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Licorice mucoadhesive patches

The effects of licorice mucoadhesive patches on the results of nasopharyngeal swab real-time polymerase chain reaction test of SARS-CoV-2; a randomized triple-blind placebo-controlled clinical trial

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Key point

In a triple-blind randomized clinical trial, 125 patients with positive polymerase chain reaction for SARS-CoV-2, we found mucoadhesive patches containing licorice extract could eradicate SARS-CoV-2 in nasopharyngeal area of infected persons.

Abstract
Licorice mucoadhesive patches

**Introduction:** Glycyrrhizin (Licorice) is believed to have antiviral action against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

**Objectives:** This study aimed to evaluate the effectiveness of oral mucoadhesive patches containing licorice extract in the eradication of this novel coronavirus in the nasopharyngeal secretions of the patients.

**Patients and Methods:** In this triple-blind randomized clinical trial, 125 patients with positive real-time PCR (polymerase chain reaction) for SARS-CoV-2 and in stage 0 and/or 1 of COVID-19 were studied in two separate groups, namely interventional and placebo, in which they used Aftogel (licorice extract) and placebo patches, respectively. At the end of the study, the results of PCR test in aforementioned groups were assessed and compared.

**Results:** Following the application of Aftogel, real-time PCR was converted to negative in 53.97% of patients which was significantly higher than that of the placebo groups. (27.40%, \( P= 0.003 \)).

**Conclusion:** It seems that Aftogel mucoadhesive patch is effective in the eradication of SARS-CoV-2, which has colonized the nasopharyngeal area. Hence, this drug product has the potential for evaluation as a prophylactic agent against COVID-19.

**Trial registration:** The trial protocol was approved in the Iranian registry of clinical trial (#IRCT20181208041886N2; https://fa.irct.ir/trial/51774, ethical code; IR.MUI.MED.REC.1399.520).

**Keywords:** Licorice; Aftogel; SARS-CoV-2; Nasopharyngeal Swab

**Introduction**

Coronaviruses (CoVs) are single-stranded RNA viruses with envelope in order Nidovirales, family Coronaviridae, and subfamily Coronavirinae (1). Coronaviruses are classified into three different categories by their genetic and antigenic criteria; \( \alpha \)-CoVs, \( \beta \)-CoVs, and \( \gamma \)-CoVs. These viruses primarily infect birds and mammals and can cause various lethal diseases in these animals, which
Licorice mucoadhesive patches

may impact the farming industry (2). These viruses can also cause diseases in humans from infection in the upper respiratory tracts such as common cold to more severe diseases such as pneumonia, bronchitis, and even severe acute respiratory syndrome (SARS) (3,4). In late December 2019, several clusters of patients with severe pneumonia of unknown cause were reported by some health facilities (5). These clusters of patients were epidemiologically linked together with a seafood market in Wuhan, in China. Finally, a novel coronavirus was detected in these patients named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) with the resulting disease being titled as coronavirus disease 2019 (COVID-19) by WHO. This virus can cause disorders in the digestive, liver, respiratory, and nervous systems (6). It is revealed that this virus belongs to β coronaviruses and is similar to SARS-CoV-1 and MERS-CoV (7). Therefore, similar to SARS-CoV and MERS-CoV, COVID-19 virus can be spread from person to person which may result in familial clustering. Although the majority of cases have improved spontaneously, some have developed severe and fatal complications with various organ failure, septic shock, and severe pneumonia, and acute respiratory distress syndrome (ARDS) and at last death. This viral disease has currently developed into both a pandemic infectious and contagious disease spreading in various countries throughout the world.

Moreover, no effective treatment is now available for this infection, and efficacious medication is urgently needed to combat the disease and the spread of the virus.

Glycyrrhizin or glycyrrhizic acid (GLR) is a triterpenoid saponin that is believed to be useful for the destruction of the COVID-19 virus in nasopharyngeal area (8). Not only does Glycyrrhizin inhibit viral replication, but it also interferes with the viral entry into the cells (8). Glycyrrhizin is mainly a substance in the roots of licorice, which is cultivated in many countries (9). This plant contains over 10 various saponins, which is thought to have antiviral effects (9,10). In addition,
Licorice mucoadhesive patches

Glycyrrhizin has some anti-inflammatory effects. It is said that GLR may inhibit cytokines release (e.g., IL-4 and IL-3). Besides, this compound can modulate the production of IL-8 as an inflammatory cytokine. The mechanism is induced by sequestering HMGB1 (high-mobility group protein B1) (8,11). As a result, this drug is used to treat some forms of skin diseases which have inflammatory bases such as erythrodermic psoriasis, atopic dermatitis, and contact dermatitis (8). Consequently, it can be argued that on one hand, this amphiphilic drug, like a saponin (such as GLR), could interfere with the entry of the virus into cells because of the well-known membrane effects of this drug. On the other hand, with respect to the target and in association with “danger-signal” in cells (like HMGB1), this drug can interfere in immune responses (8).

In Iran, there is an herbal drug made of black licorice available in drugstores which is now in the form of muco-adhesive patches named “Aftogel”. Currently, Aftogel muco-adhesive patches are registered in Iranian pharmacopeia and are mainly being used as an herbal drug to improve oral aphthous ulcers and radiotherapy-induced oral ulcers (Figure 1). This drug enjoys both good safety and economical profile.

**Objectives**

Since the prime materials of this product are licorice and GLR, in light of the antiviral effects, this study was conducted to evaluate the possible effects of Aftogel in the conversion of the positive results of COVID-19 virus PCR to negative in patients afflicted with this infection.

**Patients and Methods**

**Preparation of drug (Aftogel) and placebo patches**

Drug and placebo patches were produced by Exir Denesh Asia Pharmaceutical Company. To prepare Aftogel mucoadhesive patches, sodium carboxymethylcellulose (NaCMC) and licorice extract (Iran Kesht-o-SanatAtran company) with batch number of 211297 which contained at least 6.4% glycyrrhizic acid were used. At first, 100 g of licorice extract was dissolved in 500 ml
Licorice mucoadhesive patches
dionized water. Then, 100 g of NaCMC was also dissolved in 4500 ml of deionized water and these two solutions were mixed very slowly. The final solution was injected into sterile containers and dried up at a temperature of 60 °C. The prepared patches (weight: 15 mg; diameter: 1 cm; thickness: 0.5 mm) were packed in 25-numeral packets with the code 2020.

In order for the preparation of placebo patches, the same method and materials as for Aftogel were utilized without the addition of the licorice extract. Next, the fabricated patches were packed in 25-numeral packets with the code 4040. In terms of color, size, and weight, the patches and packets were all identical for both Aftogel and placebo.

**Standardization of the patches**
Aftogel patches were standardized based on the glycyrrhizic acid content by means of HPLC, and the subsequent content was obtained 2.6% (w/w) equivalent to 0.39 mg in each patch.

**Clinical study**
This was a triple-blind placebo-controlled randomized clinical trial conducted in outpatient clinics in Isfahan, Iran, affiliated to Isfahan university of medical sciences, from December 2020 to Jan 2021. In the present study, 125 patients with positive PCR for COVID-19 and in stages 0 or 1 of clinical disease (and without any lung involvement), were enrolled in two parallel intervention and control groups.

Inclusion criteria for this research were; a) positive COVID-19 PCR of nasopharyngeal swab, b) stage of 0 (asymptomatic) or 1 (mild) of COVID-19), and c) consent to participate in the study. Patients with the following criteria did not enroll in the study; a) below 18 years of age, b) no compliance for drug consumption, c) elderly with no cooperation, d) Mental retardation, e) use of other non-herbal and herbal drugs for COVID-19 f) underlying diseases such as hypertension, g) use of any antiseptic’s mouthwash solutions.
Licorice mucoadhesive patches

Exclusion criteria during the study were: a) hypersensitivity reaction to the study drug, b) consumption of any anti-bacterial and/or antiviral drug during the study, c) admission to the hospital during the study.

The patients with inclusion criteria were randomized (by balanced block randomization using blocks of size 4 with all possible arrangements of two 2020 and two 4040 codes in each block followed by a random selection of blocks by random number table) into drug (Aftogel) and placebo groups. Upon the first visit, each included patient was prescribed the randomly-selected patch. Next, they were asked to attach four patches on their soft palate until the drug disappeared and washed out by saliva, four times a day in a six-day time span. In addition, they were encouraged and controlled for the proper application of the patches by one of the team members every day. Furthermore, the patients were instructed to discontinue the patch application and inform the physician in case they develop any symptoms of allergic reaction (e.g., pruritus; chest tightness; shortness of breath and a cough; skin rash or hives; and swollen lips, face, eyes, and tongue). It is worth mentioning that only the pharmacist who prepared the patches was aware of the type (drug or placebo), while the physician, patients, sampler, laboratory staff and the statistical analyzer were all blind to it. Throughout the study, the signs and symptoms of the patients were monitored, so that any patient who developed the signs and symptoms of moderate or severe disease was excluded and encouraged to refer to the physician for appropriate management. On the 7th day of the intervention, all the selected patients were called back and, reverse transcription polymerase chain reaction (RT-PCR) test was repeated on the nasopharyngeal swab of the patients who had applied the patches properly and completely. At the end of the intervention and after statistical analysis, the codes were revealed by the pharmacist and the type of intervention for each patient was determined.
Licorice mucoadhesive patches

Sample collection

According to CDC guideline, nasopharyngeal/oropharyngeal specimens were collected properly by means of sterile swabs (Dacron swabs). Swabs were then placed into a 15-mL tube containing Viral Transport Medium (VTM). It should be noted that sample collection was carried out for each patient before and 5 days after the application of patches.

RNA extraction

Viral RNA extraction was performed through the QIAamp Viral RNA mini kit (Qiagene, Germany) in line with the manufacturer’s instructions. The extracted RNA was stored at -70°C until use.

Real-time PCR

In order to perform real-time polymerase chain reaction (real-time PCR) test, the kit of Pishtaz Teb Company, which is approved by the ministry of health, was used. The kit contains all the necessary enzymes for reverse transcription and DNA amplification. The reaction mixture contained 5 µL of double distilled water (ddH2O), 9 µL of the enzyme buffer mixture, 1 µL of each primer (10 mM) and probes (10 mM). The final solution was then transferred to a tube containing lyophilized enzyme mixed gently three times without bubbling. After adding 5 µL of RNA template, the mouth of the tube was closed with a cork and incubated for 15 minutes at room temperature. Notably, master mix was prepared according to the number of samples and controls (positive and negative controls). Real-time PCR was performed on Rotor-gene device (Qiagen, Germany) according to the manufacturer's instructions as follows: 50 °C for 20 min for reverse
Licorice mucoadhesive patches

transcription, a three minutes for pre-denaturation at 95 °C followed by 45 amplification cycles of three second at 94 °C and 40 second at 55 °C for each step (annealing-extension step).

Data analysis

Statistical analysis was conducted using SPSS software version 24 (SPSS Inc., Chicago, USA). The results were reported as the frequencies and the corresponding percentages. Independent-Samples t-Test and chi-square test were used to compare the groups regarding age and categorical variables, respectively. A p-value <0.05 was assumed to be statistically significant.

Results

Over the study period, from 2020/Nov to 2021/Jul, 260 patients were evaluated for eligibility that 179 of whom fulfilled the inclusion criteria and were enrolled; nevertheless, 54 patients were excluded from the study (Figure 2). Finally, a total of 125 patients completed the study including 34 males and 29 females (totally 63 patients) in drug group and 36 males and 26 females (totally 62 patients) in placebo group (P=0.645)

The mean age of the patients in both the drug and placebo groups was 335.73±11.80 and 36.21±12.33 years, respectively (P=0.834).

At the end of intervention, RT-PCR test of 53.97% (n=34) of the patients in Aftogel group converted to negative as compared to 27.4% (n=17) of patients in the placebo group with the difference being statistically significant (P= 0.003, OR: 1.968, 95% CI: 1.237 - 3.133).

Moreover, during the study no patient reported any sign of allergic reaction to the patch.
Licorice mucoadhesive patches

Discussion

This study was conducted on patients with positive COVID-19 real-time PCR, the patients who underwent Aftogel-patch treatment over a six-day period, and it had significantly higher odds of having a negative second PCR test than those receiving placebo patch. The difference in the results of tests in two groups was significant. (P = 0.003)

In this study, 125 patients were evaluated and a larger trial may be warranted. Notably, no similar study on licorice and its derivatives in patients with COVID-19 infection was conducted. Licorice is a common herbal drug with over 20 triterpenoids and 300 flavonoids along with a good therapeutic effect on viral and bacterial pathogens (12). It has been revealed that several compounds such as chrorogenic acid, rutin, hyperoside, hydroxyacephenone, scopoletin, scoparone, quercetin, luteolin, apigenin, acacetin, aristolactam, and glycyrrhizin are remarkable compounds in licorice, which could play a pivotal role in the treatment and prevention of coronaviruses infections like SARS (13).

Glycyrrhizin, the main compound in licorice, has been shown to have the ability to bind to ACE2 (angiotensin-converting enzyme 2) on the cells, an enzyme recently identified as the SARS-CoV-2 receptor. Therefore, it may be considered as a potential treatment for COVID-19 (14).

Aftogel patch is an herbal drug product that is made of black licorice. Considering the results of this study, it seems that this drug is effective in removing the viruses which are colonized on the pharynx. Therefore, this drug may be used for primary eradication of COVID-19 virus in the site of colonization (nasopharyngeal area) and may be useful in patients who have been in contact with COVID-19 patients.
**Licorice mucoadhesive patches**

There are some findings suggesting that glycyrrhizin has the activity to inhibit the inflammatory pathways by reduction of toll-like receptor 4 (TLR4) expression in the heart and the lungs (14). This reduction has been accompanied by a dramatic decline in tumour necrosis factor α, interleukin 6, and interleukin-1β (15). Other studies have indicated the TLR4 antagonistic effect of glycyrrhetinic acid (GA), one of the metabolites of glycyrrhizin, in several tissues including the lungs (16). Besides, these anti-inflammatory effects may also be important in the central nervous system (CNS) and, therefore, glycyrrhizin may protect the patients against psychiatric and neurological complications of COVID-19 infection (17,18).

Aftogel is a locally-used drug and it may not have these systemic anti-inflammatory effects. Therefore, evaluation of the systemic effects of licorice and/or glycyrrhizin is highly recommended in patients suffering from COVID-19 infection.

**Conclusion**

In conclusion, taking the results of this study into account, it seems that Aftogel mucoadhesive patch (licorice extract) is effective in the eradication of SARS-CoV-2 which has colonized the nasopharyngeal area. Therefore, this drug product has the potential for evaluation as a prophylactic agent against COVID-19, therefore it might be useful in the prevention of infection in patients who have come into contact with COVID-19 patients.

**Limitations of the study**

The main limitations of our study were small sample size and lack of assessment for clinical variables including signs and symptoms. However, as this study was placebo-controlled with a
Licorice mucoadhesive patches

triple-blind design, its results could be reliable as a basis for future clinical studies with larger sample sizes evaluating the effectiveness of Aftogel on the clinical course of COVID-19.

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Authors’ contribution

MP, MoS, and RS were the principal investigators of the study. MP, MoS, and RS were included in preparing the concept and design. MP, KS, FN, MaS and RS revisited the manuscript and critically evaluated the intellectual contents. All authors participated in preparing the final draft of the manuscript, revised the manuscript and critically evaluated the intellectual contents. All authors have read and approved the content of the manuscript and confirmed the accuracy or integrity of any part of the study.

Ethical issues

The research was conducted in accordance with the items of the Declaration of Helsinki. The Ethics Committee of Isfahan University of Medical Sciences approved this study. This committee accepted all study protocols (IR.MUI.MED.REC.1399.520). Accordingly, written informed consent was taken from all participants before any intervention. This study was a research project approved by the vice-chancellery for research and technology of Isfahan university of medical sciences. The trial protocol was approved in the Iranian registry of clinical trial (#IRCT20181208041886N2; https://fa.irct.ir/trial/51774).

Conflicts of interest

The authors declare that they have no competing interests.
Licorice mucoadhesive patches

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All costs of this work, including the costs of drug and placebo patches and the laboratory procedures, were provided by Exir Danesh Asia Pharmaceutical Company.

References


Licorice mucoadhesive patches


**Figure 1.** Aftogel patches, the patch contains licorice extract and has a sweet taste due to its glycyrrhizic acid content. After application on the oral mucosa, the patch absorbs the moisture, adheres to the area, and releases its active substance over a period of 10 to 20 minutes.
**Figure 2.** CONSORT flowchart of patients’ enrollment

- **Enrollment**
  - Assessed for eligibility (n=260)
  - Excluded (n=81)
    - Not meeting inclusion criteria (n=81)

- **Randomized (n=179)**
  - Allocated to control group (n=90)
    - Received allocated intervention (n=71)
    - Did not receive allocated intervention (due to declining to participate) (n=19)
  - Allocated to intervention (n=89)
    - Received allocated intervention (n=75)
    - Did not receive allocated intervention (due to declining to participate) (n=14)

- **Follow-Up**
  - Lost to follow-up (n=0)
  - Discontinued intervention (n=8)
  - Lost to follow-up (n=0)
  - Discontinued intervention (n=13)

- **Analysis**
  - Analysed (n=63)
    - Excluded from analysis (n=0)
  - Analysed (n=62)
    - Excluded from analysis (n=0)