INTRODUCTION

Medicinal plants and herbs are essential to developing Indonesia’s health service system.1 As a landlocked country right on the equator, Indonesia has a wide variety of medicinal plants with potential as immunomodulators and antioxidants.2 Curcuma xanthorrhiza (CX) and black cumin (BC) have been utilized extensively in Indonesia. The primary components of Curcuma xanthorrhiza and black cumin (CXBC) formulations are BC oil and CX extract. This study aimed to assess the influence of CXBC active compounds (polyphenols, flavonoids, and thymoquinone) on IFN-γ expression in HTB-183 cells. This study aimed to determine the levels of vitamins and minerals in CXBC preparation and the potential for immunomodulatory activities.

METHODS

Introduction: According to empirical evidence, Curcuma xanthorrhiza (CX) and black cumin (BC) have been utilized extensively in Indonesia. The primary components of Curcuma xanthorrhiza and black cumin (CXBC) formulations are BC oil and CX extract. This study aimed to assess the influence of CXBC active compounds (polyphenols, flavonoids, and thymoquinone) on IFN-γ expression in HTB-183 cells. This study aimed to determine the levels of vitamins and minerals in CXBC preparation and the potential for immunomodulatory activities.

Results: The results demonstrated that the CXBC supplement included vitamins (A, C, and E) and minerals (potassium, calcium). CXBC preparations boosted HTB-183 cell IFN-γ expression.

Conclusion: The study’s findings indicate that the CXBC formulation contains a high concentration of vitamins and minerals. CXBC formulations augment IFN-γ expression.

Keywords: Black cumin seeds, curcuma xanthorrhiza, herbal-immunomodulator, IFN-γ.

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ABSTRACT

The black cumin seeds oil with Curcuma xanthorrhiza extract preparation increases IFN-γ expression

Titiek Hidayati1*, Sunny Sun2, Akrom3,4

ABSTRACT

Introduction: According to empirical evidence, Curcuma xanthorrhiza (CX) and black cumin (BC) have been utilized extensively in Indonesia. The primary components of Curcuma xanthorrhiza and black cumin (CXBC) formulations are BC oil and CX extract. This study aimed to assess the influence of CXBC active compounds (polyphenols, flavonoids, and thymoquinone) on IFN-γ expression in HTB-183 cells. This study aimed to determine the levels of vitamins and minerals in CXBC preparation and the potential for immunomodulatory activities.

Methods: We engaged in experimental laboratory research. Observing the expression of interferon-gamma (IFN-γ) was used to evaluate the immunomodulatory activities of the HTB-183 human large lung cancer cell line.

Results: The results demonstrated that the CXBC supplement included vitamins (A, C, and E) and minerals (potassium, calcium). CXBC preparations boosted HTB-183 cell IFN-γ expression.

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INTRODUCTION

Medicinal plants and herbs are essential to developing Indonesia’s health service system.1 As a landlocked country right on the equator, Indonesia has a wide variety of medicinal plants with potential as immunomodulators and antioxidants.2 Curcuma xanthorrhiza (CX) and black cumin seed has been used empirically as an immunomodulator and antioxidant by Indonesians.3,4 CX has traditionally been used as an appetite stimulant in children with feeding difficulties and has been used empirically as an immune enhancer, anti-inflammatory, and antioxidant.5 Xanthorrhizol, curcumin, and curcuminoid of CX have been shown to have antioxidant activity.3 Xanthorrhizol has been shown to inhibit lipid peroxidation in rat brain homomers, glutamate-induced neurotoxicity, and generate reactive oxygen species (ROS) superfamily.6

Like CX, black cumin (BC) is a medicinal plant widely used by the community. In addition to being used as a spice in cooking, BC extracts and oils have been shown to have anti-inflammatory,7 antibacterial,8 antiviral,9 immunomodulatory,10 antioxidant effects, and chemopreventive activities.11 It BC has been shown in vivo to increase T cell counts,12 levels of interferon-γ (IFN-γ),13 and the phagocytic activity of macrophages.10 Thymoquinone, the main component of BC, can inhibit cyclooxygenase and lipoxygenase enzymes in arachidonic metabolism.14 have anti-inflammatory, anti-scavenging, antioxidant, anti-infective and antihistamine effects.

Empirically, CX and BC are used as immunomodulators and antioxidants both as prophylaxis and as adjunctive therapy.15 Antioxidants and immunomodulatory agents are thought to prevent infection infection.1 Tumor necrosis factor alpha (TNF-α) is required for the development of rapid antiviral immune responses.16 TNF-α increases the phagocytic activity of macrophages and neutrophils, to eliminate virus that survives physical trap.17 Immunomodulators are also thought to inhibit the increase in plasma concentrations of cytokine family interleukins (IL-2, IL-6, IL-7), and IL-10.18 Antioxidants reduce tissue damage caused by oxidative stress.19

The empirical combination of two or more medicinal plants has become a tradition in the use of medicinal plants.20 BC extract is considered to contain active polyphenols, flavonoids and nutrients with potential activities as antioxidant and immunomodulatory. CXBC preparations have been developed as immunomodulators and antioxidants. The CXBC preparation is said to contain micro and macronutrients that act as antioxidants and immunomodulators. This study aimed to determine vitamin and mineral levels during CXBC preparation and potential for immunomodulatory activities.

METHODS

Study Design

This study was an experimental laboratory study. We used several methods to achieve the research objectives: to determine the...
levels of flavonoids, polyphenols and nutrients of the CXBC preparation and its potential activity as an antioxidant and immunomodulator. We used the CXBC composition as the main test material. The preparation CXBC is provided by the field of traditional medicine (with permission of the Food and Drug Administration of the Republic of Indonesia) The protocol has been reviewed and approved by the health research ethics committee, Universitas Muhammadiyah Yogyakarta medical and health science faculty, 078/EC-KEPK FKIK UMY/III/2021.

Materials and Procedures

We used UV-Vis spectroscopy (Shimadzu 1240), purified quercetin and 10% aluminum chloride (Sigma Aldrich) to determine the flavonoid levels. Folin-Ciocalteu reagent, UV-Vis spectrophotometer (Shimadzu 1240), gallic acid and Na2CO3 p.a to determine polyphenol content. Purified thymoquinone and densitometry were used to determine thymoquinone levels. We used an automated chemical analyzer (Hitachi 7600-110) to determine the vitamin and mineral levels in the CXBC preparation. Fluorescein isothiocyanate IFN-γ (FITC) mAb to examine potential immunomodulatory activities.

The potential immunomodulatory activity was assayed on HTB-183 (HTB-183 or NCI-H661) cells of human lung carcinoma (Homo sapiens). Cells were cultured in DMEM (Dulbecco’s Modified Eagle Medium) containing 10 μl bovine serum with 1% penicillin-streptomycin, then incubated at 37°C in a CO incubator containing 5% CO. The cell growth status was checked regularly, the inoculum was added according to the cell growth status and cell migration was performed when the cell density reached 90%. Each trial was conducted separately and three parallel trials were set up. Examination of the potential activity of the CXBC preparation as an immunomodulator was performed by fluorescence-activated cell sorting (FACS) cytometry by observing the expression of IFN-γ in HTB-183 cells.

Data Analysis

We performed univariate analysis to obtain data on vitamin and mineral content in CXBC preparation. We performed a bivariate analysis with one-way ANOVA to determine the difference in mean IFN-γ expression as a function of CXBC preparation concentration in HTB-183 cells.

RESULTS

Examination of Vitamins and Minerals of CXBC Preparations

The results of the examination of total flavonoid levels, total polyphenols, thymoquinone levels, and nutrient content levels of CXBC preparations are presented in Table 1.

Potential Activity of CXBC Preparations as Immunomodulators by Increasing IFN-γ Expression

Figure 1 illustrates how the production of IFN-γ on HTB-183 cells was used to test the CXBC preparation’s potential immunomodulatory function. Influence of the CXBC preparation on IFN-γ growth

Three concentration levels of IFN-γ expression were seen in HTB-183 cells: IC50 (54.78 ppm), 1/2 IC50 (27.39 ppm), and 14 IC50 (13.52 ppm).

Table 1. Content of micro and macronutrients in CXBC preparations

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Result</th>
<th>Content of nutritional value per 5 ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polyphenol</td>
<td>32.00 (mg/ml)</td>
<td>160.35 mg</td>
</tr>
<tr>
<td>Flavonoid</td>
<td>47.87 mg/mL</td>
<td>239.29 mg</td>
</tr>
<tr>
<td>Thymoquinone</td>
<td>4.03 (%)</td>
<td>200.04 mg</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.36(%)</td>
<td>18 mg</td>
</tr>
<tr>
<td>Potassium</td>
<td>0.20(%)</td>
<td>10 mg</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>34789.32(mcg/100g)</td>
<td>1.74 mg</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>265,851(mcg/100g)</td>
<td>13.29 mcg</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>14879,551(mcg/100g)</td>
<td>743.95 mcg</td>
</tr>
</tbody>
</table>

Figure 1. Boxplot image of IFN-γ expression examination in HTB-183 cells after induction with CXBC preparations with concentrations according to IC50, IC50, 1/4IC50, and solvent as a control. Note: * = p<0.05 to standard control group.
The results indicated that CXBC preparations at doses corresponding to stimulated IFN-IC50 expression. IFN-γ expression was more significant in the treatment group than in the solvent control group. The IC50 concentration group had the highest IFN-γ expression. IFN-γ is one of the proinflammatory cytokines. By inhibiting inflammatory cytokines, antigen-presenting cells (APCs), and other functions, IFN-γ exerts potent inhibitory effects on myeloid cells.16,18,25

**DISCUSSION**

**Examination of Vitamins and Minerals of CXBC Preparations**

CXBC supplements contain the expected micro and macro nutrients. This herbal immunomodulator contains high levels of vitamins A, C, and E. In addition to vitamins, it also contains calcium and potassium, two minerals essential for a child’s growth and development. Vitamins A, C, and E are antioxidants and immunomodulators. Vitamins A and E are oil soluble, while vitamin C is water soluble. The CXBC formulation is one of the herbal supplements that contains enough vitamins and minerals to help children for growth and development and patients in need of health restoration.

The results of this study follow the latest trends in the use of medicinal plants.1 Phenolics and flavonoids are major antioxidant components in CX extracts and BC seed oil.21,22 Phytotherapy, “Jamu” or traditional Javanese medicine, Traditional Chinese medicine, and Ayurveda (Hinduism) are holistic, utilizing a combination of medicinal plants as opposed to a single herb.23 The clinical significance of the synergistic effect of the multicomponent herbal mixture in patients with chronic diseases such as diabetes mellitus, cancer, hypertension, and many infectious diseases (SARS COV-2, tuberculosis, acquired immune deficiency virus (AIDS), and malaria) has been established.24

**Potential Activity of CXBC Preparations as Immunomodulators by Increasing IFN-γ Expression**

The research data showed that the CXBC preparation contained 4% thymoquinone. Thymoquinone, an active compound of BC, has been shown to increase the phagocytic activity and secretion of TNF-α and IFN-γ by macrophages via Toll-like receptor-4 (TLR-4). Thymoquinone has also been shown to increase the proliferation and differentiation of CD4Th into Th1 and Th2. Decreased expression of IL-10 is associated with an increase in the number of Th1, which produces proinflammatory cytokines, thereby inhibiting Th2 activity in producing IL-10.25,26 The antioxidant activity of thymoquinone was demonstrated through activation of the aryl hydrocarbon receptor (AhR), activation of the transcription factor Nrf-2 and increased production of glutathione-S-transferase.22,27 The thymoquinone level in this study was higher than the thymoquinone level in the black cumin seed oil (BCSO) from the previous study (2.7%).28

The curcuminoid family contains the active ingredient curcumin. Similar to turmeric, CX, and other Zingiberaceae, curcuminoid chemicals are polyphenols with a yellow color. The biological effects of the substance curcumin include anti-dyslipidemia, antioxidant, anti-inflammatory, anti-viral, and anti-fungal properties,29 cancer chemoprevention, and hepatoprotective. Curcumin, unlike thymoquinone, is an antagonist of TLR-4.29

CX also contains xanthorrhizol (XNT). Previous research indicated that XNT decreased the serum levels of free fatty acid and triglyceride in obese mice produced by a high-fat diet (HFD). It has also been demonstrated to benefit cardiovascular health by anti-hyperglycemic, vasorelaxation, and LDL oxidation inhibitory actions.30 The structural alterations of polyphenols are responsible for the loss of antioxidant capacity, which is proportional to the proportion of free phenols to glycosides, with iron-phenol chelates and phenolic acids interacting with other molecules in the food matrix.8 Previous research indicates that BCSO and C. Xanthorrhiza can minimize oxidative stress and immunomodulator activity in vitro and in vivo.31

**CONCLUSION**

CXBC preparations increase IFN-γ expression. The CXBC preparation contains the expected micro and macronutrients and a high level of vitamins and minerals. This herbal immunomodulator contains high vitamins A, C, and E levels. Further studies are needed to support these findings.

**FUNDING**

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**CONFLICT OF INTEREST**

No potential conflict of interest relevant to this article was reported.

**AUTHOR CONTRIBUTION**

TH: concept, data provision, data analysis, data and result in interpretation, writing and discussion, SS: discussion, AA: data and result in interpretation n. All authors contributed to the article and approved the submitted version.

**ETHICAL CONSIDERATION**

The Health Research Ethics Committee reviewed the research protocol of the Faculty of Medicine and Health Sciences, University of Muhammadiyah Yogyakarta. The research protocol has been declared ethically appropriate with an ethical certificate no. 166/EP-FKIK-UMY/VI/2019 by the ethics committee.

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