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Research Paper

Genotoxic, Antigenotoxic and Antioxidant Properties of Methanol Extracts Obtained from *Umbilicaria cylindirica* and *Umbilicaria nylanderiana*

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Abstract

The use of lichens as food, medicine in dyes and perfumes and crude drugs is very common all over the world. It is so important to determine whether their use is safe or not and which doses should be used. In the present study, the genotoxic and antigenotoxic effects of methanol extracts of *Umbilicaria cylindirica* and *Umbilicaria nylanderiana* were investigated by using sister chromatid exchange in human lymphocytes against Aflatoxin B1 (AFB_1) and the bacterial reverse mutation assay in *Salmonella typhimurium* TA1535 and TA1537 strains and in *Escherichia coli* WP2 *uvr*A strain against Sodium azide (NaN_3), 9-Aminoacridine (9-AA) and *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), respectively. Furthermore, in order to evaluate antioxidant effect of the tested materials, the main antioxidant enzymes including superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPX) and malondialdehyde (MDA) level were determined. At the end of the study, it was observed that *Umbilicaria cylindirica* and *Umbilicaria nylanderiana* exhibit no mutagenic activity, but significantly antimutagenic activity against all the different doses of mutagens and antioxidative activity against oxidative stress caused by AFB_1.

Keywords: Umbilicaria spp.; Antimutagenicity; Antioxidative; Bacterial reverse mutation assay; SCE.

1. Introduction

Natural products used as folk remedy by humankind for ages provide a great source of compounds for potential drugs such as current antibiotics and anticancer drugs [1]. Therefore, it would be useful to search plants that have been used by folk during the ages for discovering new phytomedicines. Since lichens have been for a long time for traditional treatment of many diseases including tuberculosis, hemorrhoids, dysentery etc.), the researchers have recently focused on the lichens and their metabolites [2-3]. The lichens are self-supporting symbiotic forms between a fungus and one or several algal or cyanobacteria. They have been generally considered a type of fungus because of being unique with its fungal constituents dominating the association in that symbiosis [4]. They have evolved particularly during the past 600 million years and existed on more than 10% of terrestrial part of the earth surface [5].

About 300 genera and 18000 species of lichens are presently recognized. Up to the present more than 800 secondary metabolites have been identified from lichens [6]. Some of these lichen metabolites are unique to lichens and produced so as to protect and sustain the symbiotic association from various biotic and abiotic factors. Therefore, much attention has been recently paid to the biological activities of lichen metabolites [7]. As a result of this attention, it is revealed that many lichen species pose various biological activities such as antiviral, antimicrobial, antiallergic, antitumor, anticancer, antioxidative, anti-inflammatory [8]. A wide range of biological activities is attributed to constituents of the lichens such as chloroatranorin, atranorin, (+)-usnic acid, evernic acid, parietin, galactomannan, arabitol, mannitol, galactoside, sucrose, glycolic and aspartic acid gyrophoric acid [9].

Umbilicaria Hoffm. is a well-known genus of umbilicate, foliose, lichen-forming fungi comprising *c*. 90 currently accepted species. These species exhibit a worldwide distribution appearing mostly on siliceous

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rocks in the polar, alpine and high-alpine biomes. 31 species of *Umbilicaria* in Europe; 26 species in the continental part of Mediterranean region and 21 species in the Carpathians have been defined [10]. The previous studies demonstrated that some species of *Umbrilicaria* synthesize a great variety of secondary metabolites and have significant antioxidative activity. However, there is no satisfying literature about biological activities of *Umbilicaria* species. In the light of the foregoing, it was aimed to evaluate antigenotoxic properties of *Umbilicaria cylindirica* and *Umbilicaria nylanderiana* against strong mutagens including Sodium azide (NaN_3), 9-Aminoacridine (9-AA), *N*-Methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) and Aflatoxin B1 (AFB_1), respectively. In addition, the genotoxic properties of the lichens were tested to make sure whether their use is safe or not through using sister chromatid exchange (SCE) and bacterial reverse mutation assays. We also evaluated dose-dependent oxidative effects in human blood by determining main antioxidant enzymes activities including superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GP_X) and also MDA level. The present study showed that *U. cylindirica* and *U. nylanderiana* have significant antioxidative and antimutagenic effects but no mutagenic effect. In the consequence of this study, it could be concluded that researched lichens could supply a great resource to discover new phytomedicinal agents exhibiting antioxidative and antimutagenic properties.

2. Material and Methods

2.1. Preparation of lichen materials

Umbilicaria cylindirica and *Umbilicaria nylanderiana* samples were collected from Oltu/Erzurum, in spring. The taxonomic identification of the tested materials was carried out by Dr. Ali Aslan from the lichenology laboratory in Biology Department of Ataturk University, Erzurum-Turkey. The voucher specimens have been stored at the Herbarium of Kazım Karabekir Education Faculty, Ataturk University, and Erzurum. The collected plant materials were dried in the shade, then, grounded in a grinder with a 2 mm in diameter mesh. Dried and powdered lichen samples (20 gr) were extracted by using 200 ml methanol in room temperature for four hours via Soxhlet extractor (Isopad, Heidelberg, Germany). Then, the extracts were filtered through Whatman filter paper (no.1), concentrated at 40 °C in a rotary evaporator (Buchi Labortechnic AG, Flawil, Switzerland), lyophilized and kept in dark at +4 °C for further studies.

2.2. Bacterial strains

S. typhimurium TA1535 (ATCC® Number: 29629), *S. typhimurium* TA1537 (ATCC® Number: 29630) strains were provided by The American Type Culture Collection - Bacteria Department of Georgetown University, Washington, USA and *E.coli* WP2*uvrA* (ATCC® Number: 49979) strain was provided by LGC standarts Middlesex, UK. All strains were stored at -80 ºC. These cultures were prepared by inoculation with the frozen cultures in nutrient broth, followed by an overnight incubation at 37 ºC with gentle agitation [11].

2.3. Viability assays and determination of test concentrations

Toxic levels of the test materials for *S. typhimurium* TA1535, 1537 and *E. coli* WP2*uvrA* strains were determined as described in detail elsewhere [12]. These tests confirmed that there was normal growth of the background lawn, spontaneous colony numbers within the regular range, and no significant reduction in cell survival. Thus, for the concentrations and conditions reported here, no toxicity or other adverse effects were observed.

2.4. The bacterial reverse mutation assay for mutagenicity and antimutagenicity

The bacterial mutagenicity and antimutagenicity assays were carried out using the method reported before [13]. The used mutagens NaN_3 (in distilled water - 1 µg/plate) for *S. typhimurium* TA1535, 9-AA (in methanol - 10 µg/plate) for *S. typhimurium* TA1537 and MNNG (in 10% DMSO - 1 µg/plate) for *E. coli* WP2*uvrA* were used as positive controls and 10% DMSO was used as negative control in these researches. In the mutagenicity test performed with TA1535 and TA1537 strains of *S. typhimurium* and *E. coli* WP2 100 µl of the overnight bacterial culture, 50µl test compounds at different concentrations (20, 40, 60, 80, 100 µg/plate in 10% DMSO), and 500 µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin for TA1535 and TA1537 strains of *S. typhimurium* and the same solution containing tryptophan (0.05 mM) instead of histidine for *E. coli* WP2. The mixture was poured onto minimal glucose plates. Histidine and tryptophan independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48 or 72 h. In the antimutagenicity test, 100 µl of the overnight bacterial cultures, 50 µl mutagen, 50 µl test compounds at different concentrations (0.05, 0.5, 5 µg/plate in 10% DMSO), and 500 µl phosphate buffer were added to 2 ml of the top agar containing 0.5 mM histidine/biotin or tryptophan (0.05 mM). The mixture was poured onto minimal glucose plates. Histidine and tryptophan independent revertant colonies and viable cells were scored on plates after incubation at 37 ºC for 48 or 72 h [14]. For the mutagenicity assays, the mutagenic index was calculated for each concentration, which is the average number of revertants per plate divided by the average number of revertants per plate with the negative (solvent) control. A sample was considered as mutagenic when a dose-response relationship and a two-fold increase in the number of revertants with at least one concentration were observed [14]. For the anti-mutagenicity assays, the inhibition rate of mutagenicity was calculated by using the following equation (M: number of revertants/plate induced by

mutagen alone, S0: number of spontaneous revertants, S1: number of revertants/plate induced by the extract plus the mutagen): %Inhibition = 1-[(M-S1)/(M-S0)]×100. 25–40% inhibition was defined as moderate anti-mutagenicity; 40% or more inhibition as strong anti-mutagenicity and 25% inhibition as no anti-mutagenicity. The data were further analyzed for statistical significance using analysis of variance (ANOVA), and the difference among the means was compared by high-range statistical domain using Tukey's test. A level of probability <0.05 was taken as indicating statistical significance [15].

2.5. SCE assay for cytogenetic analysis

Peripheral blood lymphocytes were obtained from four (age: 28, 26) nonsmoking healthy individuals between the ages of 22 and 25. Lymphocyte cultures were maintained by adding 0.5mL of heparinized whole blood to RPMI-1640 chromosome medium containing 15% heat-inactivated fetal calf serum, 100 IU/mL streptomycin, 100 IU/mL penicillin, and 1% L-glutamine. Lymphocytes were induced to divide by adding 1% phytohemaglutinin. The well-known mutagen AFB_1 (5 μM) was used for positive control, peripheral blood lymphocytes without any treatments was used for negative control in the study. In antimutagenicity assay was carried out according to SCE assay. Different concentrations of the tested materials (5 μg/ml, 10 μg/ml) were used as test groups against AFB_1 (5 μM). AFB_1 (5 μM), and the tested materials UCME and UNME (in concentrations of 5 and 10 μg/ml) were added to the cultures just before incubation. After addition of 5-bromo 2-deoxyuridine (8 mg/mL), all the cultures were incubated at 37°C for 72 h with no light. 0.1 mg/mL of colcemide was added for arresting the cells at metaphase before harvesting. Then, the cultures were centrifuged at 800 g for 10 min. The supernatants were saved to use for enzyme analysis. Cells were harvested and treated for 30 min with hypotonic solution (0.075 M KCl) and fixed in a 1:3 (v/v) mixture of acetic acid/methanol. Bromodeoxyuridine incorporated metaphase chromosomes were stained with fluorescence plus Giemsa technique as described by Speit and Haupter [16]. In order to evaluate SCE, 20 satisfactory metaphases were analyzed. For each treatment condition, well-spread second division metaphases containing 42-46 chromosomes in each cell were scored, and the values obtained were calculated as SCEs per cell. Each test assays were repeated three times.

2.6. Biochemical analysis for antioxidative effect determination

2.6.1. Preparation of the cell homogenates

The cell homogenates were diluted in 10 mM potassium phosphate buffer (pH 7.4) as 1:10 (w/v). Samples were centrifuged at 3000 rpm for 10 min at 4 °C. The supernatants were collected and immediately assayed for enzyme activities. Cu, Zn-SOD and GPx activities and MDA levels in the cell culture supernatant were measured by the method of Paglia and Valentina, Ohkawa et al. and Sun et al. [17-19] respectively. All samples were measured in six times.

2.6.2. SOD assay

Cu, Zn-SOD activity of the cell culture supernatant was carried out according to Sun et al. [19] 100 μL of the sample was added in to 2.45 mL of assay reagent [0.3 mM xanthine, 0.6 mM Na_2EDTA, 0.15 mM nitro blue tetrazolium (NBT), 0.4 M Na_2CO_3, 1 g/L bovine serum albumin]. Furthermore, Xanthine oxidase (50 μL, 167 U/L was added into the mixture to initiate the reaction. The reduction of NBT by superoxide anion radicals produced by xanthine-xanthine oxidase system was spectrophotometrically determined by measuring at 560 nm. One unit activity of Cu, Zn-SOD was defined as the amounts of enzyme that inhibited the rate of NBT by 50% under the assay conditions described above.

2.6.3. GSH assay

GSH activity of the cell culture supernatant was carried out using the method of Anderson et al. [20] The final volume of the reaction mixture was 3 ml containing 750 μL 10 mM 5-5'-dithio-bis-2-nitrobenzoic acid (DTNB) solution, 100 mM KH_2PO_4, 5 mM Na_2EDTA (pH 7.5), 625 U/L GSH-RD and 100 mL sample. After incubation at room temperature for 3 min, 150 μL of 1.47 mM β-NADPH was added into the reaction mixture and mixed rapidly by inversion. The rate of 5-thio-2-nitrobenzoic acid formation meaning proportional to the amount of reduced and oxidized GSH was spectrophotometrically measured at 412 nm. The blank sample contained equal concentrations of DTNB and NADPH without sample. The values were presented as μmol per gram protein.

2.6.4. GPx assay

GPx activity in the cell culture supernatant was measured by the method of Paglia and Valentine [17]. The reaction mixture was generated with 100 μL 8 mM NADPH, 100 μL 150 mM reduced GSH, 20 μL glutathione reductase (30 units/mL), 20 μL 0.12 M sodium azide solution, 2.65 mL 50 mM potassium phosphate buffer (pH 7.0, 5 mM EDTA) and also 50 μL sample and incubated at 37°C for 30 min. To initiate the reaction, 100 μL 2 mM H_2O_2 solution was added and rapidly mixed by inversion. Then, the conversion of NADPH to NADP was measured spectrophotometrically at 340 nm. The enzyme activity was defined as units per g protein using an extinction coefficient for NADPH at 340 nm of 6.22×10^{-6}.

2.6.5. MDA assay

MDA levels in the cell culture supernatant were determined spectrophotometrically according to the method described by Ohkawa et al. [18] The final volume of the reaction mixture was 4 ml containing 8.1% sodium dodecyl sulphate, 20% acetic acid, 0.9%

thiobarbituric acid and 200 µL of the tested samples. After incubation of the mixture at 95 °C for 1 h, the tubes were left to cool under cold water. 1 mL distilled water with 5 mL *n*-butanol/pyridine (15:1, v/v) was added into the mixture and mixed up. Then, the samples were centrifuged at 4000 × g for 10 min and the absorbances of the supernatants were measured at 532 nm against a blank by using the standard graph created by 1,1,3,3-Tetraethoxypropane. Lipid peroxide levels were defined as µmol/l MDA. Protein concentrations in the cell culture supernatant were determined by Bradford method [21]. All of the spectrophotometric measurements were performed with a DU 530 spectrophotometer (Beckman Instruments, Fullerton, California, USA) in a quartz cuvette.

2.7. Statistical analysis

The results were presented as the average and standard error of three experiments with triplicate application. The analysis of SCE rates and biochemical parameters were performed through Mann-Whitney U-Test of SPSS software of version 15.0 (SPSS Inc, Chicago, Illinois, USA). A level of probability <0.05 was taken as indicating statistical significance.

3. Results and Discussion

In this study, the antigenotoxic effects of methanol extracts of UC and UN were examined against *N*-methyl-*N*'-nitro-*N*-nitrosoguanidine (MNNG), sodium azide (NaN_3), 9-aminoacridine (9-AA) and aflatoxin (AFB) mutation agents in the WP2, Ames (TA1535, TA1537) and SCE test systems, respectively. Besides, the genotoxic effects of the lichens were studied to determine whether their use is safe or not and which doses should be used. Our findings obtained from the bacterial reverse mutation assay showed that UCME and UNME had no mutagenic activity either in the strains of *S. typhimurium* (TA1535 and TA1537*)* or in the WP2*uvrA* strain of *E. coli* at any tested concentration (20, 40, 60, 80, 100 µg/plate in %10 DMSO). On the other hand, it was observed that the tested materials at some concentrations had strong antimutagenic activity in the strains of *S. typhimurium* (TA1535 and TA1537*)* and in the *E. coli (WP2)* against NaN_3, 9-AA and MNNG, respectively. However, the extracts exhibited antimutagenic activity at different levels for all the studied concentrations. UNME applied at 0.05 µg/plate showed inhibition rate of 31,12%; 26,19% was observed at 0.5 µg/plate of UNME for inhibition rate. The results were given in Table 1. 25-40% inhibition was defined as moderate antimutagenicity; 40% or more inhibition as strong antimutagenicity; and inhibition under 25% as no antimutagenicity [22]. These results show that dose adjustments should be done, when lichens are used for phytomedicinal purpose. NaN_3 affecting organisms from bacteria to animals, the positive control for TA1535 strain of *S. typhimurium*, are a very strong mutagenic compound.

Mutagenicity of NaN_3 is mediated through the production of an organic metabolite called as *L*-azidoalanine [23]. In this study, it was observed that UCME and UNME had antimutagenic activity against NaN_3 in most concentrations. The antimutagenic activity against NaN_3 might be mediated through inhibitor activity of the extracts on the production of *L*-azidoalanine [14]. 9-AA, the positive control for 1537 strain of *S. typhimurium*, might show its mutagenic effect via frameshift event. In the frameshift mutagenicity, acridines including 9-AA bind to DNA non-covalently by intercalation [24]. The both of lichen extracts exhibited significant increasing antimutagenic effect depending on increasing dose against 9-AA. It was thought that this antimutagenicity was mediated via inhibition potentials of the tested extracts through blocking of 9-AA binding to DNA. MNNG, the positive control for WP2 strain of *E. coli*, is a compound mediated its mutagenic and lethal effects through the methylation of DNA. The most particular product of MNNG, O^6- methylguanine, is responsible for mutagenic activity of MNNG [25].

In this study, both extracts showed a great antimutagenic potential ranging from 26.55% to 41.53% at tested concentrations. This antimutagenic activity might be explained through inhibitor activities of the tested materials on the formation of O^6-methylguanine. In addition, *E. coli* WP2 test system was especially used to determine the antimutagenic activities of antioxidant substances. According to literature, lichen extracts produced by many different methods were highly effective for radical scavenging and inhibition of linoleic acid oxidation which meant having high antioxidant activity [26]. As it was observed, the tested materials had high antimutagenic activity when *E. coli* WP2 test system was used. It could be explained with direct correlation between the antioxidant capacity and antimutagenic activity of the extracts. However, UCME showed no antimutagenic activity at some concentrations and the antimutagenic activity of UNME showed variable activity against MNNG. These results could be observed due to toxic effect in WP2 strain. The stress caused by extract exposure at high level might disturb the used strain. In addition to *Salmonella* and *E. coli* WP2 test system, SCI test system was performed to determine the mutagenic and antimutagenic activity of the studied lichens. According to the results, the tested materials exhibited antimutagenic activity at high level with applications of increasing doses against AFB_1 and SCE formations on peripheral lymphocytes. The results were given in Table 2. AFB_1, the positive control for SCE test system, is a mycotosin and the mutagenicity of AFB_1 is mediated by stimulating the release of free radical including reactive oxygen species which cause gene mutations, chromosomal aberrations, formation of micronuclei and SCE. Finding new antimutagen agent against AFB_1 is especially an important objective for the researchers focusing on herbal medicine.

Table 1. Antimutagenicity assay results of the methanol extract of *Umbilicaria cylindirica* and *Umbilicaria nylanderiana* for *E. coli* WP2*uvrA*, *S. typhimurium* TA1535 and TA1537 bacterial test strains

Test Items	Concentration (µg/plate)	*Umbilicaria cylindirica*						*Umbilicaria nylanderiana*					
		Number of revertants						Number of revertants					
		E. coli WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537		*E. coli* WP2*uvrA*		*S. typhimurium* TA1535		*S. typhimurium* TA1537	
		Mean ± S.E.	Inhib. %	Mean ± S.E.	Inhib. %	Mean ± S.E.	Inhib. %	Mean ± S.E.	Inhib. %	Mean ± S.E.	Inhib. %	Mean ± S.E.	Inhib. %
MNNG**	1	368.00 ± 09.61						561.00 ± 09.66					
NaN_3**	1			649.00 ± 11.19						649.50 ± 11.19			
9-AA**	40					306.20 ± 10.42						317.12 ± 10.41	
DMSO** (µl/plate)	100	34.00 ± 00.65		18.30 ± 00.94		14.50 ± 00.28		23.00 ± 00.65		18.30 ± 00.94		16.50 ± 00.28	
	2	235.26 ± 03.29	**36.14***	436.00 ± 09.60	**32.81***	204.00 ± 04.41	**33.33**	328.00 ± 04.41	**41.53**	447.00 ± 09.61	**31.12**	195.00 ± 04.48	**38.48**
	4	269.00 ± 04.10	**26.90***	448.25 ± 10.42	**30.97***	193.50 ± 03.08	**36.92**	329.50 ± 03.06	**41.53**	479.25 ± 10.41	**26.19**	178.50 ± 03.05	**43.84**
	6	339.50 ± 07.00	-	483.50 ± 11.60	**25.57***	181.00 ± 04.50	**40.84**	412.00 ± 04.53	**26.55**	453.50 ± 11.60	**30.20**	161.00 ±g 04.54	**49.21**
	8	339.00 ± 04.53	-	465.00 ± 08.09	**28.35**	156.50 ± 04.40	**49.01**	400.50 ± 04.42	**28.69**	458.00 ± 08.19	**29.42**	159.50 ± 04.48	**49.84**
	10	345.00 ± 05.84	-	545.25 ± 07.31	_	128.50 ± 06.39	**58.16**	424.50 ± 06.30	**24.42**	465.25 ± 07.31	**28.35**	1474.50 ± 06.33	**53.62**

* p<0,05, ** MNNG, NaN_3 and 9-AA were used as positive controls for *E. coli* WP2*uvrA*, S. *typhimurium* TA1535 and TA1537 strains, respectively. 10% DMSO (dimethylsulfoxide) was used as negative control

Table 2. Antimutagenic activity against SCE formations on peripheral lymphocytes of *U. cylindirica* and *U. nylanderiana*

Culture types	*Umbilicaria cylindirica*				Culture types	*Umbilicaria nylanderiana*			
	Metaphase	**Range of SCEs**	**Number of SCEs**	**SCE/Cell*** **		**Metaphase**	**Range of SCEs**	**Number of SCEs**	**SCE/Cell*** **
Control	60	4-7	396	5.32±0.82[a]	**Control**	60	3-8	416	5.10±1.50[a]
AFB_1 5 µM (84ml)		4-10	507	5.96±0.83[e]	**AFB_1 5 µM (84ml)**	60	4-11	545	5.64±1.50[d]
UC* (5µg/ml)	60	4-9	429	5.43±0.81[c]	**UN** (5µg/ml)**	60	4-10	520	5.43±2.79[c]
AFB_1 5 µM (84ml) + UC (5µg/ml)	60	4-10	474	5.56±0.81[d]	**AFB_1 5 µM (84ml) + UN (5µg/ml)**	60	5-10	498	5.33±5.29[cb]
AFB_1 5 µM (84ml) + UC (10µg/ml)	60	5-11	435	5.44±0.82[c]	**AFB_1 5 µM (84ml) + UN (10µg/ml)**	60	5-13	484	5.25±4.00[b]
AFB_1 5 µM (84ml) + UC (100µg/ml)	60	3-12	417	5.38±0.81[b]	**AFB_1 5 µM (84ml) + UN (100µg/ml)**	60	3-12	462	5.14±4.5[a]

* UC: *U. cylindirica* methanol extracts, ** UN: *U. nylanderiana* methanol extracts *** [a]p<0,001 compared with control group; [b]p<0,05 compared with control, [c]p <0.05 compared with AFB_1 (5 µM) group; [d]p <0.001 compared with AFB_1 (10 µM) group

Table 3. The activities of SOD, GPx, GSH, and MDA levels

Culture types	*Umbilicaria cylindirica**				Culture types	*Umbilicaria nylanderiana***			
	SOD (U/mL)	**GPx (U/mL)**	**GSH (µmol/L)**	**MDA (nmol/mL)**		**SOD (U/mL)**	**GPx (U/mL)**	**GSH (µmol/L)**	**MDA (nmol/mL)**
Control	1,75±0,02	0,95±0,14	3,40±0,12	2,21±0,33	**Control**	1,60±0,02	0,85±0,24	2,40±0,41	2,81±0,56
AFB_1	0,97±0,11a	0,46±0,16[a]	1,34±0,01[a]	4,69±2,03[a]	**AFB_1**	0,97±0,11a	0,46±0,16[a]	1,34±0,21[a]	4,69±2,03[a]
UC	1,78±0,01b	1,14±0,27[ab]	3,46±0,29[b]	2,02±0,73[ab]	**UN**	1,77±0,03b	0,88±0,37[b]	2,42±0,19[b]	1,74±0,11[ab]
AFB_1+UC1	1,71±0,01b	0,65±0,24[abc]	3,24±0,14[b]	3,32±0,46[abc]	**AFB_1+UN1**	1,73±0,01b	0,90±0,34[b]	2,42±0,31[b]	2,66±0,26[abc]
AFB_1+UC2	1,77±0,02b	1,02±0,64[abd]	3,32±0,23[b]	2,44±0,12[bd]	**AFB_1+UN2**	1,71±0,02b	0,82±0,84[b]	2,42±0,11[b]	2,44±0,11[abc]
AFB_1+UC3	1,78±0,01b	0,94±0,15[bd]	3,44±0,01[b]	2,37±0,62[bd]	**AFB_1+UN3**	1,78±0,01b	0,92±0,05[b]	2,45±0,01[b]	2,41±0,16[abc]

*UC: *U. cylindirica*, a: p<0,05 compare with control group, b: p<0,05 compare with AFB_1 group, c: p<0,05 compare with UC group, d: p<0,05 compare with AFB_1+UC1 group, e: p<0,05 compare with AFB_1+UC2 group ** UN: *U. nylanderiana*, a: p<0,05 compare with control group, b: p<0,05 compare with AFB_1 group, c: p<0,05 compare with UN group, d: p<0,05 compare with AFB_1+UN1 group, e: p<0,05 compare with AFB_1+UN2 group

As AFB_1 is the most abundant contaminant in many foods including wheat, barley and milk and also the most common mutagenic agent [27]. The antimutagenic activity observed by SCE test system might result from inhibitor activities of the lichen extracts on the formation of free radicals. In order to determine how the tested materials exhibited the possible antimutagenic effect, the activities of antioxidant enzymes including SOD, GSH and GP_X and MDA level were determined in presence and absence of AFB_1 and lichen extracts. According to our results, the activities of SOD, GSH and GPx decreased and MDA level increased through AFB_1 application. On the other hand, the application of increasing concentrations of methanol extracts of lichens with AFB_1 increased the antioxidant enzymes and reduced the level of MDA. The results were showed in Table 3. SOD, playing the first line defense against oxidative damage catalyzes the dismutation of superoxide to hydrogen peroxide and oxygen. GP_X and GSH are important for regulation of intracellular H_2O_2. They scavenge endogenous superoxides and H_2O_2 and thereby play a significant role in decreasing lipid peroxidation which means a decrease in MDA level [28]. GSH also plays a crucial role in the detoxification of majority of alkylating agents including diethylnitrosamine (DEN) [28]. The decrease of MDA level and increase of antioxidant enzymes can result from antioxidant activity of the tested materials through their utilization in inactivating the free radicals generated by AFB_1. Besides, the antimutagenic activity of the lichens at high level against 9-AA can be explained with activity of GSH in TA1537 strain of *S. typhimurium*. As previously reported, the genotoxic effect of lichens extracts can be explained through their antioxidant substances including polyphenolic compounds such as epigallocatechin gallate, quercetin, gallic acid, curcumin, eugenol, usnic acid, polysaccharide Ci-3, lichestrerinic acid, protolichesterinic acid and organic acids such as oxalic, fumaric, malic and lactic acids. Therefore, many lichen ingredients were commercially purchased or isolated from lichens species and tested for their biological activities by many researchers [9]. For example, Valencia-Islas et al. [26] carried out a study about determination of the anti-oxidant activity and anti-radical power of six phenolics including boninic acid, 2-*O*-methylsekikaic acid, salazinic acid, chloroatranorin, atranorin and (+)-usnic acid. According to the literature, *Umblicaria* species possess many compounds such as umbilicaric, ovoic, hiascic, gyrophoric, lecanoric acids varying according to specimens, as well as the quality of other secondary metabolites detected by HPLC method [29]. The results of this study were supported by previous studies.

4. Conclusions

In conclusion, UCME and UNME have significant antioxidative and antigenotoxic potentials against several mutagen agents. Although important dates were obtained from the performed test systems, further studies should be carried out to fractionate the methanol extracts for identification of the active compounds, to adjust accurate dose. Besides, the results about mutagenic and antimutagenic activities make the methanol extracts of lichens promising candidates for further researches focusing on phytotherapeutic agent discovery and development.

Conflict of Interest

The authors declare no conflict of interest.

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