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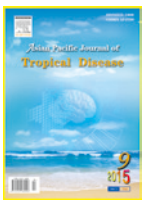

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Possible effect of tea plant parasite, *Scurrula atropurpurea* (Blume) Danser, on growth inhibition of culture HeLa cells *in vitro* through DNA repair and apoptosis intrinsic pathways mechanism

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ABSTRACT

Objective: To study the possible *in vitro* effect of tea plant parasite [*Scurrula atropurpurea* (Blume) Danser] extract on HeLa cells culture growth inhibition through DNA repair and apoptosis intrinsic pathways mechanism.
Methods: Flow cytometry assay were performed for those analyses.
Results: For DNA repair pathway mechanism, treatment of tea plant parasite extract decreased MDM2 expression and increased p21 expression. And for apoptosis markers measurement on bax and bcl-2 showed an opposite result, increasing on bax and decreasing on bcl-2 expression. Almost all doses treatment in all measurement showed significantly result.
Conclusions: This result showed an effect of tea plant parasite extract on inhibition of cells HeLa culture growth through DNA repair and apoptosis intrinsic pathways mechanism.

1. Introduction

Cervix cancer is a malignant tumour that growth on cervix and attached at vaginal peak[1,2]. Based on data from World Health Organization, to date especially at developing country, cervix cancer is placed on the top position among other kinds of cancers that causing mortality in productive women worldwide[3]. In Indonesia, it was estimated that the cervix cancer incident are on the first position with a prevalence percentage 17.2% and frequency of occurrence around 16 in every 100000 women[4]. The major cause of cervix cancer is human papilloma virus (HPV)[5-7]. HPV infection could affect every person, from women aged 20 years old until unproductive women[8]. Several risk factors for high risk HPV infection was closely related with the bad living habits[9]. Many studies found that cervix cancer incidents at young age was increased and tumour seen to be more aggressively. Proportion of women below 35 years old that diagnose with cervix cancer was increased from 9% to 25%[10].

Cervix cancer cells infected by HPV are known to express two different oncogenes, E6 and E7. Both protein were proved causing immortal characteristics on primary culture of human keratinocyte[11-13]. Deregulation of cell death pathways will induced cancer development, and induction of tumour cells apoptosis is a common basic target for cancer therapies[14].

The development of alternative anti-cancer drugs from natural sources have been used because of the inhibition effect on cancer cells development and reduce toxic effects from chemotherapy and radiotherapy[15-17]. One of the natural source that mostly used as an anti-cancer drug is *Scurulla artropurpurea* (*S. artropurpurea*), which in Indonesia well known as tea parasite tea[18]. Plant parasite itself is a parasite that grouped in family of Loranthaceae or Viscaceae. Similar with other parasite species, plant parasites are living together with their host and also absorb their nutrition[19]. Previous study reported that tea plant parasite contain several bioactive compounds, in which had a potency as anti-cancer[20]. In hypertensive patients, bioactive compound that contained in this plant parasite also have a curative

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effects by reducing endothelial cells damage and increasing endothelial progenitor cells [18,21-23].

Herein we reported the effect of tea plant parasite (*S. artropurpurea* (blume) Danser) extract on culture of HeLa cells. We observed two possibility mechanisms that could be affecting by tea plant parasite extract (TPPE), DNA repair mechanism and apoptosis intrinsic pathways. Observation was conducted by measuring the expression effect of upstream signals from each mechanism pathways.

2. Materials and methods

2.1. Cell culture

In this study HeLa cells were used which will be treated with TPPE. HeLa cells were obtained from Center of Assessment and Application Technology. Culture media for growing the HeLa cells contained RPMI medium as a basic media, then supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin. In addition, culture cells were growth by incubating in condition with temperature 37 ℃, 5% CO_2. Culture was performed using trypsin if culture cells already confluent. Treatment and measurement analysis were conducted when culture cells showed a stable growth condition.

2.2. Plant parasite [S. atropurpurea (Blume) Danser] extract

Species of tea plant parasite, *S. atropurpurea* (Blume) Danser, was extracted using similar procedure as previous study[24]. In general, TPPE was obtained through two main steps, drying and maceration. Maceration was divided into two step based on the solution that used in this process. Firstly, maceration was conducted using *n*-hexane solution, then continued with maceration using chloroform solution. Treatment of TPPE in this study was grouped into four doses: 25, 50, 100, 200, and 400 μg/mL. Before used for treatment, TPPE was diluted in 1% DMSO for making stock solution. For making a required doses, TPPE stock solutions were diluted with culture media.

2.3. Analysis of apoptosis and DNA repair marker expression

Two different pathways were observed as a possible mechanism of TPPE effect on culture HeLa cells. Firstly, apoptosis mechanism through intrinsic pathways was observed using flow cytometry method. Marker for apoptosis that measured is expression of Bax and Bcl-2. Second, mechanism that also observed is DNA repair pathways. Expression changes were measured on two marker of DNA repair pathways, murine double minute 2 (MDM2) and p21. Flow cytometry also performed as a measurement method.

2.4. Statistical analysis

One-way ANOVA followed by *post hoc* Tukey test with significance $P < 0.05$ was performed to compare the measurement result between control and treatment. All statistical analysis was conducted using SPSS for windows.

3. Results

Two pathway mechanisms, *i.e.* DNA repair and apoptosis intrinsic pathways, were suggested as a mechanism in which TPPE could affect culture of HeLa cells, thus finally inducing an increasing of apoptosis and proliferation inhibition. Two protein expressions related with the upstream mechanism of each pathways was observed in this study. Expression of MDM2 (Figure 1) and p21 (Figure 2) were measured for DNA repair pathways. Then expression of Bax (Figure 3) and Bcl-2 (Figure 4) were also measured as a marker for upstream signalling of apoptosis intrinsic mechanism.

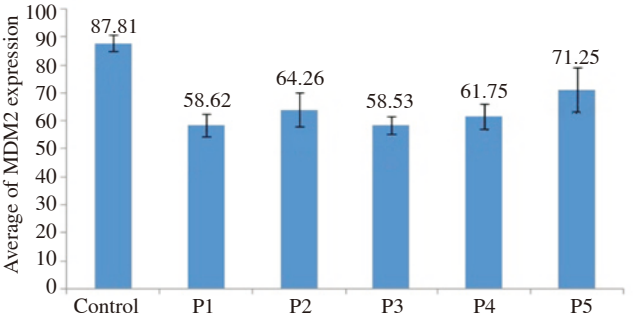

Figure 1. Flowcytometry analysis result of MDM2 expression.
MDM2 expression analysis of HeLa cells treated with TPPE showed decreasing expression compare to control. All doses showed significant decreasing of expression, with the highest reduction obtained from P2. P1: TPPE dose 25 μg/mL; P2: TPPE dose 50 μg/mL; P3: TPPE dose 100 μg/mL; P4: TPPE dose 200 μg/mL; P5: TPPE dose 400 μg/mL. One-way ANOVA followed by *post hoc* Tukey test was used with significance $P < 0.05$.

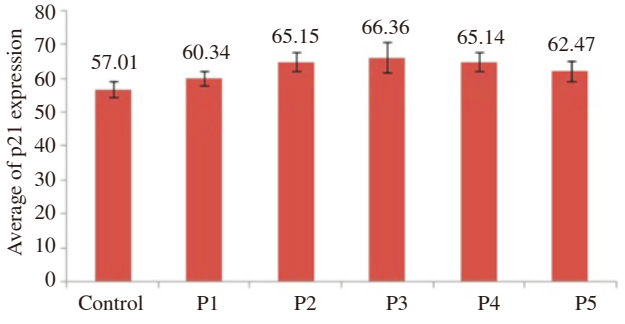

Figure 2. Flowctometry analysis result of p21 expression.
p21 expression analysis of HeLa cells treated with TPPE showed opposite result with MDM2. Almost all doses showed significant increasing of expression if we compared between control and treatment, except P1 and P5. The highest elevation was obtained from P2. P1: TPPE dose 25 μg/mL; P2: TPPE dose 50 μg/mL; P3: TPPE dose 100 μg/mL; P4: TPPE dose 200 μg/mL; P5: TPPE dose 400 μg/mL. One-way ANOVA followed by *post hoc* Tukey test was used with significance $P < 0.05$.

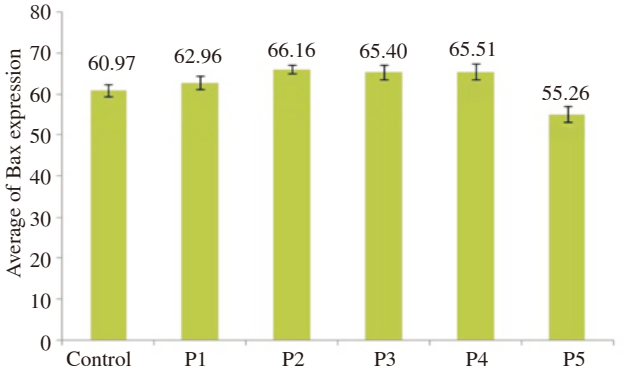

Figure 3. Flowcytometry analysis result of Bax expression.
Bax expression analysis of HeLa cells treated with TPPE showed increasing expression compare to control, with slightly different from two other measurement of apoptosis markers. Non-significant increasing was obtained in P3 and P4, with the highest elevation obtained from P2. P1: TPPE dose 25 μg/mL; P2: TPPE dose 50 μg/mL; P3: TPPE dose 100 μg/mL; P4: TPPE dose 200 μg/mL; P5: TPPE dose 400 μg/mL. One-way ANOVA followed by *post hoc* Tukey test was used with significance $P < 0.05$.

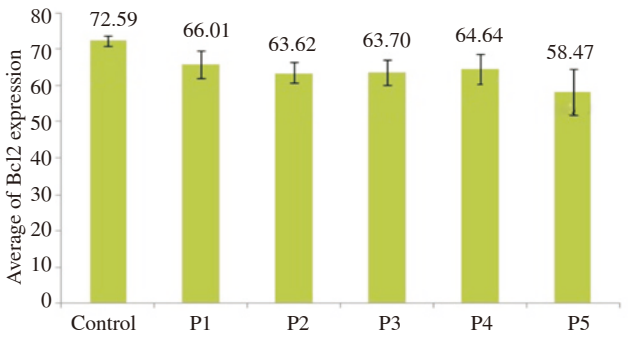

Figure 4. Flowcytometry analysis result of Bcl-2 expression.

Bcl-2 expression analysis of HeLa cells treated with TPPE showed decreasing expression compare to control. Non-significant increasing was obtained in P1 and P3, with the highest elevation obtained from P4. P1: TPPE dose 25 μg/mL; P2: TPPE dose 50 μg/mL; P3: TPPE dose 100 μg/mL; P4: TPPE dose 200 μg/mL; P5: TPPE dose 400 μg/mL; One-way ANOVA followed by *post hoc* Tukey test was used with significance $P < 0.05$.

For observation of DNA repair pathways mechanism, flow cytometry measurement of MDM2, all doses TPPE were showed significant decreasing expression compare to control. Dose 100 μg/mL was obtained the highest reduction with value 58.53. Furthermore, the lowest reduction was resulted from treatment of TPPE at dose of 400 μg/mL with value 71.25. Besides that, p21 was also measured using the same method as an upstream signal of DNA repair pathways. The results showed significant increasing of p21 expression almost for all doses treatment of TPPE, except doses 25 and 400 μg/mL. The highest increasing was obtained from dose 100 μg/mL with value 66.36. This result was similar with the MDM2 measurement. In addition, the lowest elevation of p21 expression was showed from doses 25 μg/mL with value 60.34.

As describe before, we also observed the possibility of TPPE induced apoptosis through intrinsic pathway mechanism. Two proteins expression, Bax and Bcl-2, as an upstream signal of this pathway were also measured. From Bax measurement result, almost all doses treatment showed a significant increasing of expression. Although dose 25 μg/mL also showed an increasing expression of Bax protein, but the increasing was not significant. Interesting result was shown from dose 400 μg/mL, in which this dose showed an opposite effect by decreasing Bax expression compare to control (60.97) with value 55.26. The highest expression was resulted from dose 50 μg/mL with value 66.16. Expression of Bcl-2 was also measured. From the measurement result, all doses were showed significant decreasing of Bcl-2 expression, except doses 25 and 200 μg/mL the decreasing was not significant. The highest and lowest decreasing expressions were obtained from doses 400 μg/mL (58.47) and 25 μg/mL (66.01), respectively. In general this result also showed an opposite effect of TPPE on Bax and Bcl-2.

4. Discussion

Many researches already reported TPPE as an anti-cancer drug, but only few researches that explored about the cellular mechanism effect of this plant parasite using *in vitro* cell culture. In this study we observed a cellular effect of TPPE on culture of HeLa cells. We suggested the inhibition effect of TPPE on culture of HeLa cells could be triggered through several mechanisms, thus in this study we were focusing on DNA repair and apoptosis intrinsic pathway mechanism. Cell cycle checkpoints, DNA repair and recombination are network pathways that maintain the genome integrity and cellular homeostasis in human cells. Dissociation of those mechanism pathways will resulting in uncontrolled cells growth and in turn forming a cancer cells[25].

Expressions of MDM2 and p21 protein were observed to determine the effect of TPPE on culture of HeLa cells that related with DNA repair mechanism pathway. Measurement of MDM expressions were resulted significant decreasing on almost of various TPPE doses treatment. MDM2 that also known as HDM2 in human is a member of RING-finger-containing protein family and mainly plays an important role as E3 ligase[26,27]This protein function was related with controlling and maintenance the p53 localization and/or its level by proteasome-dependent degradation. Suppressing mechanism of MDM2 to p53 function is through binding process of both proteins, thus prevent p53 to interact with basal transcriptional machinery and co-activator such as p300[28-30]. Decreasing of MDM2 expression is related with the reduction of MDM2-p53 complex formation, elevation of p53-inducible gene expression and p53 transcriptional activity, and also apoptosis[31]. Besides that, the increasing activity of p53 also triggering the activation of p21 as one of the downstream effect mechanism. Activation of p21 is closely related with the DNA repair and cell arrest mechanism[32,33]. Our measurement of p21 expression also showed similar result, in which all doses treatment were effected on significant increasing protein expression compare to control, except dose 25 and 400 μg/mL that express an increasing but not significant.

Apoptosis through intrinsic pathways was induced by permeability change of mitochondria outer membrane prior to apoptosome formation, caspase 9 activation and the caspase effectors[34]. Initiation of apoptosis mechanism through intrinsic pathways was regulated by Bcl-2 protein family and caspase[34,35]. Bcl-2 protein family is a protein family with contain of around 20 protein members and related with the mitochondrial outer membrane permeabilization event and thus lead to the releasing of protein from intermembran space. Bcl-2 protein family was grouped based on whether they promote or inhibit apoptosis. Members of this protein that have function as inhibition on apoptosis such as Bcl-2, Bcl-xL, and Bcl-w. Whereas, members that have function to promote apoptosis such as Bax, Bak, and Bok/Mtd. The basic difference between those groups lies on the domain composition, in which member that inhibit apoptosis contains BH4 domain[36].

Our measurement result of Bcl-2 and Bax expressions were in line with the function of each protein that explained above. Expression of Bcl-2 was showed a significant decreasing, vice versa, Bax measurement was resulted a significant increasing. The decreasing of Bax expression compare to control on treatment dose 400 μg/mL might be suggest that higher dose of TPPE could change the function of Bax protein as a pro-apoptosis agent become inhibition apoptosis. It could be possible, since pro-apoptosis members of Bcl-2 protein family might contain weak BH4 domain, a domain that possessed by members with anti-apoptosis function, although the exact mechanism and function about how the domain could affect the function remain unclear[36].

In conclusion, our study was revealed that TPPE treatment on *in vitro* culture of HeLa cells could trigger the DNA repair mechanism and in turn its possible to inhibit the growth process of cancer cells. Furthermore, at least in part, this result was also supported a suggestion that TPPE induce apoptosis through intrinsic pathways

by triggering the mechanism prior to mitochondrial outer membrane permeabilization. Further research is needed to revealed the molecular mechanism effect of TPPE exact, because although there is an increasing or decreasing on related protein expression, those did not stable among various doses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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