Achillea millefolium inflorescence aqueous extract ameliorates cyclophosphamide-induced toxicity in rat testis: stereological evidences

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[ABSTRACT] Cyclophosphamide (CP) is extensively used for the treatment of various cancers, as well as an immunosuppressive agent. However, CP is known to cause several adverse effects including reproductive toxicity. Achillea millefolium, a widely distributed medicinal plant, is highly regarded for its medicinal activities, including antioxidant and anti-inflammatory properties. The present study was conducted to assess whether Achillea millefolium inflorescences aqueous extract with antioxidant and anti-inflammatory activities could serve as a protective agent against reproductive toxicity during CP treatment. Male Wistar rats were categorized into four groups. Two groups of rats were administered CP at a dose of 5 mg·kg⁻¹·d⁻¹ for 28 d by oral gavages. One of these groups received Achillea aqueous extract at a dose of 1.2 g·kg⁻¹·d⁻¹ orally 4 h after cyclophosphamide administration. A vehicle treated control group and an Achillea control group were also included. The CP-treated group showed significant decreases in the body, testes and epididymides weights as well as many histological alterations. Stereological parameters, spermatogenic activities and testicular antioxidant capacity along with epididymal sperm count and serum testosterone concentration were also significantly decreased by CP treatment. Notably, Achillea co-administration caused a partial recovery in above-mentioned parameters. These findings indicate that Achillea millefolium inflorescence aqueous extract may be partially protective against CP-induced testicular toxicity.

[KEY WORDS] Achillea millefolium; Cyclophosphamide; Toxicity; Tests


1 Introduction

Cyclophosphamide (CP), is a widely used cytotoxic alkylating agent with antitumor and immunosuppressant properties. It is used for the treatment of chronic and acute leukemia, multiple myeloma, lymphomas, rheumatic arthritis and systemic lupus erythematosus and in the preparation for bone marrow transplantation[1]. Cyclophosphamide undergoes bio-activation by the hepatic microsomal cytochrome P450 mixed function oxidase system to active metabolites that enter the circulatory system. Phosphoramide mustard and acrolein are the two active metabolites of cyclophosphamide[2]. The antineoplastic effects of cyclophosphamide are associated with phosphoramide mustard, whereas acrolein is linked to toxic side-effects like cell death, apoptosis, oncrosis and necrosis[3]. In spite of its therapeutic importance, a wide range of adverse effects including reproductive toxicity has been demonstrated following cyclophosphamide treatment in humans and experimental animals[4]. Adult male patients treated with CP have demonstrated diminished sperm counts and an absence of spermatogenic cycles in their testicular tissue[5]. Previous studies on male rats have confirmed the potential of CP to cause oligospermia, azoospermia and histological alterations in the testis and epididymis[6-7]. Decrease in weight of reproductive organ, impaired fertility, growth and development of next generation was also observed in cyclophosphamide treated male rats[8]. Although the precise mechanism by which CP causes testicular toxicity is poorly understood, numerous studies have shown that CP exposure can disrupt the redox balance of tissues leading to oxidative stress[9-11]. It has been reported that oxidative DNA damage is caused by hydroperoxide derivative of CP through generation of H₂O₂[12]. Further, spermatozoa are more susceptible to peroxidative damage because of high concentra-
tion of polyunsaturated fatty acids and low antioxidant capacity\[13\]. Also, acrolein has been found to interfere with the tissue antioxidant defense system and produces highly reactive oxygen free-radicals that are mutagenic to mammalian cells\[14\]. Consequently, from these aforementioned studies, combination of the drug delivery together with potent and safe antioxidant may be the appropriate approach to reduce CP-induced reproductive toxicity.

*Achillea millefolium*, popularly known as “yarrow”, is a member of the Asteraceae family that has been used as medicine by many cultures for over 3 000 years\[15\]. The medicinal properties of *Achillea millefolium* are recognized worldwide and the plant is included in the national Pharmacopoeias of countries such as Germany, Czech Republic, France and Switzerland\[15-18\]. Different preparations of *Achillea millefolium* have been shown to have anti-inflammatory, antitumor, antimicrobial, liver protective and antioxidant properties\[19-24\]. In addition, previous studies have reported that infusions prepared from *Achillea* species had an antioxidant capacity, which is consistent with their total flavonoid and phenol contents. It was found that *Achillea* infusions are good scavengers of active oxygen species, including OH• radical, H2O2 and DPPH•\[25\]. These findings supported preliminary studies which had demonstrated that powerful antioxidant properties of this plant are associated with the presence of flavonoids such as apigenin, luteolin and rutin\[26-27\]. Based on the above findings, the present study was undertaken to assess whether *Achillea millefolium* infuses/extracts with anti-oxidant and anti-inflammatory properties could serve as a protective agent against reproductive toxicity during CP treatment in a rat model.

2 Materials and Methods

2.1 Plant material

*Achillea millefolium* plants were harvested from its natural habitat around the city of Urmia in West Azerbaijan Province, northwestern Iran during the flowering season (between May and July). The identification of collected plants was confirmed scientifically at the research laboratories of the Department of Agriculture of West Azerbaijan province.

2.2 Preparation of the aqueous extract

The aqueous extract of dried inflorescences of the plant was prepared by infusion of the finely dried material (3 × 30 min) in water at 70 °C (1 : 10, W/V). The infusion was filtered and concentrated under vacuum (at 20 °C) to 1/12 of the original volume and stored at −20 °C. The concentrated extract was diluted in distilled water immediately before use\[28\].

2.3 Animal model

Adult sexually mature male [4 months of age weighing (177.75 ± 7.68) g] albino rats of Wistar strain were obtained from animal house of Veterinary School of Urmia University. They were housed in a specific pathogen-free environment under standard conditions of temperature ([25 ± 2) °C], relative humidity ([50 ± 10]%) and light (12 h light/12 h dark). They were fed with a standard pellet diet and had free access to water. Body weights were recorded weekly during the treatment period. Clinical and behavioral observations were also recorded throughout the study. Animal work was conducted in compliance with guidelines for the humane care and use of laboratory animals using protocols approved by the university.

2.4 Experimental protocol

After 7 days of acclimation to the environment, the rats were randomly divided into four groups consisting of six animals each (n = 6). Group I served as control receiving saline vehicle throughout the experiment. Group II received CP (5 mg·kg−1·d−1) dissolved in saline, for a period of four weeks by gavage. Group III received *Achillea millefolium* aqueous extract (1.2 g·kg−1·d−1) dissolved in distilled water orally. Group IV was given orally *Achillea* solution (1.2 g·kg−1·d−1) 4 h after CP administration. The protocol for this study, including doses and duration of treatment for CP and *Achillea*, were all designed according to previous studies\[9, 28\].

2.5 Sampling

Animals were euthanized by CO2 exposure in a special device following anesthesia with Ketamine 24 h after the last *Achillea* treatment. Blood was collected without anticoagulant for serological analyses. Testes and epididymides were quickly dissected out, cleared of adhering connective tissue and weighed on a Mettler Basbal scale (Delta Range, Tokyo). Testes were fixed in Bouin’s fixative (0.2% picric acid/2% (V/V) formaldehyde in PBS) for histological evaluation.

2.6 Epididymal sperm count

In order to assess the sperm motility, one caudal epididymis was placed in 1 mL of Ham’s F10 medium. Cauda was cut into 2–3 pieces and incubated at 37 °C for 10 min in CO2 incubator to allow sperm to swim out of the epididymal tubules. The epididymal sperm count was determined by hemocytometer. After dilution of epididymal sperm to 1 : 20 in Ham’s medium, approximately 10 μL of this diluted specimen was transferred to each of the counting chambers of the hemocytometer, which was allowed to stand for 5 min in a humid chamber to prevent drying. The cells sediment during this time and were counted with a light microscope at 400 ×. The sperm count was expressed as number of sperm per milliliter\[29\].

2.7 Determination of serum testosterone concentration

Serum concentration of testosterone was measured by enzyme-linked immunoabsorbent assay (ELISA) as described in the instructions provided by manufacturer’s kit (Demeditec Diagnostics GmbH, Germany).

2.8 Assessment of testicular antioxidant capacity (TAOC)

To determine the effect of CP on oxidative stress and consequently the potential beneficial effects of *Achillea* aqueous extract, testicular antioxidant capacity was measured. The assay is based on the assessment of ferric reduction an-
tioxidant power (FRAP) assay. Briefly, at low pH which was achieved by adding of acetate buffer (300 mmol·L⁻¹, pH 3.6), reduction of 

Fe³⁺-TPTZ complex to the ferrous form produces an intensive blue color that could be measured at 593 nm. Aqueous solution of 

Fe⁢(SO₄)₃·7H₂O and appropriate concentrations of freshly prepared ascorbic acid were used as blank and standard solutions, respectively. The TAOC was expressed as mmol·L⁻¹ per mg protein of the samples.

The protein content of the samples was measured according to the Lowry method.

2.9 Histological analysis

After fixation of testes, they were directly dehydrated in a graded series of ethanol, cleared in xylol and embedded in paraffin wax. Thin sections (6 μm) perpendicular to the longest axis of the testis were cut using a microtome, stained with hematoxylin and eosin and examined using a light microscope.

2.10 Determination of histological parameters

For each testis, five vertical sections from the polar and the equatorial regions were sampled and an unbiased numerical estimation of the following histological parameters was determined using a systematic random scheme.

Seminiferous tubules Diameter (STsD) and germinal epithelium height (GEH): For measuring of seminiferous tubule and their means were calculated. Also, germinal epithelium height was measured in microscopy (Olympus Co., Germany) and their means were calculated. Also, germinal epithelium height was measured in 4 equidistance of each cross-section of seminiferous tubules and their means were calculated.

Number of profiles of seminiferous tubules in a unit area (NV): The number of profiles of seminiferous tubules per unit area was determined by using the unbiased counting frame proposed by Gundersen (1977). Using this frame, in addition to counting profiles completely inside the frame we counted all profiles with any part inside the frame provided they do not touch or intersect the forbidden line (full-drawn line) or exclusion edges or their extension.

Numerical density of seminiferous tubules (NV): This is the number of profiles per unit volume and was determined by using the modified Floderus equation: 

\[ NV = N_t / (D + T) \]

Where; \( N_t \) is the number of profiles per unit area, \( D \) is the diameter and \( T \) the average thickness of the section.

Sertoli cell index (SCI), repopulation index (RI) and mitotic index (MI): Sixty seminiferous tubules per group were randomly examined for the calculation of Sertoli cell index (SCI), repopulation index (RI) and mitotic index (MI). SCI is the ratio of the number of germ cells to the number of Sertoli cells identified by a characteristic nucleus and nucleolus in all seminiferous tubules. RI is the percentage of tubules populated with germ cells that had clearly reached the intermediate spermatogonial stage or later. MI, the number of round spermatids for each pachytyne primary spermatocytes, was calculated for determination of cell loss percentage during cell division.

Leydig cell nuclear diameter (LCND) and Sertoli cell nuclear diameter (SCND): These parameters were also determined using calibrated ocular micrometer as described by Elias and Hyde.

2.8 Statistical analysis

Results are expressed as \( \bar{x} \± s \). Differences between groups were assessed by the analysis of variance (ANOVA) using the SPSS software package for Windows. Statistical significance between groups was determined by Tukey multiple comparison post hoc test and the P-values less than 0.05 were considered to be statistically significant. In addition, between and within groups’ degree of freedom were 3 and 20, respectively.

3 Results

3.1 Clinical signs and body and organ weight changes

All animals survived the experimental period. CP-treated animals showed general signs of deterioration such as piloerection, hair loss, lethargy, hunched posture, shivers and low activity. Testes and epididymides were significantly decreased by cyclophosphamide treatment, while it was less decreased from controls with Achillea coadministration (Fig. 1). The absolute and relative weights of testes and epididymides were significantly lower than controls after cyclophosphamide treatment, whereas daily administration of Achillea caused significant increase in the absolute and relative weights of testes and epididymides of cyclophosphamide-Achillea group in comparison with cyclophosphamide group (Table 1).

3.2 Epididymal sperm count

Treatment of male rats with CP caused a significant de-

![Fig. 1 Gross appearance of testes from all groups of rats. Illustrations of representative testes in Control, CP, Achillea and CP + Achillea groups, respectively. CP group rats have obviously smaller testes as compared to the other three groups.](image-url)
Table 1  Effect of cyclophosphamide and Achillea millefolium inflorescences aqueous extract on body weight and weights of testis and epididymis ( x ± s, n = 6)

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CP</th>
<th>Achillea</th>
<th>CP + Achillea</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final body weight (BW/g)</td>
<td>226.33 ± 5.35</td>
<td>182.00 ± 6.06 a</td>
<td>226.16 ± 9.26 b</td>
<td>194.83 ± 10.72 a</td>
<td>45.491</td>
</tr>
<tr>
<td>Absolute weight (g)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>2.01 ± 0.065</td>
<td>1.49 ± 0.040 a</td>
<td>2.04 ± 0.084 b</td>
<td>1.69 ± 0.099 ab</td>
<td>72.695</td>
</tr>
<tr>
<td>Epididymides</td>
<td>1.15 ± 0.044</td>
<td>0.85 ± 0.010 b</td>
<td>1.16 ± 0.039 b</td>
<td>1.00 ± 0.081 ab</td>
<td>51.359</td>
</tr>
<tr>
<td>Relative weight (per BW/%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testes</td>
<td>0.88 ± 0.011</td>
<td>0.82 ± 0.004 a</td>
<td>0.90 ± 0.007 b</td>
<td>0.86 ± 0.008 ab</td>
<td>5.578</td>
</tr>
<tr>
<td>Epididymides</td>
<td>0.50 ± 0.019</td>
<td>0.46 ± 0.023 a</td>
<td>0.51 ± 0.017 b</td>
<td>0.51 ± 0.031 b</td>
<td>104.470</td>
</tr>
</tbody>
</table>

a P < 0.05 vs control group, b P < 0.05 vs cyclophosphamide group

crease in the sperm concentration, while coadministration of Achillea millefolium inflorescences aqueous extract caused a significant increase in epididymal sperm quantity and minimized the toxic effects of CP (Fig. 2).

Fig. 2  Effect of cyclophosphamide and Achillea millefolium inflorescences aqueous extract on epididymal sperm count. ( x ± s, n = 6), a P < 0.05 vs control group, b P < 0.05 vs cyclophosphamide group. F-value = 94.114

3.3 Serum testosterone level
Administration of CP alone significantly decreased serum level of testosterone as compared to control rats. The administration of aqueous extract Achillea millefolium inflorescences along with CP significantly restored serum testosterone level towards the control value (Fig. 3).

3.4 Testicular antioxidant capacity
Interestingly, treatment with Achillea millefolium inflorescences aqueous extract alone significantly increased antioxidant capacity in the testis of normal rats. CP administration caused a significant decrease in TAOC level when compared to that of control, while concurrent treatment with Achillea aqueous extract significantly suppressed this reduction (Fig. 4).

3.5 Histopathological findings
CP induced drastic morphologic changes in the testis (Fig. 5B). Atrophied seminiferous tubules showed severe hypocellularity (reduction in number of germ cells) and intraepithelial vacuolization. Rupture, vacuolization, vascular congestion, inflammatory cells infiltration, oedematous fluid accumulation and interstitial space widening were also observed in intertubular connective tissue. In these specimens, Leydig cells were degenerated and appeared with pyknotic nuclei. Moreover, Sertoli cells lost their junction with germ cells and looked amorphous with irregular and smaller nuclei.

Fig. 3  Effect of cyclophosphamide and Achillea millefolium inflorescences aqueous extract on serum concentrations of testosterone. ( x ± s, n = 6), a P < 0.05 vs control group, b P < 0.05 vs cyclophosphamide group. F-value = 127.198

Fig. 4  Effect of cyclophosphamide and Achillea millefolium inflorescence aqueous extract on testicular antioxidant capacity (TAOC). ( x ± s, n = 6), a P < 0.05 vs control group, b P < 0.05 vs cyclophosphamide group. F-value = 25.558
Fig. 5 Photomicrographs of testicular sections of control (A), Cyclophosphamide (B), Achillea (C) and Cyclophosphamide + Achillea (D) treated rats. Testes from control (A) and Achillea-treated (C) rats exhibit a normal feature of seminiferous epithelium and interstitial tissue with active spermatogenesis. However, a testis from a Cyclophosphamide treated rats (B) reveals markedly atrophied seminiferous tubules with severe hypocellularity and impaired spermatogenesis. Note Rupture, vacuolization, vascular congestion (black arrow), oedematous fluid accumulation (white arrows) and interstitial space widening in intertubular connective tissue. Achillea cotreated animals (D) display nearly normal architecture. Hematoxylin and eosin (× 200).

Administration of Achillea along with CP restored these changes towards normalcy (Fig. 5D).

3.6 Histological parameters

As seen in Table 2, Cyclophosphamide treatment induced deletion of germ cells during spermatogenesis, which resulted in a dramatic decrease in SCI. Due to the germ cells deletion, the number of repopulated seminiferous tubules was greatly decreased in the CP-treated animals. CP treatment also caused considerable decrease in miotic index. However, Achillea coadministration significantly attenuated the CP-induced germ cell loss from seminiferous tubules.

The seminiferous tubules diameters (STsD) and their epithelial heights (GEH), Cross-sectional area of the seminiferous tubules (A_c), Number of profiles of seminiferous tubules in a unit area of testis (N_t) and Numerical Density of seminiferous tubules (N_v) as well as Leydig cell nuclear diameter (LCND) and Sertoli cell nuclear diameter (SCND) were reduced by CP treatment. CP-induced testicular damages were mitigated by Achillea coadministration (Table 3).

4 Discussion

Many drugs used for cancer chemotherapy are known to

| Table 2 | Effect of cyclophosphamide and Achillea millefolium inflorescences aqueous extract on Sertoli cell index, repopulation index and miotic index (x ± s, n = 6) |
|-------------------|-----------------|---------------------|-----------------|-----------------|
|                   | Control         | CP                 | Achillea        | CP + Achillea   |
| Sertoli cell index| 25.22 ± 0.85    | 4.50 ± 0.17        | 25.62 ± 0.76    | 19.36 ± 0.56    |
| Repopulation index| 95.41 ± 2.03    | 19.58 ± 2.08       | 92.41 ± 2.33    | 76.41 ± 2.51    |
| Miotic index      | 2.11 ± 0.027    | 0.98 ± 0.013       | 2.18 ± 0.018    | 1.63 ± 0.013    |

*P <0.05 vs control group, †P <0.05 vs cyclophosphamide group
produce toxic side-effects in multiple organ systems including the testes. In a clinical context, testicular stem cell damage in patients exposed to chemotherapeutic drugs for a limited duration could result in long-term infertility or genetic alterations\(^{[41]}\). A strategy to diminish the side-effects of anticancer drugs with preservation of their chemotherapeutic efficacy is necessary. Effective anticancer and immunosuppressive therapy with CP is severely limited by testicular toxicity as documented in a variety of species\(^{[4]}\). An oxidant mechanism may be involved in the reproductive toxicity, wherein CP and its metabolite acrolein cause inactivation of microsomal enzymes and result in increased reactive oxygen species generation and lipid peroxidation\(^{[42]}\). In the present study, reduction in body weight, weight of the testis and epididymis and histological changes in testis were indicative of drug toxicity. Because the weight of the testis largely depends on the mass of the differentiated spermatogenic cells\(^{[43]}\), the marked reduction in organ weight by CP can be explained by diminished number of germ cells, atrophy of Leydig cells and a significant lower rate of spermatogenesis as confirmed by our findings. Reduction in the weight of testes and epididymis in CP-treated animals reflects the reduced availability of androgens\(^{[44]}\). Increased generation of free radicals is one of the possible mechanisms involved in CP-induced Leydig cell degeneration resulted in marked reduction of serum testosterone\(^{[45]}\). Chemotherapy can result in long-term or permanent azoospermia, the mechanism of which is most likely the death of germ cells\(^{[46]}\) and stereological parameters such as seminiferous tubules diameters and their epithelial heights, cross-sectional area of the seminiferous tubules, number of profiles of seminiferous tubules in a unit area of testis and numerical density of seminiferous tubules; STsD, Cross-sectional area of the seminiferous tubules; GEH, germinal epithelium height; AC, Number of profiles of seminiferous tubules; NV, Number of profiles of seminiferous tubules; LCND, Leydig cell nuclear diameter; SCND, Sertoli cell nuclear diameter. *P < 0.05 vs control group, \(P < 0.05\) vs cyclophosphamide group.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CP</th>
<th>Achillea</th>
<th>CP + Achillea</th>
<th>F-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>STsD (µm)</td>
<td>256.79 ± 4.94</td>
<td>97.23 ± 4.99(^a)</td>
<td>256.21 ± 9.53(^a)</td>
<td>193.05 ± 5.83(^{[43]})</td>
<td>780.530</td>
</tr>
<tr>
<td>GEH (µm)</td>
<td>97.90 ± 3.99</td>
<td>37.75 ± 3.14(^a)</td>
<td>99.74 ± 5.48(^a)</td>
<td>69.33 ± 3.49(^{[43]})</td>
<td>299.295</td>
</tr>
<tr>
<td>AC (×10(^3) µm(^2))</td>
<td>51.81 ± 2.00</td>
<td>7.44 ± 0.76(^a)</td>
<td>51.62 ± 3.82(^a)</td>
<td>29.29 ± 1.76(^{[43]})</td>
<td>483.736</td>
</tr>
<tr>
<td>Ns (×10(^4) µm(^2))</td>
<td>33.93 ± 2.96</td>
<td>9.73 ± 0.54(^a)</td>
<td>34.80 ± 2.55(^b)</td>
<td>22.60 ± 1.78(^{[43]})</td>
<td>176.287</td>
</tr>
<tr>
<td>Nv (×10(^8) µm(^2))</td>
<td>12.91 ± 1.16</td>
<td>9.43 ± 0.55(^a)</td>
<td>13.27 ± 0.94(^a)</td>
<td>11.35 ± 0.96(^{[43]})</td>
<td>21.064</td>
</tr>
<tr>
<td>LCND (µm)</td>
<td>6.32 ± 0.48</td>
<td>3.48 ± 0.27(^a)</td>
<td>6.36 ± 0.30(^b)</td>
<td>5.45 ± 0.44(^{[43]})</td>
<td>72.944</td>
</tr>
<tr>
<td>SCND (µm)</td>
<td>9.22 ± 0.75</td>
<td>6.44 ± 0.53(^a)</td>
<td>9.74 ± 0.40(^b)</td>
<td>8.92 ± 0.29(^{[43]})</td>
<td>46.327</td>
</tr>
</tbody>
</table>

Structural development and maturation of germ cells and spermatogenesis are important functions of Sertoli cells\(^{[48]}\). Therefore, a potential explanation for the failure of spermiogenesis in the CP-treated males is disruption of testosterone-dependent junction of Sertoli cells with germ cells leading to their disorganization and separation. In the present study, epididymal sperm count decreased by confirming a previous report that CP induces an epididymis specific effect on sperm count\(^{[49]}\). The decreased sperm count clearly shows the elimination of sperm cells at different stages of development and points to free radical attack through CP metabolism. In fact, oxidative damage to polyunsaturated fatty acids of cell membranes has long been considered to result in the impairment of membrane fluidity and permeability. This results in the damage of germ cells, spermatozoa and mature sperm\(^{[50]}\). It has also been reported that CP causes an increase in apoptosis at specific stages of germinal cycle\(^{[51]}\). Hence, the decrease in epididymal sperm count observed in CP-treated rats might reflect the spermatogenic cell death. There are several reports on the benefit of antioxidants in protecting male reproductive system from deleterious effects of reactive oxygen species and other free radicals generated during CP exposure. It was found that ascorbic acid reduces cyclophosphamide-induced reproductive toxicity\(^{[49]}\) as well as alpha-tocopherol-succinate\(^{[52]}\). There is also evidence that Yukmijihwang-tang as a multi-herbal medicinal formula can improve reproductive toxicity of CP through reduction of oxidative stress\(^{[53]}\). Two studies from the same researchers indicated that supplementation with lipoic acid as an antioxidant reduces CP-induced reproductive toxicity by the same mechanism\(^{[54-55]}\).

In the present study, it has been shown that *Achillea millefolium* inflorescences aqueous extract coadministration was effective in protection or attenuation of testicular damage following CP exposure. Increasing evidences support the fact that *Achillea* is beneficial where free radicals are known to play a predominant role in toxicity. Previous studies have shown *Achillea millefolium* protected rat stomach against
gastric ulcers induced by reactive oxygen species due to its antioxidant properties[39]. Furthermore, it has been revealed that Achillea infusions reduce H₂O₂-induced oxidative damage in human erythrocytes and leucocytes, which is consistent with their total flavonoid and phenol contents[37]. In conclusion, the finding of our study indicate that cyclophosphamide can adversely damage the testicular tissue through imposing oxidative stress, while Achillea millefolium inflorescences aqueous extract coadministration could effectively prevent these adverse effects by effective inhibition of oxidative processes and efficient scavenging of free radicals.

References

[6] Trasler JM, Hales BF, Robaire B. Chronic low dose cyclophosphamide can adversely damage the testicular tissue through imposing oxidative stress, while Achillea millefolium inflorescences aqueous extract coadministration could effectively prevent these adverse effects by effective inhibition of oxidative processes and efficient scavenging of free radicals.

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