

## Research Article

# Successfully Applied the Ethanol Extract Prepared from the Calyx for the Treatment of Genital Condylomata Acuminate

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## Abstract

We examined the clinical effects of the eggplant extract on condylomata acuminate. For those with an 80-90% reduction or the complete disappearance of condylomata acuminate, the result of treatment was determined to be effective. We confirmed the capability of eggplant extract to induce apoptosis in Caski and HeLa cells. Our current study showed the possibility of new therapeutic for condylomata acuminate.

**Keywords:** Ethanol extract prepared from the calyx; Genital condylomata acuminate; Treatment

## Introduction

Condylomata, caused by HPV6/11 infection, is a sexually transmitted disease which develops in the vulva, vaginal vestibule, perianal regions, and vagina. Laser treatment and local immunotherapy with imiquimod have recently been employed. However, both have adverse effects, such as flare, erosion, and pain, which occasionally become persistent [1-4]. In Japan, a liquid extruded from the calyx of the eggplant has traditionally used to treat warts. Tomoda et al. applied an ethanol extract, prepared from the calyx of the eggplant, to treat warts, demonstrating significant clinical effects without adverse effects. In addition, we previously demonstrated that 9-oxo-(10E, 12E)-octadecadienoic acid in the extract induced apoptosis in an ovarian cancer cell line [5]. Warts are a benign skin disease caused by HPV infection [6]. Since they are pathologically similar to condylomata, we examined the clinical effects of the extract on condylomata. In addition, to elucidate the mechanism of action of the extract was applied to cervical cancer cells (i.e., HPV-infected cells) *in vitro* for apoptosis induction.

## Materials and Methods

### Patients

After informed consent fifty-seven patients aged 18-50, visually or pathologically diagnosed with condylomata at Tomoda Clinic, were included.

### Clinical Findings

Of the patients, 36 (63.1%) and 11 (19.2%) patients were aged 20-29 and 30-39, respectively. The major development sites were the vulvae (84.2%), including labia majora and minora, and vagina (8.7%). Five patients (8%) were pregnant.

### Reagents

The calyx of the eggplant was minced (1,300 gr) and dried in air (100 gr), followed by the addition of 98% ethanol (500 mL) for extraction at room temperature. This extract was concentrated three times to be tested in the clinical research. In an *in vitro* study, we used the extract at a 10,000-times dilution.

### Treatments

The extract was applied to 57 patients with condylomata at

5-7-day intervals. The treatment was repeated 20 times. For those without >80% reduction, treatment was determined to be ineffective. Those who discontinued visits during the course of treatment were excluded as dropouts. Before the start of the clinical research on the eggplant extract, written informed consent was obtained from all patients.

### Effectiveness

For those with an 80-90% reduction or the complete disappearance of condylomata, the result of treatment was determined to be effective. For those with proliferation up to 20 times or <80% reduction or those who were switched to a different treatment, it was also determined to be ineffective.

### Cell Culture and Treatment

The human cervical cancer cell line (Caski and HeLa) was purchased from the American Type Culture Collection. These cell lines were maintained in RPMI-1640 medium (Sigma, St. Louis, Mo, USA) supplemented with 10% Fetal Calf Serum (FCS), penicillin (100 U/mL), and streptomycin (100  $\mu$ g/mL) at 37°C in a humidified 5% CO<sub>2</sub> atmosphere.

### Analysis of Apoptosis

The Caski cells were plated onto chamber plates at a density of 1.5 x 10<sup>4</sup> cells and incubated for 12 h, and the medium was replaced with the medium containing 9-oxo-(10E,12E)-octadecadienoic acid (9-EE-KODE). After 12 h of incubation, the cells were stained with Fluorescein Isothiocyanate (FITC)-labeled Annexin V (BD Pharmingen, USA) at room temperature. After 15 min of annexin V incubation at RT in darkness, 7AAD (BD Pharmingen, USA) was added to the eggplant extract (1:1000). Finally, the samples were analyzed by flow cytometry.

## Results

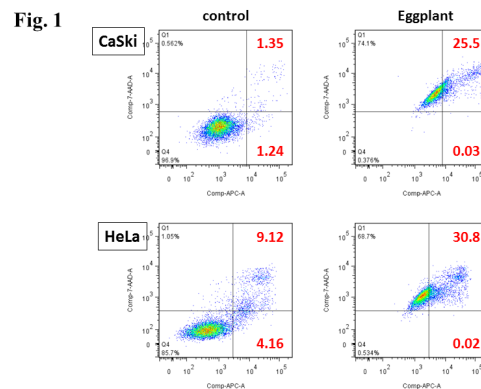
### Outcomes

Twenty-three patients (39.6%) with condylomata were histologically diagnosed before or during the treatment. Forty-five patients (77.5%) underwent cytological diagnosis of the cervix, with abnormalities frequently detected: ASC-US in 1 patient (2%) and LSIL in 11 patients (24.4%). In 51 patients (87.9%), treatment was determined to be effective, with complete disappearance in 38 patients (74.5%), 90% disappearance in 8 patients (15.6%), and 80% disappearance in 5 patients (9.8%). Twenty-eight (about 50%) and 23 (40%) patients received the treatment 9 times or less and 10-19 times, respectively. Some patients took a prolonged time until cure (mean number of treatments: 10). Nineteen patients (37.2%) could be followed up, while 2 patients (10.5%) experienced a recurrence. Of those who could not be followed up, none made a visit with a recurrence after

being cured. Three patients were switched to a different treatment, and, therefore, treatment was determined to be ineffective.

### In Vitro Study

To confirm the capability of eggplant extract to induce apoptosis in Caski and HeLa cells, flow cytometry (using 7-AAD and Annexin-V staining) was conducted. The (Figure 1) shows that Caski and HeLa cells exhibited an increase in 7-AAD and Annexin-V-positive cell populations after eggplant samples treatment compared with untreated cells.



## Discussion

Various surgical therapies, such as laser therapy, cryotherapy, electrical surgery, and resection, have been employed for condyloma treatment. Chemotherapy includes the use of anticancer agents, podophyllin, and imiquimod. Recently, laser and imiquimod therapies are mainly selected. The cure rates of condylomata are 38-97% for chemotherapy and 70-90% (recurrence rate: 0-65%) for surgery [7]. Local immunotherapy with commercially available imiquimod is based on an ingenious concept, facilitating condyloma treatment. Reportedly, the cure rate with imiquimod is 63.6%, with a recurrence rate of about 13%. However, this drug cannot be applied to the vagina, because it may cause severe skin disorders. Laser treatment is hampered by high recurrence rates.

The summary of ethanol extract prepared from the calyx for the treatment was shown in below.

### Merits:

- No adverse effects (e.g., pain at administration site, flare, sores, and ulcers)
- Applicability to vaginal lesions
- Applicability in pregnant women and
- Few recurrences.

### Demerits:

Relatively long treatment period and Physician's prescription and hospital visits.

In the future, formulations, such as cream and ointment, will be available depending on onset sites. However, the merits outweigh the demerits. The present therapy was effective for condylomata through cytotoxic effects on HPV-infected cells. However, since no histological examination was conducted in this study, immune cell induction will be examined in the future. The mechanisms of the therapeutic effects can be predicted from a basic experiment on E-CEP. Specifically, E-CEP exerts potent antitumor effects *in vitro* through apoptosis.

E-CEP is an eggplant that have been consumed since ancient times, from which herbal medicine is extracted with ethanol in Japan. Fruit, food, and fruit extracts are habitually consumed throughout the world, and, therefore, are not considered to have significant effects on the skin. We demonstrated no adverse effect in treating verruca vulgaris. Mizukami et al. and the current authors demonstrated that three kinds of conjugated linoleic acid, contained in the calyx of the eggplant, had antitumor effects. However, whether the clinical effects are attributable to the linoleic acid or additive or synergistic effects with other substances remains unclear. To examine this, the clinical effects of the conjugated linoleic acid on warts and condylomata will be investigated.

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