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Evaluation of Renaoprotective Effect of Costus Afer Leaf Extract on Rats exposed to Cyclosporine: Antioxidant and Anti-inflammatory Pathways

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Abstract

Costus afer leaf (Cost) extract used as traditional herbal therapy for various diseases. Long term treated with cyclosporine (CsA) concomitant with development of renaltoxicity. The current study aims to assess the nephroprotective mechaism effect of Cost in CsA nephrotoxic rats. Forty male rats were distributed into 4 groups; control (Cont), CsA; rats intraperitoneal (i.p) injected with CsA (twenty five mg/kg b.wt) for twenty one days, Cost 750 mg/ kg b.wt +CsA, Cost 1500 mg/ kg b.wt +CsA; rats received Cost orally for twenty one days, followed by i.p. injection with CsA. Biochemical samples were collected after 24 h from the last dose of CsA as well as renal tissue samples were collected for histopathogical examination. Renal oxidative stress biomarkers (lipid peroxides (MDA) and catalase (CAT)) and the serum anti-inflammatory biomarkers (tumor necrosis factor interleukin-1beta (IL-1β) and -alpha (TNF-α)) were derermined. Kindeny functions; serum levels of creatinine, uric acid and urea as well as serum ionic levels of sodium Na^+ and potassim K^+ were measured. The results of this study revealed that injection of CsA induced significant increased in renal MDA, serum anti-inflammatory cytokines (TNF-α and IL-1β), serum kidney function paramters and the serum ionic K+ levels, with significant decrease in the renal CAT and serum ionic Na+ compared with cont group. Renal tisuues showing congestion, focal hemorrhage and intesritial nephritis, with coagulating necrosis of the renal tubules in the CsA group. Oral adminstration of Cost extract significantly ameliorated CsA- induced renal oxidative stress. It reduced CsA-induced elevation in serum anti-inflammatory cytokines and kidney function parameters. as well as the changes in ionic Na+ and K+ levels compared with CsA group. It also protected against CsA-induced histopathological changes. Therefore, Cost extract ameliorates nephrotoxicity caused by CsA through antioxidant and anti-inflammatory mechanisms.

Keywords: Costus afer leaf, Cyclosporine, renaltoxicity, antioxidant, anti,inflammatory.

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Introduction

Kidney is the organ in the body that removes blood metabolic waste such as uric acid, urea and creatinine and excrete them by urine; the kidney also removes drugs and various toxic agents in the blood (Kunworarath et al., 2014). One of the most common kidney problems is nephrotoxicity, which is caused by toxic compounds that may be endogenous or exogenous such as chemotherapeutic agents, antiplastic agents (cyclosporine and cyclophosphamide), aminoglycosides and antibiotics, which are the most important exogenous toxic agents (Sembulingam and Sembulingam, 2010). Drugs can cause oxidative stress by producing free radicals that are mostly available as by-products or as metabolic aerobics (Goyal et al., 2016). The damage to nucleic acids, membrane lipids and tissue proteins caused by free radicals when generated excessively at the cellular level (Lakshmi, Muvvala and Shashank, 2014). The drug CsA is the most widely medicine used in organ transplantation, which has immunosuppressive effect, currently CsA had been investigated for a wide range of autoimmune diseases in clinical trials (Capasso et al., 2008). Other adverse effects of CsA are hyperactivity, encephalopathy, neurotoxicity, hypertension and nephrotoxicity, in addition to the immunosuppressive effect (Gnieszka et al., 2010). The generation of reactive oxygen species is widely attributed to nephrotoxicity due to chronic CsA treatment (Bobadilla and Gamba, 2007).

Management of nephrotoxicity is still a challenge to the modern scientific community. Unfortunately, drugs have little to offer alleviation of kidney ailments. Thus given rise to research involved in identification of safe, inexpensive and available alternatives from natural resources (Kotnis et al., 2004). For several years, plants have been used as medicine to help maintain human health (Uy et al., 2019). And, medicinal plants have also been widely used as medicinaland aromatic plants since the ancient times (Niazi et al., 2019). Due to the presence of various bioactive compounds, medicinal plants have been proved to be a major source of drugs (Pereira, Barros and Ferreira, 2016). Bioactive compounds with antioxidant property can prevent and combat oxidative stress-related diseases (Anyasor et al., 2010). Recent studies revealed the Cost extract has significant

nephroprotective properties (Gnieszka et al., 2010; Kotnis et al., 2004).

Costusafer Ker Gawl of the Zingiberaceae family, now referred to as Costaceae, is a herbaceous, rhizomatous monocotyledon. The common names for Cost include ginger lily, bush cane or spiral ginger (Iwu, 2014). The Cost leaves and stem as medicinal plant parts have received a lot of attention as a result of its antioxidant activity, as well as in treating a vast number of disease conditions including malaria, rheumatoid arthritis, diarrhea, diabetes mellitus, stomach ache, cough and cold (Anaga et al., 2004). *CostusAfer* has proven to possess hepatoprotective, nephroprotective antimicrobial, anti-inflammatory and hypolipidemic effects (Anyasor et al., 2014). The Cost active compounds are flavonoids, tannins, alkaloids; phenols and saponins, which are powerful water-soluble antioxidants that prevent oxidizing damage to cells (Anyasor et al., 2010). Fruits and vegetables have been recognized as natural sources of various bioactive compounds (Messaoudi et al., 2019). The purpose of the present work is to evaluate the potential protective role of Cost against kidney toxicity Induced by CsA.

Material and Methods

Drugs and chemicals and plant material

Cyclosporine A (CsA) soft capsules, provided by Novartis Pharaceuticals , Australia. All chemical and kits with high grade obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co. Costus afer (Cost) leaves was purchased from local marker in Jeddah, Saudi Arabia.

Preparation of Costus afer leaf extract

Costus afer leaves dried powder (500 g) was macerated in 1 L of ethanol (80%) at room temperature and mixed for 48 hrs by magnetic stirrer at 100 rpm speed. The extract was concentrated at 40°C under vacum using rotary evaporator, the condensed residue was further freeze-dried (Siddhuraju and Becker, 2003). The extract was stored in non-permeable glass containers at 4°C until used.

Experimental desgin

Forty male rats weighting 120-150 g purchased from animalhouse of King Fahd Medical Resrach Center, KAU. They were adhering under the rulrs of Canadian ethical approval from the local biomedical ethical cmmittee of KAU with free water and standard diet. After one week of acclimatization rats were distributed into 4 groups (n=10 in each group). Group I (Cont); rats received distilled water for 2 weeks then intraperitoneal (i.p) injected with olive oil (vehicle) for 21 days. Group II (CsA); rats distilled water for 2 weeks then were i.p. injected with CsA at a dose (25 mg/kg b.wt) diluted in olive oil for a peroid of twenty one days acording to Chandramohan and Parameswar (Chandramohan and Parameswari, 2013). Group III (Cost 750 mg/ kg b.wt +CsA) (Ezejiofor, Orish and Orisakwe, 2014). Group IV (Cost 1500 mg/ kg b.wt +CsA). Rats in groups III and IV received Cost orally for twenty one days, followed by i.p. injected with CsA.

Samples collection

Twenty four hours after the last injected dose of the CsA drug, rats were anesthetized then blood and renal samples were collected. The serum samples were separated and stored at -80 °C until used for biochemical anaysis. The renal samples were either prepared for biochemical analysis or preserveal in neutral buffer formaldehyde solution for histopathological studies.

Biochemical analysis

- ***Renal oxidative stress biomarkers***; the lipid peroxidation level (MDA) and the activity of catalase (CAT) enzyme were estimed in homogenated renal using ELISA kits.
- ***The anti-inflammatory cytokines;*** theserum levels of tumor necrosis interleukin-1beta (IL-1β) and -α (TNF-α) were assessed by ELISA kits.
- ***The level of kindeny functions***; blood urea nitrogen (BUN) , uric acid (UA)cand serum levels of creatinine (Cr), as well as serum levels of sodium (Na^+) and potassim (K^+) were measured using colormetric kits.
- All kits'used were obtained MyBioSource, USA followed the steps and instructions of kits.

Histopathological studies

A kidney tissue portion from each group was fixed in formaldehyde solution and prepared for examined under micrscope after stainning with hematoxylin and eosin to determine histopathogical changes.

Statistical analysis

All results were analyzed by SPSS ver. 22 , by using ANOVA, values are expressed as mean± SEM, P-value < 0.05 considered significance.

Results

Effect of Cost on renal oxidative stress in nephrotoxic rats by CsA

The levels of non-enzymatic MDA and enzymatic CAT antioxidant levels in the renal tissue in different groups are represented in Figure (1). The level of MDA was significantly ($p< 0.001$) increased with significantly ($p< 0.001$) decreased in CAT activity in CsA compared with the control group. Oral administration of Cost induced significant improvement in the antioxiant status of renal. The MDA level decreased significantly ($p< 0.001$) and the CAT enzyme activity increased significantly ($p< 0.001$) in the Cost adminstration (750 mg/kg) and the (1500 mg/kg) groups as compared with CsA group. Significant (p<

0.05) changes were observed in Cost (750 mg/kg)+ CsA and Cost (1500 mg/kg)+ CsA, which indicated the protective effect of Cost is a dose-dependent. .

Effect of Cost on serum anti-inflammatorycytokines in nephrotoxic rats by CsA

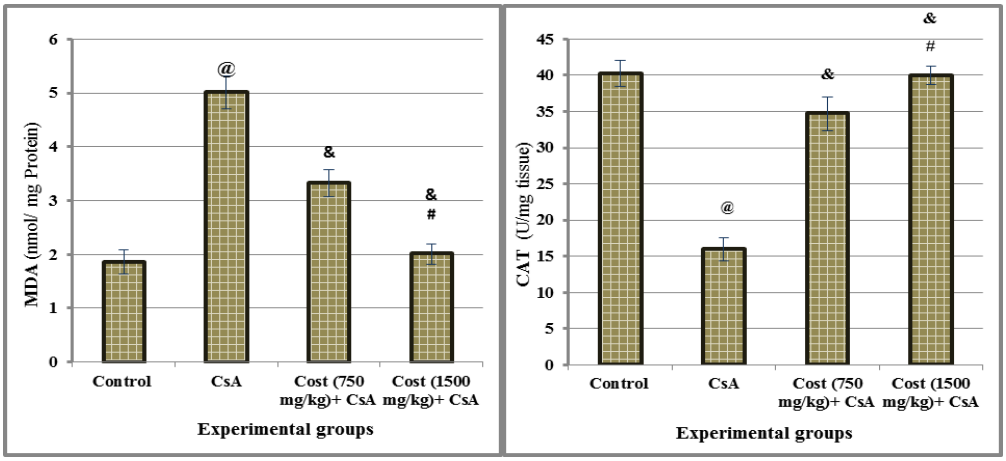
The serum levels of IL1-β and TNF-α in different groups are showed in Figure (2). The levels of TNF-α and IL1-β were significantly (p< 0.001) increased in CsA compared with the control group. Oral administration of Cost induced significant decreased in the serum anti-inflammatory cytokines status, there were significant (p< 0.001) decreases in TNF-α and IL1-β levels in both Cost groups as compared with CsA intoxicated group. Significant (p< 0.05) changes were observed in Cost (750 mg/kg) + CsA and Cost (1500 mg/kg)+ CsA.

Effect of Cost on renal functions in nephrotoxic rats by CsA

Table (1) represents the levels of serum renal functions (Cr, BUN and UA) in different groups of experiments. The serum of kidneyfunctions level in the CsA group was significantly (p< 0.001) increased in CsA group compared to control group. Cost adminstration (750 and 1500 mg/kg) reduced the serum kidneyfunction levels significantly (p< 0.001) as compared with CsA group, which may be indicator for renal toxicity induced by CsA. Significant (p< 0.05) changes were observed in Cost (750 mg/kg) + CsA and Cost (1500 mg/kg) + CsA, which indicated the protective effect of Cost is a dose-dependent.

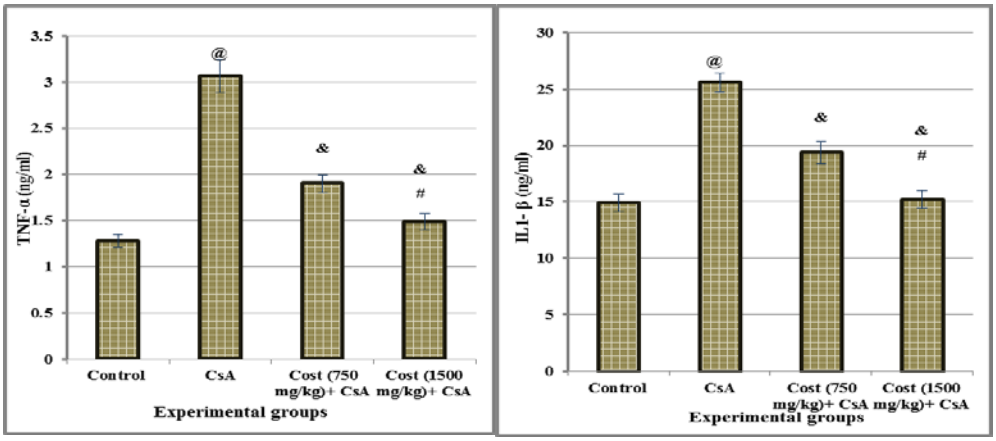
Effect of Cost on ionic sodium and potassim in nephrotoxic rats by CsA

Figure (3) shows the levels of serum ionic Na^+ and K^+ in different experimental groups. The serum level of ionic Na^+ was significantly (p< 0.001) decreased with significantly (p< 0.001) increased in the serum ionic K^+ in CsA group compared with control group. Oral Cost administration significantly (p< 0.001) increased the ionic Na^+ level with a significant (p< 0.001) increase in ionic K^+ level in the Cost adminstration groups as compared with CsA intoxicated group. Significant (p< 0.05) changes were observed in Cost (750 mg/kg) + CsA and Cost (1500 mg/kg) + CsA.



In each group N = 10, values are expressed as mean ± SEM, values. p ≤ 0.05 considered significant; @ versus Control, & versus CsA values. # between Cost (750 mg/kg) and Cost (1500 mg /kg) groups.

Figure 1: Effect of Cost extract on renal non-enxymatic (MDA) and enzymatic (CAT) levels aginst CsA-induced nephrotoxicity in rats



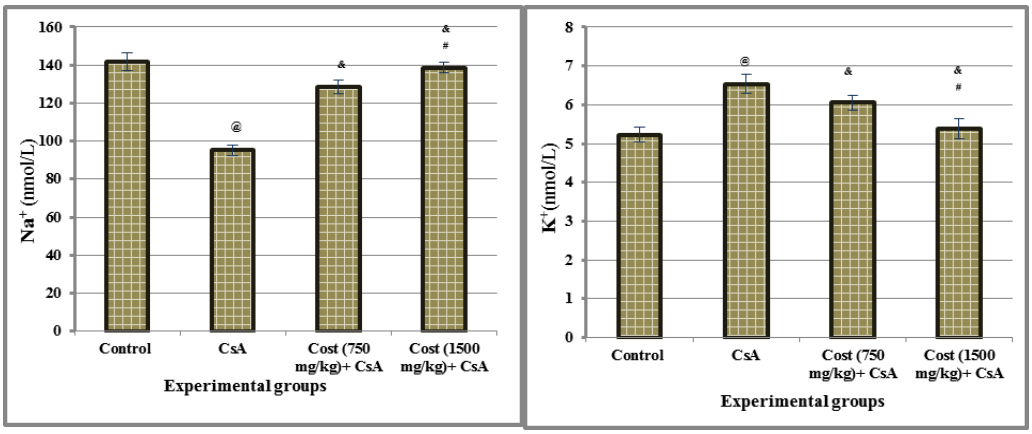
In each group N = 10, values are expressed as mean ± SEM, values. p ≤ 0.05 considered significant; @ versus Control, & versus CsA values. # between Cost (750 mg/kg) and Cost (1500 mg /kg) groups.

Figure 2: Effect of Cost extract on serum inflammatory TNF-α and IL1-β levels aginst CsA-induced nephrotoxicity in rats

Table 1: Effect of Cost extract on renal functions aginst CsA-induced nephrotoxicity in rats

Groups	Renal function Parameters		
	Cr (µmol/L)	**BUN** (mmol/L)	**UA**(umol/L)
Control	22.50 ± 1.04	7.86 ± 0.44	62.20 ± 2.62
CsA	50.48± 3.56 [a]	21.09 ± 1.48 [a]	122.59 ± 5.55 [a]
Cost (750 mg/ kg) + **CsA**	33.52 ±2.77 [b]	11.69 ± 1.15 [b]	76.95 ± 2.96[b]
Cost (1500 mg/ kg) + **CsA**	28.19 ± 1.61 [b, c]	8.50 ± 0.63 [b, c]	66.37 ± 2.69 [b, c]

In each group N = 10, values are expressed as mean ± SEM, values. p ≤ 0.05 considered significant; [a] versus Control, [b] versus CsA values. [C] between Cost (750 mg/kg) and Cost (1500mg /kg) groups.



In each group N = 10, values are expressed as mean ± SEM, values. p ≤ 0.05 considered significant; @ versus Control, & versus CsA values. # between Cost (750 mg/kg) and Cost (1500 mg /kg) groups.

Figure 3: Effect of Cost extract on serum ionic Na^+ and potassim K^+ levels against CsA-induced nephrotoxicity in rats

Histopatholohical results

Kidney of control group showing the normal histological structure. In the CsA group, renal tisuue showing congestion of renal blood vessels, focal hemorrhage and intesritial nephritis, with coagulating necrosis of the renal tubules associated with mononuclear cells imfilteration. In the Cost (750 mg/kg) + CsA grouprenal tissue slight congestion of the glomerular tufts with sliglt vacuolation of some renal tublular were seen. Apparent normal appearance of renal tisuue was revealed in the Cost (1500 mg/kg) + CsA group.

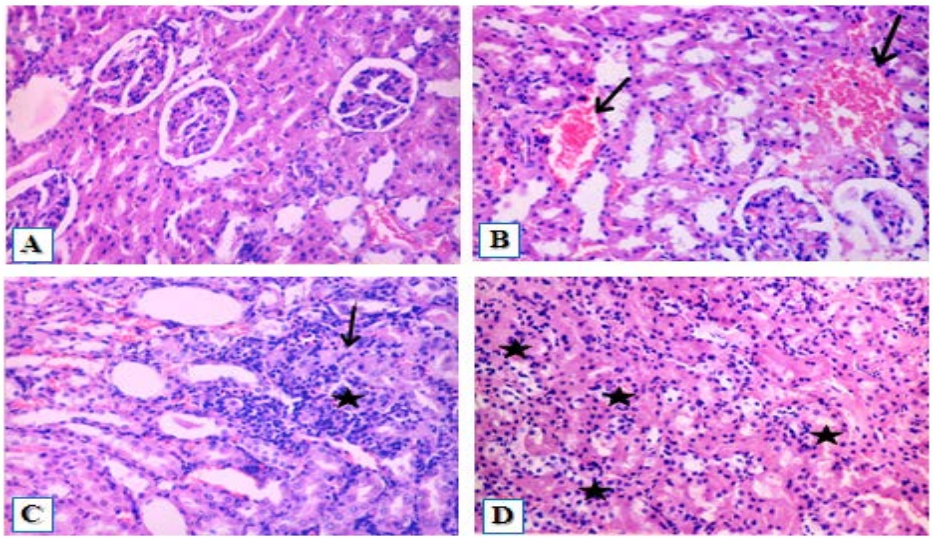


Figure 4: Effects of Costus Afer (Cost) leaves extract on the kidney tissue histopathological changes detected by H&E staining in CsA-

induced nephrotoxicity in rats (Power magnification x 66). Photo A represent control group, renal tissue showing the normal histological structure. Photo B represent CsA group, renal tisuue showing congestion of renal blood vessels (small arrow), as well as focal hemorrhage (large arrow). Photo C represent CsA group, renal tisuue showing focal intesritial nephritis (arrow), with necrosis (star). Photo D represent CsA group, renal tisuue showing coagulating necrosis of the renal tubules associated with mononuclear cells imfilteration (star).

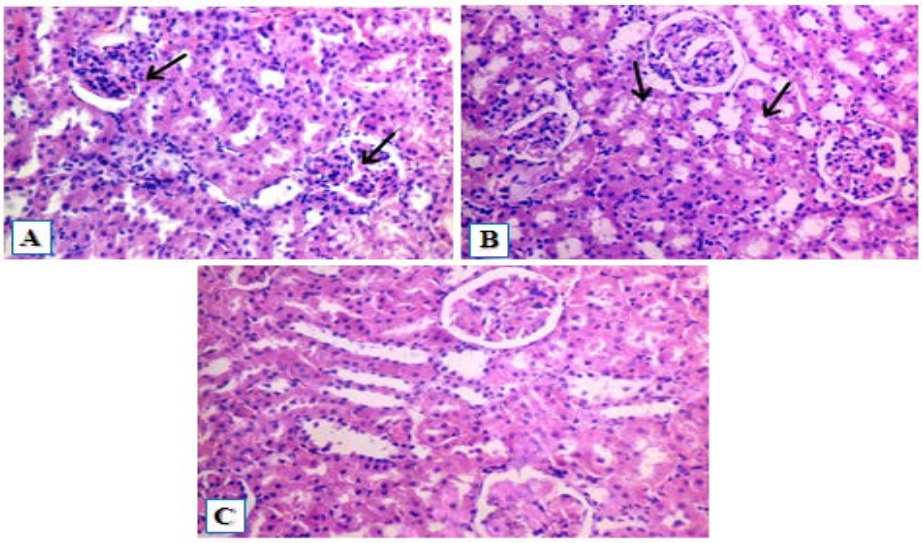


Figure 5: Effects of Costus Afer (Cost) leaves extract on the kidney tissue histopathological changes detected by H&E staining in CsA-induced nephrotoxicity in rats (Power magnification x 66). Photo A represent Cost (750 mg/kg) +CsA group, renal tissue showing slight congestion of the glomerular tufts (arrows). Photo B represent Cost (750 mg/kg) +CsA group, renal tisuue showing slighly necrobiotic changes and vacuolation of some renal tublular (arrows). Photo C represent Cost (750 mg/kg) +CsA group, renal tisuue showing the normal appearance.

Disscusion

The study was conducted to assess the protective effect and antioxidant activity of Cost against CsA induced nephrotoxicity. The use of CsA was found to be nephrotoxic in the first attempt after transplantation for immunosuppression (Lorber et al., 1987). The results showed that i.p. injection with CsA in a dose of 25 mg/kg b.wt. diluted in olive oil for a period of twenty one days, resulted in deterioration of renal function and the development of histopathological changes in the renal tissues. The results were in the same line with Gnieszka*et al.* (2010) who demonstrated that using CsA in a dose of 25 mg/kg causes a significant but unexpected nephrotoxicity that was not seen in initial animal experiments. Several studies have identified nephrotoxicity as the most common and clinically significant adverse effect of CsA (Gnieszka et al., 2010; Kotnis et al., 2004).

In the present study there were significant increases in renal MDA, anti-inflammatory cytokines (TNF-α and IL-1β), kidney function parameters and the serum ionic K+ levels, with significant decreases in the renal CAT and serum ionic Na+ in intoxicated group compared with control group. The results obtained correspond to most of the experimental procedures reported (Agnieszka et al., 2010; Ciarcia, Damiano and Fiorito, 2012; Wongmekiat et al., 2011). This could be due to an increase in renal vascular resistance, a significant reduction in renal blood flow and activation of the sympathetic renal nervous system which had been demonstrated as a concomitant stimulation of plasma renin activity (Elsayed, Bayomy and Azab, 2016). The exact cause for nephrotoxicity by CsA administration remains obscure, but several studies suggested deficiencies in magnesium, intracellular calcium, nitric oxide and oxidative stress (Parra et al., 1998). The generation of reactive oxygen species is widely attributed to nephrotoxicity due to chronic CsA treatment (Mohamadin et al., 2005).

The present study confirmed that, there has been a significant ($p<0.05$) increases in the level of renal tissue MDA as well as in the level of TNF-α and IL-1β pro-inflammatory cytokines compared with the extract pretreated groups. The results were consistent with other researchers who demonstrated that there is a significant increase in lipid peroxidation and in pro-inflammatory cytokines during CsA administration (Fong et al., 1990). Excessive MDA levels in kidney tissue and in pro-inflammatory cytokines had been taken into considered as an indicator of cellular harm because of excessive lipid peroxidation processes during antioxidant defense system malfunction (Marsoul et al., 2016).

In contrast, oral administration of Cost prevented this elevation in a dose dependent manner. The results were in agreement with Uboh *et al.*, (2014), who reported that Cost has a remarkable inhibition of lipid peroxidation and radical scavenging due to its phytoconstituents such as flavonoids, alkaloids, saponins, phenols, terpenoids, tannins, and cardiac glycosides. Alkaloids

are known to have anti-inflammatory effect while, the other water-soluble antioxidants prevent cell damage from oxidizing and suggesting anti-inflammatory properties (Ukpai et al., 2012).

Antioxidant catalase is one of the major defenses against oxidative damage caused by ROS (Hussein, Ragab and El-Eshmawy, 2014). Intoxication with CsA resulted in a significant reduction in the activity of CAT enzyme compared to the untreated group. The reduction in CAT activity level may be due to NADPH production depletion during CsA administration (Anyasor et al., 2010). Oral administration of Cost with CsA led to significant elevation in renal tissue antioxidant activity compared with intoxicated group with CsA. The same effect were reported by Anyasor*et al*. (2014) who stated that the effect could be due to phytochemicals present in the Cost such as flavonoids, polyphenols, tannins and saponins which are known as plant antioxidant metabolites. Considering all types of natural antioxidants, polyphenols can be regarded as the major potent compound, because of being abundantly applied in the food industry, cosmetic, pharmaceutical and medicinal materials (Tabet et al., 2018).

This study also included several important biochemical parameters. These biological parameters are organ toxicity indicators. For example, serum urea, creatinine, uric acid and electrolytes (Na+ and K+ levels) which were used to evaluate kidney functions. In the current study, there were an increase in concentrations of serum uric acid, urea, creatinine and K+ level while there was a decrease in Na+ level detected in the CsA group compared with the normal group. Chronic Administration of CsA for 21 days resulted in deficiencies in renal functions characterized by high serum levels of uric acid, creatinine and urea, which could be used as a rough glomerular filtration index, indicating multiple kidney disorders (Burdmann, Andoh and Bennett, 2003). Pretreatment with Cost to CsA treated rats elevate the changes caused by CsA. The same results were reported by Uboh *et al*., (2014) who stated that rats orally given Cost restored the levels of kidney function parameters (creatinine, urea and uric acid).

The present study demonstrated that administration of CsA lead to a significant decrease in potassium level and significant increase in serum sodium level in pretreated Cost groups compared to CsA group. The same results were seen in the study of Ezejiofor *et al.* (2014) who stated that rats orally given Cost significantly reduced creatinine and serum blood urea as well as sodium level and increased potassium level in gentamicin induced nephrotoxicity.

Histopathological studies had been carried out on all rats. The study results revealed a significant influence of the CsA administration on histopathological damage of CsA group. This results were in the same line with Gnieszka *et al*. (2010). On the other hand, there was an improvement in kidneys tissue of rat groups given Cost in combination with CsA.

In Conclusion, the results of the recent study demonestrate that Cost has a protective role that is likely mediated by its antioxidant proparities against CsA-induced nephrotoxicity. Further studies are needed to evaluate its potential utility of this extract in clinical conditions associated with renotoxicity.

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