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Research paper

Efficacy of whole extract of licorice in neurological improvement of patients after acute ischemic stroke

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ABSTRACT

Objective: Licorice root has been reported to contain several neuroprotective compounds. In the present study we investigated its benefit in the treatment of acute ischemic stroke for which, treatment modalities are limited.
Design: Randomized double-blind placebo controlled trial.
Subjects: 75 patients admitted to the neurology emergency department of Namazi hospital affiliated to Shiraz University of Medical Sciences, Iran, diagnosed with acute ischemic stroke.
Intervention: Patients were randomly prescribed oral 450 mg or 900 mg licorice extract or placebo capsules three times daily for 7 days. National institute of Health stroke scale (NIHSS) and Modified Rankin Scale (MRS) scores were assessed before initiation of therapy and 3 months after treatment. Improvement of these scores were compared between study and control groups.
Results: Mean NIHSS scores in 450 mg and 900 mg groups decreased from an initial score of 10.68 and 10.44 to 6.4 and 5.48 after 3 months respectively; while in the control group changed from 8.36 to 5.64. The decline in NIHSS scores were significantly greater in licorice treated groups than the control group. Similarly the decrease in MRS was greater in the licorice treated groups (4.2–2.9 in 450 mg licorice group, and 4.4–2.8 in 900 mg licorice group) versus the control group (3.9–2.8). None of the participants developed adverse reactions attributed to licorice overdose.
Conclusions: The results of this study support the beneficial effect of whole licorice extract in neurologic improvement of patients with acute ischemic stroke. Licorice may be useful as a medication for the treatment of the adverse effects caused by acute ischemic stroke.

1. Introduction

Stroke is one of the leading causes of mortality and disability both in developed and developing countries (Borhani-Haghighi et al., 2013; van der Worp and van Gijn, 2007). Strokes can be categorized by their major pathologic event as ischemic and hemorrhagic, with the ischemic type accounting for 80% of all cases. In an ischemic stroke, occlusion of an artery results in hypoxic damage to the zone of brain tissue supplied by that artery (van der Worp and van Gijn, 2007; Borhani Haghighi et al., 2010). Since neurologic tissue lacks self-regeneration capacity, a stabilized damage cannot be reversed. Immediately after an ischemic stroke reestablishment of blood supply is crucial within a period of three hours before persistent damage occurs. The only available standard and approved approach for reperfusion of the ischemic zone is clot destruction using recombinant tissue plasminogen activator (rtPA) (Wardlaw et al., 2003; van der Worp and van Gijn, 2007). Although effective, there are shortcomings in treatment with rtPA including the risk of intracranial hemorrhage and a small therapeutic window (Hacke et al., 2004). Therefore, finding effective neuroprotective drugs for the patients for whom thrombolysis is contraindicated or not feasible seemed to be mandatory in the world of cerebrovascular medicine.

Licorice is extracted from the root of the licorice plant scientifically known as *Glycyrrhiza glabra* L. which has been historically used as an herbal medicine. *G. glabra* L. is a perennial belonging to the Leguminosae/Fabaceae (commonly known as pea and bean) family. The native origin of licorice is Western Asia,

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Northern Africa and Eurasia and is widely cropped in the Middle East. The plant grows best in dry, subtropical climates with full sun and well drained and nitrogen rich soil. It grows about 1–2 m tall and has underground stems (rhizomes) and highly branching roots which grow horizontally underground. Both rhizomes and roots have biochemically active substances including glycyrrhizin (sweet tasting agent) and flavonoids (responsible for yellow color of the root cuts) (Omar et al., 2012). These active substances have been widely studied for their pharmacologic effects and medical benefits in animal models and human studies (Fiore et al., 2005). Glycyrrhizic acid is a triterpene consisting of two conjugatied active molecules, glucuronic acid and glycyrrhetinic acid. All three agents are metabolically active, with anti-inflammatory, antiviral, and anti-oxidant properties (Ming and Yin, 2013). Glycyrrhizic acid has been shown to assert its anti-inflammatory effect through suppression of necrosis factor-κB, a key component of lipopolysaccharide-induced inflammatory response (Wang et al., 2011). Glycyrrhizic acid is also a potent neuroprotective agent that protects neural tissue from hypoxic damage *in vitro* by modulation of the PI3K/Akt pathway (Kao et al., 2009) and inhibition of neurotoxic HMGB1 pathway (Kim et al., 2012). The flavonoids in licorice root include liquiritin, isoliquiritin, isoflavones and glabridin. Flavonoids have been reported to possess neuroprotective effects in live mice (Zeng et al., 2013). *In vitro* studies have reported both liquiritin (Yang et al., 2013) and isoliquiritin (Yang et al., 2012) as neuroprotective agents by inhibition of glutamate mediated cytotoxicity after hypoxic damage to neural tissue.

1.1. Hypothesis

Early after occlusion of blood supply to a zone of the brain, a core of infarction forms, surrounded by a larger area of hypoxia called penumbra. Within the penumbral zone, hypoxic stress is not sufficient to cause immediate cell death but impairs the ability of the neurons to maintain membrane potential and consequent loss of membrane integrity. If re-perfused early, these cells can heal and retrieve normal function, but in persistence of ischemia, several cascades of inflammation, oxidative stress and tissue edema result in penumbral cell death leading to a larger zone of infarction (Ramos-Cabrer et al., 2011). The authors considered that since glycyrrhizic acid and flavonoids assert their neuroprotective effect by inhibition of similar pathways of neural damage responsible for ischemic neurotoxicity in the penumbral zone, that licorice root extract could result in clinical improvement in patients with acute ischemic stroke if administered early (before neurotoxic events damage the penumbral zone cells).

The present study was conducted as a double-blind randomized controlled clinical trial to verify the efficacy of oral administration of licorice in improvement of neurologic deficits after an acute ischemic stroke.

2. Materials and methods

2.1. Preparation of medications and randomization

In the present study, licorice root preparations were purchased from Shirin Daru Inc. (Shiraz, Iran). To prepare licorice extract in powder form the licorice root was first dried, roasted and ground into smaller than 0.1 mm particles. For each 100 g of licorice powder, 1000 ml of distilled water was added and heated at 5 atmosphere pressure and 80 °C for 30 min. The resultant solution was then filtered to eliminate solid particles and concentrated to 1/40 by volume. The concentrated extract was then spray-dried to form extract powders used to fill the study capsules. For the purpose of this study identical appearing capsules of licorice extract containing 450 mg and 900 mg of spray-dried powder of licorice extract concentrate were used.

Since toxicity of licorice intake is mostly due to its glycyrrhizic acid content (Bernardi et al., 1994), the purchased (450 mg and 900 mg) capsules were analysed to measure the glycyrrhizic acid content. Both 450 mg and 900 mg licorice preparations were filled with the same source of dried licorice extract. Random "high performance liquid chromatography (HPLC)" analysis of 100 capsules revealed that all contained a mean value of 7.85% by mass glycyrrhizic acid; making the glycyrrhizic acid content of the 450 mg and 900 mg capsules, 35.3 mg and 70.6 mg respectively. The "no observed-adverse-effect level" (NOAEL) for consumption of glycyrrhizic acid is reported as 217 mg/day in previous studies (Bernardi et al., 1994), which indicates that the prescribed daily intake of licorice in this study was within safe limits.

Medication boxes were prepared to contain 450 mg or 900 mg whole licorice extract or placebo capsules filled with Avicel (microcrystalline cellulose powder) an inert filler. Each box contained 21 capsules to be used three times a day for 1 week. Capsule packs were coded according to a computer-generated random number table. All medication boxes contained capsules with identical color, size and shape. The Administrators of the project were not informed of the grouping of the codes. Only the pharmacist supervisor of the study was not 'blinded'. The codes were not broken until the study results were entirely analyzed.

2.2. Study population

During a 2 year period from July 2012 to July 2014, all patients aged between 18 and 85 years who had been referred to the Neurology Emergency Department of Namazi hospital affiliated to Shiraz University of Medical Sciences with signs and symptoms of acute ischemic stroke and Recognition of Stroke in the Emergency Room (ROSIER) scale higher than 2 were invited to participate in the study. Patients with brain CT scans without formation of ischemic lesion consistent with their clinical picture were dropped from the study. Patients were only enrolled with National Institute of Health Stroke Scale (NIHSS) scores between 5 and 20 with a motor deficit of 2 or more (for either one arm or leg). Patients outside this range were excluded.

For higher validity of the study and elimination of other possible influencing factors, the authors considered strict exclusion criteria as follows:

- Clinically relevant preexisting neurological deficit or previous cerebrovascular accident (CVA)
- Primary intracerebral hemorrhage
- Coma (level of consciousness more than 2 in NIHSS scale)
- Negative swallow test
- Patients undergoing hemicraniectomy
- History of epilepsy
- Clinical seizure at onset of stroke
- Systolic BP is >160 mmHg, diastolic BP >110 at onset of stroke (if a rise in blood pressure occurred in the course of study it was controlled according to medical guidelines)
- Atrial fibrillation or other tachy/bradyarrythmias at time of allocation or in the middle of intervention
- Ejection Fraction less than 45%
- Potassium less than 4 mEq/dl at onset of stroke
- Malignancy or premalignant state within 5 years
- Myocardial infarction in previous month
- Significant kidney disease (creatinine higher than 1.8 mg/dl)
- Significant liver disease (Bilirubin >20 mmoll/L)
- Significant lung disease (FEV1 <1.5 L, $pO_2 < 70$ in room air, pCO_2 >45)
- Psychiatric illness requiring hospital admission