPF compared to the control rats. We noticealso a significantly reduced weight gain for the group of DLM/CPF. Pretreatment with aqueous extract of *U.dioica* and mixture of vitamin CE significantly corrected these body weight disturbances for animals receiving a subchronic mixture of DLM and CPF (Fig. 1B).

Hematological parameters

In table 2, a significant decrease in the number of RBC, Hb, Ht, MCV, MCH, MCHC and WBC in DLM/CPFrats when compared to the control rats (-26.27; -21.9; -26.42; -25.31; 1.34; -0.17; 35.66% respectively). The group treated with *U.dioica*+DLM/CPF or VitCE+DLM/CPF exhibited an increase in those parameters cited comparatively with the group treated by DLM/CPF only (+34.36; +32.37; +1.43; +3.49; +25.39; +16.36; +25.06%); (+33; +37.14; +5.45; +371; +26.68; 29.23; +25.15%). Our results demonstrated that no significance deference between control and group treated with *U.dioica*.

Biochemicals parameters

Our results of biochemical parameters in the liver and kidney are summarized in table 3. Significant increases of serum ASAT (+11.06%), ALAT (+32.57%), ALP (+16.14%), and LDH (+13.16%) caused hepatotoxicity, as evidenced by the administration of DLM/CPF. However, pretreatment with the extract of *U.dioica* significantly prevented DLM/CPF induced elevation in those parameters. Contrary, the administration of *U.dioica* recovered the impaired resulting from DLM/CPF induced toxicity. No significant modifications were seen in *U.dioica* extract alone when compared to the control group. For the levels of renal markers such as urea, creati-

nine, and uric acid in serum, no significant perturbations were seen in *U.dioica* extract alone compared to control. A significant increase increatinine (+13.25%), urea (+9.89%), and uric acid (+24.91%) were detected in DLM/CPF administered rats compared to controls (table 3). However, rats treated with *U.dioica*+DLM/CPF and Vit CE+DLM/CPF showed significant reductions in the level ofcreatinine, urea, and uric acid markers compared to the DLM/CPF group (-26; -2; -14.16%); (-4; -9.71; -41.61%) respectively. It is interesting to note that the aqueous extract of *U.dioica* contains antioxidants that can decrease the degree of toxicity induced by the mixture of DLM/CPF.

Determination of protein oxidation of AOPP and CP

DLM/CPF administration caused a highly significant increase in AOPP and CP levels in both liver and kidney compared to control rats. The levels of those parameters decreased in rats who received *U.dioica*+DLM/CPF or Vit CE+DLM/CPF when compared to DLM/CPF alone. No difference was detected between the group which was treated with *U.dioica* and control, as shown in Fig. 2.

Estimation of lipid oxidation of TBARS and CD

Our results showed that there was a significant increase in TBARS and CD levels in hepatic and renal function for the group of rats receiving the mixture of CPF and DLMindicate that these pesticides caused lipid oxidation at the membrane and plasma levels. The supplementation of the extract of our plant or Vit CE reduced the increase of TBARS substances and CD content in comparison with DLM/CPF only. On the other hand, the present study shows that the administration of *U. dioica* extract did not cause a change in TBARS and CD parameters (Fig. 3).

Histopathological study

The histological study performed on the liver shows healthy liver parenchyma in control. On the other hand, there is an alteration of the architecture of the liver and the presence of inflammation and leukocyte infiltration in the group of rats receiving the mixture of pesticides. Pretreatment with *U.dioica* restored the alteration induced by the mixture of pesticides. This indicates that the combination of DLM/CPF has a remarkable hepatotoxic effect and thus the establishment of alterations in the appearance of the liver (Fig. 4). The histological study carried out on the kidneys also showed very serious nephrotoxicity caused by the mixing of pesticides by the installation of the severe inflammation, epithelial detachment. Pretreatment with *U.dioica* reduced the nephrotoxicity provoked by DLM/CPF (Fig. 5).

DISCUSSION

Our data showed that *U.dioica* possessed high contents of phenolics compounds and antioxidant activity as evaluated by FRAP and DPPH tests. DLM/CPF administration induced a decrease in hematological parameters, an increase in biochemical parameters, and oxidative stress markers in the liver and kidney. Our results were confirmed by histopathological study. The antioxidant activity of nettle may be due to the presence of flavonoid chrysoeriol; also its antioxidant activity may be attributed to its flavonoids and phenolics contents [28]. Our results were in agreement with the study of Vajic et al. [29] who showed that nettle leaves have significant antioxidant power and can modulate blood pressure and oxidative damage in hypertensive rats. As well as the work of Sadegh et al. [30] showed similar levels of phenolics compounds.

We studied the effect of subchronic exposure to a mixture of DLM/CPF pesticides, on the other hand, the pretreatment with *U.dioica* extract was used in traditional medicine. According to our results, the DLM/CPF administration caused a significant decrease in hematological parameters such as RBC, Hb, Ht, MCV, MCH, MCHC, and WBC compared to the control group. By contrast, the administration of *U.dioica* extract and Vit CE significantly reduced these perturbations of these parameters comparatively with the mixture of CPF/DLM pesticides. The reduction in the number of RBC, Hb, and Ht levels could be due to the inhibition of erythropoiesis and chemosynthesis. A significant reduction in the number of WBC in the DLM/CPF group compared to control could be explained by an attack of the defense system. However, pretreatment with the aqueous extract of *U.dioica* significantly reduces this disruption. Our study was in agreement with Abdel-Daim et al. [31] who re-

vealed that exposure to DLM induced a decrease in RBC and Hb.

Numerous epidemiological studies suggest a correlation between the professional use of pesticides and the appearance of some pathology in the populations. Many effects of pesticides have been demonstrated in animals as well as, carcinogenic and neurotoxic. Our study concerned the evaluation of the protective and antioxidant effects of the aqueous extract of *U. dioica* in rats receiving a mixture of pesticides on some liver indicators such as (ASAT, ALAT, ALP, and LDH) and renal markers (creatinine, urea, and uric acid). Our results showed a significant increase in hepatic and renal biochemical parameters in the DLM/CPF group compared with the control group. This increase is due to the alterations of tissues caused by poisoning. The administration of the aqueous extract of nettle significantly restored these hepatic and renal serum biomarkers in the group of rats receiving DLM/CPF compared to the group of rats receiving only the mixture of pesticides. In our case, pretreatment with Vit CE with the mixture of DLM/CPF also reduced toxicity induced by DLM/CPF. It is interesting to note that the aqueous extract of nettle contains antioxidants compounds that can decrease the degree of toxicity. Our results were in agreement with Ncir et al. [32] who revealed that deltamethrin treatment caused an increase in liver enzyme activities of ASAT, ALAT, ALP, and LDH.

In the present study, oxidative damage caused in hepatic and renal tissues by the mixture of DLM/CPFis also proved by the significant increase in AOPP and CP levels in the group of rats receiving DLM/CPF pesticides compared to control. This increase confirms the hepatic and renal lesions caused by these two pesticides. Several studies have shown that exposure to pesticides causes changes in the protein level. Our results are in good accordance with those of Hamdaoui et al. [33] who have shown that rat exposure to Kalach 360 SL (an herbicide) is responsible for ovarian protein damage this is shown by the increase in AOPP levels, as well as the work of Ben Amara et al. [34] who have shown that methyl thiophanate fungicide causes an increase in AOPP level and the generation of a mechanism of cellular toxicity at the hepatic and renal tissues. The study of Feriani et al. [35] has shown that the administration of DLM causes an increase in the CP level. Moreover, the works of Yazdinezhad et al. [36] showed that the administration of CPF caused an overproduction of reactive oxygen species (ROS) which is responsible for protein damage in hepatocytes. However, the supplementation of the extract of *U.dioica* or Vit CE mixture induced a significant decrease in the both AOPP and CP content which confirms the high content of antioxidants compounds, which proves that the combination of VitCE has a particularly important and protective effect against the deleterious effects of free radicals caused by the mixture of pesticides.

In the current study, it has been reported that DLM/CPF administration induced an increase in the TBARS and CD levels in liver and kidney tissues compared to the control group, indicate that these pesticides caused lipid oxidation at the membrane and plasma levels. This is consistent with many authors [37]. Also, the administration of *U.dioica* extract significantly reduced TBARS formation. It should be noted that the complex of vitamin CE reduced the level of TBARS in both liver and kidney; these results are following the work of Niki et al. [38]. Indeed, vitamin CE is known as strong natural antioxidants because of their ability to trap EROs intracellularly and extracellularly [39]. CD is one of the products of lipid peroxidation, the dosage of these products is used as a marker of oxidative stress. Our results are in agreement with the work of Ncir et al. [32] who showed that exposure to DLM causes cellular damage at the cerebral and renal levels. The treatment with the extract of our plant reduced the level of DC, alsothe application of the complex of vitamin CE significantly decreased the level of DC compared to the group receiving the mixture of pesticides only, these results being following those of Giray et al. [40] who showed that the administration of vitamin E decreased the level of CD and TBARS caused by the administration of cypermethrin.

In the present study, the protective effects of *U. dioica* extract and vitamin CE mixture were confirmed by histological examinations. Therefore, *U. dioica* could be used as a preventive and therapeutic agent in oxidative hepatic and renal diseases. Our study indicated that DLM/CPF mixture induced alteration of liver and kidney architecture and the presence of inflammation and leukocyte infiltration compared to control.

By contrast, pretreatment with *U. dioica* extract or vitamins CE recovered hepatotoxicity and nephrotoxicity induced by amixture of pesticides. Our results are in line with Abdou et al. [41] which revealed that DLM induced hepatoxicity evidenced by severe necrotic changes and inflammatory cell infiltration.

CONCLUSIONS

In conclusion, our results revealed that *U.dioica* has a therapeutic effect on some health problem and has an important antioxidant and antiradical activity by polyphenols, DPPH, and FRAP, indeed, both *U.dioica* and the mixture of Vit CE causing a decrease in biochemical parameters, protein oxidation, and lipid peroxidation. These results are in agreement with the use of *U.dioica*.

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Conflict of interest
The authors declare no conflict of interest.

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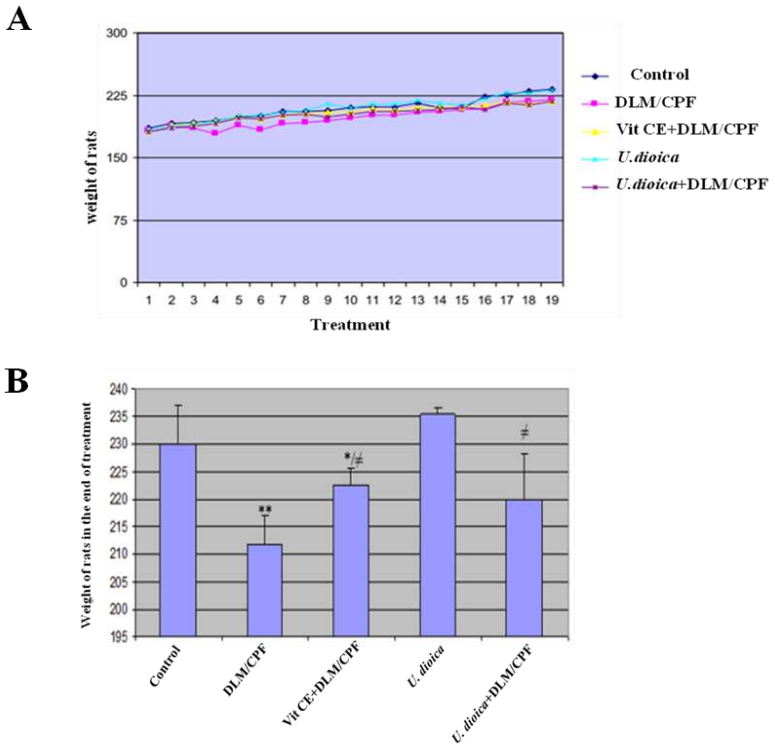

Figure 1. Growth curve and body weight in the treatment of control and treated rats

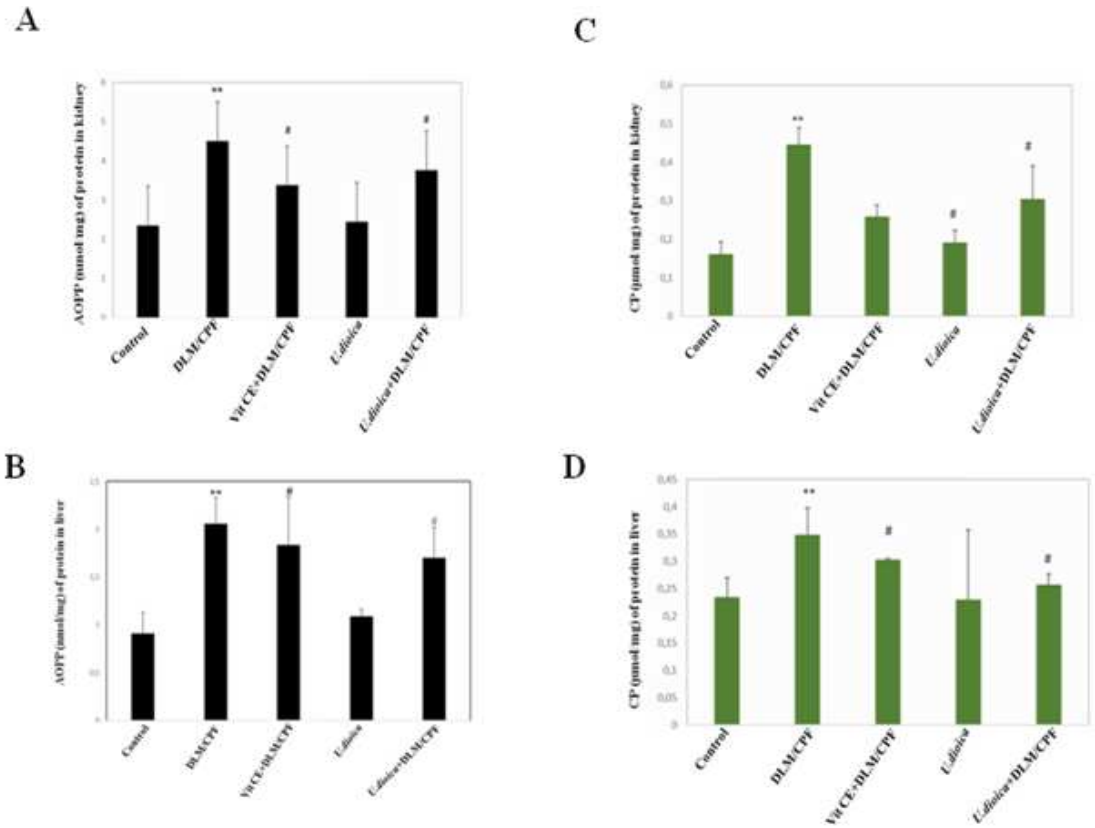

Figure 2. Effects of DLM/CPF, *U.dioica*, Vit CE and their combination (Vit CE+DLM/CPF, *U.dioica*+DLM/CPF) on Advanced oxidation of protein products(AOPP) and carbonyl protein (CP) in liver and kidney of control and experimental rats

Values were expressed as means± SEM. The number of determinations was n=5 ; * $p \leq 0.05$ vs control *U.dioica*+DLM/CPF ; Vit CE+DLM/CPF vs DLM/CPF : $^{\#}$ $p \leq 0.05$

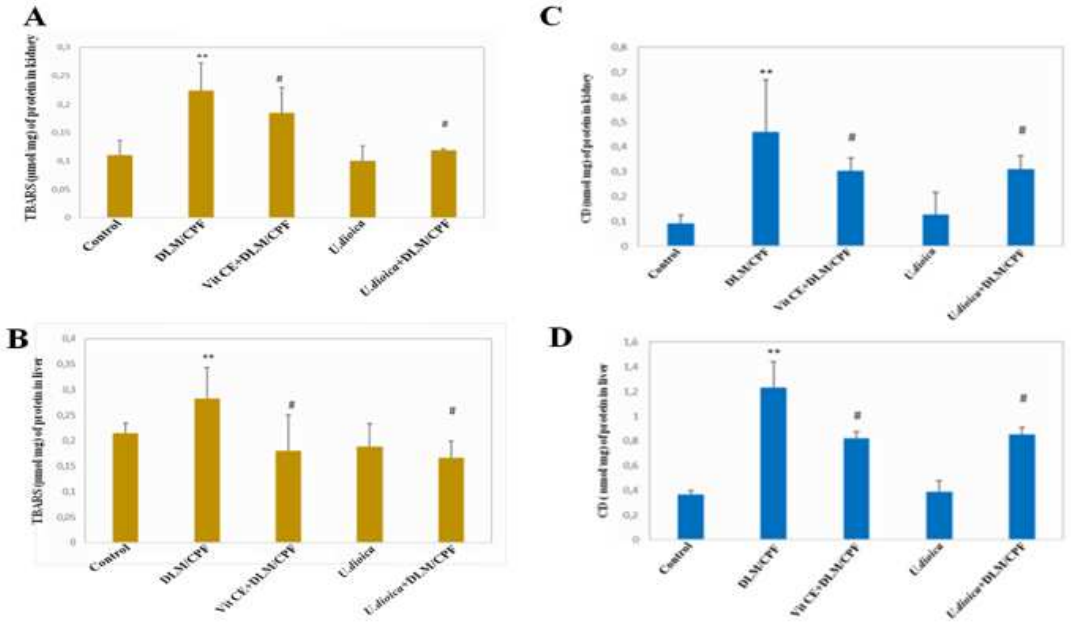

Figure 3. Effects of DLM/CPF, *U.dioica*, Vit CE and their combination (Vit CE +DLM/CPF), *U.dioica*+DLM/CPF) on thiobarbituric acid-reactive substances (TBARS) and conjugated dienes (CD) in liver and kidney of control and experimental rats

Values were expressed as means± SEM. The number of determinations was n=5 ; * $p \leq 0.05$ vs control *U.dioica*+DLM/CPF ; Vit CE+DLM/CPF vs DLM/CPF : # $p \leq 0.05$

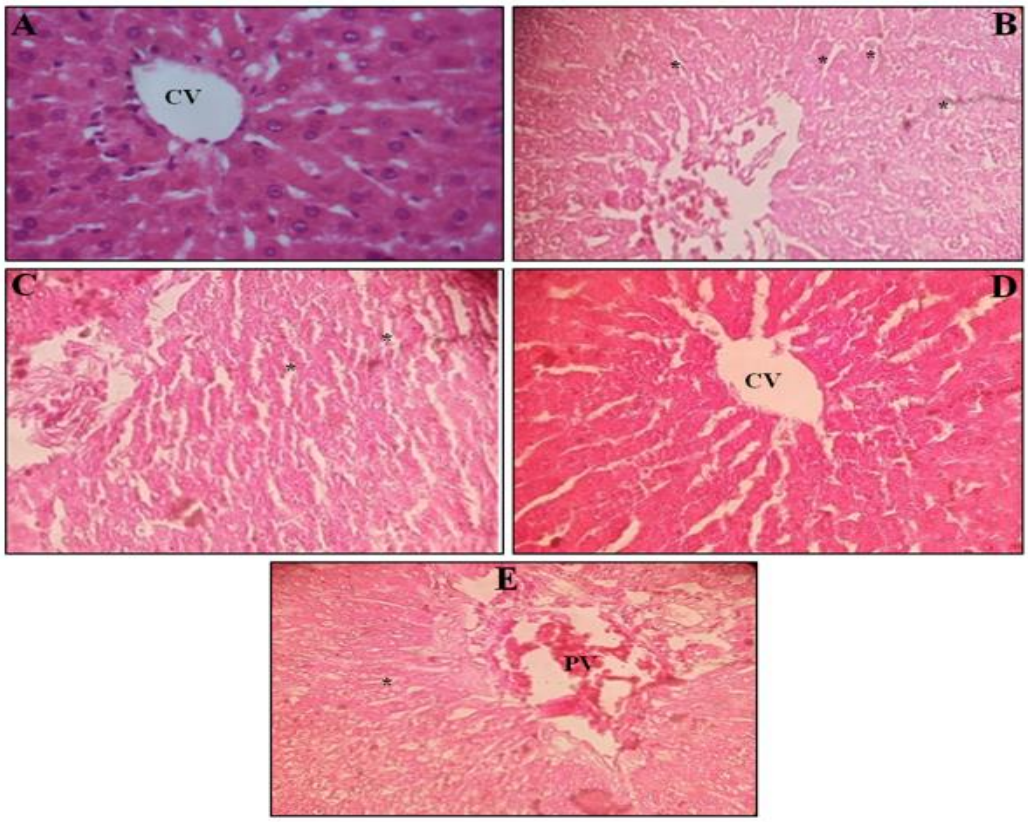

Figure 4. Representative photographs of liver showing the protective effect of *U.dioica* on DLM/CPF induced nephrotoxicity in rats. Control (A), rats treated with DLM/CPF (B), rats treated with the combination of Vitamins CE+DLM/CPF (C), rats treated with *U.dioica* (D), rats treated with the combination of *U.dioica* +DLM/CPF (E)

CV: Centrilobular Vein; * leukocyte infiltration PV: Portal Vein

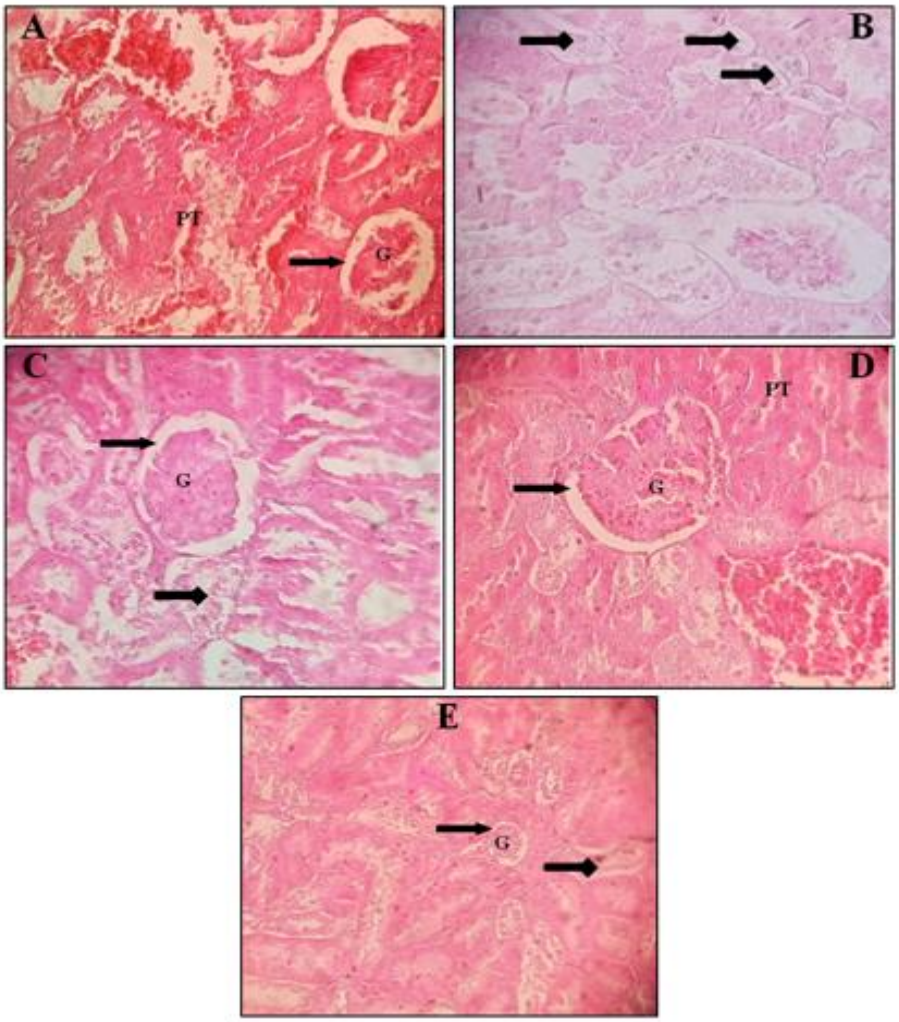

Figure 5. Representative photographs of a kidney showing the protective effect of *U.dioica* on DLM/CPF induced nephrotoxicity in rats. Control (A), rats treated with DLM/CPF (B), rats treated with the combination of Vitamins CE +DLM/CPF (C), rats treated with *U.dioica* (D), rats treated with the combination of *U.dioica* + DLM/CPF (E)

PT: Proximal Tubule; G: Glomerulus; ➞DT: Distal Tubule; ➡ Leukocyte infiltration

Table 1. Antioxidant tests of *U.dioica* by FRAP and DPPH

Parameters	IC_{50} of extract (mg/ml)	IC_{50} of ascorbic acid (mg/ml)
FRAP	0.09 ± 1.3	0.07 ± 0.05
DPPH	0.07 ± 0.005	0.02 ± 0.004

Table 2. Effects of DLM/CPF, *U.dioica*, Vit CE, and their combination (Vit CE+DLM/CPF, *U.dioica*+DLM/CPF) on some hematology parameters in blood.

Parameters &treatment	Control	DLM/CPF	Vit CE+DLM/CPF	*U.dioica*	*U.dioica*+DLM/CPF
RBC (x10^6µL)	8.03±0.3	5.92±1.98*	7.91±0.26 #	7.77±0.18	7.9±0.2#
Hb (g/dl)	13.47±0.22	10.52±3.54**	14.35±0.6 #	14.02±0.49	14.1±0.29 #
Ht (%)	43.9±1.59	32.3±10.81*	44.35±1.62#	42.52±0.91	43.4±0.67#
MCV (fL)	54.72±0.72	40.87±11.8	56.05±0.51	55.32±0.21	54.1±0.5
MCH (g/L)	17.43±0.2	17.4±0.18	18.35±0.32	17.95±0.21	17.65±0.13
MCHC (g/dt)	31.93±0.14	31.5±0.26	32.67±0.51	32.42±0.31	32.6±0.17

WBC (x10^{3}µL)	14.3±1.9	9.2±3.11*	13±1.93 #	14.75±1.79	11±0.92#

Values were expressed as means± SEM. The number of determinations was n=5 ; * $p \leq 0.05$ vs control *U.dioica*+DLM/CPF ; Vit CE+DLM/CPF vs DLM/CPF : # $p \leq 0.05$

Table 3. Effects of DLM/CPF, *U.dioica*, Vit CE and their combination (Vit CE +DLM/CPF, *U.dioica*+DLM/CPF) on serum liver and kidney of control and treated rats

Parameters & treatment	**Control**	**DLM/CPF**	**VitCE+ DLM/CPF**	***U.dioica***	***U.dioica*+ DLM/CPF**
ASAT (U/L)	338.33±12.28	375.75±28.57*	338.2±41.55#	349±14.65	329.66±8.85#
ALAT (U/L)	88.5±2.26	117.33±3.23**	133.5±2.84#	101.6±4.34	105.5±4.11#
ALP (U/L)	176.5±10.89	205±4.91*	165.66±9.63#	166.25±7.38	180±5.07#
LDH (U/L)	3100.33±64.4	3508±10.6*	2628±342.94##	3043±296.11	2890±190.87##
Creatinin (µmol/L)	17.66±0.28	20±0.7*	19.2±2.07	19.66±0.28	14.8±0.89##
urea (mmol/L)	6.37±0.19	7±0.1*	6.32±0.31#	6.1±0.14	6.86±0.16
Uric acid (µmol/L)	96.33±1.52	120.33±1.89*	70.25±10.11##	98.66±2.3	103±3. 46#

Values were expressed as means± SEM. The number of determinations was n=5 ; * $p \leq 0.05$; ** $p \leq 0.01$ vs control *U.dioica*+DLM/CPF ; Vit CE+DLM/CPF vs DLM/CPF : # $p \leq 0.05$; ## $p \leq 0.01$