Ziziphusspina-christi leaves extract alleviate renal toxicity induced by Cyclosporine in male rats

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ABSTRACT
Cyclosporine (CSP) is the drug most commonly used for organ transplants, which has immunosuppressive effect, concomitant with development of renal toxicity. Ziziphusspina-christi leaves extract (ZSCLE) has been tested to be an excellent antioxidant, thereby can play a role in the treatment of renal toxicity. The study was conducted to examine the effect of ZSCLE on CSP-induced kidney damage. Forty male albino rats have been distributed into four groups. Group I (Con); rats received distilled water for 2 weeks then intraperitoneal (i.p) injected with olive oil (vehicle) for 21 days. Group II (CSP); rats received distilled water for 2 weeks then were i.p. injected with CSP at a dose (25 mg/kg b.wt) diluted in olive oil for a period of twenty one days. Group III ZSCLE (300 mg/kg)+ CSP. Group IVZSCLE (600 mg/kg)+ CSP. Rats in groups III and IV received ZSCLE orally for twenty one days, followed by i.p. injected with CSP. Blood was collected for biochemical analysis. The kidney was also examined histopathologically. The results of the study illustrated that CSP induced significant increase in serum levels of kidneys function (serum levels of creatinine (Cr), uric acid (UA) and blood urea nitrogen), serum ionic potassim (K+) level and the renal lipid peroxides (MDA), but there were decreased in levels of serum ionic sodium (Na+) and renal superoxide dismutase (SOD) compared to Con group. Renal tisuues shows congestion, thickening of renal capsule and coagulated necrosis of epithelial lining in the CSP group. Oral administration of ZSCLE at doses of three hundred and six hundred mg/kg/day significantly ameliorated CSP- induced renal oxidative stress. It reduced CSP -induced
elevation in serum kidney function parameters, as well as the changes in ionic Na⁺ and K⁺ levels compared with CSP group. It also protected against CSP-induced histopathological changes.

**Keywords:** Renal toxicity, Ziziphus spina-christi, Cyclosporine, Rats, Lipid peroxidation.

1. **INTRODUCTION**

Cyclosporine-A (CSP), a cyclic endecapeptide, is one of the most commonly use immunosuppressive drugs in autoimmune diseases and during transplantation of organ, as a life saving procedure, which its usage rate is increasing worldwide (Chapman and Nankivell, 2006). The safety of its use remains a disputed concern. Systemic administration of CSP may induce several toxic effects as hepatotoxicity, nephrotoxicity, hypertension, cardiovascular affection and risk of malignancy (Rezzani, 2006; Hewedy et al., 2016; Raeisi et al., 2016). The CSP oxidative damage may induced pulmonary disorders (Lyu and Zamora, 2009). The optimal use of CSP is one of the major difficulties in therapy (Kokuhu et al., 2013). Several studies have been performed to assess the added protection of natural plants for the renal toxic effect of CSP (Shin et al., 2012; Mostafa et al., 2015; Raeisi et al., 2016). Natural antioxidants have been documented to alleviate intoxication induced by free radicals generated from drugs in several researches (Almeer et al., 2018; Al Omairi et al., 2018).

Ziziphus spina-christi (Rhamnaceae family), known as Sidr, Jujube, Chirst’s thorn, and Nabka, is from the core vital plants of Saudis’ practices (Saied et al., 2008; Ali et al., 2017). The ZSC tree is highly revered by Muslims because it is mentioned in the Holy Qur’an and the Sunna, thus indicate its’ curative and health properties (Farooqi, 1997, Ishrak et al., 2006). The ZSCLE contains various phytochemical constituents as saponins, triterpenoid, phytosterols, alkaloids, flavonoids, erols, ceanothic acids and tannins betulinic (Ali and Hamed, 2006; Kadioglu et al., 2016; Almeer et al., 2018). The ZSCLE has been shown to possess antioxidant (Abalaka et al., 2011), anti-diabetic via an action comparable to sulfonylurea (Farooqi, 1997; Abdel-Zaher et al., 2005; Adamu et al., 2006; El-Kamali and Mahjoub, 2009), antispasmodic (Ads et al., 2017), anticholinergic (Mohammed et al., 2012) activities. It was used to increase blood flow to the heart and control irritability, palpitations and insomnia (Ads et al., 2017). Cytotoxic effects of ZSCLE against cervical, breast and colon cancers have been proven (Ads et al., 2017). Sidr leaves have defensive impact against aflatoxicosis (Abdel-Wahhab et al., 2007), cure scores and some skin diseases (Adzu et al., 2001), Alcoholic ZSCLE has been shown potent antibacterial activities and could be used in the urinary tract infection, diarrhea, wound infection (Motamedi et al., 2009; Alsaimary, 2012). Water ZSCLE prevents liver fibrosis induced by carbon tetrachloride in rats (Amin and Mahmoud-Ghoneim, 2009). Recently, ZSCLE has been proved to suppress heavy metals as mercury chloride-induced nephrotoxicity and prevent or minimize the renal pathological changes (Almeer et al., 2019).

To best of our knowledge, the possible renal protective effect of ZSCLE against renal toxicity caused by CSP has not been explored. Therefore, this study aimed to evaluate whether ZSCLE has the ability to cure kidney tissue from the intoxication induced by CSP via the evaluation of renal functions, antioxidant status, ionic electrolytes homeostasis, and histopathological changes of renal in male rats.

2. **MATERIAL AND METHODS**

**Drug, plant and chemicals**

Cyclosporine (CSP) soft capsules, provided by Novartis Pharaceuticals, Australia. All chemical and kits with high grade obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co. Ziziphus spina-christi (ZSC) leaves was purchased from local marker in Jeddah, Saudi Arabia.

**Extraction of Ziziphus spina-christi leaves**

The leaves of the ZSC were dried under shade at room temperature and finely powdered with an electric drill. Using maceration process the powdered sample was extracted using 70 percent ethanol. After keeping shaking for 7 days, the mixture was drained out. The filtrate was condensed through a rotary evaporator into dryness (Abdel-Zaher et al., 2005).

**Ethical approval**

This experimental protocol was approved by the Biomedical Committee, Faculty of Medicine, KAU, no (HA-02-J-008).
Experiment protocol
Forty male rats weighing 140-160 g purchased from King Fahd Medical Research Center, KAU’s animal house. They were adhering with free water and standard diet under Canadian ethical approval from KAU’s local biomedical ethical committee. After one week of acclimatization rats were distributed into four groups (n=10 in each group). Group I (Con); rats received distilled water for 2 weeks then intraperitoneal (i.p) injected with olive oil (vehicle) for 21 days. Group II (CSP); rats distilled water for 2 weeks then were i.p. injected with CSP at a dose (25 mg/kg b.wt) diluted in olive oil for a period of twenty one days according to Chandramohan and Parameswar (2013). Group III ZSCLE (300 mg/kg)+ CSP (Rafa et al., 2019). Group IVZSCLE (600 mg/kg)+ CSP. Rats in groups III and IV received ZSCLE orally for twenty one days, followed by i.p. injected with CSP.

Biochemical measurements
Seven days after CSP injection, renal and blood samples were collected. Serum samples were held at -80°C, until renal function levels were assessed (creatinine, blood urea (BUN) and uric acid), ionic sodium and potassium concentrations were determined using colorimetric kits, purchased from Abcan, USA, following the manufactures’ procedure.

Estimation of renal oxidative stress biomarkers
The MDA and the activity of SOD enzyme were estimated in homogenated renal using ELISA kits.

Histopathological hematoxylin and eosin staining
Stained renal sections were examined and photographed using light microscope.

Statistical
All results were analyzed by SPSS ver. 24, by using ANOVA, values are expressed as mean± SD, P-value <0.05 considered significance.

3. RESULTS
Serum renal function markers (Cr, BUN and UA)
Rats treated with CSP revealed appreciably significant (P<0.05) renal damage, which noticed through elevates in renal function markers (Cr, BUN, and UA) as compared with Con, which might be indicator for renal toxicity induced by CSP. Significant (P< 0.05) decreases were observed in kidneys function of rats treated with ZSCLE (300 mg/kg)+ CSP and ZSCLE (600 mg/kg)+ CSP compared with CSP group, which indicated the protective effect of ZSCLE is a dose-dependent Figure (1).
Figure 1 Effect of *Ziziphus spina-christi* leaves extract (ZSCLE) on [A] creatinine, [B] uric acid and [C] blood urea nitrogen against Cyclosporine (CSP)-induced nephrotoxicity in rats. Values are stated as mean ± SMD in each group N = 10. Values within a column of different superscript letters are substantially different at P<0.05.

**Serum ionic electrolyte levels (Na+ and K+)**

Figure 2 show the levels of serum ionic Na⁺ and K⁺ in different experimental groups. The group treated with CSP revealed noticeably significant decrease in ionic Na⁺ level (p<0.005) with significant increase in ionic K⁺ level (P<0.05) as compared to Con. The ZSCLE administration significantly (P< 0.05) increased the ionic Na⁺ level with a significantly (P< 0.05) decrease in ionic K⁺ level in both groups (ZSCLE (300 mg/kg)+ CSP and ZSCLE (600 mg/kg)+ CSP) compared with CSP group.
Figure 2  Effect of Ziziphus spina-christi leaves extract (ZSCLE) on serum ionic [A] Na$^{+}$ and [B] potassium K$^{+}$ levels against Cyclosporine (CSP)-induced nephrotoxicity in rats. Values are stated as mean ± SMD in each group N = 10. Values within a column of different superscript letters are substantially different at P<0.05.
Figure 3 Effect of *Ziziphus spina-christi* leaves extract (ZSCLE) on renal [A] non-enzymatic MDA and [B] enzymatic SOD levels against Cyclosporine (CSP)-Histological results. Values are stated as mean ± SMD in each group N = 10. Values within a column of different superscript letters are substantially different at P<0.05.

Figure 4 Photomicrography illustrating H&E-stained sections of kidney in different groups. The Con group revealed normal glomerular and tubular histology Photo A. Photo B represents the CSP group, which demonstrated thickening of renal capsule and coagulated necrosis of epithelial lining. Extract therapy of ZSCLE (300 mg/kg) +CSP shows slight congestion of the glomerular tufts with slight vacuolation of some renal tubular Photo C. Photo D presents the normal appearance of renal tissue in the ZSCLE (600 mg/kg) +CSP group.

Renal oxidative status markers and enzymatic antioxidants

Rats treated with CSP revealed appreciably significant renal oxidative stress, which observed through significant (P<0.05) elevation in renal MDA, with significant (P<0.05) decline in renal SOD compared with Con. Oral administration of ZSCLE induced significant improvement in the antioxidant status of renal. The MDA level decreased significantly (P< 0.05) and the SOD enzyme activity increased significantly (P< 0.05) in the ZSCLE (300 mg/kg)+ CSP and ZSCLE (600 mg/kg)+ CSP, groups as compared with CSP group, which indicated the protective effect of ZSCLE is a dose-dependent Figure (3).
**Histopathological results**

Renal section of Con rats showed normal histological parenchyma structure (Fig.4.A). The CSP group showed thickening of renal capsule and coagulated necrosis of epithelial lining (Fig. 4.B). The group of ZSCLE (300 mg/kg)+ CSP showing slight congestion of the glomerular tufts with slight vacuolation of some renal tubular (Fig. 4.C). Apparent normal appearance of renal tissue was shown in the ZSCLE (600 mg/kg)+ CSP group (Fig. 4.D).

4. DISCUSSION

Cyclosporine-A, a potent immunosuppressive drug, widely prescribed in autoimmune disorders therapy (Hariharan et al., 2000; Zizhang et al., 2014). However, its usage induced severe toxicity especially nephrotoxicity (Nakamura et al., 2007; Zizhang et al., 2014). Many researches proved the protective effect of natural antioxidant against CSP nephrotoxicity (Durak et al., 2007; Wongmekiat et al., 2008). Therefore, this study explored whether ZSCLE can protect renal toxicity caused by CSP in rats.

In the present study the serum level of kidney function parameters was increased significantly after treated by CSP, while these parameters were inhibited significantly in the groups pretreated with ZSCLE in a dose dependent manner. The same results proved by many authors, who found that serum creatinine, uric acid and blood urea nitrogen levels were significantly elevated in group treated with CSP compared with control animal group (Durak et al., 2007; Wongmekiat et al., 2008; Zizhang et al., 2014). This could explained via a disturbance in renal functions. Uric acid, urea and creatinine levels reflected kidney function and structure integrity, the increment of these kidney indices may be explained tubular renal damage induced by CSP (Kaya et al., 2008; Yuce et al., 2008). The underlying mechanisms of this reflect is to stimulate of the proliferative genes as osteopontin, collagen I and IV, and transforming growth factor-beta, as well as an alter release of vasoactive substances as endothelin, prostaglandins and nitric oxide (Busauschina et al., 2004; Corrêa-Costa et al., 2009). The protective effect of ZSCLE confirmed by significantly inhibited the elevated renal indices induced by CSP. The usage of ZSCLE restores the renal functions in mice (Dkhil et al., 2018). Furthermore, ZSCLE showed hypoglycemic and decline creatinine and urea in diabetic rats (Jarald et al., 2009). The nephroprotective effect of ZSCLE could explain by its potent antioxidant compounds (Abdel-Wahhab et al., 2007; Abalaka et al., 2011, Ads et al., 2017).

Renal is a metabolically active organ, it is highly challenged with oxidizing response and toxicants. In the present study, CSP intoxication induced significant disturbance in the balance of ionic electrolyte Na+ and K+ compared with control rats. This effects could be explained through functional changes accompanied by decreased expression of aquaporins, Na+ depletion further promote CSP induced tubulointerstitial damage (Montagnino et al., 2004; Ponticelli, 2005; Magnasco et al., 2008). Elevation the potassium concentration could be attributed to decline efficiency of urinary K+ excretion (Chang and Gheun-Ho, 2007). Pretreatment with ZSCLE ameliorate the effect of CSP on ionic electrolyte, there were significant increase in serum Na+ with significant decrease in K+ compared with CSP group, the high dose was effectively higher than low dose. This could explain via ZSCLE antioxidant and chelating activities (Rafa et al., 2019).

The MDA is a marker of oxidative stress, and the SOD is an indicator of antioxidant capacity (Li et al., 2004; Yoon and Yang, 2009). The renal oxidative statuses were assessed in this study. There was significant change in the renal oxidant status balance which proved by significant depletion ofrenal SOD with significant increase in renal MDA contents compared with control rats, while ZSCLE especially in high dose group could reduce CSP induced oxidative damage, there were significantly improved in the renal antioxidant status. The SOD showed significant elevation and the MDA showed significant reduction compared with CSP group. These results could be explained through CSP generate the ROS of renal c

Administration of ZSCLE restrained renal oxidative stress and damage compared to CSP treatment. Antioxidant capacity of the ZSCLE has been reported, it suppress MDA and NO production, concomitant with enhance antioxidant enzymes and GSH in renal tissue of experimental animals, via the overexpression of their respective genes (Dkhil et al., 2018; Rafa et al., 2019). Shen et al. (2009) reported that ZSCLE potentiated the hepatic SOD, GSH and CAT content after carbon tetrachloride intoxication. This effect was explained by the up-regulation of antioxidant enzyme expression by mRNA after ZSCLE administration. The other explanation could be due to ZSCLE bioactive compounds (Kadioglu et al., 2016; Almeer et al., 2018).

Microscopically examined renal tissue showed severe changes in section from CSP group as necrosis, thickening of capsule and coagulated of epithelial lining. Administration of ZSCLE, especially high dose group alleviate these changes. In a previous study, after treatment with CSP for two week there was necrosis, fibrosis with vacuolar degeneration in renal tubular epithelia cells (Zizhang et al., 2014). The protective effect of ZSCLE could result from its ability to activate the antioxidant pathway, and quench ROS as proved by several studies (Almeer et al., 2018; Dkhil et al., 2018; Singh et al., 2018; Almeer et al., 2019).
5. CONCLUSION

The present research provides an evidence for CSP-induced nephrotoxicity. However, ZSCLE has exercised a defensive function against CSP-mediated renal toxicity by restoring all changes to near normal values; this could explain via its antioxidant activity. Therefore, for patient underlining CSP therapy, ZSCLE could be minimize or prevent the nephrotoxicity induced as side effect of CSP.

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**Conflicts of Interest:** The authors declare no conflict of interest.

**REFERENCE**


