Activity, acute and sub-acute toxicity and safety assessment of the hydroalcoholic root extract of *Diplotaenia turcica*

U. Özdek*, Y. Başıbüşan, S. Yıldırım, M. Boğa, M. Firat and Y. Değer

Van Yuzuncu Yil University, Vocational School of Health Services, 65080 Van, Turkey.

Received: 27-01-2018  Accepted: 08-04-2018

**ABSTRACT**

*Diplotaenia turcica* is widely utilized in conventional treatment in the east of Turkey. Due to the insufficient data on the safety profile, the acute and sub acute toxicity of *Diplotaenia turcica* was determined. Furthermore, total phenolic content, flavonoid content and antioxidant activity of the extract were determined. The acute toxicity of hydroalcoholic root extract of *Diplotaenia turcica* (HREDT) was evaluated in mice after administration of single oral dose at the 5000 mg/kg (acute model) and rat after 28 days orally administration at the dose range of 250, 500 and 1000 mg/kg (subacute model). According to result, the LD50 value of HREDT was found to be greater than 5000 mg/kg. In sub acute toxicity study, no statistically significant differences were observed in the values of hematological and pathological parameters in comparison with control group. It was determined that the glucose, triglyceride, cholesterol and LDL levels exerted a significant effect depending on the HREDT doses. The results from the present study found out that HREDT did not produce any toxic effects or deaths in animals for both single and chronic administration. Additionally, HREDT showed moderate antioxidant activities and exhibited relatively notable total phenolic content.

**Key words:** Antioxidant capacity, Acute toxicity, *Diplotaenia turcica*, Sub chronic toxicity.

**INTRODUCTION**

*Diplotaenia* is a genus belonging to the Umbelliferae (Apiaceae) family. It has five breeds: *Diplotaenia bingolensis*, *Diplotaenia cachrydifolia*, *Diplotaenia damavandica*, *Diplotaenia cachrydifolia* and *Diplotaenia turcica* (Pimenov et al., 2011). As elaborated at the floristic studies, it was found that *Diplotaenia turcica*, which is also named as Siyaboyn, spreads in Hakkari, Şırnak, Van and Bitlis regions. It is a perennial herb of around 150-200 cm in height; base leaves are split and 3-4 pinnate, and white flowers (Firat, 2013). The most important feature of *Diplotaenia turcica* is to be being used in herbal cheese, meals and traditional treatments in the East Anatolian provinces. *Diplotaenia turcica* is used for protection from the bites of poisonous animals. Besides, the root part of the plant in among people has been being used for treatment of rheumatism, diabetes and blood pressure balancing since ancient times (Kaval et al., 2014, Uce and Tunçtürk 2014). Although the plant is widely used in traditional medicine, no sufficient data has been generated on pharmacological activities and toxicity of the plant. The objective of this study was to determine the total phenolic content, the total flavonoid content, antioxidant activity, acute and subacute toxicity of hydroalcoholic root extract of *Diplotaenia turcica* (HREDT).

**MATERIALS AND METHODS**

**Plant material:** The plant of *Diplotaenia turcica* was collected and identified by research assistant Mehmet Fýrat (YuzuncuYil University, Faculty of Science and Arts, Dept of Biology) from east of Turkey (Hakkari) in May-June.

**Collection Location:** C9, Hakkari, VareValto region, 29.07.2016, step. M. Firat 32858 (VANF).

**Preparation of the extract:** Roots were dried up and powdered by using an electrical mill. A 100 g powdered sample was added to 1000 ml of alcohol. Initially, 96% ethanol was utilized and after 24 hours of time period, the solution was filtered. Secondly, 70% ethanol was added to the rest dry materials. After 24 hour, the solution was filtered and then both filtered solutions were mixed together and then evaporated repeatedly by rotary evaporator at 50° C and 70 rpm. Concentrated extracts were lyophilized to yield 6% w/w dry extract, and stored at -20°C until further used (Farkhad et al., 2012).

**Animals:** Swiss albino female mice (25-30 g) and male Wistar albino rats (200-250 g) selected from the

---

*Corresponding author’s e-mail: ugurozdek@yyu.edu.tr*

1Van Yuzuncu Yil University, Vocational School of Health Services, 65080 Van, Turkey.
2Department of Internal Diseases, Faculty of Veterinary Medicine, University of Van Yüzüncü Yil TR-65080 Van-Turkey.
3Department of Pathology, Faculty of Veterinary Medicine, Ataturk University, TR-25240 Erzurum-Turkey.
4Department of Pharmaceutical Technology, Faculty of Pharmacy, Dicle University, 21280 Diyarbakir, Turkey.
5Department of Biology, Faculty of Education, University of Van Yüzüncü Yýl, TR-65080 Van-Turkey.
6Department of Biochemistry, Faculty of Veterinary Medicine, University of Van Yüzüncü Yil, TR-65080 Van-Turkey.
Experimental Research Laboratory of Yüzüncü Yıl University, Faculty of Medicine, were maintained in an environmentally controlled room (22 ± 2°C) with a 12-h light/12-h dark cycle. Laboratory animal was administered normal chow and allowed tap water ad libitum. The protocol of the experiment was confirmed by the Animal Use Ethics Commission (02-2016) of Van Yüzüncü Yıl University.

**Acute toxicity:** Acute toxicity study was performed on 5 female Swiss albino mice by use of the guidelines of the Organization for Economic Co-operation and Development (OECD) guideline 425 (Limit Test at 5000 mg/kg). Mice were administrated single orally at the dose 5000 mg/kg of HREDT. The symptoms of toxicity and mortality were monitored after treatment for the first four (critical) hours, then over a period of 24 hours, after daily for 14 days (Lohith et al., 2013).

**Sub chronic toxicity:** Sub chronic toxicity study was performed on 32 male Wistar albino rats by use of the guidelines of the Organization for Economic Co-operation and Development (OECD) guideline 407. 32 male Wistar albino rat were divided in four groups (n = 8). For a period of 4 weeks, control group was treated with saline vehicle. The HREDT (Group 1: 250 mg/kg/day, Group 2: 500 mg/kg/day, Group 3: 1000 mg/kg/day) dissolved in saline vehicle was given orally to the eight rats in each group. All the animals were observed for mortality, behavioral and clinical signs. Body weights of the rats were recorded at the end of the first day and at weekly intervals throughout during the treatment period.

On the final day of the study (on 29th day), rats were anaesthetized by ketamine HCL (75 mg/kg, I.P) and blood samples were collected by cardiac puncture and immediately submitted for hematological and biochemical analyzes.

**Hematological and biochemical parameters:** Heparinized samples were used for the estimation of hematological parameters such as red blood cell count (RBC), white blood cell count (WBC), hematocrit (HCT), and hemoglobin (Hb) by automated hematology analyzer. The serum was separated from blood and the serum biochemical parameters like glucose, insulin, albumin, globulin, total protein, bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), cholesterol, triglycerides, high-density lipoprotein (HDL), low-density lipoprotein (LDL), blood urea nitrogen (BUN), creatinine, uric acid and urea were assayed using commercial kits by utilizing automatic biochemical analyzer.

**Histopathology:** Necropsia was performed immediately after sacrifice and the tissues from vital organs, liver, kidney and heart were taken. Organ weight per 100 g body weight (relative weight) was assayed according to the body weight of the fasted animals. All tissues were preserved in 10% neutral phosphate-buffered formalin and studied macroscopically. Tissues were embedded in paraffin blocks after the process for making routine histopathological examination. Sections of 4 mm thickness were stained with hematoxylin-eosin (H&E) and were held under light microscope for histomorphological changes (Luna Lee 1968, Taylor and Cote 2006).

**Total phenolic content, total flavonoid content and antioxidant activity of HREDT:** The total phenolic content was carried out with Folin–Ciocalteu colorimetric technique with some modification (Slinkard and Singleton 1977). The flavonoid content was identified in relation to the aluminum chloride technique with some modifications (Moreno et al. 2000). The antioxidant activity was determined by 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) radical scavenging assay (Kosanic et al., 2012) and cupric ion reducing capacity in the presence of neocuproine (CUPRAC) for the evaluation of reducing power (PR) (Apak et al., 2004).

**Statistical analyzes:** The conclusions were stated as the mean ± SD and statistical analysis was conducted by One way ANOVA followed by Duncan test. Differences were thought important at p < 0.05. All the analyses were made utilizing SPSS (20.0) software.

**RESULTS AND DISCUSSION**

**Acute toxicity:** In the toxicity assay, single orally administraton of HREDT did not produce any death and adverse effects in mice in the 14-days. So, LD₅₀ value of HREDT was found to be greater than 5000 mg/kg.

**Subacute toxicity:** No mortality and symptoms of adverse effects were recorded in rats treated at 250, 500 and 1000 mg/kg orally during 28 days of treatment. No significant difference in mean initial and final body weight and organ weight were observed between control and treated groups (Table 1).

**Hematological and biochemical parameters:** The hematological profile of experimental and control group are summarized in Table 2. The hematological parameters did not differ significantly in different doses of HREDT. The biochemical parameters of experimental and control group

<table>
<thead>
<tr>
<th>Organ</th>
<th>Control group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.86 ± 0.04</td>
<td>4.92 ± 0.03</td>
<td>4.87 ± 0.04</td>
<td>4.91 ± 0.01</td>
</tr>
<tr>
<td>Kidney</td>
<td>1.31 ± 0.01</td>
<td>1.33 ± 0.01</td>
<td>1.31 ± 0.01</td>
<td>1.32 ± 0.01</td>
</tr>
<tr>
<td>Heart</td>
<td>0.91 ± 0.02</td>
<td>0.92 ± 0.02</td>
<td>0.91 ± 0.02</td>
<td>0.92 ± 0.01</td>
</tr>
</tbody>
</table>

Values are expressed as means ± S.D., (n= 8). Control group; untreated group, Group 1; orally administered 250 mg/kg HREDT, Group 2; orally administered 500 mg/kg HREDT, Group 3; orally administered 1000 mg/kg HREDT.
The results did not reveal any statistically significant differences among the group averages bearing different letters in the same sequence. The difference among the group averages bearing different letters in the same sequence is statistically significant, p<0.05. Values are expressed as means ± S.D., (n=8). Control group; untreated group, Group 1; orally administered 250 mg/kg HREDT, Group 2; orally administered 500 mg/kg HREDT, Group 3; orally administered 1000 mg/kg HREDT.

Table 2: The effect of hydroalcoholic root extract of *Diplotaenia turcica* on hematological parameters after 28 days treatment in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC (x10^12)/L</td>
<td>7.63 ± 0.093</td>
<td>7.38 ± 0.110</td>
<td>7.73 ± 0.160</td>
<td>7.50 ± 0.13</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>42.56 ± 0.470</td>
<td>40.73 ± 0.524</td>
<td>42.88 ± 0.916</td>
<td>40.56 ± 0.78</td>
</tr>
<tr>
<td>WBC (x10^3)/L</td>
<td>1.78 ± 0.29</td>
<td>1.90 ± 0.40</td>
<td>2.31 ± 0.27</td>
<td>2.09 ± 0.65</td>
</tr>
<tr>
<td>Hb (g/dL)</td>
<td>15.06 ± 0.14</td>
<td>14.47 ± 0.22</td>
<td>15.27 ± 0.31</td>
<td>14.70 ± 0.23</td>
</tr>
</tbody>
</table>

Red blood cells (RBC), white blood cells (WBC), hematocrit (HCT) and hemoglobin (Hb), values are expressed as means ± S.D., (n=8). Control group; untreated group, Group 1; orally administered 250 mg/kg HREDT, Group 2; orally administered 500 mg/kg HREDT, Group 3; orally administered 1000 mg/kg HREDT.

Table 3: The effect of hydroalcoholic root extract of *Diplotaenia turcica* on biochemical parameters after 28 days treatment in rats.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mg/dl)</td>
<td>172.00 ± 4.1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin (µU/ml)</td>
<td>5.20 ± 0.19</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.69 ±0.13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Globulin (g/dl)</td>
<td>2.69 ±0.09</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TP (g/dl)</td>
<td>6.38 ± 0.21</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>0.12 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST (U/L)</td>
<td>257.25 ± 32.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT (U/L)</td>
<td>46.75 ± 3.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALP (U/L)</td>
<td>117.25 ±13.26</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>80.00 ± 2.9</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dl)</td>
<td>55.83 ± 7.44</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>48.86 ± 1.85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LDL (mg/dl)</td>
<td>10.4 ± 1.33</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>0.83 ± 0.20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dl)</td>
<td>41.00 ± 2.38</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.52 ± 0.02</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dl)</td>
<td>1.78 ± 0.43</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Total phenolic content, total flavonoid content and antioxidant activity: The total phenolic content of the HREDT was higher than flavonoid content, assessed from the calibration curve, was 72.65 µg PEs/mg, and the total flavonoid content was 8.30 µg KEs/mg (Table 4).

**Total phenolic content, total flavonoid content and antioxidant activity:** The total phenolic content of the HREDT was higher than flavonoid content, assessed from the calibration curve, was 72.65 µg PEs/mg, and the total flavonoid content was 8.30 µg KEs/mg (Table 4).

**DPHH free radical scavenging activity** was 82.11% at a concentration of 100 µg/ml root extract while that of the standards, butylated hydroxyanisole (BHA) and butylated hydroxyl toluene (BHT), were 98.30% and 71.42%, respectively (Fig 1).

**Reduction of Cu²⁺ ion to Cu⁺** was shown to rise with enhancing concentrations of the HREDT. Cupric reducing antioxidant capacities (CUPRAC) were 0.951 for 100 µg/ml of the root extract and 3.987, 1.171 for the

Table 4: The total phenolic and flavonoid contents of the hydroalcoholic root extract of *Diplotaenia turcica*.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control group</th>
<th>Group 1</th>
<th>Group 2</th>
<th>Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic content (µgPES/mg extract)</td>
<td>72.65 ± 2.20</td>
<td>8.30 ± 0.61</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flavonoid content (µgQES/mg extract)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values expressed are means ± S.D. of three parallel measurements.

PEs, pyrocatecholequivalents (y=0.0316x + 0.0422 R²=0.99520)

QEs, quercetinequivalents (y=0.0269x – 0.0393 R²=0.9989)

Histopathological study: The results did not reveal any significant changes in color or texture of the liver, kidney and heart when compared with control groups animals (Fig 4-5-6). The No-Observed Adverse Effect level (NOAEL) of the extract was thought to be bigger than 1000 mg/kg/day in rats.
Fig 1: The effect of hydroalcoholic root extract of *Diplotaenia turcica* on body weights after 28 days treatment, values are expressed as means ± S.D., (n=8).

Fig 2: The inhibition (%) of DPPH free radicals cavenging of the hydroalcoholic root extract of *Diplotaenia turcica*, BHT and BHA, values are expressed as means ± S.D. of three parallel measurements.

Rodents are among the favorite animal model to be used in biological research due to their easy availability, small size, low cost, ease of handling, and fast reproduction rate (Yaqub *et al.*, 2017). In general, the first toxicity test conducted a compound is acute toxicity, identified from the utilization of a single exposure. The main objective of acute toxicity testing is to supply an approximation of the intrinsic toxicity of the substances often stated as median lethal dose (LD$_{50}$). It is indicated that substances which present LD$_{50}$...
greater than 2000 mg/kg when administered orally can be categorized practically non-toxic (Curtis, 2007). The results of present study revealed that the LD_{50} value of HREDT orally administration in mice is more than 5000 mg/kg and may be considered nontoxic.

Exposure to drugs and chemicals often induce toxicity to living organisms (El Shahat et al., 2017). The changes in body and organs weights are important markers of toxic effects of drugs and chemicals, and this is considered statistically significant if there is a 10% loss of first weight (Prasanth et al., 2015). In the present study, mortality and symptoms of adverse health effects were not found. Also the body weight gain and internal organs (heart, liver and kidney) weight were not significantly different from control group during 28 days administration.

The changes in the blood parameters in animals have been considered useful indicator for predicting human toxicity (Prasanth et al., 2015). In the present study, hematological parameters in HREDT treated groups showed no significant changes in comparison with control group after 4 weeks. This indicated that the HREDT did not affect the blood cellular components or their production. In this study, it has been determined that the glucose levels were decreased significantly in group 1 compared to the other treated groups and the control group. This result can be showed that the HREDT has hypoglycemic property, this may be reason that people consume this as antidiabetic. For multiple mechanisms in hypoglycemia are possible, further experiments are needed to establish the mechanism of the observed hypoglycemic activity. The significant change in the concentrations of serum triglyceride, cholesterol, and LDL levels in the all treatment groups as dose dependent. Therefore HREDT may be useful in treatment of hyperlipidemia. All other liver and kidney function parameters tested were non significantly different in all the groups compared to control (Table 3). No change in histopathology also showed the absence of toxic effects on vital organ examined and supported the biochemical and hematological results (Fig 3-4-5).

Plants rich in secondary metabolites, such as phenolics, flavonoids and carotenoids, have antioxidant activity due to their redox properties and chemical structures. The presence of antioxidants in plants protect against a large number of diseases. Therefore, the medicinal plants are being investigated for their antioxidant properties. Phenolic and flavonoid compounds are widespread in plant kingdom where they act as antioxidants and free radical scavengers (Krithika and Naik, 2015). As this is the first report on the antioxidant activity of hydroalcoholic root extract of

![Fig 3: Cupric reducing antioxidant capacity of the hydroalcoholic root extract of *Diploptaeum turcica*, BHT and BHA, values are expressed as means ± S.D. of three parallel measurements.](image1)

![Fig 4: Liver tissue, A: Control group, B: group 1 (250 mg/kg), C: Group 2 (500 mg/kg), D: Group 3 (1000 mg/kg), normal histological appearance, H & E, Bar: 20μm](image2)
Diplotaenia turcica. It was determined that the total phenolic content (72.65 ± 2.20 μgPEs/mg) of HREDT was found to be higher than its flavonoid content (8.30 ± 0.61 μgQEs/mg). The DPPH radical is widely used in assessing free radical scavenging because of the ease of the reaction (Alam et al., 2013). HREDT exhibited higher activity than BHT, but lower activity than BHA, which were used standard in the DPPH free radical scavenging assay, at 100 μg/ml.

Antioxidants can not only allow scavenging of free radicals by their electron donating capability, but also reduce higher valiant elements such as copper, iron to their lower valence state. The redox potential of an antioxidant is an important indicator of its efficacy (Shahidi and Zhong 2015). The CUPRAC assay is a redox potential-based method in which the copper (II)-neocuproine complex, as a chromogenic oxidant, is reduced to copper (I)-neocuproine.
chelate by antioxidants (Arttuluk et al., 2016). HREDT exhibited moderate activity than BHT and BHA which were used standard CUPRAC method, at 100 μg/ml.

Thus, this study showed that the root extract of *Diplotaenia turcica* at different levels tested did not cause toxicity. Moreover, the extract has antioxidant properties.

**ACKNOWLEDGEMENT**  
We thank to the Scientific Research Projects Presidency of Yuzuncu Yil University (TSA-2016-5067) for their financial support.

**REFERENCES**


