Effect of *Camellia oleifera* essential oil on transdermal delivery of aconitine from carbopol gel

Yang Zaichang *, Qiao Chong, Niu Yule, Ma Xiaoyan

Laboratory of Pharmacology, Chemical Engineering College of Guizhou University, PR China


**Abstract**

Aconitine is well known for its high toxicity that induces severe arrhythmias leading to death. However, its analgesic effect has proven to be more potent than morphine without any habit-forming potential. Based on carbopol 940, aconitine gels were developed as a topical analgesic agent in this research. *Camellia oleifera* essential oil which contains α-terpineol (13.179 %), linalool (12.959 %), trans-geraniol (6.172 %) and other components, was used as penetration enhancer. The results show that aconitine gel (0.1% w/w) can produce topical analgesic effects at low dose (5 mg/cm²) in the tests in mice. The therapeutic index (TI) of the gel (No. IV) is more than 16. These data suggest that aconitine gel may be a safe local analgesic agent and *camellia oleifera* essential oil is a suitable penetration enhancer for aconitine gel.

**Keywords**: Aconitine; Carbopol gel; Topical analgesic agent; *Camellia oleifera* essential oil.

**INTRODUCTION**

Aconitine, a C₁₉ diterpenoid alkaloid, is isolated from the extracts of *Aconitum* species that are used in traditional Chinese medicine predominantly for the treatment of pain and as an anti-inflammatory agent. Activation of Na⁺ channels by aconitine is responsible for its analgesic properties and cardiotoxic properties which prevent the therapeutic use of aconitine as an analgesic (Chan *et al.*, 1994; Gutser *et al.*, 1998).

Aconitine is well known for its high toxicity that induces severe arrhythmias leading to death, but its analgesic effect had been proven to be more potent than morphine without any habit-forming potential (Ameri, 1998). When aconitine is administrated orally, it is absorbed from gastrointestinal tract into bloodstream, which carries aconitine to heart and other organs. Conversely, if aconitine is applied directly onto local disorder site, it is distributed, first into its effector site instead of being taken into bloodstream, so that it can produce more potent analgesic activity at a relative low dose at topical site. We propose that aconitine can be developed as a topical analgesic agent, such as topical-gel, for the treatment of neuralgia, rheumatism, shoulder pains, wrench, arthralgia and muscle pains.

The skin is very effective as a selective penetration barrier for topical agents. Penetration enhancers are usually used in topical agent to facilitate the absorption of drug through the skin by temporarily diminishing the impermeability of the skin (Sinha *et al.*, 2000). Some essential oils are reported to enhance the permeation of drugs. *Camellia oleifera* Abel, commonly known as Tea Oil Camellia, is a plant in the family Theaceae. It is widely cultivated in China specifically for the seeds, from which commercial tea oil is extracted. In this study the *Camellia oleifera* essential oil was obtained by steam distillation from leaf shoot of *Camellia oleifera* Abel and analyzed by GC-MS. The main components of the essential oil are linalool, α-terpineol, and geraniol which were reported to increase the penetration of drugs (Kararli *et al.*, 1995; Donald *et al.*, 1999; Arellanoa *et al.*, 1996).

The present study was conducted to determine the possibility of aconitine being used as topical agent in gel base, and to investigate whether *Camellia oleifera* essential oil can enhance the transdermal delivery of aconitine from gel.

*Corresponding author:*
Yang Zaichang, Ph.D.
Laboratory of Pharmacology, Chemical Engineering College of Guizhou University, Guiyang 550003, PR China
Email: yangzaichang@126.com
MATERIALS AND METHODS

Chemicals

Carbopol 940 (Tianjin Well-Real Chemical Technology Co., Ltd., Tianjin, China), aconitine, and triethanolamine (Sigma, St Louis, MO, USA) were purchased from Guiyang Dongxin Chemical Co., Ltd., Guiyang, China.

Plant material

Leaf shoots of *Camellia oleifera* Abel were collected in Guiyang, Guizhou province, China and identified by authors. A voucher specimen has been deposited at the author’s laboratory in Guizhou University.

Chemical analysis of essential oil

The essential oil of *Camellia oleifera* was obtained by steam distillation from leaf shoot of *Camellia oleifera* Abel in Clevenger apparatus for 5h. GC-MS analyses were performed using HP 5890 GC and HPMSD 5973 GC-MS instruments with an HP 5 column (30 m × 0.25 mm × 0.25 μm) and using helium as a carrier gas at a linear flow rate of 1 ml/min. The injector temperature was set to 280°C, and the interface was at 280°C. The oven temperature was programmed from 60-280°C at 6°C per min. The electronic impact energy was set at 70eV, and mass spectra were collected in the range of 10-550 atomic mass units.

Preparations of aconitine gel

An accurately weighed amount of Carbopol 940 (1.0 g) was added in an appropriate amount of purified water with continuous stirring using porcelain mortar and pestle until uniform consistency was achieved. An accurate amount of essential oil was slowly added to the dispersion with continuous stirring. The aconitine solution prepared by dispersing 0.1g of aconitine in 2g of ethanol was subjected to dispersion, and the mixture was stirred continuously until it was homogeneous. An accurate amount of triethanolamine (8g) was added and stirred to obtain the gel. Purified water was added to attain the total weight of 100g with continuous stirring. The gels were then transferred into clean glass vials and centrifuged at 3000rpm for up to 8min to remove air bubbles. The blank gels (no aconitine and no oil content) were also prepared by the above procedure. The compositions of the formulations are shown in Table 1 [Supplementary data].

Animals for analgesic test and acute dermal toxicity test

The *in vivo* experiments were conducted using male Kunming mice (20-22g), which were purchased from Animal Center of Guiyang Medical College, Guiyang, China. The animal room was maintained at 20 ± 2°C with a 12 h light/dark cycle. Food and tap water were supplied. All experiments were done in compliance with the internationally accepted standard guidelines for use of animals and have been approved by the Guizhou University.

Tail clip test

This analgesic test was based on a modified method as described by Bianchi and Adeyemi (Bianchi *et al.*, 1954; Adeyemi *et al.*, 2004). An artery clip is applied to the root of the tail (approximately 1cm from the body) to induce pain. The animal quickly responds to the noxious stimuli by biting the clip or the tail near the location of the clip. The time between stimulation onset and response is measured by a stopwatch. Control and treated groups each consisted of 8 mice selected by random procedures. On the day prior to testing, an area on the back of each mouse (2 cm × 2 cm) was shaved and depilated with sodium sulfide. The dose at 5mg/cm² of respective aconitine gels (Table 1) were rubbed onto the root of tail or the back of the mice. An artery clip was placed to the root of tail, and the mice that responded to the clip placement by biting at the clip over 16s were not used in this test. The presence or absence of analgesia was determined at 1, 2, 3, 5, 7, and 12 h after application of the aconitine gels. The artery clip is applied for 30sec. The results are expressed as the percentage of mice showing analgesia after a given dose of aconitine gels.

Writhing test in mice

The writhing test was based on a modified method as described by Collier (Collier *et al.*, 1968). Control and treated groups each consisted of 8 mice selected at random. On the day prior to testing, an area on the abdomen of each mouse (2 cm×2 cm) was shaved and depilated with sodium sulfide. The respective aconitine gels (Table 1), at dose of 5 mg/cm², were rubbed onto the abdomen of the mice and maintained in contact with the mice skin for 3h, at which the writhing response was induced by intraperitoneal injection of 1.0% acetic acid solution at the dose of 0.1 ml/10 g body weight. The number of stretching movements was counted for 10min, starting 10min after acetic acid injection.

LD50 test by topical application of aconitine gels in mice and identification of aconitine in blood plasma of mice by HPLC

The mice were divided into 5 groups (n=11). An area on the back of each mouse (2 cm × 2 cm) was shaved and depilated with sodium sulfide prior to dosing. Aconitine gel of No. IV, at dose of 20, 32, 51, 82, and 131 mg/cm², were rubbed evenly onto the back of the
mice. The gel was kept in place by applying a gauze patch over the test substance. A clean plastic wrap was then applied over the entire midsection and was held in place with elastoplast tape. The test gel remained in contact with the mice skin for 24h, at which time the tape, plastic wrap, and gauze were removed and the residual gel was wiped from the skin. Records were kept on signs of toxicity and mortality.

One mouse was selected randomly from each group for identification of aconitine in blood plasma. Blood samples (about 50 μl) were collected from the eye socket vein at 5h after the administration. Each collected sample was immediately centrifuged at 1500g for 10 min to obtain plasma, which was transferred to a polypropylene plastic vial and mixed with 50μl of ammonia and 400μl of aether. After shaking extraction, about 350μl of the supernatant was obtained and dried by nitrogen gas blowing. The residue was dissolved in 40μl of methanol to obtain sample solution, which was loaded to the HPLC. Standard solution (1mg/ml) of aconitine was prepared by dissolving 5mg of aconitine in 5 ml methanol. Then 10μl of standard solution was added to 40μl of blank plasma of mice. The plasma containing standard solution was processed by the same procedure described as above. The injection volume was 3μl for all HPLC runs.

A HP1100 HPLC system was used in the study, which consisted of a G1315A DAD, a G1311A quaternary pump, a G1322A vacuum degasser, and a G1367A well plate autosampler. Zorbax C8 reverse phase column (ID 4.6 × 250mm, particle size 5µm) was used as the stationary phase. The mobile phase consisted of glacial acetic acid (0.2%, adjusted to pH 5.43 with triethylamine): methanol (55:45) at a flow rate of 0.8 ml/min. Detection was carried out at 231nm at 25°C.

Statistical analysis
The data were analyzed for statistical significance using Student's t-test. P-value of less than 0.05 was considered to be significant.

RESULTS AND DISCUSSIONS

Chemical constituents of Camellia oleifera essential oil
The volatiles from Camellia oleifera leaf shoots were studied by GC-MS. Twenty-seven components were identified (Table 2 [Supplementary data]), representing 67.058% of the total oil. Yield of the oil was 0.5% (v/wet weight). The major components were α-terpineol (13.179 %), linalool (12.959%), trans-geraniol (6.172%), and vitispirane (4.550%).

Tail clip test
In tail clip test the aconitine gels were applied to the back or the root of tail of the mice. When the gels were applied onto the root of tail of the mice, the aconitine gels of No. II, III and IV, using Camellia oleifera essential oil as penetration enhancer, showed analgesic effect. The control gel without Camellia oleifera essential oil (No. V) and the gel without aconitine (No. I) did not exhibit any analgesic effect.
analgesia. The analgesia rate decreased to 25% at 12 hrs after administration. The aconitine gel of No. IV, contains 8\% (w/w) of *Camellia oleifera* essential oil, showed potent analgesic effect. After the gel of No. IV was applied to the mice for 1 hr, 50\% of mice showed analgesia. Two hours late 100\% of mice showed analgesia and the analgesic effect lasted for 5 hours. 12 hrs later the analgesic effect of the gel begins to decrease.

The results demonstrated that *Camellia oleifera* essential oil does not exhibit analgesic effect on mice by itself, and the skin of mice is a barrier for aconitine to be delivered into subcutaneous tissue. However *Camellia oleifera* essential oil can enhance the transdermal delivery of aconitine from carbopol gel in a dose-dependent manner.

When the aconitine gels were applied to the back of the mice, all of them did not show analgesia in the test. It is obvious that the aconitine gels did not have systemic analgesic effect. The aim of this study is to evaluate whether aconitine can be used as topical analgesic agent. The results presented here indicated that the topical application of aconitine gel only leads local activity.

A report indicated that the ED50 for aconitine to exhibit systemic analgesic effect on mice is about 0.06mg/kg by i.v. administration (Gutser et al., 1998). It can be suggested from the data that 1.2\mu g of aconitine is sufficient to make mice (20g body weight) show analgesia. In this study the dose of gel applied to the back of mice is 5mg/cm². The percentage of aconitine in gels is 0.1\%. About 20\mu g of aconitine was in contact with the skin of mice.

It is difficult for us to answer the question as to ‘Why the aconitine gel does not exhibit systemic analgesic activity at such a high dose?’ To answer this, we tried to use HPLC to detect the amount of aconitine in the subcutaneous tissue of the mice. However, HPLC is not insensitive enough to detect it. It is not clear, how aconitine is distributed in the subcutaneous tissue or in blood after it passes through the skin of the mice. However, the data shows that aconitine gel, using *Camellia oleifera* essential oil as penetration enhancer, is a good topical analgesic agent.

**Writhing test in mice**

Pain was induced by injection of acetic acid into the peritoneal cavity of mice in this study. The results are summarized in Figuer 2. The numbers of abdominal constrictions for group I (no aconitine content) and V (no essential oil content) were 31.13±2.42 and 31.62±2.44, respectively. The aconitine gels of No. III and No. IV caused significant inhibition (P < 0.01) of the pain induced by acetic acid compared to the gel of No. V. The numbers of abdominal constrictions for group III and IV were 5.64±1.06 and 4.13±1.24, respectively. The gel of No. II could reduce the number of abdominal constrictions slightly, but there is no significant difference compared to No. V (P > 0.05).

Writhing test in mice is usually used to detect both central and peripheral analgesics. There is a good relationship that exists between the potencies of analogesics in writhing assays and their clinical potencies (Siegmund et al., 1957). The results demonstrate that aconitine gel produces significant blockade of abdominal constriction when applied 3hrs prior to acetic acid challenge. This is clearly a local effect. In general, the results of writhing test are consistent with the results of tail clip test.

**Acute dermal toxicity and aconitine in blood plasma of mice**

The acute dermal toxicity was performed based on the aconitine gel of No. IV in mice. Because the gel of No. IV contains 8\% (w/w) of *Camellia oleifera* essential oil, so the test can detect not only the toxicity of aconitine gel but the toxicity of *Camellia oleifera* essential oil too. The result of the acute toxicity study and calculated LD50 are shown in Table 3 [Supplementary data]. The results display that there was no mortality in the group that received 20mg /cm² of the aconitine gel. Mortality occurred in groups that received 32, 51, 82, and 131mg /cm² of the aconitine gel within 24hrs. LD50 of the aconitine gel is 81.28±22.75 mg /cm², indicating that the aconitine of the gel can be transferred from skin to circulatory system when at high doses.
At the end of the 24 h period of exposure the residual gel was wiped from the skin, and the skin reactions are observed immediately. There are no erythema and other pathological change appearing on the skin of the mice that died or survivied, indicating that the aconitine gel is in conformance with the skin of mice. The results indicate that aconitine gel did not damage the skin tissue of the mice. The previous experiment (data not showed in here) displayed that when applied to the mice at dose of 5mg/cm² the gel of No. IV could make 100% of mice to show topical analgesic effect. So we can speculate that the therapeutic index of the gel of No. IV is more than 16. The therapeutic index of aconitine by i.v. administration is less than 3 (Gutser et al., 1998). Therefore the aconitine gel is safe for topical application.

HPLC analysis was conducted to identify aconitine in the plasma of mice. It should be pointed out that we do not intend to analyze the exact amount of aconitine in the plasma of the mice in this study. Firstly, we tried to use TLC to detect the aconitine in plasma. But TLC is not sensitive enough to detect aconitine in plasma. The results (Figure 3) show that there is no peak signal of aconitine detected by HPLC in the plasma of the mice selected from the groups that received 32mg/cm² of aconitine detected by HPLC in the plasma of the mice that received more than 82mg/cm² of the aconitine gel. It is clear that the aconitine gel is safe for topical application.

The authors acknowledge Mr. Wang Daoping who performed the GC-MS analysis of *Camellia oleifera* essential oil.

**CONCLUSION**

*Camellia oleifera* essential oil can enhance aconitine to permeate skin. The aconitine gel, using *Camellia oleifera* essential oil as penetration enhancer is a potent analgesic agent when used topically and does not exhibit toxic effects to skin. It also produces systemic toxicity at high doses, but the therapeutic index (TI) of the gel (No. IV) is more than 16. These data suggest that aconitine gel may be a safe local analgesic agent.

**References**


