Indian Journal of Traditional Knowledge
Vol 23(9), September 2024, pp 869-872
DOI: 10.56042/ijtk.v23i9.13617

The effect of different doses of *Tarantula-Logoplex*® on oxidative status and biochemical parameters in Japanese quails

Oznur Tufan[a], Mustafa Sedat Arslan[b], Muhittin Uslu[a] & Ayse Er[a,*]

[a]Department of Pharmacology and Toxicology & [b]Department of Anatomy,
Veterinary Faculty, Selcuk University, Konya 42031, Turkey
[*]E-mail: aer@selcuk.edu.tr

Received 05 April 2022; revised 08 December 2023; accepted 27 August 2024

Two commercial forms of *Tarantula cubensis* (TC) extract are used as homeopathic agents in veterinary medicine. These are *Tarantula-Logoplex*® (TL) and *Theranekron*® (THR). This study aims to evaluate the effect of TL on albumin (ALB), aspartate aminotransferase (AST), alkaline phosphatase (ALP), cholesterol (CHO), creatinine (CRE), blood urea nitrogen (BUN), malondialdehyde (MDA), superoxide dismutase (SOD) and catalase (CAT) values in healthy quails. A total of 24 Japanese quails were divided into 2 equal groups TL0.6 (n: 12, 0.6 mL/animal, IM) and TL0.8 (n: 12, 0.8 mL/animal, IM). Before the application, control blood was taken from the medial metatarsal vein of 6 quails in each group. Blood was drawn from the medial metatarsal vein at the 2nd, 4th, 8th, 24th and 48th hours after administration (6 quails were used at each sampling time). Animals were euthanized by decapitation after blood collection at the last sampling time. ALB, AST, ALP, CHO, CRE and BUN levels were measured with an auto-analyzer, whereas MDA, SOD and CAT values were determined with an ELISA reader. TL caused increases in hepatic damage markers (AST, ALP) and fluctuations in oxidative stress markers (MDA, SOD). Due to the increase in AST and ALP values in Japanese quail, it is recommended to be careful when using TL at these doses.

Keywords: Biochemical, Logoplex, Oxidative stress, Quail

IPC Code: Int Cl.[24]: A61K 9/00, A61K 36/00

Homeopathy is a system of alternative medicine defined by Samuel Hahnemann[1]. Extract of *Tarantula cubensis* (TC) has two commercial forms Tarantula-Logoplex® (TL) and Theranekron® (THR). Both are used as homeopathic agents in veterinary medicine. TC extract caused regression of benign mammary tumours and cytotoxic effect on different cancer cell lines[2-4]. Anti-inflammatory, antioxidative, demarcative, regenerative and resorptive effects of THR have been reported in many previous studies. THR has been used successfully in the treatment of oral lesions in the bluetongue disease of cattle, canine mammary tumors and oral papillomatosis, indolent ulcers of cats, foot-and-mouth disease in cattle and endometriosis[2,5-11]. In addition, there are studies examining the biological effects of venom, extracted from a *Philippine tarantula*, κ-theraphotoxin-Cg2a, extracted from the venomous glands of the Chinese earth tiger tarantula *Chilobrachys guangxiensis*, and gomesin, isolated from the haemocytes of the Brazilian tarantula *Acanthoscurria gomesiana*, as well as the contribution of spider web to wound healing[12-15]. Regarding TL, there is only one *in vitro* study. In this *in vitro* study, it was concluded that TL showed a cancer cell-specific effect as a result of its lower cytotoxic effect against normal cells compared to cancer cells[4]. No information was found on the use of THR or TL in Japanese quails.

Free radicals, which are short-lived unstable structures that can be harmful to living tissues, are constantly produced in biological systems. These are produced in large amounts during mitochondrial respiration in cells. Free oxygen radicals produced in the living body are detoxified by enzymatic or non-enzymatic antioxidants to prevent damage to cells. Superoxide dismutase (SOD), one of the enzymatic antioxidants, provides the conversion of superoxide radicals to hydrogen peroxide and oxygen. Produced hydrogen peroxide is inactivated by converting it into water and molecular oxygen by glutathione peroxidase (GPX) and catalase (CAT) enzymes. In cases where the produced free oxygen radicals cannot be sufficiently detoxified, they cause lipid peroxidation in cells, especially the cell membrane,

[*]Corresponding author

and disrupt their structure. As a biomarker of developing lipid peroxidation, the measurement level of malondialdehyde (MDA) or thiobarbuturic acid reactive products (TBARS) is used[10,16,17]. It has been reported that THR shows antioxidant activity in healthy sheep and does not cause significant serological/hematological side effects in healthy horses and sheep[10,18].

Organ damage can be defined by some biomarkers in the blood. The measurement of these parameters in serum or plasma provides information about the safety of the drugs being taken or the course of the disease. Aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin (ALB) concentrations are used as liver damage biomarkers, while blood urea nitrogen (BUN) and creatinine (CRE) concentrations are accepted as kidney damage biomarkers. Cholesterol (CHO) level is accepted lipid metabolism parameters[19].

This study aimed to evaluate the effect of TL on oxidative stress parameters like MDA, SOD and CAT values and biochemical parameters like ALB, AST, ALP, CHO, CRE, and BUN in healthy Japanese quails.

Methodology

Animals used

In the study, 24 (male, 250-290 g) healthy Japanese quails (*Coturnix coturnix japonica*) were used, and the procedure was approved by the Ethics Committee of Experimental Medical Practice and Research Center of Animal Experiments of Selcuk University, Konya, Turkey (2020-80).

Experimental design

Animals were distributed randomly into 2 cleanable cages and each cage contained 12 birds. The animals were given drug-free commercial poultry feed and water ad libitum. Animals were divided into 2 groups TL0.6 (n: 12, 0.6 mL/animal, IM) and TL0.8 (n: 12, 0.8 mL/animal, IM). Before the IM administration of drugs, control blood (1 mL) was taken from the medial metatarsal vein of 6 quails in each group. Then TL (Richter Pharma AG, Austria) was administered to all animals. Blood was drawn from the medial metatarsal vein at the 2nd, 4th, 8th, 24th and 48th hours after administration. Six animals in each group were used for 3 sampling times (0, 4 and 24 h or 2, 8 and 48 h). Animals were euthanized by decapitation after blood collection at the last sampling time. Blood samples were centrifuged at 4,000 g for 10 min and plasma samples were stored at −80°C until analysis. Biochemical parameters (ALB, AST, ALP, CHO, CRE, BUN) were measured with an auto-analyzer (BT-300 plus, Rome, Italy), while oxidative status parameters (MDA, SOD, CAT) were determined with ELISA reader (MWGt Lambda Scan 200, Bio-Tek Instruments, Winooski, VT, USA).

Statistical analysis

The results of the research were evaluated by one-way analysis of variance ANOVA and Tukey test as a posthoc test (SPSS22.0). $p<0.05$ value was considered statistically significant.

Results and Discussion

The oxidative status and biochemical parameters were presented in Table 1 and Table 2, respectively. AST and ALP values increased in both groups. Although there was no statistical change in MDA value in the TL0.8 group, it decreased in the first 24 h, and a statistically insignificant decrease was observed at the 4th hour in the TL0.6 group. Statistical fluctuation was determined in the SOD value of the TL0.8 group.

Although the use of THR has been reported in many animal species[2,5,6,10] there is no information on the use of THR and TL in quail. Statistical fluctuations ($p<0.05$) in MDA were detected in the TL0.6 group but were similar to the control at all sampling times. Although there was no statistical change in the TL0.8 group, a decrease in MDA value was determined in the first 24 h. The SOD activity showed statistical fluctuations in the group applied only TL0.8 group, but the whole sampling times were similar to the control. CAT activity did not show a

Table 1 — The effect of TL application in two different doses on oxidative status parameters

	0. h	2. h	4. h	8.h	24. h	48. h
$MDA_{0.6}$ (µM)	1.988±0.100^{ab}	2.296±0.212^{ab}	1.464±0.144^{b}	2.276±0.267^{ab}	2.499±0.490^{ab}	3.076±0.329^{a}
$MDA_{0.8}$ (µM)	3.514±0.208	3.297±0.209	3.063±0.252	2.802±0.174	2.873±0.245	3.428±0.292
$SOD_{0.6}$ (U/mL)	0.059±0.004	0.052±0.001	0.053±0.002	0.052±0.003	0.062±0.004	0.058±0.003
$SOD_{0.8}$ (U/mL)	0.057±0.002^{ab}	0.062±0.004^{ab}	0.072±0.007^{a}	0.046±0.003^{b}	0.056±0.002^{ab}	0.057±0.003^{ab}
$CAT_{0.6}$ (nmol/min/mL)	1.422±0.180	1.150±0.250	0.910±0.191	0.842±0.107	1.220±0.173	1.252±0.286
$CAT_{0.8}$ (nmol/min/mL)	1.168±0.201	0.692±0.082	1.038±0.214	0.722±0.071	0.770±0.088	1.722±0.716

Table 2 — The effect of TL application in two different doses on biochemical parameters

	0. h	24. h	48. h
$ALB_{0.6}$ (g/L)	7.3±0.6	6.6±0.5	7.3±0.6
$ALB_{0.8}$ (g/L)	7.2±0.5	6.2±0.5	7.0±0.5
$AST_{0.6}$ (U/L)	214±10^{b}	509±50^{a}	522±92^{a}
$AST_{0.8}$ (U/L)	217±11^{b}	518±68^{a}	572±59^{a}
$ALP_{0.6}$ (U/L)	157±6.9^{b}	154±22^{b}	289±54^{a}
$ALP_{0.8}$ (U/L)	157±7.3^{b}	169±23^{b}	312±43^{a}
$CHO_{0.6}$ (mg/dL)	185±11	153±17	195±12
$CHO_{0.8}$ (mg/dL)	186±12	185±16	180±9.5
$CRE_{0.6}$ (mg/dL)	0.29±0.01	0.26±0.02	0.27±0.01
$CRE_{0.8}$ (mg/dL)	0.29±0.01	0.26±0.01	0.26±0.01
$BUN_{0.6}$ (mg/dL)	2.0±0.0	2.5±0.2	1.8±0.5
$BUN_{0.8}$ (mg/dL)	2.0±0.0	1.7±0.3	2.5±0.2

statistical change in both groups (Table 1). While there are studies on the effect of TH on blood/tissue oxidative parameters in healthy or experimental model animals, there is no information about the effect of TL on these parameters when applied to healthy animals. It has been reported that after THR application, TBARS level decreased in healthy sheep[10], while the MDA value was like the control value[20] or increased[21] in healthy rats. In this study, the not statistically significant decrease in MDA value at the first 24 h and change in SOD activity in the TL0.8 group indicates the presence of the antioxidant effect of TL. Accordingly, it can be stated that TL may also have an antioxidant effect as it does in THR.

In both groups, increases in AST at 24 and 48 h were statistically significant, while increases in ALP at 48 h were significant (Table 2). Although there were fluctuations after THR application in horses and sheep, no difference was detected in AST and ALP values[10,18]. Aksit *et al.*[22] stated that there was a statistical increase in these values after THR administration in healthy rats, but when given together with gentamicin, it prevented the increase caused by gentamicin. It is reported that AST and ALP values in cadmium and treatment groups in chickens were not different from healthy animals[23]. When the ALB values of both groups were compared with the control group, no statistical change was determined, but the decrease in the 24th hour values increased at the 48th hour and returned to the control values. It has been reported that THR application does not affect the albumin value in horses and sheep[10,18]. It was reported that there was a statistical decrease in the ALB value after THR administration in healthy rats, but when given together with gentamicin, it also prevented the decrease caused by gentamicin[22]. CHO and CRE values were not affected in both groups (Table 2), similar results were found in studies at horses and sheep[10,18]. In this study, the BUN value showed a non-statistically significant increase in the TL0.6 group at the 24th hour and in the TL0.8 group at the 48th hour. While a significant decrease was reported at the 48th hour in horses, a statistically insignificant increase was reported over time in sheep[10,18]. This study demonstrated that TL liver damage is evidenced by the elevation of blood AST, ALP, and a decrease in albumin. In addition, the fact that there are different results depending on the dose in the BUN value, which is considered a biological marker of kidney damage, shows that further experimental studies are needed at the molecular level.

Conclusion

Although it was observed that 0.8 mL of TL may also have an antioxidant effect in Japanese quails, it is recommended to be careful during its use due to its harmful effects on hepatocytes as reflected by enhancing liver enzymes in blood.

Acknowledgements

The authors would like to thank Prof. Dr. Enver YAZAR for his scientific contributions.

Conflict of Interest

The authors declare that no conflict of interest exists.

Author Contributions

AE contributed in designing and conducting the study; reviewing, analyzing results and preparing manuscript; OT, MSA and MU guided in planning the study, reviewing the data and approval of manuscript.

Ethical Approval

The procedure was approved by the Ethics Committee of Experimental Medical Practice and Research Center of Animal Experiments of Selcuk University, Konya, Turkey (2020-80).

Data Availability

Data will be made available on request.

References

1 Pekmezci D & Gultiken N, Principles of homeopathy and applications in veterinary practice, *J Fac Vet Med Erciyes Univ*, 12 (1) (2015) 49-56.

2 Gultiken N & Vural M R, The effect of *Tarantula cubensis* extract applied in pre and post-operative period of canine mammary tumors, *J Istanbul Vet Sci*, 2 (2007) 13-23.
3 Pulat C C, *In vitro* cytotoxic activity of Tarantula cubensis alcoholic extract on different human cell lines, *Cumhuriyet Sci J*, 42 (2) (2021) 252-259.
4 Ilhan S, Can a veterinary drug be pepurposed for human cancers?: Cytotoxic effect of *Tarantula cubensis* venom on human cancer cells, *J Inst Sci Technol*, 11 (3) (2021) 1763-1769.
5 Yardımcı C & Yardımcı B, Indolent ulcer in a cat, *Ankara Univ Vet Fak Derg*, 55 (1) (2008) 65-67.
6 Albay M K, Sahinduran S, Kale M, Karakurum M C & Sezer K, Influence of *Tarantula cubensis* extract on the treatment of the oral lesions in cattle with bluetongue disease, *J Fac Vet Med Univ Kafkas*, 16 (4) (2010) 593-596.
7 Icen H, Sekin S, Simsek A, Kochan A & Tunik S, The efficacy of *Tarantula cubensis* extract (Theranekron) in treatment of canine oral papillomatozis, *Asian J Anim Vet Adv*, 6 (7) (2011) 744-749.
8 Lotfollahzadeh S, Alizadeh MR, Mohri M & Dezfouli M R M, The therapeutic effect of *Tarantula cubensis* extract (Theranekron®) in foot-and-mouth disease in cattle: A randomised trial in an endemic setting, *Homeopathy*, 101 (3) (2012) 159-164.
9 Dolapcioglu K, Dogruer G, Ozsoy S, Ergun Y, Ciftci S, *et al*., Theranekron for treatment of endometriosis in a rat model compared with medroxyprogesterone acetate and leuprolide acetate, *Eur J Obstet Gyn Reprod Biol*, 170 (1) (2013) 206-210.
10 Dik B, Er A & Corum O, Effect of alcoholic extract of *Tarantula cubensis* (Theranekron®) on serum thiobarbituric acid-reactive species concentrations in sheep, *Eurasian J Vet Sci*, 30 (2) (2014) 68-71.
11 Abdullah A, Çiğdem Y, Tuğrul E E & Erhan A, Effect of intravesical tarantula cubensis extract (Theranekron) on inflammation in an interstitial cystitis rat model, *Low Urin Tract Symptoms*, 15 (2) (2023) 63-67.
12 Kumari P, Chahar M K, Veerapur V P, Spandana G, Thippeswamy B S, *et al*., Spider web ointment: A traditional based approach in cutaneous wound healing, *Indian J Tradit Know*, 12 (4) (2013) 657-663.
13 Tanner J D, Deplazes E & Mancera R L, The biological and biophysical properties of the spider peptide Gomesin, *Molecules*, 23 (7) (2018) 1733.
14 Lopez S M M, Aguilar J S, Fernandez J B B, Lao A G J, Estrella M R R, *et al*., The venom of Philippine Tarantula (Theraphosidae) contains peptides with pro-oxidative and nitrosative-dependent cytotoxic activities against breast cancer cells (MCF-7) *In vitro*, *Asian Pac J Cancer Prev*, 21 (8) (2020) 2423-2430.
15 Zaheer Z A & Sankaranarayanan K, *In silico* analysis of κ-theraphotoxin-Cg2a from *Chilobrachys guangxiensis*, *Indian J Biochem Biophys*, 57 (4) (2020) 458-466.
16 Yazar E & Tras B, Free oxygen radicals, antioxidant enzymes and antibiotics, *J Turk Vet Med Assoc*, 14 (2002) 42-44.
17 Mayne S T, Antioxidant nutrients and chronic disease: use of biomarkers of exposure and oxidative stress status in epidemiologic research, *J Nutr*, 3 (2003) 933S-940S.
18 Sardari K, Mohri M, Sabzevari S & Fathi B, Effects of the Theranekron® an alcoholic extract of the Tarantula cubensison hematology and serum biochemical properties in horses, *Iran J Vet Sci Technol*, 3 (2) (2011) 9-16.
19 Turgut K, Veterinary Clinic Laboratory Diagnosis, (Bahcivanlar Press, Konya, Turkey), 2000.
20 Eren C & Aksit D, Investigation of the protective effects of *Tarantula cubensis* extract in rats with experimental gentamicin nephrotoxicity, *Anim Health Prod Hyg*, 12 (1) (2023) 31-39.
21 Guler F, Kozlu T, Ergun Y, Alan S B & Tutar T, The effect of Theranekron® in intact and with ischemia-reperfusion injury rat ovary, *J Etlik Vet Microbiol*, 34 (1) (2023) 23-29.
22 Aksit D, Aksit H, Altun E, Celebi C & Celebi M, Investigation of the protective effect of Tarantula cubensis extract on the liver and brain of rats exposed to gentamicin, *Acta Vet Brno*, 92 (2023) 205-211.
23 Patel U D, Bhatt P R, Pandya K B, Patel H B & Modi M, Cadmium induced oxidative stress-mediated pathophysiological alterations in chickens and their amelioration by polyherbal mixture enriched feed, *Indian J Tradit Know*, 20 (1) (2021) 41-53.