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Nephroprotective Effect of *Malva sylvestris* Extract against CCl_4 Induced Nephrotoxicity in Albino Rats

Hanaa S. S. Gazwi and Magda E. Mahmoud

Department of Agricultural Chemistry, Faculty of Agriculture, Minia University, El-Minia, Egypt

ABSTRACT

The aim of the study was to evaluate the potential effect of *Malva sylvestris*(*M. sylvestris*) extract on nephrotoxicity caused by CCl_4 in rats. Forty rats were divided into four equal groups. Group 1: negative control group (normal).Group2: positive group, treated with CCl_4 (1ml/kg body weight (b.w)), twice a week for 8 weeks. Groups 3 and 4 were treated with the same dose of CCl_4 co-administered with *M. sylvestris* at dose 150 or 300 mg/kg b.w, respectively. Results appeared that rats treated with CCl_4 showed a significant increment in WBCs, kidney function (urea and creatinine), total lipid, cholesterol, triglycerides, glucose, malondialdehyde (MDA), and nitric oxide (NO) levels, but a significant decline in the mean values of weight gain, RBCs, PCV, Hb, uric acid, and catalase (CAT) as comparing with the control group. The treatment with *M. sylvestris*(150 and 300 mg/kg b.w) improved the hematological and biochemical parameters. These protective effects were dose dependent. The histological results confirmed these parameters that enhanced by CCl_4 Thus, the extract of *M. sylvestris* represents an encouraging chance for the curative renal injury.

Keywords: *Malva Sylvestris*, Nephrotoxicity, Kidney function, Malondialdehyde, Nitricoxide.

INTRODUCTION

Kidneys are one of the key organs of the body which carry out several important roles in the body. Removal of waste from the bloodstream (urine formation) is considered the main function of the kidney. Also, the kidney perform many homeostatic functions example maintain volume, pH and ionic balance. Also, toxic metabolic by-products such as urea, ammonia, and uric acid are excrete (Alam *et al*.,2016).There are numerous therapeutic agents such as aminoglycoside antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs), antitubercular and chemotherapeutic drugs can badly influence the kidney causes severe renal failure, nephrotic syndrome and chronic interstitial nephritis (Hoitsma *et al*.,1991).Renal failing is the disorder where the with holding of metabolic products in reaction to the weakening of function (Pydi, 2011). Prevalence of chronic kidney disease in worldwide is estimated 8-16% in 2013 (Jha *et al*.,2013). In the 2015 worldwide Burden of ailment observe, kidney sickness changed into the 12th most commonplace cause of demise, accounting for 1.1 million deaths worldwide(Wang *et al*., 2016). Until date, available options for a remedy against renal disorders revolve around dialysis and kidney transplant. Both of them are often outside the reach of many people, especially in developing countries. Therefore, there is need to increase research toward finding a safe, facilely available and effective remedy against kidney disorder. Since this solution is the usage of plants in medicine being an age-long practice in several parts of the world for both preventive and curative. Today, it is estimated that about 80% of the planet's population rely on botanical preparations as medication to meet their health needs (Ogbera *et al*., 2010).

Malva sylvestris L. (Malvaceae) has been used medicinally throughout the world since 3000 BC (Henry and Piperno, 2008). It is commonly used as medicinal plant and in nurturing both animals and humans. Edible uses are attentive with folk gastronomy and with those uses covered by so-called simple nourishment (Barros *et al*., 2010 and Guarrera, 2003). Raw young leaves are eaten in salads, shoots and leaves are consumed in soups and as boiled vegetables. Medical applications of the plant are numerous such as treating specific disorders of the respiratory system, gastrointestinal tract, musculoskeletal system, as well as skin injuries. It also has diuretic effects, laxative, lenitive , spasmolytic and choleretic impacts. Also , is used as bronchodilator , anti-diarrheal, anti-cough and highly recommended for the treatment of acne and skin care (Carvalho, 2005 and DellaGreca *et al*., 2009).

Additionally, effective against mouth and throat diseases and can reduce swelling and soothe tooth pain, and gingivitis (Passalacqua *et al*., 2007). Many compounds in this plant carry the properties of analgesic and anti-inflammatory (Gasparetto *et al*., 2012),including kaempferol (De Melo *et al*., 2009), apigenin (Funakoshi-Tago *et al*., 2011), quercetin (Kleemann *et al*., 2011), scopoletin (Moon *et al*., 2007) and ferulic acid (Kim *et al*., 2012).The biological activity of this plant might be ascribed to antioxidants, for example, polyphenols, vitamin E, β-carotene, vitamin C, unsaturated fatty acids (e.g. α-linolenic acid), minerals and other vital phytochemicals (Barros *et al*., 2010). Leaves of *Malva sylvestris* have exceptionally antioxidant properties including the activity of radical-scavenging, diminishing force and lipid peroxidation hindrance in liposomes and brain cells homogenates.

To date, there are no data concerning the *in vivo* effect of *M. Sylvestris* extract on nephrotoxicity damage and oxidative stress induced by CCl_4. Thus, the present study evaluates the protective effect of *M. Sylvestris* against CCl_4-induced oxidative stress and nephrotoxicity in rats.

MATERIALS AND METHODS

Materials

Carbon tetrachloride were obtained from El-Gomhorya Pharmaceutical Company, Cairo, Egypt and Chemical kits for uric acid, blood urea nitrogen (BUN),creatinine, protein, albumin, total lipids total cholesterol (TC), triacylglycerol (TG),glucose, malondialdehyde (MDA), catalase (CAT) and nitric oxide (NO) were purchased from Biodiagnostic Company, Cairo, Egypt. All other chemicals were considered among the best available commercial grades. Leaves of *M. Sylvestris* were collected from Faculty of Agriculture Farm.

Preparation of aqueous extract of *M. Sylvestris* leaves

M. Sylvestris leaves were dried in shade at room temperature for two weeks and ground into fine powder. 100 g of powder was mixed with 1L of boiling distilled

water for 10 min under continuous stirring. The mixture was filtered twice through a mesh and through whatman No .1 filter paper, and the obtained liquid was evaporated to dryness under vacuum using a rotary evaporator, then collected in glass Petri dishes, dried in a vacuum desiccator and finally stored in airtight glass containers in a refrigerator at 4°C for use in the experiments.

Animals and Experimental Design

Forty female Sprague-Dawley albino rats (150 ± 5g) were obtained from the Animal House of Faculty of Agriculture, Minia University. They were housed under standardized environmental conditions, fed with standard diet and left to acclimatize to the environment (22 ± 2°C under a 12/12 hr light/dark cycle) for one week before the experiment was beginning .

Animals were divided into four groups (ten rats each) and treated as follows: Control group (negative), rats were injected with the respective vehicle (0.5 ml/kg b.w paraffin oil in saline). Rats of the second group (positive) were injected with CCl_4 (1 ml/kg, 1:1 mixture with paraffin oil, i.p. (Marsillach *et al.,* 2009)), each third days for 8 weeks to induce liver fibrosis. The third and fourth groups (CCl_4+*M. Sylvestris*150 and 300 mg/kg respectively) were received *M. Sylvestris* extract (150 and 300 mg/kg respectively) daily for two weeks. Then injected with CCl_4 as described in positive group.

After eight weeks rats were fasted overnight and anesthetized by diethyl ether to take the blood samples from the retro-orbital plexus (Schermer, 1967) from all animals of each group. Each sample was divided into two portions: the first portion was immediately taken in heparinized tube for hematological study and the second portion of the sample was taken in glass tube and left for 20 min to coagulant at room temperature and then centrifuged at 3000 rpm for 15 min, to obtained serum samples which kept at -20°C until used for the assessment of kidney function and lipid profile tests. Then, rats were sacrificed and kidney tissues were dissected, washed with ice-cold saline, weighed and stored at −20◦C. Thereafter, kidney tissues were homogenized in saline and the homogenate was used for assessment of oxidative stress markers catalase (CAT), NO and lipid peroxides (MDA). In addition, specimens from kidney tissues were fixed in 10% formalin for histopathological examination.

Determination of Hematological Parameters

The red blood cells (RBC) and white blood cells (WBC) counts were carried out by Neubauerhemocytometer method (Dacie and Lewis 1991). The hemoglobin (Hb) concentration was determined according to Jain (1986), using the cyanmethemoglobin method. The packed cell volume (PCV) was determined by the microhaematocrit method according to Dacie and Lewis (1991).

Biochemical estimations

Kidney function tests

Serum uric acid, urea and creatinine levels were assayed in the samples by a colorimetric method (Fossati *et al*. 1980; Fawcett and Scott 1960 and Szasz*et al*., 1979, respectively).Serum albumin level was assayed using the method described by Dumas *et al*, (1972).Creatinine /albumin ratio (C/A) was calculated from the results obtained.

Determination of total lipids, cholesterol, triacylglycerol and glucose.

Serum samples were used for determination of total lipids (Zollner and Kirsch, 1962) total cholesterol (TC) (Allain *et al*., 1974), triacylglycerol (TG) (Fossati and Prencipe, 1982) and glucose Trinder (1969).

Determination of lipid peroxide level

Lipid peroxidation level in the kidney homogenate was determined as thiobarbituric acid reactive substances (TBARS) by measuring malondialdehyde (MDA) level spectrophotometrically in kidney homogenates according to Mihara *et al*.,(1978) and catalase (CAT) and nitric oxide (NO) were determined according to Yoshioka *et al*.,1979. and Green *et al*., (1982)

Histopathological examination

Kidney specimens were fixed in 10% formalin and processed for paraffin sections of 4 µm thickness. Sections were stained with hematoxylin and eosin (H&E) for routine histopathological examination and Masson's trichrome for demonstration of collagen fibers.

Statistical analysis

The results obtained in the present study were evaluated by One Way ANOVA test followed by Tukey Dunken SPSS. The results were expressed as mean ± standard error and values of $P<0.05$ were considered statistically significant (Snedecor and Cochran, 1986).

RESULTS AND DISCUSSION

Effect of *M. sylvestris* on weight gain and average kidney weight in albino rats

Body weight changes may provide an indicator of drug effect and are used for assessment of responses to the drug therapy (Asuquo *et al*., 2012).The effect of *M. sylvestris* on weight gain (g) and average kidney weight (g) are represented in Table 1. Treatment of rats with CCl_4 lead to a significant reduce in weight gain comparing with control group ($P< 0.05$). Treatment with *M. sylvestris* markedly improved the growth. The average weight of the kidney was significantly increase in CCl_4 group comparing with normal group ($P<0.05$). Administration of *M. sylvestris* leads to a significant reduce in the average kidney weight comparing with CCl_4 group ($P< 0.05$).

This increase in weight of the kidney may be imputed to lesions and injuries related to xenobiotics (Wong *et al.,* 2010) like CCl_4 which peroxidizes proteins of cell that way stimulating pathway of the inflammatory. Also, these results came in agreement with Abdel Moneim and El-Deib, (2012) and Sahar and Dalia, (2014) who found that CCl_4caused a significant decrease in body weight while increases kidney weight and relative weight of kidney. The enlargement of the kidney was significantly reduced in *M. sylvestris* groups, suggesting that the *M. sylvestris* includes some protecting phytomedicinals. This observation of the effect on body weights of *M. sylvestris* groups can be explicated by its effect on appetite center in the hypothalamus.

Table 1. Effect of *M. sylvestris* on weight gain and average kidney weight in albino rats

Parameter	Control	CCl_4	*M. sylvestris* (150 mg/kg b.w)	*M. sylvestris* (300 mg/kg b.w)
Weight gain (g)	52.45 ±4.81	22.24[a]±1.32	38.17[ab]±3.82	40.28[b]±4.69
Average kidney weight (g)	1.37±0.04	1.59[a]±0.08	1.35[b]±0.06	1.32[b]±0.07

Data represent the mean ± S.E. of observation from 10 rats .[a] significantly different from control group at P ≤ 0.05.[b] significantly different from CCl_4 group at P ≤ 0.05.

Effect of *M. sylvestris* on the hematological parameter

Hematological parameters are used to provide useful information for diagnosis in the routine clinical evaluation of the state of patient health. This parameters (RBC, PCV, Hb, and WBC) are used to evaluate the effects of *M. sylvestris* on the blood of rats (Table 2). The group was injected with CCl_4 significantly decreased the levels of RBC, PCV, and Hb, while increased the level of WBC compared to the control group (Table 2). These could be attributed to CCl_4 toxicity and its direct effect on the hematopoietic system (Zeynab and Shereen, 2012 and Al-Mashhadani, 2017). And decreasing hemoglobin in injected rats with CCl_4 is an indication of hemolysis and the decreasing in hemoglobin has a corresponding elevation in methemoglobin content which affects the oxygen-carrying the blood, caused by the toxicant(Tilak *et al*, 2007).The increasing in WBC can be due to the stimulation of the immune defense system(Kashinath 1990) or increasing antigen concentration in the body (Hoeney, 1985), the low values PCV may be attributed to anemic conditions. While groups pretreated with 150 and 300 *M. sylvestris* significantly increased (p≤ 0.05) RBC, PCV, and Hb levels while decreased in WBC levels when compared with untreated rats (Table2).

Table 2. Effect of *M. sylvestris* on a hematological parameter in albino rats

Parameter	Control	CCl_4	*M. sylvestris* (150 mg/kg b.w)	*M. sylvestris* (300 mg/kg b.w)
RBCs ($x10^6$/cmm)	6.92±0.17	6.75±0.16	6.9±0.00	6.92±0.14
PCV%	49.1±1.38	41.0[a]±2.69	45.5±0.00	45.8±2.26
Hgb g/dl	15.85±0.53	13.50[a]±0.00	13.3[a] ±1.06	14.78[b]±0.72
WBCs ($x10^3$/cmm)	10.15±0.85	13.6±2.78	10.0±1.06	10.9±2.98

Data represent the mean ± S.E. of observation from 10 rats .[a] significantly different from Control group at P ≤ 0.05.[b] significantly different from CCl_4 group at P ≤ 0.05.

Effect of *M. sylvestris* on renal function:

Table (3) show that urea, uric acid, creatinine, albumin levels and creatinine/ albumin ratio were used as biochemical markers for evaluation of kidney injury and these parameters were significantly increased in CCl_4-treated animals (P < 0.05).This result agrees with that of Al-Seeni *et al*., (2016). These increases could be attributed to impairment in renal functions. Uric acid, the metabolic end outcome of purine metabolism, has tried to be a selective antioxidant, capable particularly of reacting with free radicals and hypochlorous acid (Hasugawa and Kuroda, 1989). The increasing levels of creatinine and urea may be due to a diminish in glomerular filtration rate caused by acute renal dysfunction (Rahmat *et al.,* 2014).

In addition, reduced albumin concentration in CCl_4-treated rats resulted in significant leakage due to hyperplasia in glomeruli and tubules (Adewole *et al*., 2007). Whereas, treated rats with *M. sylvestris* concomitantly with CCl_4 afforded significant protection against CCl_4-intoxication (Table 3). The ameliorative effect against renal toxicity may be ascribed to high levels of polyphenols and other antioxidants like flavonoids.

Table 3. Effect of *M. sylvestris* on renal function

Parameter	Control	CCl_4	*M. sylvestris* (150 mg/kg b.w)	*M. sylvestris* (300 mg/kg b.w)
Uric acid (mg/dl)	2.78 ± 0.17	1.64[a] ±0.07	2.24[ab] ±0.05	2.67[b] ±0.01
urea(mg/dl)	22.13± 2.67	42.26[a] ±3.59	28.87[b] ±3.59	25.13[b] ±2.21
Creatinine (mg/dl)	0.72 ± 0.02	1.51[a] ±0.08	1.04[ab] ± 0.05	0.98[ab] ± 0.03
albumin(mg/dl)	5.02 ± 0.42	3.77[a] ±0.23	3.93[a] ±0.36	4.41[a] ±0.21
Creatinine/Albumin ratio	0.14 ± 0.01	0.4[a] ±0.01	0.27[ab] ±0.03	0.22[ab] ±0.02

Data represent the mean ± S.E. of observation from 10 rats .[a] significantly different from Control group at P ≤ 0.05.[b] significantly different from CCl_4 group at P ≤ 0.05.

Effect of *M. sylvestris* and CCl_4 on total lipid, cholesterol, triglycerides and glucose.

The data in Table 4 shows that treatment with CCl_4 to rats significantly raised the levels of total lipid, triglycerides, cholesterol, and glucose comparing with control.

These results came in agreement with Nwidu *et al*.,(2017) who found that oxidative stress caused by CCl_4 increased the lipid profile levels. On the other hand, it may be presumed that hypercholesterolemia in rats treated with CCl_4 resulted from the damage of hepatic parenchyma cells, leading to an imbalance of lipid metabolism (Havel *et al*.,1986).However, *M. sylvestris* significantly improved the lipid profile of rats treated with CCl_4.

Table 4. Effect of *M .sylvestris* and CCl_4 on total lipid cholesterol, triglycerides, and glucose.

Parameter	Control	CCl_4	*M. sylvestris* (150 mg/kg b.w)	*M. sylvestris* (300 mg/kg b.w)
Total Lipid (mg/dl)	430.15±17.67	670.16^{a} ± 28.91	500.61^{ab} ±11.72	450.21^{b} ± 11.89
Cholesterol (mg/dl)	6.27± 95.25	136.62^{a} ± 7.41	112.87± 8.52	101.69^{b} ±7.26
Triglycerides (mg/dl)	66.63 ± 4.65	87.26^{a} ± 3.65	979. 1^{a} ± 2.4	73.84^{b} ± 2.39
Glucose (mg/dl)	70.76 ± 4.99	98.78^{a} ± 5.01	80.65^{b} ± 5.62	72.43^{b} ± 6.16

Data represent the mean ± S.E. of observation from 10 rats .[a] significantly different from Control group at P ≤ 0.05.[b] significantly different from CCl_4 group at P ≤ 0.05.

Effect of *M. sylvestris* on malondialdehyde, catalase and nitric oxide in albino rats

Table 5, indicate that the level of kidney malondialdehyde in CCl_4 group was significantly higher than the control group. Our result came in agreement with other reporters who demonstrated that CCl_4 significantly increased the renal MDA levels comparing with control group (Abdel Moneim and El-Deib, 2012; Sahar and Dalia, 2014 and Hamid *et al*.,2018). The increase in the level of MDA indicates that peroxide enhancement leads to tissue injury and the failure of antioxidant mechanisms to stop the production of excessive free radicals (Furfaro *et al*., 2012; Satoh *et al.,*2013 and Garcia-Nino and Pedraza-Chaverri , 2014) .Noorah and Mousa, (2014) reported that the increase in MDA concentration due to increased oxidative stress .Concomitant *M. sylvestris* with CCl_4 markedly improved levels of MDA compared with CCl_4 group

As shown in Table 5, the activity of CAT was decreased in rats treated with CCl_4 (0.367±0.03U/g) compared to control group (0.979 ±0.02 U/g). However, treatment with *M. sylvestris* markedly increased its activity but not significant compared to CCl_4 group. It seems that *M. sylvestris* protect the kidney rats.

Oxidative stress is an imbalance between the antioxidant mechanisms and the production of Reactive oxygen species (ROS) (Frei, 1994).The results of the present study have appeared that oxidative stress caused by CCl_4 is also evident from the significant depletion of the antioxidant catalase enzymes. Decreased CAT activity in the kidney may be caused by accumulation of ROS in kidney tissue in this study. Similar results were reported (Ottu *et al*., 2013 and Sylvia *et al*., 2017). The protective effects of *M. sylvestris* against the CCl_4 could be ascribed to its high concentration of phenolic compounds and other antioxidants

Treated with CCl_4 increased NO activity (14.29±1.23) compared to control group (4.84±0.59), treatment with *M. sylvestris* significantly decreased the activity of NO compared to the CCl_4 group. Our findings came in agreement with Khan *et al*.,(2009) and Abdel-Moneim and El-Deib (2012) who showed that NO renal levels significantly increased in the CCl_4 group comparing with control group.

Table 5. Effect *of M. sylvestris* on hepatic lipid peroxide as (MDA) and catalase activities (CAT) in CCl_4-induced hepatoxicity in rats.

Parameter	Control	CCl4	*M. sylvestris* (150 mg/kg b.w)	*M. sylvestris* (300 mg/kg b.w)
nmd (nmol/g wet tissue)	5.07±0.49	10.54^{a} ±0.45	6.84^{ab} ±0.44	8.21^{ab} ±0.30
Catalase (U/g)	0.979 ±0.02	0.367^{a} ±0.03	0.613^{ab} ±0.02	0.776^{ab} ±0.02
NO(μmol/L)	4.84±0.59	14.29^{a} ±1.23	12.91^{a} ±1.82	9.56^{ab} ±1.24

Data represent the mean ± S.E. of observation from 10 rats .[a] significantly different from Control group at P ≤ 0.05.[b] significantly different from CCl_4 group at P ≤ 0.05.

Effect of *M. sylvestris* on histopathological changes in the kidney

Several studies showed that plants possessing the properties of free radical scavenging can play an important role in protecting oxidative damage in various organs such as kidney, liver, and brain; caused by environmental and chemical toxic substances through metabolic activation to highly reactive substances such as free radicals (Kengar *et al.,* 2017).

Fig 1 and 2 show the histopathological changes of kidney section. A photomicrograph of kidney section of group (A) showed renal corpuscles, glomeruli (G) formed of capillary tufts is surrounded by Bowman's capsules (arrows). Note the proximal (P) and distal (D) convoluted tubules cells intact with acidophilic cytoplasm and vesicular nuclei.CCl_4 intoxicated rat kidney showed renal structural disruption with empty areas of completely degenerated renal corpuscles (*) (B).Renal structural disruption with extensive collagen fibers deposition around renal tubules and blood vessels(arrows). These results agree with Majno and Joris, (2004) and Kengar *et al*.,(2017) who showed CCl_4 caused significant glomerular and tubular degenerations. Glomerular congestion has been observed with disintegrated Bowman's capsules. Vacuolar and degenerative changes were also evident in the endothelial lining of the glomerular tuft and in the epithelial lining of renal tubules. Swollen proximal tubules and dilated Bowman's capsule were detected. Swollen collecting dust, distinct intertubular connective tissue and thickening of collecting tubules are an indicator of CCl_4 toxicity .While administration of *M. sylvestris* regained the histological alternations produced by CCl_4 into normal histological kidney structure with a small dilatation in some tubules (H&E, scale bar=50μm). Nephroprotective effects of *M. sylvestris* related to the existence of active components such as phenolics and flavonoids which can control the oxidation and peroxidation process of lipids, resulting in reduction of tissue damage and help to continue the blood flow in the kidney and improvement of kidney functionality (Rafieian-Kopaei *et al*.,2013).

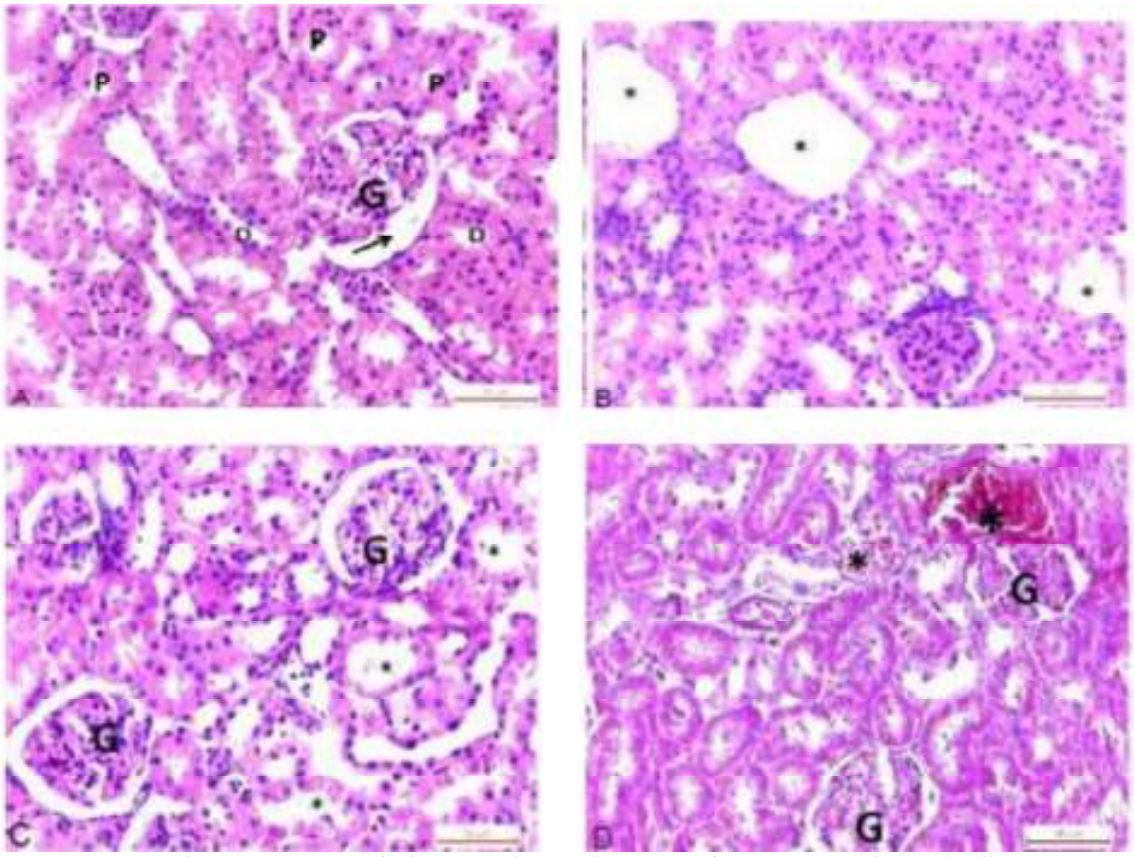

Fig. 1. Representative photographs of kidney sections stained by H&E(scale bar=50µm).(A) Control, (B) rats treated with CCl_4, (C) rats treated with *M. sylvestris* (150mg/kg b.w) + CCl_4 and (D) rats treated with *M. sylvestris* (*M. sylvestris* 300mg/kg b.w) + CCl_4

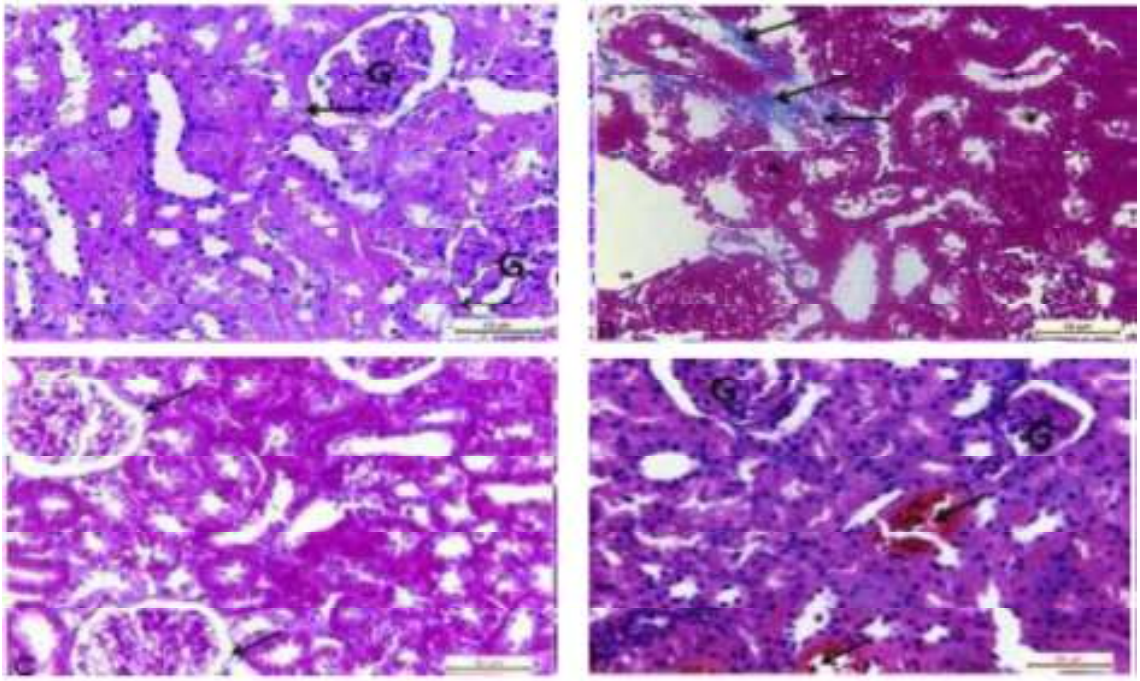

Fig. 2. Representative photographs of kidney sections stained by Masson trichrome (scale bar=50µm).(A) Control, (B) rats treated with CCl_4, (C) rats treated with *M. sylvestris* (150mg/kg b.w) + CCl_4 and (D) rats treated with *M. sylvestris* (*M. sylvestris* 300mg/kg b.w) + CCl_4

CONCLUSION

The current study indicates that *M sylvestris* supplements prevent biochemical change and pathological dissection caused by CCl_4. This renal protective effect of *M. sylvestris* can be ascribed to the presence of antioxidant contents, for example, phenol compounds and flavonoid that cause a significant reduction of the oxidative threat leading to a normal physiological function. The results support the use of *M. sylvestris* to treat kidney disease.

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تأثير مستخلص الخبيزة على التسمم الكلوي الناجم عن CCl_4 في الجرذان البيضاء

هناء سالم صالح جازوي وماجدة عويس محمود

قسم الكيمياء الزراعية – كليه الزراعة –جامعه المنيا –المنيا- مصر

يسبب التعرض لـ CCl_4 التخليق المفرط لأنواع الأكسجين التفاعلية (ROS) في العديد من الأنسجة ، مثل الكلية. تؤدى أحيانا الشقوق الحرة إلى العجز الكلوي وربما الفشل الكلوي. الهدف من هذه الدراسة تقييم التأثير المحتمل لمستخلص الخبيزة (*Malva Sylvestris* (M. *sylvestris* على السمية الكلوية الناتجة عن رابع كلوريد الكربون في فئران التجارب. تم تقسيم أربعين جرذ إلى أربع مجموعات متساوية. المجموعة ١: المجموعة الضابطة (الكنترول). المجموعة ٢: المجموعة الممرضة وهى المعاملة بـ CCl_4 (١مل / كجم من وزن الجسم)، مرتين في الأسبوع لمدة ٨ أسابيع. المجموعتين ٣ و ٤ تم إعطائهم نفس الجرعة من CCl_4 بالإضافة إلى مستخلص الخبيزة بتركيز 150أو ٣٠٠ ملجم / كجم من وزن الجسم يوميا ، على التوالي. وفي نهاية التجربة ، تم إجراء بعض التقديرات في الدم والسيرم والكلى بالإضافة إلى إجراء الفحص الهستولوجى لأنسجه الكلى. أظهرت النتائج أن الفئران المعاملة بـ CCl_4 حدث لها زيادة كبيرة فيWBC، وظائف الكلى (اليوريا والكرياتينين)، والدهون الكلية، الكولسترول، الجلسيريدات الثلاثية ، والجلوكوزو(Malondialdehyde (MDA، وأكسيد النيتريك (NO) ، بينما انخفض معدل الزيادة في الوزن، كرات الدم الحمراء، PCV، Hb، حمض اليوريك ، والكتاليز (CAT) بالمقارنة مع المجموعة الضابطة.أدت المعاملة بالخبيزة بتركيز (٣٠٠ملجم/كجم من وزن الجسم) إلى أن معظم المعاملات البيوكيميائية والدموية المقاسة كانت قريبة من قيم المجموعة الضابطة (الكنترول). وأكد ذلك نتائج الفحص الهستولوجى لأنسجة الكلى. بناء على هذه النتائج ، يمكن اعتبار المستخلص المائي للخبيزة مفيد لعلاج أمراض الكلى.