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RESEARCH ARTICLE

The Alteration of SNAIL2 and MMP-9 Protein Expression of JEG-3 Choriocarcinoma Cell by Methanolic Extract of *Tamarindus Indica* Fruit

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Abstract

Objective: *Tamarindus indica* is one of the famous natural plants that have various effects on health. Recent research has begun to examine the effect of *Tamarindus indica* on cell malignancy. One of the things that happen in cell malignancy is an increasing ability of cell migration, which is characterized by increasing expression of SNAIL 2 and MMP-9. This study was conducted to determine the effect of extract of *Tamarindus indica* Fruit Extract (TIFE) towards Snail 2 and MMP-9 expression in JEG-3 cells. Methods: TIFE were extracted by using methanol. JEG-3 cells were grown in culture medium treated with TIFE (at 0, 25, 50 and 100 µg/ml). Immunofluorescence staining was carried out, and observed under Confocal Laser Scanning Microscope. Result: SNAIL 2 expression after treatment with TIFE 50 and 100 µg/ml higher than control-(P=0.015; P=0.001) whereas at MMP-9 expression there was a biphasic pattern, in which the MMP-9 expression peaked at concentration of 50 µg/ml (P=0.002), then MMP-9 expression decreased at 100 µg/ml concentration. Conclusion: The expression of SNAIL2 influence MMP-2 expression after treat with TIFE, so it might increase the potential for JEG-3 cell migration

Keywords: *Methanolic extract, Tamarindus indica, SNAIL2, MMP-9*

Introduction

Tamarindus indica is believed to have various properties in health [1]. Some studies mention the presence of various antioxidant content in *Tamarindus indica* fruit extract .Phytochemical research shows that *Tamarindus indica* contains various contents, such as phenol compounds, glycosides, mallic acid, tartaric acid, sap, pectin, arabinose, xylose, galactose, glucose, and uronic acid.

Tamarindus indica extract also shows the presence of fatty acids and various essential elements such as arsenic, calcium, cadmium, copper, iron, sodium, manganese, magnesium, potassium, phosphorus, zinc and a little vitamin A [2, 3].

Tamarindus indica can inhibit various types of cell malignancy [4]. In the process of cell malignancy, there is a condition called the epithelial-mesenchymal transition where there is a change in cell properties characterized by increased cell migration ability [5]. Meanwhile, in other studies there has also been an increase in cell migration after administration of *Tamarindus indica* [6].

This study was conducted to investigate the effect of TIFE toward Snail2 and MMP-9 proteins expression in JEG-3 choriocarcinoma cells, which play a role in the process of cell migration.

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Materials and Methods

Preparation of Methanolic Extract of the *Tamarindus Indica* Fruit (TIFE)

Tamarindus indica was obtained from UPT Materia Medica (Batu, East Java, Indonesia), in powder form where the powder was obtained from the entire *Tamarindus indica* fruit (including skin, pulp and seed) which is dried, then mashed. The extract process was carried out by modification from other previous research by Chong (2012) [7]. *Tamarindus indica* powder was soaked in methanol at room temperature, then shaken with a shaker and then left in a dark room for 24 hours. The extract was filtered and then the filtrate was putted into the pumpkin evaporator and was evaporated. The crude extract was stored in a freezer with a temperature of -20˚C until it was used.

Cell Culture

JEG-3 Choriocarcinoma Cell line was obtained from the American Type Culture Collection (ATCC). This cell line was cultured according to ATCC protocol with little modification. Cells were incubated at 95% humidity, 5% CO2, and MEM medium with 10% activated fetal bovine serum, 1% penicillin and streptomycin, also 0.1% gentamycin were added.

Treatment of JEG-3 cells with TIFE

The JEG-3 cell line was cultured in the medium and had reached 50-60% confluence then incubated with four different concentrations of TIFE (25 µg/ml, 50 µg/ml and 100 µg/ml, respectively) for 48 hours [8].

Expression of Snail2 and MMP-9 Proteins

Immunofluorescense staining was performed by using Polyclonal antibody Snai2 Cy3 Conjugated (for SNAIL2) and polyclonal antibody FITC Conjugated (for MMP-9) from Bioss USA. The staining results were observed with the Olympus FV1000 Brand Confocal Laser Scanning Microscope at 400x magnification. Data was processed and quantified using Olympus Fluo view Ver 4.2 a software.

Statistical Analysis

All data obtained were repeated at least 3 times with almost qualitative results which were almost the same, followed by tests to check normality data. Then a one-way ANOVA test was performed using Minitab software. To find out which groups are different, a post ANOVA Tukey test was carried out. The difference was found to be significant if $P < 0.05$

Result

There was a significant difference from the Snail2 expression taken from four groups treated with TIFE ($P = 0.000$). The result of post ANOVA Tukey test showed that Snail2 expression in control group without *Tamarindus indica* extract turned out to be not significantly different compare with 25 µg /ml TIFE.

Snail2 expression of 50 and 100 µg/ml TIFE significantly higher than control (P=0.015 and P=0.012 respectively) whereas, Snail2 expression of both concentrations almost similar. Snail2 expression of 50 and 100 µg/ml TIFE also significantly higher than 25 µg/ml (P=0.001 and P=0.002, respectively). These result indicated that the increasing of TIFE concentration tends to increase Snail2 expression (Figure 1)

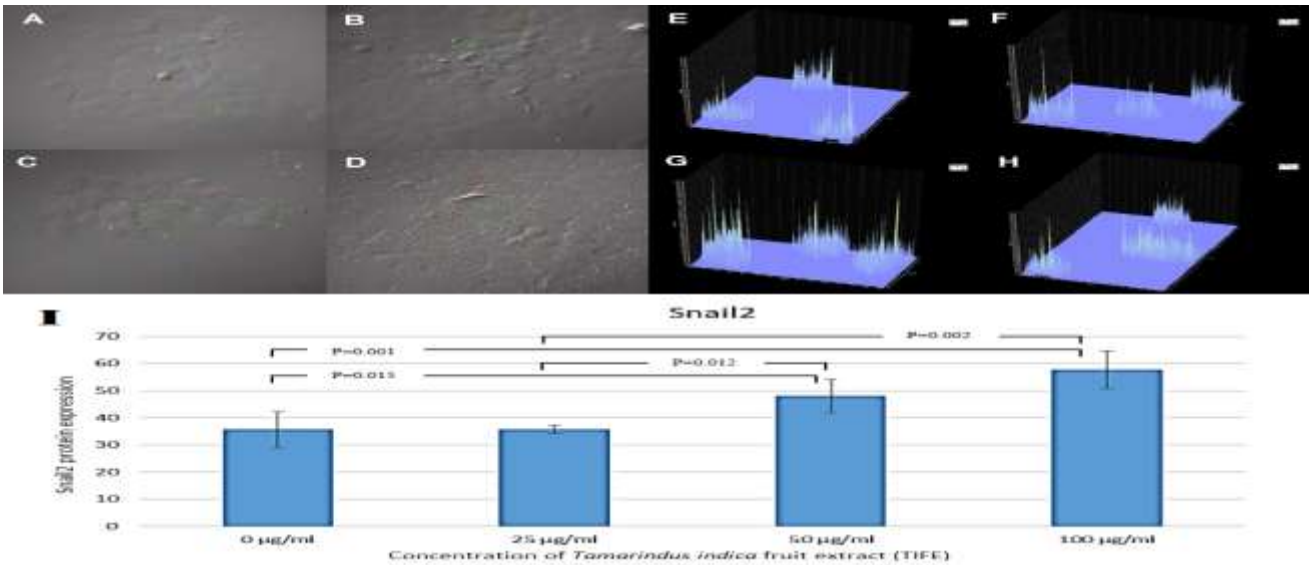

Figure 1: Snail2 Protein Expression in JEG-3 Choriocarcinoma Cell Line with Immunofluorescence Staining (A)

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Result of Staining with 0 µg/ml TIFE treatment (B) Result of Staining with 25 µg/ml TIFE treatment (C) Result of Staining with 50 µg/ml TIFE treatment (D) Result of Staining with 100 µg/ml TIFE treatment (D) Profile intensity with 0 µg/ml TIFE treatment (E) Profile intensity with 25 µg/ml TIFE treatment (F) Profile intensity with 50 µg/ml TIFE treatment (G) Profile intensity with 100 µg/ml TIFE treatment (I) The average of Snail2 protein expression

There was a significant difference from the MMP-9 expression taken from the four dose groups treated with *Tamarindus indica* methanolic extract (P=0.002) (Figure 2). Result of MMP-9 expression treated with 25, 100 µg/ml and without TIFE were not significantly different meanwhile, the expression of MMP-9 in 50 µg/ml significantly higher compare with others.

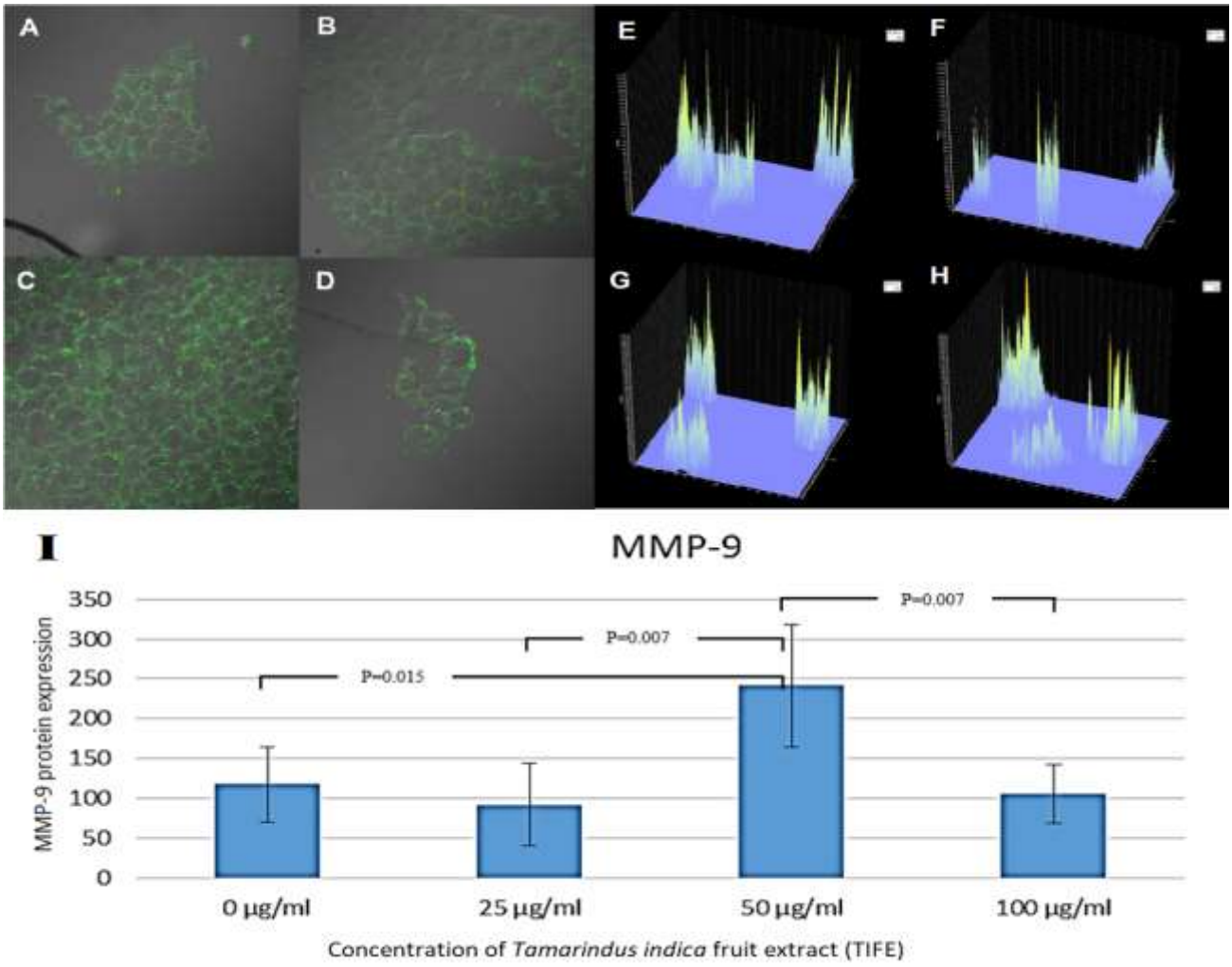

Figure 2: MMP-9 Protein Expression in JEG-3 Choriocarcinoma Cell Line with Immunofluorescence Staining (A) Result of Staining with 0 µg/ml TIFE treatment (B) Result of Staining with 25 µg/ml TIFE treatment (C) Result of Staining with 50 µg/ml TIFE treatment (D) Result of Staining with 100 µg/ml TIFE treatment (D) Profile intensity with 0 µg/ml TIFE treatment (E) Profile intensity with 25 µg/ml TIFE treatment (F) Profile intensity with 50 µg/ml TIFE treatment (G) Profile intensity with 100 µg/ml TIFE treatment (I) The average of MMP-9 protein expression.

Discussion

Snail2 or also called Slug is a transcription factor that plays a role in regulating cell migration during the embryogenesis process and in the process of cell invasion and migration. Its expression is usually associated with tumor recurrence and a poor prognosis [9]. In this study, increasing concentration of *Tamarindus indica* methanolic extract able to induce the expression of Snail2.

It seems that only at doses of 50 µg / ml and 100 µg / ml which show a significant difference compared to other groups, but if change in trend observed, there appears to be an increase in Snail2 expression according to the increased dose of methanolic extract *Tamarindus indica* given, shows the possibility of the influence of *Tamarindus indica* which actually increases the potential of ability of cell invasion and migration. This

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result is different from the research conducted by various previous researchers,

where the results of these studies indicate that *Tamarindus indica* contains many flavonoids and has the ability as an antioxidant [10-12], so it is estimated that it can also play a role in reducing malignancy [4, 13]. However, it turns out that the results of this study are consistent with the results of a study that conducted by Nie and Deters (2013) [6], which states that *Tamarindus indica* can increase cell proliferation and migration.

In the study by Nie and Deters, the ingredients that given to keratinocyte and fibroblast cell cultures were two types of xyloglucan that extracted from *Tamarindus indica* seeds. Nie and Deters also found that xylogucan in *Tamarindus indica* was able to increase ß-catenin phosphorylation in keratinocyte and fibroblast cells. Whereas as written by Steinestel *et al.* (2014) [14], an increase in ß-catenin phosphorylation in the cytosol will cause activation of the Snail2 transcription factor.

Another study by Conacci-Sorrell *et al.* (2003) [15] also states that the activation of transcription Snail2 can be induced by ß-catenin, but not so with Snail1. This may explain the increase in Snail2 expression in the administration of *Tamarindus indica*, although this requires further research, because in this study no b-catenin examination was carried out. In addition, the research carried out this time using extracts from all parts of *Tamarindus indica*, from the skin to the seeds which are then made into powder and then extracted using methanol, thus there is also an active compound xyloglucan which can have an effect in the form of increasing ß-phosphorylation catenin as is the case with studies by Nie and Deters (2013) [6].

However, further research is still needed on this matter, especially whether xyloglucan is the dominant active compound in the crude methanolic extract of *Tamarindus indica*. The increasing of TIFE concentrations proved to have a biphasic effect on MMP-9 expression. This mechanism might be caused by antioxidant of TIFE has role as NO inhibitor. According to Eagleton (2002) [16] reported NG-monomethyl-L-arginine (L-NMMA) as NO inhibitor.

Tamarindus indica itself was also known to have the ability as a NO inhibitor [10], so the effect of *Tamarindus indica* in MMP-9 expression seems to be due to its role as a NO inhibitor. Sanyal *et al.* (2000) [17] have found that neuronal NOS (nNOS) was detected in JEG-3 cells, whereas other NOS isoforms, eNOS (endothelial) and iNOS (specific macrophages), were not found. The presence of nNOS in JEG-3 showed the ability of JEG-3 to produce endogenous NO.

NO itself was known to inhibit MMP-9 expression by inducing TIMP-1 as inhibitor of MMP-9, through activation of the TGF-ß and Smad signaling pathways [18]. That might explain the increasing of MMP-9 expression at 50 µg / ml TIFE that was caused by decreasing of NO. The increasing of TIFE concentration caused a decreasing of endogenous NO levels, so the effect of TIMP-1 inhibition decreased, and consequently the expression of MMP-9 increased.

This needs further research to measure the level of NO. Our data suggested that Snail2 and MMP-9 have independent mechanism. The increasing of Snail2 expression was caused via ß-catenin phosphorylation by xylo glucan in TIFE, whereas MMP-9 expression was influenced by NO level.

Conclusion

The treatment of methanolic extract of *Tamarindus indica* fruit can cause an increase in Snail2 expression and cause modulation in MMP-9 expression. Both of these proteins are important proteins for the occurrence of Epithelial Mesenchymal Transition.

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