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Tribulus Terrestris L Extract Effects on Slow Down Ageing Process

Xiao Tian Mei[1, 2], Xu Liang[2], Amgalanbaatar Dorjkhuu[3], Sumaya Tsegmid[1], Bai Mei Rong[2]

[1]Department of Chemistry, College of Mathematics and Technology, Mongolian National University of Education, Ulaanbaatar, Mongolia; [2]Department of Chemistry, College of Chemistry and Material, Inner Mongolian MINZU University, Tongliao, China; [3]Department of Anatomy, School of Biomedicine, Mongolian National University of Medical Sciences, Ulaanbaatar, Mongolia

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Corresponding Author
Xiao Tian Mei, MS, Associated Professor
Department of Chemistry, College of Mathematics and Technology, Mongolian National University of Education, Ulaanbaatar 14120, Mongolia
E-mail: xtm1234@126.com

Objective: Tribulus Terrestris is a medicinal plant cultivated in temperate regions of the world. Inner Mongolia is one of the main producing areas of Tribulus Terrestris. We aimed in this study to examine the effect of Tribulus Terrestris extract on the aging process. **Methods:** A total of 50 mice were used for the experiment. They were divided randomly into 5 groups (10 animals in each group): normal control group and four aging groups. **Results:** Compared to the normal aging group, the parameters of the thymus gland and spleen of an aging group fed with a high and medium amount of Tribulus has visibly increased ($p < 0.05$). The content of blood catalase (CAT) and superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) of blood serum of an aging group fed with a high and medium amount of Tribulus Terrestris has visibly increased ($p < 0.05$) compared to that in the aging group. Also, the content of malondialdehyde (MDA) in blood serum has visibly lowered. We further have measured the SOD, GSH-Px content in brain tissue of each group of mice. **Conclusion:** Compared with the normal control group, the SOD content was decreased significantly and MDA content was significantly increased in the aging group of mice.

Keywords: Zygophyllaceae, Tribulus Terrestris, Larrea, Aging Process

Introduction

The world population is rapidly going through an aging process and this process also tends to be increased in the future. Today almost 1 in 10 people are over 60 years old and this number is estimated to double by 2050. Aging is a physiological process associated with morphological as well as functional changes in cellular components. It results to the progressive imbalance of the hormonal, autocrine, neuroendocrine, and immune homeostatic mechanisms of the organism. As population ageing continues

to accelerate, health expenditures tend to grow rapidly and health care spending is directed towards the elderly population. Especially, aging associated disorders such as diabetes, cancer, neuronal disorders are the burning question to be solved by scientists. It has been revealed that aging accounts for more than 50% of the cases of sporadic Alzheimer's disease [1].

It has been speculated that reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, hydroxyl radicals are the primary determinant of aging. As a metabolically active organ, the brain consumes a large amount of oxygen and produces ROS. Then, produced ROS contributes to the imbalance of physiological functions, increase of the pathological conditions as well as reduction of the life span. The mitochondrial theory of Wallace hypothesizes that ROS produced by the electron transport chain can damage its own deoxyribonucleic acid (DNA) in the mitochondria where essential genes for energy production are encoded. As mitochondrial function decreases, it results to wide range of age-related disorders and various forms of cancer [2].

There is a broad spectrum of antioxidant compounds found in foods, spices, herbs, and medicinal plants which have significant effects on antioxidants to prevent the aging process [3 - 8]. Aliahmat et al. revealed that Piper betle extract increases the activities of catalase, glutathione peroxidase, and superoxide dismutase in erythrocyte of young, middle, and old age mice [9]. Further, Terminalia chebula (T. chebula) aqueous extract was also shown to be effective against oxidative stress and enhance antioxidant status in the liver and kidney of aged rats [10]. Zhou et al. demonstrated that oral administration of Glycyrrhiza glabra preparation increased superoxide dismutase (SOD), glutathione peroxidase (GSH-Px) and catalase (CAT) activity and synchronously decreased the content of malondialdehyde (MDA) in the hippocampus of aging rats induced by D-galactose (300 mg/kg−1). It also ameliorated the dysfunction of the cholinergic system induced by D-galactose, and significantly reduced the expression of the Bax/Bcl-2 protein ratio, caspase-3 and Cyt-C protein in the hippocampus [11].

Tribulus Terrestris is an herb belonging to the Zygophyllaceae family distributed across the regions of southern Europe, southern Asia, throughout Africa, New Zealand, and Australia. It possesses various pharmacological properties and roots and fruits have been used for thousands of years in China as a folk medicine [12]. Tribulus Terrestris is rich in steroidal saponins, flavonoids, glycosides, phytosterols, tannins, terpenoids, and among them, steroidal saponins (spirostanol, furostanol) and flavonoids (quercetin, kaempferol, isorhamnetin) are considered to be the most important metabolites with various bioactivities. The extract of Tribulus Terrestris has been shown to increase testosterone level and improves fertility potential [13 - 15]. Ko et al. showed that ethanolic extract of the Tribulus Terrestris has significant anti-inflammatory effect and inhibits the production of nitric oxide (NO), TNF-α, IL-6 and IL-10 in lipopolysaccharide (LPS) stimulated RAW264.7 cells [16].

Despite advances in the understanding of the above-mentioned activities of Tribulus Terrestris, no research has yet answered the question of how Tribulus Terrestris extract can benefit the aging process. In this study, therefore, we aimed to study the effects and mechanisms to inhibit and slow down the aging process with Tribulus Terrestris by the requirements of clinical use in Mongolian medicine. This study is the first step in establishing the anti-aging action and mechanism of dehydrated Tribulus Terrestris aqueous extract on animal experiments. We have used dehydrated Tribulus Terrestris aqueous extract and determined the content of blood CAT, SOD, and MDA of blood serum, and SOD, MDA, and GSH-Px of brain tissue in an aging mouce model induced by D- galactose.

Materials and Methods

Preparation of the aqueous extract

For this clinical usage and property, 2 kilograms of Tribulus Terrestris was dehydrated in low heat until it changes into a yellow color, then ground. An appropriate amount of Tribulus Terrestris was soaked in water for 2 hours, adjusted with 10 times the amount of water and boiled for 1 hour at a low temperature. Next, we removed the solution and again added 8 times the amount of water to the drug slag and boiled for 1 hour. In the last extraction, again we removed the solution, added 6 times the amount of water to the remaining drug slag and heated for 1 hour on low heat. The extracted solution was mixed three times, then thickened to 1 mg/ml using a rotary evaporator.

Research design

A total of 50 mice were used for this experiment. They were divided randomly into 5 groups (10 animals in each group): a normal control group and four aging groups. All animals were

supplied with standard food during the experiment with access to water. Experimental procedures were conducted according to the regulations of the Animal Ethical Committee. The aging mouse model induced by D-galactose was prepared according to Guo et al [17]. Briefly, D-galactose (100 mg/kg) was injected every day in the aging group, while a physiological saline solution was injected into the normal group. After the last injection on the 60th consecutive day, we injected a 1% pentobarbital sodium ($C_{11}H_{17}O_3N_2Na$) into the abdominal cavity, anesthetizing the animals, and collect a blood sample from the abdominal aorta. The blood sample was centrifuged at 3500 rpm and relevant parameters were measured including malondialdehyde (MDA) and activities of superoxide dismutase (SOD), catalase (CAT), and Glutathione Peroxidase (GSH-Px). At the same time, the thymus gland and spleen were weighted and the organs' parameters were determined [18 - 19].

Effects of tribulus terrestris on the aging process

A total of 50 mice were used for the experiment. They were divided randomly into 5 groups: normal control group, aging group, aging group fed with a high amount of Tribulus Terrestris, aging group fed with a medium amount of Tribulus Terrestris, and an aging group fed with a small amount of Tribulus Terrestris based on their body weights. From the first day of the experiment, saline solution with D - Galactose (100 ml/kg) was injected every day into the aging group of mice, while the same amount of saline solution was injected once a day into the normal control group of mice for 60 consecutive days. At the same time, from the first day of the experiment, an appropriate amount Tribulus Terrestris solution was injected into the aging groups with high, medium and low amounts of Tribulus Terrestris. After the last injection, 1% of pentobarbital sodium was injected into the abdominal cavity, and a blood sample was collected from the abdominal aorta. Further, the thymus gland and spleen were weighted, and a sample was taken from brain tissue, washed, kept in liquid nitrogen for the further experiment.

Anti-necrosis of mouse liver cells in Tribulus Terrestris retardation model

Liver tissue was washed once with phosphate-buffered saline (PBS) and centrifuged at 3500 rpm for 5 minutes. Released cells (1 x 106 cells/ml) were mixed with 5 µl Annexin V and 5 µl Nucleic acid dye, gently mixed and kept dark at room temperature for 10 minutes, and the necrosis was measured with a cytometer apparatus.

Statistical analysis

The average value of continuous variables which are thymus gland, spleen, SOD, MDA, CAT, GSH and cell necrosis in the normal, aging and tribulus groups was compared using Kruskal-Wallis test. Multiple comparisons between the control and aging were made using Dunn's test. Significant values have been adjusted by the Bonferroni correction for multiple comparison tests. Mann-Whitney U test was used to compare the average values in two groups. A critical p-value of < 0.05 was used. SPSS version 24 software (SPSS Inc., Chicago, IL, USA) was used for statistical analyses.

Ethical statement

Our animal study was carried out following the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80-23), revised in 1996. Formal approval to conduct the experiments was obtained from the Ethical Committee of the Baotou Medical College (Protocol No. BTMY201901). All efforts were made to minimize the number of animals used and their suffering.

Results

In Table 1, we described the differences between the organ parameters as well as SOD and MDA changes in normal and aging group of mice. The aging group of mice's organ parameter were visibly lowered compared to the normal controlling group ($p < 0.05$). Moreover, SOD has visibly decreased ($p < 0.05$) in the aging group and MDA in blood serum was visibly increased ($p < 0.05$) comparing to a normal control group.

Next, we have examined the effects of Tribulus Terrestris to the parameters of thymus gland and spleen of an aging group of mice. Compared with the normal control group, the parameters of the thymus gland and spleen of an aging group were visibly lowered ($p < 0.05$). On the other hand, compared to the aging group, the parameters of the thymus gland and spleen of an aging group fed with a high and medium amount of Tribulus has visibly increased ($p < 0.05$).

However, the parameters of the thymus gland and spleen of an aging group that were fed with a small amount of Tribulus was only slightly changed ($p < 0.05$). Further, effects of Tribulus Terrestris on the quantitative concentrations associated with all blood serum in the aging model was determined (Table 2).

Table 1. Different group of mice's organ parameter and SOD, MDA changes.

	Groups		
	Control (n = 10)	Aging (n = 10)	*p-value
Parameters	**Mean ± SD**	**Mean ± SD**	
Thymus gland	0.042 ± 0.003	0.023 ± 0.001	0.061
Spleen	0.053 ± 0.004	0.031 ± 0.008	0.057
SOD (nmol/mg)	439.87 ± 56.10	211.04 ± 60.53	0.032
MDA (nmol/mg)	2.95 ± 0.71	6.06 ± 0.99	0.010

*Mann-Whitney U test

Table 2. Changes of organ parameters, serum CAT, SOD and GSH-Px in these groups of mice.

				Tribulus		
	Control (n = 10)	Aging (n = 10)	Small (n = 10)	Medium (n = 10)	High (n = 10)	
Variables	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	***p-value**
Thymus gland	0.040 ± 0.002	0.021 ± 0.009	0.027 ± 0.014	0.035 ± 0.008	0.038 ± 0.003	0.067
Spleen	0.051 ± 0.003	0.030 ± 0.002	0.036 ± 0.0219	0.043 ± 0.008	0.05 ± 0.01	0.430
SOD (nmol/mg)[a, b, c]	465.0 ± 87.5	218.30 ± 65.01	240.14 ± 88.12	348.18 ± 64.87	351.12 ± 94.15	0.045
MDA (nmol/mg)	2.48 ± 0.35	5.674 ± 1.25	4.95 ± 1.59	3.55 ± 0.97	3.10 ± 0.69	0.078
CAT (ug^{-1}·Hb)[d, e]	98.07 ± 17.98	60.13 ± 14.20	69.27 ± 15.87	80.46 ± 12.32	83.04 ± 13.81	0.021
GSH-Px ($u·mg^{-1}$)	4258.1 ± 1776.5	2603.13 ± 1114.2	2969.29 ± 1395.8	3480.41 ± 1028.4	3835.2 ± 1309.8	0.081

*Kruskal-Wallis test; [a]control vs. aging, $p < 0.041$; [b]control vs. medium, $p < 0.050$; [c]small vs. high, $p < 0.042$; [d]aging vs. high, $p < 0.014$, [e]small vs. high, $p < 0.051$.

Table 3. SOD, GSH-Px activity of a brain tissue and the content change of MDA, CAT in this group.

				Tribulus		
	Control (n = 10)	Aging (n = 10)	Small (n = 10)	Medium (n = 10)	High (n = 10)	
	Mean ± SD	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	**Mean ± SD**	***p-value**
SOD (nmol/mg)[a, b, c]	163.032 ± 47.32	88.302 ± 45.20	100.103 ± 48.24	128.064 ± 40.08	131.405 ± 34.50	0.013
MDA (nmol/mg)	10.465 ± 3.21	20.542 ± 4.28	17.992 ± 4.97	13.041 ± 2.10	12.162 ± 3.95	0.821
CAT ($u·g^{-1}$·Hb)[d]	8.06 ± 3.98	12.18 ± 6.21	11.02 ± 5.89	10.46 ± 6.32	10.04 ± 5.82	0.050
GSH-Px ($u·mg^{-1}$)	424.07 ± 197.8	330.1 9 ± 124.1	359.74 ± 115.6	370.65 ± 122.9	403.36 ± 213.6	0.133

*Kruskal-Wallis test; [a]control vs. small, $p < 0.010$; [b]control vs. high $p < 0.031$; [c]small vs. high, $p < 0.022$; [d]control vs. high, $p < 0.031$.

The content of blood CAT, SOD, and GSH-Px of blood serum of the aging group had visibly lowered ($p < 0.05$) compared with normal control group, and MDA in blood serum had visibly increased. The content of blood CAT, SOD, and GSH-Px of blood serum of the aging group fed with a high and medium amount of Tribulus Terrestris had visibly increased ($p < 0.05$) compared to that in the aging group fed lower amounts. Also, the content of MDA in blood serum had visibly lowered. There was little change in the content of blood CAT, SOD, and GSH-Px of blood serum of the aging group that has fed with a small amount of Tribulus Terrestris ($p > 0.05$).

We further measured the SOD, and GSH-Px content in brain tissue of each group of mice (Table 3). Compared with the normal control group, the SOD content was decreased significantly and

Table 4. Effects of Tribulus Terrestris aquaeous extract in a cell necrosis on an aging model induced by D- Galactose.

	Control (n = 10)	Aging (n = 10)	Tribulus Small (n = 10)	Tribulus Medium (n = 10)	Tribulus High (n = 10)	
	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	*p-value
Cell necrosis[a, b]	0.64 ± 0.14	3.04 ± 0.47	0.98 ± 0.19	1.54 ± 0.28	1.94 ± 0.67	0.001

*Kruskal-Wallis test; [a]control vs. aging, $p < 0.001$; [b]control vs. high, $p < 0.012$.

MDA content was significantly increased in the aging group of mice. However, when mice were treated with Tribulus Terrestris extract, SOD and GSH-Px content were increased dependent on the dose in each group of mice. Further CAT and MDA concentration were also decreased dose-dependently, and significant change was observed at high and medium Tribulus Terrestris extract groups.

In Table 4, we showed the effect of aqueous extract of Tribulus Terrestris on necrosis of the aging group of mice. As can be seen here, cell necrosis was highly observed in the aging group of mice. However, when treated with a low dose of Tribulus Terrestris, we could not observe a high amount of cell necrosis. On the other hand, when medium as well as high amount of the extract were used, cell necrosis got higher, but not higher than non-treated aging mice.

Discussion

By the cellular theory, aging is a process characterized by progressive loss of tissue and organ function. Studies have revealed that there are several aging mechanisms including telomere shortening, mitochondrial dysfunction, and cellular senescence. Further, it has been suggested that free radicals play a significant role in age related disease such as cardiovascular diseases, neurodegenerative diseases, and cancer [20]. When the cell loses or accepts a single electron, it produces ROS and reactive nitrogen species. Especially mitochondrial ROS contribute significantly to the aging process through the mitochondrial dysfunction from oxidative stress [21, 22].

Recent pharmacological studies have shown many capabilities of medicinal plant extracts on the aging process. For example, Zhao et al. demonstrated that a walnut oil capsule improved the pathologic lesions caused by oxidative stress and significantly enhance the total antioxidant capacity (T-AOC), increase the activities of SOD, GSH-Px and decrease the contents of MDA in serum, liver and brain of galactose induced aging mice [23]. Another study of Mu et al. also showed that Ilex kudingcha C.J. Tseng tea and insect tea regulate viscera indices of major organs, improve liver, skin, and spleen tissue morphology; decrease inflammatory cytokine production; up regulate SOD, CAT, GSH, GSH-PX, and T-AOC and down regulate NO and MDA levels in serum and liver tissue. These preparations also upregulate gene and protein expression of GSH-PX, GSH1, SOD1, SOD2, and CAT [24]. Ficus vasculosa ethanol extract also significantly increased the level of GSH in serum, hepatic tissue and kidney, significantly decreased MDA production in hepatic tissue and kidney. In addition, the phytochemical investigation discovered six compounds from the extract: naringenin; vanillic acid; 9, 16-dioxo-10, 12, 14-octadeca-trienoic acid; 2, 6-dimethoxy-1, 4-benzoquinone; apigenin; and norartocarpetin [25].

Tribulus terrestris is an herb belonging to the Zygophyllaceae family. Its root and fruits are widely used in traditional Chinese and Indian medicine. Chemical composition of this plant revealed that steroids, saponins, flavonoids, sterols, Harman alkaloids, minerals, lignan- amides, and cinnamic acid amides are rich in this plant [26, 27]. Li et al. demonstrated that Tribulus Terrestris significantly reduced the level of serum glucose in diabetic mice and the level of serum triglyceride was reduced 23.35%. Saponin of Tribulus Terrestris decreased the content of serum cholesterol and increased serum SOD activity [28]. Another study by Amin et al. also showed that arabian Tribulus Terrestris extract significantly decreased the levels of alanine amino transferase (ALT) and creatinine in the serum of diabetic rats and lowered the MDA level in liver, which in turn indicates that Tribulus Terrestris could be an important preparation for the recovery of the liver [29].

Tribulus Terrestris is also a part of Mongolian traditional medicine that has a long history [29]. This study is the first step in establishing the anti-aging action and mechanism

of dehydrated Tribulus Terrestris aqueous extract on animal experiments. Based on previous studies, a D-galactose-induced accelerated aging model was developed. As expected, the group of mice fed with high concentrations of D-galactose showed significantly decreased organ parameters for thymus gland and spleen. Moreover, SOD was significantly decreased in the aging group (211.04 ± 60.53) compared with normal mice (439.87 ± 56.10), and MDA was significantly increased in aging mice (6.06 ± 0.99 vs 2.95 ± 0.71). When we treated aging mice with different amount of Tribulus Terrestris aqueous extract, especially medium and high amount of the extract, it resulted in significantly recovered organ parameters. Moreover, serum SOD and MDA activity was also recovered with high and medium amount of the extract feeding. We have also measured SOD and GSH-Px activity in brain tissue of treated and non-treated groups of mice. The high and medium amount of feeding in aging mice resulted in increased SOD and GSH-Px activity, while CAT and MDA content of the brain tissue was significantly decreased compared with non-treated aging mice. The result of the present study is expected to be used for the optimal use of clinical and further studies of Tribulus Terrestris.

The major limitation of the present study was that we examined crude plant extract of Tribulus Terrestris. As usual, hot water extraction gives significant number of polar compounds on the extract, which may act synergistically on the model animals. On the other hand, extraction by nonpolar solvents such as hexane or chloroform could be important to distinguish more bioactive compounds of Tribulus Terrestris. Therefore, due to the significance of Tribulus Terrestris extract on the aging model, active compound determination from the both hot water and nonpolar solvent extraction, and other anti-oxidant activity measurement will be the future direction of this study.

Conclusion

The quantitative parameters of the organ in an aging group have visibly decreased comparing to the normal control group. The content of SOD has visibly decreased, but the content of MDA has visibly increased. Tribulus Terrestris has been affective in visibly increasing quantitative parameters of organs, also it led blood CAT and the content of SOD, GSH-Px in blood serum to a high level. However, it led the content of MDA to visibly decreased levels. Tribulus Terrestris's effect on SOD, GSH-Px, and CAT in brain tissue had visibly increased to a high level, yet the content of MDA has decreased. Aqueous extracts of Tribulus Terrestris have the effect of inhibiting the necrosis of liver cells in the aging model.

Conflict of Interest

The following authors have no conflict of interest.

Asknowledgement

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