The Effect Of Ethanol Extract From Lingzhi Fungi (*Ganoderma Lucidum*) Cianjur Isolate On Syndecan-1 Expressions In Kb CCL17 Oral Cancer Cell

Efek Ekstrak Ethanol Dari Jamur Lingzhi (*Ganoderma Lucidum*) Isolat Cianjur Terhadap Ekspresi Syndecan-1 Pada Sel Kanker Rongga Mulut Kb Ccl-17

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Abstract

Intercellular adhesion plays a role in cancer formation and protein has a key potential in maintaining cell adhesion, including syndecan-1. Meanwhile, oral cancer originates from the oral epithelium, which has an invasive and metastatic level. Its treatments involving chemotherapy and radiotherapy commonly leave unfavorable side effects, hence, suitable alternatives are needed. Natural ingredients are widely used as an alternative treatment for cancer, for example, *Ganoderma lucidum* (*G. lucidum*) which has anti-cancer and anti-angiogenic properties, induces apoptosis, stimulates an immune response, inhibits the degradation of Extracellular matrix (ECM), reduces inflammation, affects cell cycles, cytotoxic, and acts as an antioxidant. This study aims to determine the effect of ethanol extract from *Ganoderma lucidum* Cianjur isolate on syndecan-1 expression in KB CCL-17 oral cell cancer. This was an experimental study with a post-test only control group design, the treatment group used *G. lucidum* ethanol extract with a concentration of 2.12 μg/ml (P1), 4.24 μg/ml (P2), and 8.49 μg/ml (P3), while the positive control group used cisplatin with a concentration of 11.5 μg/ml (K1). In contrast, the negative control used aquadest (K0), while syndecan-1 expression was observed using the immunohistochemical examination. The highest syndecan-1 expansion rate was found in the treatment group with a concentration of 8.49 μg/ml. A significant difference was indicated by one-way ANOVA (p<0.05) between K0 - K1, K0 - P1, K0 - P2, K0 - P3, K1 - P1, K1 - P2, K1 - P3, P1 - P2, as well as P1 and P3. The administration of ethanol extract from *G. lucidum* Cianjur isolate increases syndecan-1 expression in KB CCL-17 oral cell cancer.

Keywords: Syndecan-1, oral squamous cell carcinoma, *Ganoderma lucidum*, KB CCL-17

Abstrak

Adhesi sel antar sel memainkan peran terhadap pembentukan kanker. Protein yang berperan dalam mempertahankan adhesi sel adalah Syndecan-1. Kanker rongga mulut berasal dari epitel rongga mulut yang memiliki tingkat invasif dan metastasis. Perawatan kanker yang mengalami invasif dan metastasis dilakukan perawatan kemoterapi dan radioterapi, namun efek dari perawatan tersebut memiliki efek samping yang tidak menguntungkan, untuk itu dibutuhkan perawatan...
INTRODUCTION

Oral cancer had an incidence of 354,864 cases in 2018, with a mortality rate of 177,384.1 The increased incidence is influenced by poor prognosis, low survival rates, and poor radiotherapy treatment.2 Besides, cancer has an invasive and metastatic ability, which accounts for 90% of the deaths. Protein and gene expression are used to predict the invasiveness and metastasis, hence, they are used as a reference in determining tumor prognosis.3 Syndecan-1 is commonly applied as a diagnostic standard biomarker by analyzing the number of expressions which often decrease due to the downregulation mechanism of cancer.4 Cancers with a decreased expression of syndecan-1 are commonly found in the lungs, kidneys, as well as head and neck.5

Cancer treatment uses surgical therapy combined with radiotherapy and/or chemotherapy, but these treatments have side effects on the patients.5,7 Therefore, suitable and safe alternatives such as natural ingredients are needed to minimize the side effects. Ganoderma lucidum (G. lucidum) is a fungus that is cytotoxic, has anti-cancer properties by inducing apoptosis, suppressing invasive cancer cells, regulating key signaling molecules,8 inhibiting progressive cell cycles, and suppressing angiogenesis.8,9

Studies on the effect of G. lucidum Cianjur ethanol extract and Syndecan-1 expression on KB CCL-17 oral cancer cell have not been conducted. Therefore, this study aims to determine the potential of this natural ingredient in inhibiting the develop-ment of oral cancer.

MATERIALS AND METHODS

This study used a post-test group-only design with a purely experimental approach. The treatment group was given G. lucidum ethanol extract with a concentration of 2.12, 4.24, and 8.49 μg/ml, while the positive control group was treated using cisplatin at a concentration of 11.5 μg/ml and the negative control was administered with aquadest. Each treatment and control was repeated four times, while the G. lucidum fungus used in the study was obtained from the CV Asa Agro Corporation. It was harvested after four months of planting, and three months after the fruit body sprang out. The fungus lives in the lowlands with a temperature of 30°C, humidity of approximately 80%, and a pH of 7. It was collected in September 2019 and the part used was 300 grams containing 10 mL DMEM serum medium and centrifuged for 10 minutes at 1200 rpm. The supernatant was separated between sediment and liquid, while the precipitate formed along with DMEM, was collected and the mixture was stirred and tightly closed with aluminum foil, left for 3x24 hours by stirring, and filtered every day.

KB CCL-17 cancer cells obtained from ATCC with epithelial morphology were taken from liquid nitrogen tanks and then diluted with water baths at 37°C. The cells were inserted into centrifuge tubes containing 10 mL DMEM-serum medium and centrifuged within 10 minutes at 1200 rpm. The supernatant was separated between sediment and liquid, while the precipitate formed with DMEM, was kept for 20 minutes, then the centrifuge was performed at 1200 rpm for 10 minutes. Further-more,
the next suspension was placed into Tissue Culture Flask (TCF) with a growth medium containing 20% FBS and then viewed under an in-verted microscope. Living cells appeared round, glowing, and clear. The TCF was incubated in an incubator at 37°C and 5% CO₂ with a non-tense lid, while the cells were given treatment when 80% con-fluent occurred, which was marked with a yello-wing medium. Moreover, the cells were treated with the extracts and incubated for 24 hours on a 24 well microplate. They were further washed with 500 μL Phosphate buffer saline (PBS), 7% ethanol and 3% hydrogen peroxide at 100 μL, and kept for 10 minutes. The solution was then discarded with a micropipette, and the coverslip was washed with 500 μL PBS. PBS was taken with a 1 mL micro-pipette and transferred to a glass preparation. Pre-diluted blocking serum of 100 μL was dropped and kept for 10 minutes, then, an immunohistochemistry examination was performed to observe syndecan-1 expressions.

Subsequently, data were tabulated and tested for normality by Shapiro-Wilk, then, the analysis was performed using the one-way ANOVA test. When the data is normally distributed with a confidence level of 95% (p <0.05), a post-hoc Least Significance Difference (LSD) test was performed to determine the presence of significant differences between the groups.

RESULTS

Immunohistochemical observations were performed on 10 random Microscope fields of view with a magnification of 400 times. The results showed positive brown Syndecan-1 on cytoplasm and negative with no staining. The syndecan-1 expression on immunohistochemical examination is shown in Figure 1.

The analysis results of Syndecan-1 expression in oral cancer cell KB CCL-17 culture are shown in Table 1, shows the mean of syndecan-1 expression in the KB CCL-17 oral cancer cell culture from each group. Syndecan-1 expression was mostly observed in the treatment group at a concentration of 8.49 μg/ml, with a mean of 34.59%. In contrast, the smallest expression was found in the non-treatment group with a mean of 1.51%. Based on the results, the mean of syndecan-1 expression in the oral cancer cell culture with ethanol extract increased proportionally with the increasing concentration.

After the mean was obtained, statistic tests were performed, including normality test using Shapiro-Wilk and homogeneity using the Levene test, then the one-way ANOVA was used to analyze the data. The normality test showed a significance value of 0.125, while Shapiro-Wilk results showed that the data were normally distributed with p>0.05. Furthermore, the homogeneity test obtained a significance value of 0.233 after the data were transformed. A value of p>0.05 indicates homogeneity, hence, the data were used.

The one-way ANOVA parametric statistical test requires that data have normal distribution and homogeneity. It was used to analyze differences in syndecan-1 expression between groups and treatments, Table 2 depicts the test results.

Based on the results, the expression of syndecan-1 in oral cancer cell KB-CCL-17 cultures between the non-treatment group, as well as others treated with cisplatin, and G. lucidum ethanol extract of 2.12 μg/ml, 4.24 μg/ml and 8.49 μg/ml had significant differences with a value of 0.000 (p<0.05). Furthermore, there was a significant difference in the one-way ANOVA parametric test, while the LSD test was conducted to identify significant differences between the groups. Table 3 depicts the LSD parametric test results.

The LSD parametric test results in Table 3 showed that there were differences in the expression of syndecan-1 between each group, except the P2 and P3 groups. The difference was categorized as significant when the p-value is <0.05. The significance value of the P2 and P3 groups was 0.40.

DISCUSSION

Based on the results, there were differences between the groups without treatment, as well as cisplatin, G. lucidum ethanol extract at 2.12 μg/ml (P1), 4.24 μg/ml (P2), and 8.49 μg/ml (P3). Further tests using LSD showed that there were significant differences in the expression of syndecan-1 between the non-treatment, cisplatin, and treatment groups of P1, P2, P3. Ethanol extract of G. lucidum increased the syndecan-1 expression in oral cancer cell KB CCL-17. Meanwhile, the expression is related to cell growth regulation, pro-liferation, the interaction between heparin and growth factor groups, angiogenesis induction, growth promotion, tumor progression, and contributes directly to invasive and metastatic abilities in malignant tumors.

A correlation between metastases in invasive ductal carcinoma and the number of syndecan-1 expressions in primary tumors was found in previous studies. This occurred because syndecan-1 is expressed in more than 90% of epithelium tumors. The high expression is related to the maintenance of epithelial cell morphology and increasing the interaction of cell adhesion with matrix components. Aside from maintaining cell adhesion as a physio-
logical function, syndecan-1 also regulates pathological processes, such as invasive tumor cells, angiogenesis, and metastasis. Based on the above data, the lowest mean of syndecan-1 expression was found in the group without treatment. This is because the expression is reduced in epithelial tumors in relation to the prognosis as well as due to an increase in MMP-9 which has opposite effects with syndecan-1, hence, a decrease in MMP-9 expression increases the syndecan-1 expression.

Syndecan-1 expression in ameloblastoma shows that Desmoplastic Ameloblastoma has a smaller number compared to other types. Multicystic types which have invasive properties from weak to strong are follicular, akantomatos, granular, plexiform, and desmoplastic types, respectively. Furthermore, in odontogenic keratocyst, unicystic ameloblastoma, and dentigerous cyst, low syndecan-1 expression increases the invasive and destructive ability of neoplastic cells in unicystic ameloblastoma and extends to surrounding tissues. The mean of expression in epithelial tissues with ameloblastoma showed a decrease of 26.3%, keratocystic odontogenic tumor (KCOT) 92.3%, and dentigerous cyst 100%. There was an association with cell differentiation and invasive ability into deeper tissues on malignancy. Al Otaibi, et al. found that the mean of syndecan-1 expression in normal cells was more than mild, moderate, and severe dysplasia in the oral cancer cell, but there was no significant difference between tumor grades.

Syndecan-1 also increases in human fibrosarcoma cell line HT-1080. It stimulates malignancy by increasing proliferation, metastatic potential, and migration ability. Overexpression of the branch bypasses the ectodomain, but it has amino acids in the juxta-membrane that promote proliferation and chemotaxis. The ectodomain increases tumor cell proliferation by the migratory ability of syndecan-1, which is enhanced by syndecan-2. Increased syndecan-1 expression triggers IGF1R activation and elevates Ets-1 proliferation. Moreover, syndecan-2 transfection with an upregulated mechanism increases the chemotaxis migration of HT-1080 cells to the ECM protein. The proliferation of HT-1080 cells occurs due to hyper-phosphorylation of Rb (retinoblastoma) by the CDK2 (cyclin-dependent kinase 2) -cyclin E1 complex. This process is also stimulated by syndecan-1, hence, increasing its expression also raises syndecan-2.

Increased expression of syndecan-1 reduces the ability of cell proliferation by prolonging G1 and S phases in the cell cycle and retarded migration. The results show that syndecan-1 work simultaneously in inhibiting the motility and cycle of mesenchymal cells that are not present in the ectodomain, hence, it has a dual role.

There was no significant difference in the mean of syndecan-1 expression in the P2 and P3 treatment groups. In other words, G. lucidum ethanol extract of 4.24 μg/ml and 8.49 μg/ml had a comparable ability to increase syndecan-1 expression. Based on the LSD parametric test, there was a significant difference in the expression between P1 and P3 groups, as well as between P2 and P3 groups. These results showed that the ethanol extract with a concentration of 4.24 μg/ml and 8.49 μg/ml was stronger against the increased syndecan-1 expression compared to 2.12 μg/ml.

The treatment groups, as well as negative and positive controls, had syndecan-1 below the normal value which is greater than 66%. Moreover, there was a significant difference in the syndecan-1 expression in the K1 group (cisplatin) with P2 as well as the K1 Group with P3. These results indicate that the effectiveness of G. lucidum ethanol extract was higher than cisplatin as a chemotherapy agent.

Decreased expression of more than 80% of oral cancer is a risk factor for recurrence and death after 24 months of surgery, hence, syndecan-1 in the stroma is a reliable indicator of oral cancer prognosis. The increased expression associated with cytotoxic treatment shows clear predictive factors and a good prognosis in oral cancer. Furthermore, the loss of syndecan-1 in breast cancer causes reduced metastasis in the brain, therefore, when the expression increases, the potential risk of metastasis to the brain is elevated. Also, the loss of syndecan-1 triggers changes in breast cancer cells to secrete cytokines/chemokines that affect BBB (Blood-brain barrier). The cytokines / chemokines regulated by syndecan-1 are IL-8, IL-6, and IL-34. Altogether, they influence the integrity and transmigration of BBB mediated by CCL5, ICAM-1, IL-6, IL-8, and GM-CSF. The syndecan-1 expression on fibroblast stroma and endothelial cells enhances growth, angiogenesis, and forms a microenvironment that favors invasive breast cancer. Syndecan-1 expression in fibroblast stroma is needed to perform metastasis in 4T1 breast cancer mice.

Nevertheless, the expression by some cancers is not always a risk factor for increasing metastasis to the brain. Prostate cancer characterized by an increased syndecan-1 expression has a low incidence of metastasis to the brain namely 0.2-2%. The syndecan-1 expression also increases in lung cancer, but the presence of a correlation with a good or poor prognosis is unclear.

These results indicate that the ethanol extract of G. Lucidum effectively increases the number of syn-
decan-1 expressions, therefore, *G. Lucidum* needs to be developed as a potential anti-cancer agent to suppress the invasive and metastatic levels of oral cancer. In conclusion, the administration of ethanol extract from *G. lucidum* Cianjur isolate increases syndecan-1 expression in KB CCL-17 oral cancer cells.

ACKNOWLEDGEMENTS

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TABLES

Table1. Analysis Results of Syndecan-1 Expression in oral cancer cell KB CCL-17 Culture.

<table>
<thead>
<tr>
<th>No</th>
<th>Group</th>
<th>Mean (%)± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Without Treatment</td>
<td>1.51 ± 0.52</td>
</tr>
<tr>
<td>2</td>
<td>Cisplatin 11.5 μg/ml</td>
<td>25.53 ± 2.45</td>
</tr>
<tr>
<td>3</td>
<td>Ethanol Extract of <em>G. lucidum</em> – Concentration of 2.12 μg/ml</td>
<td>10.77 ± 1.33</td>
</tr>
<tr>
<td>4</td>
<td>Ethanol Extract of <em>G. lucidum</em> – Concentration of 4.24 μg/ml</td>
<td>32.29 ± 6.05</td>
</tr>
<tr>
<td>5</td>
<td>Ethanol Extract of <em>G. lucidum</em> – Concentration of 8.49 μg/ml</td>
<td>34.59 ± 1.66</td>
</tr>
</tbody>
</table>

Source: Primary data was processed, 2020

Table2. One-way ANOVA test on Syndecan-1 Expression

<table>
<thead>
<tr>
<th></th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean squares</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>2048.128</td>
<td>4</td>
<td>512.032</td>
<td>48.641</td>
<td>.000</td>
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<tr>
<td>Within Groups</td>
<td>94.741</td>
<td>9</td>
<td>10.527</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>2142.869</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Source: Primary data was processed, 2020
Table 3. Results of LSD Parametric Tests between Groups on Syndecan-1 Expressions

<table>
<thead>
<tr>
<th>No.</th>
<th>Group</th>
<th>K0</th>
<th>K1</th>
<th>P1</th>
<th>P2</th>
<th>P3</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>K0</td>
<td>24.031** (p=0.000)</td>
<td>-9.265* (p=0.012)</td>
<td>-30.875** (p=0.000)</td>
<td>-33.092** (p=0.000)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>K1</td>
<td>24.031** (p=0.000)</td>
<td>14.766** (p=0.000)</td>
<td>-6.753* (p=0.031)</td>
<td>-9.060* (0.008)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>P1</td>
<td>9.265* (p=0.012)</td>
<td>-14.766** (p=0.000)</td>
<td>-21.520** (p=0.000)</td>
<td>-23.826** (0.000)</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>P2</td>
<td>30.785** (p=0.000)</td>
<td>6.753* (p=0.031)</td>
<td>21.520** (p=0.000)</td>
<td>-2.306 (p=0.407)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>P3</td>
<td>33.092** (p=0.000)</td>
<td>9.060* (0.008)</td>
<td>23.826** (0.000)</td>
<td>2.306 (p=0.407)</td>
<td></td>
</tr>
</tbody>
</table>

Description:
- * = significant (p<0.05)
- ** = very significant (p<0.01)
- K0 = negative control (without treatment)
- K1 = positive control (cisplatin 11.35 μg/ml)
- P1 = ethanol extract of G. lucidum with concentration of 2.12 µg/ml
- P2 = ethanol extract of G. lucidum with concentration of 4.24 µg/ml
- P3 = ethanol extract of G. lucidum with concentration of 8.49 µg/ml

Source: Primary data was processed, 2020

FIGURES

![Figure 1. Expression of Syndecan-1 with A. treatment ethanol extract; B. Cisplatin; C. Aquades on immunohistochemical examination](image)

ARROW: cells that did not express syndecan-1
ARROW: cells that expressed syndecan-1

Source: Primary data was processed, 2020

REFERENCE


