Effects of the Theranekron®

an alcoholic extract of the

Tarantula cubensis” on hematology and serum biochemical properties in horses

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Abstract

Theranekron® is commercially available, alcoholic extract of the tarantula cubensis (brown spider). Ten healthy thoroughbred mare racehorses were used at the present study. Blood samples were taken 30 minutes before and 8, 24, 48, 72 and 168h after subcutaneous administration of 10ml Theranekron (1mg/48kg or 0.02mg/kgbw) via a jugular catheter. The results of this study showed that sampling time had a significant effects on the amount of PCV, hemoglobin concentration, RBC number, total protein, albumin, glucose, cholesterol, BUN, creatinine, bilirubin, activity of ALT, and ALP (p<0.05) while, had no significant effects on MCV, MCH and MCHC amounts, WBC numbers, fibrinogen concentration, AST, CPK and GGT activities (p>0.05). In conclusion, most of the observed changes in hematological and serum biochemical parameters were statistically and not clinically significant. Thus it seems that administration of Theranekron has no adverse reaction in experimental horses.

Keywords: brown spider, venom, horse, theranekron, tarantula cubensis
Introduction

Venom is a part of defensive or prey capture apparatus of venomous creatures which also assist in chemical digestion (Cousin and Bon, 1999; Honkanen and Golden, 2002; Dounay and Forsyth, 2002). Venom is a complex mixture of various compounds such as proteins, enzymes, ions, biogenic amines, polyamines, cytolytic peptides, and variety of toxins. Most of these materials have been shown to have specific and diverse pharmacological activities (Li-Smerin and Swartz, 2001; Milne et al., 2003). They interfere with important physiological function of cells, result in organ injury, dysfunction or ultimately death (Olivera et al., 1984). For example, toxins which target, ion channels and receptors in different cells have been isolated from spiders, marine snails, snakes, scorpions, and some other venomous animals (Fry et al., 2005). In addition, several reports indicated that some toxins can affect blood and plasma biochemical parameters (El-Asmar et al., 1986; Aguiyi et al., 2001; Al-Jammaz, 2003 and Muhammad, 2009).

Moreover, many of these toxins have proven to be invaluable research tools and have provided leads for potential new therapies (Diochot et al., 2003). A number of these toxins have already been used in vivo for proof of concept studies. While some of them have pre or clinical proof for pain management, others use to treat diabetes, cancer, multiple sclerosis, and cardiovascular disease (Lewis and Garcia, 2003).

Among the venomous spiders, *tarantula cubensis* is the famous one and many therapeutic effects have been reported for its venom (Stampa, 1986). Theranekron is commercially available as an alcoholic extract of the whole *tarantula cubensis*. In 1977 Mezger described the homeopathic effects of Theranekron (Mezger, 1977). Theranekron® remains active in pharmaceutical compounds for a considerable time. Many therapeutic effects have been described for Theranekron such as; antiphlogistic, demarcative, necrotizing action and wound healing (Stampa, 1986, Koch and Stein, 1980; Stampa, 1986; Sardari et al., 2007).

Theranekron has been used in cattle, horse, sheep, goat and dog for different purposes. Literature confirmed that Theranekron can be used successfully in cow cases with necrotic wounds, retained placenta and pododermatitis circumscripta (Koch and Stein, 1980; Stampa, 1986). In dogs Theranekron was used to stop growth of mammary tumors (Koch and Stein, 1980).

The aim of the present study was to evaluate the effects of the Theranekron on hematology and serum biochemical properties in clinically normal horses.

Materials and Methods

Horses

Ten healthy thoroughbred mare racehorses of age 6±1 years and weighing 460±30 kg were used. The horses were housed in stable, fed with a maintenance ration three times per day and had free access to water. They walked 30 minutes twice a day during the study period.

Theranekron® alcoholic extract (1:100) of *Tarantula cubensis* in alcoholic solution 1mg/ml, purchased from Richter-Pharma AG, Wels, Austria.

Experimental set-up

Blood samples were taken 30 minutes before and 8, 24, 48, 72 and 168h via a jugular catheter after administration of 10 ml Theranekron subcutaneously, based on the advised dose by manufacturer for a 485 kg horse almost 0.02mg/kg bw. Blood was collected into ethylenediaminetetraacetic acid (EDTA) and plane tubes for biochemical and hematological analysis respectively.

Hematological and biochemical analysis

Anti-coagulated blood was used for CBC determination using automated veterinary hematology analyzer (Nihon Kohden, Cell Tac a, MEK 6108, Tokyo, Japan). Differential
leukocyte count was performed microscopically on Giemsa stained blood film using cross sectional method. Plane tubes were centrifuged at 1800g for 10 min followed by removal of serum. Serum was stored at -20 °C until analyzed. The amounts of total serum protein (tp), albumin (alb), urea, creatinine (cre), glucose (glu), cholesterol (chol), total bilirubin (bil), alkaline phosphatase (ALP), creatin kinase (CK), gamma glutamyltransferase (GGT), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured by commercial kits (Pars Azmoon, Tehran, Iran) using an autoanalyser (Biotecnica, Targa 3000, Rome, Italy). Control serum (Randox control sera, Antrim, UK) was used for accuracy.

**Statistical analysis**

Statistical analyses were performed using the SPSS 9 program for windows (SPSS. Inc., Chicago IL, USA). Sampling time effects were examined using ANOVA. All of the analysis was corrected for repeated measurements, time of sampling as fix and horses as random factor were used. In addition paired t-test was used for the comparison of sampling stages with first sampling time. P<0.05 was considered as significant.

**Results**

The results are presented in tables 1 and 2. Sampling time had a significant effect on PCV amount (p=0.01), there were significant differences between hours 48, 72 and 168 compared with the amount at first sampling time (p<0.05). Sampling time had a significant effects on HB concentration (p=0.01), but there were no significant differences between various sampling time compared with the amount at first sampling time (p>0.05). Sampling time had a significant effect on RBC number (p=0.014), there were significant differences between hours 8, 72, and 168 compared with the amount at first sampling time (p=0.05). Sampling time had no significant effect on MCV (p=0.267) and MCH (p=0.069) value, but there were significant differences in MCH levels between hours 8 and 72 compared with the amount at first sampling time (p<0.05). Although, sampling time had no significant effect on MCHC (p=0.07), but there were significant differences between hours 8 and 72 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on WBC numbers (p=0.2), but there were significant differences between hours 72 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on neutrohpil (p=0.25), eosinophil (p=0.64), lymphocyte (p=0.49), monocyte (p=0.11) and platelet (p=0.47), there were no significant differences between various sampling time compared with the number at first sampling (p>0.05). Sampling time had a significant effect on TP amounts (p=0.00), there were significant differences between hours 24, 48 and 72 compared with the amount at first sampling time (p<0.05).Sampling time had a significant effect on albumin concentration (p=0.00), but there were no significant differences between various sampling time compared with the amount at first sampling (p>0.05). Sampling time had no significant effect on fibrinogen concentration (p=0.86) and there was no significant difference between various sampling time to compared with the amount at first sampling (p>0.05). Sampling time had a significant effect on glucose concentration (p<0.05), there were significant differences between hours 8 compared with the amount at first sampling time (p<0.05). Sampling time had no significant effect on cholesterol concentration (p=0.034), but there was no significant difference between sampling time compared with the amount at first sampling (p>0.05). Sampling time had a significant effect on BUN concentration (p=0.00), there were significant differences between hours 48 compared with the amount at first sampling time (p<0.05). Sampling time had a significant effect on creatinin concentration (p=0.00), but there were no significant differences between
various sampling time to compared with the amount at first sampling ($p>0.05$). Sampling time had a significant effect on bilirubin concentration ($p=0.005$), but there were no significant differences between sampling time to compared with the amount at first sampling ($p=>0.05$). Sampling time had a significant effect on AST activity ($p=0.05$), but there were no significant differences between sampling time compared with the amount at first sampling ($p>0.05$). Sampling time had no significant effect on GGT activity ($p=0.3$), but there were significant differences between hours 8, 72 and 168 compared with the amount at first sampling ($p<0.05$). Sampling time had no significant effect on ALP activity ($p=0.03$), but there was a significant difference between sampling time compared with the amount at first sampling ($p>0.05$). Sampling time had significant effect on GPT activity ($p=0.03$), but there was no significant difference between sampling time compared with the amount at first sampling ($p>0.05$).

Table 1. Mean±SE of hematological parameters of horses after injection of 10ml Theranekron® subcutaneously (n=10).

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>0</th>
<th>8 hours</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematocrit (%)</td>
<td>38.7±0.8</td>
<td>34.1±1.1</td>
<td>34.8±1.4</td>
<td>35.7±0.9*</td>
<td>33.8±0.7*</td>
<td>35.4±0.9*</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.97±0.6</td>
<td>9.57±0.6*</td>
<td>9.75±0.6*</td>
<td>10.27±0.5</td>
<td>9.13±0.5*</td>
<td>11.4±0.6</td>
</tr>
<tr>
<td>RBC (10^6/μl)</td>
<td>8.3±0.2</td>
<td>7.2±0.2*</td>
<td>7.4±0.4</td>
<td>7.7±0.2</td>
<td>7.4±0.3*</td>
<td>7.6±0.3*</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>46.4±0.8</td>
<td>46.3±0.8</td>
<td>46.4±0.7</td>
<td>46.3±0.8</td>
<td>46.5±0.7</td>
<td>46.4±0.8</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>14.3±0.7</td>
<td>13±0.7*</td>
<td>13.1±0.8</td>
<td>13.4±0.7</td>
<td>12.4±0.8*</td>
<td>14.9±0.8</td>
</tr>
<tr>
<td>MCHC (%)</td>
<td>30.9±1.2</td>
<td>28±1.2*</td>
<td>28.1±1.4</td>
<td>28.9±1.4</td>
<td>26.7±1.5*</td>
<td>32.2±1.7</td>
</tr>
<tr>
<td>WBC (10^9/μl)</td>
<td>5.56±0.32</td>
<td>5.55±0.38</td>
<td>4.9±0.2</td>
<td>5.3±0.55</td>
<td>4.6±0.24*</td>
<td>5.74±0.41</td>
</tr>
<tr>
<td>Neutrophil (10^9/μl)</td>
<td>2.75±0.23</td>
<td>2.63±0.17</td>
<td>2.55±0.21</td>
<td>2.55±0.16</td>
<td>2.30±0.15</td>
<td>2.92±0.19</td>
</tr>
<tr>
<td>Lymphocyte (10^9/μl)</td>
<td>2.35±0.18</td>
<td>2.55±0.29</td>
<td>2.07±0.15</td>
<td>2.34±0.41</td>
<td>2.07±0.21</td>
<td>2.48±0.32</td>
</tr>
<tr>
<td>Monocyte (10^9/μl)</td>
<td>0.36±0.049</td>
<td>0.36±0.1</td>
<td>0.19±0.04</td>
<td>0.31±0.074</td>
<td>0.17±0.046</td>
<td>0.24±0.062</td>
</tr>
<tr>
<td>Eosinophil (10^9/μl)</td>
<td>0.1±0.03</td>
<td>0.09±0.01</td>
<td>0.06±0.02</td>
<td>0.09±0.04</td>
<td>0.07±0.009</td>
<td>0.1±0.04</td>
</tr>
<tr>
<td>Platelet (10^9/μl)</td>
<td>159±7.3</td>
<td>172±9</td>
<td>164±9.1</td>
<td>165±5.7</td>
<td>171±5.9</td>
<td>167±6.5</td>
</tr>
</tbody>
</table>

* Significant difference with first sampling time ($p<0.05$).

Table 2. Mean±SE of serum biochemical parameters of horses after injection of 10ml Theranekron® subcutaneously (n=10).

<table>
<thead>
<tr>
<th>Sampling time</th>
<th>0</th>
<th>8 hours</th>
<th>24 hours</th>
<th>48 hours</th>
<th>72 hours</th>
<th>168 hours</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total protein (g/dl)</td>
<td>7.06±0.17</td>
<td>7.1±0.18</td>
<td>6.44±0.16*</td>
<td>6.57±0.13*</td>
<td>6.36±0.08*</td>
<td>6.62±0.14</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>3.38±0.09</td>
<td>3.37±0.09</td>
<td>3.13±0.06</td>
<td>3.32±0.07</td>
<td>3.46±0.06</td>
<td>3.48±0.08</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>288±68</td>
<td>300±69</td>
<td>322±70</td>
<td>322±49</td>
<td>222±55</td>
<td>289±42</td>
</tr>
<tr>
<td>Bilirubin (mg/dl)</td>
<td>2.33±0.11</td>
<td>2.12±0.12</td>
<td>1.88±0.08</td>
<td>2.01±0.1</td>
<td>2.09±0.1</td>
<td>2.12±0.15</td>
</tr>
<tr>
<td>BUN (mg/dl)</td>
<td>31.75±1.36</td>
<td>29.33±1.16</td>
<td>29.22±1.32</td>
<td>28.8±1.59*</td>
<td>30.32±1.35</td>
<td>31.96±1.57</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>1.35±0.04</td>
<td>1.33±0.03</td>
<td>1.34±0.02</td>
<td>1.26±0.02</td>
<td>1.31±0.02</td>
<td>1.33±0.03</td>
</tr>
<tr>
<td>Glucose (mg/dl)</td>
<td>105±1.9</td>
<td>100±4*</td>
<td>108±2</td>
<td>108±2</td>
<td>106±3</td>
<td>106±2</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>82.35±4.2</td>
<td>86.5±4</td>
<td>79.5±3</td>
<td>81.1±3.2</td>
<td>78.7±3</td>
<td>80±3</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>108±10</td>
<td>107±8</td>
<td>115±9</td>
<td>112±9</td>
<td>118±8</td>
<td>121±10</td>
</tr>
<tr>
<td>ALT (IU/L)</td>
<td>7±0.9</td>
<td>8.1±0.6</td>
<td>7.7±0.5</td>
<td>7±0.4</td>
<td>7.5±0.4</td>
<td>7.4±0.4</td>
</tr>
<tr>
<td>ALP (IU/L)</td>
<td>190.7±23.5</td>
<td>220±11.9</td>
<td>209.4±9.4</td>
<td>210±10.4</td>
<td>213±9.4</td>
<td>219±10.2</td>
</tr>
<tr>
<td>GGT (IU/L)</td>
<td>15.6±1.9</td>
<td>17.8±1</td>
<td>17.1±0.8</td>
<td>17.9±1.1</td>
<td>17±0.8</td>
<td>16.1±0.5</td>
</tr>
<tr>
<td>CPK (IU/L)</td>
<td>233±30</td>
<td>322±32</td>
<td>296±22</td>
<td>269±20</td>
<td>282±61</td>
<td>255±48</td>
</tr>
</tbody>
</table>

* Significant difference with first sampling time ($p<0.05$).
Discussion

Venom is a great source of biochemical compounds with considerable pharmacological effects. Venomous animals with this property are a potential source for therapeutic investigations. Tarantula cubensis (Cuban tarantula) is a homeopathic remedy with several therapeutic properties. The effect of its alcoholic extract on wound healing (Sardari et al., 2007), bovine papillomatosis (Cam et al., 2007), and chronic endometritis in dairy cows (Emberg and Sensen, 2007) have been reported, but there is no report of its effect on hematological and serum biochemical parameters.

In the present study, Theranekron administration caused a significant decrease of RBC parameters during the experiment. These decreases reached the lowest level at 8 hours post administration and then increased very slowly toward the pre-administration level. Generally, this RBC decreasing might be a manifestation of a condition that increased erythrocyte destruction, erythrocyte loss through hemorrhage, decreased production of red cell or some combination of these conditions. In the current study, the not really significant changes of WBC, granulocytes and monocytes were in contrast to production disorders of bone marrow.

The not-really-significant changes of platelet ruled out production disorders and hemorrhage while the level of bilirubin was in contrast with RBC destruction. According to the significant decrease of the amount of total protein and albumin, it seems the decrease of RBC parameters was probably related to the shift of interstitial fluid to blood and/or sequestration of RBC in spleen.

Da Silva and colleagues (2003), studied the effects of brown spider (Loxosceles intermedia) venom on hematological parameter of rabbit. They revealed no significant changes in RBC parameters although the number of nucleated red blood cells significantly decreased in bone marrow of experimental rabbits. Futrell, (1992) suggested no evidence of hemolytic anemia in rabbits following spider venom administration.

In our study, the administration of alcoholic extract of tarantula cubensis had no significant effects on the value of WBC, and any type of leukocytes. In contrast, da Silva 2003, reported significant changes in the values of WBC, and neutrophils in blood of rabbits were received brown spider venom. They believed these changes attributed to transient bone marrow depression, influx of neutrophils to tissue and tissue necrosis due to the venom. In agreement with our results, no significant changes were reported for eosinophil numbers in rabbits following brown spider envenomation (da Silva et al., 2003).

The venom of brown spider is able to promote thrombocytopenia (da Silva et al., 2003). They believed this effect could be due to bone marrow depression of megakaryocytes and also extensive consumption of platelets at the site of bite and direct effect of venom. In our study, we did not see any significant changes on platelet number. Morphological changes of human RBC as spherocytosis caused by red – back spider (Latrodectus mactans) venom was reported (Flachsenberger et al., 1995). We did not observe such an abnormal morphologic change of RBC in horses following administration of alcoholic extract of tarantula cubensis. Also, fibrinogen concentration was not changed significantly during the current study. This indicated that Theranekron did not have any inflammatory effects in experimental horses.

In the present study, the levels of urea and creatinine significantly decreased up to 48 hours after drug administration and then slowly increased. It seems that increased renal clearance due to the higher glomerular perfusion caused these changes. Similarly, the decreased levels of bilirubin and cholesterol could be attributed to the higher amounts of hepatic uptake and excretion via bile. Absence of significant changes of AST and GGT activity indicated that Theranekron did not have any adverse effects on liver. The significant changes of CK and ALT activity were probably originated from striated muscle.

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but the exact mechanism was not clear. Also there was no clear explanation for cause of significant changes of ALP activity; however it may originate from the liver.

In conclusion most of the observed changes in hematological and serum biochemical parameters were statistically and not clinically significant. Thus it seems the administration of alcoholic extract of *Tarantula cubensis* (brown spider) has not any adverse reaction in experimental horses.

**References**


تأثیر ترانکرون، عصاره الکلی تراناتولا کوبنیسیس بر همآبندی و خواص بوتیومایی سرم در اسب

کامران سرداری٠، مهرداد مهری١، صدف سبزواری٢، بهروز فتحی٣

چکیده

ترانکرون بطور عصاره الکلی تراناتولا کوبنیسیس (عنکبوت قهوه‌ای) در بارز موجود است. در مطالعه حاضر ده‌سب سالم نزدیک توزیع مسابقه ای مورد استفاده قرار گرفت. نمونه‌های خون ۳۰ دقیقه قبل و بعد از تزریق زیر جلده به سه لیتر (۱:۱:۱) گرم به‌راهنما ۴۸ کیلوگرم وزن دهنده و ۱۰۰۰ میلی‌گرم از میوه کارانیه به ثبت رسید. نتایج این مطالعه شان داشت که زمان نمونه کردن بر مقدار ظرفیت بالایی (۱۸۸/۱۸) دراز است. تأثیر دردسر گرم ۳۰ در در ضرورت است. خون‌های سه گروه از قارچی و همگونی نیز نشان دادند. این مطالعه نشان داد که تأثیر آموزش گونه‌ای در این گروه‌ها ممکن است باشد. در نظر گرفتن نتایج این مطالعه در استفاده از الکلیه در طب سالمندی ممکن است خواص ویژه این گونه از نظر دارویی مورد توجه قرار گیرد.

واژگان کلیدی: عنکبوت قهوه‌ای، زهر، اسب، ترانکرون، تراناتولا کوبنیسیس