Anti-inflammatory activities of Brazilian licorice (Periandra mediterranea) extract

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Abstract

Anti-inflammatory effects of Brazilian licorice (Periandra mediterranea or P. dulcis, local name: aracacuz da terra) extract on animal models for inflammatory or autoimmune diseases were examined comparing them to those of Chinese licorice (Glycyrrhiza glabra) extract. Brazilian licorice extracts potently inhibited the elevation of tumor necrosis factor-α and interleukin-6 concentrations, and glutamate-oxaloacetate transaminase activity in the sera of mice treated with P. acnes and lipopolysaccharide. Brazilian licorice extracts apparently inhibited the clinical symptom and bone destruction associated with the induction of arthritis in mice, and the body weight change in mice treated with Brazilian licorice extract was minimal. Brazilian licorice extracts apparently inhibited the clinical symptom and inflammatory cell accumulation in the parenchyma of spinal cord in rats immunized with bovine myelin basic protein. The inhibitory effects of Brazilian licorice extract on these disease models were apparently less pronounced than those of Brazilian licorice extract. Furthermore, the IC50 value of Brazilian licorice extract against 11β-hydroxysteroid dehydrogenase activity, one of the metabolizing enzymes for corticosteroids, was more than 3 times of Chinese licorice extract. These results clearly indicate that Brazilian licorice possesses potent anti-inflammatory activities with little adverse effect when compared to Chinese licorice.

Key words Brazilian licorice, Chinese licorice, inflammation, autoimmune disease, 11β-hydroxysteroid dehydrogenase, cytokine.

Introduction

Brazilian licorice (Periandra mediterranea, local name: aracacuz da terra), an indigenous leguminous plant in Brazil, has been used for phlegm removal, liver protection, virus infection, urinary disease, abdominal inflammation and taste correction similar to Chinese licorice (Glycyrrhiza species obtained in Central Asia, East Asia and Europe) in Asian countries, North America and Europe. A major active constituent in Chinese licorice is glycyrrhizin which is well known as a sweet triterpenoid glycoside possessing anti-inflammatory and anti-allergic activities, and there is a number of reports on the action of glycyrrhizin. In contrast, very few investigations on Periandra species have been performed. Hashimoto and his colleagues have reported some periandrins, constituents of Periandra mediterranea. Periandrins are sweet triterpenoid glycosides similar to glycyrrhizin, but there is no carbonyl group on C-11 site in perianandrins distinct from glycyrrhizin. It is well known that the carbonyl group in glycyrrhizin is essential for its medicinal activities. Glycyrrhizin prevents the degradation of corticosteroid through inhibiting 11β-hydroxysteroid dehydrogenase (HSD) activity. A similar inhibitory effect is observed in aldosterone metabolism and administration of glycyrrhizin for a long period results in the pseudo-aldosteronism characterized by edema and hypertension. Activities of glycyrrhizin independent of HSD inhibition are also recognized. As the carbonyl group in C-11 position of glycyrrhizin is an important pathogenic determinant for pseudo-aldosteronism, periandrins lacking the carbonyl group seem to be beneficial.

In the present study, therefore, we investigated the anti-inflammatory activities of Brazilian licorice extract and compared them to those of Chinese licorice extract. We employed lipopolysaccharide (LPS)-induced mouse hepatitis model, collagen-induced mouse arthritis (CIA) model and rat experimental autoimmune encephalomyelitis (EAE) model for the observation of licorice actions.

Materials and Methods

Licorice roots and reagents. Dried root of Brazilian licorice on the market was purchased from Quimer ervas e espesias (Vila Maria, Brazil). Dried root of Chinese licorice was purchased from Matsuura Yakugyo Co. (Nagoya, Japan).

Prednisolone acetate (prednisolone, Shionogi, Osaka, Japan), Propionibacterium acnes (P. acnes, Van Kampen Group Inc.), lipopolysaccharide (LPS, prepared from E. coli, 0.55:B5, phenol extract, Sigma, St. Louis, Mo., USA), bovine type II collagen (CII, Cosmo Bio, Tokyo, Japan), bovine myelin basic protein (MBP, Sigma), Mycobacterium tuberculosis (M. tuberculosis, H37.Ra, Japan Becton Dickinson, Tokyo) and Freund's complete adjuvant (FCA, Sigma) were obtained commercially.

Licorice extracts. Extracts from Brazilian and Chinese licorices were prepared as follows. Dry roots were ground

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and passed through a 60-mesh sieve. The dry root powder (300 g) was extracted with 4 times the weight of an ethanol aqueous solution (20%, w/w) at 60°C for 2 hours and then filtrated. The residue was extracted again in the same manner. The filtrates were combined and concentrated at 50°C under reduced pressure up to a concentration of 10%. Then the concentrate was dried using a spray dryer. Finally 24.4-25.3 g of Brazilian licorice extract and 27.0-28.5 g of Chinese licorice extract were obtained.

**Animals.** Female ddY mice, 7 weeks of age, male DBA/1J mice, 8 weeks of age, and female DA rats, 8 weeks of age, were purchased from Japan SLC (Hamamatsu, Japan) and used. They were housed in an air-conditioned room at 22 ± 1°C and fed a standard laboratory diet and water *ad libitum*. All experiments were carried out following a guideline for the care and use of experimental animals made by Gifu Pharmaceutical University.

**LPS-induced hepatitis in mice.** ddY Mice were sensitized with an intravenous injection of 0.5 mg/head *P. acnes*. After 7 days, hepatitis was evoked by injecting 0.01 mg/kg of LPS into the tail vein, and blood samples were taken from the orbital sinus at 2 hours after the LPS injection. Serum concentrations of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were measured using commercial enzyme-linked immuno-sorbent assay kits (Endogen, Cambridge, MA, USA). Serum glutamate-oxaloacetate transaminase (GOT) activity was measured using a GOT assay kit (GOT-UV Test Wako, Wako Pure Chemical, Osaka, Japan).

Brazilian licorice extract or Chinese licorice extract at a dose of 25 mg/head was administered intraperitoneally everyday from the day of sensitization. Saline as a negative control and 5 mg/kg of prednisolone as a positive control were administered similarly.

**CII-induced arthritis (CIA) in mice.** CIA was induced in DBA/1J mice according to a previously described method. CII was dissolved in phosphate buffered saline containing 0.01 M acetic acid at a concentration of 8 mg/ml and emulsified with an equal volume of FCA supplemented with 4 mg/ml *M. tuberculosis*. DBA/1J mice were immunized by injecting 50 μl of the emulsion 2 times on day 0 into the dorsal skin and on day 21 into the base of the tail.

Evaluation of clinical symptoms of arthritis was carried out before the initial immunization (week 0) and 3, 4, 5, 6, 7 and 8 weeks after the initial immunization by the same observer. The severity of arthritis in the metacarpophalangeal wrist, metatarsophalangeal and ankle joints was scored as: 0: normal, 1: swelling and erythema of 1-2 digits, 2: swelling and erythema of 3-5 digits, 3: mild swelling and erythema of the limb, 4: gross swelling and erythema of limb, and 5: gross swelling and erythema of the limb with joint rigidity. The paw volume was measured using a plethysmometer (TK-101, UNICOM, Chiba, Japan). At the end of the experiment (week 8), bone changes were assessed radiographically using a Softex X-ray apparatus (PK-5, Shimadzu Rika Instruments Co., Kyoto, Japan). The change was graded as: 0: negative, 1: subtle erosions, 2: mild erosions, and 3: severe erosions in multiple joints. The sum of each value from the four joints of the hind limbs was indicated as Softex index (score range: 0-12). For the histopathological examination, the limbs of all mice were amputated and immersed in 10% formalin. The joints were decalcified, embedded in paraffin, sectioned and stained with hematoxylin and eosin. A histopathological analysis was carried out to observe degeneration of cartilage, formation of pannus and periosteal tissues.

After the primary immunization, 6.25 or 12.5 mg/head of Brazilian licorice or Chinese licorice extract, which was dissolved in saline, was intraperitoneally injected every other day for 8 weeks. Saline was used for a negative control and 5 mg/kg of prednisolone was used for a positive control.

**EAE in rats.** EAE was induced in DA rats by injecting bovine MBP as previously described. MBP dissolved in phosphate-buffered saline at a concentration of 2 mg/ml and mixed with an equal volume of FCA supplemented with 4 mg/ml *M. tuberculosis*. Fifty microliters of the emulsion was injected subcutaneously into both hind footpads of the animals (100 μg MBP/head). The clinical symptom of EAE was evaluated macroscopically everyday until day 25 after the immunization by the same observer. The severity of EAE was graded as follows: 0: no change, 1: motor-paralysis of tail, 2: partial paralysis of hind legs, 3: complete paralysis of hind legs and partial paralysis of front legs, 4: paralysis of four legs, and 5: death. In order to confirm the symptom, histopathological examination of the spinal cord was carried out. Under anesthesia, rats were sacrificed on day 13 after the immunization, because control animals showed maximum clinical symptoms on day 13. The tissues were treated in a similar manner as in the case of CIA.

To evaluate the efficacy of the extracts, Brazilian or Chinese licorice extracts (12.5 or 25 mg/head) was administered intraperitoneally everyday from day 0 to day 11 after the immunization with MBP. As a positive control, 5 mg/kg of prednisolone was administered in a similar manner.

**Assay of 11β-hydroxysteroid dehydrogenase (HSD) activity.** Freshly prepared rat liver homogenate was centrifuged at 10,000×g for 10 minutes and the supernatant was then centrifuged again at 105,000×g for 60 minutes. The obtained precipitate (rat liver microsome) was suspended in 0.25 M sucrose and used as an HSD active fraction. The enzyme activity was measured in a 0.8 ml assay mixture containing 100 mM Tris-HCl, pH 8.0, 1.0 mM NADP, 125 mM cortisol and an adequate amount of rat liver microsome fraction. The extract of Brazilian or Chinese licorice was added to the reaction mixture as an inhibitor ranging from 2 mg/ml to 12.5 mg/ml. The reaction was started by adding cortisol and terminated by addition of ethyl acetate (0.5 ml) under vigorous mixing. Then cortisol and produced cortisone in the reaction mixture were extracted two times with the ethyl acetate. The amount of the cortisone was determined with reverse phase HPLC and the relative activities were calculated and compared with the activity without inhibitor which was estimated as 100%.

**Statistical analysis.** Most results were indicated as mean values and standard error. The statistical significance of differences was evaluated by Student’s t-test, Mann-Whitney’s
Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on lipopolysaccharide (LPS)-induced hepatitis in ddY mice

Seven days after an intravenous injection of *P. acnes*, hepatitis was evoked by injecting LPS intravenously. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at a dose of 25 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally everyday throughout the experiment. Two preparations of each extract (a and b) were examined. A: serum TNF-α concentration, B: serum IL-6 concentration, C: serum GOT activity. Each value represents the mean ± S.E.M. of 8 mice. *p<0.05, **p<0.01

![Fig. 1](image)

**Results**

**Effects on LPS-induced hepatitis.** Effects of Brazilian and Chinese licorice extracts on the elevation of cytokine concentrations and GOT activity in the sera of mice treated with *P. acnes* and LPS were examined. As shown in Fig. 1, after induction of hepatitis, elevated serum TNF-α and IL-6 concentrations, and elevated serum GOT activity were detected. Brazilian licorice extract significantly reduced the elevated serum concentrations of the cytokines and the reduction was comparable to that by prednisolone. In contrast, Chinese licorice extract showed a tendency to decrease the concentration of the cytokines (Fig. 1 A and B). Similarly, Brazilian licorice extract depressed the elevated serum GOT activity significantly, although Chinese licorice extract and prednisolone failed to reduce the elevated activity significantly (Fig. 1 C).

A single dose of Brazilian licorice extract, 10 mg/head, given 30 minutes before LPS injection diminished the elevated concentration of serum TNF-α, but not serum IL-6. In contrast, a single dose of Chinese licorice extract did not reduce the elevated serum cytokine levels (data not shown).

Administration of Brazilian and Chinese licorice extracts did not affect the body weight gain and intake of food and water in mice (data not shown).

**Effects on CIA.** Effects of Brazilian and Chinese licorice extracts on CIA were investigated. Changes in the arthritis index and the foot pad volume are shown in Fig. 2. Brazilian licorice extract at doses of 6.25 and 12.5 mg/head inhibited the elevation of the arthritis index, whereas Chinese licorice extract had no effect (Fig. 2 A). Furthermore, Brazilian licorice extract showed a tendency to inhibit the increase in foot pad volume, although Chinese licorice extract had no effect (Fig. 2 B). Prednisolone inhibited the changes of both parameters completely.

Results of arthritis-associated bone destruction are shown in Fig. 3. Softex index elevated according to the progression of arthritis. Although Brazilian licorice extract at a dose of 12.5 mg/head tended to inhibit the elevation, Chinese licorice extract had no effect. Prednisolone completely inhibited the elevation of Softex index. In histopathological study, formation of pannus, proliferative synovitis, degeneration and erosion of cartilage, and infiltration of inflammatory cells have been observed.

![Fig. 2](image)
cells were observed with limbs taken at the end of experiment (week 8). As shown in Fig. 4 A, severe proliferative synovitis and degeneration and erosion of cartilage were observed in the control mice. Brazilian licorice extract (12.5 mg/head) and prednisolone (Fig. 4 C and F) completely prevented the establishment of these symptoms and the extract administered at a dose of 6.25 mg/head apparently reduced the symptoms. Chinese licorice extract was less effective in preventing these symptoms than Brazilian licorice extract (6.25 mg/head) even when the Chinese licorice extract was administered at a dose of 12.5 mg/head (Fig. 4 E).

The change in body weight in the whole experimental period is indicated in Fig. 5. In control mice, body weight loss accompanying the progression of arthritis was observed from week 4. In mice which received Brazilian licorice extract, the body weight loss was prevented in a dose-dependent manner. The body weight change in Chinese licorice extract-administrated mice was similar to that of control mice.

**Effects on EAE.** Effects of Brazilian and Chinese licorice extracts on clinical symptoms of EAE were investigated. The results are shown in Fig. 6. Clinical scores in control mice elevated from day 9 after the onset of immunization, reached the maximum on day 11, and declined thereafter. Brazilian licorice extract or Chinese licorice extract (at a dose of 12.5 mg/head) delayed the onset and reduced the duration of EAE, and slightly improved the symptoms. Although the elevation of clinical scores was clearly inhibited by 25 mg/head of Brazilian licorice extract, the recovery was slightly later than that observed in the other treatment. No clinical symptoms were observed in mice treated with prednisolone.

In the histopathological study on day 13 after the onset of immunization with MBP, a marked accumulation of inflammatory cells to parenchyma of the spinal cord and the increase of glial cells were observed in control mice (Fig. 7 A). Brazilian licorice extract at a dose of 25 mg/head and prednisolone at a dose of 5 mg/kg completely inhibited the inflammatory cell accumulation (Fig. 7 B and D), whereas Chinese licorice extract at a dose of 25 mg/head did not (Fig. 7 C).

**Fig. 3** Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on bone destruction associated with bovine type II collagen (CII)-induced arthritis (CIA) in DBA/1J mice. Mice were immunized by CII emulsified in FCA to cause arthritis. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at doses of 6 and 12.5 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally every other day throughout the experiment. The bone change at week 8 was expressed as Softex index. ***p<0.001

**Fig. 4** Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on histological change in knee joints of DBA/1J mice immunized with bovine type II collagen (CII) to cause arthritis (CIA). Mice were immunized by CII emulsified in FCA to cause arthritis. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at doses of 6 and 12.5 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally every other day throughout the experiment. Tissue sections at week 8 were stained with hematoxylin and eosin. A: control, B: BL 6 mg/head, C: BL 12.5 mg/head, D: CL 6 mg/head, E: CL 12.5 mg/head, F: Pred 5 mg/kg
Fig. 5 Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on body weight change in DBA/1J mice immunized with bovine type II collagen (CII) to cause arthritis (CIA). Mice were immunized by CII emulsified in FCA to cause arthritis. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at doses of 6 and 12.5 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally every other day throughout the experiment. Each value represents the mean of 6 mice.

Fig. 6 Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on experimental autoimmune encephalomyelitis (EAE) in DA rats. EAE was evoked in rats by immunization with bovine myelin basic protein (MBP) emulsified in FCA. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at doses of 12.5 and 25 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally everyday from day 0 to day 11. Each value represents the mean ± S.E.M. of 6 mice. *p<0.05, **p<0.01

Fig. 7 Effects of Brazilian licorice extract, Chinese licorice extract and prednisolone on histological change in spinal cord of DA rats immunized with bovine myelin basic protein (MBP) emulsified in FCA. EAE was evoked in rats by immunization with MBP emulsified in FCA. Brazilian licorice extract (BL) and Chinese licorice extract (CL) at doses of 25 mg/head, and prednisolone (Pred) at a dose of 5 mg/kg were administered intraperitoneally everyday from day 0 to day 11. Tissue sections at day 13 were stained with hematoxylin and eosin. A: control, B: BL 25 mg/head, C: CL 25 mg/head, D: Pred 5 mg/kg
Inflammation and Brazilian licorice

**Fig. 8** Inhibition of 11β-hydroxysteroid dehydrogenase (HSD) activity by Brazilian licorice extract and Chinese licorice extract. The relative activity of rat microsomal HSD was compared in the presence of Brazilian licorice extract (○) and Chinese licorice extract (●). The value of 50% inhibitory concentration of each extract was estimated from the relative activity-concentration curves.

**Effects on HSD activity.** Results of HSD activity are shown in Fig. 8. Although Brazilian licorice extract exhibited an inhibitory action on HSD activity, the inhibition was much lower than that of Chinese licorice extract. The IC50 values were estimated as 320 and 90 μg/ml for Brazilian and Chinese licorice extracts, respectively.

**Discussion**

In the present study, we demonstrated potent anti-inflammatory effects of Brazilian licorice extract on animal models for autoimmune diseases. Brazilian licorice extract apparently attenuated the clinical symptoms in mouse CIA as a model for rheumatoid arthritis (RA) and rat EAE as a model for multiple sclerosis. Furthermore, Brazilian licorice extract potently inhibited the elevation of serum TNF-α and IL-6 concentrations, and GTO activity in hepatitis mice. However, Chinese licorice extract was less effective in inhibiting these increases.

Chinese licorice has been used for a long period for the treatment of gastric and duodenal ulcer, tussiculation, inflammation and allergy, and various flavonoids and saponins involved in the herb have been reported as effective constituents. Glycyrrhizin, a major active constituent of Chinese licorice is well known to be effective against ulcer and hepatitis. As shown in the present results, however, Chinese licorice extract was less effective in attenuating arthritis, encephalomyelitis and hepatitis induced in experimental animals than Brazilian licorice extract. It is suggested, therefore, that Brazilian licorice contains some active constituents that may exhibit distinct mechanisms of anti-inflammatory action from those in Chinese licorice. Although the active components in Brazilian licorice were not specified in the present study, periandrins, sweet triterpenoid glycosides, whose structures are closely resemble to glycyrrhizin, are suggested to be candidates.

It is well known that long-term administration of glycyrrhizin shows pseudo-aldosteronism characterized by edema, hypertension and hypopotassemia, and that one of the pathogenic mechanisms is the inhibition of HSD, a corticosteroid metabolizing enzyme, by glycyrrhizin. In the present study, we examined and compared the inhibitory effects of Brazilian and Chinese licorice extracts on HSD activity. The IC50 value of Brazilian licorice extract was almost 3 times higher than that of Chinese licorice extract. Therefore, the incidence and degree of pseudo-aldosteronism by prolonged administration of Brazilian licorice seem to be lower than those of Chinese licorice. Although the carbonyl group in C-11 position of glycyrrhizin is an important determinant for pseudo-aldosteronism, periandrins, putative active constituents in Brazilian licorice, lack the carbonyl group. The weak inhibitory effect of Brazilian licorice on HSD activity may be explained by the presence of periandrins instead of glycyrrhizin. In addition, we confirmed that Brazilian licorice extract did not affect the body weight gain and that it inhibited the body weight loss associated with the onset of inflammatory diseases in experimental animals. These results indicate that the incidence and degree of adverse effects of Brazilian licorice could be low.

TNF-α is one of the most attractive targets for RA treatment and has been reported to be responsible for inflammatory cell accumulation, neo-vascularization and other processes in RA. Although it had been considered that anti-cytokine antibodies such as anti-IL-1β, anti-IL-6 and anti-TNF-α antibodies might be effective for RA, only anti-TNF-α antibodies have proved to be effective in the treatment of RA. Although, at present, infliximab, a chimeric anti-TNF-α antibody preparation, has been introduced in clinical use in the United States, gradual decrease in effectiveness is recognized because of the formation of anti-infliximab antibodies, and co-medication with other drugs such as methotrexate seems to be essential. In the present study, we demonstrated that Brazilian licorice extract potently inhibited TNF-α production in mice with hepatitis. The potency of 25 mg/head of Brazilian licorice extract was comparable to that of 5 mg/kg of prednisolone. Brazilian licorice may be a good remedy for RA.

In summary, Brazilian licorice possesses potent anti-inflammatory activities with little adverse effect when compared to Chinese licorice. Brazilian licorice is, therefore, expected to be a remedy for inflammatory diseases including RA and multiple sclerosis. Although some saponins such as periantradulcin A, B and C as well as periandrins exhibit phosphodiesterase inhibition, it has not been examined yet whether the inhibition of phosphodiesterase is involved in the anti-inflammatory action of Brazilian licorice extract. We need further study to elucidate the active constituents of Brazilian licorice and the mechanisms of their anti-inflammatory actions.

**References**


**Japanese abstract**

プラジル固有のマメ科植物であるブラジルカノソの抽出物の抗炎症作用を検討し，中国カノソ抽出物の作用と比較した。ブラジルカノソ抽出物はlipopolysaccharideで肝炎を誘発したマウスに与われる血中サイトカイン濃度およびGOT 活性の上昇を強く抑制し，collagenで誘発したマウス関節炎の症状および骨破壊を軽減し，関節炎病変に伴う体重減少を抑制した。また，ウサ myelin basic proteinで免疫したラットの脳脊髄炎症状および炎症性細胞浸調を明らかに抑制した。これらの炎症抑制効果は中国カノソ抽出物では軽度であった。さらに，中国カノソによる偽アルドステロン症症には腎臓皮質ホルモンを代謝する11-ヒドロキシステロイド脱水素酵素の阻害が関与すると言われているが，ブラジルカノソ抽出物の脱水素酵素活性は低下，そのIC50 値は中国カノソ抽出物の3倍以上であった。以上の成績から，ブラジルカノソは中国カノソよりも抗菌力の抗炎症効果を発揮するが，偽アルドステロン症などの副作用は軽度であると推定される。

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