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Protective capability of Astragalus (Huangqi) on auditory function of rats with estrogen deficiency

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To the Editor: The estrogen level of females plays an important role in the maintenance of auditory integrity.[1] In laboratory studies, a worsened auditory function was reported on the ovariectomized rats relative to the result on the ovary-intact animals.[2] However, contradictory findings were also presented.[3] Therefore, the direct impact of estrogen level on the auditory function is still uncertain. Meanwhile, estrogen therapy in the ovariectomized rats has been used to restore the changes in auditory function due to estrogen deficiency.[2] Nonetheless, conflicting findings on the benefits of estrogen replacement were also reported.[4] In fact, the impact of estrogen deficiency on the auditory function can be increased by various added factors such as the administration of Cisplatin, an anti-cancer drug.[5] This finding may suggest that females with estrogen deficiency (e.g., ovariectomy, menopause, ovarian failure) can experience a noticeable change in their auditory function when they are treated with an ototoxic drug such as Cisplatin. Since many studies have documented the clinical benefits of Astragalus (also known as Huangqi in the traditional Chinese medicine), including the reduction of side effects due to chemotherapy for cancer patients,[6] we report on the protective capability of Astragalus on the auditory function of the ovariectomized rats when they were treated with Cisplatin. In addition, the performance with estrogen therapy (Estradiol alone) was also obtained with the same procedures as applied to the study of Astragalus. Thus, the benefits between Astragalus and estrogen therapies in protecting the auditory function could be compared.

Forty-eight female, Sprague-Dawley rats were used to report the results. They were 2-month old, weighing 180-200 grams. All rats were assigned randomly into 4 groups (Sham, ovariectomy [OVX], OVX+H, OVX+E). Each group comprised 12 rats. The rats in the Sham group did not receive ovariectomy or Cisplatin treatment. This group served as a control. All rats in the other three groups received OVX and 3 weeks later they were administered with Cisplatin. Moreover, the rats in the OVX+H and OVX+E groups received Huangqi (H) and estrogen (E) therapies, respectively. A university animal ethics committee (China) reviewed and approved all the procedures. The auditory function of all rats was measured with DPOAE (Distortion Product Otoacoustic Emissions) and ABR (Auditory Brainstem Responses), DPOAE is a test to record the acoustic responses elicited when the cochlea is stimulated simultaneously with two pure tones, while ABR is a test to examine how well a sound travels along the auditory nerve to the brainstem by measuring the electrical responses at the scalp. To perform ovariectomy or DPOAE / ABR tests, the rats were sedated with 10% chloral hydrate ketamine (3ml/kg body weight) by an intra-peritoneal injection. All animals
were monitored daily to ensure they were well hydrated and their bodies were kept warm during anesthesia. The ovariectomy was administered to the rats in the OVX, OVX+H and OVX+E groups. Each animal’s abdominal skin and peritoneum were cut open, the ovarian arteries were then ligated and the ovaries were excised bilaterally before the muscle wall and skin were sutured. The animals in the Sham group also went through the same surgical procedures except their ovarian arteries and ovaries were kept intact. The sham surgery should not cause the estrogen level to change. Astragalus or estrogen therapy started after ovariectomy and lasted for 3 weeks. The rats in the OVX+H group were injected intra-peritoneally with Huangqi extract (Chiatai Qingchunbao, China) at a daily dose of 5 ml/kg body weight, while the rats in the OVX+E group were given Estradiol Valerate (Bayer, China) by gavage at a daily dose of 0.1 mg/kg body weight. The daily dose for Huangqi or Estradiol administered to the rats was comparable to approximately a dose of 1 mg/kg body weight given to a 60 kg woman. Though the rats in the OVX and Sham groups did not receive any Astragalus or estrogen therapy, they were injected intra-peritoneally daily with saline water of 5 mg/kg body weight. After 3 weeks, all the rats in the OVX, OVX+H and OVX+E groups were administered intra-peritoneally with Cisplatin for 4 days at a daily dose of 5 mg/kg body weight. Typically, a cycle of 4 days is used in the combination chemotherapy. The auditory function of individual animals was then tested at 24 hours after the Cisplatin treatment was ended. The rats in the sham group were also tested on the same day as the other ovariectomized rats.

Figure 1a shows the average SNR (signal-to-noise ratio) obtained in the DPOAE test for the four groups at the end of the experiment. A higher SNR suggests a better hearing function (i.e., higher cochlear responses) in the peripheral auditory system. The SNRs for the three groups of ovariectomized rats (OVX, OVX+H, OVX+E) are significantly lower than the SNRs for the Sham group across the stimulus frequencies 2 – 8 kHz, $p<0.05$. To examine the therapeutic effects of Huangqi and Estradiol, the SNRs for the OVX+H and OVX+E groups are compared with the SNRs for the OVX group. The SNRs for the OVX+H are significantly better than those for the OVX group at 2 kHz and 8 kHz, while the SNRs for the OVX+E group are significantly better than those for the OVX group at 2, 4, 6, and 8 kHz, $p<0.05$ (shown with an asterisk in Figure 1a). In Figure 1b, it plots the average hearing thresholds obtained in the ABR test at the end of the experiment. An elevated hearing threshold indicates a worsened hearing function in the central auditory pathway. The hearing thresholds for the three groups of rats with ovariectomy (OVX, OVX+H, OVX+E) are
significantly higher than the hearing thresholds for the Sham group across the stimulus frequencies, \( p<0.05 \). Moreover, the hearing thresholds for the OVX+H and OVX+E groups are significantly lower than those for the OVX group across all stimulus frequencies, \( p<0.05 \) (shown with an asterisk in Figure 1b), except at 2 kHz where the hearing thresholds for the OVX+H and OVX groups are similar.

Therefore, the current study shows a significant damage to the auditory function in the OVX group relative to the sham group when the ovariectomized rats are challenged with the ototoxic drug, Cisplatin. However, when the performance of the ovariectomized rats with Huangqi therapy (OVX+H) is compared to that of the ovariectomized rats without any therapy (OVX), the SNR in DPOAE test and the hearing threshold in ABR measurement are significantly improved. This finding suggests that Astragalus, comprised primarily with polysaccharides, astragalosides and flavonoids, can be used as an agent to protect the auditory function of ovariectomized rats (estrogen deficiency) under the treatment of Cisplatin. Specifically, the benefits of Astragalus therapy in protecting the auditory function can be observed in both the peripheral (cochlear) and central auditory systems. Moreover, the current data indicates that the capability of Astragalus in protecting the auditory function is similar to that of estrogen therapy (Figure 1). As the use of Astragalus usually has milder side effects, the current findings may suggest that this Chinese herbal medicine can be considered as an alternative therapy in protecting the auditory function of patients with estrogen deficiency. In China, Astragalus is frequently combined with platinum-based chemotherapy in the treatment of cancer.\(^6\) However, depending on the health background of individual patients, Astragalus is seldom used alone as an agent therapy in clinical practice. Since the results reported in the current research are based on the design that Astragalus is used as a single-agent therapy, the benefits of Astragalus in protecting the auditory function may not be the same when it is combined with other herbal medicines; further investigation is needed.

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**Author contribution:** Hu XJ contributed the study conception and design, acquisition of data, analysis and interpretation of data, drafting of manuscript, and critical revision.

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References


Figure Legend

Figure 1: The average SNRs in DPOAE (a) and hearing thresholds in ABR (b) obtained for different groups. OVX = Ovariectomy, OVX+H = OVX + Huangqi, OVX+E = OVX + Estradiol. An asterisk (*) denotes that the difference between the OVX+H group (and the OVX+E group) and the OVX group is significant at a particular stimulus frequency.