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The Effectivity of IC_{50} Test between Green Tea and Curcumin Extracts from Mt. Lawu as an Antioxidant for SOD and MDA Levels in a Cisplatin Rat Model

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Abstract

AIM: This study aims to determine the relationship of the effective dose between Mt. Lawu green tea and Mt. Lawu curcumin as antioxidants based on superoxide dismutase (SOD) and malondialdehyde (MDA) levels.

METHODS: Blood was undertaken through the orbital sinus and allowed to stand until serum was formed. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was used to observe the effective dose of Mt. Lawu green tea and curcumin by quantifying the color change of each sample after incubation. The color change measured the absorbance value through a spectrophotometer. The data were analyzed using a correlation test to measure the effective concentration of Mt. Lawu green tea and Mt. Lawu curcumin toward SOD and MDA as antioxidant parameters in a cisplatin (CN) rat model.

RESULTS: The study results show that the IC_{50} green tea concentration of 0.75 ± 0.16 μg/mL and the curcumin concentration of 5.3 ± 0.4 μg/mL can reduce 50% of DPPH. Based on the graphs, SOD increased and MDA decreased.

CONCLUSION: This study demonstrates that the IC_{50} parameter of Mt. Lawu green tea and curcumin extracts is a good indicator for assigning the SOD and MDA levels in a CN rat model. Extracts derived from Mt. Lawu green tea and curcumin have ideal lowering DPPH, and the dosage has a strong relationship with the increase in SOD and the decrease in MDA.

Introduction

Cisplatin (CN) is a chemotherapeutic agent generally used to treat solid malignancies. However, multiple organ toxicities have limited its usefulness, for instance, in the head and neck, kidneys, liver, and reproductive system. Cellularly, CN promotes replication-related DNA damage and, therefore, apoptosis [1]. The primary cytotoxic mechanism of CN in proliferating cells binds to DNA. Toxic levels of reactive oxygen species and protein dysregulation have resulted in ototoxicity within numerous cellular compartments [2], [3]. CN-induced ototoxicity causes intrinsic apoptotic pathway activation mediated by the change in mitochondrial membrane permeability [1]. Oxidative stress usually exists when the antioxidant level is lower than the free radical level (CN). Antioxidants in our body are assigned a SOD level. Meanwhile, oxidative stress is assigned an MDA level.

An endogen antioxidant level deficiency cannot inhibit free radicals. Thus, our bodies need exogenous antioxidants such as multivitamins, polyphenol flavonoids, and non-flavonoids to prevent oxidative damage. Indonesia is one of the largest biodiverse countries, having several species of native plants that are known for their antioxidant efficacy, such as green tea and curcumin from Mount Lawu, Central Java. The human body benefits from tea polyphenols in preventing oxidative damage by regulating different oxidase and antioxidant enzyme activities. In addition, the expression of specific antioxidant enzymes is also upregulated by tea polyphenols, like SOD, while simultaneously reducing the production of malondialdehyde (MDA) [4], [5]. Superoxide dismutase (SOD) is the most endogenous antioxidant enzyme in our body that provides protection, especially in inhibiting oxidative stress and detaining superoxide anion damage by converting superoxide anion into hydrogen peroxide [6]. MDA is a radical parameter that

reacts with nucleophilic or electrophilic components. It can bind with other biological molecules, such as protein, nucleic acid, and aminophospholipid, so free radical and electrophilic metabolites will decrease. In other words, the MDA level relates to the antioxidant level [7].

This study determines the antioxidant effectivity of green tea and curcumin extract derived from Mt. Lawu by B2P2TOOT in Central Java on the expression of MDA and SOD levels on the 14th day after being induced by CN.

Methods

The method for observing the effective doses for green tea and curcumin extract by the Centre of Research and Development of Medical Plants and Traditional Medicine (B2P2TOOT) was DPPH. The Health Research Ethics Commission (KEPK) of Dr. Moewardi Regional General Hospital, Surakarta (417/IV/HREC/2021), has granted an ethical clearance letter for experimental animal research. Using carbon dioxide, all treated animals were euthanized as scheduled on the 14th day. This research used *Rattus norvegicus* Wistar strain, aged 2–3 months, 150–250 g weight which were treated according to the guidelines for the care and use of experimental animals at the Inter-University Center Laboratory (PAU) Faculty of Medicine, Gadjah Mada University, Yogyakarta. We treated four rats in each group and randomly distributed them into two groups: Group A1 was given Mt. Lawu green tea extract after cisplatin 20 mg/kgBW/iv for 14 days and Group A2 was given Mt. Lawu curcumin extract after cisplatin 20 mg/kgBW/iv for 14 days.

Blood sampling was undertaken through the orbital sinus using the ELISA method. The blood samples were allowed to stand until serum was formed. Then, the serum was collected for measuring MDA and SOD level expression. The antioxidant activity of samples was measured by the DPPH method using a UV–Visible Spectrophotometer. The DPPH method is used because it is simple, fast and requires only a few test samples. This DPPH method was conducted by observing the color change of each sample after being incubated with DPPH [8].

Furthermore, the color change was the absorbance value measured using a spectrophotometer. The IC_{50} data were analyzed using a regression test to identify their median. On the other hand, this study employed a correlation test to determine the relationship between the dose and SOD and MDA as antioxidant parameters in a CN rat model. Pearson's correlation test was used if the data were normally distributed, whereas Spearman's correlation test was used when the data were not normally distributed. The SPSS software version 25 was used in this study.

DPPH method of antioxidant activity testing

A total of 1.0 mL of 0.04 mM DPPH were mixed with the extracts, and methanol was added at a volume of 5.0 mL. The solution was homogenized with a vortex, then allowed to stand in a dark room for 20 min. The absorbance of the solution was then measured at the maximum wavelength, 514 nm.

The blank solution, which consisted of 1.0 mL of 0.04 mM DPPH and methanol, was measured at the maximum wavelength, 514 nm. The concentration series of the green tea extract solution was 1–4 g/mL, and the curcumin extract solution was 1–8 g/mL. The % inhibition was calculated by subtracting the absorbance of the blank by the absorbance of the sample solution, divided by the absorbance of the blank, and multiplied by 100% using the formula below:

$$\textit{The \% Inhibition} = \left\{\frac{(\textit{Absorbance control} - \textit{Absorbance Samples})}{\textit{Absorbance Control}}\right\} \times 100$$

The results of the calculations were then made into a linear regression equation between the percent inhibition versus the sample concentration. The IC_{50} value was calculated based on the linear regression equation obtained by entering the value 50 on the y-axis, and the x value (IC_{50}) was obtained. The IC_{50} value indicates the concentration that can neutralize 50% of radicals (DPPH). The MTT assay is based on the mitochondrial reductase involving the conversion of the yellow, water-soluble tetrazolium dye MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] to the purple-colored, insoluble formazan. Formazan is then solubilized, and the concentration is quantified spectrophotometrically by optical density at 570 nm. This results in a sensitive assay with excellent linearity up to ~10^6 cells per well.

The DPPH-free radical scavenging method is a widely accepted mechanism that allows for evaluating potential antioxidant compounds, extracts, or other biological sources. This method is the simplest way to introduce a DPPH solution to the candidate compound or extract. Then, the absorbance is measured after a given amount of time [9]. The color of DPPH is purple, and it reacts with antioxidants to form non-radical DPPH, which then turns yellow. The radical form of DPPH has a longer chromophore group than the non-radical form, which is why the color changes from purple to yellow. The intensity of the purple color reduction

is measured at the maximum wavelength of DPPH, which is about 514 nm [8].

Results

The effectiveness of the concentration of Mt. Lawu green tea and from Mt. Lawu curcumin toward the IC_{50} value

Based on Figure 1, the results of the IC_{50} assessment of Mt. Lawu green tea extract show that the mean IC_{50} is 0.75 ± 0.16 μg/mL. The following chart shows the IC_{50} of Mt. Lawu green tea extract using the DPPH method.

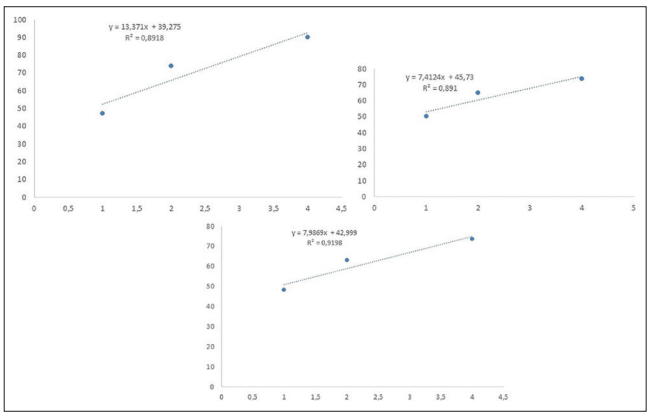

Figure 1: IC_{50} value from Mt. Lawu green tea extract

Based on Figure 2, the average IC_{50} of Mt. Lawu curcumin is 5.3 ± 0.4 μg/mL, indicating that Mt. Lawu green tea is better than Mt. Lawu curcumin. However, both still demonstrate effectiveness as an antioxidant.

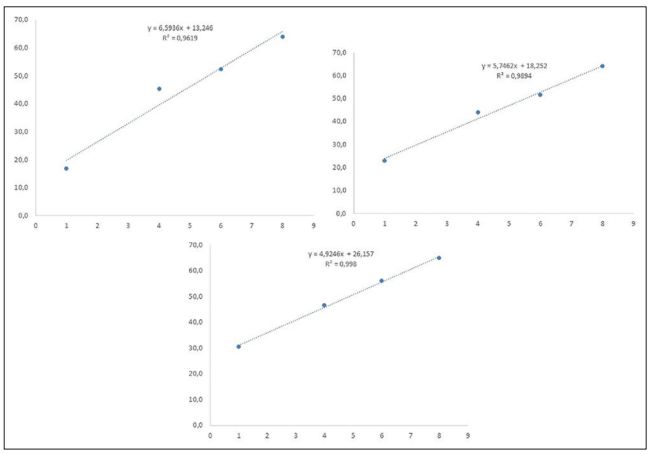

Figure 2: IC_{50} value from Mt. Lawu curcumin extract

The expression of MDA and SOD levels of green tea and curcumin extracts after CN administration

The magnitude of the relationship between the dose of green tea extract and curcumin with MDA and SOD was determined using a correlation test, with the test results in Table 1.

Table 1: A correlation test between antioxidants to MDA and SOD

Description	Correlation coefficient		*p*-value	
	MDA	SOD	MDA	SOD
Green tea extract dosage	−0.995	0.938	<0.001	<0.001
Curcumin extract dosage	−0.986	0.984	<0.001	<0.001

Based on the Table 1, the correlation coefficient value for the relationship between Mt. Lawu green tea and Mt. Lawu curcumin extract dosage and MDA is −0.995 and −0.986, respectively, with p = 0.000 (p < 0.05). Therefore, these are negative correlations. It means that the higher the Mt. Lawu green tea and Mt. Lawu curcumin extract dosage, the lower the MDA level. In contrast, the lower the Mt. Lawu green tea and Mt. Lawu curcumin extract dosage, the higher the MDA.

The correlation test results for the relationship between Mt. Lawu green tea and Mt. Lawu curcumin extract dosages toward SOD show a correlation coefficient of 0.938 and 0.984, respectively, with p = 0.000 (p < 0.05). In other words, there is a positive correlation. This means that the higher the Mt. Lawu green tea and Mt. Lawu curcumin extract dosage, the higher the SOD level. In contrast, the lower the Mt. Lawu green tea and Mt. Lawu curcumin extract dosage, the lower the SOD.

Discussion

CN is widely used to treat tumors, but its use has been limited due to its toxic side effects, including nephrotoxicity, neurotoxicity, ototoxicity, and hepatotoxicity. When the human body cannot produce and eliminate free radicals to reach an equilibrium, it is prone to oxidative stress, which can cause further damage [10]. Signaling pathways enable the transfer of various extracellular stimuli. One possible mechanism is suppressing the antioxidant (SOD) and causing oxidative stress injury (MDA) [6]. As a consequence, we need an answer to overcome these side effects. In recent years, flavonoids found in polyphenols have demonstrated their potential as prophylactic and therapeutic agents in many medical conditions associated with oxidative stress and free radical damage [11], [12]. Green tea and curcumin grown in Mount Lawu, Indonesia, contain polyphenolic compounds. This research utilized extracts from the Centre of Research and Development of Medical Plants and Traditional Medicine (B2P2TOOT) in Tawangmangu, Karanganyar, Central Java. Then, we measured the IC_{50} level to assign the antioxidant level, collaborating with Muhammadiyah University Surakarta. This research is aligned with a previous study by Noha

et al., 2013, investigating the potential role of curcumin-derived antioxidants against CN-induced cytotoxicity and oxidative stress [13], [14].

The graphs in Figures 1 and 2 show that the increase in concentration is directly proportional to the percentage in reduction. This is in accordance with the Lambert-Beer Law, which states that absorbance is directly proportional to the concentration of the sample. Hence, the higher the concentration, the smaller the absorbance and the higher the percentage of antioxidant activity. After being included in the linear equation, the IC_{50} value of 66.08 μg/mL was obtained, which was included in the "strong" category. The IC_{50} value indicates the concentration of soursop leaf infusion (ppm), which can deter the oxidation process by 50%. The smaller the IC_{50} value, the higher the antioxidant activity. The antioxidant group is the appropriate group for preventing CN-related cytotoxicity. Non-enzymatic antioxidants – also known as exogenous antioxidants – play a role in preventive defense systems. Because there is no cytotoxicity treatment, preventive measures become necessary [15]. Examples of available antioxidants to prevent CN cytotoxicity include flavonoid and non-flavonoid polyphenols, tocopherol, α-carotene, turmeric, Vitamin E, Vitamin C, N-acetylcysteine, and lycopene [16]. Tea polyphenols can protect the body from oxidative damage by regulating different oxidase and antioxidant enzyme activities. Moreover, tea polyphenols upregulate the expression of certain antioxidant enzymes, such as SOD, and can reduce malondialdehyde (MDA) production [15].

This finding is aligned with our study results, which intend to compare the potential for the antioxidant effect of Mt. Lawu green tea and Mt. Lawu curcumin. The results suggest that both Mt. Lawu green tea and Mt. Lawu curcumin effectively reduce MDA and increase SOD. Polyphenols produce the antioxidant effect by increasing antioxidant enzyme activity, inhibiting lipid peroxidation, and reducing oxidation through the chelation of metal ions.

Conclusion

This study's findings show that the IC_{50} parameter of Mt. Lawu green tea and curcumin indicates a good indicator for assigning SOD and MDA levels in a CN rat model. The dosage of Mt. Lawu green tea extract and Mt. Lawu curcumin extract has a strong correlation with the increment of SOD and the decrement of MDA. However, further *in vivo* and clinical studies are recommended.

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