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Research Article

The Effect of Combination between Green Tea Extract and Curcumin Extract from Mt. Lawu on BAX, Bcl-2 and Caspase-3 in Cisplatin-Induced Rat Models

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ABSTRACT

Introduction: The study determines effect of Combination between Green Tea and Curcumin Extract from Mount Lawu on BAX, Bcl-2 and Caspase-3 in Cisplatin (Cis)-induced rat models. **Methods:** We treated four rats in each group and randomly distributed them into four groups: group C (–) was the negative control group with no treatment given, group C (+) was the positive control group given Cis only, group A1 was given green tea extract and curcumin extract combination after Cis, and group A2 was given Ginkgo biloba after Cis. Expression levels of BAX, Bcl-2, and Caspase-3 were assessed by ELISA. An ANOVA, a parametric test, was used if the data were normally distributed. If there were significant differences between the three groups regarding BAX, Bcl-2 and Caspase-3, a post hoc test was performed to determine the differences between treatments. **Results:** The results of the study show that combination between green tea and curcumin extract can increase Bcl-2 levels with an average value of 15.42 + 0.76 ng/mL, better than Ginkgo biloba extract with a value of 13.50 + 0.47 ng/mL, reduce BAX and Caspase-3 levels with a value of 6.57 + 0.38 ng/mL and 2.89 + 0.19 ng/mL, better than Ginkgo biloba with a value of 7.34 + 1.06 ng/mL and 3.86 + 0.34 ng/mL. **Conclusion:** This research shows that Combination between Green Tea and Curcumin Extract can increase Bcl-2 levels and reduce BAX and Caspase-3 in Cis rat models after fourteen days of treatment, better than Ginkgo biloba.

Key words: Antioxidant, Antiapoptotic, Ototoxicity.

INTRODUCTION

Cisplatin (Cis-diamminedichloroplatinum; Cis) is a chemotherapy medication widely used for treatment of numerous cancers in human including head and neck, lung, ovarian, bladder, and testicular cancers. Despite demonstrating confident results in treating malignancy cases, some limitations during its clinical use are mainly because it destroys cancer cells and healthy body cells.[1] Expanded histological harm that accompanies Cis administration is diminished in treatment, particularly in combination treatment. Casp-3 and BAX proteins were fundamentally expanded, yet Bcl-2 was somewhat diminished in the Cis group compared with the control. This imbalance can cause toxicity, one of which is ototoxicity. Cisplatin will elevate the regeneration of ROS and induce DNA damage; this process also activates the apoptotic in the cochlea and lead to hearing loss.[2]

Medicinal plants and their principal compounds have been recognised as useful in the carcinogenesis cycle, like initiation, promotion, and progression. In addition, active compounds of natural herbal products play a substantial role in decreasing the harmful effects of chemotherapy medication. Medication combinations that include dietary enhancements and natural products have been relied upon to accomplish similar results as traditional chemotherapeutic medications with diminished unfavourable outcomes.[3] Green tea and curcumin are Indonesian medicinal plants known for their antioxidant efficacy. Meanwhile, Ginkgo biloba is not native to Indonesia, although it decreases Cis-induced ototoxicity. Therefore, from an economic standpoint, the price of these materials is more affordable and easier to obtain. Dietary intercession is a common way to counteract anti-cancer-related toxicity to limit the detrimental impacts of Cis. Natural products or cures with a wide range of therapeutic properties, including anti-carcinogenic, cancer preventive, antimutagenic, and anti-apoptotic agents, have attracted specialists' advantage to balance the effects of Cis. For example, an assortment of complementary and traditional treatments has been displayed to limit the symptoms of anti-cancer drugs.[4] Combination between green tea and curcumin extract is used as an anti-apoptotic agent against the side effects of Cis.[5]

Green tea and curcumin are a phytochemical that consists of flavonoids and non-flavonoids. The flavonoid group comes from green tea, whereas the non-flavonoid comes from curcumin. Alas, its use in medical practice—also as a functional and nutraceutical food component—has been a severe obstacle caused by the rapid degradation and poor oral bioavailability of curcumin. Combinations of curcumin and other bioactive phytochemicals could be the way to resolve this issue, but not curcumin alone. When there is a synergism between them, the dosages of each compound needed to produce any potential biological effect can be lowered through this method.[4] Many synergistic biological effects can be produced from curcumin and green tea combinations. The equimolar mixture of curcumin and green tea extracts can be examined as an effective antioxidant for practical applications, especially

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in polar systems. These combinations of green tea and curcumin, a lipid-soluble antioxidant, displayed apparent synergism in heterogeneous systems (a phosphatidylcholine-based liposome system and oil-in-water emulsion) but antagonistic or additive effects in bulk oil.[6] This study determines the anti-apoptotic effect of combination between green tea and curcumin. Therefore, apoptosis in other organs can be inhibited.

MATERIALS AND METHODS

This research was a randomised, post-test-only, control group, experimental laboratory study investigating combination of Mt. Lawu green tea extract and curcumin extract by the Centre of Research and Development of Medical Plants and Traditional Medicine (B2P2TOOT). This research used adult male and healthy Rattus norvegicus Wistar-strain rats weighing 200–300 grams 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. Based on the provisions of the WHO, we treated four rats in each group and distributed them into four groups: group C (−) was the negative control group with no treatment given, group C (+) was the positive control group given Cis only, group A1 (curcumin extract and green tea extract combination after Cis), and group A2 (Ginkgo biloba after Cis). Groups C (+), A1 and A2 were induced with Cis 20 mg/kg BW/iv for 14 days, while groups A1 were given additional 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract therapy and A2 were given additional 1.44 mg/kg of Ginkgo biloba therapy *via* nasogastric tube (NGT).

The Health Research Ethics Commission has granted this research an ethical clearance letter for experimental animal research. Using carbon dioxide, all treated animals were euthanised on the 14th day. Blood sampling was taken from the orbital sinus and allowed to stand until serum was formed. Then, it was collected to estimate the levels of B-cell lymphoma (Bcl-2), Bcl-2 Associated X-Protein (BAX) and Caspase-3 using the Enzyme-linked immunosorbent assay (ELISA) method.

Preparation method for plants

Extraction is defined as the process of separating and isolating solids or liquids. The process will be complete if the solute is separated from the solvent, for example by distillation / evaporation, using the Soxhlet method.[7] Combination between curcumin and green tea extraction process employed the Soxhlet method. The surface of the extract was expanded by blending it. 95% ethanol solvent was used because the extract produced tends to increase in line with the increase in solvent concentration. The ethanol solvent was heated using a temperature of 60^0 C. The vapor then gets into the condenser through a small pipe and exits in the liquid phase. Then the solvent gets into the sleeve containing the solid. The solvent will wet the sample and be retained in the sleeve until the solvent level in the siphon was equal to the solvent level in the sleeve. Then all the solvent will be pushed back into the boiling flask and so on. Withdrawal of chemical components carried out by means of simplicial powder was placed in a cloning that had been coated with filter paper in such a way, the filter liquid was heated in a round bottom flask so that it evaporates and was condensed by a spherical condenser into molecules of the filter liquid which fall into the cloning to extract the active substance inside simplicial and if the filter liquid had reached the surface of the siphon, all the liquid will fall back into the round bottom flask through the capillary tube until circulation occurs. Complete extraction was indicated when the liquid in the siphon was colorless, there was no visible stain on TLC, or the circulation had reached 20-25 times.[8]

ELISA method to assess the expression of BAX, Bcl-2, and Caspase-3

The kits were taken and incubated for 30 minutes at 370C. The blood sample is placed at room temperature. Samples were added to each plate repeatedly for 3 times, and 50 µl of streptavidin solution was added, followed by incubation at 37°C for 1 hour. Then, the plate was washed with washing-up liquid and dried. Fifty µl sample from each substrate were added for incubation at 37°C for 10 min in the dark, and the reaction was stopped by stop solution. After that, measure the absorbance of the sample. Primary wavelengths were recorded by the spectrometer at 450nm and reference wavelengths are at 610 nm to 650 nm.[9]

The ANOVA parametric test is used if the data is normally distributed established from the results of the data normality test. Between these three treatments on BAX, Bcl-2 and Caspase-3, a post hoc test will establish the differences if the ANOVA test produces a significant difference.

RESULTS

The effect of combination between green tea extract and curcumin extract with ginkgo biloba on Bcl-2, BAX and Caspase-3 Levels in Cis-induced rat models

The Bcl-2 level in the control group (+) treated with Cis was very low, and the highest Bcl-2 level was in the A1 group with a value of 15.42 + 0.76 ng/mL. The Bcl-2 levels between various antioxidant preparation treatments were significantly different. They show that 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract increase Bcl-2 levels more than 1.44 mg/kg of Ginkgo biloba.

The BAX level in the control group (+) treated with Cis is very high, and the lowest BAX level is in the A1 group (Cis 20 mg/kg → 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract with a value of 6.57 + 0.38 ng/mL. There is a significant difference in BAX levels between various groups. It shows that the combination of 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract decreases BAX levels better than 1.44 mg/kg of Ginkgo biloba.

The Caspase-3 level in the control group (+) treated with Cis was very high, and the lowest Caspase-3 level was in the A1 group (Cis 20 mg/kg → 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract) with a value of 2.89 + 0.19 ng/mL. There were significant differences with various Caspase-3 treatments. The combination of 75 mg/kg of green tea extract and 100 mg/kg of curcumin extract decreases Caspase-3 levels more than 1.44 mg/kg of Ginkgo biloba. A further test was performed, namely the post hoc LSD test, with the results shown in Table 2.

The comparison of Bcl-2 in the C (−) group shows a significant difference between the A2 and C (+) groups ($p < 0.05$), but not with the Bcl-2 of the A1 group ($p = 0.088$). The comparison of Bcl-2 in the C (+) group shows a significant difference between the A1 and A2 groups (p

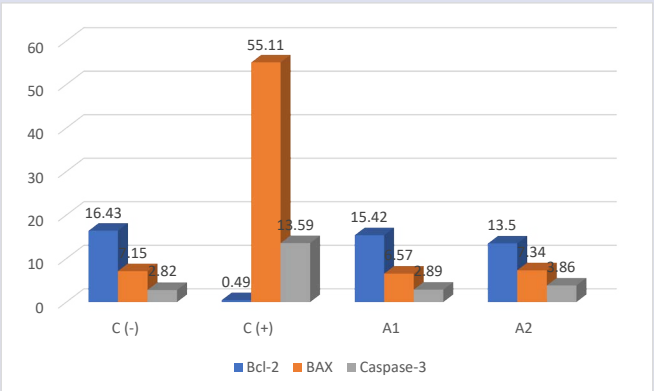

Figure 1: Comparison between the results of Bcl-2, BAX, and Caspase-3 in the four groups.

Table 1: ANOVA test (Simultaneous difference test) of the variables Bcl-2, BAX and Caspase-3 after fourteen days of treatment.

Group	N	Bcl-2 (ng/mL) (Mean ± SD)	BAX (Mean ± SD)	Caspase-3 (Mean ± SD)
C (−)	5	16.43 ± 0.52	7.15 ± 0.19	2.82 ± 0.27
C (+)	5	0.49 ± 0.30	55.11 ± 1.29	13.59 ± 0.33
A1	5	15.42 ± 0.76	6.57 ± 0.38	2.89 ± 0.19
A2	5	13.50 ± 0.47	7.34 ± 1.06	3.86 ± 0.34
p-value		<0.001	<0.001*	<0.001*

C (-) = Normal
C (+) = Cis 20 mg/kg
A1 = Cis 20 mg/kg → 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract
A2 = Cis 20 mg/kg → 1.44 mg/kg of Ginkgo biloba
One Way ANOVA Test;
* Significant p < 0.05

Table 2: Post Hoc test (Partial difference test) of variables BAX, Bcl-2 and Casp-3 after fourteen days of treatment.

Group	BAX (ng/mL) (p-value)	Bcl-2 (ng/mL) (p-value)	CASP-3 (ng/mL) (p-value)
C (−) *vs* C (+)	<0.001*	<0.001*	<0.001*
C (−) *vs* A1	0.265	0.088	0.754
C (−) *vs* A2	0.703	<0.001*	<0.001*
C (+) *vs* A1	<0.001*	<0.001*	<0.001*
C (+) *vs* A2	<0.001*	<0.001*	<0.001*
A1 *vs* A2	0,141	0.003*	<0.001*

C (-) = Normal
C (+) = Cis 20 mg/kg
A1 = Cis 20 mg/kg → 75 mg/kg of green tea extract + 100 mg/kg of curcumin extract
A2 = Cis 20 mg/kg → 1.44 mg/kg of Ginkgo biloba
One Way ANOVA Test;
* Significant p < 0.05

< 0.05). The comparison of Bcl-2 in the A1 group shows a significant difference with the A2 group (p < 0.05).

The comparison of BAX in the C (−) group shows a significant difference with the C (+) group (p < 0.05), but not with the BAX in the A1 (p = 0.265) and A2 groups (p = 0.703). The comparison of BAX in the C (+) group shows a significant difference between the A1 and A2 groups (p < 0.05). The comparison of BAX in the A1 group does not show a significant difference with the A2 group (p = 0.141).

The comparison of Caspase-3 in the C (−) group shows a significant difference with the C (+) and A2 groups (p < 0.05), but not with the Caspase-3 in the A1 group (p = 0.754). The comparison of Caspase-3 in the C (+) group shows a significant difference with the groups A1 and A2 (p < 0.05). The comparison of Caspase-3 in the A1 group shows a significant difference with the A2 group (p < 0.05).

The results presented in table 2 indicate that green tea extract combine with curcumin extract demonstrates promising therapeutic results compared with Ginkgo biloba. This result is proven by the values of Bcl-2, BAX and Casp-3 that are not significantly different from those of untreated group. Hence, it can be concluded that the therapeutic results shown in the green tea extract combine with curcumin extract group produced a clinical outcome similar to the untreated group. Meanwhile, we can see that Ginkgo biloba is not significantly different from untreated group in the BAX parameter.

The results of the study show that combination between green tea extract and curcumin extract can increase Bcl-2 levels as an anti-apoptosis and reduce BAX and Caspase-3 levels as a pro-apoptosis better than Ginkgo biloba extract.

DISCUSSION

Cis is a powerful antineoplastic agent used in cancer therapy and has been the primary agent in germ cell cancer treatment since the 1970s. In 1978, the U.S. Food and Drug Administration accepted its utilisation. From that point onward, it has become one of the most successful anti-cancer medications for many patients with solid tumours. In any case, the utilisation of Cis is disallowed because of its harmful side effects.[10] It has become crucial to reduce the side effects of Cis, which ultimately restricts its use. Cis toxicity induces mitochondrial membrane permeability changes that mediate the activation of the intrinsic apoptotic pathway.[11] So, we decided to use combination between green tea and curcumin extract on Cisplatin-Induced Rat Models and at the same time asses post treatment apoptosis regulator levels.

This study evaluated the expression of Bcl-2, BAX and Caspase-3 from the intrinsic apoptotic pathway. Anti-cancer inhibition *via* the induction of apoptosis is an essential effect of natural/active compounds from medicinal plants. Antioxidant, anti-carcinogenic and anti-apoptosis effects are included in the broad range of medicinal properties from natural remedies or products. Interfering with the side effects of Cis has been the focus of researchers. Various complementary and traditional therapies have minimised the side effects of anti-cancer drugs.[12]

The antioxidant activities of green tea extracts and their main bioactive compounds, flavan-3-ols, are very well known. The chemo-preventive effects of an active compound in green tea (Epigallocatechin-3-gallate) are used to kill cancer cells. It enhances anti-cancerous activity and reduces toxicities through its combination with chemotherapeutic drugs. Epigallocatechin-3-gallate (EGCG) is an abundant polyphenol in green tea extract, which possesses antioxidant, anti-inflammatory and antitumorigenic properties. EGCG effectively prevents apoptosis and the destruction of hair cell arrays. Also, EGCG is compelling in neutralising ototoxicity by suppressing Caspase-1 initiation and NF-kB. In a study by Borse *et al.*, the authors showed that EGCG is a prototypic agent exhibiting properties of an effective otoprotective agent.[13] The synergistic effect of EGCG with chemo-preventive agents

has been demonstrated to promote anti-cancerous activity and decrease toxicity levels.[14]

The increase in apoptotic proteins (Casp-3, BAX) and anti-apoptotic Bcl-2 protein expression against Cis-induced apoptosis are the protective qualities of curcumin.[15] Curcumin is a flavonoid antioxidant that contains antioxidant, anti-inflammatory, antibacterial, anti-allergic and anti-apoptotic qualities. Regarding carcinogenesis and toxicity, flavonoids interfere with various signal transduction pathways. Therefore, they not only increase apoptosis but also restrict angiogenesis, metastasis and proliferation.[16] Curcumin-combination treatment was shown to be safe and tolerable in clinical trials of breast cancers, chronic myeloid leukaemia, colorectal cancer, pancreatic cancer and prostate cancer. In addition, patients experienced decreased toxicity effects with curcumin-combination therapy and improved quality of life.[3]

A study by Zang *et al.*, highlighting the number of polyphenols possessed by green tea, a natural antioxidant. Compared with vitamins C and E, green tea, which contains polyphenol, has been reported to display free radical scavenging activity many times higher.[17] In addition, study Molecular analysis by Zhao *et al.* demonstrated that curcumin treatment significantly increased Bcl-2 expression and decreased BAX, increasing the ratio of Bcl-2/BAX.[16] In a study by Gevrek and Erdemir, Caspase-3 and BAX apoptotic protein pathways were achieved by Cis-induced testicular apoptosis and were detected immunohistochemically.[18] Possible cell death decreased before the toxic effects of Cis occurred. This is when antioxidant molecules, like curcumin, are given individually or especially in combination.15 In a study by Haryuna, the results showed that curcumin could reduce the apoptotic index of fibroblasts in the lateral wall of the cochlea in rats.[19]

CONCLUSION

This finding is aligned with our study results that green tea and curcumin combination extract displays a positive impact on increasing Bcl-2 levels and reduce BAX and Caspase-3 in Cis rat models after fourteen days of treatment, better than Ginkgo biloba. Combination between green tea and curcumin has shown synergistic effect to restrict the development of cancer cell lines. In addition, green tea and curcumin combination extract can reduce the side effects of cisplatin as a chemotherapeutic agent. However, further *in vivo* and clinical studies are recommended.

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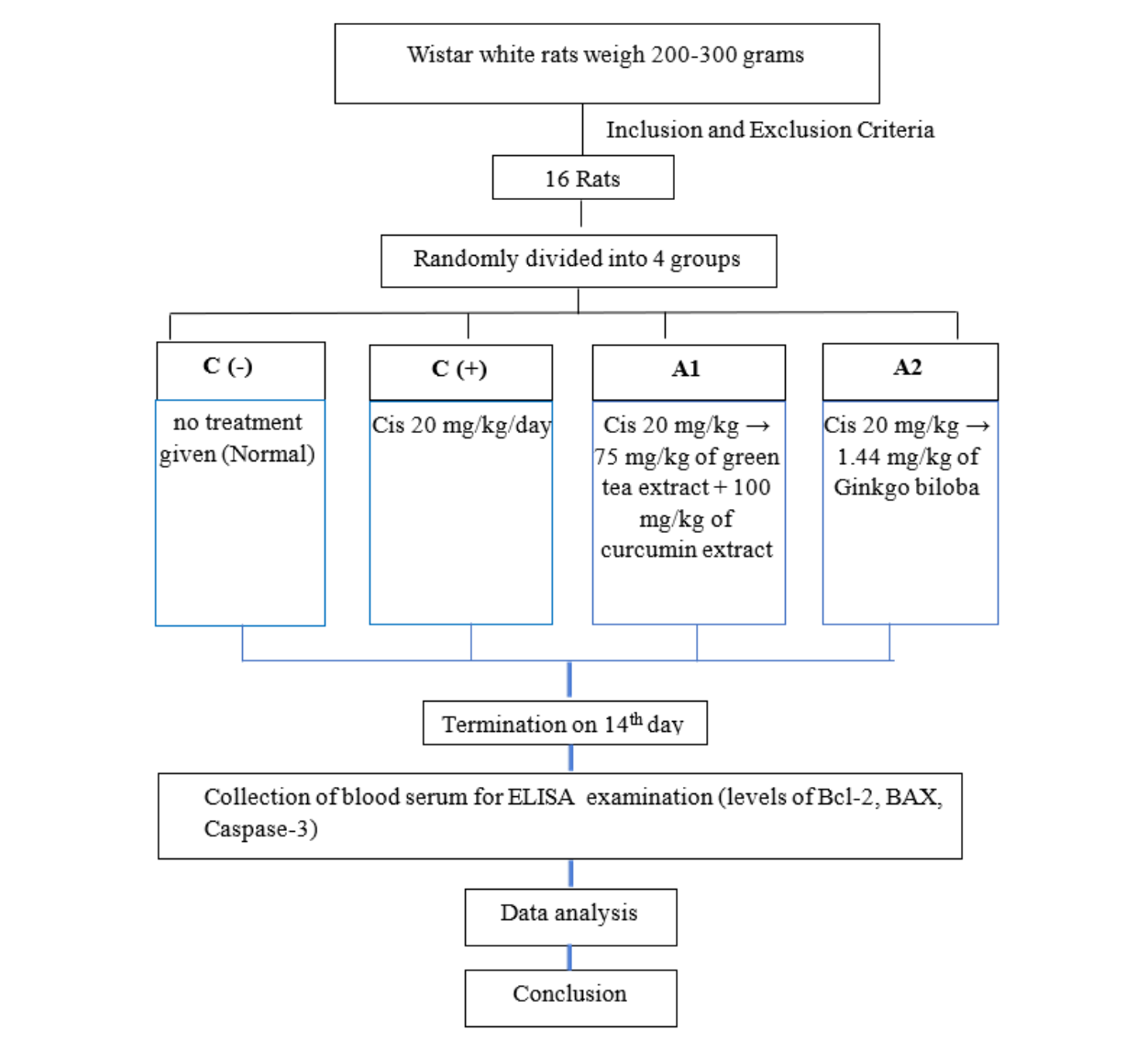
GRAPHICAL ABSTRACT



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