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Full Length Research Paper

Aqueous extract of *Astragalus mongholicus* ameliorates high cholesterol diet induced oxidative injury in experimental rats models

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Astragalus mongholicus **is a Chinese traditional medicine. In this study, we sought to explore potential benefits in cardiovascular disorders associated with excess cholesterol and hyperlipidemia. We have investigated the effects of *A. mongholicus* extract as a dietary supplement on hyperlipidemia and oxidative stress in rats maintained on a high- cholesterol diet. Diets were supplemented with *A. mongholicus* extract at 0.4 and 0.8% for five weeks, while control animals received no supplement. *A. mongholicus* extract administration to hyperlipidemic rats resulted in a significant decline in serum levels of total cholesterol, triglycerides and low density lipoprotein-cholesterol, with an increase in serum high-density lipoprotein-cholesterol levels. Furthermore, *A. mongholicus* extract improved serum and heart antioxidant status as assessed by superoxide dismutase and glutathione peroxidase activities and reduced levels of lipid peroxidation. These results suggest that *A. mongholicus* extract consumption can improve lipid profiles, inhibit peroxidation, and increase the activity of antioxidant enzymes, and is thereby likely to reduce the risk of coronary heart disease associated with hyperlipidemia and oxidative stress.**

Key words: *Astragalus mongholicus* extract, coronary heart disease, high- cholesterol diet.

INTRODUCTION

Astragalus is a perennial legume plant; its root has been of medicinal use for centuries within the traditional Chinese system. *Astragalus mongholicus* and *Astragalus membranaceus* are the two species most often prescribed, for general debility, chronic illnesses, and increase of the overall vitality of patients. The main ingredients of *A. membranaceus* var. mongholicus (synonym *A. mongholicus*, AM) are polysaccharides (Kitagawa et al., 1983a), saponins, and flavonoids (Kitagawa et al., 1983b; Kitagawa et al., 1983c; Aldarmaa et al., 2010). In traditional medicine, AM has been used for the treatment of general weakness, chronic illness, and to increase overall vitality. Different peripheral effects such as improved sensitivity to insulin (Lin et al., 2000), immune modulation, antiviral activity, antineoplastic activity, and enhancement of cardiovascular functions have been described (Monograph, 2003). The protection of cardiovascular function might be explained in terms of protection against membrane lipid peroxidation (Chen et al., 1995; Wang et al., 1996; Toda and Shirataki, 1999; Shuai et al, 2010).

Oxidative stress is currently suggested as a mechanism underlying hypercholesterolemia. Free radicals are continually produced in the body as the result of normal metabolic processes and interaction with environmental stimuli. Enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT). Nonenzymatic antioxidants are represented by ascorbic acid (vitamin C), α-tocopherol (vitamin E), glutathione (GSH), carotenoids, flavonoids, and other antioxidants. Under normal conditions, there is a balance between both the activities and the intracellular levels of these antioxidants. This

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Table 1. Effect of *A. mongholicus* extract on serum MDA level.

Group	MDA (μmol/ml)
NC	6.38 ± 0.32
UMC	12.54 ± 0.84$^{\$\$}$
AME (0.4%)	9.12 ± 0.47$^{\#\#}$
AME (0.8%)	7.26 ± 0.43$^{\#\#}$

$^{\$\$}p < 0.01$, vs NC group; $^{\#\#}p < 0.01$, vs UMC group, NC: normal control; UMC: untreated model control; AME: *A. mongholicus* extract.

balance is essential for the survival of organisms and their health (Valko et al., 2007; BeMiller, 2011; Shiga, Cordenunsi and Lajolo, 2009). Oxidative stress results from imbalance between radical-generating and radical-scavenging systems, that is, increased free radical production or reduced activity of antioxidant defenses or the both.

Hypercholesterolemia, high-cholesterol diet, and oxidative stress increase serum total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol levels, resulting in increased risk for atherosclerosis development (Hakimoglu et al., 2007). In such conditions, antioxidants play an important role in inhibiting and scavenging radicals, thus, providing protection to humans against infectious and degenerative diseases. In the present experiment, we examined effect of *A. mongholicus* extract on oxidative injury in rats fed with a high-cholesterol diet.

MATERIALS AND METHODS

Plant material and extraction

A. mongholicus used in this study were purchased in a local market. Plant materials were extracted in boiling water for 2 h. After 2 h of extraction, the procedure was repeated. After centrifugation, the combined extract was evaporated in a rotary evaporator and freeze-dried.

Animals and dietary treatment

Twelve male Wistar rats weighing 178 ± 10 g were housed in stainless steel cages in a room with controlled lighting (12 h light:dark cycle), constant temperature (24°C) and relative humidity (60%).

The animals were randomly divided into four groups of six rats each and fed a different diet for 5 weeks, as follows: one group (normal control) fed a standard diet; one group (model control) fed a diet containing 1% cholesterol and 0.5% cholic acid, that is, high cholesterol diet (HCD) and the other two groups (AME-treatment) fed the HCD diet supplemented with 0.4 and 0.8% *A. mongholicus* extract, respectively. After 5 weeks of treatment, the animals were fasted overnight and then sacrificed under diethyl ether anesthesia. All experiments were carried out according to the guidelines for the care and use of experimental animals and approved by state veterinary administration of China. Blood and heart tissue samples were taken from the animals of all groups. The heart tissue was immediately washed with saline, blotted on filter paper, weighted and then cut into small pieces and homogenized in Tris–HCl buffer (0.025 M, pH 7.5) with a homogenizer to give a 10% (w/v) heart homogenate. The homogenate was then centrifuged at 12,000 rpm for 15 min and the supernatant obtained was frozen until use. Serums were separated from the blood samples and were stored at –70°C pending biochemical analyses.

Biochemical analysis

Blood malondialdehyde (MDA) level was estimated according to method of Draper and Hadley (1990), which is based on the coupling MDA with thiobarbituric acid. Triglycerides (TG), TC, HDL-c and LDL-c in serum were determined with enzymatic kits. The activities of SOD (Sun et al., 1988), CAT (Aebi, 1974) and GPx were measured in blood and heart tissue homogenates. Tissue protein content was assayed by colorimetric method of Lowry et al. (1951).

Statistical analyses

Data were analyzed using Student's t-test and $p < 0.05$ was considered significant. Values are expressed as means ± standard deviation (S.D.) for six rats per group.

RESULTS AND DISCUSSION

Oxidative stress and lipid peroxidation have been implicated in atherogenesis. One of the byproducts of lipid peroxidation is MDA. Modification of proteins with MDA changes antigenicity, function, and turnover kinetics of various proteins and has been implicated as a pathogenetic mechanism of atherosclerosis (Haberland et al., 1988; Mooradian et al., 1995, 1996; Lung et al., 1993; Shah et al., 1994).

As shown in Table 1, the untreated model control group exhibited significantly higher concentrations of serum MDA than normal control group, whereas HCD supplemented with 0.4 and 0.8% *A. mongholicus* extract groups displayed significantly lower concentrations of serum MDA than the untreated model control group.

The association of serum TC and low-density lipoprotein cholesterol (LDL-c) with developing coronary heart disease (CHD) has been well established, and low serum high-density lipoprotein cholesterol (HDL-c) is considered a major risk factor for CHD. There are some evidences that serum TG may be an independent risk factor for cardiovascular diseases (CVD) (Hokanson and Austin, 1996). Elevated TG and low HDL-c are basic characteristics of insulin resistance and the metabolic syndrome (MetS), which are strongly associated with CHD (McLaughlin et al., 2003). TG/HDL-c ratio, as a relatively novel lipoprotein index, indicates the presence of small dense LDL particles and could serve as a good predictor of CHD (Barzi et al., 2005; Gaziano et al., 1997). In the present study, serum TC, TG and LDL-c concentrations were markedly higher in untreated model

Table 2. Effect of *A. mongholicus* extract on serum TC, TG, LDL-c and HDL-c levels.

Group	TC (mmol/ml)	TG (mmol/ml)	LDL-c (mmol/ml)	HDL-c (mmol/ml)
NC	3.28 ± 0.11	2.01 ± 0.14	0.94 ± 0.05	1.93 ± 0.09
UMC	5.27 ± 0.21 $^{\$\$}$	3.92 ± 0.12 $^{\$\$}$	1.73 ± 0.09 $^{\$\$}$	0.94 ± 0.05 $^{\$\$}$
AME (0.4%)	4.05 ± 0.19 $^{\#\#}$	3.15 ± 0.17 $^{\#\#}$	1.48 ± 0.08 $^{\#\#}$	1.48 ± 0.08 $^{\#\#}$
AME (0.8%)	3.17 ± 0.13 $^{\#\#}$	2.27 ± 0.12 $^{\#\#}$	1.02 ± 0.06 $^{\#\#}$	1.87 ± 0.09 $^{\#\#}$

$^{\$\$}p < 0.01$, vs NC group; $^{\#\#}p < 0.01$, vs UMC group, NC: normal control; UMC: untreated model control; AME: *A. mongholicus* extract.

Table 3. Effect of *A. mongholicus* extract on serum SOD, CAT and GPx activities.

Group	SOD (U/ml)	CAT (U/ml)	GPx (U/ml)
NC	98.43 ± 3.76	21.65 ± 2.04	18.59 ± 1.28
UMC	56.21 ± 2.64$^{\$\$}$	12.57 ± 1.11$^{\$\$}$	11.09 ± 0.94$^{\$\$}$
AME (0.4%)	70.84 ± 4.02$^{\#\#}$	17.05 ± 1.53$^{\#\#}$	15.37 ± 1.33$^{\#\#}$
AME (0.8%)	87.43 ± 5.38$^{\#\#}$	20.51 ± 1.59$^{\#\#}$	18.68 ± 1.57$^{\#\#}$

$^{\$\$}p < 0.01$, vs NC group; $^{\#\#}p < 0.01$, vs UMC group, NC: normal control; UMC: untreated model control; AME: *A. mongholicus* extract.

Table 4. Effect of *A. mongholicus* extract on heart SOD, CAT and GPx activities.

Group	SOD (U/mg)	CAT (U/mg)	GPx (U/mg)
NC	121.36 ± 10.37	27.04 ± 1.42	20.16 ± 1.42
UMC	77.39 ± 5.38$^{\$\$}$	14.63 ± 1.74$^{\$\$}$	14.29 ± 1.19$^{\$\$}$
AME (0.4%)	97.38 ± 4.02$^{\#\#}$	20.82 ± 1.88$^{\#\#}$	17.99 ± 1.35$^{\#\#}$
AME (0.8%)	117.32 ± 9.47$^{\#\#}$	26.37 ± 2.05$^{\#\#}$	21.27 ± 1.71$^{\#\#}$

$^{\$\$}p < 0.01$, vs NC group; $^{\#\#}p < 0.01$, vs UMC group, NC: normal control; UMC: untreated model control; AME: *A. mongholicus* extract.

control groups compared with the normal control group. In contrast, serum HDL-c concentration was markedly lower in untreated model control groups compared with the normal control group. Supplementation of the drinking water of the HCD rats with *A. mongholicus* extract significantly decreased serum TC, TG and LDL-c concentrations relative to that of the untreated HCD group ($p < 0.05$, Table 2).

There is an ongoing debate as to whether soy dietary supplements are of therapeutic use against a variety of cardiovascular diseases in humans (Anderson et al., 1995; Clarkson, 2002; Sacks et al., 2006). In a number of rat models, however, improvements in several cardiovascular risk factors are striking (Mahn et al., 2005; Park et al., 2005; Trujillo et al., 2005; Douglas et al., 2006).

In recent years, antioxidants have gained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. Human antioxidant defense is equipped with enzymatic scavengers like superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase; hydrophilic scavengers like urate, ascorbate, glutathione and flavonoids; lipophilic radical scavengers such as tocopherols, carotenoids and ubiquinol (Pan and Mei, 2010; Alim et al., 2009; Abdelhalim, 2010). The defense also comprises enzymes involved in the reduction of oxidized forms of molecular antioxidants like glutathione reductase, dehydroascorbate reductase. Apart from these scavengers, there exists cellular machinery, which maintains a reducing environment, for example regeneration of NADPH by glucose-6-phosphate dehydrogenase (Sefi et al., 2010). Some of these agents synthesized by cell itself; however, majority including ascorbic acid, lipoic acid, polyphenols and carotenoids are derived from dietary sources. In disease conditions, the defense against ROS is weakened or damaged and the oxidant load increases. In such conditions, external supply of antioxidants is essential to countervail the deleterious consequences of oxidative stress (Rocha et al., 2009).

As shown in Tables 3 and 4, the untreated model control group significantly reduced the activities of CAT,

SOD and GPx when compared to the normal control group. The results showed that HCD supplemented with 0.4 and 0.8% *A. mongholicus* extract significantly increased serum and heart CAT, SOD and GPx activities when compared to the untreated model control group.

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