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# PORTULACA OLERACEA LEAVES EXTRACT ATTENUATES CYCLOPHOSPHAMIDE TOXICITY IN MICE

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*Cyclophosphamide (CTX) is a well-known broad-spectrum anticancer agent however; it has severe side effects on vital organs. Portulaca oleracea that belongs to family Portulacaceae showed promising nutritional and biomedical properties. This research aimed to estimate the effect of P. oleracea leaves extract (POLE) on CTX toxicity in male CD-1 mice. Total phenolic, flavonoids contents, total antioxidant capacity (TAC), and DPPH scavenging activity (DPPH %) were determined in POLE by phytochemical quantitative analysis. Thirty-six male CD-1 mice were divided into three groups (n=12), as negative control, CTX (200 mg/kg) injected, and CTX/POLE (200 mg/kg) injected groups. Blood samples, liver tissues and kidney tissues were collected for hematological, biochemical and histopathological investigations. The total phenolic, flavonoid content, saponin, DPPH %, and total antioxidant capacity (TAC) were 1368 µg/ml, 302 µg/ml, 0.1250 µg/g, 65%, 193 µg/ml, respectively. Treatment with POLE augmented the hematological, biochemical, and histopathological changes induced by CTX of hepatic and renal tissues. Collectively, POLE reduced the hepato-renal toxicities caused by CTX in mice by improving the antioxidants/oxidants hemostasis*

***Keywords:*** *Portulaca oleracea leaves, Portulacaceae, Antioxidants, Phytochemicals, Cyclophosphamide, Hepato-renal, Toxicity*

## INTRODUCTION

Chemotherapy is used for cancer treatment; however, undesirable side effects on normal organs were associated[1]. Therefore, there is a significant challenge to find new treatment agents with low toxicities[2]. CTX is an alkylating anticancer drug used for different cancer types[3]. CTX can be injected prior to the immunotherapeutic modalities for cancer treatment[4]. CTX treatment led to neutropenia and lymphopenia[5]. Previous studies have demonstrated that CTX cause liver and kidney toxicity[6,7]. CTX inside the human body generates acrolein, which promotes lipid peroxidation, DNA damage, protein oxidation, and severe toxicities[8,9].

Natural products play a significant role in the eradication of several adverse effects that caused by exposure to contaminants in the environment and food[10]. Natural antioxidants have been shown to be beneficial in CTX-induced toxicity[11]. Lowering the side effects of the chemotherapy by natural constitutes may permit to safely administer more effective dose[12]. *Portulaca oleracea* is commonly called purslane that belongs to *Portulacaceae* family[13]. Pervious study reported that *P. oleracea* exhibited bioactive phytochemicals and ethnopharmacological potentials suggesting its nutraceutical and biomedical benefits[14&15]. Previous studies have shown that *P. oleracea* is rich sources of essential fatty acids, tocopherols, ascorbic acid, glutathione, and β-carotene[16,17] Several biomedical

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applications of *P. oleracea* have been found such as antioxidant, anti-diabetic, antiobesity, anti-inflammatory, and anticancer activities[18&19]. Therefore, this research sought to evaluate the potentiality of the *P. oleracea* leaves extract (POLE) against the hematological and the hepatorenal toxicities induced by CTX in mice.

## MATERIALS AND METHODS

### Chemicals

Cyclophosphamide (CTX) was purchased from local pharmacy in Tanta city, Egypt. Gallic acid, quercetin, aluminum chloride, saponin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), phosphomolybdenum were purchased from Sigma (St. Louis, Mo., USA). Kits for the determination of biochemical parameters were purchased from Bio-diagnostic Company, Egypt.

### Preparation of *P. oleracea* leaves extract

*Portulaca oleracea* leaves were purchased from local market in Tanta city, Egypt. The plant materials were identified and authenticated by taxonomist at Botany department, Faculty of Science, Tanta University. The leaves were washed, cut into very fine pieces, and dried. Then, ground in a mortar, and 50 g of the powder was mixed with 500 ml of 70% ethanol. Then, filtered, and left to get the hydro-alcohol POLE for further use.

### Phytochemicals analysis of POLE

Total phenolic, flavonoids, saponin, DPPH activity, and TAC were determined either by quantitative analysis in POLE. Total phenolic content was determined according to Miliauskas *et al.*[20]. Total flavonoids content was determined according to Zhishen *et al.*[21]. Saponin content was assessed due to Hiai *et al.*[22]. DPPH activity and total antioxidant activity were determined according to Prior *et al.*[23].

### Mice and experimental design

From the National Research Center, 36 male albino mice (20 ± 2 g) were purchased (NRC, Cairo, Egypt). Mice were housed with 12 h/12 h dark/light cycle, in a laboratory environment with controlled humidity and temperature. Animals were treated according to guidelines for experimental animal's uses in research which was approved by Ethical committee at the Faculty of Science, Tanta University, with ethical approval license (IACUC-SCI-TU-0241). Mice were split into three groups (n=12). The 1st group (Gp1) was used as a negative control that i.p injected with saline. Gp2 had injected with CTX (200 mg/kg b.wt) i.p[24]. Gp3 had injected with CTX as in Gp2, followed by treatment with POLE (200 mg/kg b.wt)[25] every day for 28 days[26]. All mice were sacrificed, blood, sera, and tissues were collected for investigations.

### Determination of the total leucocytes count and the biochemical parameters

The electronic blood counter was used to determine leucocytes. Sera aspartate aminotransferase (AST) (AS106145), alanine aminotransferase (ALT) (AL103145), urea (UR2110), creatinine (CR1250), superoxide dismutase (SOD) (SD2521), catalase (CAT) (CA2517), and malondialdehyde (MDA) (MD2529) were determined according to the manufacturer's protocols.

### Investigation of hepato-renal histopathological changes

Tissue samples of liver and kidney were fixed, cut from paraffin blocks, stained with hematoxylin and eosin; the histopathological changes were investigated under light microscope (Optika light microscope (B-350)[27].

### Statistical analysis

All data are presented as mean ± SD. The significant differences between the groups were ascertained using one-way analysis of variance. The criterion for statistical significance was set at $p \leq 0.05$.

## RESULTS AND DISCUSSION

### Results

### Phytochemical quantitation of POLE

The results showed that the total phenolic and flavonoids contents in the POLE were 1368 and 202 µg/ml, respectively. Saponin content and the total antioxidant capacity (TAC) were 12500 µg/g and 193 µg/ml, respectively. DPPH radical scavenging activity

was 65% and its $IC_{50}$ value was 9.32 mg/ml **(Table 1).**

**Treatment with POLE after CTX injection improved the alterations in WBCs induced by CTX injection**

Cyclophosphamide treatment induced a significant increase ($p < 0.05$) in the total WBCs count after 28 days in comparing with the control group. Treatment with POLE after CTX injection restored the total WBCs count close to their normal values in the control group. CTX injection caused a marked increase ($p < 0.05$) in the neutrophils percentages after 28 days in comparing with the control values. Treatment with POLE after CTX injection significantly restored the neutrophils percentages **(Table 2).**

**Treatment with POLE post CTX injection ameliorated the hepato-renal toxicities induced by CTX**

The result showed that CTX injection resulted in a significant increase the serum ALT and AST activities compared to the values of the control group. POLE treatment after CTX injection led to a decreased in ALT and AST activities when compared to CTX-treated group **(Fig. 1A and B).** CTX led to significant increase in urea and creatinine levels, however, POLE treatment after CTX injection led to significant decline in the levels of these parameters **(Fig. 1C and D)**.

**Treatment with POLE ameliorated CTX-induced oxidative stress in mice**

CTX treatment led to significant decline in the hepatic SOD and CAT activities accompanied by a significant increase in MDA levels in sera, when compared to their values in the normal control group. Treatment with POLE after CTX injection resulted in a significant increase in the SOD and CAT activities, with a significant decrease in the MDA level **(Fig. 2).**

**Table 1:** Quantitative phytochemical analysis of *Portulaca oleracea* leaves extract.

| **Phytochemical analysis** | ***P. oleracea* leaves extract (POLE)** |
|---|---|
| Total phenolic (µg/ml) | 1368 |
| Total flavonoids (µg/ml) | 202 |
| Saponin (µg/g) | 12500 |
| TAC (µg/ml) | 193 |
| DPPH scavenging% | 65 % |
| $IC_{50}$ of DPPH (mg/ml) | 9.32 |

**Table 2:** Total WBCs count, and their differential count in different groups after 28 days of CTX injection.

| **Groups** | **WBCs ($\times 10^3$/ul)** | **Differential count** | | |
|---|---|---|---|---|
| | | **Neut. (%)** | **Lymph. (%)** | **Mono. (%)** |
| **Control** | 7.9 ± 1.13 | 33.01 ± 2.5 | 72.65 ± 5.24 | 2.67 ± 0.68 |
| **CTX alone** | 4.6 ± 0.95 | 40.05 ± 5.13* | 50.06 ± 4.14* | 8.67 ± 1.22 |
| **CTX/POLE** | 6.5 ± 0.85 | 19.75 ± 1.15* | 69.37 ± 5.82 | 7.97 ± 2.13 |

The values represented means ± S.D.; CTX: Cyclophosphamide, POLE: *P. oleracea* extract, WBCs: White blood cells *$p$- < 0.05, significantly different from CTX group.

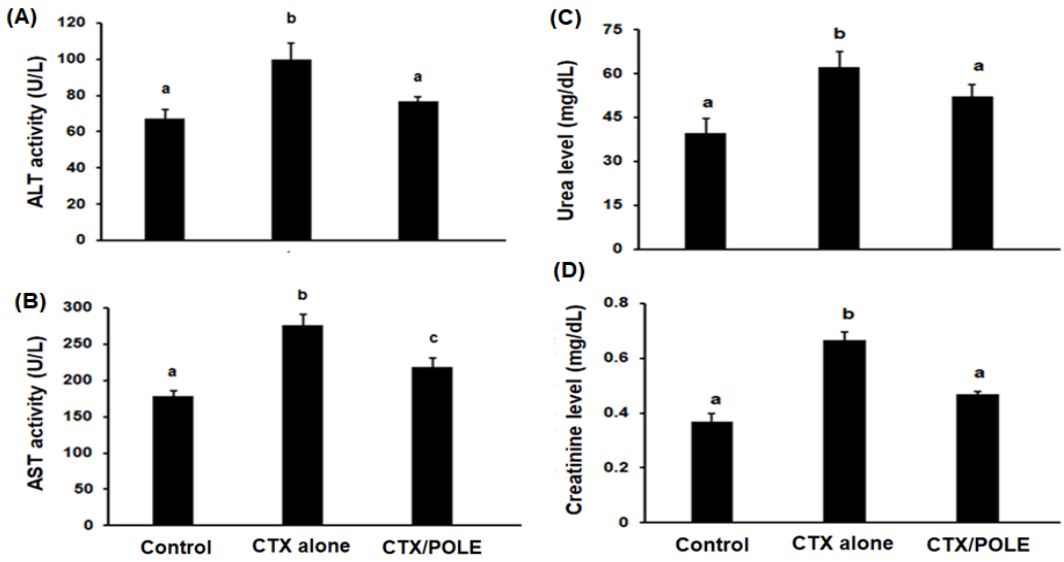


**Fig. 1:** Sera ALT **(A)**, AST **(B)** activities, urea **(C)**, and creatinine level **(D)** post CTX and POLE treatment. CTX: Cyclophosphamide, POLE: *P. oleracea* extract. All data were represented as mean ± S.D. Means that do not share a letter are significantly different. *P* value < 0.05 was considered to be statistically significant.

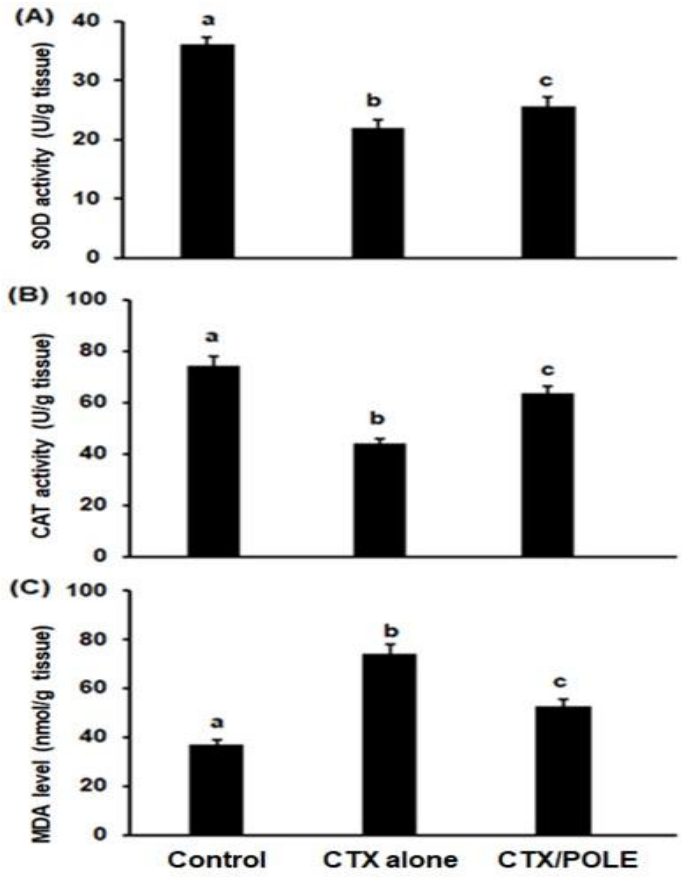


**Fig. 2:** Hepatic antioxidants/oxidants biomarkers post CTX and POLE treatment. SOD activity (A), CAT activity (B), and MDA levels (C). CTX: Cyclophosphamide, POLE: *P. oleracea* extract. All data were represented as mean ± S.D. Means that do not share a letter are significantly different. *P* value < 0.05 was considered to be statistically significant.

**Administration of POLE improve CTX-induced histopathological changes in mice**

Liver sections from control group that stained with H & E displayed normal architecture with normal polygonal hepatocytes radiating from the central vein **(Fig. 3A)**. Liver sections from CTX-treated group showed remarkable degenerative changes including abnormal vacuolated hepatocytes, lymphocytic infiltration, and degenerated blood sinusoid and pyknotic cells. Also, a considerable number of hepatocytes were binucleate **(Fig. 3B)**. In CTX/PLOE-treated group, the liver sections revealed remarkable restoring in their damaged histological structures induced by CTX despite presence of some vacuolated and pyknotic cells **(Fig. 3C).**

The kidney sections of control group showed the normal histological features of renal corpuscles in the form of normal well-organized glomeruli, where Bowman's space is surrounded by regular simple squamous epithelium of Bowman's capsule. Proximal convoluted tubules lined with brush bordered cubical epithelial whereas distal convoluted tubules lined with cubical epithelium with little microvilli **(Fig. 4A)**. In CTX-treated group, the kidney sections showed severe deleterious histological alterations in their architectures. Such alterations included an irregular Bowman's capsule, vacuolization and swelling of the endothelial cells lining the tufts of the glomeruli, tubular epithelium of both proximal and distal convoluted tubules showed clusters of hypertrophied cells with vacuolated cytoplasm. There were also some degenerated tubules with small darkly stained pyknotic cells **(Fig. 4B)**. In CTX/PLOE-treated group, the kidney sections showed an obvious amelioration in their architectures in spite of vacuolated tubular epithelium still found **(Fig. 4C)**.

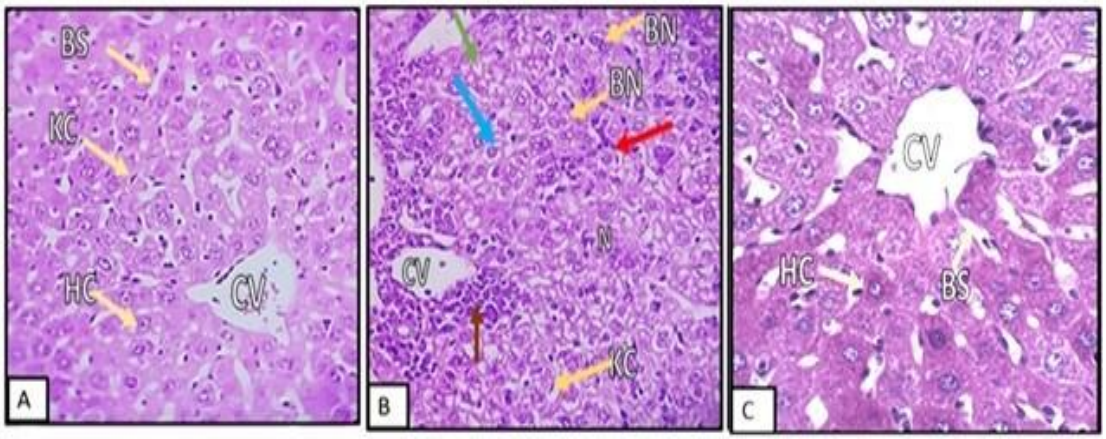


**Fig. 3:** Photomicrograph of liver sections. **(A):** Control group showed normal hepatic lobule with normal polygonal hepatocytes (HC) radiating from the central vein (CV), blood sinusoids (BS) lined by endothelial cells and distinct phagocytic Kupffer cells (KC), **(B):** CTX–treated mice showed necrotic cells (N), vacuolated hepatocytes (Blue arrow), lymphocytic infiltration (brown arrow), degenerated blood sinusoid (Green arrow), pyknotic cells (Red arrow) and binucleated hepatocytes (BN), **(C):** CTX/ POLE-treated mice showed remarkable restoring in their damaged histological structures induced by CTX despite presence of some vacuolated and pyknotic cells.

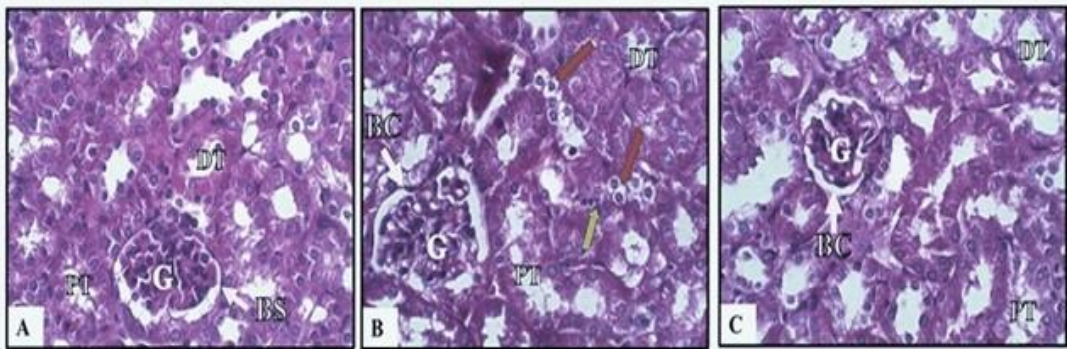


**Fig. 4:** Photomicrograph of kidney sections. **(A):** Control group showed normal histological features of renal corpuscles in the form of normal well-organized glomeruli (G), where Bowman's space is surrounded by regular simple squamous epithelium of Bowman's capsule (BC). Proximal convoluted tubules (PT) lined with brush bordered cubical epithelial whereas distal convoluted tubules (DT) lined with cubical epithelium with little microvilli, **(B):** CTX-treated mice showed an irregular Bowman's capsule (BC), vacuolization and swelling of the endothelial cells lining the tufts of the glomeruli, tubular epithelium of both proximal and distal convoluted tubules showed clusters of hypertrophied cells with vacuolated cytoplasm (red arrow). There were also some degenerated tubules with small darkly stained pyknotic cells (yellow arrow) and **(C):** CTX/ POLE-treated mice showed an obvious amelioration in their architectures in spite of vacuolated tubular epithelium still found.

**Discussion**

In herbal therapy, a number of plants are frequently utilized for the treatment and amelioration of various ailments. An earlier investigation found that POE had various pharmaceutical effects[28]. A well-known chemotherapy drug called CTX is used to treat many cancers; however, it can have negative consequences on important organs[29]. The current study assessed the potency of *P. oleracea* leaves extract (POLE) to mitigate the hepato-renal dysfunctions induced by CTX in mice. The phytochemical analysis showed that POLE has phenolics and flavonoids contents. The secondary metabolites may have pharmacological and biochemical actions as well as antioxidant potential.[30].

The results showed that the total WBCs count in the CTX-treated groups has increased after 28 days when compared to their count in the normal control. This increase could be due to the impact of the CTX on the hematopoietic system. Previous study by Salem *et al.* [5] reported that these significant increases in the WBCs count be due to the synthesis of WBCs by bone marrow progenitors. The restoration of the total WBCs count by the treatment with POLE after CTX-injection could be due to its phytochemical composition, which decreased the toxic effects of CTX, which in turn restore the WBCs count close to their normal levels. CTX injection caused a marked decrease in the % of neutrophils in comparing with their control group. Treatment with POLE post CTX injection increased the % of neutrophils.

Injury or damage of normal tissues is the major limitation of using CTX as potential chemotherapeutic drug. CTX-injected mice showed significantly increase in hepatic ALT and AST activites in comparing with normal control group. Treatment with POLE after 28 days of CTX injection attenuated the toxic effect of CTX on the liver transaminases activity. This result goes parallel with previous studies reported the CTX-induced liver toxicity in mice and how the natural products were attenuated these toxicities[31&32]. Furthermore, urea and creatinine levels were increased in mice injected with CTX. These finding go in agreement with the previously published study by Rehman *et al.*[33] who reported that elevation in the serum ALT and AST activities might be due to the leakage of these enzymes into the bloodstream brought on by CTX-induced kidney injury and increased membrane permeability.

Treatment with POLE for 28 days after CTX injection led to significant decline in the levels of urea and creatinine. This result was in the line with previous studies showed that the

treatment with natural products attenuated the renal injury induced by CTX[32&33]. The increased therapeutic dose of CTX caused cellular and tissue toxicity. Herbal medicine reduced CTX-induced oxidative stress and toxicities to vital organs. Treatment with POLE post CTX ameliorated its oxidative stress in mice by increasing in the SOD and CAT and decreasing the level of MDA. These finding hypothesized that the POE could have bioactive compounds that may help to protect normal tissues from CTX-induced ROS[32].

The histopathological investigations showed that CTX causes damage to the liver tissues. These pathological changes are consistent with previous studies reported that CTX caused liver injury[7]. The disordered microstructural changes induced by CTX were improved after treatment with POLE. *P. oleracea* was found to have hepatoprotective effects against a variety of toxins causing hepatotoxicity as Carbon tetrachloride, acetaminophen and Cd[34&35].

Cyclophosphamide caused severe histological alterations of the kidney. The degenerative changes observed in renal tissue following CTX administration could be attributed to oxidative stress caused by the loss of anti-oxidant defenses. Here, treatment with both CTX/POLE resulted in a significant improvement in the histological structure of the kidney. This finding comes in accordance with Seif *et al*.[35] who claimed that *P. oleracea* has nephroprotective qualities and may be helpful in the alleviation of acute renal injury.

**Conclusion**

The present study demonstrates that the POLE has a significant antioxidant activity and was able to ameliorate the CTX-induced hepatorenal toxicities evidenced by the improvement of the hematological, the biochemical, and hepatorenal alterations induced by CTX.

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نشرة العلوم الصيدلية
جامعة أسيوط

# يخفف مستخلص أوراق بورتولاسا اوليراسي ( الرجلة) من سمية السيكلوفوسفاميد في الفئران

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[٣] شعبة الكيمياء الحيوية ، قسم الكيمياء ، كلية العلوم ، جامعة طنطا ، مصر

[٤] قسم علم الحيوان ، كلية العلوم ، جامعة دمنهور ، مصر

سيكلوفوسفاميد هو عامل معروف مضاد للسرطان واسع المفعول. ومع ذلك ، فإنه له آثار جانبية شديدة على الأعضاء الحيوية. أظهر بورتولاسا اوليراسي ( الرجلة) الذي ينتمي إلى عائلة الرجلية خصائص غذائية وطبية حيوية واعدة. يهدف هذا البحث إلى تقدير تأثير مستخلص أوراق الرجلة على سمية سيكلوفوسفاميد في ذكور الفئران. تم تحديد إجمالي محتويات الفينول والفلافونويد والقدرة الكلية المضادة للأكسدة ونشاط مضاد الاكسدة (DPPH) ٪ (في مستخلص أوراق الرجلة عن طريق التحليل الكمي الكيميائي النباتي. تم تقسيم ستة وثلاثين فأر ذكر إلى ثلاث مجموعات (ن = ١٢) ، كعنصر تحكم سلبي ، سيكلوفوسفاميد (٢٠٠ مجم / كجم) محقون ، سيكلوفوسفاميد مستخلص أوراق الرجلة ٢٠٠ ) مجم / كجم) مجموعات محقونة. تم جمع عينات الدم وأنسجة الكبد وأنسجة الكلى لإجراء فحوصات الدم والكيمياء الحيوية والتشريح المرضي. بلغ إجمالي محتوى الفينول ، الفلافونويد ، الصابونين ، DPPH٪ ، والقدرة الكلية لمضادات الأكسدة ١٣٦٨ ميكروجرام / مل ، ٣٠٢ ميكروجرام / مل ، ٠.١٢٥٠ ميكروجرام / جرام ، ٦٥٪ ، ١٩٣ ميكروجرام / مل على الترتيب. أدى العلاج باستخدام مستخلص أوراق الرجلة إلى تحسين التغيرات الدموية والكيميائية الحيوية والتغيرات النسيجية التي يسببها سيكلوفوسفاميد للأنسجة الكبدية والكلوية. وبالتالي قلل مستخلص أوراق الرجلة من السمية الكبدية الكلوية التي تسببها سيكلوفوسفاميد في الفئران عن طريق التحسين بمضادات الأكسدة / المؤكسدات.