

Original article

## Effectiveness of capsaicin nanoparticle gel of *Capsicum frutescens L.* on oral squamous cell carcinoma in *Rattus norvegicus*

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### ABSTRACT

**Background:** Oral squamous cell carcinoma (OSCC) is an oral cancer with a low life expectancy, less than five years after diagnosis. The drug therapy often used for OSCC patients is cisplatin, but it is considered to cause tumour persistence, drug resistance, and high toxicity. Therefore, it is important to test the development of alternative drugs from natural ingredients. One potential ingredient is green chilli pepper (*Capsicum frutescens L.*). It contains capsaicin that functions as an anticancer agent by suppressing BCa tumorigenesis so that proliferation is inhibited, as well as increasing and preventing p53 antibody mutations that play a role in cancer cell apoptosis.

**Purpose:** This study aimed to compare effectiveness using capsaicin nanoparticle gel from green chilli pepper extract levels of 1% and 3.3% to reduce OSCC nodules. **Methods:** This study used 20 *Rattus norvegicus* that were randomly divided into five groups; C- (rat without treatment), C+ (rat induced to 7,12-Dimethylbenz(a)anthracene (DMBA)), E1 (DMBA exposed and given cisplatin), E2 (rat induced to DMBA and capsaicin extract nanoparticle gel with a concentration of 1%), and E3 (rat induced to DMBA and capsaicin extract nanoparticle gel with a concentration of 3.3%). The data were analysed statistically with the one-way ANOVA and least significance difference (LSD) test. **Results:** The comparison of mean nodule volume between C+ ( $5.834 \pm 2.77 \text{ mm}^3$ ) with E1 ( $1.75 \pm 0.37 \text{ mm}^3$ ), E2 ( $1.747 \pm 0.36 \text{ mm}^3$ ), and E3 ( $1.812 \pm 0.11 \text{ mm}^3$ ) had a significant difference ( $p = 0.00$ ,  $p \leq 0.05$ ). **Conclusion:** Capsaicin nanoparticle gel with green chilli pepper extract at levels of 1% (E2) reduces OSCC nodules by more than gel with green chilli pepper extract at 3.3% (E3) concentration.

**Keywords:** anticancer; capsaicin; OSCC

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### INTRODUCTION

Cancer is one of the leading causes of death in the world. The prevalence of cancer in Indonesia has increased from 1.4% in 2013 to 1.8% in 2018.<sup>1</sup> Of the different types of oral cancer, 95% is oral squamous cell carcinoma (OSCC). OSCC is an oral cancer with a low life expectancy, less than 5 years after diagnosis.<sup>2</sup> OSCC occurs due to a multitude of factors such as smoking, betel chewing, and alcohol consumption.<sup>3</sup>

The drug therapy often used for OSCC patients is cisplatin, but cisplatin is considered to cause tumour persistence, drug resistance, and high toxicity.<sup>4</sup> It is

important to test the development of alternative drugs from natural ingredients, such as fruits and plants, to create anticancer benefits of price and applications that are more affordable for OSCC patients. One such potential ingredient is green chilli pepper (*Capsicum frutescens L.*) because it contains anticancer, high-antioxidant, pain-reliever, and anti-inflammatory benefits. These benefits are mainly due to the content of capsaicin. Capsaicin (8-methyl-N-vanillyl-6-noneamide) is an alkaloid compound that is responsible for the spicy taste of green chilli peppers.<sup>4</sup> Green chilli pepper (*Capsicum frutescens L.*) has the highest capsaicin content of all the chilli varieties at 2.11%. Capsaicin functions as an anticancer agent by suppressing BCa

tumorigenesis so that proliferation is inhibited, as well as increasing and preventing p53 antibody mutations that play a role in cancer cell apoptosis.<sup>6,7</sup>

Green chilli pepper (*Capsicum frutescens L.*) will be applied using a nanoparticle gel on the object of research, Wistar rats (*Rattus norvegicus*). Wistar rats were chosen as research material because they have an anatomy similar to humans.<sup>8</sup> Nanoparticle gel preparations were selected because the particles are small, making it easier to reach the target. In addition, the use of nanoparticles will achieve higher bioavailability than oral preparations and is accepted by patients because it is practical, safe, and painless.<sup>9</sup>

With the low life expectancy of OSCC patients, drugs treatment such as cetuximab and cisplatin has high resistance and toxicity. Utilization of natural resources as the latest treatment developments is needed to overcome this problem. This study aimed to compare the effectiveness of using capsaicin nanoparticle gel from green chilli pepper extract at levels of 1% and 3.3% to reduce OSCC nodules. The hope is that the presence of green chilli pepper extract nanoparticles will inhibit the growth rate of OSCC with the right concentration of nanoparticle gel and facilitate the curing of OSCC.

## MATERIALS AND METHODS

The research was performed in an experimental laboratory using the method of randomized post-test only control group design for four months at Jenderal Soedirman University. The research had ethical clearance agreement number 125/KEPK/VII/2021 and applied strict health protocols.

Wistar rats aged two months with an initial body weight of 150–200 grams were the test animals obtained from the Gadjah Mada University, Medical Faculty Research Laboratory. The rats were divided into five groups. *Capsicum frutescens* was obtained from Kemutug Kidul, Baturraden, Banyumas, with 22 grams of urea fertilization in the plantation and got determination tests in the Environmental Laboratory of the Faculty of Biology, Jenderal Soedirman University.<sup>10</sup>

Capsaicin extraction was performed using 96% ethanol solvent, at a stirring speed of 200 rpm, a temperature of 50°C, and 4 hours by maceration method. The extract evaporated to form a thicker extract and filtrate. The sample, formed as *Capsicum frutescens* powder, was dissolved in a single solvent of n-hexane and methanol.<sup>11</sup> Then, 5 grams of the *Capsicum frutescens* powder was dissolved

in 100 ml of methanol, with 10 ml taken for isolation. The solution had 10 ml of distilled water and n-hexane added to form a fraction containing purer capsaicin. After shaking the solution, the fraction that formed was placed in a vial. It was then placed in a desiccator for 24 hours in the refrigerator until crystals formed that proved it contained purer capsaicin.<sup>11</sup>

The nanoparticles were prepared in 100-gram extract concentrations of 3.3% and 1%. The concentration used was based on research that stated that the capsaicin content of 333 µg/mL has a significant effect and the highest cytotoxic activity on the OSCC cell line.<sup>12</sup> Materials were all weighed according to the calculation. The gel was prepared by mixing 0.5 grams of a gelling agent (Carbopol) with heated water that was then allowed to form and expand the gel mass.<sup>13</sup> Then 0.3 grams of methylparaben was dissolved in 15 grams of propylene glycol, and 1 gram of sodium metabisulfite was added. This was stirring until it was homogeneous. The solution was placed on a Carbopol base and homogenized. Extracts of 1 gram and 3 grams were mixed into the resultant base and homogenized. The remaining water was added to this base and again homogenized.<sup>14</sup> The manufacture of nanoparticles was carried out by stirring on a magnetic stirrer for 4 hours.

Carcinogen induction in rats used a formula of 96 mg 7,12-Dimethylbenz(a)anthracene (DMBA) dissolved in 24 mL corn oil, vortexed for ± 15 minutes until homogeneous. Carcinogen induction was performed two times a week for two weeks by the right buccal mucosa injection route. The induction dose given was 20 mg/kg of a rat's weight.<sup>15</sup> Rats were anaesthetized using ketamine intramuscularly at a dose of 0.2 mL/200 gr of a rat's weight and observed by the method of palpation of the buccal mucosa four times a week for two weeks. Rats were diagnosed with cancer if there were palpable nodules on the buccal mucosa.<sup>16</sup> After observation and rats were diagnosed with cancer, treatment commenced and was carried out once a day for seven days. Each test group of Wistar rats received a marker, and the rats were acclimatized for one week. The rats then received an induced DMBA solution by injection of the right buccal mucosa. The rats were sampled using the method random sampling. They were divided into five treatment groups, and the treatments were carried out once a day for seven days (Table 1).

The Wistar rats were weighed after being divided into the five groups. Weighing occurred three times: after the acclimatization process, before DMBA was induced, and before treatment. These weights were recorded to help

**Table 1.** Experiment group

Experiment group	Treatment
Control – (C -)	Rat without treatment
Control + (C+)	Rat induced to DMBA
Experimental control 1 (E1)	Rat induced to DMBA and cisplatin
Experimental control 2 (E2)	Rat induced to DMBA and capsaicin extract nanoparticle gel with a concentration of 1%
Experimental control 3 (E3)	Rat induced to DMBA and capsaicin extract nanoparticle gel with a concentration of 3.3%

track the symptoms of cancer.<sup>16</sup> A surgical procedure was performed on each rat three months after it was diagnosed with cancer. The rat was given a capsaicin extract nanoparticle gel using the cervical dislocation method. The rat's head was cut off to observe the tumour tissue, then stained using toluidine blue, cleaned using saline, and stored in a sample pot containing 10% neutral buffered formalin (NBF). The variables obtained were nodule volume, length and width. The volume of the nodules was calculated by the formula ( $p \times l^2$ ), and the length and width were measured in millimetres using callipers.<sup>17</sup> The analysis used in this research are the normality and homogeneity tests, the one-way ANOVA test, and post-hoc LSD. The ANOVA test and post-hoc LSD are completed if the results reach  $p \leq 0.05$ .

**Table 2.** Weight change comparison

Weight measurement time	n	Mean weight (gr) $\pm$ SD
Weight before acclimatization	20	200.29 $\pm$ 24.3
Weight before DMBA induction	20	214.05 $\pm$ 24.7
Weight before treatment	20	186.81 $\pm$ 40.6

**Table 3.** Comparison of weight change between groups

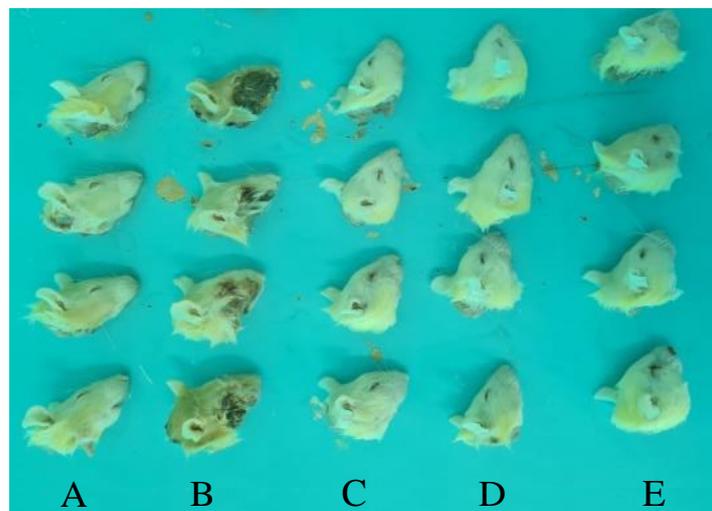
Weight measurement time	Mean difference	P
Weight before acclimatization vs Weight before DMBA induction	-13.762	0.174
Weight before acclimatization vs Weight before treatment	13.476	0.513
Weight before DMBA induction vs Weight before acclimatization	13.762	0.174
Weight before DMBA induction vs Weight before treatment	27.238	0.012*
Weight before treatment vs Weight before acclimatization	-13.476	0.513
Weight before treatment vs Weight before DMBA induction	-27.238	0.012*

\*ANOVA, significant difference ( $p \leq 0.05$ )

## RESULTS

The rats' weight results in Table 2 showed that there was a considerable weight loss before the inducement of DMBA and before treatment ( $p \leq 0.05$ ) in 3 weeks, with a difference in body weight between the two periods of 27.23 g (Table 3). Cancer patients can experience the condition cachexia, and one of the symptoms of this is weight loss.

Observation of OSCC nodules was carried out for two weeks after DMBA induction through palpation of the buccal mucosa four times a week. The results of clinical observations showed the formation of OSCC nodules on the right buccal of the rats in the form of nodules that extended to the outer cheek. The round nodules felt rubbery and contained tissue. They appeared purplish-red to black and were of exophytic, endophytic, or ulcerative types. The extension was on the extraoral, to the infraorbital, to the buccal anterior. Group 1 (C-) had no buccal nodules. Group 2 (C+) had exophytic-typical nodules with the largest volume, but some rats also had ulcerative-type lesions. Lesions in this group appeared to contain tissue in some parts that were necrotic and purplish-red in colour, spreading to the infraorbital and buccal areas of the front. Clinically, OSCC had degraded the tissue to the extent it



**Figure 1.** Intergroup rat head morphology; A. Negative control group (C-); B. Positive control group (C+); C. The cisplatin treatment group (E1); D. 1% nanoparticle gel treatment group (E2); E. 3.3% nanoparticle gel treatment group (E3)

could be seen externally on the rat's head. Group 3 (E1) had exophytic nodules with volumes that were not as large as the other group and felt supple and filled with tissue, but externally there was no sign of redness. A difference in size and texture could be felt on both sides of the buccal. Group 4 (E2) had an exophytic type of nodule, almost the same nodule volume as Group 3, felt supple, and external inspection showed no redness on the outer buccal skin of the rats. Group 5 (E3) had an exophytic type of nodule, with a larger nodule volume than the other two treatment groups. External inspection revealed a reddish colour on the buccal outer skin of the rats, which indicates the progression of OSCC to almost the external structure of the rat (Figure 1).

Another indicator in determining the development of cancer cells is the measurement of nodule volume. The volume of the nodule was calculated by the formula ( $p \times l^2$ ), and the length and width were measured in millimetres using callipers. The measurement of nodule volume occurred in all groups. A one-way ANOVA test performed on the nodule volume data of the five groups showed a significant difference in nodule volume between the five groups during the two-week observation ( $p \leq 0.05$ ). This difference indicates the effect of DMBA administration in inducing OSCC (C+) and the effect of capsaicin nanoparticle gel administration (in treatment groups E2 and E3).

The LSD post hoc test that compared between groups showed that the positive control group (C+) and the treatment groups (groups E1, E2, and E3) had a significant difference ( $p \leq 0.05$ ). Comparison between treatment groups (groups E1, E2, and E3) showed no significant difference,

but clinically there was a difference mean nodule volume in the measurement results (Table 4). Comparison indicated the difference in the volume of nodules between the groups that were not given any treatment (C+), with the groups treated with cisplatin (E1) and capsaicin nanoparticle gel separately (groups E2 and E3) (Table 5).

## DISCUSSION

Patients with OSCC have several manifestations in the development of cancer cells, two of which are weight loss and the formation of OSCC nodules. There was considerable weight loss in the rats used in this study, before DMBA was induced and before treatment. This shows that weight loss is one of the symptoms of pain in cancer patients. Generally, cancer patients experience a decrease in their quality of life, and major weight loss is one of the most prominent signs of this reduction.<sup>18</sup> Inducing DMBA in rats to cause breast cancer showed a decrease in body weight in rats, especially in the DMBA-induced group who died in the middle of the study. In addition to the weight loss of rats, this group also showed symptoms in the form of decreased appetite, then reduced movement, until their death.<sup>19</sup> There was a substantial difference in body weight after the inducement of DMBA between the control group and the treatment group. This is because cachexia, a cancer-induced weight loss condition, is initiated by cachectin, a tumour necrosis factor that breaks down fat and reduces fat-storing enzymes. In addition, a decrease in the rats' feed intake could be a factor in their weight loss. This decrease

**Table 4.** Comparison of mean volume of nodules

Groups	n	Mean volume nodule $\pm$ SD	Sig.
Negative control	4	0	0.000*
Positive control	4	5.834 $\pm$ 2.77	0.000*
Cisplatin	4	1.75 $\pm$ 0.37	0.000*
1% capsaicin nanoparticle gel	4	1.747 $\pm$ 0.36	0.000*
3.3% capsaicin nanoparticle gel	4	1.812 $\pm$ 0.11	0.000*

\*ANOVA significant difference ( $p \leq 0.05$ )

**Table 5.** Comparison of mean volume of nodules each group

Groups	Negative control	Positive control	Cisplatin	1% capsaicin nanoparticle gel	3.3% capsaicin nanoparticle gel
Negative control					
Positive control	0.000*				
Cisplatin	0.097	0.001*			
1% capsaicin nanoparticle gel	0.098	0.001*	0.998		
3.3% capsaicin nanoparticle gel	0.087	0.001*	0.951	0.949	

\*post hoc LSD, a significant difference ( $p \leq 0.05$ )

could be due to difficulty in accessing food due to lumps in the rats' buccals.<sup>20</sup> The mechanism of action of capsaicin on cachexia is not fully understood, but some literature states that the relationship between giving capsaicin to patients with cachexia affects the amount of food intake. Capsaicin is thought to increase food intake by induced vagal afferent signalling, which results in weight gain and decreased metabolism.<sup>21,22</sup>

The OSCC nodule formation that extends extra-orally looks purplish-red to blackish and reaches the infraorbital to the anterior buccal area. This supports the description of cancerous lesions that OSCC can be exophytic, endophytic or ulcerative. Lesions can also spread to several parts of the oral cavity and tend to be more common in areas with non-keratinized mucosa because it has a thinner barrier than other mucosa.<sup>23</sup>

Measurement of nodule volume is one indicator to detect the progression and spread of cancer. Changes in the size of cancer tissue indicate the development of cancer. This study measured the administration of the extract to cancer and noted whether a change occurred in the nodule volume.<sup>17</sup> In general, cancer develops constantly, and the nodule volume increases gradually from day to day. This characteristic shows that the measurement of nodule volume can be an indicator of cancer development.<sup>17</sup> Development of nodule volume also supports differences in post-DMBA induction in rats. Observations were made post-induction by palpation of the buccal mucosa. Nodules with an average size of 1.9 mm<sup>3</sup> that developed after two weeks of induction were found in the DMBA-induced group, and no nodules were found in the control group. The results of the nodule volume measurement also showed a notable difference between the control group and the treatment group ( $p \leq 0.05$ ). This difference indicates that the nodule volume in the treatment group was smaller than the positive control group. Comparison between the three treatment groups showed no major difference, but in the mean volume of nodules, there were differences in numbers between groups. There were differences of 0.01 between groups E1 and E2, and 0.1 between groups E2 and E3. Groups E1 and E2 had no real difference in their nodule volume measurements, indicating there is potential for a study to be developed on 1% capsaicin nanoparticle gel, used in E2, due to its anticancer effect, which was comparable with the standard OSCC treatment with cisplatin used in E1. The comparison between groups E2 and E3, namely the 1% and 3.3% capsaicin nanoparticle gel treatment groups, showed that the 1% group had better results. This better result in the 1% group is because the use of capsaicin at a dose of 3% can cause saturation of the capsaicin concentration, and so the absorption power of rats is not optimal.<sup>24</sup> Previous in vivo studies state that capsaicin has mutagenic potential, depending on the dosage and the treatment period, which is why lower doses were non-mutagenic when given for several days. The 1% concentrate showed the same anticancer mechanism as in previous studies, with action induced G0/G1 phase cell arrest, inhibited tumour growth

and promoted apoptosis.<sup>25,26</sup> Therefore, administering anticancer agents to a nodule can slow the progression and development of cancer.<sup>17</sup>

Generally, normal cells have apoptotic ability with different triggers. Cancer cells form due to DNA damage that means cell division and replication cannot be controlled also do not have regulation in apoptosis. However, in preventing uncontrolled replication that causes cancer, the body naturally has tumour suppressor genes, one of which is the p53 gene in the p53 protein. The concentration of p53 protein in normal cells is low, and if the cell is damaged by DNA causing uncontrolled replication, activation of p53 protein is required to prevent further cell division. The p53 protein can inhibit the G1 phase in cell division. The inhibited G1 phase can provide an opportunity for cells to repair DNA or carry out apoptosis to suppress the further development of cancer cells.<sup>27</sup> Capsaicin is thought to be able to phosphorylate p53 to inhibit the growth of existing tumours. The cytotoxic effect appears by giving various doses of capsaicin from the extraction of *Capsicum annum L. var. angulosum* against human squamous cell carcinoma and human submandibular gland carcinoma. The administration of capsaicin can activate p53 protein that will increase apoptosis in cancer cells.<sup>28</sup>

DMBA induction could lead to serious differences in body weight between the groups and the growth of cancerous nodules on the buccal mucosa. Administration of the capsaicin nanoparticle gel with 1% and 3.3% concentrations, along with cisplatin, can suppress the growth of cancer cells when viewed from the morphology of the nodules and the measurement of the nodule volumes between groups. Nevertheless, capsaicin nanoparticle gel from green chilli pepper extract at levels of 1% reduces OSCC nodule more than 3.3% concentration.

We suggest further research using the same green chilli pepper (*Capsicum frutescens L.*) or another variety of chilli pepper. We also suggest that future research use various concentrations and testing using histopathology and other apoptosis cell activities.

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