ANTI-PROLIFERATION ACTIVITY OF NANOENCAPSULATED BIOADHESIVE VAGINAL GEL OF ISOLATED ACTIVE COMPOUND (BVI03) FROM *Boehmeria virgata* (FORST) GUILL LEAVES AGAINST HUMAN CANCER CERVIX HEla CELLS

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ABSTRACT

To investigate the anti-proliferation of BVI03 which was formulated in Nanoencapsulated Bioadhesive Vaginal Gel (NBVG) form, an isolated active compound from *B. virgata* using MTT method. The anti-proliferative effects of nanoencapsulated and NBVG were tested against HeLa cells compared with BVI03 un-formulated. The result showed that this formula had less anti-proliferation effect against cervical cancer of HeLa cells.

Keywords: BVI03, nanoencapsulated, NBVG, anti-proliferation; HeLa cells

INTRODUCTION

Worldwide annually two to three percent of deaths recorded arise from different types of cancer, continues to increase largely because of the aging and growth of the world population alongside an increasing adoption of cancer-causing behaviors, particularly smoking, in economically developing countries [1, 2]. In our previously study we found, that Makassar Traditional Healer used the *Boehmeria virgata* leaves to treated cancer. Isolated active compound (BVI03) of *B.virgata* leaves showed anti-proliferative activity against HeLa cell line [3, 4].

For cancer therapy in the future use nanoparticles as drug delivery systems for anticancer therapy has a great potential. For hydrophilic drugs and hydrophobic nanoparticles are an efficient delivery system [5], paclitaxel nanoparticles able to reduce its toxic effects [5] and nanocurcumin dispersion in liquid media that could increase its potential in the treatment of cancer [6].

Vaginal delivery system is an important route of drug delivery for local and systemic disease. Traditional dosage forms such as creams, foams, gels, irrigation and tablets which are used through in vaginal cavity has a relatively short of time residence due to the self-cleaning action of vagina and required many times in a day to get a therapeutic effect. To overcome these problems, drug delivery systems have been developed to extend the residence time of bio-adhesive drug in the vaginal cavity [7, 8].

As an alternative treatment of cervical cancer and to improve compliance and convenience for application, the study is intended to determine anti-proliferation activity of NBVG-BVI03-containing using MTT assay.
MATERIALS AND METHODS

Reagents
Dimethyl sulfoxida (DMSO), 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-tetrazolim bromide (MTT), ethanol 96%, n-hexane, ethyl-acetate, butanol and silica F245 gel were purchased from Sigma Aldrich (USA). Fetal bovine serum (FBS), RPMI-1640 medium, penicillin-streptomycin and trypsin-EDTA were purchased from Gibco-Brl (USA). Membrane filters 0.2 µm and 96 well microplate were purchased from Nunc (Denmark).

Isolation of BVI03
The isolation of BVI03 from B. virgata leaves was done [9] (Figure 1).

Preparation of vaginal nanocapsules
Nanoencapsulated formulation of BVI03 from B. virgata leaves was done previously described with modification [10]. Chitosan solution in acetic acid 1% and BVI03 in acetone were mixed. Tween 80 was added while stirred at 1,000 rpm for 150 minutes. Sodium tripolyphosphate was added gradually during stirring, centrifuged at 5,000 rpm for 20 minutes. The precipitate was re-suspended in aquadest to remove un-trapped drug and nanocapsules were freeze-dried.

Preparation of nanocapsule-containing vaginal bioadhesive gels (NBVG)
BVI03 nanocapsules incorporated gels were prepared by mechanical stirring method using various grades of carbopol such as carbopol 934, 940, 974 and 980 with other formulation additives. Nanocapsules were mixed with prepared bioadhesive gels [11, 12]. The gel preparations were packed in wide mouth plastic jars covered with screw capped plastic lid after covering the mouth with an aluminum foil and were kept in cool place for further study.

Cell Culture
HeLa (human cervical cancer) cell line was provided in Biofarmaka Laboratory in Hasanuddin University. The cells were cultured in a RPMI-1640 medium (supplemented with 10% fetal bovine serum; 100 IU/mL penicillin and 100 UI/mL streptomycin). The cell culture was maintained at 37°C in humidified air with 5% CO2.

Anti-proliferative Activity Assay
The anti-proliferative activity assay of BVI03 compound was measured using MTT assay [13]. The assay detects the reduction of MTT by mitochondrial dehydrogenase to blue formazan product, which reflects the normal function of mitochondria and cell viability. Exponentially growing cells were washed and seeded at 1x10^5 cells/well (in 100 µL of growth medium) in 96 well microplate. After 24 h incubation, a partial monolayer was formed then the media was removed and 100 µL of the growth medium containing the BVI03 (initially
dissolved in DMSO) were added and re-incubated for 24 h. Then 100 µl of the medium were aspirated and 100 µl of the MTT 0.5 mg/mL solution were added in each well. After 4 h contact with the MTT solution, blue crystals were formed. One 100 µl of the stop solution were added and incubated further for 24 h. Reduced MTT was assayed at 559 nm using a Microplate Reader (Biorad). Control groups received the same amount of DMSO (0.1%). Untreated cells were used as a negative control, while doxorubicin as a positive control.

The cell viability or percentage of control was calculated by the following equation:

\[
\text{Anti-proliferation} \% = \frac{\text{Absorbance}_{\text{control}} - \text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \times 100\%
\]

Statistical Analysis
We use the Software to determine the significance of the difference between the treated and untreated groups. The results are presented as means±SD of three independent experiments. The differences were considered significant at \( p<0.05 \).

RESULTS AND DISCUSSION

Nowadays, natural products and its related drugs are used to treat 87% of all human diseases [14], about 25% of prescribed drugs in the world originate from plants and there many species of plants have been reported to have anticancer properties [15, 16]. *B. virgata* leaf have been widely used in Traditional Makassar Medicine to treat cancer [3]. *B. virgata* is classified in the family of Urticaceae (genus of Boehmeria). The Boehmeria genus have been widely studied by several author, in search of answers to their cytotoxic effect [17].

BVI03 is alkaloid compounds previously isolated and identified from *B. virgata* was determined by comparing the spectroscopic data of the isolated compounds with the relevant data which have been published (references). BVI03 was preferable to make a formula of the compound in to Nanoencapsulated Bioadhesive Vaginal Gel (NBVG). Further tests were also performed to prove the anti-proliferative effect on HeLa cell.

Our study describes investigations into the anticancer potential of NBVG formula that contain nanocapsule of BVI03, an isolated active compound from *B. virgata*.

Cell viability was measured using MTT assay. The viable cell number/well is directly proportional to the production of formazan, which is able to be dissolved in DMSO. After HeLa cells were incubated with indicated concentrations of BVI03-nanocapsule and NBVG (0.19; 0.39; 0.78; 1.56; 3.12; 6.25; 12.50 and 25.00 mg/mL) for 24 h, cell viability significantly reduced. The all samples were showed that the percentage of anti-proliferation activity to be increasing with increasing concentration of test compounds (Figure 1).

![Figure 1. Anti-proliferative effect of (A) BVI03 with concentrations 0.07; 0.15; 0.29; 0.59; 1.17; 2.34; 4.69 and 9.38, (B) BVI03-nanocapsule with concentrations 0.19; 0.39; 0.78; 1.56; 3.12; 6.25; 12.50 and 25.00 mg/mL, (C) NBVG with concentrations 0.19; 0.39; 0.78; 1.56; 3.12; 6.25; 12.50 and 25.00 mg/mL. Cell viability of HeLa cells was determined by MTT assay using ELISA reader. Values were presented as means±SD of three independent experiments.](image-url)
The IC50 is a measure of how effective a drug. It indicates how much of a drug is needed to inhibit a given biological process by half. In other words, it is the half minimal (50%) inhibitory concentration (IC) of a substance (50% IC, or IC50).

Table 1  IC50 of BVI03 isolated active compound, nanoencapsulated and NBVG on HeLa cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>IC50 (µg/mL)</th>
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<tbody>
<tr>
<td>BVI03</td>
<td>2.88</td>
</tr>
<tr>
<td>Nanoencapsulated</td>
<td>59.26</td>
</tr>
<tr>
<td>NBVG</td>
<td>725.46</td>
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</tbody>
</table>

The IC50 of BVI03, nanoencapsulated and NBVG are 2.88; 59.26 and 725.46, respectively. Base on The American National Cancer Institute category; BVI03 was categorized as expertly (IC50 value > 30); nanoencapsulated was categorized as moderately (IC50 value = 30-100 µg/mL) but after formulated in to NBVG, its activity decrease and categorized as nontoxic (IC50 value > 250 µg/mL) [18].

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References