Research Article

Artemisia campestris Leaf Aqueous Extract Alleviates Methidathion-Induced Nephrotoxicity in Rats

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ABSTRACT

Methidathion (MD) is an organophosphate insecticide and acaricide used in both agricultural and urban areas throughout the world including Algeria. The objective of this study was to evaluate the protective effect of *Artemisia campestris* (Ac) leaf aqueous extract on sub-acute four-week's oxidative damage induced by MD on the hematological system and renal integrity of rats. The animals were randomly divided into four groups of seven each: group I served as control which received standard diet; group II received only Ac (5g/l) while the third group received only MD (5mg/kg b.w.by gavage using corn oil as vehicle). Rats in the fourth group (MD+Ac) were treated with MD and Ac extract. Results showed that lipid peroxidation increased significantly in MD-treated rats, as evidenced by high renal malondialdehyde (MDA) levels. As well as, the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and glutathion-s-transferase (GST), catalase (CAT), and the level of reduced glutathione (GSH) content in renal tissue decreased by MD. On the other hand, a significant increase in plasmatic levels of total protein, urea and creatinine levels was observed in MD-treated group. However, the treatment of Ac extract with MD-treated rats has maintained the biochemical parameters cited above. The changes in hematological and biochemical parameters were substantiated by histological data. In conclusion, our results indicated a possible mechanism of MD-induce nephrotoxicity and plasma biomarkers were disturbed. *Artemisia campestris* leaf aqueous extract ameliorated the toxic effects of this pesticide in renal tissue suggesting their role as potential antioxidants.

Keywords: Methidathion, Artemisia campestris, Biochemical studies, Kidney, Oxidative stress, Rat.

INTRODUCTION

The widespread use of pesticides around the world in large amount, specifically in public health and agricultural programs has caused environmental pollution and led to a variety of negative effects in human health. Organophosphate insecticides (OPIs) represent one of the major classes of insecticides in use since the mid 1940s[1]. Commonly OPIs are used for their relative low toxicity and their low persistence in mammalian system[2]. Furthermore, the major acute mammalian toxicity associated with exposure to OPIs results from the irreversible inhibition of acetyl cholinesterase enzyme[3,4], which cause accumulation of acetylcholine at cholinergic synapses resulting in muscarinic and nicotinic syndromes[4]. Chronic and subchronic exposure of OPIs may induce oxidative stress leading to generation of free radicals and alteration in antioxidants or reactive oxygen species (ROS) scavenging enzymes in the target tissue[5]. This led an imbalance between antioxidant system and pro-oxidant state generated by OPIs. In addition, the cells have various mechanisms to repair oxidative stress by enzymatic antioxidant comprising, superoxide dismutase (SOD), Catalase (CAT), Glutathion peroxidase (GPx), or non enzymatic systems by the action of reduced glutathione (GSH), and glutathione-S-transferase (GST)[6].

Methidathion (MD) S-[(2, 3-dihydro-5-methoxy-2-oxo-1,3,4-thiadiazol 3(2H)-yl) methyl] O,O dimethyl phosphorodithioate), is one of the most important OPIs used worldwide in the pest control of crops[7]. It has been reported that the major route of the metabolism of methidathion in rat liver was through glutathion-s-transferase and the predominant metabolite was desmethyl methidathion[8]. In addition, the sub chronic administration of MD may induce biochemical and histopathological changes in different tissues such as liver, kidney and heart. However, the supplementation of antioxidants can be useful to inhibit oxidative damage[7].

Recently, it has been reported to find natural antioxidant in plant materials which play a significant function in protecting living organisms from the toxic effect of pesticides[9]. Moreover, plants often contain substantial amounts of antioxidants including alpha tocophérol, carotenoids; ascorbic acid, flavonoids and tannins[10]. In this context, *Artemisia campestris* (Ac) represent a peremial scarcely aromatic herb belonging to *Asteraceae* family, and it is used against a variety of diseases in Northern Africa[11,12].

The leaves of this plant collected in summer (july-august 2012) from Tebessa (Eastern Algerian) commonly called in Algerian folk medicine (T gouft). Several recent studies revealed after extraction of A. campestris (aqueous or ethanol) different pharmacological activities such as, antioxidants, insecticidal, antimicrobial, antirheumatic and relaxation[11-14]. In fact, Ac extract was reported to be a potent free radical scavenger of 1,1-diphenyl-2-picryl-hydrozyl (DPPH), hydroxyl and superoxide anion radicals[15]. Recently, Ghorab[14] have found the main

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essential oil of hydrodistilled fresh aerial parts of Ac which exhibited the best antibacterial activity against *Pseudomonas aeruginosa* and *Escherichia coli*. Furthermore, the recent study of Akrout[11] reported that the essential oils extracted from Ac (100µg/ml) ethanol-water (50%) and infusion (5g/100ml) can inhibit the human adenocarcinoma cell growth. In addition, Sefi[12] suggested that Ac leaf extract (200mg/kg) can prevent diabetic nephropathy by attenuating oxidative and nitroactive stress.

Behind liver, the kidneys are the most sensitive organ against pesticide and toxicity damage, as they play a key role in the metabolism and elimination of xenociotics[16]. To our knowledge, there are no previous data about the nephroprotective effect of Ac against MD toxicity. For this reason, the present study was undertaken to examine the role of an aqueous extract (5g/l) of Ac against MD toxicity in kidney of male rats. Accordingly, the antioxidants status of kidney was determined by measuring the activity of glutathion-s-tranferase (GST), glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase (CAT), as well as the level of reduced glutathione GSH, and MDA. In addition, Urea and creatinine level were also estimated.

MATERIALS AND METHODS

Chemicals and Reagents

Methidathion is an organophosphate insecticide and acaridae (Figure 1). CAS chemical name is (S-2, 3-dihydro-5-methoxy-2-oxo-1, 3, 4-thiadiazol-3-ylmethyl O, O-dimethyl phosphorodithioate); CAS registry number is *950-37-8*. A commercial formulation of MD, named 'Supracide®' was used in the experiments. All other chemical products used in this study were purchased from Sigma Chemical Co. (St. Louis, France).

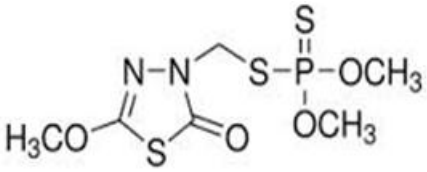

Figure 1: Chemical structure of Methidathion

Plant

Preparation of A. campestris Extract

Areal parts of *A. campestris* were collected in summer (2012) from Tebessa (Eastern Algerian). Samples were air dried during 20 days in the laboratory at ambient temperature (20-25°). After that the samples were ground, washed and put in boiling water (5g/l) for 15-20min. The aqueous extracts were filtrated using Whatmann filter paper and directly used for experiment. The extract should be prepared daily at the moment of experiment.

Determination of Total Phenolic Content

The concentration of total phenolic in the extract was determined with Folin-Ciocalteu reagent and external calibration with gallic acid, using the method of Chen.[17] Briefly, 100 µl of diluted sample was shaken for 5 min with 750 µl of diluted Folin-Ciocalteu reagent (x10). Then, 750 µl of 20% Na_2CO_3 was added. The final mixture was shaken and then incubated at room for 90 min. The absorbance was measured at 750 nm with UV-VIS Bueco S-22 spectrophotormeter. Gallic acid was used to prepare a standard curve (0.05–0.5 mg/ml, y=0.011x+0.04, R^2=0.987, where y is the absorbance and x is the standard concentration). The phenolic content was expressed as milligram gallic acid equivalent per gram extract (mg GAE/ g extract).

Determination of Total Flavonoids Content

The total flavonoid content in the extract was determined according to Djeridane[18], using the method based on the formation of a complex flavonoid-aluminium. About 1 ml of diluted sample was mixed with 1 ml of 2% aluminium trichloride (AlCl3) methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm with UV-VIS Bueco S-22 spectrophotometer. Quercetin was used for standard curve construction (0.05–0.5 mg/ml, y=0.036x+0.275, R^2=0.97, where y is the absorbance and x is the standard concentration). The results were expressed as milligram quercetin equivalents (QE)/g extract.

Determination of Total Tannin Content

The method of Julkunen-Tiitto[19] was followed in this assay. An aliquot (50 µl) of diluted sample or standard solution was mixed with 3 ml of 4 % vanillin (prepared with methanol), then 100 µl of HCl were added. The well-mixed solution was incubated in the dark at ambient temperature for 20 min. The absorbance against blank was read at 500 nm. Catechin was used to make the standard curve (0.05–0.5 mg/ml, y=0.002x+0.006, R^2=0.98, where y is the absorbance and x is the standard concentration). The results were expressed as milligram catechin equivalents (CE/g extract).

Antioxidant Activity

DPPH Essay

Scavenging activity against the diphenyl-picrylhydrazyl (DPPH) radical was determined by Blois[20] with some modifications. Various dilutions of samples were mixed with ethanolic DPPH solution. After an incubation period of 30 min at 25°C, the absorbance against blank was read at 520 nm with UV-VIS Bueco S-22 spectrophotometer. The free radical scavenging of each solution was then calculated as follows:

$$\% \text{ Inhibition} = 100 (A_{Blanc} - S_{Sample}) / A_{Blanc}$$

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The antioxidant activities of Ac were expressed as IC50, defined as the concentration of the test material required to cause a 50 % decrease in initial DPPH concentration.

ABTS Essay

ABTS radical scavenging assay was conducted according to the method reported by Re[21]. The assay is based on ABTS radicals, which are generated by the reaction of 9.5 mL ABTS solution (7 mmol/L) with 245 µL potassium persulfate (100 mmol/L). The volume was then made up to 10 mL with distilled water. The solution was kept in dark at room temperature for 18 h prior to use. After that, the solution was diluted with 0.1 mol/L potassium phosphate buffer so as to obtain an absorbance of 0.70 =0.02 at 745 nm at 30 °C. For the assay, 10 µL plant extract/fraction (0.5 mg/mL) was mixed with 2.99 mL ABTS radical solution. The absorbance was measured at 734 nm after 10 min.

Animals and Treatment

Twenty eight male Wistar rats (weighing 270–290g) were obtained from the Pasteur institute (Algiers, Algeria). Animals were acclimated for 1 week under the same laboratory conditions of photoperiod (12-h light: 12-h dark cycle), a minimum relative humidity of 40% and room temperature 24±2°C. Food (standard diet, supplied by the ''ONAB, El-Harrouch'', Algeria) and water were available *ad libitum*. The rats were randomly divided into four groups of seven individuals each as follows. The first group of rats served as the control; the second group (Ac) received via drinking an aqueous extract of Ac at the dose of 5g/ml. While the third group (MD) was administered at a dose of 5mg/kg b.w.by gavage using corn oil as vehicle. Rats in the fourth group (MD + Ac) were treated with MD plus an aqueous extract of A. campestris at the dose of 5g/l. Only corn oil was given in the same way to the control group. The dose of MD used in this study represents 1/15 of LD_{50} (5mg/kg bw) which has been used previously by other investigators since it is toxic but not lethal to rats[7,22].

The Ac dose (5mg/kg b.w.) used in our experiment and in other findings gave high protection against stress conditions in several tissues[23,6,12]. Rats were administered their respective doses every day for four weeks. The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care and approved by the Ethics Committee of our Institution.

The amount of ingested diet was calculated as the difference between the weight of feed that remained in the food bin (D_1) and the amount placed 1 day before (D_2). These data were then used to calculate the daily average feed intake, according to the formula:

Average feed intake: D_2-D_1

At the end of the experiment, body weights of rats were recorded. Animals were sacrificed by cervical decapitation without anesthesia to avoid animal stress. Kidneys, removed from adipose tissue and adrenal glands, were taken and their absolute and relative weights were recorded. Blood samples were collected in EDTA tubes and centrifuged at 2200g for 15 min at 4°C. Plasma samples were stored at -20°C until biochemical analysis of total protein, creatinine and urea contents.

Hematological Variables

Red blood cells (RBCs) count, hemoglobin (Hb) concentration, hematocrit, mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration were measured by electronic automate coulter MAXM (Beckman Coulter, Inc., Fullerton, California, USA).

Tissue preparation

Kidneys removed from control and tested rat were homogenized in 2 ml of buffer solution of phosphate buffered saline 1:2 (w/v; 1g tissue with 2ml PBS, pH 7.4). Homogenates were centrifuged at 10.000g for 15 min at 4°C, and the resultant supernatant was used for the determination of malondialdehyde (MDA) and reduced glutathione (GSH) levels in the hand, and measuring the activity of GPx, CAT, SOD and GST in the other hand.

Estimation Renal Plasma Biomarkers

Plasma protein, creatinine, urea levels were assayed using commercial reagent kits (Spinreact spain Ref: 41010, 1001110 and 1001332 respectively).

Estimation of Lipid Peroxidation

Lipid peroxidation level in kidney homogenate was estimated by measuring thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content which is the end product of lipid peroxidation, according to Buege and Aust (1984)[24]. 125µl of supernatants were homogenized by sonication with 50µl of PBS, 125µl of TCA-BHT (trichloroacetic acid- butylhydroxytoluene) in order to precipitate proteins and then centrifuged (1000*g*, 10min, and 4°C). 200µl of supernatant were mixed with 40µl of HCl (0.6 M) and 160µl of TBA dissolved in Tris, and the mixture was heated at 80°C for 10min. The absorbance of the resultant supernatant was read at 530nm. The amount of MDA was calculated using a molar extinction coefficient of 1.56×10^5 M/cm.

Estimation of Reduced Glutathione

Kidney GSH content was estimated using a colorimetric technique, as mentioned by Ellman (1959)[25] modified by Jollow[26], based on the development of a yellow colour when DTNB [(5,5 dithiobis-(2-nitrobenzoic acid)] is added to compounds containing sulfhydryl groups. In brief, 0.8ml of liver supernatant was added to 0.3ml of 0.25% sulphosalycylic acid and tubes were centrifuged at 2500g for 15min. Supernatant was mixed with 0.01 M DTNB and phosphate buffer (0.1M, pH 7.4). Finally, absorbance at 412nm was recorded. Total GSH content was expressed as nmol GSH/mg protein.

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Estimation of Glutathione-s-transferase Activity

Glutathione-S-transferase (GST) (EC 2.5.1.18) catalyzes the conjugation reaction with glutathione in the first step of mercapturic acid synthesis. The activity of GST was measured according to the method of Habig[27]. P-nitrobenzylchloride was used as substrate. The absorbance was measured spectrophotometrically at 340nm at 30s intervals for 3 min.

Measurement of Antioxidant Enzyme Activities

Measurement of Glutathione Peroxidase Activity

Glutathione peroxidase (GPx) *(E.C.1.11.1.9)* activity was measured by the procedure of Flohe and Gunzler (1984)[28].

Supernatant obtained after centrifuging 5% kidney homogenate at 1500g for 10 min followed by 10000g for 30 min at 4°C was used for GPx assay. One ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1M, pH 7.4), 0.2ml of GSH (2mM), 0.1 ml of sodium azide (10mM), 0.1 ml of H_2O_2 (1mM) and 0.3 ml of liver supernatant. After incubation at 37°C for 15 min, reaction was terminated by addition of 0.5 ml 5% TCA. Tubes were centrifuged at 1500g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1M pH7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant.

After mixing, absorbance was recorded at 420 nm.

Measurement of Catalase Activity

The catalase (*E.C.1.11.1.6*) activity was measured according to Aebi (1974)[29]. Assay is based on the ability of the enzyme to induce the disappearance of hydrogen peroxide. The reaction mixture consist of 780µl phosphate buffer (pH 7.5), 200µl of hydrogen peroxide (500mM) and 200µl supernatant in a final volume of 1ml. Absorbance was recorded at 240 nm every 15 second for 1min. The enzyme activity was calculated by using an extinction coefficient of 0.043mM cm^{-1}.

Measurement of Superoxide Dismutase Activity

The method of the determination of SOD (*E.C.1.15.11*) activity by the NBT test is a method of photo reduction of riboflavin/methionine complex that generates superoxide anion. The oxidation of the NBT by the anion superoxide O_2^- is used as the basis for the presence of SOD detection. In aerobic environments the riboflavin, methionine, and NBT mixture gives a blue color. The presence of SOD inhibited the oxidation of the NBT. SOD activity was estimated by Beauchamp and Fridovich (1971)[30]. Procedure, briefly, 5µl of the supernatant was combined with 1ml of EDTA/methionine (0.3mM), 1890 ml phosphate buffer (pH 7.8), 85µl of 2.6mM NBT and 22µl of riboflavin (0.26mM) was added as the last and switching on the light.

The reaction changes in absorbance at 560nm were recorded after 20min.

Measurement of Protein Content

The protein contents of various samples were determined by the method of Bradford (1976)[31] using bovine serum albumin as a standard.

Histopathological Analysis

For histopathological analysis, the kidneys tissues were dissected and the tissue samples were immediately fixed in formalin solution, embedded in paraffin.

The paraffin sections were cut into 5µm thick slices and stained with hematoxylin and eosin (H&E)[32] or light microscopic examination. The sections were viewed and photographed.

Statistical Analysis

Data are expressed as means ± SEM. Data comparisons were carried out by using one way analysis of variance followed by Student's t-test to compare means between the different treatment groups. Differences were considered statistically significant at $p \leq 0.05$.

RESULTS

Polyphenol, Flavonoids and Tannin Content of Infusion Extract

The total phenolic content of an infusion extract of Artemisia campestris was 16.88 mg of CAE/100 g of extract as also shown in Table 1. The total flavonoid content was expressed as 1.9 mg of quercetin equivalents per gram of Ac. Besides, the results showed that Ac Infusion contained 426.5 mg of condensed tannins, which expressed as milligrams of catechin equivalents per gram.

Antioxidant Activity of Artemisia Campestris

The results of the determination of the antioxidant activity of Ac extract using two methods (DPPH, ABTS) are presented in Table 1.

The assessment of antioxidant activity (DPPH) showed an IC50 value of 74.62% (Table 1). However, the IC50 value of Ac on ABTS radical-scavenging activity was 26.01 %.

Table 1: Amounts of antiradicalar DPPH, antiradicalar ABTS, total phenols content, total flavonoids and condensed tannins in infusion extract of Artemisia campestris.

50 % scavenging concentration (mg/ml) on DPPH radical	74.62 ± 5.81
50 % scavenging concentration (mg/ml) on ABTS radical	26.01 ± 5.74
Total phenolic content (mg GAE/g of Ac	16.88 ±0.01
Total flavonoid content (mg QE/g Ac)	1.90 ± 0.108
Condensed tannins (mg CE/g Ac)	426.5 ± 0.128

GAE gallique acid equivalents; QE quercetin equivalents; CE catechin equivalents

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Effects of Treatment on General Rat Health

In our study, there were no deaths in any of the groups.

While more severe fatigue symptoms were observed in MD group compared to control group.

However, fatigue symptoms were less severe in MD+Ac group as compared to control group.

As shown in (Table 2), the decrease in body weight of MD group was associated with the reduction in food intake by 21%.

Beside, the administration of Ac extract with MD showed an improvement of the consumption in food intake by 28.49% as compared to the MD-treated group.

Effects of Treatments on Body and Kidney Weights

The variations in the body and relative kidney weights of male rats in control and treatment groups are given in (Table 2). During the course of present work, it was observed that the body weights of control and Ac extract treated group, increased progressively throughout the study. However, the exposure of MD resulted in the loss of body weight of the rats by 15%. Treatment with Ac extract resulted an improvement in the body weight of the MD-treated rats by 8% as compared to the control one. Furthermore, a significant increase of MD-treated rats in absolute and relative kidney weights was recorded (12% and 11% respectively) as compared to the case of controls, and by 12.16% and 10.9% respectively as compared to the MD-treated group.

Table 2: Effects of methidathion (MD), Artemisia campestris (Ac) and their combination (MD+Ac) on body weight (g), relative and absolute kidney weights and daily food intake in rats after 4-week treatment.

Parameters Studied	Treatment Groups			
	Control	Ac	MD	MD+Ac
Initial body weight (g)	315.63 ± 31.90	323.63 ± 45.40	316 ± 38.97	308.5 ± 28.14
Final body weight (g)	347.50 ± 58.62	337.63 ± 45.70	296.6 ± 38.94*	321.8 ± 28.20
Absolute kidney weight (g)	1.90 ± 0.13	1.90 ± 0.22	2.12 ± 0.30*	1.9 ± 0.20
Relative kidney weight [a]	0.63 ± 0.04	0.62 ± 0.10	0.71 ± 0.10*	0.64 ± 0.10
Food intake [b]	22.45 ± 3.29	22.93 ± 1.87	17.83 ± 1.67*	22.91 ± 1.25#

[a] Absolute kidney weight measured as gram per 100 gram body weight (g/100 g b.w); [b] Food intake measured as gram per day per rat (g/day/rat); Values are given as mean ± SEM for group of 7 animals each.; * MD and MD+Ac groups compared with control group (*p≤0.05; **p≤0.01); # MD+Ac group compared with MD group (#p≤0.05)

Table 3: Effects of methidathion (MD), Artemisia campestris (Ac) and their combination (MD+Ac) on hematological parameters in rats after 4-week treatment.

Parameters Studied	Treatment Groups			
	Control	Ac	MD	MD+Ac
RBCx 10^6 mm^3	6.74 ± 0.60	5.60 ± 0.70	4.10 ± 0.60**	5.42 ± 1.20
WBCx10^6 mm^3	4.61 ± 1.01	5.90 ± 1.30	4.83 ± 1.83	4.9 ± 1.61
Hb (g/dl)	9.50 ± 1.30	9.73 ± 1.10	8.70 ± 2.14*	9.34 ± 1.99
Plt (10^3/mm^3)	355.10 ± 32.23	422.65 ± 25.74	298.32 ± 19.45*	389.95 ± 27.84
Ht (%)	25.21 ± 5.02	28.20 ± 3.60	28.6 ± 7.50	26.00 ± 7.20
VMC(μ^3)	50.04 ± 1.10	50.50 ± 0.90	50.50 ± 0.80	50.63 ± 1.62
TCMH(pg)	16.80 ± 0.40	18.00 ± 0.33	17.20 ± 0.20	17.20 ± 0.60**
CCMH (%)	34.53 ± 0.50	35.60 ± 0.60	34.03 ± 0.30	33.80 ± 0.42

Values are given as mean ± SEM for group of 7 animals each.; * MD and MD+Ac groups compared with control group (*p≤0.05; **p≤0.01); # MD+Ac group compared with MD group (#p≤0.05)

Table 4: Effects of methidathion (MD), Artemisia campestris (Ac) and their combination (MD+Ac) on serum biochemical parameters, antioxidant enzyme activities in kidneys of control and treated rats after 4-week treatment.

Parameters Studied	Treatment Group			
	Control	Ac	MD	MD+Ac
Total protein (mg)	66.69 ± 4.50	67.72 ± 2.30	78.44 ± 6.33**	70.95 ± 5.60#
Urea (mmol/l)	6.27 ± 0.54	6.31 ± 0.73	7.12 ± 0.48**	6.32 ± 0.70##
Creatinine (mmol/l)	34.13 ± 5.70	35.38 ± 6.60	40.50 ± 6.74*	39.60 ± 3.30
Glutathione peroxidase [a]	9.89 ± 0.21	10.72 ± 0.26	6.94 ± 0.16*	10.91 ± 0.65
Superoxyde dismutase [b]	2.69 ± 0.07	0.88 ± 0.04	1.43 ± 0.05**	2.08 ± 0.03*
Catalase [c]	2.53 ± 0.01	1.69 ± 0.07	0.93 ± 0.01*	2.19 ± 0.05***

[a] Glutathione peroxidase: nmoles of GSH/min/mg protein; [b] Superoxyde dismutase : units represents the amount of enzyme that inhibits the oxidation of NBT by 50%/mg de protein.; [c] Catalase: µmoles H_2O_2 degraded/min/mg protein; Values are given as mean ± SEM for group of 7 animals each.; *MD and MD+Ac groups compared with control group (*p≤0.05); # D+Ac group compared with MD group (#p≤0.05)

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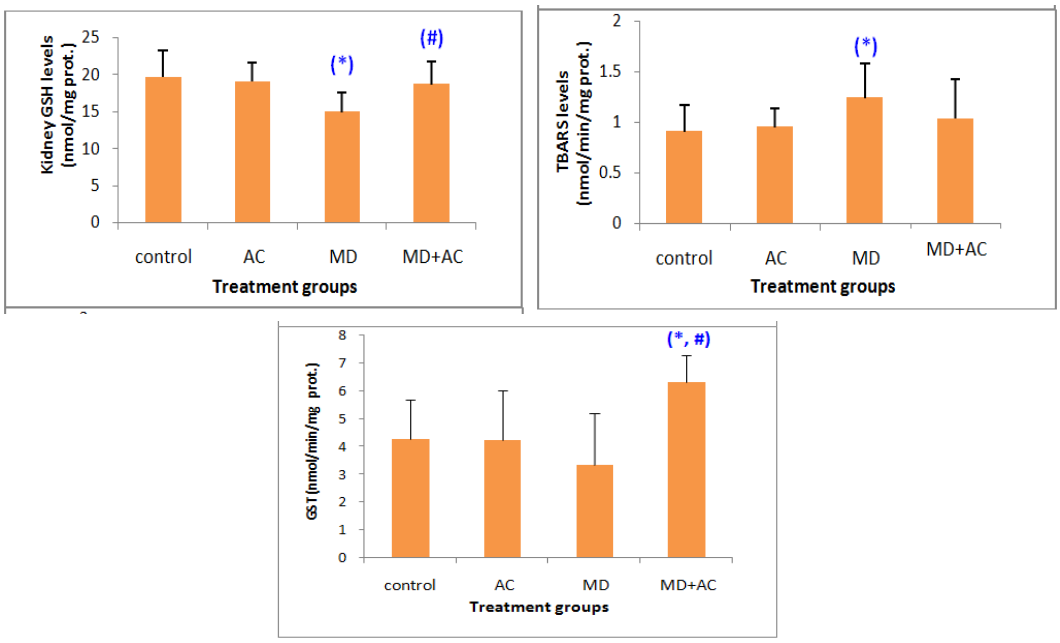

Values are given as mean ± SEM for group of 7 animals each. *MD; MD+Ac groups versus control group (*p≤0.05). # MD+Ac group versus MD group (#p≤0.05).

Figure 2: Reduced glutathione (nmol/mg protein), TBARS (nmol MDA/mg protein) levels and Enzyme activity of GST (nmol/min/mg prot) in kidney of control and rats treated with methidathion (MD), Artemisia campestris (Ac) and their combination (MD+Ac) after 4-weeks of treatment.

Table 6: Semiquantitative scoring of architectural damage on histopathological examination of the rat kidneys in the different treatment groups.

Parameters Studied	Treatment groups			
	Control	Ac	MD	MD+Ac
Tubular dilatation	-	-	++	-
Hemorrhage	-	-	+++	++
Necrosis in epithelial cells of the proximal tubules	-	-	+++	-
Leucocyte infiltration	-	-	+++	-
Glomerular sclerosis	-	-	++	-

(–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.

Effects of Treatments on Hematological Parameters

As shown in (Table 3), hematological parameters in control and treated groups. Red blood cells (RBCs), haemoglobin (Hb) content in MD-treated group were significantly decreased compared to those in the controls. While, administration of Ac extract with MD showed an improvement of RBCs and Hb levels by 32.8% and 8% respectively as compared to the MD-treated group**.**

Effects of Treatments on Plasma Biochemical Parameters

Biochemical parameters were investigated to assess the effects of MD on renal functions and to determine whether Ac extract could ameliorate the damage induced by this compound, so total protein, creatinine and urea are indicators of renal function. As shown in (Table 4) compared to the control group, the MD-treated group had significantly higher total protein, creatinine, and urea levels. However, the administration of Ac extracts to MD-treated group was significantly reversed these change to near normal values.

Effects of Treatments on Renal Oxidative Stress Parameters

Table 4; Figure 2 summarizes the changes of kidney oxidative injury. To explore the oxidative consequences of methidathion treatment in kidney and to determine the possible protective effects of Ac extract, we analyzed the formation of TBARS which showed a significant increase (36.26%) in MDA level compared to the control group. Conversely, Exposure to Ac extract in rats prevented the TBARS production induced by MD. A significant decrease of SOD (-47%), GSH-Px (-30.5%), GST (-22.01%), and CAT (-63%) activities was found in Kidney of MD-Treated animals compared to control. These changes were

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accompanied by a significant decrease (- 24%) in GSH content. On the other hand, the administration of Ac extract with MD produced recovery in the above mentioned renal oxidative stress parameters.

Histopathological Changes in the Kidneys

The hispathological alterations in kidney observed under light microscopy in methidathion treated rats were objectified by tubular dilatation, hemorrhage, glomerular sclerosis, leukocyte infiltration, and necrosis in epithelial cells of the proximal tubules (Figure 3C-F)). In fact, there were no histological changes in the kidney of a positive group treated only with Ac extract (Figure 3B) when compared with a control group (Figure 3A). Whereas, in MD+Ac group kidney injury was remarkably reduced in comparison to the MD treated group. The histopathological changes are graded and summarized in Table 6. Histological grading was made according to five severity grades: (–) indicates normal, (+) indicates mild, (++) indicates moderate, (+++) indicates severe, and (++++) indicates extremely severe.

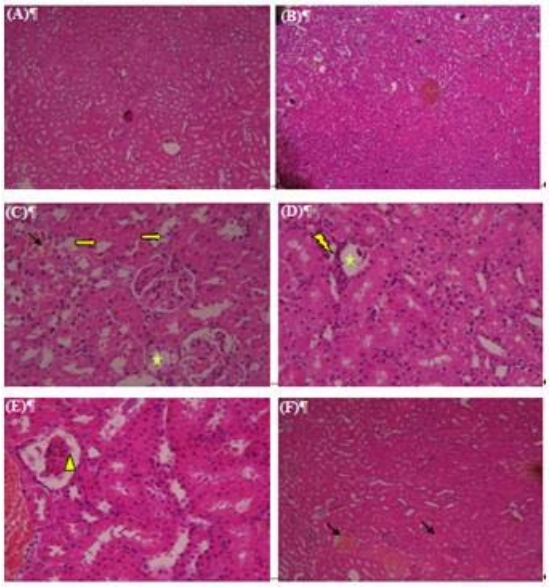

Figure 3: Effect of methidathion (MD) and Artemisia campestris (AC) administrated with MD on histopathological damages in the kidney. Controls (A), treated with AC (B), MD (C-D-E) and AC administrated with MD (F) after 4 weeks of treatment, as revealed by light microscopy.

Tubular dilatation (⇨), leukocyte infiltration (✎), necrosis in epithelial cells of the proximal tubules (☆), hemorrhage (→), and glomerular sclerosis (△) .

DISCUSSION

Oxidative stress is characterized by the imbalance between production of oxygen free radicals (OFRs) and antioxidants defenses in the body which may cause at the time chronic permanent damage[33]. Furthermore, treatment of OPIs induced-oxidative stress with medicinal plants has proved to be much safer than synthetic drugs, and gained an importance in recent years[12]. Medicinal plant contains a large variety of free radicals scavenging molecules such as vitamins, phenolic compounds, flavonoids, and other endogenous metabolites rich in antioxidants activity[34] In the current study, Preliminary phytochemical analysis showed that phenols, flavonoids and condensed tannin existed in the infusion extract of Artemisia campestris. The stable DPPH radical is widely used to evaluate the free radical-scavenging activity in many plant extracts. The assessment of antioxidant activity showed that the infusion extract of Ac was able to scavenge this radical.

MD is widely used throughout the world as a wide-spectrum insecticide for numerous harmful agricultural crops. Although MD is well known to have general health effects little is known of how MD contributes to the oxidative stress on higher animals. Many studies suggest that MD could cause oxidative stress in liver, kidney and heart[35,36]. In addition, OPIs cause a reduction of body weight in experimental animals[22,37,38]. In the present study, oral administration of MD (5mg/kg b.w) resulted in significant reduction in body weight. This might probably attributed to the reduction of feed consumption and/or malabsorption of nutrients induced by MD effects on the gastro-intestinal tract and/or inhibition of protein synthesis. MD exposure may account to reduced food intake seen in the MD-treated group. Hence, these findings were similar to the results published by Ogutcu[38] and Messarah[39], who reported that OPs exposure have significantly induced disturbances of the total body weight. MD treatment resulted also in a significant increase in relative and absolute kidneys weights of rats. This may probably due, according to Kamath[40], and Mahjoubi-samet[41], to oedema and inflammation.

In the current study, the exposure of rats to MD induced hematological changes (essentially RBC and Hb). Our results corroborated with the findings of Ojezele and Abatan[42] who reported a reduction in RBC and Hb in MD-treated group, which can be attributed by intravascular haemolysis, anemia, or depression of the haemopoeisis. Paradoxaly, the kidneys are responsible for the production of erythropoietin, so the finding may be due to the dysfunction of kidney and inhibition in the syntheses of erythropoietin.

On the other hand, haemoglobin in erythrocytes, is a major source of radical production when it interacts with redox drugs or xenobiotics giving rise to superoxide radicals, hydrogen peroxide and in certain cases peroxy radicals leading to membrane lipid peroxidation and hemolysis[43]. Similar results have been found in rat erythrocytes exposed to diazinon for 3 weeks[39]. Prasanthi[44] reported that oxidative damage, induced by pesticides, might be due to their lipophilicity, whereby they could penetrate easily to the cell membrane and caused membrane lipid peroxidation.

The co-administration of Ac (5mg/kg bw) attenuated the *in vivo* effects of MD by scavenging or neutralizing reactive oxygen species (ROS). These results indicated that Ac might have a beneficial role in lowering OPIs toxicity probably due to its radical scavenging property.[45]

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The treatment of rats with Ac alone did not cause alterations in erythrocytes.

The kidney is the critical target organ for xenobiotic compounds which produce a variety of renal toxic effects involving tubular cells and glomerulus[46]. In the other hand, urea and creatinine level represent the most sensitive markers of kidney damage and it is known that the elevated of may be correlated to an increase protein catabolism in mammals and/or the conversion of ammonia to urea blood urea[47]. The increase in plasma urea and creatinine in MD-treated rats support these findings and suggest a reduction in glomerular filtration. The administration of Ac extract protects the kidney function from MD intoxication as indicated by significant restoration of urea and creatinine levels. These findings corroborated with previous studies investigated in adult's rats treated by dimethoate[41].

The mentioned biochemical alterations are correlated with kidney histological changes. In fact, histological changes seen in the kidneys of rats are characterized by a glomerular sclerosis, and leucocyte infiltration which is in good accordance with previous study of Sulak[36] and Mahdjoubi samet[41], who had found degenerative changes in kidney of adult rats exposed to methidathion and dimethoate respectively. Moreover, necrosis in epithelial cells of the proximal tubules and tubular dilatation were observed. This could be due to the accumulation of free radicals as the consequence of increased lipid peroxidation by cyanides and aldehydes in the renal tissues of MD-treated rats[47]. Whereas, administration of oral Ac extract reduce degenerative damage of the kidneys, so, it protect the histological aspect of kidney which is confirmed by previous findings of Sefi[12].

To improve biochemical and histological changes, antioxidant play major role against harmful injuries[6]. GSH represent a major compound of defense against oxidative stress, so, its play a role as a cofactor with different antioxidant enzymes especially GPx[48]. It is known that mammalian cells have a comprehensive set of antioxidants defense mechanism to prevent free radicals formation and to limit their damaging effect[49]. In the present finding, the significant decrease in GSH levels promoted by MD leads to a significant decrease in the effectiveness of the antioxidant enzyme defense system sensitizing the cells to ROS[35]. In fact, GSH is known to participate in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen species intermediates[50]. Thus, depletion of GSH levels may be due to their consumption in quenching free radicals probably generated by DM. Indeed, GSH is considered as the essential compound that maintains cell integrity due to its reducing properties and its participation in the cell metabolism[3].

Co-administration of Ac after MD treatment improved GSH levels to reach normal values and ameliorated the antioxidant defense system. These data are similar to those reported in diabetic rats and suggested that Ac increase the biosynthesis of GSH and reduce the oxidative stress leading to less degradation of GSH[45]. Furthermore, administration of MD contribute to a significant decrease in GPx, SOD and CAT activities. Lowered activities of GPx, SOD and CAT may be due to excess production of O_2^-, and H_2O_2 or the product of decomposition which therefore triggered deleterious reactions[41]. Similar results have been found in rat erythrocytes exposed to triazophos[16] or lambda cyhalothrin[47]. Our findings, confirm previous investigations of Mahdjoubi-samet[41] and Sefi[45].

GST may play an important role in metabolism of xenobiotics substances especially in cellular detoxification of ionizing radiation in human[51]. A significant decrease of GST activity in rats treated with MD was observed. This could be explained according to Kostaropoulos[52], by rapidly metabolizing the insecticide to nontoxic products, or by rapidly binding and very slowly turning over the insecticide. Such results are in a good accordance with those obtained by Larsen[53]. In Ac extract treated group we found a significant higher in GST activity, it can due to the protective effect of Ac extract which stimulate the production of this enzyme.

ROS were a part of normal oxidative metabolism, when it produced in excess, they caused tissue injury including lipid peroxidation, protein and DNA damage[54]. However, lipid peroxidation is a basic cellular deteriorating process induced by oxidative stress and occurs readily in the tissues rich in highly oxidizable polyunsaturated fatty acids[55]. MDA, the end product of lipid peroxidation, has also been measured to indicate the presence of free radicals[56]. Organophosphorus compounds cause increase of lipid peroxidation level[56,41,36]. In the current study, the increased levels of MDA, the major product of lipid peroxidation, suggested that MD treatment induced significant oxidative stress in kidney tissue of rats. Howerver, lipid peroxidation were scavenged by the Ac extract which are rich in flavonoids and polyphenols. Several studies have shown that these compounds (flavonoids and polyphenols) inhibited the formation of free radicals and propagation of free radical reactions through hydrogen-donation and aromatic hydroxylation[55,57].

The mechanism by which Ac extract induced its nephroprotective activity against oxidative damage of methidathion, is based on the phytochemical composition of this extract (essential oil, tannins, polyphenol, flavonoids, saponosids) which have the ability to detoxify free radicals H, OH, O_2, and to its capacity to inhibits lipid peroxidation in the kidney tissue.

Furthermore, the recent previous study of Saoudi[23] showed after phytochemical tests on Ac extract infusion (5mg/ml) that the extract has a potential source of antioxidant. Moreover, it could take place as oxide radical scavenging activity and/or superoxide scavenging, as well as, our finding proved and support the antioxidant activity of Ac extract.

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CONCLUSION

In conclusion, this study demonstrates that exposure to MD provoked nephrotoxicity by inducing lipid peroxidation and depletion in antioxidant enzyme activities of rats.

However, Ac extract treatment could protect kidney against MD toxicity by reducing MDA level and increasing the activities of antioxidant enzymes. Further biochemical investigations are needed to ascertain the precise mechanisms of its action.

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