Protective effects of the wild blackberry on cyclosporine-induced renal injury in rats: implications for veterinary and agricultural toxicology

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Abstract

Immunosuppressant nephrotoxicity limits safe use. This study tested whether an aqueous extract of *Rubus ulmifolius* protects against cyclosporine-induced renal injury in rats, of clinical, agricultural, and veterinary relevance worldwide. Twenty-four male rats were randomized into four groups (n=6): G1 vehicle; G2 cyclosporine 0.2 mg/kg by oral gavage every other day; G3 extract 400 mg/kg daily for 30 days; G4 extract 400 mg/kg daily with Cyclosporine 4 h later on alternate days. Serum urea, creatinine, albumin, globulin, and total protein were assessed; kidneys were examined histologically. Biochemical assays used standard colorimetric methods, and statistical significance was set at $P<0.05$. Cyclosporine increased urea and creatinine and decreased albumin, globulin, and total protein versus G1. Extract alone matched control with no observable adverse effects. Pre-treatment prevented cyclosporine changes, normalizing values; G4 remained comparable to G1. Histology: G2 showed glomerular atrophy, widened Bowman's space, tubular sloughing, congestion, hemorrhage, inflammatory infiltration, and tubular degeneration/necrosis; G4 appeared near normal with preserved glomeruli and tubules. *R. ulmifolius* aqueous extract confers renoprotection against cyclosporine nephrotoxicity in male rats, normalizing renal biomarkers and preserving histology. These findings support its potential as a phytogenic adjunct to mitigate drug-induced kidney injury; dose–response and mechanistic studies are warranted.

Keywords: Ciclosporin, *Rubus ulmifolius*, kidney tissue.

Introduction

Medicinal plants have been used since ancient times and can be considered the origin of modern medicine. Compounds of plant origin have been and continue to be an important source of compounds used in medicines [1]. Since ancient times, people have searched for drugs in nature to relieve their ailments. The beginnings of the use of medicinal plants were instinctive; treatment with medicinal plants is as old as humanity itself. The connection between humans and their search for drugs in nature goes back a long way. Awareness of the use of medicinal plants is the result of many years of struggle against diseases, during which humans have learned to search for drugs in

the bark, seeds, fruit bodies, and other parts of plants. Contemporary science has recognized their effectiveness, and a group of drugs of plant origin has been included in modern pharmacological treatment [2].

Medicinal plants contain a wide variety of chemical compounds. The World Health Organization reported that 80% of the developing world still benefits from the use of traditional medicines derived from medicinal plants and described medicinal plants as one of the potential sources of new medicines. Medicinal plants produce an almost unlimited source of biologically active compounds, and their use as antimicrobial agents has been exploited in various ways [3].

Rubus ulmifolius is a perennial shrub commonly known as cranberry and is widely distributed in Asia, North Africa, and Europe, especially in the Iberian Peninsula. The flowering season occurs between May and June, followed by the ripening and development of the fruits, which are characterized by a cluster of numerous fleshy drupes that change color during ripening from green to black. These fruits are consumed fresh or as derivative products such as jams, juices, liqueurs, and preserves, due to their flavor and pleasant taste. Their bioactive compounds have been the focus of numerous scientific studies [4]. They are clustered, spherical in shape, with an acidic flavor and a black color at the end of ripening [5].

Plants typically bear biennial or semi-woody stems called canes. They range from sprawling shrubs to semi-erect, spreading, thorny plants with leaves. The stems grow up to 7 meters tall and are green, purple, or red in color. The color of the blackberry fruit and its juice depends on the natural pigments present in it. The flowers and fruits occur in inflorescence-like clusters or panicles. Blackberries bear fruit twice a year, in spring and autumn. These blackberries are thorny and have many spines. [6]

The *Rubus ulmifolius* genus comprises 900-1,000 species distributed worldwide. Archaeologists have found evidence of Rubus use dating back to around 8000 BC, suggesting that Rubus species have long been used as food and medicinal plants. Rubus species are rich sources of bioactive compounds [7].

These fruits are rich in sugars, fat, vitamin C, vitamin E, ellagic acid, flavones, anthocyanins, etc. Moreover, these berries have antibacterial, anti-inflammatory, antioxidant, antiaging, and other effects [8, 9] and are favored by many consumers on the market. The main free antioxidant component, ferulic acid, is the main binding component leading to the antioxidant capacity of blackberry [10]. Moreover, related studies have also found that a large amount of active phenolic substances can be extracted from raspberry leaves [11, 12].

Rubus ulmifolius is used in folk medicine to treat digestive problems and skin diseases in many countries. Different parts of the plant have long been used in herbal medicine to treat many ailments such as diabetes, high blood pressure, gastrointestinal disorders, mouth ulcers, heart disease, menstrual pain, hemorrhoids, tonsillitis, skin diseases, etc. The fruits are consumed fresh or in jams, juices, and preserves. The buds are used in omelets and soups [13]. Raspberry extracts have been shown to have anti-inflammatory, antioxidant, anticancer, antimicrobial, antihelminthic, and anti-

Alzheimer's activities [14].

Cyclosporine (CsA) is one of the most potent immunosuppressive drugs used in organ transplantation to reduce the risk of organ rejection [15]. Cyclosporin, with the chemical formula $C_{62}H_{11}N_{11}O_{12}$, is a potent immunomodulator and antibiotic produced by fungi. It is a lipophilic and hydrophobic cyclic peptide compound consisting of 11 amino acids [16]. The most important activity of CsA is to inhibit antibody formation and the cellular immune response. Its primary mode of action is to inhibit the production of cytokines involved in regulating T cell activation. In particular, it inhibits the transcription of interleukin 2 (IL-2), selectively inhibiting immune responses mediated by T helper lymphocytes [17]. It can suppress the immune system by changing the activity of genes that encode immune factors [18]

However, despite Cyclosporine's effectiveness, its use causes numerous side effects resulting from increased free radical production. Cyclosporine's metabolism by cytochrome CYP450 directly produces free radicals. It also inhibits mitochondrial respiration in cells, causing hypoxia, which also leads to free radical production [19]. The side effects of CSA depend on several factors, such as the type of use (organ transplantation or other), the dose and duration of treatment, the presence of various medical conditions, concomitant drug use, genetics, age, and gender, leading to significant individual variation among patients [20]. Immune suppression by Cyclosporine makes the body vulnerable to many diseases and infections and increases the risk of cancer, such as leukemia and bladder cancer, and inflammation. The main adverse effects associated with CSA are neurotoxicity, nephrotoxicity, hypertension, hyperglycemia, and gastrointestinal disturbances [21]. Side effects of CsA are usually accompanied by histopathological changes in various organs, such as the liver, kidneys, and heart [22], and testicular and prostate tissue [23].

This may be due to the increase in reactive oxygen species and the decrease in antioxidant levels caused by CSA, which disrupts the oxidative balance within the cell. High levels of oxidative stress can damage DNA and proteins, as CSA inhibits DNA synthesis, and free radicals affect the regulation of signaling pathways in the cell [24]. Free radicals attack nucleic acids, and when they penetrate membrane barriers, they cause damage and disrupt cellular activity. They enter the cell nucleus, which can cause genetic mutations and cell death resulting from the breakdown of DNA and lipids. They also lead to enzyme dysfunction and protein synthesis [25].

The current study explained the impact of Cyclosporine on kidney functions and kidney tissue, while and elucidating the protective role of *Rubus ulmifolius*.

Materials and Methods

Experimental Animals

In this study, 24 adult male white rats were used, weighing between 190-230g and aged between 9-12 weeks. They were obtained from private breeding fields in Baghdad Governorate. They were raised in the animal house of the College of Pharmacy/University of Karbala. Respectively, covered with metal covers, and in suitable laboratory

conditions in terms of temperature and lighting duration (12 hours of light - 12 hours of darkness), and good ventilation. The animals were left for two weeks before the start of the experiment to acclimatize and ensure that they were free from diseases.

The fruits of the *Rubus ulmifolius* plant were collected from the Islamic Republic of Iran in Isfahan province and classified by Prof. Dr. Nepal Matar, a specialist in plant taxonomy. Preparation of the aqueous extract of the *Rubus ulmifolius* plant fruits according to the method of [26].

Experimental Design

Twenty-four experimental animals were divided systematically into four groups, with six male rats in each group, as follows:

- **Negative control group (G1):** Animals in this group were orally dosed with a standard water solution for 30 days
- **Positive control group (G2):** Animals were dosed between one day and another with Cyclosporine at a concentration of 0.2 mg/kg of B. W. for 30 days
- **third group (G3):** Animals were dosed daily with the aqueous extract of the fruits of the plant *Rubus ulmifolius* at a concentration of 400 mg/kg of B. W. for 30 days.
- **fourth group (G4):** The animals of this group were given a daily dose of the aqueous extract of the fruits of the plant *Rubus ulmifolius* (400 mg/kg) of B. W. 4 hours before being given an oral dose of the drug Cyclosporine (0.2 mg/kg) between (one day and another day).

Statistical analysis using the t-test and a significance threshold of $P \leq 0.05$, the statistical tool Graph Pad Prism 8.0 was utilized. The information was displayed as mean ± SD.

Results and Discussion

Through the results of the Physiological examination of our experimental animals, which lasted for 30 days, it was found that Cyclosporine resulted in a significant decrease in the level of Albumin, Globulin, and Total protein compared to G1. The results showed that the aqueous extract of *Rubus ulmifolius* had a protective effect when given before Cyclosporine, as it worked to curb the harmful effects of the Cyclosporine and proved to be and restore Albumin, Globulin, and Total protein to their normal state and remained at their normal level compared to G1.show Table 1.

Table (1): Effect of Cyclosporine and aqueous extract of *Rubus ulmifolius* on the concentrations of Albumin, Globulin, and Total protein in male albino rats.

Treatments	Albumin dl/g	Globulin dl/g	Total protein dl/g
Control G1	2.533 0.13 B	2.986 0.18 B	4.520 0.22 A
Control (positive) Cyclosporine 0.2 mg/kg G2	1.883 0.11 C	1.561 0.19 C	3.216 0.36 B

***Rubus ulmifolius* aqueous extract 400mg/kg G3**	3.278 0.14 A	3.276 0.29 A	4.026 0.18 A
***Rubus ulmifolius* aqueous extract 400 mg/kg + Cyclosporine 0.2 mg/kg G4**	2.529 0.08 B	2.812 0.16 B	4.728 0.26 A
LSD	0.2245	0.2055	0.7874

*** P value Significant ≤ 0.05**

It was found that Cyclosporine resulted in a significant increase in the level of urea and creatinine compared to G1, while the group of animals that were dosed with the aqueous extract of *Rubus ulmifolius* showed a positive effect compared with G2. The results showed that the aqueous extract of *Rubus ulmifolius* had a protective effect when given before Cyclosporine, as it worked to curb the harmful effects of the Cyclosporine and proved to be and restored urea and creatinine to their normal state and remained at their normal level compared to G1. Show Table 2.

Table (2): Effect of Cyclosporine and aqueous extract of *Rubus ulmifolius* on the concentrations of urea and creatinine in male albino rats

Standards Groups	**CREA mg/dl**	**UREA mg/dl**
Control	0.271 0.03 B	35.54 3.92 B
Control (positive) Cyclosporine 0.2 mg/kg G2	0.315 0.02 A	122.80 9.42 A
***Rubus ulmifolius* aqueous extract 400mg/kg G3**	0.270 0.02 B	32.86 1.97 B
***Rubus ulmifolius* aqueous extract 400 mg/kg + Cyclosporine 0.2 mg/kg G4**	0.281 0.03 B	34.63 2.42 B
LSD	0.0542	10.355

*** P value Significant ≤ 0.05**

The results of histological examinations indicate that the kidney tissue in the second group showed the presence of glomerular atrophy, increased Bowman's space, and destruction of the walls of the urinary tubules, with congestion of the blood vessels between the renal tubules, hemorrhage between the renal tubules, sloughing of the lining epithelium, and showing severe infiltration of inflammatory cells with degeneration of the urinary tubule cells and necrosis of the tubule lining, Figure (2,3). Also showing in the kidney tissue of the third and fourth groups, the glomerulus and tissue are observed to be closer to normal, and the presence of Bowman's capsule and Bowman's space with the typical structure of the urinary tubules, Figure (4,5).

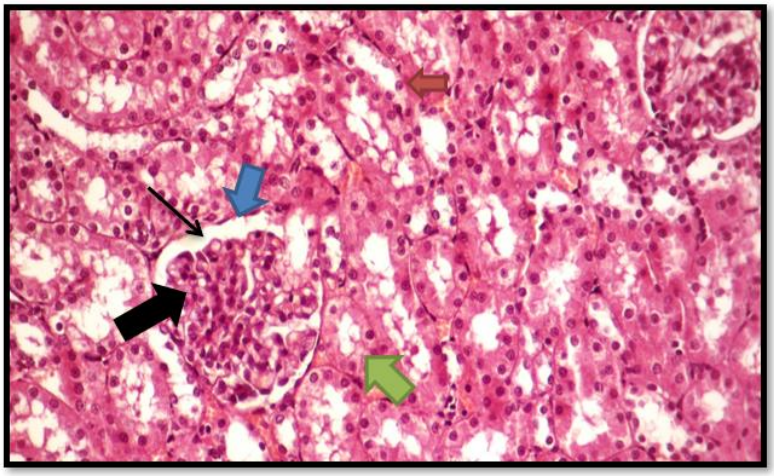

Figure (1): A cross-section of a rat kidney tissue from the negative control group showing the presence of a normal glomerulus (⬅) and Bowman's capsule (⬅) with the proximal convoluted tubule (⬅) and distal tubule (⬅) and Bowman's space (⬅) (H & E 200 X).

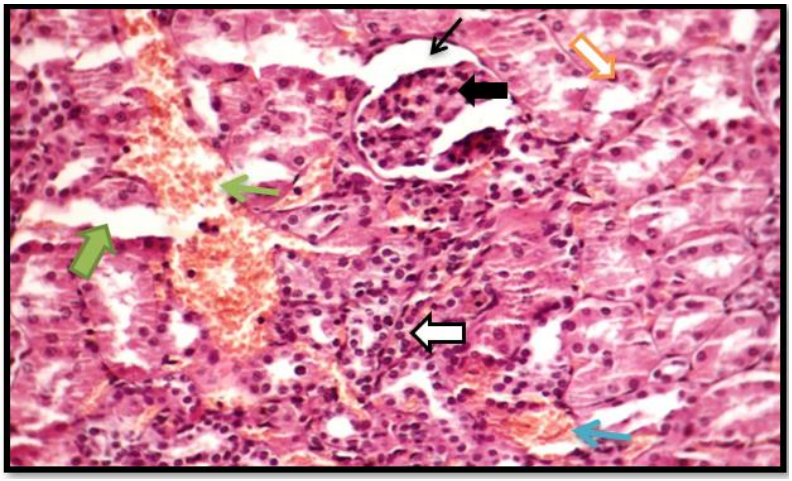

Figure (2): A cross-section of a rat kidney tissue in the group treated with Cyclosporine at a concentration of 0.2 mg/kg of body weight, showing glomerular atrophy (⬅), increased Bowman's space (⬅), and destruction of the walls of the urinary tubules (⬅), with congestion of the blood vessels between the renal tubules (⬅), hemorrhage between the renal tubules (⬅), sloughing of the lining epithelium (⬅) , and infiltration of inflammatory cells (⬅) (H & E Stain 200X).

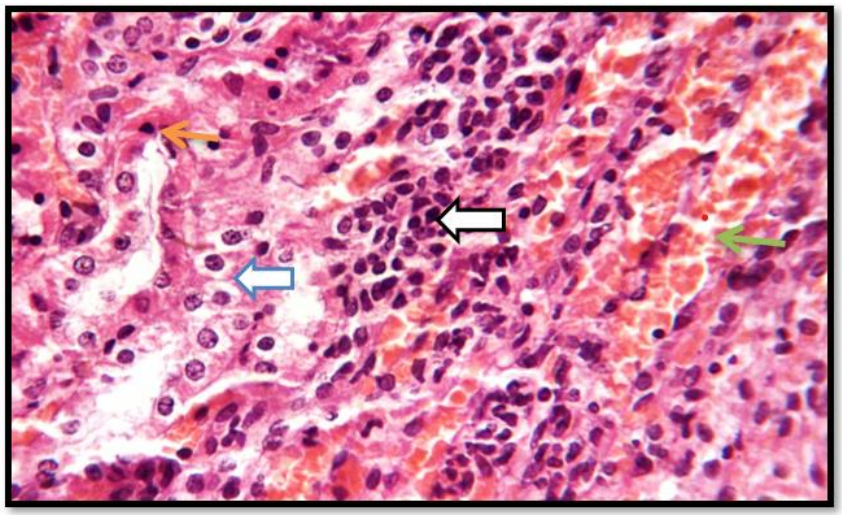

Figure (3): A cross-section of a rat kidney tissue in the group treated with Cyclosporine at a concentration of 0.2 mg/kg of body weight, showing severe infiltration of inflammatory cells (⇦) with hemorrhage between the renal tubules (←) with degeneration of the urinary tubule cells (⇦) and necrosis of the tubule lining (←) (H & E Stain 400 X)

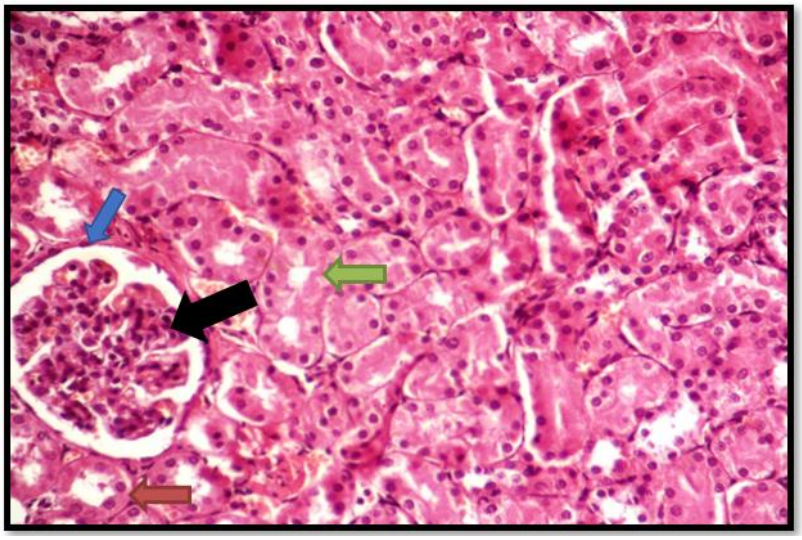

Figure (4): A cross section of the kidney tissue in the group treated with the aqueous extract of *Rubus ulmifolius* at a concentration of 400 mg/kg of body weight, in which the typical structure of the glomerulus (⇦), the proximal tubule (⇦), and the distal tubule (⇦) is observed with Bowman's capsule (⇦) (H & E 200 X).

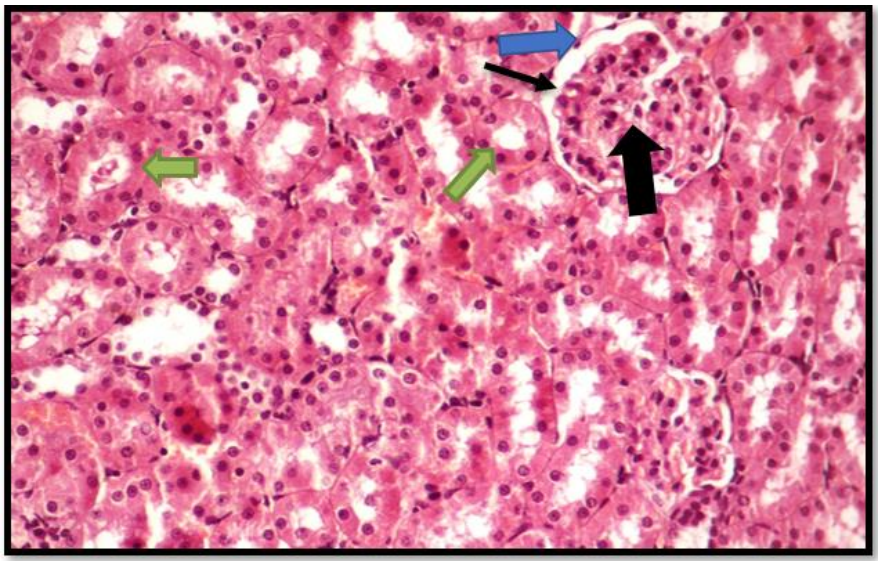

Figure (5): A cross-section of the rat kidney tissue in the preventive group treated with the aqueous extract of *Rubus ulmifolius* at a concentration of 400 mg/kg with the drug cyclosporine at a concentration of 0.2 mg/kg of body weight. The glomerulus and tissue are observed to be closer to normal (⬅) and the presence of Bowman's capsule (⇦) and Bowman's space (⬅) with the typical structure of the urinary tubules (⇦) (E & H Stain 200X)

The results we have reached in the table regarding the effect of the Cyclosporine on the albumin, Globulin, Total protein, urea, and creatinine levels, and histological examinations are consistent with [27, 28, 29]

The most common and potential limiting consequence of Cyclosporine therapy is renal dysfunction. Coupled with renal pathological changes and systemic hypertension, this adverse effect can lead to chronic and even end-stage kidney failure. Throughout the extensive metabolism, Cyclosporine is metabolized to multiple metabolites by hepatic cytochrome P-450, mixed-function-oxidase enzymes. This leads to clinically relevant drug interactions. Drugs that inhibit cytochrome P-450 enzymes increase the concentration of parent cyclosporine and may cause nephrotoxicity, hypertension, and other side effects. Conversely, drugs that induce these enzymes reduce parent cyclosporine concentration and lead to reductions in the drug's immunosuppressive activity to the unchanged drug. However, the use of CsA is associated with major nephrotoxicity, very likely being a consequence of its toxic effect on vascular endothelium, consistently documented in experimental animals and humans. Evidence is now available that in kidney transplant recipients, each oral dose of CsA is followed by a transient reduction in renal plasma flow and glomerular filtration rate that results from a form of acute reversible renal hypoperfusion [27]. Treatment of rats with CsA induced lipid peroxidation in the spleen as indicated by an increase in the by-product of lipid peroxidation, MDA [30].

Cyclosporine could inhibit hepatic protein synthesis and then cause depression in protein [31]. Besides, overproduction of Reactive oxygen species after CsA exposure [32] may be accompanied decrease in liver protein level and then depression in serum. It has been shown that CsA toxicity in the kidney, liver, and nervous system is accompanied by increased both H_2O_2 production and lipid peroxidation, and concomitantly decreased cellular level of reduced glutathione [33]. Reduction in the content of protein sulfhydryl groups and formation of protein thiol oxidation by CsA, leading to GSH depletion, could be another possible mechanism. It has been demonstrated that CsA-induced local production of hydroxyl radical, a highly active and detrimental radical, with elevation in superoxide radical, could be attributed to the production of peroxynitrite radical. [28]

The antioxidant activity of flavonoids in the plant (*Rubus ulmifolius*) is related to the number and position of hydroxyl groups. These compounds prevent oxidation and protect cells by donating hydrogen atoms to reactive species of free radicals [34]. The previous study [35] demonstrated the therapeutic potential of the active compound of berries and tissue protection by reducing MDA levels and increasing SOD and GSH levels [36].

Antioxidant and inflammation reduction action of medicinal plants has been verified. They can lower oxidative stress and regulate inflammation via modulation of signaling pathways, scavenging of oxidants directly, or generation of antioxidant enzymes, thereby helping to prevent chronic inflammation-related illnesses caused by Cyclosporine [37].

In a rat model, Rubus ulmifolius aqueous extract prevented cyclosporine-induced renal dysfunction and histological lesions; these findings support follow-up dose–response and species-specific studies aimed at agricultural/veterinary use.

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