Determination of Antigentoxic Properties of Friedelin, an Organic Compound

Hamit Emre KIZIL, Selçuk CEKER and Güleray AGAR

Abstract— The excess of side effects of chemical agents, the infections of antibiotic-resistant disease-causing microorganisms and the presence of antimicrobial activity have led to the search for many plant secondary metabolites. Initially, it was suggested that lichens were a plant, but later it turned out that there were no organisms on their own. They are symbiotic associations that combine fungi and algae to form a morphological and physiological whole. Friedelin is a triterpenoid, which is a special group of lichens and other secondary groups of plants that they specifically produce. It is aimed to find promising drug candidate molecules in many different diseases by investigating the antigenotoxic activity of friedelin. In this study, it was aimed to determine the antigenotoxic properties of the friedelin compound against genotoxic damage induced by AFB_1 in human lymphocyte cells. Results: In our study, it was found that friedelin causes a decrease in micronucleus (MN) frequencies at increasing doses of 5, 10, 20, 40, and 80 µg/mL, therefore, it can be evaluated that friedelin exhibits high antigenotoxicity.

Index Terms— AFB_1, antigenotoxic activity, genotoxic damage, Friedelin, lichen metabolite, micronucleus assay, triterpenoid

1 INTRODUCTION

FRIEDELIN is a triterpenoid which isolated from several plants such as *Elaeocarpus floribundus, Annona muricata, Garcinia prainiana, Aucuba jabonica* and *Azima tetracantha* and some lichens such as *Alectoria ochroleuca, Cetraria cucullata, Cetraria delisei, Cladonia alpestris* and *Stereocaulon paschale* [1,2,3]. It was also determined that friedelin has antioxidative, cytotoxic and apoptotic properties [1]. In a study Susanti et al. also showed that friedelin stimulated glucose uptake and adipocytes differentiation in 3T3-L1 adipocytes [4]. Additionally, Antonisamy et al. indicated that friedelin is a natural preservative compound against gastric ulcer.

Aflatoxin B1 (AFB_1) causes liver damage, congenital malformations, immunodeficiency, bowel bleedings and infant births and mutagenic properties on humans and animals. Aflatoxins show acute effects at high doses, and have chronic toxic effects at doses below the deadly dose [5]. Despite the strong toxicological effect of AFB_1, some of the human chromosomes were resistant to AFB_1, although some chromosomes were found to be non-resistant [6]. Although the mechanism of damage caused by AFB_1 in cells is not fully known, reactive oxygen species (ROS), lipid peroxidation (LPO), and direct DNA binding toxicity have been accepted as the main mechanisms [5]. In this study, the anti-genotoxic effect of friedelin was investigated against AFB_1 by using sister micronucleus (MN) test in human lymphocyte cell culture *in vitro*.

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2 MATERIALS AND METHODS

Friedelin produced by various plant and lichen species was were purchased from Sigma-Aldrich. The chemical formula of friedelin is $C_{30}H_{50}O$.

2.1 Cytogenetic Analysis

Four donors who were aged between 24 and 28 without any health problems and who wanted to participate voluntarily were selected. The donors were selected from healthy individuals who were non-smokers (also determined not to be passive smokers) and who did not have any inherited, acute or chronic diseases. The previously prepared medium was removed from -20 ° C and allowed to reach room temperature. The following chemicals were added to the room temperature and the following chemicals were added under sterile conditions. After this procedure, 1 mL of blood samples of donors were added to each tube. This experimental setup was repeated for a total of 4 donors. The experiments were performed in 7 groups as follows:

Culture 1: Control
Culture 2: Blood (1 ml) + 5 µM AFB_1
Culture 3: Blood (1 ml) + 80 µg/ml Friedelin
Culture 4: Blood (1 ml) + 5 µM AFB_1 + 5µg/ml Friedelin
Culture 5: Blood (1 ml) + 5 µM AFB_1 + 10µg/ml Friedelin
Culture 6: Blood (1 ml) + 5 µM AFB_1 + 20µg/ml Friedelin
Culture 7: Blood (1 ml) + 5 µM AFB_1 + 40µg/ml Friedelin
Culture 8: Blood (1 ml) + 5 µM AFB_1 + 80µg/ml Friedelin

For MN analysis, at 44 hours of lymphocyte culture 6 µg / ml

of cytokalazine B was added to each tube. The tubes cultured at 72 hours were centrifuged at 1000 rpm for 10 minutes and the supernatant fraction was separated for biochemical analysis. A hypotonic solution (0.075 M KCl) was added onto the pellet and the tubes were incubated for 25 minutes at 37°C. The supernatant portion was discarded by centrifugation at 1000 rpm for 10 minutes and a 1: 3 cold acetic acid methanol mixture was added to the pellet to determine the fixation. The detection was repeated three times in total. At the end of the fixation, the supernatant was discarded, except for the 1-1.5 mL lower portion. After resuspension of the remaining pellet by shaking, 5 to 6 drops of each of the slides waiting in the cold fixation were dripped from this suspension and 5 slides were prepared. The prepared preparations were aged by standing at room temperature for three days. At the end of this period, each preparation was stained with giemsa stain. For the MN scoring, the micronucleus criteria described by Countryman and Heddle were used [7].

3 RESULTS

As seen in **Table 1**, AFB_1 caused the formation of MN in peripheral lymphocyte cells. MN frequencies increased more and more with increased aflatoxin AFB_1. The amounts of MN were reduced by friedelin addition and the antigenotoxic properties are as follows: 80 μg/ml >40 μg/ml >20 μg/ml >10 μg/ml >5 μg/ml ($P < 0.05$).

TABLE 1
MN FREQUENCIES

Culture types	Counted of MN	MN/1000 cell
Control(-)	1008	4.31+0.85
Control (+)	1050	9.5+0.90
Friedelin (80 μg/mL)	1010	4.37+0.91
AFB_1 + Friedelin (5 μM + 5 μg/mL)	1028	8.51+0.70
AFB_1 + Friedelin (5 μM + 10 μg/mL)	1015	7.78+0.60
AFB_1 + Friedelin (5 μM + 20 μg/Ml	1023	6.16+0.47
AFB_1 + Friedelin (5 μM + 40 μg/mL)	1035	5.89+0.33
AFB_1 + Friedelin (5 μM + 80 μg/mL)	1017	4.97+0.44

4 DISCUSSIONS

In recent years, many *in vivo* and *in vitro* studies have attempted to utilize different herbal and plant parts as a medicinal product [8]. Researchers have developed various test systems in public health that can be used to determine the mutagenic antimutagenic properties of plants for medical purposes for centuries [9].

It has been shown by many researches that it is a triterpenoid, found in the content of many plants and lichen and has high biological activity. For instance, in a recent study, the cytotoxic and apoptotic effect of friedelin on breast cancer cells was investigated. It was also determined that friedelin, which inhibited the growth of MCF 7 cells by 78%, also had high apoptotic properties [10].

AFB_1 ($C_{17}H_{12}O_6$) is a mycotoxin that is frequently encountered in foods and animal feeds. High doses cause toxic health problems and economic losses. Low doses are potent hepatocarcinogen, mutagenic and teratogenic and also suppress the immune system. In addition, AFB_1 suppresses the immune sytem as well as causes intestinal bleeding and birth defectsf. Aflatoxins may contaminate many food products such as hazelnuts, groundnuts and dried fruits. Aflatoxin contamination usually occurs during food transport and storage. In previous studies, aflatoxins have been known to increase chromosomal aberrations and SCE [11].

According the results of MN analysis, it was determined that friedelin have shown antigenotoxic effects against AFB_1 which has been shown to increase MN in human peripheral lymphcytes.

Therefore, our near future experiments will be focused on determining the *in vivo* activities of friedelin. Then, as a result of advanced pharmacological studies, drug development studies will be planned.

ACKNOWLEDGMENT

This work was supported by Bayburt University, Scientific Research Projects Unit (No: 2017/01-69001-28)

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