



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

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


Manal MS Mansoury. Renoprotective influence of *Teucrium polium* leaf extract on Rats Intoxicated by Cyclophosphamide. *Medical Science*, 2020, 24(101), 249-258

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 renal toxicity. 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-1 β), 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-1 β), 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; renal toxicity; 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 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 human diseases including 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 vitro* and *in vivo* 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 (Kadifkova 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 Pharmaceuticals, 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 Research Center, KAU's animal house. They adhered to free water and standard diet under Canadian ethical approval from KAU's local biomedical ethical committee. 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 preserved 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-1 β were assessed by ELISA kits.
- The level of kidney function; serum levels of Cr, BUN and UA, as well as serum levels of Na⁺ and K⁺ were measured using colorimetric 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 microscope after staining with hematoxylin and eosin to determine histopathological changes.

Statistical analysis

Results analysis by ANOVA one-way analysis of variance, values were presented as mean \pm 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).

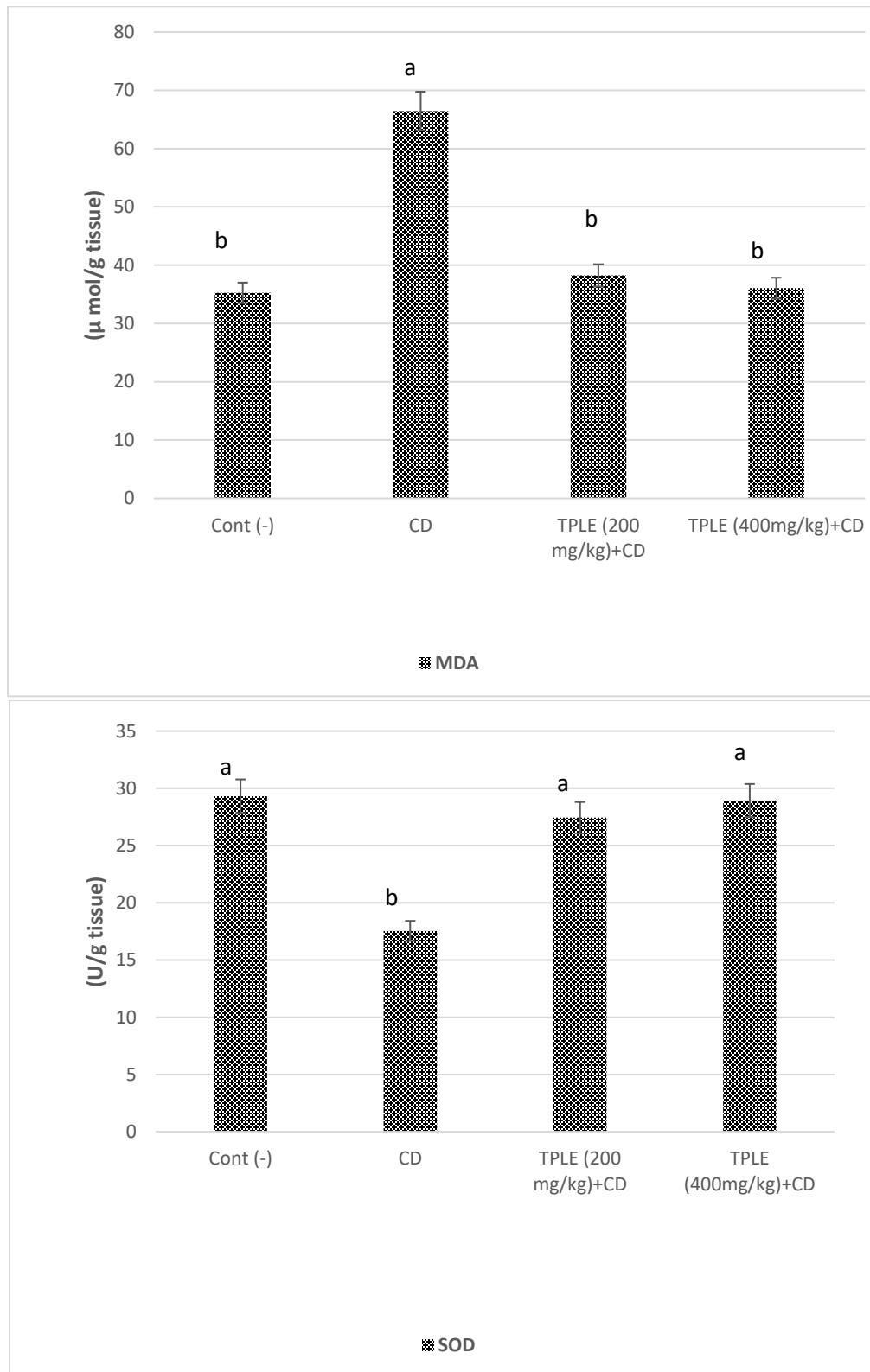
Oral administration of TPLE induced significant improvement in the antioxidant status of renal. The MDA level decreased significantly ($p < 0.05$) and the SOD enzyme activity increased significantly ($p < 0.05$) in the TPLE administration (200 mg/kg) and the (400 mg/kg) groups as compared with CD group, 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 groups against renal toxicity in male rats. 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 potassium in nephrotoxic rats by CD

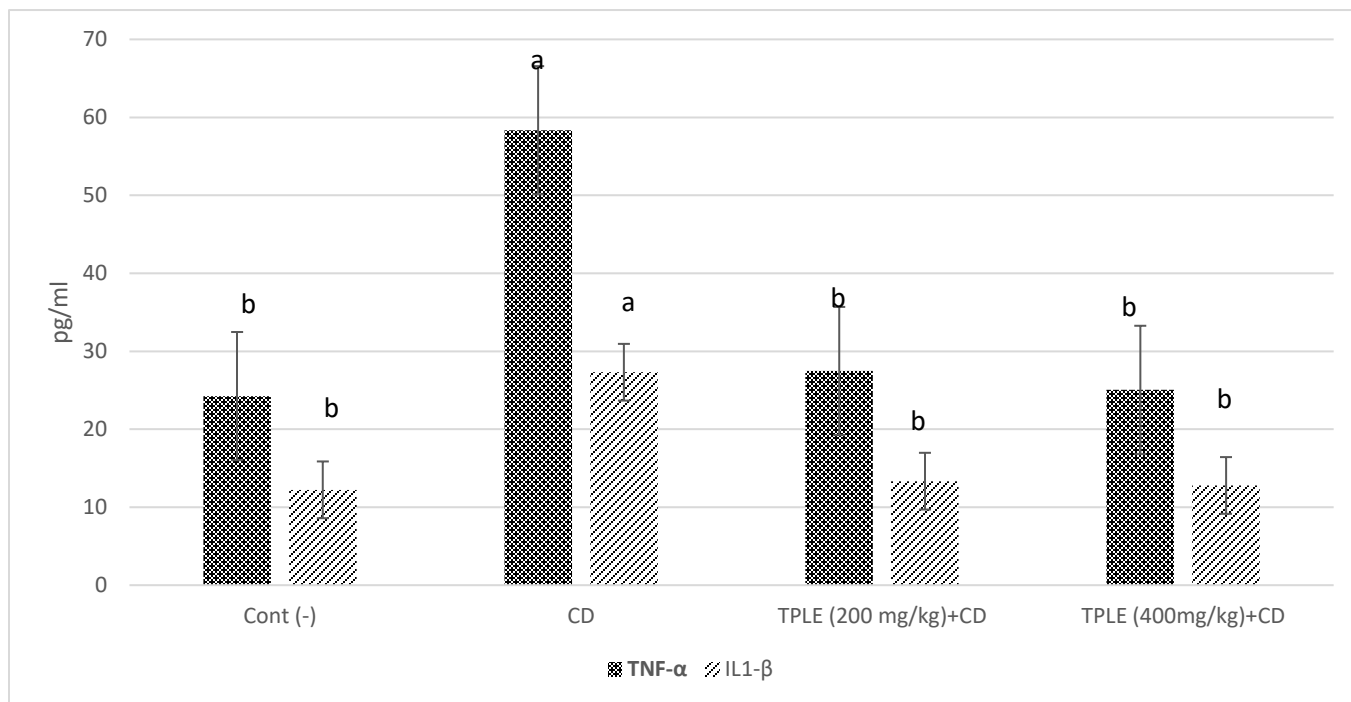
Figure (3) showed the levels of serum ionic Na⁺ and K⁺ in different experimental groups. The serum level of ionic Na⁺ was significantly ($p < 0.05$) decreased combined with significant ($p < 0.05$) elevation in the serum ionic K⁺ of CD group in contrast to normal group. Oral TPLE administration significantly ($p < 0.05$) 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 \pm 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 \pm 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

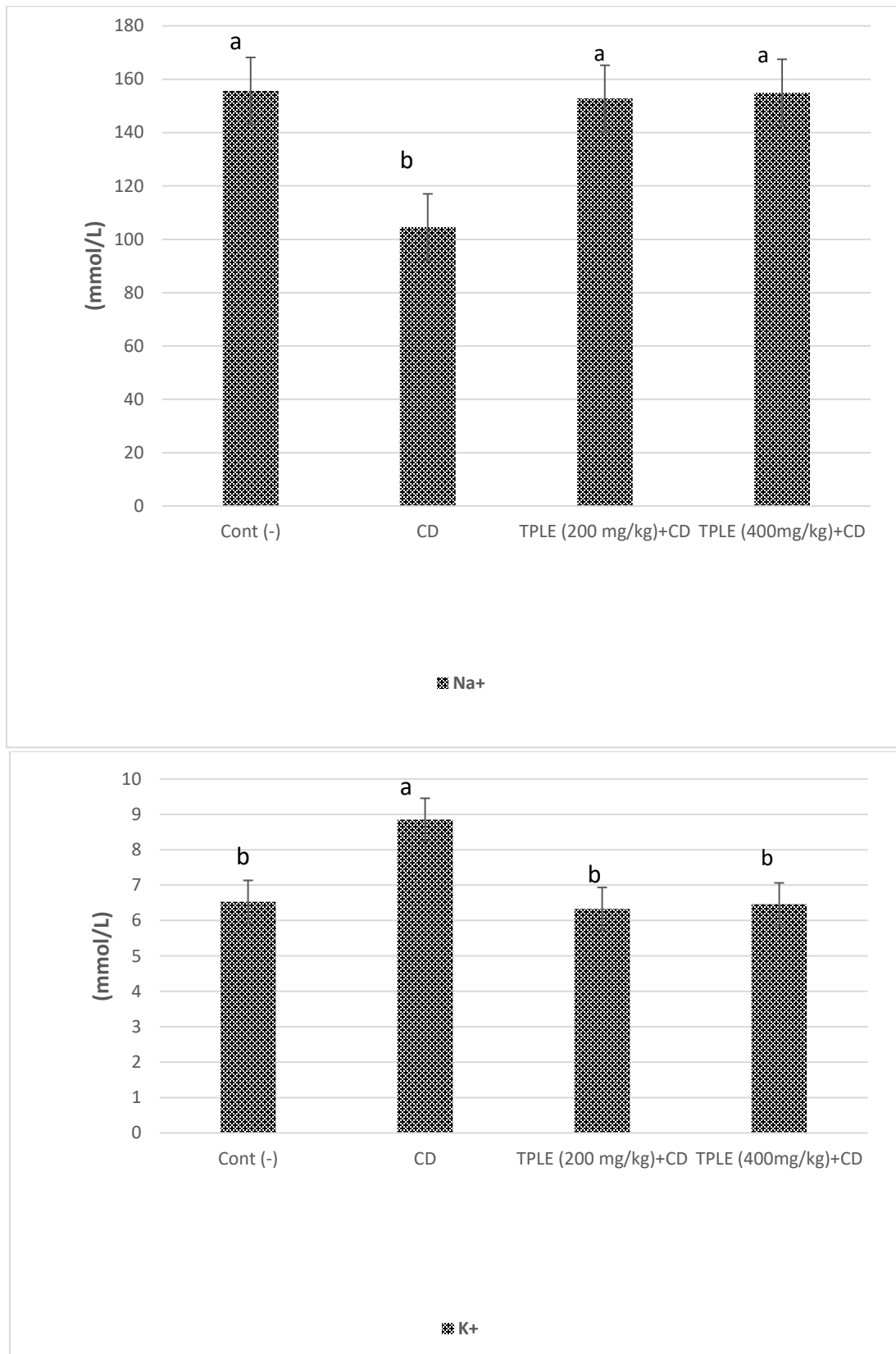
Groups	Creatinine ($\mu\text{mol/L}$)	Urea nitrogen (mmol/L)	Uric acid ($\mu\text{mol/L}$)
Cont (-)	26.34 \pm 1.12b	8.65 \pm 0.22b	69.45 \pm 1.32b
CD	51.22 \pm 1.44 a	18.82 \pm 0.23a	127.71 \pm 2.62 a
TPLE (200 mg/kg)+CD	27.29 \pm 1.81b	9.06 \pm 0.13b	68.97 \pm 1.42 b
TPLE (400mg/kg)+CD	26.75 \pm 1.75 b	8.85 \pm 0.02b	69.98 \pm 1.71 b

- Values are presented as mean \pm 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 group renal 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

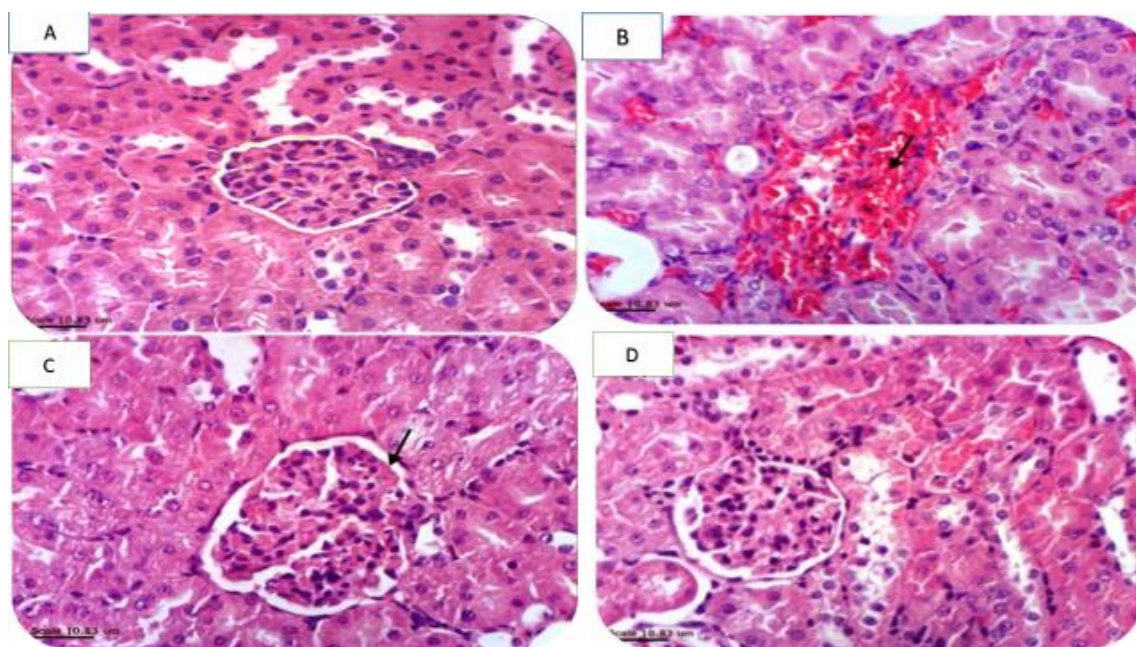


Figure 4 Photomicrography illustrating H & E-stained sections of kidney in different groups. Negative control rats' kidney with normal histological parenchyma in Photo A. Photo B represent the CD group, kidney's tissue showed atrophy of glomerular tuft local interstitial nephritis and karyomegaly of 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-1 β , 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 led 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 (Pettia 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-1 β , 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 nbutanol) were effective as β -carotene 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 (Esmaili 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 properties against CD-induced nephrotoxicity. Further studies are needed to evaluate the potential utility of this extract in clinical conditions associated with renotoxicity.

Funding: This research received no external funding.

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

List of abbreviations

Acute Kidney injury	AKI
Control	Cont
Creatinine	Cr
Cyclophosphamide	CD
Gomerular filtration rate	GFR
Interleukin-1beta	IL-1 β
Intraperitoneal	i.p.
Lipid peroxides	MDA
Nonsteroidal anti-inflammatory drugs	NSAIDs
Potassium	K ⁺
Sodium	Na ⁺
Superoxide dismutase	SOD
Teucrium polium	TP

Teucrium polium leaves extract	TPLE
Tumor necrosis factor- α	TNF- α
Blood Urea Nitrogen	BUN
Uric Acid	UA

REFERENCE

- Abrahama P, Indiranib K, Sugumar E. Effect of cyclophosphamide treatment on selected lysosomal enzymes in the kidney of rats. *Exp Toxicol. Pathol* 2007; 59:143-9.
- Anyasor GN, Olusola-Ogunwenmo K, Oyelana OA, Akpofunure BE. Phytochemical constituents and antioxidant activities of aqueous and methanol stem extracts of *Costus afer* Ker Gawl. (Costaceae). *Afr J Bioethanol* 2010; 31:4880-4.
- Arif K, Ejaj A. Protective effect of liposomal formulation of tufts in (a naturally occurring tetrapeptide) against cyclophosphamide-induced genotoxicity and oxidative stress in mice. *Indian J Biochem Biophys* 2009; 46:45-52.
- Azar B, Yahya M, Alireza M, Mahmoud R, Mehdi N, Azam A, Zahra K, Farshad H, Hamid N. Nephrotoxicity of hydroalcoholic extract of *Teucrium polium* in Wistar rats. *Pak J Med Sci* 2013; 29 (1):329-33.
- Balmekki N, Bendimerad N, Bekhechim C, Fernandez X. Chemical analysis and antimicrobial activity of *Teucrium polium* L. essential oil from Western Algeria. *J Med Plants Res* 2013; 7: 897-902.
- Beg S, Swain H, Hasan MA, Barkat MS. Systematic review of herbals as potential anti-inflammatory agents: Recent advances, current clinical status and future perspectives. *Pharmacog Rev* 2011; 5: 120-37.
- Blobaum AL, Marnett LJ. Structural and functional basis of cyclooxygenase inhibition. *J Med Chem* 2007; 50(7): 1425-41.
- Dhodi DK, Bhagat SB, Pathak D, Patel SB. Drug-induced nephrotoxicity. *Int J Basic Clin Pharmacol* 2014; 3:591-7.
- Dipica R, Aditya G, Divya B, Manvendra SK, Nazneen D. Protective effect of *Curculigo orchoides* extract on cyclophosphamide-induced neurotoxicity in murine model. *Toxicol International* 2014; 21(3):232-6.
- Esmaili MA, Zohari F, Sadeghi H. Antioxidant and protective effects of major flavonoids from *Teucrium polium* on beta-cell destruction in a model of streptozotocin-induced diabetes. *Planta Med* 2009; 75: 1418-20.
- Everest A, Ozturk E. Focusing on the ethnobotanical uses of plants in Mersin and Adana provinces (Turkey). *J Ethnobiol Ethnomed* 2005; 6: 1-6.
- Hosseinian S, Khajavi A, Hadjzadeh MR, Mohamadian Roshan N, Havakhah S, Shafiee S. The protective effect of *Nigella sativa* against cisplatin-induced nephrotoxicity in rats. *Avicenna J. Phytomed* 2016; 6:44-54.
- Kadifkova-Panovska T, Kulevanova S, Stefova M. In vitro antioxidant activity of some *Teucrium* species (Lamiaceae). *Acta. Farm* 2005; 55: 207-14.
- Kasabri V, Afifi FU, Hamdan I. In vitro and in vivo acute antihyperglycemic effects of five selected indigenous plants from Jordan used in traditional medicine. *J Ethnopharmacol* 2011; 133: 888-96.
- Khajavi Rad A, Ghazi L, Boroushaki MT. Effect of commercial (vimang) and hydroalcoholic extract of *Mangifera indica* (Mango) on gentamicin-induced nephrotoxicity in rat. *Avicenna J Phytomed* 2011; 1:98-105.
- Khleifat K, Shakhaneh J, Tarawneh K. The chronic effects of *Teucrium polium* on some blood parameters and histopathology of liver and kidney in the rat. *Turk J Biol* 2002; 26: 65-71.
- Khorsandi L, Orazizadeh M. Protective effect of *Curcuma longa* extract on acetaminophen induced nephrotoxicity in mice. *Daru* 2008; 16:155-9.
- Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. *Cell Biol Toxicol* 2007; 6:23-303.
- Kotnis MS, Patel P, Menon SN, Sane RT. Renoprotective effect of *Hemidesmus indicus*, a herbal drug used in gentamicin-induced renal toxicity. *Nephron* 2004; 9(3):142-52.
- Markowitz GS, Perazella MA. Drug-induced renal failure: a focus on tubulointerstitial disease. *Clin Chim Acta* 2005; 351:31-47.
- Mehrabani D, Rezaee A, Azarpira N, Fattahi MR, Amini M, Tanideh - Panjehshahin, MR, Saberi-Firouzi M. The healing effects of *Teucrium polium* in the repair of indomethacin-induced gastric ulcer in rats. *Saudi Med J* 2009; 30(4): 494-9.
- Milosevic-Djordjevic O, Radovic Jakovljevic M, Markovic A. Polyphenolic contents of *Teucrium polium* L. and *Teucrium scordium* L. associated with their protective effects against MMC-induced chromosomal damage in cultured human peripheral blood lymphocytes. *Turk J Biol* 2018; 42(2):152-62.
- Nagai J, Takano M. Molecular-targeted approaches to reduce renal accumulation of nephrotoxic drugs. *Expert Opin Drug Metab Toxicol* 2010; 6:1125-38.
- Nash K, Hafeez A, Hou S. Hospital-acquired renal insufficiency. *Am J Kidney Dis* 2002; 39 (5): 930-6.

25. Naughton CA. Drug-induced nephrotoxicity. *Am Fam Physician* 2008; 78 (6): 743-50.
26. Panovska TK, Kulevanova S, Gjorgoski I, Bogdanova M, Petrushevska G. Hepatoprotective effect of the ethyl acetate extract of *Teucrium polium* L. against carbon tetrachloride induced hepatic injury in rats. *Acta Pharm* 2007; 57(2): 241-8.
27. Pereira C, Barros L, Ferreira IC. Extraction, identification, fractionation and isolation of phenolic compounds in plants with hepatoprotective effects. *J Sci Food Agric* 2016; 96:1068-84.
28. Petti S, Scully C. Polyphenols, oral health and disease: a review. *J Dent* 2009; 37: 413-23.
29. Proestos C, Sereli D, Komaitis M. Determination of phenolic compounds in aromatic plants by RP-HPLC and GCMS. *Food Chem* 2006; 95(1):44-52.
30. Rafieian-Kopaei M, Baradaran A, Rafieian M. Plant's antioxidants: from laboratory to clinic. *J Nephropathol* 2013; 2:152-3.
31. Rahmouni F, Saoudi M, Amri, N. Protective effect of *Teucrium polium* on carbon tetrachloride induced genotoxicity and oxidative stress in rats. *Arch Physiol Biochem* 2018; 124(1):1-9.
32. Rahmouni F, Hamdaoui L, Badraoui R, Rebai T. Protective effects of *Teucrium polium* aqueous extract and ascorbic acid on hematological and some biochemical parameters against carbon tetrachloride (CCL4) induced toxicity in rats. *Biomed Pharmacoter* 2017; 91: 43-48.
33. Rasekh HR, Khoshnood-Mansourkhani MJ, Kamalinejad M. Hypolipidemic effects of *Teucrium polium* in rats. *Fitoterapia* 2001; 72: 937-9.
34. Ren S, Yang JS, Kalthorn TF, Slattery JT. Oxidation of cyclophosphamide to 4-hydroxycyclophosphamide and deschloroethylcyclophosphamide in human liver microsomes. *Cancer Res* 1997; 57(19):4229-35.
35. Schetz M, Dasta J, Goldstein S, Golper T. Drug-induced acute kidney injury. *Curr Opin Crit Care* 2005; 11:555-65.
36. Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Beneficial effects of DL-alpha-lipoic acid on cyclophosphamide-induced oxidative stress in mitochondrial fractions of rat testis. *Chem Biol Interact* 2005; 152:59-66.
37. Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Protective effect of DL-alpha-lipoic acid in cyclophosphamide induced oxidative injury in rat testis. *Reprod Toxicol* 2004; 19:163-7.
38. Suboh SM, Bilito YY, Aburjai TA. Protective effects of selected medicinal plants against protein degradation, lipid peroxidation and deformability loss of oxidatively stressed human erythrocytes. *Phytother Res* 2004; 18(4): 280-4.
39. Tepe B, Degerli S, Arslan S. Determination of chemical profile, antioxidant, DNA damage protection and antiamebic activities of *Teucrium polium* and *Stachys iberica*. *Fitoterapia* 2011; 82(2):237-246.
40. Tiong HY, Huang P, Xiong S, Li Y, Vathsala A, Zink D. Drug-induced nephrotoxicity: Clinical impact and preclinical in vitro models. *Mol Pharm* 2014; 11:1933-48.
41. Tripathi DN, Jena GB. Intervention of astaxanthin against cyclophosphamide-induced oxidative stress and DNA damage: a study in mice. *Chem Biol Interact* 2009; 180:398-406.
42. Uren N, Yuksel S, Onal Y. Genotoxic effects of sulfur dioxide in human lymphocytes. *Toxicol Ind Health* 2014; 30(4):311-5.
43. Viaud S, Saccheri F, Mignot G, Yamazaki T, Daillere R, Hannani D. The intestinal microbiota modulates the anticancer immune effects of cyclophosphamide. *Science* 2013; 4:342-9.
44. Yuksel S, Tasdemir S, Korkmaz S. Protective effect of thymoquinone against cyclophosphamide induced genotoxic damage in human lymphocytes. *Bratisl Lek Listy* 2017; 118(4):208-11.
45. Zabihi NA, Mousavi SM, Mahmoudabady M, Soukhtanloo M, Sohrabi F, Niazmand S. *Teucrium polium* L. improves blood glucose and lipids and ameliorates oxidative stress in heart and aorta of diabetic rats. *Int J Prev Med* 2018; 9: 110-118.
46. Zhao H, Liang J. Folic acid and soybean isoflavone combined supplementation protects the post-neural tube closure defects of rodents induced by cyclophosphamide in vivo and in vitro. *Neurotoxicology* 2010; 31:180-87.
47. Zulkipli IN, David SR, Rajabalaya R, Idris A. Medicinal Plants A Potential Source of Compounds for Targeting Cell Division. *Drug Target Insights* 2015; 9:9-19.