ORIGINAL RESEARCH ARTICLE EXPERIMENTAL

# Antinociceptive activity of *Astragalus gummifer* gum (gum tragacanth) through the adrenergic system: A *in vivo* study in mice

*Seyyed Majid Bagheri, Leila Keyhani[1], Mehrangiz Heydari[1], Mohammad Hossein Dashti-R[2]*

*Department of Physiology, Shahid Sadoghi University of Medical Sciences, Yazd, [1]Department of Biology,Azad Islamic University of Research Sciences, Shiraz, [2]Neurobiomedical Research Center, Shahid Sadoghi University of Medical Sciences, Yazd, Iran*

## ABSTRACT

**Background:** In Iranian traditional medicine, gum obtained from *Astragalus gummifer* and some other species of *Astragalus* was used as analgesic agent. **Objective:** In this study, we investigated the antinociceptive effect of several concentrations (125, 250, and 500 µg/kg body weight) of *Astragalus gummifer* gum (AGG) on thermal and acetic acid induced pain in mice. **Materials and Methods:** AGG was dissolved in distillated water and injected i.p to male mice 15 minute before the onset of experiment. Writhing and hot-plate tests were applied to study the analgesic effect of AGG and compared with that of diclofenac sodium (30 mg/kg, i.p.) or morphine (8 mg/kg, i.p). To investigate the mechanisms involved in antinociception, yohimbine, naloxone, glibenclamide, and theophylline were used in writhing test. These drugs were injected intraperitoneally 15 min before the administration of AGG. The number of writhes were counted in 30 minutes and analyzed. **Results:** AGG exhibited a significant antinociceptive effect and the most effective dose of AGG was 500 µg/kg. The most maximum possible effect (%MPE) was observed (117.4%) 15 min after drug administration. The %inhibition of acetic acid-induced writhing in AGG 125, 250 and 500 was 47%, 50% and 54% vs %15 of control and 66.3% of diclofenac sodium group. The antinociceptive effect induced by this gum in the writhing test was reversed by the systemic administration of yohimbine ($\alpha_2$-adrenergic antagonist), but naloxone, glibenclamide, and theophylline did not reverse this effect. **Conclusions:** The findings of this study indicated that AGG induced its antinociceptive through the adrenergic system.

**Key words:** *Astragalus gummifer*, *Astragalus gummifer g*um, gum tragacanth, hot-plate, mechanism action, pain, writhing test

## INTRODUCTION

In humans, pain has injurious effects on sleep, cognitive abilities such as learning,[1] attention,[2] and the capacity for work.[3] Pain is caused following tissue or peripheral nerve damage or injuries to different parts of the central nervous system in humans and animals. Although there are different effective analgesic drugs and widely used, but these drugs have side-effects and making their clinical use problematic.[4] In the recent years, tendency to herbal medicine has been increased and people have recognized and used of many cultivated or wild plants. Plant products have less toxic effects than synthetic ones and are a good source for novel therapeutic agent.[5] *Astragalus* L. is the largest genus of flowering plants and is a member of the Fabaceae family. Species of this genus are known to have numerous pharmacological activities and used for medicinal purposes in some countries such as Iran and China.[6,7] The roots of *Astragalus* species represent a very old and well-known drug in Traditional Chinese Medicine for its usage as an antiperspirant, tonic, and diuretic, and immune modulating and immune restorative agent.[8] It has also been used in the treatment of diabetes mellitus, nephritis, leukemia,[9] and uterine cancer.[10] Extracts of *Astragalus* roots are used in multi-herbal mixtures, as an immunostimulant to prevent, and treat a wide variety of illness ranging from the common cold to cancer.[11] *Astragalus* species contain saponins, polysaccharides, and phenolics. Behavioral

**Address for correspondence:**
Prof. Mohammad Hossein Dashti-R, Department of Physiology, Shahid Sadoughi University of Medical Sciences, Prof. Hesabi Bulvd Shohadaye Gomnam Bulvd, Yazd-8915173149, Iran.
E-mail: dashti-r@ssu.ac.ir

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studies demonstrated that some saponins in *Astragalus* such as astragaloside IV have analgesic effects.[12] In Iranian traditional medicine, tragacanth is used as a demulcent for treating sore throat and hair loss due to seborrhea.[13] *Astragalus gummifer* is one the *Astragalus* species that grows in different parts of Iran. In Iranian ancient medical books, several therapeutic effects including therapeutic effect on respiratory diseases has been described.[14] Modern pharmacological studies have been reported that *Astragalus gummifer* has anti-inflammatory effect on airway inflammation, protective effect on pulmonary epithelial damage, and immune regulatory process.[14] In Iranian tradition, it is usually used as topical therapy to relieve the arthritis rheumatoid, tooth, and neck pain or orally for treating stomach pain.[7] In this study, we experiment antinociceptive effect of *Astragalus gummifer* gum (AGG) and its mechanism by chronic and acute pain tests. To our searches, there is no comprehensive study to investigation the antinociceptive activity of AGG in an animal model. The present study was performed as a starting point for examining the folklore claims of beneficial effects of this gum in chronic and acute pain.

## MATERIALS AND METHODS

### Animals

Ninety-eight male albino mice (25-30 g) with 6-8 weeks old that bred in animal house of Shahid Sadoughi Medical School were selected. Animals were housed at controlled temperature (22 ± 2°C) with a 12 h-light/dark cycle and with standard lab chow and tap water *ad libitum*. Each animal was used only once. The experiments reported in this study were carried out in accordance with current ethical guidelines for the investigation of experimental pain in conscious animals.[15] The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

### Plant gum extract

Dried ribbon-like of AGG was purchased from market in Yazd. The powdered dried gum (0.5 g) was soaked overnight in distilled water (100 ml) at room temperature for daily use. Concentrations and doses of the aqueous extract are expressed as total amount of the dried gum used in preparing the extract.

### Drugs administration

Morphine hydrochloride was intraperitoneally (i.p.) administered in a final dose of 8 mg/kg. Diclofenac was administered at 30 mg/kg intraperitoneally (i.p.) and naloxone (5 mg/kg), theophylline (5 mg/kg, i.p.), yohimbine (5 mg/kg, i.p.), and glibenclamide (8 mg/kg, p.o) was also used for investigation of action mechanism intraperitoneally.

### Hot-plate test

The hot-plate test was carried out according to the method previously described.[16] Briefly, before the initial of experiment, mice were habituated to a Plexiglas cylinder for 5 min. In these experiments, the hot-plate apparatus was maintained at 54 ± 0.1°C. Animals were placed into an acrylic cylinder (20 cm in diameter) on the heated surface, and the time (in seconds) between placement and licking of their hind paws or jumping (whichever occurred first), was recorded as the response latency (reaction time). Each mouse served as its own control. A 45-s cut-off was used to prevent tissue damage. After baseline behavior tests, mice were immediately administered with drugs. The animals were intraperitoneally (i.p.) received vehicle (saline, 10 ml/kg), AGG dissolved in distilled water at three doses (125, 250, and 500 μg/kg), and morphine (8 mg/kg) 15 min before the test. The reaction time of each mouse was again valuated at 15, 30, 45, and 60, min after treatment. This was pooled for the mice in each treatment group and the final test mean value for each treatment group at each measurement was calculated. This final test mean value represented the after treatment reaction time and was subsequently used to determine the percentage of maximum possible effect (%MPE) by applying the following formula:

$$\%\text{MPE} = \frac{\text{Test latency} - \text{Control latency}}{\text{Cut off-Control latency}} \times 100$$

### Acetic acid-induced writhing test

The abdominal constriction test described by Collier *et al*.[17] was used to measure the analgesic activity of AGG. Male mice pre-treated with AGG (125, 250, and 500 μg/kg) or diclofenac (30 mg/kg). Fifteen minutes later, all mice were treated with intraperitoneal injection of 0.6% acetic acid to cause a typical stretching response. Five min after acetic acid injection, mice were kept in individual cages and writhing or stretching of each mouse was counted for a period of 30 min by an individual who was unaware of the pretreatment type, to ensure an assessor blind evaluation. The analgesic effect was measured by calculating the mean reduction in the number of abdominal constrictions for each drug as compared to saline control. Percentage inhibition of writhing was calculated by using the following formula:

$$\%\ \text{Inhibition} = \frac{\text{Mean number of writhes (control)} - \text{Mean number of writhes (test)}}{\text{Mean number of writhes (control)}} \times 100$$

### Assessment of some mechanisms involved in antinociceptive activity

To investigate the possible mechanisms by which AGG inhibits acetic-acid-induced nociception, mice were pre-treated with different drugs include naloxone (5 mg/kg, i.p.), theophylline (5 mg/kg), yohimbine (5 mg/kg, i.p.), and glibenclamide (8 mg/kg, p.o). After 15 min, the animals received an injection of AGG (500 μg/kg, i.p.), and 15 min later acetic acid was injected. The number of writhes was counted to analyze. The doses of antagonists were selected on the basis of earlier literature data[18-20] and in pilot experiment in our laboratory. The writhing test was chosen for this purpose because of the specificity and sensitivity in nociception transmission of this model.

### Acute toxicity study

At end of experiment, the mice were observed for symptoms of toxicity for the following 10 days in terms of mortality, behavioral changes, and weight loss.[21] Mice were observed for 10 days to see if AGG had acute toxicity in mice. This had been evidenced by the absence of lethargy, tremor, fatigue, paralysis, and loss of weight. There was also no mortality observed in the study period.

### Data analysis

All data are expressed as the mean ± standard error of the means (S.E.M.). Graph pad prism 5 was used to analyze behavior studies. Statistically significant differences were determined using one-way analysis of variance (ANOVA) with the Tukey Kramer post-test for multiple comparisons. The values of $P < 0.05$ were regarded as statistically significant.

## RESULTS

### Hot-plate test

In this study, we investigated the effect of AGG on acute and chronic pain. Latency responses for animals in different groups are shown in Table 1. The latencies for time 0 (base line latency) were statically analyzed by one-way ANOVA and there was no significant difference between the groups. Our data analysis showed that all doses of AGG have a maximum analgesic effect against thermally induced pain at 15 min ($P < 0.01$). The most effective dose of AGG was 500 μg/kg and its maximum effect was observed 15 min after drug administration. As shown in Figure 1, the % MPE at 15 min post-treatment time point for all doses of AGG was significantly greater than that of the control group.

### Acetic-acid-induced writhing test

The effect of AGG on acetic acid induced writhing is presented in Table 2. All doses of the AGG reduced acetic acid-induced writhing significantly. These results showed that with increasing the AGG concentration, the number of writhing was decreased. The smallest dose of the AGG showed relatively moderate analgesic activity with 47% ($P < 0.01$) inhibition of acetic acid-induced writhing compared to controls, and the AGG 250 and 500 showed 50% and 54% inhibition and lower to that of the standard drugs.

**Table 1: Hot plate latency in different groups (*n*=7)**

| Groups | Latency time (s) | | | | |
|---|---|---|---|---|---|
| | 0 | 15 | 30 | 60 | 90 |
| control | 9.7±2.1 | 11.1±3.2 | 10.0±1.7 | 10.2±1.8 | 10.1±1.2 |
| AGG125 (μg/kg) | 9.0±1.6 | 17.6±3.8* | 9.3±1.5 | 10.3±2.1 | 9.7±1.1 |
| AGG250 (μg/kg) | 7.6±0.9 | 15.1±3.1* | 9.6±1.3 | 7.1±0.8 | 7.4±0.7 |
| AGG500 (μg/kg) | 9.1±1.4 | 18.3±3.9* | 12.1±2.1 | 9.7±0.9 | 10.9±0.9 |
| Morphine 8 (mg/kg) | 8.5±1.6 | 15.9±2.9* | 16.9±3.1* | 13.4±2.3* | 16.8±3.4* |

*Indicates the significant difference ($P$<0.01) between the latency times of each group and the baseline as control time, using one way ANOVA followed by Tukey's post test. AGG=*Astragalus gummifer* gum

**Table 2: Effect of AGG on acetic acid-induced writhing in mice**

| Group | Number of writhing | Percentage inhibition (%) |
|---|---|---|
| Control | 108.4±2.1 | |
| AGG125 (μg/kg) | 56.3±2.5* | 47 |
| AGG250 (μg/kg) | 53.7±1.9* | 50 |
| AGG500 (μg/kg) | 49.3±2.7* | 54 |
| Diclofenac 30 (mg/kg) | 35.7±2.9* | 66.3 |

*$P$<0.01 compare to the control group. Values are the mean±SEM for at least 8 mice per group. AGG=*Astragalus gummifer* gum

### Assessment of some mechanisms involved in the antinociceptive activity

We also investigated some mechanisms related to chronic induced antinociception. As shown in Figure 2, Pre-treatment of animals with the $\alpha_2$ adreno receptor antagonist, yohimbine, (8 mg/kg, i.p.) significantly prevented the anti nociception action produced by AGG 500 mg/kg. Other antagonist, naloxone, glibenclamide, theophylline, could not reverse anti-nociception effect of AGG.

### Acute toxicity

Mice were observed for 10 days to see if AGG had acute toxicity in mice. This had been evidenced by the absence of lethargy, tremor, fatigue, paralysis, loss of weight, and autonomic behavioral changes. There was also no mortality observed in the study period.

## DISCUSSION

The results from present study showed that AGG exhibits analgesic effects. The dried exudation gum obtained

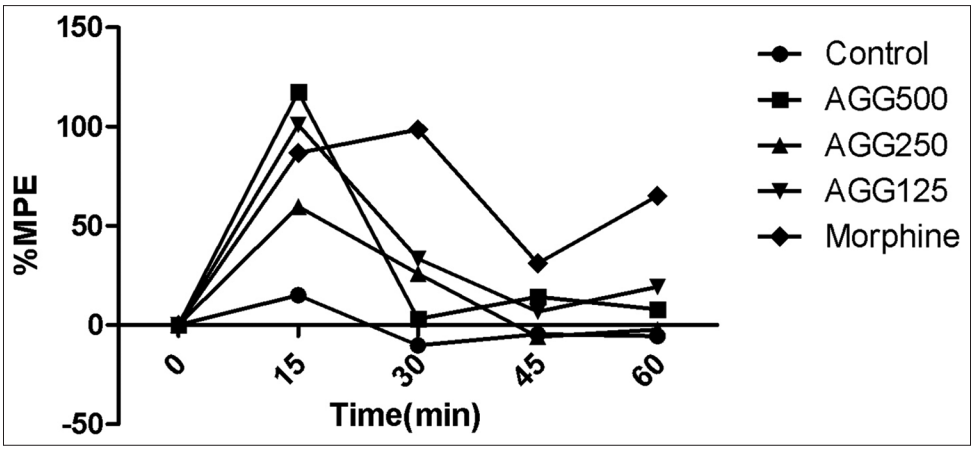


**Figure 1:** The percentage of maximum possible effect (%MPE) of different treatments on acute pain inhibition in hot plate test ($n = 7$)

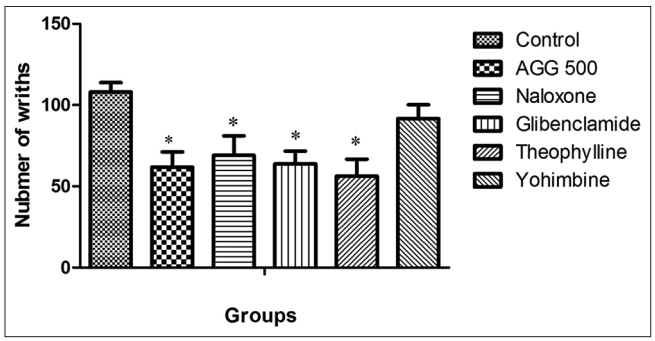


**Figure 2:** Effect of intraperitoneal injection of naloxone, theophylline, glibenclamide and yohimbine on the antinociceptive effect of AGG 500 mg/kg. *$P < 0.05$ compared the number of writhes in different groups to control

from trunk and branches of *Astragalus gummifer* and some other species of *Astragalus* (Leguminosae) that found in western Asia and was defined gum tragacanth.[22] This gum is approved as a food additive within the class of thickeners, stabilizers, emulsifiers, and gelling agents. Several studies have established that gum tragacanth is nontoxic, nonmutagenic, nonteratogenic, noncarcinogenic, and has a potential application as a food additive for immobilizing agent in viral plaque assay, superabsorbent hydrogel, and carrier for controlled release of verapamil hydrochloride.[12] It is also used as vehicle for insecticide,[23] antidepressant,[24] and antilipemic drugs.[25] In addition, this gum showed hypolipidemic activity in rats and white leghorn cockerels.[26] In modern medicine, antiviral and antibacterial effects have been claimed for tragacanth.[27] This biopolymer is a high-arabinose, protein containing, acidic heteropolysaccharide, which occurs in nature as mixed calcium, magnesium, and sodium salts.[28] Although pharmacological effects of gum tragacanth have been investigated in different diseases, its analgesic effect has not been studied adequately. In this study, gum tragacanth showed a dose dependent and significant inhibition of pain in both the chronic acetic acid writhing test and acute pain induced by hot plate in mice. However, this effect was more pronounced in reducing chronic pain. In hot plate test, most effective of gum tragacanth was observed 15 minutes after injection and significantly increased latency time. Therefore, we also investigated the mechanism of action of analgesic activity using several different drugs. Our findings indicate that AGG produces antinociception action via blocks of α2-adrenoceptor. When we used AGG combination with yohimbine (α2-adrenergic receptor antagonist), its analgesic activity of the gum was reversed that indicating the involvement of α2 -adrenergic receptors in its analgesic activity. However, with combination of the non-selective opioid antagonist, naloxone, the adenosine triphosphate (ATP)-sensitive K channel inhibitor, glibenclamide and the non-selective adenosine receptor, theophylline no significant difference was observed in antinociceptive effect compared to AGG500 alone.

## CONCLUSION

This study demonstrated the analgesic activity of gum tragacanth and this effect is relative to the adrenergic pathway. However, its active components and their mechanism of actions need to be elucidated by further studies.

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