

Alleviation of cisplatin-induced nephrotoxicity in rats by aqueous extract of *Salvia officinalis* leaves

Kawa Dizaye

Department of Pharmacology, College of Medicine, Hawler Medical University, Irbil

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ABSTRACT

Objectives: To evaluate the protective effects of the aqueous extract of *Salvia officinalis* leaves against nephrotoxicity induced by cisplatin in rats.

Methods: Albino rats were divided into three groups, each group consist of six rats. Group I treated with vehicle (distilled water) was kept as a control. Group II injected with a single dose of cisplatin (12 mg/kg body weight; i.p.). Rats in Groups III were received a single daily dose of aqueous extract of *S. officinalis* 100 mg/kg (P.O.), for 7 days. On the seventh day, cisplatin (12 mg/kg body weight; i.p. was administered half an hour after the last dose of the plant extract. The rats in all groups were sacrificed 72 h after treatment. Renal injury was assessed using serum biochemical markers (creatinine and urea).

Malondialdehyde (MDA) concentration was measured as a marker of lipid peroxidation. The renoprotective activity of *S. officinalis* was supported by histopathological studies of the kidney.

Results: Aqueous extract of *S. officinalis* leaves significantly protected rat kidneys from cisplatin-induced histopathological changes. This extract also normalized cisplatin induced increases in serum creatinine and blood urea. In vitro studies revealed that the *S. officinalis* leaf extract possessed significant oxidative radical scavenging activities.

Conclusion: Both biochemical findings and histopathological evidence showed the renoprotective potential of aqueous extract of *S. officinalis* leaves against cisplatin-induced oxidative stress and renal dysfunction in rats.

Key Words: Cisplatin, nephrotoxicity, extract of *Salvia officinalis*.

الخلاصة

الهدف: دراسة التأثير الوقائي للمستخلص المائي لأوراق نبات *Salvia officinalis* للتسمم الكلوي بعقار cisplatin في الجرذان.

طرق العمل: قسمت جرذان بيضاء الى ثلاثة مجاميع، كل مجموعة مكونة من ستة جرذان. عوملت المجموعة الأولى بمادة التخفيف (ماء مقطر) كمجموعة سيطرة. أما المجموعة الثانية فقد تم حقنها بعقار cisplatin في الخلب (12 ملغم/كغم من وزن الجسم). اعطيت المجموعة الثالثة جرعة يومية من مستخلص *S. officinalis* في الفم (120 ملغم/كغم) ولمدة سبعة أيام، ثم اعطيت الحيوانات في اليوم السابع عقار cisplatin (12 ملغم/كغم في الخلب) بنصف ساعة بعد اخر جرعة من المستخلص النباتي. وقتلت جيع الحيوانات بعد 72 ساعة من المعاملة. وتم قياس مصل الكرياتينين واليوريا دلالة على كفاءة الكلية ومصل المالوندايديهايد دلالة على زناخة الدهون. وكان النشاط الوقائي للمستخلص النباتي على الكلية قد تم بالدراسة النسيجية.

النتائج: لوحظ ان المستخلص المائي لنبات *S. officinalis* قام بحماية كلى الجرذان من التسمم بعقار cisplatin وعدل المستخلص النباتي من زيادة مصل الكرياتينين ويوريا الدم بعقار cisplatin. وفي الزجاج بينت الدراسة أن المستخلص النباتي على فعالية التهام الجذور الحرة.

الاستنتاج: بينت الدراسات الكيميائية والحياتية والنسيجية التأثير الوقائي الكامن لمستخلص نبات *S. officinalis* ضد الإجهاد التأكسدي والأذى الكلوي لعقار cisplatin.

Medicinal and poisonous plants always play an important role in the health among people all over the world. However, it has been estimated that only 6-8 % of the world's flora (approximately 250000 plants) and less than 10% of the organic constituents are known and have been investigated chemically, and 90% remains for discovery and investigations¹. Medicinal plants have various effects on living systems. Some are sedatives, analgesics, antipyretics, antibacterials, antivirals antiprotozoals, cardioprotectives, hepatoprotective and nephroprotective agents².

For centuries, many herbs have been used as natural remedies for the prevention and/or treatment of kidney diseases. Various herbs and herbal products are believed to have nephroprotective effects and widely used in clinical practice in many parts of the world, for example *Silybum marianum* attenuated nephrotoxicity induced by gentamicin in dogs³.

Aqueous extract of *Kalanchoe pinnata* leaves significantly protects rat kidneys from gentamicin-induced histopathological changes in rats⁴. *Salviae Radix* Extract exerts a protective effect against renal cell injury induced by cisplatin, and its effect might be attributed to its antioxidant action⁵.

The name *Salvia officinalis* derives from the Latin 'salveo', which means to be saved. *Salvia* is a perennial herbaceous to shrubby herb growing up to 50cm in height⁶. Herb is highly regarded for its healing qualities.. *Salvia officinalis* has a very

long history of effective medicinal use. The ancient Greeks used it to treat consumption, ulcers and snake bites, and is an important domestic herbal remedy for disorders of the digestive system⁷. Its antiseptic qualities make it an effective gargle for the mouth where it can heal sore throats, ulcers etc. The leaves applied to an aching tooth will often relieve the pain⁸.

The leaf of *S. officinalis* has antihistaminic activity which was not associated with anticholinergic activity. It had antifungal effects against *Candida albicans*⁹.

The present study was performed to evaluate the protective effects of the aqueous extract of *Salvia officinalis* against nephrotoxicity induced by cisplatin in rats.

Materials and methods

Experimental animals

Male waster albino rats weighing 150-200 g were kept in the animal house of College of Medicine, Hawler Medical University. The room temperature was maintained at 25 °C. A 12 hr light/dark cycle was set. Rodent food rich in nutrient and tap water were used as bedding.

The animals were divided into three groups, each group consist of six rats. Group I was treated with vehicle (distilled water) and kept as control. Group II injected with a single dose of cisplatin (12 mg/kg body weight; i.p.). Group III received a single daily dose of aqueous extract of *S. officinalis* (100 mg/kg body wt; p.o), for 7 days. Cisplatin (12 mg/kg body weight; i.p. was administered half an hour after the last dose of the plant extract. Rats in

all groups were sacrificed 72 h after treatment.

Blood samples

Blood samples (1-2 ml) were collected from the rats in sterilized dry centrifuge tubes after carotid bleeding and allowed to coagulate for 30 min at 37 °C. Serum was separated at 2500 rpm for 10 min and subjected to biochemical investigations.

Measurement of hepatocellular enzyme levels in serum

Serum creatinine, urea and Malondialdehyde (MDA) concentration were determined using a standard clinical automatic analyzer (Beckman, Brea, CA).

Histological examination of kidney

Anatomy of the kidney was studied immediately after sacrificing the animals. The kidney was excised from the animals and washed with normal saline. The materials were fixed in 10% buffered neutral formalin for 48 hr and with bovine solution for 6 h and processed for paraffin embedding. Sections of 5 mm thickness were taken using a microtome, processed in alcohol-xylene series and stained with special stain PAS (periodic acid Schiff) Bankroft 1996¹⁰ and subjected to histopathological examination.

Plant preparation

An aqueous extraction of *Salvia officinalis* was prepared by using of ultrasonication¹¹. A sample of 50 g of powdered herb was suspended in 1000 ml distilled water in a glass beaker, mixed well, sonicated for 2.5 hr, in an ultrasonic machine bath (Decon FS 200 Frequency Sweep) at a constant

temperature (25 °C). Extract was separated by simple filtration, residual material washed with 20 ml, of pure water, the soluble extracts were concentrated ten-folds by rotary vacuum evaporator at 45-50 °C preserved in refrigerator for further studies.

The entire plant of *S. officinalis* was sun dried for ten days before the final drying in an oven at 50 °C for 24 hours. The dried plants were powdered in both manual grinder and electric grinder. Fifteen grams of the *S. officinalis* powder were soaked with 350 mL of distilled water in a beaker and the mixture shaken on the laboratory bench for 24 hours before filtering. The filtrate was evaporated by hot-air oven treatment at 40-50 °C. Appropriate weights of the residue were prepared in distilled water to obtain the various concentration used for the experiments.

Results were expressed as means \pm SE. Statistical analyses were carried out using ANOVA and Duncan test. Level of significance was set at $p < 0.05$.

Results and discussion

In this study, compared to the control group the level of serum urea and creatinine in cisplatin-induced nephrotoxicity in rats were found to be significantly increased in rats treated only with cisplatin, whereas treatment with the aqueous extract of the leaves of *S. officinalis* found to protect the rats from such effects of cisplatin as shown in Table 1.

Table 1. Serum creatinine and urea level in control, cisplatin and cisplatin with aqueous extract of *S. officinalis* rats

Parameters	Control group	Cisplatin Group	Cisplatin + <i>S. officinalis</i> group
Urea mg/dl	43.3 ± 3.22 a	56.8± 4.34 B	45.2±6.25 A
Creatinin mg/dl	0.65 ±0.02 a	1.24±0.12 B	1±0.15 Ab
Malondialdehyde μ mol/L	0.74±0.018 a	2.51±0.0528 B	1.86±0.043 Ab

Different letters indicate there is a significant difference at $P < 0.05$

The concentration of malondialdehyde (MDA) is observed to be significantly increased in the cisplatin-treated animals compared to the normal group. Administration of *S. officinalis* extract for 7 days before and a long with cisplatin decreased MDA to the normal level compared to cisplatin-treated animals (Table 1), this indicates the presence of antioxidant substances which protects the kidney damage via free radicals induced by cisplatin⁵.

To determine the morphologic changes of the kidney after cisplatin treatment, we performed histologic analyses of kidneys that were isolated from animals that survived to determine tubular necrosis using established histologic criteria¹².

Compared to the control group the measurements of the thickness of basement membrane in glomerular capillary and kidney tubules and PAS +ve granules in distal convoluted tubule in rats treated with ip injection of cisplatin were significantly increased. In normal condition the

basement membrane of glomeruli and renal tubules were normal (1-2 μ m), while in pathological conditions of renal diseases there was an increase in basement membrane thickness (3-5 μ m) with presences of PAS positive particles indicated that there will be damage to renal tissue which lead to an accumulation of glycoproteins within renal tubules¹³, as shown in Tables 2 and Figures 1 and 2.

Cisplatin and its analogues are widely used in the chemotherapy of a variety of human malignancies including head, neck, ovarian testicular and lung cancers¹⁴. However, the use of these compounds is limited by their side effects, cisplatin appear to be directly toxic to the renal tubules, usually most severe in the proximal tubules. After a single injection of cisplatin, 28-36% of patients develop dose-dependent nephrotoxicity. Cisplatin has been shown to accumulate in the kidney to a greater degree than in other organs^{15,16}.

Table 2. The effects of *S. officinalis* (PO) on the histological changes of kidney in cisplatin treated rats

	Control	Cisplatin	Cisplatin + <i>S. officinalis</i>
GBM (µm)	1.26±0.19 a	3.29 ± 0.32 B	1.99 ± 0.22 A
TBM (µm)	1.66 ± 0.17 a	3.71 ± 0.42 B	2.23 ± 0.15 Ab
PAS +ve	0.05 ± 0.04 a	5.34 ± 0.67 B	2.26 ± 0.23 Ab

GBM = Glomerular Basement Membrane

TBM = Tubular Basement Membrane

PAS = Periodic acid Schiff reagent, special stain used for the staining of glycoproteins such as basement membrane components.

The different letters indicate there is a significant difference at P<0.05

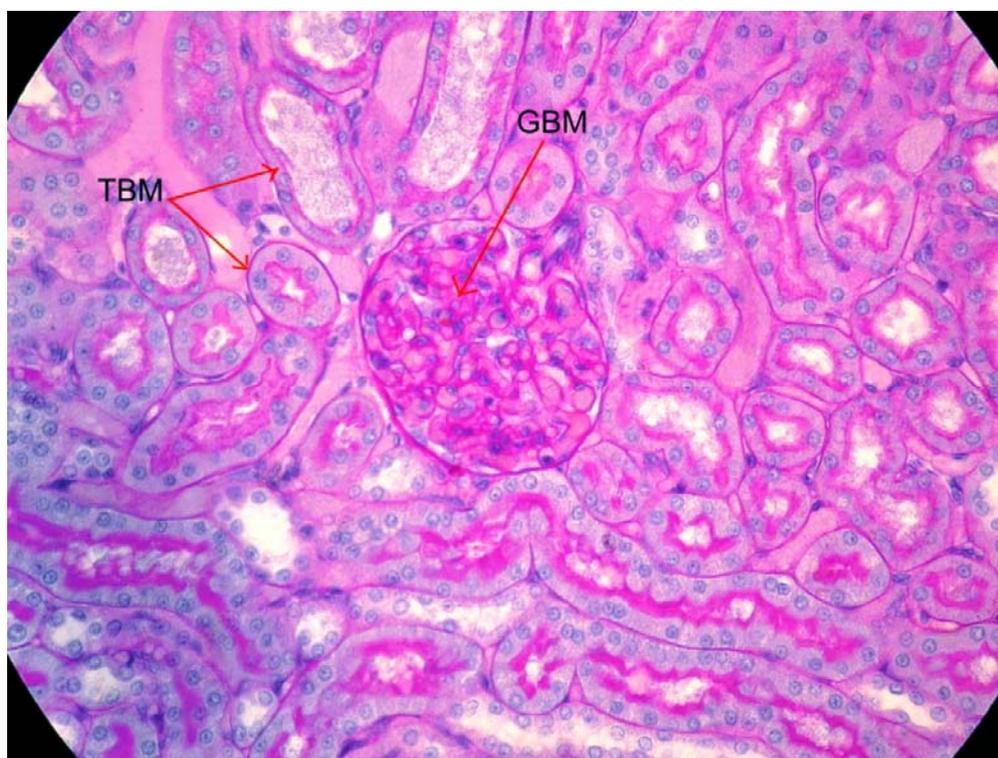


Figure 1. Renal cortex of normal rats stained by PAS. X100.

GBM = Glomerular Basement Membrane

TBM = Tubular Basement Membrane

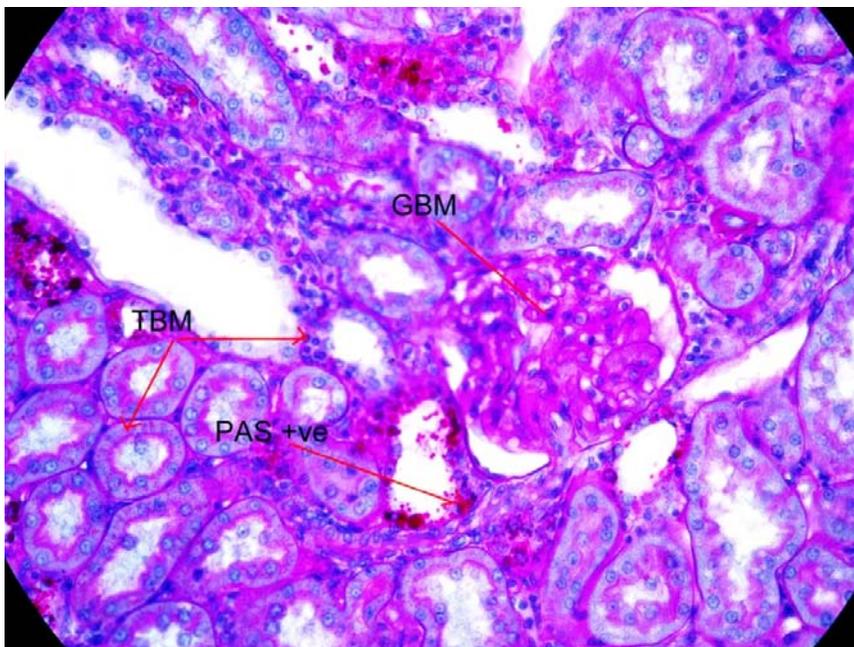


Figure 2. Renal cortex of rats treated with cisplatin stained by PAS. X100.

GBM = Glomerular Basement Membrane

TBM = Tubular Basement Membrane

PAS = Periodic acid Schiff reagent, special stain used for the staining of glycoproteins such as basement membrane components.

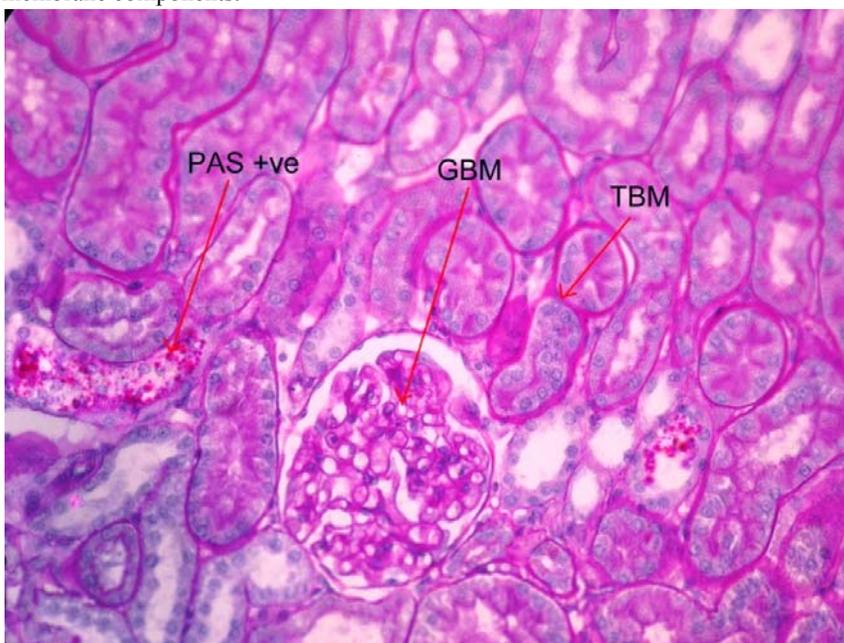


Figure 3. The effects of *S. officinalis* extract on the renal toxicity induced by cisplatin, stained by PAS. X100.

GBM = Glomerular Basement Membrane

TBM = Tubular Basement Membrane

PAS = Periodic acid Schiff reagent, special stain used for the staining of glycoproteins such as basement membrane components.

The nephrotoxicity associated with cisplatin is well documented and constitutes a dose-limiting side effect of cisplatin therapy. Morphological and physiological studies have identified the renal tubule system as the site of maximum cisplatin damage, with the proximal tubules being most affected

The mechanism of the cisplatin-induced cytotoxicity is not fully understood. It was shown that cisplatin is capable of binding to several cellular components, including membrane phospholipids, thiols, cytoskeletal microfilaments, proteins, RNA and DNA¹⁵. Apparently, this may involve more than one mechanism of cell death. Several lines of evidence indicate that free radicals are involved in the nephrotoxicity caused by cisplatin, and the damage is suggested to be the consequence of decreased renal anti-oxidant enzyme activity with enhanced lipid peroxidation. Administration of antioxidants has been shown to ameliorate cisplatin-induced nephrotoxicity in animals¹⁶.

A significant decrease in the level of blood urea and creatinin were observed in cisplatin treated rats after administration of *S. officinalis* extracts. In the present study, this reduction of blood urea seems to be due to its ability to reduce renal dysfunction. The stabilization of these renal parameters by the aqueous extract was a clear indication of the improvement of the functional status of the kidney, since most histological and histochemical changes seen before treatment with the extract were restored to nearly normal forms, such as a reduction in the thickness of glomerular and tubular basement

membranes. The results of the present study showed that plant extracts bring back the progression of renal damage in cisplatin treated rats to near normal levels, this indicates that the protective effect of flavonoid in the plant extracts have repairing effects on the organ tissue¹⁷. Moreover, there is a significant reduction in PAS +ve granule in distal convoluted tubules through decreasing rate of glycoprotein as shown in Tables 2 and Figure 3.

We suggest that the plant extract contains constituents having nephroprotective and antioxidant which acts in the kidney as a potent scavenger of free radicals to prevent the toxic effects of cisplatin.

In conclusion, both biochemical findings and histopathological evidence showed the renoprotective potential of aqueous extract of *S. officinalis* against cisplatin-induced oxidative stress and renal dysfunction in rats.

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