

Antihyperlipidaemic Activities of *Agriophyllum squarrosum* (L.) Moq. *in vivo*

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Abstract

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Agriophyllum squarrosum (L.) Moq. is medicine herb used as a traditional Mongolian drug. Its pharmacological efficacy studies are rarely reported. In the present study, we tested and confirmed the antihyperlipidaemic activity of *A. squarrosum* and its extracts in rats for the first time. The results of the antihyperlipidaemic experiment *in vivo* showed that *A. squarrosum* and its extracts had significant lipid-lowering activities. The significances of *A. squarrosum* and its extracts on decreasing blood levels of total cholesterol, triglyceride, and low density lipoprotein cholesterol were equal to that of simvastatin, which is a drug for the treatment of hyperlipidaemic.

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Introduction

Agriophyllum squarrosum is a species of the family Chenopodiaceae. It is commonly known as “Chuliger” in Mongolian. The full herb is light green therophyte and between 15 -50 centimeters of high with stiff stem. *A. squarrosum* grows in flowing sand dunes or lowland dunes, and distributed from north parts of China to Mongolia, Russia. It is widely grown in Khorchin, Liaohe plain, Huxi plateau, Wulanchabu, Ordos and Alashan of Inner Mongolia autonomous region, China. In Mongolian folk medicine, *A. squarrosum* was used for removing disease, detoxication, reducing fever and causing dieresis; Its decoction or pill was used to treat plague, headache, red eye, yellow gangrene, kidney fever, urethral burning pain, stomach “Hei”, mouth sores and tooth fester among other thing (Erdenebilig, 2014). In the

previous studies, the flavonoids, triterpenoids, sterides, coumarins, alkaloids and fatty acids as the main compounds of *A. squarrosum* were reported (Gong, 2012; Zhou, 2012; Liu, 2013). The antidiabetics activity of *A. squarrosum* were studied (Bao *et al.*, 2016), however, other pharmacological efficacy studies are rarely reported and haven't been included in to national pharmacopoeia and local medicinal standards. In the research of active parts of *A. squarrosum*, we studied that *A. squarrosum* and its extracts had an anti-hyperlipidemia activity on high fat rats model for the first time.

Experimental Procedures

A total of 60 males of Wistar rats (Research Centre for Laboratory Animal Science, Beijing,

China) were used in the following in vivo experiments. The rats were housed in plastic cages under controlled conditions ($24\pm 0.5^{\circ}\text{C}$; humidity $55\pm 5\%$; 12h light/dark schedule) and maintained according to the Guide for the Care and Use of Laboratory Animals established by Inner Mongolia University (IMU, Hohhot, China). After acclimation for one week, the rats were randomly divided into six groups ($n = 10$), including two control groups (normal diet (ND) and high-fat diet (HFD)), and four treatment groups (simvastatin, *A. squarrosum*, aqueous extracts of *A. squarrosum* and ethanol extracts of *A. squarrosum*). The ND group was fed a standardized laboratory chow diet, while the 50 rats in the other 5 groups were fed an HFD supplemented with 3% cholesterol, 0.5% sodium cholate and 10% lard (Cho, 2006; Han, 2008; Erdenebaatar, 2013).

The four treatment groups were given a daily oral administration of samples. Simvastatin (10mg/kg), *A. squarrosum* (385 mg/kg), Aqueous extracts of *A. squarrosum* (60 mg/kg) and Ethanol extracts of *A. squarrosum* (60 mg/kg) samples were prepared as water. The control ND group and HFD group were orally administered water.

The body weights of the rats were measured daily. Serum TC, TG, HDL-C and LDL-C were measured with a biochemistry analyzer using standard enzymatic assay kits and the data are shown in Table 1. After 14 days of oral administration, the rats were fasted with free drinking for 16 hours, blood samples were collected from arteria femoralis and placed clotting 30 min after centrifuge (3000 r/min, 15 min) for separating serum. Serum TC and TG were

measured enzymatically. HDL-C was measured after PTA-Mg²⁺-selective precipitation of LDL-C. The LDL-C value was obtained by subtracting cholesterol, which was measured enzymatically after PVS-selective precipitation of LDL-C from TC.

The data were expressed as the mean \pm standard deviation (SD) and analyzed by the analysis of variance technique (ANOVA) followed by Student's t-test. A value of $p < 0.05$ was chosen as the criterion of statistical significance.

Results and Discussion

We analyzed the lipid profile of animals treated with vehicle only or chemicals. Compared to the control ND-fed group, the TC, TG and LDL-C levels in the serum of the HFD group were significantly increased, while HDL-C levels in the serum were significantly decreased at the end of the experiment, indicating that a hyperlipidaemic animal model was successfully created by the ingestion of an HFD. As shown in Table 1, simvastatin, *A. squarrosum*, aqueous extracts of *A. squarrosum* and ethanol extracts of *A. squarrosum* are significantly decreased the TC, TG, and LDL-C levels in the serum, and had no significant effects on HDL-C, but they had increasing tendency.

The results showed that the aqueous extracts group and ethanol extracts group had extremely significant differences with the control HFD-fed group ($p < 0.01$); the *A. squarrosum* group also had a significantly lower total cholesterol than the control group ($p < 0.05$) in the decreasing total.

Table 1. Total cholesterol (TC), triglyceride (TG), lipoproteins (HDL-C and LDL-C) in serum of the rats.

Groups ^a	Daily dose ^b (mg/kg)	TC (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
ND	0	2.11 \pm 0.14**	1.11 \pm 0.29**	1.05 \pm 0.09**	1.56 \pm 0.20**
HFD	0	14.40 \pm 1.80	1.92 \pm 0.19	0.40 \pm 0.09	5.12 \pm 0.78
Simvastatin	10	11.32 \pm 1.24**	1.40 \pm 0.28**	0.46 \pm 0.06	4.20 \pm 0.41**
<i>A. squarrosum</i>	385	12.45 \pm 1.72*	1.52 \pm 0.33**	0.43 \pm 0.08	4.37 \pm 0.57*
Aqueous extracts	60	11.62 \pm 1.45**	1.49 \pm 0.33**	0.44 \pm 0.08	4.26 \pm 0.65*
Ethanol extracts	60	11.24 \pm 1.40**	1.55 \pm 0.25**	0.46 \pm 0.09	3.85 \pm 0.80**

Values are mean SD of 10 rats.

^a Five groups of rats were tested, each group used 10 rats. Four treatment groups (simvastatin, *A. squarrosum*, aqueous extracts of *A. squarrosum* and ethanol extracts of *A. squarrosum*), two control groups (ND and HFD).

^b Samples listed in groups were orally administrated once a day for 14 days.

* $p < 0.05$ significantly different from HFD (Student's t-test).

** $p < 0.01$ significantly different from HFD (Student's t-test).

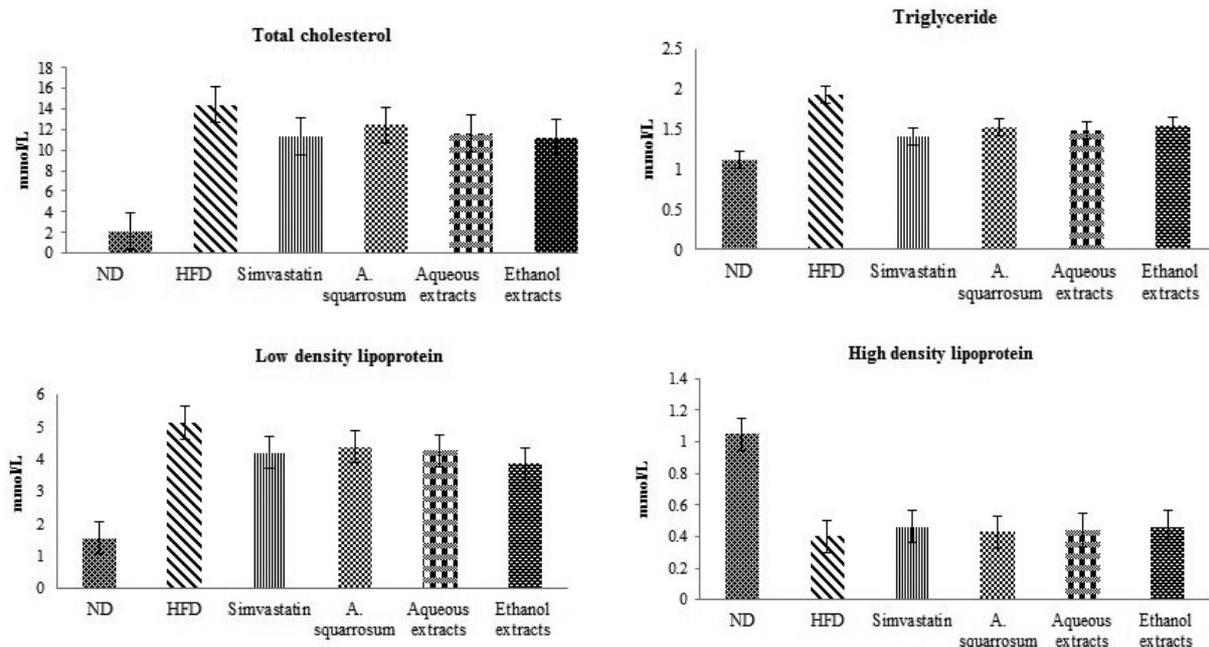


Fig. 1. Total cholesterol, triglyceride, low density lipoprotein cholesterol (LDL-C) and high density lipoprotein cholesterol (HDL-C) in serum of two control groups (ND and HFD) and four treatment groups (simvastatin, *A. squarrosom*, aqueous extracts of *A. squarrosom* and ethanol extracts of *A. squarrosom*).

It indicated that *A. squarrosom* might be used to prevent the increase of total cholesterol.

In the triglycerides test, the *A. squarrosom* group, aqueous extracts group and ethanol extracts group had extremely significant differences with the control HFD-fed group ($p < 0.01$), and it indicated that *A. squarrosom* was obviously preventing the increase of triglycerides.

In the low density lipoprotein test, the *A. squarrosom* group, the aqueous extracts group and ethanol extracts group had significant differences with control HFD-fed group ($p < 0.05$), and the results indicated that *A. squarrosom* was preventing the increase of low density lipoproteins. In the high-density lipoprotein cholesterol test, the *A. squarrosom* group, the aqueous extracts group and ethanol extracts group had no significant differences compared with the control HFD-fed group ($0.05 < p < 1$), but they had increasing tendency.

As shown in Figure 1, the significances of *A. squarrosom* and its extracts on decreasing blood levels of total cholesterol, triglyceride, and low density lipoprotein cholesterol were equal to that of simvastatin, which is a drug for the treatment of hyperlipidaemic.

Agriophyllum squarrosom and its extracts showed excellent antihyperlipidaemic activity in rats. These findings demonstrated the

antihyperlipidaemic activity of *A. squarrosom* and its extracts and the results of the present studies indicated that *A. squarrosom* is a potential antihyperlipidaemic drug candidate.

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