**Introduction**

*Scutellaria baicalensis* Georgi (Labiatae), called ‘Hwang-Gum’ in Korea and as ‘Huang-Qin’ in China, is one of the most widely used herbal medicines against bacterial infection of the respiratory and gastrointestinal tracts and various inflammatory diseases\(^1,2\).

Prostaglandins (PGs) are key inflammatory mediators that are converted from arachidonic acid by cyclooxygenase (COX). There are two isoforms of COX: cyclooxygenase-1 (COX-1) is constitutively expressed in nearly all tissues and provides PGs to maintain physiological functions such as cytoprotection of the stomach and regulation of renal blood flow\(^3,4\). In contrast, cyclooxygenase-2 (COX-2) is inducible in the immune cells such as macrophages and synoviocytes in response to infection, injury or other stresses and COX-2 produces excessive amount of PGs that sensitize nociceptors and induce inflammatory states\(^5,6\). Prostaglandin E2 (PGE2) is overproduced at sites of inflammation as a result of the activation of inducible COX-2\(^7,8\).

The writhing test is a well-known animal test for the model of inflammatory and visceral pain.

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**Backgrounds**: *Scutellaria baicalensis* has been used as a medicinal plant to treat various disease conditions accompanying inflammatory response and oxidative stress.

**Objectives**: The aim of this study is to evaluate the effects of *Scutellaria baicalensis* against inflammatory, pain and edema.

**Methods**: In vitro, the effects of *Scutellaria baicalensis* against lipopolysaccharide-induced inflammation were investigated in mouse BV2 microglial cells. In vivo, the effects of *Scutellaria baicalensis* on acetic acid-induced writhing response, carrageenan-induced edema and the plantar test (nociceptive thermal stimulation) were investigated using rats and mice.

**Results**: The present results showed that pre-treatment with the aqueous extract of *Scutellaria baicalensis* suppressed the lipopolysaccharide-stimulated cyclooxygenase-2 expressions in mouse BV2 microglial cells. The aqueous extract of *Scutellaria baicalensis* inhibited acetic acid-induced abdominal pain in mice and also reduced thermal pain in rats. However, no significant inhibition on carrageenan-induced edema in rats.

**Conclusions**: The present study showed that *Scutellaria baicalensis* possesses anti-inflammatory and analgesic effects.

**Key Words**: *Scutellaria baicalensis*, analgesic, anti-inflammatory
It is useful test for studying anti-nociceptive effect. Carrageenan induced local inflammation is commonly used to evaluate analgesic effect. Therefore, carrageenan induced local inflammation (paw edema) is a useful model to assess the contribution of mediators involved in vascular changes associated with acute inflammation. Plantar test (Hargreaves method) is used to determine thermal pain threshold by exposing the animals to heat.

The effect of herbal medicine on inflammatory disease and pain is widely studied. For example, anti-inflammatory of Corydalis turtschaninovii, Clematis mandshurica are generally evaluated. Scutellaria baicalensis is also studied about acute renal failure, hepatocytes cytotoxicity, ischemia-reperfusion injuries of hearts, neuroprotective of the brain ischemia and so on. But, anti-inflammatory and analgesic actions of the Scutellaria baicalensis have not been clarified yet. In the present study, we investigated the effects of Scutellaria baicalensis against LPS-stimulated expressions of COX-1 and COX-2 in mouse BV2 microglial cells by using Western blot analysis in mouse BV2 microglial cells, in vitro. Moreover, the antinociceptive and anti-inflammatory effects of Scutellaria baicalensis using several experimental pain models, acetic acid-induced writhing response, carrageenan-induced for edema and response on thermal pain were also evaluated, in vivo.

### Materials and Methods

1. Preparation of the aqueous extract of Scutellaria baicalensis

To obtain the aqueous extract of Scutellaria baicalensis, 100 g of Scutellaria baicalensis was added to distilled water and extraction was performed by heating at 90°C for 2 h, concentrating with a rotary evaporator and lyophilization (Eyela, Tokyo, Japan). The resulting powder, weighing 20.58 g, was dissolved in saline solution and filtered through a 0.22 μm syringe before use.

2. In vitro experiments

1) Reagents

The Scutellaira baikalis was purchased from Oriental Medicine Hospital, Kyungwon University (Seoul, Korea). Rats and mice were purchased from Oriental Bio Co (Kyunggido, Korea).

2) Cell culture

Mouse BV2 macroglia cells were cultured in Dulbecco's modified Eagle's Medium (DMEM; Gibco BRL, Grand Island, NY, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA) at 37°C in 5% CO2-95% O2 in a humidified cell incubator. The cells were plated onto culture dishes at a density of 2 × 104 cells/cm² in culture dish, 24 h prior to drug treatments.

3) MTT cytotoxicity assay

Mouse BV2 macroglia cells were grown in a final volume of 100 μl culture medium per well in 96-well plates. In order to determine the cytotoxicity of Scutellaria baikalis, the cells were treated with Scutellaria baikalis at concentrations of 1 μg/ml, 10 μg/ml, 100 μg/ml, 1,000 μg/ml and 10,000 μg/ml for 24 h. Cultures of the cells of the control group were left untreated. After adding 10μl of the MTT labeling reagent containing 5 mg/ml 3-(4,5-dimethyl thiazol-2-y1)-2,5-diphenyl tetrazolium bromide in phos-
phate-buffered saline to each well, the plates were incubated for 2 h. Solubilization solution 100 µL containing 10% sodium dodecyl sulfate in 0.01 M hydrochloric acid was added to each well and the cells were incubated for another 12 h. The absorbance was then measured with a microtiter plate reader (Bio-Tek, Winooski, VT, USA) at a test wavelength of 595 nm with a reference wavelength of 690 nm. The optical density (O.D.) was calculated as the difference between the absorbance at the reference wavelength and that observed at the test wavelength. Percent viability was calculated as (O.D. of drug-treated sample/control O.D.) × 100.

4) Western blot analysis

Mouse BV2 microglial cells were lysed in an ice-cold whole cell lysate buffer containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1.5 mM magnesium chloride hexahydrate, 1 mM ethyleneglycol-bis-(β-aminoethyl ether)-N,N'-tetraacetic acid (EGTA), 1 mM phenylmethylsulfonyl fluoride (PMSF), 2 µg/ml leupeptin, 1 µg/ml pepstatin, 1 mM sodium ortho vanadate and 100 mM sodium fluoride and the mixture were incubated 30 min at 4°C. Cell debris was removed by microcentrifugation, followed by quick freezing of the supernatant. The protein concentration was measured using a Bio-Rad colorimetric protein assay kit (Bio-Rad, Hercules, CA, USA). Protein of 40 µg was separated on SDS-polyacrylamide gels and transferred onto a nitrocellulose membrane (Schleicher & Schuell GmbH, Dassel, Germany). Goat COX-1 antibody (1:1000; Santa Cruz Biotech, CA, USA) and Goat COX-2 antibody (1:1000; Santa Cruz Biotech, CA, USA) were used as a primary antibody. Horseradish peroxidase-conjugated anti-goat antibody (1:4000; Santa Cruz Biotech) for COX-1 and COX-2 was used as a secondary antibody. Band detection was performed using the enhanced chemiluminescence (ECL) detection system (Amersham Pharmacia Biotech GmbH, Freiburg, Germany).

3. In vivo experiments

1) Animals and treatments

Male ICR mice weighing 28-30 g and male Sprague-Dawley rats weighing 150-160 g were used for the experiments. The experimental procedures were performed in accordance with the animal care guidelines of the National Institute of Health (NIH) and the Korean Academy of Medical Sciences. The animals were housed under laboratory conditions at a controlled temperature (20±2°C) and maintained under light-dark cycles, each consisting of 12 h of light and 12 h of darkness (lighting from 07:00 to 19:00 h) with food and water made available ad libitum.

2) Acetic acid-induced writhing response in mice

Male ICR mice were divided into five groups: the control group, the acetic acid injection group, the acetic acid injection and 50 mg/kg Scutellaria baicalensis-treated group, the acetic acid injection and 100 mg/kg Scutellaria baicalensis-treated group and the acetic acid injection and 200 mg/kg Scutellaria baicalensis-treated group (n = 10 in each group). Scutellaria baicalensis and control vehicle were orally administered 1 h before the acetic acid injection. Then, the mice received intraperitoneally with 0.15 ml of 1.0% acetic acid as an irritant stimulus and placed in an individual plastic cage (20 × 30 × 12 cm high) for observation. The number of writhes was counted for 30 min after acetic acid injection.
3) Carrageenan-induced edema in rats

The volume of the paw edema in male Sprague-Dawley rats was measured in each animal using a plethysmometer (Ugo Basile, Italy) with a precision of two decimal places. To induce edema in the experimental animals, a single subplantar injection of carrageenan (1%, 0.05 mL Sigma Chemical Co., St. Louis, MO, USA) was given to each animal and the animals of the control group received injections of equivalent doses of normal saline as a same method.

Animals of Scutellaria baicalensis-treated groups received orally with 1 mL of the aqueous extract of Scutellaria baicalensis at the respective doses before 1 h of carrageenan injection and those of the control group and the carrageenan-induced edema group received equivalent amount of drinking water before 1 h of carrageenan injection. The paw volume was measured immediately before, 1 h, 2 h, 3 h, 4 h and 5 h after carrageenan injection. The animals were divided into five groups: the control group, the carrageenan-induced edema group, the carrageenan-induced edema and 100 mg/kg Scutellaria baicalensis-treated group, the carrageenan-induced edema and 200 mg/kg Scutellaria baicalensis-treated group and the carrageenan-induced edema and 400 mg/kg Scutellaria baicalensis-treated group (n = 10 in each group). The percentage of edema was calculated as follows:

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\text{Percentage of edema (\%)} = \frac{(V_t-V_n)}{V_n} \times 100
\]

\[V_t = \text{The paw volume of each time after the injection of carrageenan}\]

\[V_n = \text{The paw volume before the injection of carrageenan}\]

4) Plantar test (Hargreaves's method)

To assess nociceptive responses to thermal stimuli, paw withdrawal latency of male Sprague-Dawley rats was tested using the procedure previously described by Hargreaves et al. The center of a focused beam of radiant heat was applied to the plantar surface of the hind paw in rats and the withdrawal latency time was recorded. The infra-red intensity of the heat stimulus was 60 and stimulation was adjusted so that the baseline latency was 6 sec and 20 sec cut-off time was imposed to avoid tissue damage. Three minutes were allowed for the next test. The animals in the Scutellaria baicalensis-treated groups received orally with 1 mL of the aqueous extract of Scutellaria baicalensis at the respective doses before 1 h of test and those of the control group received equivalent amount of saline before 1 h of test. The withdrawal latency time was measured immediately before, 1 h and 2 h after drug administration. The animals were divided into four groups: thermal stimulation-induced nociception and drinking water-treated group, thermal stimulation-induced nociception and 50 mg/kg Scutellaria baicalensis-treated group, thermal stimulation-induced nociception and 100 mg/kg Scutellaria baicalensis-treated group and thermal stimulation-induced nociception and 200 mg/kg Scutellaria baicalensis-treated group (n = 6 in each group).

4. Data analysis

Results are presented as the mean ± standard error means (SEM). Statistical analysis was performed using one-way ANOVA followed by Duncan post-hoc test. Duncan's Test can be used to determine the significant differences between group means in an analysis of variance setting. Duncan's test is based on the range statistic for a detailed discussion of different post hoc tests. Difference was considered significant at p<0.05.
Results

1. Cell viability of *Scutellaria baicalensis* on BV2 microglia cells

In order to assess the cytotoxic effect of the aqueous extract of *Scutellaria baicalensis* on BV2 microglia cells, the cells were cultured with the aqueous extract of *Scutellaria baicalensis* at final concentrations of 0.001, 0.01, 0.1, 1 and 10 µg/ml for 24 h and MTT assays were then carried out. The cells cultured in *Scutellaria baicalensis*-free media were used as the control. The viability of cells incubated with *Scutellaria baicalensis* at concentrations of 0.001, 0.01, 0.1, 1 and 10 µg/ml for 24 h was 105.10 ± 0.94, 103.83 ± 1.70, 106.51 ± 1.71, 77.31 ± 2.06 and 30.07 ± 0.47 % of the control value, respectively.

The present results show that the *Scutellaria baicalensis* exerted no significant cytotoxicity until it was at a concentration of 0.1 µg/ml. However, a high concentration (1 µg/ml and 10 µg/ml) of *Scutellaria baicalensis* reduced cell viability. Then, we used the aqueous extract of *Scutellaria baicalensis* at concentration of 0.01 and 0.1 µg/ml for the next experiment (Fig. 1).

2. Effect of *Scutellaria baicalensis* on protein expression of COX-1 and COX-2

The level of COX-1 protein was 1.06 ± 0.02 following a treatment with 1 µg/ml LPS for 24 h. The level of COX-1 protein was 0.97 ± 0.01, 0.86 ± 0.04 and 0.80 ± 0.05 in the cells pre-treated with the aqueous extract of *Scutellaria baicalensis* at 0.01 mg/ml, 0.1 mg/ml and 500 µM acetylsalicylic acid (ASA), one hour before LPS treatment.

The level of COX-2 protein was markedly increased to 5.28 ± 0.81 following a treatment with 1 µg/ml LPS for 24 h. The level of COX-2 protein was decreased to 3.64 ± 0.58, 1.27 ± 0.16 and 1.49 ± 0.08 in the cells pre-treated the aqueous extract of *Scutellaria baicalensis* at 0.01 mg/ml, 0.1 mg/ml and 500 µM ASA one hour before LPS treatment.

The present results show that LPS enhanced COX-2 protein expression in mouse BV2 microglia cells and the aqueous extract of *Scutellaria baicalensis* suppressed LPS-induced COX-2 protein expression. However, LPS and the aqueous extract of *Scutellaria baicalensis* exerted no significant effect on the expression of

![Fig. 1. Cell viability of Scutellaria baicalensis on BV2 microglia cells. (A) Control group, (B) 0.001 mg/ml Scutellaria baicalensis-treated group, (C) 0.01 mg/ml Scutellaria baicalensis-treated group, (D) 0.1 mg/ml Scutellaria baicalensis-treated group, (E) 1 mg/ml Scutellaria baicalensis-treated group, (F) 10 mg/ml Scutellaria baicalensis-treated group. * represents p < 0.05 compared to the control group.](image-url)
COX-1 protein, except the aqueous extract of *Scutellaria baicalensis* at concentration of 0.1 mg/ml (Fig. 2).

3. Effect of *Scutellaria baicalensis* on acetic acid-induced writhing response in mice

The number of the writhing response in the control group was 0.25 ± 0.25. The number of writhing response in the acetic acid injection group was 39.88 ± 8.18. The number of writhing response in the acetic acid injection and *Scutellaria baicalensis* (50, 100 and 200 mg/kg)-treated group was 27.33 ± 1.61, 29.33 ± 6.10

Fig. 3. Effect of *Scutellaria baicalensis* on the number of writhing response. (A) control group, (B) 1% acetic acid-induced writhing response group, (C) 1% acetic acid-induced writhing response and 50 mg/kg *Scutellaria baicalensis*-treated group, (D) 1% acetic acid-induced writhing response and 100 mg/kg *Scutellaria baicalensis*-treated group, (E) 1% acetic acid-induced writhing response and 200 mg/kg *Scutellaria baicalensis*-treated group. * represents p < 0.05 compared to the control group. # represents p < 0.05 compared to the 1% acetic acid-induced writhing response group.
and 25.63 ± 3.04.

The present results showed that acetic acid injection into the abdominal cavity induced writhing response. The acetic acid injection and Scutellaria baicalensis-treated group suppressed acetic acid-induced writhing response (Fig. 3).

4. Effect of Scutellaria baicalensis on the volume of carrageenan-induced paw edema

After 1 h, paw volume in the control group was 0.00 ± 0.00 %. Paw volume in the 1 % carrageenan-induced edema group was increased to 5.96 ± 0.28 %. Paw volume of 1 % carrageenan-induced edema and Scutellaria baicalensis-treated groups at concentrations of 100, 200 and 400 mg/kg was 4.68 ± 1.79, 9.40 ± 1.52 and 7.87 ± 2.17 %.

After 2 h, paw volume in the control group was 0.00 ± 0.00 %. Paw volume in the 1 % carrageenan-induced edema group was increased to 17.57 ± 2.11 %. Paw volume in the 1 % carrageenan-induced edema and Scutellaria baicalensis-treated groups at concentrations of 100, 200 and 400 mg/kg was 14.61 ± 1.23, 22.51 ± 3.97 and 14.92 ± 2.26 %.

After 3 h, paw volume in the control group was 0.00 ± 0.00 %. Paw volume in the 1 % carrageenan-induced edema group was increased to 24.21 ± 2.21 %. Paw volume in the 1 % carrageenan-induced edema and Scutellaria baicalensis-treated groups at concentrations of 100, 200 and 400 mg/kg was 27.86 ± 1.77, 28.00 ± 4.78 and 20.67 ± 2.52 %.

After 4 h, paw volume in the control group was 0.00 ± 0.00 %. Paw volume in the 1 % carrageenan-induced edema group was increased to 26.20 ± 3.39 %. Paw volume in the 1 % carrageenan-induced edema and Scutellaria baicalensis-treated groups at concentrations of 100, 200 and 400 mg/kg was 21.80 ± 3.21, 30.38 ± 3.74 and 20.67 ± 2.52 %.

After 5 h, paw volume in the control group was 0.00 ± 0.00 %. Paw volume in the 1 %

![Fig. 4. Effect of Scutellaria baicalensis in response to doses and time on carrageenan-induced paw edema.](image)

carrageenan-induced edema group was increased to 15.71 ± 4.56 %. Paw volume in the 1 % carrageenan-induced edema and *Scutellaria baicalensis*-treated groups at concentrations of 100, 200 and 400 mg/kg was 8.24 ± 2.95, 14.83 ± 5.51 and 21.19 ± 6.63 %.

The present results showed that the paw volume in the control group was maintained at constant level and that paw volume in the carrageenan-induced edema group was increased as time-dependently during 4 h.

First of all, the concentrations of *Scutellaria baicalensis*-treated groups are 50, 100, 200 mg/kg. However, carrageenan-induced edema and *Scutellaria baicalensis*-treated groups exerted no significant inhibition on carrageenan-induced paw edema. And this time, the concentrations of *Scutellaria baicalensis*-treated groups changed 100, 200, 400 mg/kg, but the result was also no significant inhibition (Fig. 4).

5. Effect of *Scutellaria baicalensis* on the plantar test (nociceptive thermal stimulation)

After 1 h, paw withdrawal threshold in the pre-treated value was considered as 1.00. The withdrawal latency in the thermal stimulation-induced nociception and drinking water-treated group was 1.17 ± 0.17. The withdrawal latency in the thermal stimulation-induced nociception and 50 mg/kg *Scutellaria baicalensis*-treated group was 1.39 ± 0.13. The withdrawal latency in the thermal stimulation-induced nociception and 100 mg/kg *Scutellaria baicalensis*-treated group was 1.13 ± 0.16. The withdrawal latency in the thermal stimulation-induced nociception and 200 mg/kg *Scutellaria baicalensis*-treated group was 1.28 ± 0.15. After 1 h, the withdrawal latency of thermal stimulation-induced nociception was not significantly different in the animals treated with *Scutellaria baicalensis*.

After 2 h, the withdrawal latency in the thermal stimulation-induced nociception and drinking water-treated group was 0.96 ±0.09. The withdrawal latency in the thermal stimulation-induced nociception and 50 mg/kg *Scutellaria baicalensis*-treated group was 1.22 ± 0.15. The

![Graph](image-url)  
*Fig. 5. Effect of *Scutellaria baicalensis* in response to doses and time on thermal pain. (□) thermal stimulation-induced nociception and drinking water-treated group (control), (■) thermal stimulation-induced nociception and 50 mg/kg *Scutellaria baicalensis*-treated group, (○) thermal stimulation-induced nociception and 100 mg/kg *Scutellaria baicalensis*-treated group, (●) thermal stimulation-induced nociception and 200 mg/kg *Scutellaria baicalensis*-treated group. * represents p < 0.05 compared to the control group.
withdrawal latency in the thermal stimulation-induced nociception and 100 mg/kg *Scutellaria baicalensis*-treated group was 1.03 ± 0.08. The withdrawal latency in the thermal stimulation-induced nociception and 200 mg/kg *Scutellaria baicalensis*-treated group was 1.36 ± 0.16. The present results showed that the withdrawal latency in the thermal stimulation-induced nociception and 200 mg/kg *Scutellaria baicalensis*-treated group was increased after 120 min. *Scutellaria baicalensis* showed significant inhibitory effect on thermal stimulation-induced nociception (Fig. 5).

**Discussion**

*Scutellaria baicalensis* is known to have the anti-bacterial activity against staphylococci, cholera, typhoid, paratyphoid, dysentery, diphtheria, hemolytic streptococci, Escherichia coli, pneumococci and spirochaeta and it also exerts anti-viral effect against influenza virus. The properties of *Scutellaria baicalensis* is ‘bitter and cold’. It is credited with ‘damp-heat’-clearing, ‘heat’-lower, detoxicant and anti-inflammatory actions and is known to prevent abnormal fetal movements. It has been used for the treatment of fever, cough, hemoptysis, jaundice, hepatitis, dysentery, acute conjunctivitis, carbuncle and furuncle.

*Scutellaria baicalensis* is known to contain a number of flavone derivatives. The first flavone isolated from its root was wogonin. Wogonin is present only in small amounts in the root; the flavone glycoside named baicalin. Acid hydrolysis of baicalin yields glucuronic acid and a flavone aglycone named baicalein. Anti-inflammatory effects of these three major constituents-baicalin, baicalein and wogonin-are well documented. Baicalin, baicalein and wogonin were found to inhibit acetic acid-induced increase in vascular permeability in mice and to reduce acute paw edema in rats induced by compound 48/80. They also suppressed development of secondary lesion in adjuvant-induced arthritis in rats. *Baicalein* and wogonin have been shown to inhibit lipopolysaccharide (LPS)-induced nitric oxide (NO) production in macrophages. Flavonoids can inhibit 5-lipoxigenase and cyclooxygenase activities and act as phospholipase A2 inhibitors. These evidences indicate that flavonols can modulate the prostanooid biosynthetic pathway. Arachidonic acid which is accumulated in the membrane lipid, can be selectively released from the phospholipids pool by chemical or mechanical stimulation and is subsequently converted to prostaglandins (PGs) by two enzymes, COX-1 and COX-2. COX-2 is primarily responsible for PGs produced in inflammation and COX-1 for PGs involved in normal homeostasis. In this regard, COX-2 is up-regulated in the air pouch and catalyzes the production of large amounts of PGE2. The up-regulation of COX-2 associated with the increase of PGE2 may be major event in acetic acid-induced writhing response and carrageenan-induced inflammation. Alternatively, the decrease of PGE2 may be induced by the inhibition of the release of TNF-α, because stimulation of macrophages/monocytes, fibroblasts and epithelial cells with cytokines such as IL-1 and TNF-α leads to PGE2 production. In the present results, the aqueous extract of *Scutellaria baicalensis* suppresses LPS-induced COX-2 protein expression in mouse BV2 microglia cells.

The analgesic effect of *Scutellaria baicalensis*
was evaluated by acetic acid-induced writhing response. In acetic acid-induced abdominal writhing which is the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism. In the present results, pre-treatment with the aqueous extract of *Scutellaria baicalensis* revealed an analgesic effect on acetic acid-induced writhing response in mice.

The carrageenan test was selected because of its sensitivity in detecting orally active anti-inflammatory agents particularly in the acute phase of inflammatory. The intraplantar injection of carrageenan in rats leads to paw edema. In the present results, however, *Scutellaria baicalensis* exerted no significant inhibition on carrageenan-induced paw edema.

Thermal hyperalgesia can be explained by central convergence of afferents from deep tissues and the skin. In the present results, administration of *Scutellaria baicalensis* suppressed the thermal stimulation-induced nociception.

Here in this study, we have demonstrated that the aqueous extract of *Scutellaria baicalensis* have analgesic and anti-inflammatory activities and could be the reason for its wide use in traditional medicine to treat different types of pain. Although pharmacological activities have been carried out, the detailed mechanisms of actions on each respective effect should be further investigated.

References

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