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# A study on *Astragalus mongholicus* heterosaccharides affecting contractions of isolated bladder detrusor strips

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ABSTRACT

The aim of this study was to examine the effect of *Astragalus mongholicus* heterosaccharides (AMH) on urinary system. The results of gas chromatography (GC) quantitative analysis of the acetylated of monosaccharides revealed that galactose, arabinose and glucose were the sugars in *A. mongholicus* heterosaccharides. The Fourier transform infrared (FT-IR) spectra indicated that the band in the region of 3258 $cm^{-1}$ is due to the hydroxyl stretching vibration of the polysaccharide. The band in the region of 2927 $cm^{-1}$ is due to C–H stretching vibration. *A. mongholicus* heterosaccharides administration resulted in reduced lipid peroxidation products and increased antioxidant enzyme activities in medicine-treated mice's kidney and urinary bladders. Furthermore, *A. mongholicus* heterosaccharides administration significantly increased mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips in medicine-treated mice. From these results, it was demonstrated that *A. mongholicus* heterosaccharides have the ability to protect against oxidative damage and improve urinary bladders function. We suppose that *A. mongholicus* heterosaccharides improve urinary bladder function partly through enhancing antioxidant status in kidney and urinary bladders.



## 1. Introduction

In human anatomy, the urinary bladder is the organ that collects urine excreted by the kidneys before disposal by urination. A hollow muscular, and distensible (or elastic) organ, the bladder sits on the pelvic floor. Bladder dysfunction is common in the elderly. A major factor that promotes the progressive deterioration of bladder function may be reactive oxygen species that are released by the cyclical ischemia/reperfusion that is the result of recurrent bladder overdistension and emptying in patients with bladder outlet obstruction (Aikawa, Leggett, & Levin, 2003; Madersbacher et al., 1996; Parekh, Lobel, O'Connor, Leggett, & Levin, 2001). Several studies have showed that oxidative injury was closely associated with many diseases, including urinary bladders cancers (Chiou et al., 2003). The correlation between oxidative stress and formation of stone diseases in urinary system is well known (Huang, Ma, Chen, & Chen, 2002; Tugcu et al., 2007). In another study on rats, it was shown that green tea had preventive effects on calcium oxalate urolithiasis because of its antioxidant effects (Itoh et al., 2005).

Under normal conditions, there is a steady redox balance between the production of reactive oxygen species (i.e. free radicals) and their destruction by cellular antioxidant systems, such as the α-tocopherol–ascorbic acid, GSH–GSSG and $NADP^+$–NADPH systems. However, this redox balance can be broken either by an increase in the production of reactive oxygen species or by the inhibition of the defense system (Khan, Sobti, & Kataria, 2005; Hirata, Kawamoto, & Nishimoto, 1991; Kumar, Joo, Choi, Koo, & Chang, 2004). Under conditions of increased ROS production or when the antioxidant system is compromised, cells are unable to efficiently scavenge the free radicals, leading to ROS accumulation (Dhalla, Elmoselhi, Hata, & Makino, 2000; Keith et al., 1998; Martindale & Holbrook, 2002; Tsutsui, 2001; Ma & Xiao, 1998; Nitika, Abhigyan, Prabhjot, Amlesh, & Alpana, 2010). Most free radicals are highly reactive and extract electrons from neighboring molecules to complete their own orbital, which results in oxidation of the biological molecules (Dhalla et al., 2000; Keith et al., 1998; Martindale & Holbrook, 2002; Tsutsui, 2001; Sun et al., 2009; Tang, 1967). Results of a variety of studies on the mammalian system have shown that oxidative stress can be reliably measured by oxidative-damage biomarkers, such as lipid peroxides, protein carbonyls, and GSSG levels (Beal, 2002; Ceballos-Picot et al., 1996; Chevion, Berenshtein, & Stadtman, 2000; Jerca, Busuioc, Nastasia, & Haulica, 1996; Valenzuela, 1991).

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There is a great interest in many herbal medicinal plants due to their antioxidant and medicinal activities. The main ingredients of *Astragalus membranaceus var. mongholicus* (synonym A. mongholicus, AM) are polysaccharides (Kitagawa, Wang, & Saito, 1983), saponins, and flavonoids (Aldarmaa et al., 2010; Kitagawa, Wang, Saito, & Yoshikawa, 1983; Kitagawa, Wang, & Takagi, 1983; Kitagawa, Wang, & Yoshikawa, 1983). The root of the *Astragalus* plant is typically used in soups, teas, extracts, or capsules. There is no recent clinical evidence to guide dosages of *Astragalus* products. However, typical recommendations are 2–6 g of the powdered root (Monograph, 2003). In traditional medicine, *A. membranaceus* 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). *Astragalus mongholicus* heterosaccharides have been reported to be effective in modulating immune functions and inhibiting tumor growth (Ma et al., 1996; Zhou et al., 1995). The immunomodulatory effects of AMH were extensive, including promoting humoral immunity and cellular immunity, improving the immune functions of the immunosuppressive model mice (Mao, 1998; Pan & Song, 1977), and modulating the production of cytokines (Tu, Yang, Wang, Zhang, & Shen, 1995).

Contractile activity can be initiated in the smooth muscle of the urinary bladders of most mammals by stimulation of the parasympathetic nerves, or by field stimulation of intrinsic excitatory nerves in smooth muscle strips. The contractile response has, however, long been known to be partially resistant to blockade with atropine (Henderson & Roepke, 1934; Langley & Anderson, 1985; Ursillo & Clark, 1956).

The present study was aimed to estimate the antioxidant potentials of *A. mongholicus* heterosaccharides in mice' urinary bladder. In addition, effect of *A. mongholicus* heterosaccharides on mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips was also evaluated. These oxidative and antioxidative markers were correlated with urinary bladder dysfunction.

## 2. Materials and methods

### 2.1. Material

*A. mongholicus* were purchased from Chinese medicine market in ShanTou city.

### 2.2. Preparation of A. mongholicus heterosaccharides

Samples (500 g) were first grind into fine powder. The powder was extracted in boiling water under mechanical stirring (500 t/min) for 2 h and then filtered in vacuum through a Go sintered glass funnel. The extraction was carried out two times. The resulting filtrate was condensed and dried to obtain *A. mongholicus* heterosaccharides.

### 2.3. Chemical composition and structure

Purified polysaccharide sample was hydrolyzed in 2 M HCl for 2.5 h at 105 °C in a sealed glass tube. The residual acid was removed under vacuum, followed by co-distillation with water. Then the hydrolyzates were converted to acetylated aldononitrile derivatives according to conventional protocols and analyzed by gas chromatography (GC). The polysaccharides extracts were analyzed by combined gas chromatography. The gas chromatographic conditions were as follows: column DB-5 30 m long, 0.25 mm i.d.; film thickness 0.25 μm; injection temperature: 250 °C; temperature program: 60 °C hold for 1 min, then 3.3 °C/min up to 250 °C, hold for 15 min, then 8 °C/min up to 300 °C, hold for 15 min. All the samples were injected twice, using the split technique, with a split ratio of 1:10. The percentage composition of the polysaccharides compound was computed from gas chromatography (GC) peak areas without any correction for the relative response factors. As references, the following neutral sugars were converted to their acetylated derivatives and analyzed: rhamnose, arabinose, xylose, mannose, glucose, ribose and galactose. The compounds were identified by comparing their retention times with those of pure standard samples.

All analyses were carried out using a Varian (formerly BioRad) FTS 6000 FT-IR spectrometer. Spectral collection was conducted under ambient conditions. The operating range was from 4000 to 400 $cm^{-1}$ with a resolution of 4 $cm^{-1}$ using a DTGS detector. Following the pellet preparation, KBr pellets were immediately placed into the sample compartment of the spectrometer, left to purge with dry air for 15 min to remove ambient water vapor and FT-IR spectra were subsequently recorded. All spectra were measured with a blank KBr pellet as the background and were ratioed against the background.

### 2.4. Experimental protocols

Mice weighing 27–33 g were used throughout the experiments. Animals were housed under standard environmental conditions (23 ± 1 °C, 55 ± 5% humidity and a 12-h light: 12-h dark cycle) and maintained with free access to water and a standard laboratory diet ad libitum. Animal care and the experimental protocols were approved by the National Research Centre Animal Care and Use Committee and were in accordance with the guidelines of the International Association for the Study of Pain Committee for Research and Ethical Issues (Zimmermann, 1983).

Fifty mice were divided into five groups (10 mice each). Mice ($n$ = 10 per group) were treated with the vehicle only and feed with standard diet 30 days. The mice in the AMH groups (I, II, III and IV) were fed with standard diet containing 200, 300, 400 and 500 mg/kg/day doses of *A. mongholicus* heterosaccharides, respectively, for 30 days. The experiment was stopped 12 h after the last administration of the drugs. Mice were stunned. Urinary bladders were taken out for measuring mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips. Then the mice were killed. Kidneys were removed rapidly.

### 2.5. Determination and methods

The activity of glutathione (GSH), malondialdehyde (MDA), and superoxide dismutase (SOD) in blood, kidneys of mice were examined with detection kits. Blood GSH level was measured using the commercial kits (Jiancheng Institute of Biotechnology, Nanjing, China) (Qu et al., 2009). Glutathione peroxidase (GPx) activity were determined following the methods of Gunzler, Flohe, and Clairbone (1986), respectively.

### 2.6. Measurement of mean contractile amplitude increase, contractile frequency and contractile tension

Mice were used in this study. The lower abdomen was opened. The urinary bladder was held at its apex and the coat and connective tissue were cut away. The bladder was then removed and washed in Krebs-Henseleit solution. Two lateral incisions were made in the bladder wall, and the tissue was opened into a rectangular sheet. Strips 1- 1.5 × 0.2 cm were prepared with a pair of fine scissors. Sin-

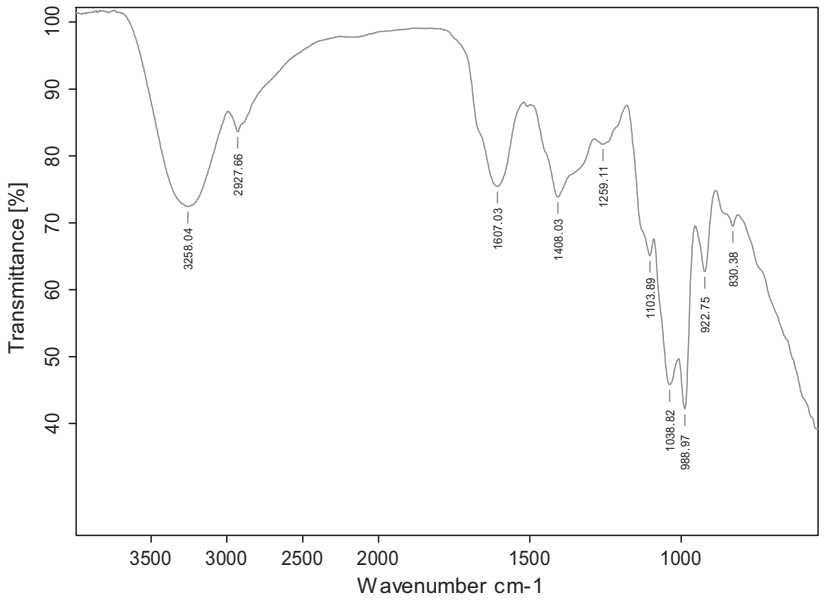


**Fig. 1.** FT-IR analysis of *Astragalus mongholicus* heterosaccharides.

**Table 1**
GC analysis of Astragalus mongholicus heterosaccharides.

| Standard sample | Glucose | Rhamnose | Arabinose | Galactose | Xylose | Mannose |
|---|---|---|---|---|---|---|
| AMH | + | + | + | + | – | – |

*Note*: + (detection); – (no detection).

gle strips were suspended between parallel platinum electrodes in a 2 ml jacketed organ bath containing Krebs-Henseleit solution. Mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips after *A. mongholicus* extract treatment were measured according to the literatures (Huang et al., 2009).

### 2.7. Statistical analysis

Data were expressed as mean ± S.D. and compared using the double-tail Student's *t* test; $p < 0.05$ was taken as statistically significant.

## 3. Result

### 3.1. Chemical characterization of *A. mongholicus* heterosaccharides

The yield of *A. mongholicus* heterosaccharides was 10.73% of the plant raw material. The results of GC quantitative analysis of the acetylated of monosaccharides revealed that galactose, arabinose and glucose were the major sugars in *A. mongholicus* heterosaccharides. Namely, *A. mongholicus* heterosaccharides were composed of galactose, arabinose and glucose (Table 1).

In Fig. 1, a representative FT-IR spectrum (3258–830 $cm^{-1}$) of *A. mongholicus* heterosaccharides is shown and the region containing the fundamental C–H, N–H and O–H stretching vibrations is highlighted. The band in the region of 3258 $cm^{-1}$ is due to the hydroxyl stretching vibration of the polysaccharide. The band in the region of 2927 $cm^{-1}$ is due to C–H stretching vibration. Previous IR studies on pectins have suggested that the esterified $CH_3$ group presents bands in the 1350–1450 $cm^{-1}$ range, one at 1380 $cm^{-1}$ corresponding to the symmetric stretching of $CH_3$ and one at around 1440 $cm^{-1}$ corresponding to the asymmetric stretching modes of $CH_3$ (Synytsya, Copikova, Matejka, & Machovic, 2003).

### 3.2. In vivo pharmacological activities

Table 2 represents the levels of MDA and GSH in urinary bladder of mice after supplementation with *A. mongholicus* heterosaccharides. The level of blood MDA was significantly decreased in the urinary bladder of mice compared to normal mice. Administration of *A. mongholicus* heterosaccharides for 30 days significantly reduced the MDA level in urinary bladder of mice when compared to normal control mice. Supplementation of *A. mongholicus* heterosaccharides increased the GSH level in mice compared to that of normal control mice in a dose-dependent manner.

The results are shown in Table 3. When compared to normal control groups, *A. mongholicus* heterosaccharides significantly enhanced kidney and urinary bladder antioxidant enzymes activities ($p < 0.05$). According to Table 3, the increase in kidney and urinary bladder antioxidant enzymes activities in AMH-treated mice showed a dose-dependent manner.

When mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips of mice were determined after *A. mongholicus* heterosaccharides treatment, the results shown in Fig. 2 were obtained. Mean contractile amplitude increase, contractile frequency and contractile

**Table 2**
MDA and GSH levels in mice's urinary bladder after *Astragalus mongholicus* heterosaccharides treatment.

| Group | MDA (nmol/mg) | GSH (mg/g) |
|---|---|---|
| NC | 5.83 ± 0.47 | 46.48 ± 4.03 |
| AMH I | 4.01 ± 0.25* | 59.73 ± 3.83** |
| AMH II | 3.47 ± 0.28** | 73.52 ± 5.66** |
| AMH III | 3.52 ± 0.22** | 60.39 ± 3.81** |
| AMH IV | 2.54 ± 0.17** | 77.04 ± 5.72** |

NC: normal control.
* $P < 0.01$, compared with NC group.
** $P < 0.01$, compared with NC group.

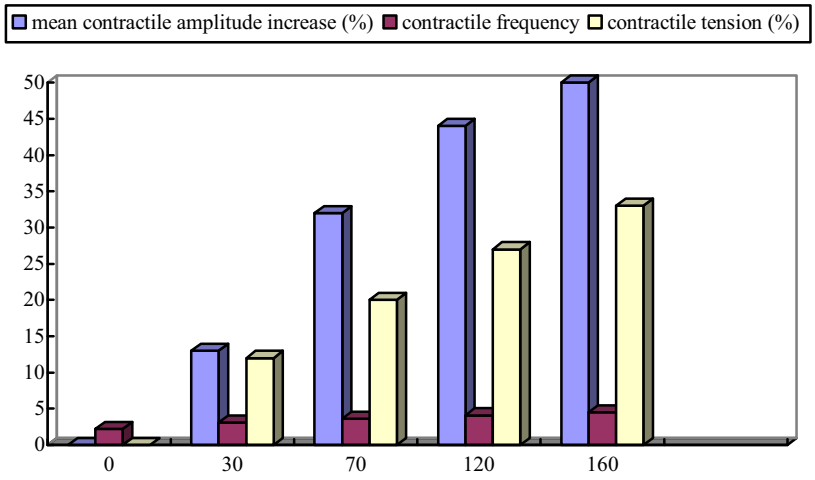


**Fig. 2.** Mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips after *Astragalus mongholicus* heterosaccharides treatment.

**Table 3**
SOD, CAT and GSH-Px activities in kidney and urinary bladder in mice after *Astragalus mongholicus* heterosaccharides treatment.

| Group | SOD (U/mg) | | CAT (U/mg) | | GSH-Px (U/0.1 mg) | |
|---|---|---|---|---|---|---|
| | Kidney | Urinary bladder | Kidney | Urinary bladder | Kidney | Urinary bladder |
| NC | 0.35 ± 0.09 | 30.33 ± 3.03 | 7.32 ± 1.21 | 0.87 ± 0.13 | 604.81 ± 53.42 | 69.28 ± 5.92 |
| AMH I | 0.47 ± 0.07** | 50.38 ± 4.38** | 9.39 ± 1.04** | 1.47 ± 0.31** | 828.37 ± 77.09** | 83.41 ± 6.91* |
| AMH II | 0.59 ± 0.08** | 74.31 ± 3.95** | 14.52 ± 1.44** | 1.94 ± 0.29** | 974.38 ± 72.59** | 109.39 ± 7.04** |
| AMH III | 0.60 ± 0.07** | 85.37 ± 7.05** | 8.26 ± 1.11** | 2.05 ± 0.32** | 962.07 ± 97.14** | 93.28 ± 4.12** |
| AMH IV | 0.86 ± 0.12** | 153.94 ± 11.04** | 17.41 ± 1.53** | 3.13 ± 0.41** | 1284.33 ± 105.36** | 173.39 ± 11.52** |

NC: normal control.
* $P < 0.01$, compared with NC group.
** $P < 0.01$, compared with NC group.

tension in isolated bladder detrusor strips of mice increased with increasing *A. mongholicus* heterosaccharides concentration. Namely, the effect was displayed in a dose-dependent manner.

## 4. Discussion

Several disease states or pathological situations in the urinary bladder are characterized by the formations of reactive oxygen species (ROS). Bratslavsky, Kogan, Matsumoto, Aslan, and Levin (2003) showed that reperfusion injury of the bladder is more detrimental than ischemia alone. Especially during the reperfusion period, ROS are formed. Examples of ROS are superoxide anion ($O_2^-$), hydroxyl radical ($OH^-$), hydrogen peroxide ($H_2O_2$), hypochlorous acid (HOCl), and peroxynitrite ($ONOO^-$). It has been reported that bladder outlet obstruction and acute urinary retention followed by bladder decompression enhances lipid peroxidation (Lin, Yang, Chen, & Chang, 2005; Saito & Miyagawa, 2001; Zhang et al., 2010; Karagöz, Doğruöz, Zeybek, & Aslan, 2009; Biskup & Lojkowska, 2009).

*Astragali Radix*, the roots of *A. membranaceus* (Huangqi) are amongst the most popular health-promoting herbs in China, their use dated back more than 2000 years, and was recorded in Shen Nong's Materia Medica written in the Han dynasty (Mckenna, Hughes, & Jones, 2002). Previous research about the immune modulatory effect of *A. membranaceus* was limited to normal or chemopreventive agent-challenged animals (Huang, Wu, Chen, Yang, & Wang, 2007). The root of *Astragalus* species is known to be rich in polysaccharides, saponins, and flavonoids. The former two compounds of several *Astragalus* species are reported to possess anticancer, antioxidant and immunity-stimulating effects (Lee & Jeon, 2005; Sun, Hu, Wang, Zhang, & Liu, 2006; Verotta et al., 2001; Yesilada, Bedirb, Calıs, Takaishic, & Ohmoto, 2005).

Oxidative stress associated with increase in the formation of TBARS in the body which was indicative of lipid peroxidation has been adduced to be a crucial step in oxidative injury (Bouderbala, Lamri-Senhadji, Prost, Lacaille-Dubois, & Bouchenak, 2008). The antioxidant constituents present in the extracts might have been responsible for their ability to reduce the lipid peroxidation (Banerjee et al., 2001). Thus, enhanced antioxidant enzyme activities (CAT and GPx) could be a part of a compensatory mechanism against oxidative stress and/or it might be the result of an over expression of antioxidant genes (Solomon, Raosaheb, & Nazma, 2002). GSH is a major non-enzymatic antioxidant molecule that is involved in the second line of defense against free radical damage in the body. GSH donates an electron in the reduction of peroxides catalyzed by GSHpx as a component of the enzyme system containing GSH oxidase and reductase (Hollander et al., 1998). MDA and GSH levels in the AMP groups were significantly decreased and increased compared to NC group.

In the research field of free radicals in biological samples it still is a major problem to determine the amount of free radical damage. Oxygen-derived free radicals are very important mediators of cell injury and cell death. Not only are these highly reactive chemical species important in the aging process, but they are also, either directly or indirectly, involved in various clinical disorders, such as atherosclerosis, reperfusion injury, cancer, etc. (de Zwart, Meerman, Commandeur, & Vermeulen, 1999). In addition, they play an important role in cellular injury induced by chronic inflammatory processes and several disorders of the central nervous system. Continuous exposure of aerobic organisms to prooxidant challenges has endowed living cells with efficient and sophisticated antioxidant systems. These can be divided into enzymatic antioxidant and non-enzymatic antioxidant systems. Superoxide dismutase (scavenges superoxide anions) and GPx (removes $H_2O_2$

and lipid peroxides) are considered primary antioxidant enzymes involved in the direct elimination of reactive oxygen species. Glutathione (GSH) is known to participate in the cellular defense system against oxidative stress by scavenging free radicals and reactive oxygen intermediates (Nicotera & Orrenius, 1986). In our study, increased CAT, SOD, GPx and GR activity and decreased lipid peroxidation, antioxidant enzyme activities in animals' kidney and urinary bladder was restored as an effect of *A. mongholicus* heterosaccharides supplementation, indicating the efficacy of plant extract in heightening antioxidant status in urinary system.

*A. mongholicus* extract had been used in therapy of urinary bladders' diseases (Jing & Zhang, 2005; Pei & Wang, 2008). In our study, *A. mongholicus* heterosaccharides supplementation could markedly enhance mean contractile amplitude increase, contractile frequency and contractile tension in isolated bladder detrusor strips of mice. Subsequently, the polysaccharides improved urinary bladders' function.

In conclusion, our study demonstrate that the administration of *A. mongholicus* heterosaccharides reduced lipid peroxidation and increasing antioxidant enzymes activities in animals' kidney and urinary bladders. This implies that *A. mongholicus* heterosaccharides treatment can prevent or be helpful in improving the urinary bladders. We suppose that *A. mongholicus* heterosaccharides improve urinary bladder function partly through enhancing antioxidant status in urinary bladders. However, further investigations to fully identify the biologically active ingredients and to define the precise molecular mechanism of these effects are required.

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