Renoprotective influence of *Teucrium polium* leaf extract on Rats Intoxicated by Cyclophosphamid

Manal MS Mansoury

Food and Nutrition Department, Faculty of Home Economics, King Abdulaziz University, Jeddah, Saudi Arabia

Article History
Received: 11 September 2019
Reviewed: 14/September/2019 to 28/October/2019
Accepted: 30 October 2019
Prepared: 01 November 2019
Published: January - February 2020

Citation

Publication License
This work is licensed under a Creative Commons Attribution 4.0 International License.

General Note
Article is recommended to print as color digital version in recycled paper.

ABSTRACT

*Teucrium polium* leaves extract (TPLE) used as traditional herbal therapy for various diseases. Long term treated with Cyclophosphamide (CD) concomitant with development of renotoxicity. The present study aims to assess the mechanism of renal protective effect of TPLE on rats intoxicated with CD. Forty male rats were separated into 4 groups; control (Cont), CD; rats intraperitoneal (i.p) injected with CD (fifty mg/kg b.wt) for twenty one days, TPLE two hundred mg/kg b.wt +CD and TPLE four hundred mg/kg b.wt +CD; rats received TPLE orally for 21 days, followed by i.p. injected with CD. Following 24 hours of the last dose of CD blood and renal samples were collected for biochemical and histopathological investigations. Renal oxidative stress biomarkers; lipid peroxides (MDA) and superoxide dismutase (SOD), serum anti-inflammatory biomarkers, tumor necrosis factor-alpha (TNF-α) and interleukin-1beta (IL-1β), kidney function; serum levels of creatinine(Cr), urea (BUN) and uric acid (UA), as well as serum ionic levels of sodium (Na⁺) and potassium (K⁺) were determined. The current study demonstrated that injection of CD caused significant increases in renal MDA, serum anti-inflammatory cytokines (TNF-α and IL-1β), serum kidney function parameters and the serum ionic K+ levels, with significant decreases in the renal SOD and serum ionic Na+.
against control group. Renal tissues showing congestion and coagulating necrosis of the renal tubules in the CD group. Oral administration of TPLE significantly ameliorated CD-induced renal oxidative stress. It reduced CD-induced elevation in serum anti-inflammatory cytokines, kidney function parameters and the changes in ionic Na+ and K+ levels as well as it ameliorate the changes in kidney tissues compared with CD group. Therefore, TPLE ameliorates the effect of CD-induced nephrotoxicity.

**Keywords:** *Teucrium polium* leaf; cyclophosphamide; renaltoxicity; antioxidant; anti-inflammatory.

1. INTRODUCTION

Nephrotoxicity is "renal disease or dysfunction that arises as a direct or indirect result of exposure to medicines and industrial or environmental chemicals". It happens when a drug or toxin causes harm to the kidneys and it cannot eliminate surplus urine and waste (Nagai and Takano, 2010). A common problem in clinical medicine is drug-induced nephrotoxicity and the incidence of drug-induced acute kidney injury (AKI) may be as high as 60 percent (Schetz et al., 2005). Drugs can trigger nephrotoxicity by changing hemodynamics intraglomerular-large and reducing glomerular filtration rate (GFR) (Markowitz and Perazella, 2005). Cyclophosphamide (CP) is widely used clinically as a chemo-therapeutic and immunosuppressant agent (Yuksel et al., 2017). Long-term use of CD causes damage to renal tubular cells by loss of mitochondrial function and interference with tubular transport, oxidative stress and free radicals (Uren et al., 2014) The generation of reactive oxygen species is widely attributed to nephrotoxicity due to chronic CD treatment (Anyasor et al., 2010).

Management of nephrotoxicity is still a challenge to the modern scientific community. Unfortunately, drugs have little to offer alleviation for kidney ailments. Thus given rise to research involved in identification of safe, inexpensive and available alternatives from natural resources (Kotnis et al., 2004). Due to the presence of various bioactive compounds, medicinal plants have been proved to be a major source of drugs (Pereira et al., 2016).

*Teucrium polium* (TP) is distributed throughout Africa, Asia and Europe, but primarily in the region of Mediterranean, more than three hundred species included (Rahmouni et al., 2017). Traditionally, TP has been used to treat multiple gastrointestinal disorders (liver and kidney disorders and intestinal pain), eczema, inflammation of the urinary tract, rheumatism and diabetes, as well as it improve mental performance (Everest and Ozturk, 2005). Studies conducted in vivo and in vitro have verified various biological activities of TP such as anti-rheumatoid, anti-inflammatory (Milosevic et al., 2018), antihypertensive hypolipidemic (Rasekh et al., 2001), antimicrobial (Balmekki et al., 2013) and hypoglycemic effect (Kasabri et al., 2011). In addition, many studies have revealed that various TP extracts have important hydroxyl radical scavenging, free radical scavenging and antioxidant activity due to its polyphenolic compounds (Kadikova et al., 2005). Recently, the anticancer, cytotoxic and antimutagenic effects of TP on various cell lines have been studied (Rahmouni et al., 2017). The aim of this work is to assess TPLE’s possible protective role against CD-induced kidney toxicity.

2. MATERIAL AND METHODS

**Drug, chemicals and plant material**

Cyclophosphamide (CD) soft capsules, provided by Novartis Pharaceuticals, Australia. All chemical and kits with high grade obtained from Sigma-Aldrich (St. Louis, MO) Chemical Co. *Teucrium polium* (TP) leaves was purchased from local market in Jeddah, Saudi Arabia.

**Preparation of *Teucrium polium* leaf extract**

*Teucrium polium* leaves were air-dried in darkness at ambient temperature for a short period of time, dried powder (500 g) was macerated in 1 L of methanol (80%) at room temperature. The maceration was repeated 3 times. Filter paper was used to filter the extract (Whatman No. 1) and then evaporated to dryness under vacuum using rotary evaporator. The extract was stored in non-permeable sterile bottle at 4°C until used (Rahmouni et al., 2018).

**Experimental design**

Forty male rats weighing 130-150 g bought from King Fahd Medical Resrach Center, KAU’s animal house. They adhered to free water and standard diet under Canadian ethical approval from KAU’s local biomedical ethical cmmittee. After one week of acclimatization rats were separated into four 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 (CD); rats distilled water for 2 weeks then were i.p. injected with CD at a dose (50 mg/kg b.wt) diluted in olive oil for a period of 21 days according to Dipica et al., (2014). Group III (Teucrium polium leaves extract 200 mg/ kg b.wt +CD) (Azar et al., 2013). Group IV (Teucrium polium leaves extract 400 mg/ kg b.wt +CD). Rats in groups III and IV received Teucrium polium leaves extract orally for 21 days, followed by i.p. injected with CD.

Ethical approval
The experimental study was adhering under rules of Candian ethic upon approval for biomedical committee, KFMRC, KAU, KSA.

Samples collection
Twenty-four hours after the last CD drug administered, rats were anesthetized then blood and renal samples were collected. The serum samples were separated and stored at -80 °C until biochemical analysis was performed. The renal samples were either prepared for biochemical analysis or preserveal in neutral buffer formaldehyde solution for histopathological studies.

Biochemical analysis
- The levels of MDA and the activity of SOD enzyme were estimated in homogenated renal using ELISA kits.
- The anti-inflammatory cytokines; the serum levels of TNF-α and IL-1β were assessed by ELISA kits.
- The level of kideny function; serum levels of Cr, BUN and UA, as well as serum levels of Na⁺ and K⁺ were measured using colormetric kits.

All kits'used were obtained from 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 microsopre after stainning with hematoxylin and eosin to determine histopathogical changes.

Statistical analysis
Results analysis by ANOVA one-way analysis of variance, values were presented as mean ± SMD for 10 rats/ group.

3. RESULTS
Influence of TPLE on renal oxidative stress in nephrotoxic rats by CD
The levels of non-enzymatic MDA and enzymatic SOD antioxidant in the renal tissue in different groups are represented in Figure (1). The level of MDA was elevated significantly (p< 0.05) along with significant (p< 0.05) reduction in SOD activity (66.44 ± 2.34 and 17.54 ± 0.77 respectively) of CD group against the healthy group (35.25 ± 1.45 and 29.31 ± 1.12 respectively).

Oral administration of TPLE induced significant improvement in the antioxiant status of renal. The MDA level decreased significantly (p< 0.05) and the SOD enzyme activity increased significantly (p< 0.05) in the TPLE administration (200 mg/kg) and the (400 mg/kg) groups as compared with CD group, which indicated the protective effect of TPLE is a dose-dependent.

Figure (2) demonstrated the effect of TPLE on the serum levels of TNF-α and IL1-β in different experimental groups. The CD group caused a significant (P<0.05) increases in both TNF-α and IL1-β (58.35± 1.24 and 27. 31 ± 0.84 respectively) against the normal group (2.03 ± 0.02 and 0.91± 0.01). Oral TPLE administration at both dosage levels resulted in a significant reduction (p<0.05) in both TNF-α and IL1-β levels compared to the CD group.

Table (1) represented the levels of serum renal function (Cr, BUN and UA) in different groups of experiment. The serum of kidney functions level in the CD group was significantly (p< 0.05) raised contrary to normal group. The TPLE administration (two hundred and four hundred mg / kg) decreased the level of serum renal functions (p<0.05) compared to the CD group, which may be indicator for renal toxicity induced by CD. Significant (p< 0.05) reduction were detected in TPLE (200 mg/kg)+ CD and TPLE (400 mg/kg)+ CD for all kidney functions, which indicated the protective effect of TPLE is a dose-dependent.

Influence of TPLE on ionic sodium and potassim in nephrotoxic rats by CD
Figure (3) showed the levels of serum ionic Na⁺ and K⁺ in different experimental groups. The serum level of ionic Na⁺ was significantly (p< 0.05) decreased combined with significant (p< 0.05) elevation in the serum ionic K⁺of CD group in contrast tonormal group. Oral TPLE administration significantly (p< 0.05) elevated the ionic Na⁺ level combined with a significant (p< 0.05) reduction in ionic K⁺level as compared with CD intoxicated group.
Values are presented as mean ± SMD.
Values with different superscript letters within a column are significantly different at P<0.05.

**Figure 1** Influence of TPLE on renal non-enzymatic (MDA) and enzymatic (SOD) levels against CD-induced nephrotoxicity in rats.
Values are presented as mean± SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

**Figure 2** Influence of TPLE on serum inflammatory TNF-α and IL1-β levels against CD-induced nephrotoxicity in rats

**Table 1** Influence of TPLE on serum level of kidney function against CD-induced nephrotoxicity in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Creatinine (µmol/L)</th>
<th>Urea nitrogen (mmol/L)</th>
<th>Uric acid (umol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cont (-)</td>
<td>26.34 ± 1.12b</td>
<td>8.65 ± 0.22b</td>
<td>69.45 ± 1.32b</td>
</tr>
<tr>
<td>CD</td>
<td>51.22± 1.44 a</td>
<td>18.82 ± 0.23a</td>
<td>127.71 ± 2.62 a</td>
</tr>
<tr>
<td>TPLE (200 mg/kg)+CD</td>
<td>27.29 ± 1.81b</td>
<td>9.06 ± 0.13b</td>
<td>68.97 ± 1.42 b</td>
</tr>
<tr>
<td>TPLE (400mg/kg)+CD</td>
<td>26.75 ±1.75 b</td>
<td>8.85 ± 0.02b</td>
<td>69.98 ± 1.71 b</td>
</tr>
</tbody>
</table>

- Values are presented as mean± SMD.
- Values with different superscript letters within a column are significantly different at P<0.05.

**Histopathological results**
The control group of the kidneys displayed normal histological parenchyma. In the CD group, kidney’s tissue showed atrophy of glomerular tuft local interstitial nephritis and karyomegaly of nuclei of renal tubular epithelium. In the TPLE (200 mg/kg) + CD grouprenal tissue showing slight congestion of the glomerular tufts with slight vacuolation of some renal tubular. Apparent normal appearance of renal tissue is showing in the TPLE (400 mg/kg) + CD group.
- Values are presented as mean ± SMD.
- Values with different superscript letters within a column are significantly different at P < 0.05.

**Figure 3** Influence of TPLE on ionic sodium and potassium against CD-induced nephrotoxicity in rats
Figure 4 Photomicrography illustrating H & E-stained sections of kidney in different groups. Negative control rats’ kidney with normal histological parenchyma in Photo A. Photo B represent the CD group, kidney’s tissue showed atrophy of glomerular tuft, local interstitial nephritis and karyomegaly of nuclei of renal tubular epithelium. Photo C In the TPLE (200 mg/kg) +CD group renal tissue showing slight congestion of the glomerular tufts with slight vacuolation of some renal tubular. Photo D present normal appearance of renal tissue in the TPLE (400 mg/kg) +CD group.

4. DISCUSSION

The kidney is extremely prone to poisonous insults, and medications, which are a prevalent cause of acute kidney injury (Naughton, 2008). Researchers proposed that medications are accountable for up to 20% of acute renal injuries acquired by the community and hospital (Nash et al., 2002). Acute renal injury (AKI) is a sudden loss of kidney function that causes waste products such as creatinine and urea to accumulate in the body, water and sodium retention, decrease in glomerular filtration rate, hyperkalemia, and metabolic acidosis are other features of AKI (Tiong et al., 2014).

Drug induced nephrotoxicity is a common condition and the cause of around 8% to 60% of all AKI cases in the intensive care unit (Schetz et al., 2005). Various therapeutic drugs, including aminoglycoside antibiotics, chemotherapeutic agents, angiotensin II receptor blockers, angiotensin-converting enzyme inhibitors, radio contrast media, and nonsteroidal antiinflammatory drugs (NSAIDs), exert nephrotoxic effects via different pathogenic mechanisms (Dhodi et al., 2014). Cyclophosphamide (CD) is a nitrogen-alkylating agent used for various types of cancer and some autoimmune diseases (Viaud et al., 2013). A CD is converted to its active metabolites in liver (Ren et al., 1997). At higher doses, the cell cycle is interrupted by the formation of DNA cross-linking and DNA lesions (Korkmaz et al., 2007). This anti-neoplastic drug leads to apoptosis and toxicity of cells (Arif, and Ejaj, 2009). Recent studies strongly evidence that the co-administration of different medicinal plants along with various nephrotoxic drugs may reduce the incidence of kidney injury (Khorsandi, and Orazizadeh, 2008, Khajavi et al., 2011 and Hosseinian et al., 2016). This research was carried out to evaluate TPLE’s protective impact and antioxidant activity against nephrotoxicity caused by CD.

In the current study there were significant increases in renal MDA, TNF-α and IL-1β, kidney function parameters and the serum ionic K+ levels, with significant decreases in the renal SOD and serum ionic Na+ in intoxicated group compared with control group. The results obtained correspond to most of the experimental procedures reported (Arif and Ejaj, 2009; Tripathi and Jena, 2009 and Zhao and Liang, 2010). This could be due to the disturbance of the balance between free radicals and antioxidants which lead to injures of the renal tissue (Selvakumar et al., 2005). It is believed that the mechanisms of renal toxicity induced by CD are mediated through free radical generation; iron-dependent oxidative damage of biological molecules, membrane lipids peroxidation, and protein oxidation, these effects can increase glomerular capillaries permeability and tubular atrophy (Selvakumar et al., 2004).
Cyclophosphamide-mediated genotoxicity either occur induction of microtubule damages, DNA reactive intermediates or endogenous mutagenic agents (Zulkipli et al., 2015).

Antioxidants can reduce oxidative stress, inhibit oxygen free radicals and reduce associated complications (Rafieian et al., 2013). Phenolic acid and flavonoids are the main polyphenolic compounds in plants (Pettiad and Scully, 2009). There were five phenolic acids and six flavonoids in the TPLE; chlorogenic acid was the most concentrated phenolic acid. However, catechin was the most abundant flavonoid that plays an important role against the toxic effect of CD (Tepe et al., 2011). This study verified that treatment of rats with TPLE induced significant declines in the level of renal MDA, TNF-α and IL-1β, kidney function parameters and the serum ionic K+ levels, with significant elevation in the renal SOD and serum ionic Na+ compared to intoxicated group. This could be due to its phyto constituents that inhibit scavenging free radicals and lipid peroxidation. The TPLE protective effects found in this research paper are in the same line with the studies previously published (Zabihi et al., 2018 and Suboh et al., 2004). In this respect, a TPLE methanolic extract protected RBCs against hydrogen peroxide-induced lipid peroxidation (Suboh et al., 2004). In another study, Khleifat et al., (2002) demonstrated that the TPLE prepared using various organic solvents (ethyl acetate, diethyl ether and n-butanol) were effective as β-cryptoxanthin oxidation inhibitors. In an earlier study, it was shown that the extract prepared from TPLE suppressed lipid peroxidation in vitro (Suboh et al., 2004). Also, previous studies indicated that TPLE prevents oxidative damage in the liver (Panovska et al., 2007), stomach (Mehrabani et al., 2009) and pancreas (Esmaeili et al., 2009). This high antioxidant activity was indicated because of the phenolic compounds found in this herb such as hydroxybenzoic acid derivatives, ferulic acid, caffeic acid and flavonoid derivatives such as quercetin and luteolin (Proestos et al., 2006). Polyphenolic compounds showed powerful antioxidant activity (Kasabri et al., 2011). Because of their ability to prevent lipid peroxidation and chelate redox-active metals, these antioxidants have beneficial effects. These phyto constituents could be responsible for anti-inflammatory activity. It was established that flavonoids inhibited the enzyme prostaglandin synthetase, more precisely endoperoxidase, and developed important anti-inflammatory effects due to inhibition of chemical inflammation mediators (Blobaum and Marnett, 2007). Phenolic compounds have strong anti-inflammatory activity in a similar study (Beg et al., 2011). Flavonoids and tannins are responsible for the anti-inflammatory effect of extract.

Histopathological studies had been carried out on all rats. The study results revealed a significant influence of the CD administration on histopathological damage of CD group. These results were in the same line with Abrahama et al. (2007). On the other hand, there was an improvement in kidneys tissue of rat groups given TPLE in combination with CD.

5. CONCLUSION

the results of the recent study demonstrate that TPLE has a protective role that is likely mediated by its antioxidant proparities against CD-induced nephrotoxicity. Further studies are needed to evaluatethe potential utility of this extract in clinical conditions associated with renotoxicity.

Conflict of interest
The author declares that no conflict of interest

Financial resources of the study
None

List of abbreviations

<table>
<thead>
<tr>
<th>Acute Kidney injury</th>
<th>AKI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Cont</td>
</tr>
<tr>
<td>Creatinine</td>
<td>Cr</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>CD</td>
</tr>
<tr>
<td>Gomerular filtration rate</td>
<td>GFR</td>
</tr>
<tr>
<td>Interleukin-1beta</td>
<td>IL-1β</td>
</tr>
<tr>
<td>Intraperitoneal</td>
<td>i.p.</td>
</tr>
<tr>
<td>Lipid peroxides</td>
<td>MDA</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory drugs</td>
<td>NSAIDs</td>
</tr>
<tr>
<td>Potassium</td>
<td>K+</td>
</tr>
<tr>
<td>Sodium</td>
<td>Na+</td>
</tr>
</tbody>
</table>
Superoxide dismutase | SOD
Teucrium polium | TP
Teucrium polium leaves extract | TPLE
Tumor necrosis factor-alpha | TNF-α
Blood Urea Nitrogen | BUN
Uric Acid | UA

REFERENCE