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RAJAA DHUNOON MARSOUL*, RIYADH MUHI ABBOOD, MOHAMMED TALAT ABBAS*****

**Department of Medical basic sciences, College of Dentistry, Kerbala, Iraq.*

***Department of Internal medicine, College of Medicine, Kerbala, Iraq.*

****Department of Physiology and pharmacology, College of Veterinary medicine, Kerbala, Iraq.*

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ABSTRACT

Cyclosporine A (CsA) is a powerful immunosuppressant drug most widely used in the management of organ transplantation and autoimmune diseases. However, in therapeutic doses, CsA induces several side effects including renal and liver toxicity. The aim of this study is to investigate the protective role of garlic oil (GO) against cyclosporine-induced nephrotoxicity by investigating abnormalities in some of the metabolic biochemical parameters in serum and tissues of male adult rats. In this study, we used 24 male adult rats weighing 230-250g. The animals were randomly divided into four groups. Each experimental group consisted of six animals. Control group: Rats were fed with only standard rat diet and tap water for 4 weeks, GO group: Rats were treated with 5 ml/kg everyday dose GO via gavage, CsA group: Rats were treated with 12.5 mg/kg everyday dose CsA via gavage, CsA + GO group: They were treated with synchronized CsA (12.5 mg/kg) plus GO (5ml/kg) everyday via gavage. A significant increase in serum levels of urea and creatinine as well as a significant increase in Malondialdehyde (MDA) level in kidney tissue, and a significant decrease in Superoxide dismutase (SOD) and Catalase (CAT) levels in kidney tissue were observed in rats treated with CsA for a period of 28 days. However, supplementation of CsA-intoxicated rats with GO ameliorated the cyclosporine adverse effects as evidenced by a significant decrease in serum urea and creatinine levels, decreasing of the tissue level of MDA, and increasing of the tissue level of SOD and CAT. Histopathological changes kidney were not observed in animals treated with GO when compared with the control group. However, animals treated with CsA showed necrosis and degeneration of the glomerulus of kidney. This effect was significantly decreased in animals treated with synchronized CsA + GO.

INTRODUCTION

Cyclosporine A (CsA) is a polypeptide that was first identified in 1976 as a novel antibiotic. CsA is a frequently used immunosuppressive agent that used to prevent graft rejection in transplant medicine. Following liver, renal, pancreatic, bone marrow and cardiac transplantation; CsA can improve graft survival significantly [1]. Really, after introducing cyclosporine to transplant medicine, survival of transplanted organs increased considerably to more than 80% at 2 years post-transplantation. In the present time, a large number of transplanted patients receive cyclosporine as an essential immunosuppressant drug [2].

CsA was shown to be also an effective treatment option in autoimmune and inflammatory diseases like rheumatoid arthritis, uveitis, psoriasis, atopic dermatitis, inflammatory bowel disease, nephritic syndrome and primary biliary cirrhosis [1,3]. CsA have many important immunologic properties that make it an attractive agent for immunosuppression: it is found to inhibit both lymphocyte sensitization by allogeneic target cells in addition to *in vitro* cell-mediated lysis [4]. Clinically, as summarized by Hariharan et al. in 2000, who reviewed 93,000 transplants from 1988 and 1996, CsA obtained one-year graft survival rates in 94% and 88% in living related and deceased donor allografts respectively [5]. More recent data from the United Network for Organ Sharing (UNOS) from 1998 to 2007 show one-year adjusted survival rates of 96.6% and 91.6% in living related and deceased donor allografts respectively [6-10].

However, CsA has also side effects including renal, hepatic, cardiac and neural toxicity. CsA hepatotoxicity is one of the most disquieting side effects. It has been reported that hepatic function is impaired in 20 to 50% of CsA treated patients [2]. Increased alkaline phosphatase, elevated transaminases, inhibition of protein synthesis, hypoproteinemia, cholestasis, hyperbilirubinemia and disturbed lipid secretion in both human and experimental animals was found to characterize hepatotoxicity that caused by CsA administration [11]. Hepatotoxicity usually occurs in the first 90 post-transplant days and can limit CsA clinical application [12].

Nephrotoxicity of CsA was discovered early after its initial use, when Calne et al. found a significant and unexpected nephrotoxicity that had not been observed in animal experiments in their first attempt to use CsA following transplantation using a dose of 25 mg/kg [13]. Currently, it is well known that renal damage may be an important side effect of CsA therapy, but it is also

known that most persistent renal dysfunction is related to prolonged therapy, or doses of greater than 5 mg/kg/day, both of which can result in structural renal changes. Furthermore, it has been reported that nephrotoxicity is also related to individual susceptibility [14].

Garlic is a member of the lily family, contains more than 200 chemical compounds. Some of its more important ones include volatile oil with sulphur containing compound: (allicin, allin and ajone), and enzymes (allinase, peroxidase and myrosinase) [15,16]. Ancient Egyptian records mentioned that use of garlic as a remedy for a variety of disease [15]. Garlic, an antioxidant, has been shown to inhibit lipid peroxidation [17], and dose-dependent induction of endogenous antioxidants in rat kidney and liver [18]. Garlic has antioxidant, antimutagenesis, xanthine oxidase inhibitor, anticarcinogenesis, anti-inflammatory, anticancer, antiviral, antifungal, antiatherogenic and antithrombotic effects [19-23].

The aim of this study is to investigate the protective role of garlic oil against cyclosporine-induced nephrotoxicity in male adult rats.

MATERIALS AND METHODS

Chemicals

Cyclosporine (100 mg/ml) was obtained from the Essential Drug Company (Baghdad, Iraq), and given orally via gavage at a dose of 12.5 mg/kg body weight as previously described [24]. Garlic oil was purchased from local market (Karbala, Iraq). Garlic oil was given by gavage at a dose of 5 ml/kg as described [25].

Animals

In this study, we used 24 adults 230-250 g male rats which were fed by free diet and tap water with a 12 h light/ dark cycle for 4 weeks. The experimental protocol and procedures used in this study were approved by the Ethics Committee of the Karbala University, Karbala, Iraq for the care and use of laboratory animals. The animals were randomly divided into four groups. Each experimental group consisted of six animals.

Group 1 (n= 6): Control Group, they were fed with only standard rat diet and tap water for 4 weeks.

Group 2 (n= 6): CsA group, rats were treated with 12.5 mg/kg everyday dose of CsA orally via gavage.

Group 3 (n= 6): GO group, rats were treated with 5 ml/kg everyday dose GO orally via gavage.

Group 4 (n= 6): (**CsA + GO) group,** rats were treated with synchronized oral CsA plus GO 5 ml/kg everyday via gavage.

At the end of the experiment, rats were sacrificed 24h after the last GO and CsA received, and blood samples were collected in centrifuge tubes. Serum was separated from coagulant blood by centrifugation at 860 g for 20 min and then frozen at -20°C for biochemical analysis. 1 gram liver and kidney tissue samples were homogenized with 4 ml 0.9% NaCl at 4000 rpm and then extract were centrifuged at 20000 rpm for 20 min and then stored at -20°C for subsequent measurements. Liver and kidney were excised. Liver and kidney tissue samples were rinsed with 0.9% cold NaCl solutions and stored at -80°C. Tissues were embedded in paraffin for histopathologic research.

Biochemical Analysis

Serum urea and creatinine assays were performed according to Reflotron methods [26]. Tissue MDA assays were performed according to the guidelines of Ohkawa et al [27]. MDA is a product of lipid peroxidation that reacts with Thiobarbituric acid (TBA) under acidic conditions at 95°C, forming a pink complex that absorbs at 532nm. 1,1,3,3-Tetraethoxypropane was used as the standard. The results are expressed as nmol/g tissue. Tissue CAT assay was performed by using ELISA kit from Elabscience company. This ELISA kit used Sandwich-ELISA as the method [28]. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm ± 2nm. The OD value is proportional to the concentration of CAT, the concentration of CAT in the samples was calculated by comparing the OD of the samples to the standard curve. SOD level was determined by using Elabscience company ELISA kit which used Competitive-ELISA as the method [29]. The enzyme-substrate reaction is terminated by the addition of sulfuric acid solution and the color change is measured spectrophotometrically at a wavelength of 450nm ± 2nm. The concentration of SOD1 in the samples was then determined by comparing the OD of the samples to the standard curve.

Histopathological Study

The kidneys were excised and fixed in 10% formalin and stained with hematoxylin and eosin and then observed under microscope for histopathological changes [30].

Statistical Analysis

The data was analyzed using the Statistical Package for Social Science program (SPSS 12). For comparison between different experimental rat groups, one way analysis of variance (ANOVA) was used followed by Tukey's test. The results were expressed as means ±SD and $P < 0.05$ was considered to be statistically significant.

RESULTS

As shown in Table 1, urea and creatinine levels were examined in the serum collected from each group. Rats fed on standard diet supplemented with garlic oil did not show any significant changes in the parameters above. A significant increase in serum levels of urea and creatinine levels was observed in rats treated with cyclosporine for a period of 28 days. However, supplementation of cyclosporine-intoxicated rats with garlic oil ameliorated the cyclosporine adverse effects as evidenced by a significant decrease in urea and creatinine levels.

Table (1). The concentrations of serum urea and creatinine in different rat groups.

	Group 1	Group 2	Group 3	Group 4
Parameters	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
Urea	21.56±2.39 [a]	61.18±5.23 [b]	20.26±2.01 [a]	21.91±1.91 [a]
Creatinine	0.76± 0.15 [a]	1.81±0.16 [b]	0.72±0.05 [a]	0.77±0.07 [a]

Values are expressed as mean ± SD (n=6). P values were calculated by student t-tests, mean with different superscripts (a and b) differ significantly, $P<0.05$.

In the kidney tissue, MDA was significantly increased in cyclosporine-treated group as compared to control group and this rise in MDA was decreased by garlic oil. Antioxidant enzymes (SOD and CAT) activities significantly differed between the CsA group and the

CsA+GO group. Compared to the control group, CsA administration significantly decreased SOD and CAT activities in the kidney tissue, while GO administration increased them compared with that in the CsA group (Table 2).

Table (2). The levels of CAT, SOD and MDA in the kidney tissue homogenate samples in different rat groups.

	Group 1	Group 2	Group 3	Group 4
Parameters	Mean±SD	Mean±SD	Mean±SD	Mean±SD
MDA	102.68 ± 13.8^{a}	169.36 ± 16.36^{b}	100.53 ± 9.51^{a}	108.21 ± 11.80^{a}
CAT	0.88 ± 0.08^{a}	0.21 ± 0.06^{b}	0.95 ± 0.08^{a}	0.85 ± 0.12^{a}
SOD	11.81 ± 1.40^{a}	3.26 ± 0.45^{b}	12.35 ± 1.45^{a}	11.19 ± 2.03^{a}

Values are expressed as mean ± SD (n=6). P values were calculated by student t-test, mean with different superscripts (a and b) differ significantly, $P<0.05$.

Histopathological changes in kidney were not observed in animals treated with garlic oil when compared with the control group (Figures 1 and 2). However, animals treated with cyclosporine (12.5 mg/kg orally) showed necrosis and degeneration of glomerulus of kidney (Figure 3). This effect was significantly decreased in animals treated with garlic oil (Figure 4).

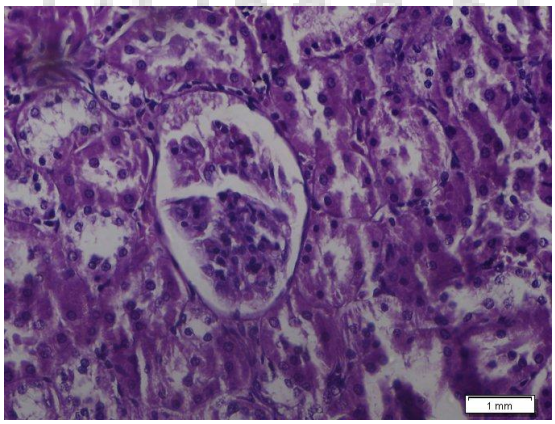

Figure (1). Kidney section from control group showing the normal structure of glomerulus

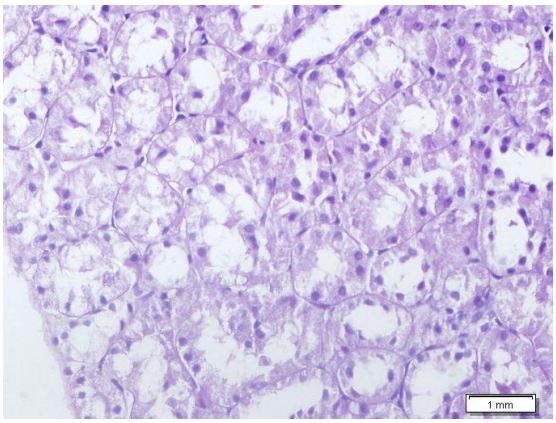

Figure (2). Kidney section from rat treated with GO showing normal morphology when compared with control rat

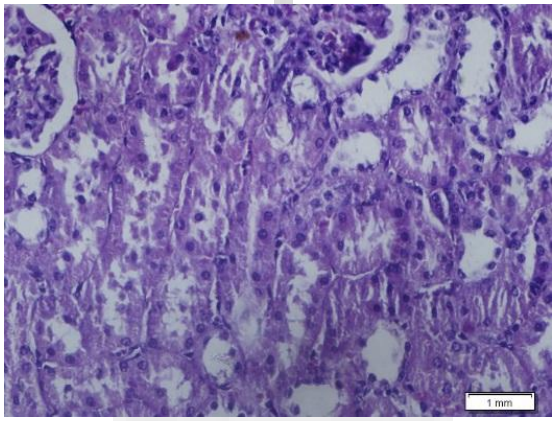

Figure (3). Kidney section from rat treated with CsA showing necrosis and degeneration of glomerulus

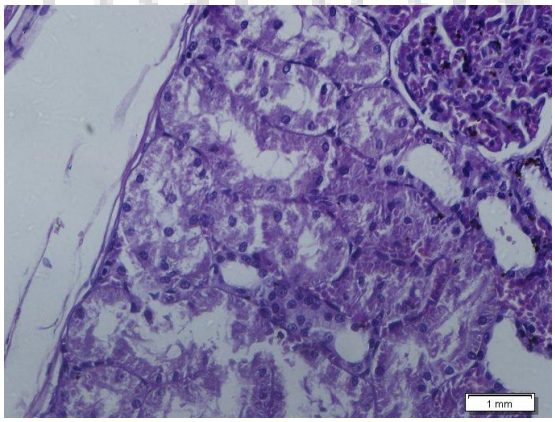

Figure (4). Kidney section from rat treated with CsA and GO showing a reduction of necrosis and degeneration of glomerulus

DISCUSSION

This study aimed to evaluate the protective effects of GO against CsA induced renal toxicity and oxidative stress in rats. This study evaluated kidney function by measuring serum creatinine and urea values; and determined oxidative stress by measuring activities of SOD, CAT, and MDA levels in the tissue.

CsA administration is found to elevate creatinine and urea levels in serum as compared to control group. These observations are generally in agreement with other studies on CsA nephrotoxicity [31-36]. Creatinine and urea are waste products of protein metabolism that need to be excreted by the kidney, therefore, a marked increase of these parameters, as observed in this study, confirms an indication of functional damage to the kidney [37]. Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney is the only significant factor that increases the serum creatinine level [38]. The increase in creatinine recorded in this work might be due to impaired kidney function by the used CsA. This view was supported by Kluwe [39], who indicated that an elevation of creatinine level in the blood is indicative of impaired kidney function.

According to the findings obtained, MDA levels in the liver and kidney tissues were significantly higher in the CsA-treated group when compared to the control group. This enhancement is a result of oxidative stress and lipid peroxidation. MDA is an indicator of lipid peroxidation [40]. Lipid peroxidation is regarded as one of the basic mechanisms of tissue damage caused by free radicals [41]. Free radicals are highly reactive molecules with odd number of electrons. They are constantly being generated in the body through various mechanisms and also being removed by endogenous antioxidant defense mechanism that acts by scavenging free radicals, decomposing peroxides and binding with pro-oxidant metal ion. Free radical mediated oxidative stress results usually from deficient natural antioxidant defense. In case of reduced or impaired defense mechanism and excess generation of free radicals that are not counterbalanced by endogenous antioxidant defense exogenously administered antioxidants have been proven useful to overcome oxidative damage [42,43]. In recent years, a number of studies have showed that CsA administration increases lipid peroxidation in the rat tissues [44,45].

However, administration of GO along with CsA caused significant decrease in MDA levels suggested the protective effects of GO. This protection offered by garlic may be attributed to its free radical scavenging property [46]. Garlic compounds have the ability to protect cell from oxidative stress, it has antioxidant properties [47]. Antioxidants act as radical scavenger and inhibit lipid peroxidation and other free radical-mediated processes, thereby protecting the human body from various diseases. There is a natural dynamic balance between the output of free radicals generated in the body and the antioxidant defense system that quenches or scavenges them and thereby protecting the body against pathogenesis. The first line of defense against free radicals are endogenous enzymatic antioxidants such as SOD, CAT, and glutathione peroxidase and the second line of defense are the non-enzymatic antioxidants such as glutathione, Vitamin-C and Vitamin-E [48-51].

The present study showed a significant decrease in SOD and CAT activities in the liver and kidney tissues treated with cyclosporine. This is due to CsA-generated free radicals, disturbing the antioxidant status and ultimately leading to oxidative stress. This results agree with others [52-55].

On the other hand, there was a significant increase in SOD and CAT activities in the kidney tissues of rats treated with GO. It has been reported that administration of GO significantly decreased lipid peroxidation and increased endogenous antioxidants, such as SOD and CAT [56,57].

Oxidative stress-induced tissue damage can be prevented or ameliorated by favoring the balance towards a lower oxidative stress status. It appears that the protective effect of GO involves the maintenance of antioxidant capacity in protecting the tissues against oxidative stress. Our study shows that treatment with GO improved the activities of SOD and CAT in rat tissues. This improvement may have resulted from GO provided a significant recovery in the level of ROS in CsA exposed animals in blood and tissues. The protection offered by GO may be attributed due to changes in the redox state of the cellular environment and altering the antioxidant defense system [58-62].

CONCLUSION

The administration of GO to the experimental animals which had an oxidative stress induced by CsA reduces the production of MDA which is positively correlate with lipid peroxidation. On the other hand, GO causes an increase in the activities of SOD and CAT in the kidney homogenate and ameliorate the histopathological defects that caused by CsA. Also GO administration has the ability to decrease the elevated serum levels of urea and creatinine in CsA-treated rats. So GO has the potential protective effect against CsA nephrotoxicity.

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