Investigation of in-vitro Antioxidant and in-vivo Protective Effects of Hypericum triquetrifolium Seed Methanol Extracts Against Cyclophosphamide-Induced Acute Myelotoxicity, Hemotoxicity and Hepatotoxicity in Rats

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Abstract:
The aim of this study was to investigate in-vitro antioxidant properties and in-vivo protective effects of different concentrations of Hypericum triquetrifolium Turra. (HT) seed methanol extracts against acute hepatotoxicity, myelotoxicity and hematotoxicity in rats exposed to overdose of cyclophosphamide (CP). HT seed methanol extracts were tested in view of its in-vitro antioxidant activities as total phenolic contents and DPPH free radical-scavenging activity. To investigate in-vivo protective effects of HT seed methanol extracts on rat tissues; tested animals were divided into nine groups. Three groups only were treated with HT extracts (25, 50 and 100 mg/kg HT) for 6 days. Three groups were pre-treated with the extract of HT (25, 50 and 100 mg/kg HT) for 6 days and on the last day they were injected with...
single dose of CP (150-mg/kg body weight). Two groups were used as control groups and one
group was only treated with CP (150 mg/kg) on the 6th day. The toxic effects of CP and
protective effects of HT extracts on the nucleated cells which were produced by bone marrow
and serum alanine transaminase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase
(LDH), oxidative stress index (OSI) levels were investigated biochemically. Additionally,
liver tissue samples were examined histopathologically. Our results show that HT seed
methanol extract has high total phenolic content and antioxidant activity. Over dose CP
administration caused hepatotoxicity, myelotoxicity and hematotoxicity on rat. Whereas, 25,
50 and 100 mg/kg HT plus CP administered groups showed significant protective effects on
nucleated cells. And 25, 50, 100 mg/kg HT plus CP treated groups showed an important
decrease on serum ALT, ALP, LDH and OSI levels when compared with CP treated group.
Our results showed that the administration of different HT doses with high doses of CP
significantly reduced hepatotoxicity, myelotoxicity and hematotoxicity on rats.

Keywords: Cyclophosphamide, cytoprotective, hepatotoxicity, hematotoxicity, Hypericum
triquetrifolium Turra., myelotoxicity, rat.

1. Introduction

Chemotherapeutic drugs are generally toxic to healthy cells and multiple organs so they cause
critical side effects such as myelosuppression [1]. In spite of the wide spectrum of application,
the usage of Cyclophosphamide (CP) is generally limited due to its undesirable toxic side
effects, including hepatotoxicity [2,3] and myelosuppression/immunotoxicity which limit its
usefulness [2-4]. Moreover, CP causes a significant reduce on erythrocyte, leucocyte,
thrombocyte and bone marrow nucleated cells [5].

CP, an alkylating chemotherapeutic prodrug, is metabolized by liver cytochrome P450
enzymes namely, CYP3A4 and CYP2B6 demonstrate active therapeutic and cytotoxic metabolites [6] and diffuse out of the hepatocytes into the plasma and are distributed throughout the body and generates two active metabolites; phosphoramide mustard (PAM) and acrolein (ACR). While CP’s immunosuppressive and antineoplastic effects are related with PAM, ACR is responsible for its undesirable toxicity [7]. ACR produces highly reactive oxygen species (ROS) and oxidative stress in hepatocytes [8,9]. Therefore; it interacts with protein amino acids and causes changes in enzyme’s structure and function, and effects to the tissue antioxidant defense mechanism [10]. CP-depended toxicity is a consequence of a mitochondrial dysfunction and ends up with a decrease of adenosine triphosphate owing to nitrosative and oxidative stress [4].

CP is used in combination with different detoxifying and protective agents with the aim of decreasing or removing its adverse toxic effects [11]. Extracts of herbal meds are indicated with beneficial therapeutic impressions such as antioxidant, anticancer, antiinflammatory, antimicrobial effects [12]. The antioxidants may minimize the toxic side effects of chemotherapeutic agents and may supply the use of more effective high doses of the anticancer drugs [4].

The Hypericum species known as “Clusioid clade” are a member of the Hypericaceae family, belonging to the large clade of generally tropical plants [13]. The methanolic extract of Hypericum species are particularly rich in bioactive compounds, such as pseudohypericin, hypericin, naphthodianthrones, phloroglucinol derivatives, flavonoids, adhyperforin, hyperforin, tannins, essential oils, xanthenes, procyanidins, and some other water-soluble components which possess a broad order of biological features [14,15].

Hypericum triquetrifolium Turra. (HT) belongs to the Hypericaceae family and it has been used for its sedative, antiseptic, anti-inflammatory, antihelminthic and effects with traditional
methods [16]. Moreover, HT includes hyperoside, chlorogenic acid, quercetin, quercitrin, rutin, kaempferol, phenolic and flavonoid compounds which are important for antioxidant capacity [17,18] That's why, HT possesses significant antioxidative activities that might be beneficial in inhibiting or decreasing the progress of various oxidative stress-induced diseases. Even so, its effects against hepatotoxicity, myletotoxicity and hematotoxicity have not been unspecified in detail. For these reasons, we examined the protective effects of HT seed methanol extract on CP-related toxicity of the hepatic cells, bone marrow nucleated cells and peripheral blood cells of rats in the present study.

2. Materials and Methods

2.1. Drug and chemicals

CP (Endoxan, Cyclophosphamide Monohydrate, C0768) was acquired commercially from Sigma-Aldrich, Taufkirchen, Germany. CP (500 mg) was dissolved in 25 mL bidistilled water to its own appropriate concentration prior to the injection, sequentially, and was stocked at 4 °C before use. Single dose CP (150 mg/kg b.w.) was intraperitoneally administered rats.

2.2. Herbal material

The herbs were reaped at seeding stages in August and September, 2015 from where its wild population is located Zinar, Mardin, Turkey. Voucher specimens were conserved at the Herbarium of Mardin Artuklu University (2015-3-MAU). 20 g of seed were ground into powder. Seed powder was preserved in a glass flask with 200 mL (99%) methanol at room temperature. Extraction process was repeated 3 times under magnetic stirrer. Before the solvent was removed on rotary evaporator under vacuum it had been filtrated. Then about 4 g of the crude methanol extracts of HT seeds were supplied and saved in light-hindered glass vials at -20 °C prior to the experiments.
The test samples (crude methanol extract) were prepared by dissolving them in 0.2% dimethyl sulfoxide (20 ml in 10 ml saline (0.9% NaCl)) (DMSO) (Sigma-Aldrich, Germany) solution to obtain concentration of 25, 50 and 100 mg/kg in a flask. The flask was partially immersed into the ultrasonic bath for 3 minutes at 30 ºC to provide effective dissolving. 0.5 ml 0.2% DMSO and serum physiologic (SF) were used as positive controls.

2.3. Determination of Antioxidant Capacity

2.3.1. Total phenolic assay

The amount of total phenolic content in seed methanol extract was determined according to the Folin-Ciocalteu method [19]. Briefly, 0.2 mL of sample solution (2 mg/mL) was added into a test tube containing 1 mL of Folin–Ciocalteu reagent and 2 mL of Na2CO3 (7.5%). The final volume was brought up to 7 mL with deionized water. After 2 h of incubation at room temperature, the absorbance was measured at 765 nm with a spectrophotometer (perkin elmer lambda 25 UV/Vis). The results were expressed as gallic acid (standard) equivalents (GAE). The equation calculated as y = 0.002X-0.051(R² = 0.995) according to linear calibration curve.

2.3.2. Scavenging Activity on DPPH Radical

The free radical scavenging activities were quantitatively tested with using a 2.2-diphenyl-1-picrylhydrazyl (DPPH) based on Shimada et al., (1992) method [20]. Briefly, 0.1 mM solution of DPPH in ethanol was prepared. Then, 1 ml of this solution was added to 3 ml of each extract solution at different concentrations (25–500 μg). The mixture was shaken vigorously and allowed to stand at room temperature for 30 mins. Then the absorbance was measured at 517 nm with a spectrophotometer (Perkin Elmer lambda 25 UV/Vis). Lower absorbance of the reaction mixture was indicated as higher free radical scavenging activity. Butylated Hydroxyanisole (BHA) and Butylated hydroxytoluene (BHT) were used as the
standard antioxidant (positive controls) for free radical scavenging activity test. The radical scavenging activity was calculated with used below formula:

DPPH scavenging activity (%) = \((A_{517\,\text{of\ control}} - A_{517\,\text{of\ sample}}/A_{517\,\text{of\ control}})\times100\) [21].

2.4. Experimental design

Experimental materials (wistar albino rats) were provided commercially from Kobay Experimental Animals Lab. San. Tic. A.S.S., and our study was applied in accordance with the approbation of ESOGU Experimental Animals Ethic Comitte's (ethical approval code: 444-1/2015). The rats were nourished in a suitable environment as it should be (12 hours light/dark) with drinkable water tap and pellet feed. Experimental albino rats (healthy, male, Wistar, 3-4 months old, weight 200 - 240 gr) were divided in 9 groups (7 per grup) including the control group. 1st group (control) was administered with 0.5 ml serum physiologic (SF); 2nd group was administered with single dose 150 mg/kg CP on the 6th day of experiment. 3, 4 and 5th groups were administered with 25, 50 and 100 mg/kg HT extracts respectively for six days; 6, 7 and 8th groups were administered with 25, 50 or 100 mg/kg HT for six days and on 6th day a single dose of CP was injected, group 9 was treated with 0,5ml - 0.2% DMSO). On 7th day, all the rats were sacrificed. Before the injections and sacrifice, the animals were weighed again. Then, according to ethical guidelines, the rats were sacrificed by cardiac puncture with ketamine/xylazine anesthesia and the samples of blood were collected.

2.5. Histological measurements

Hepatic tissues were stained in 10% formaldehyde solution. Through routine histologic preparation, tissue samples were embedded in paraffin, then 5.0 thick serial sections, which were kept with Hematoxylin–Eosin, were done. After all, histopathological properties were examined. The consequences were assayed with Variance of One Way Analysis and Kruskal-Wallis test to score varying unnatural distribution. The differences between the
groups were found important if $p < 0.001$ and if $p > 0.05$, not statistically important.

2.6. Immunohistochemistry

Sections of cardiac tissues were deparaffinized and rehydrated routinely. Antigen retrieval by citrate buffer (pH 6.0) was done by heating the sections in a microwave at 700 W for 10 min. After blocking with 3 mL/L H$_2$O$_2$ and swine serum, sections were incubated with the primary antibodies, directed against Bcl-2 (Abcam), Bax (Abcam) and caspase-3 (Thermo) at dilutions of Ultravisionquanto detection system (Thermo Scientific), respectively.

2.7. Analysis of blood and bone marrow samples

The samples of blood were taken with cardiac puncture via the ketamine/xylazine anesthesia then the rats were killed on the 7th day. After rat femurs dissection bone marrow was taken with serum physiologic into a test tube, bone marrow samples were homogenized. Then, bone marrow nucleated cells and peripheric blood cells were calculated with a cell counter (Coulter).

2.8. Measurement of hepatic marker enzymes level

Rats blood samples were centrifuged at 3000 rpm for 10 mins and examined for alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) hepatic enzymes. The activities of ALT, ALP and LDH were measured in serum by commercial kits provided by spectrum diagnostic (Cairo, Egypt), seriatim.

2.9. Measurement of Oxidative Stress Index (OSI)

Oxidative Stress Index (OSI) was calculated by taking total oxidant level (TOL)/ Total antioxidant level (TAL) proportion. For this, the unit of TAL value was changed from the mmol Trolox equivalent/L type to $\mu$mol Trolox equivalent/L type. OSI was calculated as below: OSI = [(TOL, $\mu$mol H$_2$O$_2$ equivalent/L) / (TAL, $\mu$molTrolox equivalent/L) x 100] formula [22].
2.10. Statistical analysis

The antioxidant test data were expressed as mean±standard deviation (SD). The result of the biochemical and histological test were signified as means ± S.E.M. Statistical analysing was performed by One Way Analysis of Variance and Kruskal-Wallis One Way Analysis of Variance on Ranks Test. $p<0.001$ was received as considerable statistical importance. All experiment was applied at least three times.

3. Results and Discussion

The total phenolic content of seed methanol extract of HT was found as 179.52±0.52 µg GAE/mg extract. Inhibition (%) of DPPH free radical scavenging activity of seed methanol extract of HT (25 to 500 µg/mL concentrations) and positive controls (BHA and BHT) were shown in detail in Table 1.

The decrease in the concentration of DPPH radicals was due to the scavenging ability of the rich antioxidant contents of the *Hypericum triquetrifolium* Turra. (HT) seed methanol extract. Linear increase in activities was observed with high concentrations (250, 500 µg/mL). These values were found very close to the BHT and even higher than BHA which are most known syntetic antioxidants. Our results show that the crude seed methanol extract of HT has strong anti-oxidant activity when compared with BHA and BHT. These activities of plant extracts mainly attribute to the presence of rich phenolic compounds.

Table 1: Effect of seed methanol extract of *Hypericum triquetrifolium* Turra. on the inhibition of DPPH free radical.

<table>
<thead>
<tr>
<th>Concentrations (µg/mL)</th>
<th>Standards</th>
<th>Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BHA</td>
<td>BHT</td>
</tr>
<tr>
<td>25</td>
<td>67.60±0.08*</td>
<td>53.36±0.06</td>
</tr>
<tr>
<td>50</td>
<td>79.34±0.09</td>
<td>78.71±0.09</td>
</tr>
<tr>
<td>100</td>
<td>85.13±0.13</td>
<td>85.13±0.11</td>
</tr>
<tr>
<td>250</td>
<td>85.91±0.06</td>
<td>87.01±0.10</td>
</tr>
<tr>
<td>500</td>
<td>85.91±0.06</td>
<td>87.94±0.09</td>
</tr>
</tbody>
</table>

*Data are presented as mean values; ±standard deviation (SD) of triplicate values.
Histopathological assessments of our study show that hepatic tissues of the 100 mg/kg HT and 0.2% DMSO groups have normal histology like control (0.5 ml saline) group. Although there are no major defects in the liver of 150 mg/kg CP rats, some of the rats have small bleeding, edema sites, vacuolization, ballooning and focal area of hepatic necrosis in the hepatocytes (Fig. 1A, 2A, 3A). Similar results were demonstrated in former researches [23,24]. No obvious disturbances are seen in liver tissues of HT plus 150 mg/kg CP rat groups and on the contrary, the more HT dose increases, the liver has more normal histology like control. We could infer that HT importantly improved CP caused cellular damage and inflammation of hepatocytes with antioxidant and cytoprotective properties (Fig. 3A). Parallel to our study, experimental evidences show that oxidative stress is the reason for CP hepatotoxicity and also histopathological examinations demonstrated that CP causes serious damage to liver tissue and increases in liver injury score [25,26].

Cancer chemotherapy is the most frequent method to treat malignant cells but hepatotoxicity and myelosuppression are undesirable widespread side effect. Chemotherapy works on active cells, such as cancer cells and also on some healthy blood cells. Side effects happen when chemotherapy damages these healthy cells. So, we need to have new and more effective ways to minimize the side effects of chemotherapeutic drugs without losing chemotherapeutic effectiveness. Parallel to this report, in a research on the effects of cancer therapy on cancer-related side effects it is found that 80-96% of patients receive chemotherapy [17]. Some of clinical and preclinical studies report that a combination regimen of chemotherapeutic drugs with chemopreventive drugs can be helpful to relieve chemotherapy induced systemic toxicity, likewise to ensure recommended administration of chemotherapeutics for inquired process [4,27]. Also at our previous study we demonstrated that antioxidant compounds have pharmacological activity and limit toxicity [4].
Apoptosis assessments showed that Bcl-2, caspase-3 and bax immunohistochemistry results have differences in liver tissue sections. Caspase-3 activity, elevations of Bax and a decline of Bcl-2 were observed in CP induced rat livers when compared with control. In the earlier investigations, parallel to the present results, was seen CP induced liver tissue apoptosis [24,28]. In the livers of rats that received HT was seen dose-dependent reductions of Bax, and caspase-3 density, and rise in Bcl-2, when compared with the rats that received CP plus HT (Fig. 2B,C,D; 3B,C,D). Also, in the CP plus HT treated rats, apoptotic cells were observed more common than in the other groups. We may infer that the anti-apoptotic activity of HT is generally a reflection of its anti-inflammatory and antioxidant properties (Fig. 1,2,3).

CP is a cytostatic drug which non-specifically affects to not only cancer cells but also normal healthy cells with high proliferation capacity by binding to DNA and interfering with the cell cycle [29]. Chemotherapeutic drugs-induced damage on the immune system is a major side effect. In agreement, our results showed a significant ($p<0.001$) fall in the 150 mg/kg CP
group when compared with control [Table 2, Fig. 4]. The bone marrow’s damaged or loss of stem cells cannot regenerate new blood cells and results in thrombocytopenia and leucopoenia. 25, 50, 100 mg/kg HT+CP (specially 50 mg/kg HT+CP) groups were found to have a significant increase in the bone marrow nucleated cells values (p<0.001) in comparison to CP group [Table 2, Fig. 4]. According to these results, we may assert that HT and its active compounds are great candidates for alternative adjuvant chemotherapy in decreasing the CP immunotoxicity. In the DMSO control group, bone marrow nucleated cell values showed no significant differences from the control group (0.5 ml saline) (p>0.05) (Table 2, Fig. 4).

Table 2. The effects of 25, 50,100 mg/kg doses of Hypericum triquetrifolium Turra.’ s (HT ) numbers of erythrocytes, leucocytes, thrombocytes and bone marrow nucleated cells assessments on 150 mg/kg CP toxicity in rats in multiple comparision

<table>
<thead>
<tr>
<th>Group</th>
<th>Erythrocytes (X10^3 ml)</th>
<th>Leucocytes (X10^3 ml)</th>
<th>Thrombocytes (X10^3 ml)</th>
<th>Bone marrow (X10^3 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (SF)</td>
<td>8.42±0.51</td>
<td>6.5±C80</td>
<td>718.5±57.71</td>
<td>33.9±2.50</td>
</tr>
<tr>
<td>2. 150 mg/kg CP</td>
<td>7.8±0.32</td>
<td>2.0±C22</td>
<td>340.0±17.36</td>
<td>5.9±0.44</td>
</tr>
<tr>
<td>3. 25 mg/kg HT</td>
<td>8.04±0.33</td>
<td>10.98±1.33</td>
<td>666.29±38.42</td>
<td>37.65±1.20</td>
</tr>
<tr>
<td>4. 50 mg/kg HT</td>
<td>7.7±0.38</td>
<td>13.8±1.03</td>
<td>666.57±52.68</td>
<td>38.4±1.39</td>
</tr>
<tr>
<td>5. 100 mg/kg HT</td>
<td>7.62±0.36</td>
<td>10.99±1.18</td>
<td>693.29±23.80</td>
<td>33.0±1.63</td>
</tr>
<tr>
<td>6. 25 mg/kg CP+HT</td>
<td>7.4±0.30</td>
<td>4.29±0.57</td>
<td>546.00±7.43</td>
<td>10.9±1.34</td>
</tr>
<tr>
<td>7. 50 mg/kg CP+HT</td>
<td>7.97±0.40</td>
<td>5.23±0.64</td>
<td>671.71±48.58</td>
<td>12.35±1.27</td>
</tr>
<tr>
<td>8.100 mg/kg CP+HT</td>
<td>7.21±0.30</td>
<td>4.87±0.64</td>
<td>576.71±58.41</td>
<td>9.9±0.34</td>
</tr>
<tr>
<td>9. Control (DMSO)</td>
<td>7.74±0.34</td>
<td>7.51±0.53</td>
<td>741.00±48.34</td>
<td>35.4±0.99</td>
</tr>
</tbody>
</table>

(p<0.001: statistically significant differences, (p>0.05): no statistically significant differences).

In the present study, we observed that the number of erythrocytes decreased in CP group in comparison control. In the 50 mg/kg HT+CP group, the number of erythrocytes increased, furthermore it got closer to the control despite of CP toxicity (p<0.001). According to the results it could be concluded that dose of 25 mg/kg HT wasn't enough to prevent CP toxicity when compared with CP group (Table 2, Fig. 5). On the other hand dose of 100 mg/kg HT is toxic for erytrocyte cells because of showing no cytoprotective effect. We could conclude...
from the results that dose of 50 mg/kg HT+CP is the optimum protective dose on erythrocyte
cells (Table 2, Fig. 5).

There was a statistical significant decrease in the number of leucocytes in the groups of CP
and we thought that it could be because of high dose CP (Table 2, Fig. 6). In agreement with
our result, it was obtained that there was seen a significant ($p<0.001$) decrease in the number
of leucocyte cells in the CP administered rat group [30]. The number of leucocyte cells
showed a significant increase in the CP plus 25, 50, 100 mg/kg HT groups when compared
with 150 mg/kg CP ($p<0.001$). From these results it could be inferred that all dose of CP+HT
increased the number of leucocyte and 50 mg/kg HT+CP is the optimum dose on leucocyte
cells (Table 2, Fig. 6).

The number of thrombocytes was decreased ($p<0.001$) in the CP group in comparison with
control and 25, 50, 100 mg/kg HT groups (Table 2, Fig. 7). Similar to our study Merwid et al.
(2011) reported that CP which is an anticancer or immunosuppressive drug significantly
decreased thrombocyte and leucocyte cells, however the usage of high dose CP is limited
because of its toxicity [30]. The effect of CP on the erythrocyte cells was not so clear as in the
case of leucocyte and thrombocytes. The current study made it clear that CP lessened the
number of leucocyte and thrombocyte more than erythrocyte cells. Whereas the number of
thrombocyte cells increased in the 25, 50 and 100 mg/kg CP plus HT and this rise was
especially in the 50 mg/kg HT+CP group. And these results were found to be statistically
significant ($p<0.001$) (Table 2, Fig.5,6,7). In the DMSO group, the number of erythrocyte,
leucocyte and thrombocytes showed no significant change from the control group ($p>0.05$)
(Table 2, Fig.5,6,7).
Fig. 4, 5, 6, 7- Number of erythrocyte (Fig. 5), leucocyte (Fig. 6), thrombocyte (Fig. 7) cells of 150 mg/kg CP, 25-50-100 mg/kg HT, 25-50-100 mg/kg HT+ 150 mg/kg CP, % 0.2 DMSO applied experimental groups 28

The usage of CP, which is used widely in clinical settings as a chemotherapeutic agent, is severely limited by its physiological side effects, such as hepatotoxicity and myelosuppression [31]. CP-treated hepatotoxicity causes cell injury as a result of the cytosolic enzymes (ALT, ALP, LDH) and leaches out leading to their raised levels in the blood (Table 3; Fig. 8, 9, 10). The serum values of ALT, ALP and LDH are most widely used clinical markers of hepatocellular toxicity. So, the effect of CP metabolism on the liver function enzymes was evaluated and serum ALT, ALP and LDH levels were found high in the CP groups. In several researches it was demonstrated that, CP hepatotoxicity was associated with elevated ALT levels on mice [3] and rats [25,32]. It was demonstrated that there was an increase in ALT, ALP and LDH in CP-induced hepatocyte injury as well. And this was evidenced by the significant ($p<0.001$) increase in serum ALT and ALP when compared with the control group [26]. On the other hand, CP plus 25, 50, 100 mg/kg HT treatment,
especially CP plus 25 and 50 mg/kg HT doses, reduced the serum levels of ALT, ALP and LDH (Table 3, Fig. 8,9,10).

Table 3. The demonstration of the effects of 25, 50,100 mg/kg doses of Hypericum triquetrifolium Turra.’s (HT) biochemical assessments (ALT, ALP, LDH, OSI) on 150 mg/kg CP toxicity in rats in multiple comparison.

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>p&lt;0.001</th>
<th>ALP (U/L)</th>
<th>p&lt;0.001</th>
<th>LDH (U/L)</th>
<th>p&lt;0.001</th>
<th>OSI (TOS/TASx100)</th>
<th>p&lt;0.001</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Control (SF)</td>
<td>12.77±0.98</td>
<td>1-2, 1-4, 1-6, 1-7</td>
<td>49.02±23.31</td>
<td>1-2, 1-4, 1-5, 1-6, 1-7, 1-8, 1-9</td>
<td>598.7±32.28</td>
<td>1-2, 1-3, 1-4, 1-5, 1-6</td>
<td>0.0155±0.0009</td>
<td>1-2, 1-3, 1-4, 1-5, 1-6</td>
</tr>
<tr>
<td>2. 150 mg/kg CP</td>
<td>26.17±2.28</td>
<td>2-3, 2-4, 2-5, 2-7, 2-8, 2-9</td>
<td>89.88±33.32</td>
<td>2-3, 2-4, 2-5, 2-7, 2-8</td>
<td>314.29±15.04</td>
<td>2-3, 2-4, 2-5, 2-7, 2-8, 2-9</td>
<td>0.2431±0.0155</td>
<td>2-3, 2-4, 2-5, 2-7, 2-8, 2-9</td>
</tr>
<tr>
<td>3. 25 mg/kg HT</td>
<td>13.09±1.54</td>
<td>3-4, 3-5, 3-6, 3-7</td>
<td>50.66±4.39</td>
<td>3-4, 3-5, 3-6, 3-7, 3-8</td>
<td>397.76±24.04</td>
<td>3-5, 3-6</td>
<td>0.0164±0.0014</td>
<td>3-4, 3-5, 3-6, 3-7</td>
</tr>
<tr>
<td>4. 50 mg/kg HT</td>
<td>11.94±1.99</td>
<td>4-6, 4-7</td>
<td>67.87±3.60</td>
<td>4-5, 4-6, 4-8, 4-9</td>
<td>305.01±17.44</td>
<td>4-5, 4-6</td>
<td>0.0164±0.0006</td>
<td>4-5, 4-6, 4-8</td>
</tr>
<tr>
<td>5. 100 mg/kg HT</td>
<td>9.21±0.91</td>
<td>5-6, 5-7, 5-9</td>
<td>28.61±2.70</td>
<td>5-6, 5-7, 5-8, 5-9</td>
<td>293.42±17.54</td>
<td>5-6, 5-7, 5-8, 5-9</td>
<td>0.0132±0.0006</td>
<td>5-6, 5-7, 5-8, 5-9</td>
</tr>
<tr>
<td>6. 25 mg/kg CP+HT</td>
<td>23.54±2.32</td>
<td>6-7, 6-8, 6-9</td>
<td>83.92±9.71</td>
<td>6-7, 6-8, 6-9</td>
<td>472.39±22.03</td>
<td>6-7, 6-8, 6-9</td>
<td>0.1948±0.0074</td>
<td>6-7, 6-8, 6-9</td>
</tr>
<tr>
<td>7. 50 mg/kg CP+HT</td>
<td>17.65±1.38</td>
<td>7-8, 7-9</td>
<td>72.61±0.94</td>
<td>7-8, 7-9</td>
<td>312.95±17.76</td>
<td>7-8, 7-9</td>
<td>0.1321±0.0055</td>
<td>7-8, 7-9</td>
</tr>
<tr>
<td>8. 100 mg/kg CP+HT</td>
<td>11.72±1.62</td>
<td>58.65±2.77</td>
<td>290.86±21.97</td>
<td>5-8, 5-9</td>
<td>0.0099±0.0040</td>
<td>5-6</td>
<td></td>
<td></td>
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| 9. Control (DMSO) | 12.35±1.71 | 52.76±23.37 | 353.46±28.87 | 0.0155±0.0009 | (p<0.001: statistically significant differences, (p>0.05): no statistically significant differences).

Cellular antioxidant defense system is a major defense against CP-induced oxidative stress [33,34]. ACR, which leads to a breach in the normal antioxidantive defense system and can result in uninterrupted ROS accumulation, attacks to the tissue antioxidant enzymes. In the 150 mg/kg CP group, level of OSI increased when compared with the control group. This might result from a decrease in antioxidant status, as it was evidenced by the substantial decline in the CP-treated group. 25, 50, 100 mg/kg HT prevented the CP toxicity and we can explain this with the decrease in OSI level (Table 3, Fig. 11). So, the prevention of OSI increment and enhancement of the antioxidant enzymes play a central role in the cytoprotective activity of HT against CP. In the same way our study is supported by numerous studies and in all these studies the herbs are obviously play a crucial role with antioxidant and antitumoral features.
Fig. 8,9,10,11 - ALT, ALP, LDH, OSI levels of 150 mg/kg CP, 25-50-100 mg/kg HT, 25-50-100 mg/kg HT+150 mg/kg CP and % 0.2 DMSO applied experimental groups

4. Conclusion

Therapeutic effects of anticancer agents are associated with adverse side effects because of the toxicity they cause. Even though CP is very commonly preferred as a chemotheputic drug, its toxic side effects limit high dose usage. So, we are in need of new effective agents in order to protect the normal tissue from chemotherapy-related toxicity without protecting the tumour and tumour growth stimulation features. Therefore, the combination of the treatment regimens with antioxidative and cytoprotective features can be useful in the protection of healthy cells and tissue against CP-treated oxidative damage. From our results, it could be concluded that HT seed methanol extracts show high antioxidant activity and protective effect on blood, bone marrow nucleated cells and liver tissue despite of high dose CP without limiting its chemotheputic effectiveness. Based on our study findings, it could be proposed that all dose of HT especially 50 mg/kg HT plus CP are strong candidates in preventing the CP-induced myelotoxicity, hemotoxicity and hepatotoxicity.
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References


22. Aycicek A, Erel O, Kocyigit A. Decreased total antioxidant capacity and increased


fragmentation. Exp Toxicol Pathol 2013; 65:27-34. DOI: 10.1016/j.etp.2011.05.005


