Effect of Andaliman (Zanthoxylum Acanthopodium) Fruit Extract on Anticancer Activity of HEPG2 Liver Cancer Cell Lines

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Abstract

BACKGROUND: Hepatocellular carcinoma (HCC) or hepatoma is the most common primary liver cancer and causes many deaths. Andaliman extract is an antioxidant against several cancer cells such as breast cancer cells and cervical cancer cells.

AIM: The aim of this study was to investigate the effect of andaliman fruit extract (Zanthoxylum acahantopodium) on the anticancer activity of HepG2 liver cancer cell lines.

METHODS: This research is an experimental study, exploratory laboratory to prove andaliman fruit extract to inhibit proliferation rate, inhibit growth, and triggers apoptosis of HepG2 liver cancer cell lines in vitro. HepG2 liver cancer cell lines were cultured on a suitable and controlled medium to grow. The n-hexane extract, ethyl acetate extract, and ethanol extract of andaliman fruit were dissolved in dimethyl sulfide (DMSO) solution, vortexed, and made up with to obtain a test solution with a concentration of 500 ug/mL, 250 ug/mL, 125 ug/mL, 62.5 ug/mL, and 31.2 ug/mL. The cytotoxicity test results of andaliman fruit against HepG2 cells were carried out using the MTT assay.

RESULTS: In this study, we found that the IC50 of andaliman fruit extract was 122,656 mg/mL.

CONCLUSIONS: The results show that there is a cytotoxic activity from the andaliman extract on HepG2 liver cancer cell. Our further study is to assess and compare the anticancer activity from the andaliman extract and doxorubicin on HepG2 liver cancer cell.

Introduction

Hepatocellular carcinoma (HCC) or hepatoma is primary liver cancer. HCC is an abnormal growth of liver cells which are characterized by an increase in the number of cells in the liver that has the ability to divide/mitosis accompanied by changes in liver cells that become malignant [1]. Hepatoma or liver cancer is a malignant primary liver tumor that is frequently found in Indonesia. HCC is a malignant tumor with a very poor prognosis, in which patients generally die within 2–3 months after the diagnosis is made [2], [3]. When cancer cells metastasize, the morbidity and mortality rates will increase, due to the cancer cells being less responsive to available chemotherapy. Therefore, alternative therapies are needed to provide better results in increasing patient safety. Several compounds isolated from plants have been used as anticancer agents. Phenolic compounds such as flavonoids have various pharmacological activities such as antioxidant, antimutagenic, anti-inflammatory, and anticancer activities [4]. Andaliman (Zanthoxylum acanthopodium) is a plant that is often used as a spice in traditional Batak cuisine, North Sumatra. Andaliman fruit extract is known to contain alkaloids, flavonoids, glycosides, steroids, and terpenoids. The n-hexane fraction of andaliman also contains bioactive compounds and is effective as an anticancer against T47D cell line through cell cycle inhibition, apoptosis induction, and downregulation of D1 cyclin expression [5]. This study aimed to determine the effect of andaliman fruit extract (Zanthoxylum acanthopodium) on the anticancer activity of HepG2 liver cancer cell lines.

Hepatocellular carcinoma (HCC)

Hepatocellular carcinoma (HCC) is a cancer that arises from the liver. It is also known as primary liver cancer or hepatoma [1]. HCC or hepatoma is the most common primary liver cancer found than other liver tumors such as malignant lymphoma, fibrosarcoma, and hemangioendothelioma [6]. In the United States, approximately 80%–90% of primary liver malignant tumors are hepatomas. On the other hand, in Africa and Asia, hepatoma is the most common carcinoma with an incidence rate of 100/100,000 population [7]. In Indonesia, the incidence of liver cancer in men occupies the second position with a mortality rate of 12.4 per 100,000 population [8]. HCC is associated with several risk factors such as hepatitis B and C virus infection, liver
cirrhosis, alcohol, and aflatoxins [9]. Several risk factors for the occurrence of HCC differ in different regions. In Indonesia and in other Asian countries, the highest cause of HCC is associated with chronic hepatitis B infection, while, in Europe, the cause of HCC is mostly associated with chronic hepatitis C infection [10]. Some of these risk factors are also associated with the occurrence of liver cirrhosis with frequent clinical symptoms disguised and in accordance with HCC [9], [11], [12].

The criteria for the diagnosis of HCC according to the Indonesian Association for the Study of the Liver, namely [13]:

1. An enlarged liver with nodes with/without arterial murmurs on ultrasound examination.
2. AFP (Alpha-fetoprotein) increased by more than 500 mg per ml.
3. Ultrasonography (USG), nuclear medicine, computed tomography scan (CT Scan), magnetic resonance imaging (MRI), angiography, or positron emission tomography (PET) which shows the presence of HCC.
4. Peritoneoscopy and biopsy revealed the presence of HCC.
5. The results of the biopsy or fine needle biopsy aspiration show HCC.

Diagnosis was made when there are two or more from the five criteria or only one, namely, fourth or fifth criteria. The choice of liver cancer therapy is very dependent on the results of radiological examinations in the form of cancer size, location of cancer, number of lesions, presence or absence of capsules, or cancer, which is evenly distributed throughout the liver, as well as the presence or absence of metastases to other parts in the patient’s body or whether there is a tumor thrombus in the portal vein and whether liver cirrhosis is present. The treatment steps are divided into three stages; liver surgery combined with radiological; non-surgical procedures; and liver transplantation. Surgery is performed on early-stage cancer followed by radiological measures in the form of Transarterial Embolization (TAE) and Transarterial Chemoembolization (TACE) to suppress the growth of cancer cells. Non-surgical measures are usually performed in patients with advanced liver cancer. This action is usually done with radiology or chemotherapy. Liver transplantation is usually performed on HCC patients who already have liver cirrhosis [2], [3].

**HepG2 cell culture**

HepG2 (ATCC® HB-8065™) is a well differentiated cell culture of hepatocellular carcinoma from a 15-year-old Caucasian male. Base medium for this cell line is ATCC-formulated Eagle’s Minimum Essential Medium, Catalog No. 30-2003. To make a complete growth medium, fetal bovine serum was added to a final concentration of 10% [14].

**Andaliman fruits**

Andaliman plant (Zanthoxylum acanthopodium) is a type of shrub that is well known in North Sumatra, because it is a spice that is often used in traditional Batak cuisine [15]. The spicy taste vibrates the taste buds and causes the tongue to feel numb [4]. Apart from being used as a cooking spice, andaliman fruit is also used as an aromatic ingredient, tonic, and to treat dysentery, while India uses it for paralysis and skin diseases such as abscesses and leprosy [16]. Local residents of Darjeeling Himalaya, South Bengal, India also use andaliman fruit (Zanthoxylum acanthopodium DC) as a dietary supplement, and it is also used to treat gastritis [17]. Furthermore, research using andaliman ethanol extract in the form of a peel-off gel facial mask on 12 women aged 20–30 years concluded that andaliman ethanol extract can be used as an effective anti-aging and anti-acne [18]. Figure 1 shows andaliman fruits.

**Phytochemical activity**

The previous research stated that Andaliman fruit has antioxidant and antimicrobial activity. The phytochemical test for the content of andaliman fruit was positive for alkaloids, steroids, and saponins as well as flavonoids [6]. The results of phytochemical screening of extracts of water, methanol, ethyl acetate and hexane from andaliman fruit contain alkaloids (except for aqueous extracts), flavonoids, glycosides, saponins, tannins (except in hexane extract), triterpenes/steroids (only in methanol and hexane extracts), and anthraquinone glycosides (only in methanol extracts), while the dominant chemical compound in the ethanol extract of andaliman fruit is abscisic acid which is a terpenoid group [15].

**Methods**

This research is an experimental study, exploratory laboratory to prove andaliman fruit extract (Zanthoxylum acanthopodium) inhibits proliferation rate, inhibits growth, and triggers apoptosis of HepG2 liver cancer cell lines in vitro. All samples were treated in parallel using post-test-only control group design. This research was conducted at H. Adam Malik Hospital, Pharmacy Laboratory, University of North Sumatra, Department of Biology, Faculty of Mathematics and Natural Sciences, after obtaining approval from the Health Research Ethics Commission of USU Medical Faculty/H. Adam Malik Hospital Medan. This research was conducted in 2020–2021. The research sample was HepG2 liver cancer cell lines obtained from the Laboratory of the Department of Parasitology, Faculty of Medicine, University of North Sumatra. This study used an
in vitro study, in which HepG2 liver cancer cell lines were cultured on a suitable and controlled medium to grow these cells.

The choice of the in vitro method was due to the difficulty of conducting similar studies in vivo. HepG2 liver cancer cell line is a liver cancer cell line derived from C57BL/6J mice. The number of samples was determined using the rule of thumb, as written by Murti, 2006 states that the sample size in a number of study groups based on the level of treatment should not be <5 subjects. The dependent variable in this study was the protein concentration of mTor, cytochrome C, P53, and Ki67 in the HepG2 liver cancer cell line. The independent variable in this study was andaliman fruit extract (Zanthoxylum acanthopodium). Inclusion criteria in this study were HepG2 liver cancer cell lines that had grown. The exclusion criteria in this study were contaminated HepG2 liver cancer cell lines.

Results

The results of the cytotoxicity test of andaliman fruit against HepG2 cells were assessed using the MTT method. The MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] method is a quantitative cytotoxicity test. This test is based on the measurement of color intensity (colorimetry) that occurs as a result of the metabolism of a substrate by living cells into a colored product [20].

Figure 3 shows the anticytotoxic effect of andaliman on HepG2 cells. The results showed that andaliman reduced the viability of HepG2 cells and the results depended on the concentration of the extract. Andaliman's anticytotoxic effect on HepG2 cells can be seen from the IC50 value, which means that the concentration of extract required to cause HepG2 cell death is as much as 50% of the population [21].

Discussion

From the graph in the results, the IC50 value of andaliman against HepG2 cells is 122,656 µg/mL. The National Cancer Institute (NCI) US classifies the cytotoxicity of a compound as high cytotoxic activity if IC50 <20 µg/mL, moderate cytotoxic activity if IC50 ranges from 21 to 200 µg/mL, weak cytotoxic activity if IC50 ranges from 201 to 500 µg/mL, and there is no

Data obtained from observations are recorded and presented in the form of mean ± standard deviation (mean ± SD) if the data are normally distributed. If the data are not normally distributed, then the data will be presented in quartiles. To test the normality of the data, the Saphiro–Wilk test was used, because the number of samples was <5. If the data were normally distributed and homogeneous, then an ANOVA test was performed to test the relationship between parameters. If the data are not normally distributed, then the Kruskal–Wallis test is performed. All data analysis was performed using SPSS software. In this study, for statistical test decisions, the 5% significance level (p = 0.05) was taken, which was considered significant or significant.
cytotoxicity if IC50 > 500 µg/mL [22]. Based on this criterion, the cytotoxic effect of andaliman includes moderate cytotoxicity.

The cytotoxic effect of andaliman extract is related to the active phytochemical components in andaliman fruit, namely, flavonoids, alkaloids, saponins, and tannins. These compounds can be developed into cochemotherapeutic agents that can be used in conjunction with conventional chemotherapeutic agents [23]. The role of andaliman extract as an adjuvant in HCC management can be seen in Figure 2. Several other studies have focused on the toxic effects produced by well-known alkaloids such as taxol, vincristine, and vinflunine which are used clinically in the treatment of cancer in the world. Benzohenanthridine and furoquinoline are alkaloid compounds present in Zanthoxylum buesgenii, which have cytotoxic effects in several cell lines. In a previous study, it was said that the fruit alkaloid fraction of Zanthoxylum acanthopodium DC proven effective as anticancer against several cell lines [16]. Compound percentage and active compounds can be seen in Tables 1 and 2.

Conclusions

The results reveal that Zanthoxylum acanthopodium fruits extract has a moderate cytotoxicity on HepG2 liver cancer cell line.

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