TÜBİTAK

Original Article

Turk J Med Sci
2011; 41 (6): 1043-1049
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1011-1315

Protective activity of the methanol extract of *Usnea longissima* against oxidative damage and genotoxicity caused by aflatoxin B_1 *in vitro*

Güleray AĞAR[1], Ali ASLAN[2], Elif KOTAN SARIOĞLU[1], Lokman ALPSOY[3], Selçuk ÇEKER[1]

Aim: To investigate the effects of methanol extracts obtained from *Usnea longissima* (UME) on the genotoxicity and oxidative stress of aflatoxin B_1 (AFB_1) in cultured human blood cells.

Materials and methods: Sister chromatid exchange (SCE) and micronucleus (MN) tests were used for estimation of genotoxic influences. Activities of superoxide dismutase (SOD) and glutathione peroxidase (GPx) and the malondialdehyde (MDA) level were also measured to evaluate the antioxidative effect of UME.

Results: In the SCE and MN test systems, it was observed that UME suppressed the mutagenic effects of AFB_1. Furthermore, an increase of MDA level and a decrease of SOD and GPx activities were observed after AFB_1 treatment. UME eliminated the genotoxicity of AFB_1 and lipid peroxidation by increasing the level of antioxidant enzymes activities.

Conclusion: It was shown here for the first time that UME modulates the adverse effects of AFB_1 in human blood cells. The results of the present study have also clearly shown that UME has strong antioxidative and antigenotoxic effects, and the role of these enzymes on the mechanism of antigenotoxic activity may be due to its antioxidant potency.

Key words: *Usnea longissima*, antigenotoxicity, antioxidant enzymes, aflatoxin B_1

Usnea longissima metanolik ekstraktının *in vitro* da aflatoksin B_1'in neden olduğu oksidatif stress ve genotoksik etkiye karşı koruyucu etkisi

Amaç: Bu çalışmanın amacı insan kan kültürü hücrelerinde aflatoksin B_1 (AFB_1) in neden olduğu genotoksisite ve oksidatif stress üzerine /*Usnea longissima*/ (UME)'dan elde edilen metanolik ekstrakların etkisini araştırmak idi.

Yöntem ve gereç: Genotoksik etkilerin tahmini için kardeş kromatid değişimi (SCE) ve mikro nukleus (MN) testleri kullanıldı. Aynı zamanda UME'nin antioksidatif etkisini belirlemek için superoksit dismutaz (SOD) ve glutatyon peroksidaz (GPx) enzimlerinin aktiviteleri ve malondialdehit (MDA) seviyesi ölçüldü.

Bulgular: SCE ve MN test sistemlerinde, UME'nin AFB_1'in genotoksik etkilerini baskıladığı gözlendi. Ayrıca AFB_1 muamelesinden sonra MDA seviyesinde artma, SOD ve GPx enzimlerinin aktivitelerinde azalma gözlendi. UME, AFB_1'in neden olduğu bu enzim aktivitelerindeki azalmayı ve lipid peroksidasyonundaki artışı anlamlı düzeyde engellediği görüldü.

Sonuç: UME, insan lökosit hücrelerinde AFB_1 in neden olduğu SOD ve GPx enzimleri üzerindeki olumsuz etkileri azaltan bir aktivite göstermektedir. Aynı zamanda UME, kuvvetli anti-genotoksik etkiye sahiptir ve UME'nin bu etkisi antioksidant potensiyelinden kaynaklanıyor olabilir.

Anahtar sözcükler: *Usnea longissima*, antigenotoksik etki, antioksidan enzimler, aflatoksin B_1

Received: 03.12.2010 – Accepted: 25.01.2011

[1] Department of Biology, Faculty of Science, Atatürk University, 25240 Erzurum - TURKEY
[2] Department of Biology Teacher Training, Education Faculty of Kazım Karabekir, Atatürk University, 25240 Erzurum - TURKEY
[3] Department of Biology, Faculty of Science and Arts, Fatih University, 34500 İstanbul - TURKEY
Correspondence: *Lokman ALPSOY, Department of Biology, Faculty of Science and Arts, Fatih University, 34500 İstanbul - TURKEY*
E-mail: lalpsoy@fatih.edu.tr

Introduction

Lichens are complex organisms living in symbiotic relationships involving fungi and algae, and the pertinent partners are defined as mycobionts and phycobionts, respectively (1). Slow growth rates and often harsh living conditions force the lichens to produce various protective metabolites (2). Several lichen extracts and their compounds have been used in traditional medicine in Europe, Asia, and North America (3). Previous studies reported that secondary metabolites isolated from lichens show a wide range of a variety of biological activities, including antibiotic, antibacterial, antiviral, antiinflammatory, analgesic, antipyretic, antiproliferative, antigenotoxic, antioxidant, and cytotoxic effects (4). In the folk medicine of different countries of the world, *Usnea longissima* has also been widely used as an expectorant, as a wound dressing, and to stanch nose bleeding, as well as in the treatment of ulcers. It has also been used in the treatment of injuries of the legs and loins, bone fractures, and skin eruptions (5). Usnic acid isolated from *Usnea* species has been widely used in the pharmaceutical and cosmetic industries because of its high antimicrobial, antitermite, and antioxidant activities (6). In addition, preclinical studies have permitted researchers to hypothesize about its possible use as an antineoplastic agent (7).

Aflatoxin B_1 (AFB_1) is known to cause hepatotoxicity, teratogenicity, immunotoxicity, genotoxicity, and even death in animals and humans (8). Although the mechanism of cellular damage caused by AFB_1 has not been fully elucidated (9), reactive oxygen species (ROS), lipid peroxidation (LPO), and direct binding to DNA have been considered to be main mechanisms in the toxicity of AFB_1 (10). ROS damage membrane proteins by causing lipid peroxidation in membranes by attaching to unsaturated fatty acids, which damages the membrane proteins and causes a decrease of membrane permeability, enzymes and receptor activities, and activation of cells. When free radicals attack DNA, cancer-causing mutations may occur. Therefore, antioxidants have attracted much interest with respect to their protective effect against the free radical damage that may be a reason for many diseases, including cancer (10).

Thus, antioxidant molecules may also play an important role in the prevention of genotoxic damage. In this study, the antigenotoxic effect of UME was investigated against AFB_1 by using sister chromatid exchange (SCE) and micronucleus (MN) tests, which provide sensitive and rapid monitoring of induced genetic damage as primary DNA damage in human lymphocyte cell culture *in vitro*. Additionally, the superoxide dismutase (SOD) and glutathione peroxidase (GPx) enzyme activities and the malondialdehyde (MDA) levels in the human blood culture were measured to determine the role of enzyme systems in the basis of antigenotoxic effects.

Materials and methods

Plant material

Lichen samples (*Usnea longissima*) were collected from Giresun Province in the eastern part of Turkey during the spring/summer period of 2009. Samples were dried at room temperature. Identification of samples was conducted by Dr. Ali Aslan (Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey) by using various flora books (11-14). The voucher specimens were deposited in the herbarium of Kazım Karabekir Education Faculty, Atatürk University, Erzurum, Turkey.

Preparation of methanol extracts

Air-dried and powdered lichens (10 g) were extracted with 250 mL of methanol using a Soxhlet extractor (Isopad, Heidelberg, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent (15). The extract was filtered using Whatman filter paper No. 1 and then concentrated in a vacuum at 40 °C using a rotary evaporator (Büchi Labortechnik AG, Flawil, Switzerland), yielding a waxy material. The extract was then lyophilized and kept in the dark at 4 °C until tested.

Cytogenetic analysis

Peripheral blood lymphocytes were taken from 4 nonsmoking healthy individuals (ages: 28, 26, 25, and 28). Lymphocyte cultures were set up by adding 0.5 mL of heparinized whole blood to RPMI-1640 chromosome medium supplemented with 15% heat-inactivated fetal calf serum, 100 IU/mL streptomycin, 100 IU/mL penicillin, and

1% L-glutamine. Lymphocytes were stimulated to divide by 1% phytohemagglutinin (PHA). AFB_1 (in concentrations of 5 and 10 μM) and *U. longissima* methanolic extract (UME) in concentrations of 5 μg/mL (UME1) and 10 μg/mL (UME2) were added to the cultures just before incubation. The experiments were performed in 7 groups, as follows.

Group 1: Control

Group 2: 5 μM AFB_1

Group 3: 10 μM AFB_1

Group 4: 5 μM AFB_1 + UME1 (5 μg/mL)

Group 5: 5 μM AFB_1 + UME2 (10 μg/mL)

Group 6: 10 μM AFB_1 + UME1 (5 μg/mL)

Group 7: 10 μM AFB_1 + UME2 (10 μg/mL)

For SCE demonstration, the cultures were incubated at 37 °C for 72 h, and 5-bromo 2-deoxyuridine was added at 8 mg/mL at the initiation of cultures. All cultures were maintained in darkness, and then 0.1 mg/mL of colcemide was added 3 h before harvesting to arrest the cells at metaphase. The cultures were centrifuged at 800 × *g* for 10 min. Cells were harvested and treated for 30 min with hypotonic solution (0.075 M KCl) and fixed in a 1:3 mixture of acetic acid/methanol (v/v). Bromodeoxyuridine-incorporated metaphase chromosomes were stained with the fluorescence plus Giemsa technique, as described by Perry (16). In the SCE study, by selecting 20 satisfactory metaphases, the results of SCE were recorded on the evaluation table. 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.

For MN analysis, cytochalasin B was added 44 h after PHA stimulation to a final concentration of 3 g/mL. After 28 h (i.e. after a total of 72 h of culture), the cells were harvested by centrifugation (900 × *g*, 10 min). The supernatant was removed, the cells were mixed thoroughly, and 5 mL of cold hypotonic solution (0.05 M KCl) was added. The cells were subsequently incubated at 37 °C for 20 min and centrifuged again (900 × *g*, 10 min). The pellet was mixed thoroughly and 5 mL of fresh fixative (1:3 acetic acid:methanol) was added dropwise. This fixation procedure was repeated 3 times and the tube was centrifuged again. The cell pellet was then resuspended in 1 mL of fresh fixative, dropped onto a clean microscope slide, incubated at 37 °C or at room temperature overnight, and stained with Giemsa dye. Coded slides were scored blind by 2 independent individuals. Only binucleated cells were scored for MN analysis. For each subject, at least 2000 binucleated cells were analyzed for the presence of MNs. For the MN scoring, the MN criteria described by Countryman and Heddle were used: a diameter less than one-third of the main nucleus, nonrefractivity, not touching, and with the same color as the nucleus or lighter (17).

Biochemical analysis

The cultured human blood cell homogenates were prepared at a 1:10 (w:v) dilution in 10 mM potassium phosphate buffer, pH 7.4. Samples were centrifuged at 3000 rpm for 10 min at 4 °C, and the supernatants were collected and immediately assayed for enzyme activities. All samples were measured 6-fold.

SOD assay

Cu/Zn-SOD activity in the cell culture supernatant was detected by the method of Sun et al. (18). First, 2.45 mL of assay reagent [0.3 mM xanthine, 0.6 mM Na_2EDTA, 0.15mM nitroblue tetrazolium (NBT), 0.4 M Na_2CO_3, 1 g/L bovine serum albumin] was combined with 100 μL of the sample. Then 50 μL of xanthine oxidase, 167 U/L, was added to initiate the reaction, and the reduction of NBT by superoxide anion radicals, which are produced by the xanthine-xanthine oxidase system, was determined by measuring the absorbance at 560 nm. Cu/Zn-SOD activity was expressed in units of SOD per milligram of protein, where 1 U is defined as the amount of enzyme causing the half-maximal inhibition of NBT reduction.

GPx assay

GPx activity in the cell culture supernatant was measured by the method of Paglia and Valentine (19). Briefly, 50 μL of sample was combined with 100 μL of 8 mM NADPH, 100 μL of 150 mM reduced GSH, 20 μL of glutathione reductase (30 units/mL), 20 μL of 0.12 M sodium azide solution, and 2.65 mL of 50 mM potassium phosphate buffer (pH 7.0, 5 mM EDTA), and the tubes were incubated for 30 min at 37 °C. The reaction was initiated with the addition of 100 μL of 2 mM H_2O_2 solution, mixed rapidly by

inversion, and the conversion of NADPH to NADP was measured spectrophotometrically for 5 min at 340 nm. The enzyme activity was expressed as units per gram of protein using an extinction coefficient for NADPH at 340 nm of 6.22×10^{-6}.

MDA assay

MDA levels in the cell culture supernatant were determined spectrophotometrically according to the method described by Ohkawa et al. (20). A mixture of 8.1% sodium dodecyl sulfate, 20% acetic acid, and 0.9% thiobarbituric acid was added to 0.2 mL of sample, and distilled water was added to the mixture to bring the total volume up to 4 mL. This mixture was incubated at 95 °C for 1 h. After incubation, the tubes were left to cool under cold water and 1 mL of distilled water with 5 mL of *n*-butanol:pyridine (15:1, v/v) was added, followed by mixing. The samples were centrifuged at 4000 × *g* for 10 min. The supernatants were removed and absorbances were measured with respect to a blank at 532 nm. 1,1,3,3-Tetraethoxypropane was used as the standard. Lipid peroxide levels were expressed as μmol/L of MDA. Protein concentrations in the cell culture supernatant were determined by the Bradford method (21). All photometric measurements were performed with an ELISA reader.

Statistical analysis

The statistical analysis of MN frequencies was performed by use of the chi-square test. For statistical analysis of biochemical parameters and analysis of SCE values, the Mann-Whitney U test was used. $P < 0.05$ was accepted as statistically significant. Results were expressed as mean ± SD. For these procedures, SPSS 11.5 for Windows (SPSS Inc., Chicago, Illinois, USA) was used.

Results

AFB_1 caused significant SCE and MN formations on peripheral lymphocytes, as seen in Table 1. SCE and MN frequencies increased progressively with increased AFB_1 concentration. This increase was found to be statistically significant ($P < 0.001$ and $P < 0.05$). On the other hand, the numbers of SCEs and MN were reduced by UME addition ($P < 0.001$ and $P < 0.05$).

Table 2 represents the results of the biochemical part of the present study, including the SOD and GPx activities and the MDA level in the control and experimental groups. Decreases in the activities of SOD and GPx and increases in the MDA level were observed after treatment with different

Table 1. The frequencies of MN and SCEs in blood lymphocytes after exposure to different doses of AFB_1 and UME.

Groups	Metaphase	Range of SCEs	SCE/cell	MN/cell
Control	20	2-9	5.70 ± 1.69	1.75 ± 0.21
AFB_1 (5 μM)	20	7-12	7.20 ± 2.06^{a}	3.00 ± 0.50^{f}
AFB_1 (10 μM)	20	6-15	8.05 ± 2.14^{a}	4.90 ± 0.50^{f}
AFB_1 (5 μM) + UME1	20	5-10	6.70 ± 1.47^{b}	2.82 ± 0.30
AFB_1 (5 μM) + UME2	20	5-9	$6.75 \pm 1.42^{b,d}$	2.70 ± 0.20
AFB_1 (10 μM) + UME1	20	4-11	5.75 ± 2.03^{e}	1.80 ± 0.15^{g}
AFB_1 (10 μM) + UME2	20	6-13	5.65 ± 1.67^{e}	1.77 ± 0.40^{g}

For SCE: $^{a}P < 0.001$ compared with control group, $^{b}P < 0.05$ compared with control, $^{c}P < 0.001$ compared with AFB_1 (5 μM) group, $^{d}P < 0.05$ compared with AFB_1 (5 μM) group, $^{e}P < 0.001$ compared with AFB_1 (10 μM) group. For MN: $^{f}P < 0.001$ compared with control group, $^{g}P < 0.001$ compared with AFB_1 (10 μM) group.

Table 2. SOD and GPx activities and the level of MDA in human lymphocytes incubated with AFB_1 and UME.

Groups	SOD (U/mg protein)	GPx (U/g protein)	MDA (μmol/L)
Control	6.27 ± 0.9	350 ± 26.56	1.18 ± 1.66
AFB_1 (5 μM)	4.81 ± 0.28[a]	220 ± 6.89[a]	1.51 ± 1.65[a]
AFB_1 (10 μM)	2.5 ± 0.13[a]	160 ± 5.33[a]	1.97 ± 2.38[a]
AFB_1 (5 μM) + UME1	5.15 ± 0.25[b,d]	290 ± 7.43[d]	1.27 ± 1.55[b]
AFB_1 (5 μM) + UME2	5.5 ± 0.47[b,c]	295 ± 5.66[d]	1.23 ± 1.76[d]
AFB_1 (10 μM) + UME1	4.85 ± 0.42[b,f]	308 ± 8.09[e]	1.27 ± 2.52[e]
AFB_1 (10 μM) + UME2	5.26 ± 0.13[b,f]	345 ± 7.20[e]	1.28 ± 2.78[e]

[a]$P < 0.001$ compared with control group, [b]$P < 0.05$ compared with control, [c]$P < 0.001$ compared with AFB_1 (5 μM) group, [d]$P < 0.05$ compared with AFB_1 (5 μM) group, [e]$P < 0.001$ compared with AFB_1 (10 μM) group, [f]$P < 0.05$ compared with AFB_1 (10 μM) group.

concentrations of AFB_1. However, the observed effect of AFB_1 at higher concentrations decreased after treatment with different concentrations of UME. Statistical analysis showed a significant difference in SOD and GPx activities and the MDA level between all groups treated with AFB_1, either combined with or separately from UME ($P < 0.01$ or 0.05) (Table 2).

Discussion

In this study, the protective role of UME against the toxic effects of AFB_1, which can cause genetic damage by decreasing antioxidant capacity, was studied. The genetic damage of AFB_1 on MN and SCE frequencies was decreased by treatment with different concentrations of UME. This is the first scientific report on the antigenotoxic and protective potential of UME, although recent studies have reported that several lichens' extracts have strong antigenotoxic activity in human lymphocytes *in vitro* (22,23). Zeytinoglu et al. showed that an extract from *Cetraria aculeata* had significant antigenotoxic activity in bacterial systems and cytotoxic activity in some mammalian cancer types (24). Furthermore, Uysal et al. reported that the water extract of *U. longissima* had a positive effect on the lifespan of *Drosophila melanogaster* (25). Previous studies showed that the usnic acid isolated from *Usnea* species has antimicrobial and antimitotic activities (6,26). In addition, usnic acid, barbatic acid, diffractaic acid, demethylbarbatic acid, and evernic acid were isolated from *U. longissima* and their inhibitor activities on the Epstein-Barr virus were shown by Yamamoto et al. (27). Halici et al. reported that the water extract of *U. longissima* had a protective effect in indomethacin-induced ulcers, and this protective effect was linked to the antioxidant effect of the lichen extract (28)

Our results also showed that AFB_1 decreased the activities of SOD and GPx and increased the MDA level. However, the observed effect of AFB_1 at higher concentrations decreased after treatment with different concentrations of UME.

Previous research has reported that some lichens have antioxidant capacities, and the antioxidant effects of the lichen extracts have been linked to polyphenolic compounds such as epigallocatechin, gallate, quercetin, gallic acid, curcumin, eugenol, usnic acid, polysaccharide Ci-3, lichestrerinic acid, protolichesterinic acid, and nonpolyphenolics (28-31). Polyphenolic substances are known to have more

or less antioxidant activity. Likewise, it was reported that the phenolic compounds do not react covalently with AFB_1, and the inhibitory effect of phenolic compounds on AFB_1-induced mutagenicity could be due to the increase of antioxidant enzymes (23,32). Again, antimutagenic activities of natural phenolic compounds were established in some plant species, including common bean (32) and various teas (33), against AFB_1. In summary, the antigenotoxic effect of UME may be, at least in part, attributed to the antioxidant activity of the mentioned compounds, as AFB_1 is known to induce mutagenic damage through oxidative stress. Our future studies will therefore be focused on the fractionation and isolation of the crude extract of the lichen samples containing the active components responsible for the antigenotoxic and antioxidant activities.

Acknowledgement

The authors are grateful to the volunteers for blood samples. This investigation was supported by Atatürk University (Project No: 2009-85) and Fatih University (Project No: P50031005-2).

References

1. Ahmadjian V. The lichen symbiosis. New York: Wiley; 1993.
2. Ingolfsdottir K. Usnic acid. Phytochemistry 2002; 61: 729-736.
3. Richardson D. Medicinal and other economic aspects of lichens. Boca Raton (FL): CRC Press; 1988.
4. Huneck S. The significance of lichens and their metabolites. Naturwissenschaften 1999; 86: 559-570.
5. Lal B, Upreti DK. Ethnobotanical notes on three Indian lichens. Lichenologist 1995; 27: 77-79.
6. Cansaran D, Çetin D, Halıcı MG, Atakol O. Determination Of usnic acid in some *Rhizoplaca* species from middle Anatolia and their antimicrobial activities. Z Naturforsch C 2006; 61: 47-51.
7. Bezivin C, Tomasi S, Rouaud I, Delcros JG, Boustie J. Cytotoxic activity of compounds from the lichen: *Cladonia convoluta*. Planta Med 2004; 70: 874-877.
8. Guindon KA, Bedard LL, Massey TE. Elevation of 8-hydroxydeoxyguanosine in DNA from isolated mouse lung cells following in vivo treatment with aflatoxin B_1. Toxicol Sci 2007; 98: 57-62.
9. Rastogi R, Srivastava AK, Rastogi AK. Long term effect of aflatoxin B_1 on lipid peroxidation in rat liver and kidney: effect of picroliv and silymarin. Phytother Res 2001; 15: 307-310.
10. Sohn DH, Kim YC, Oh SH, Park EJ, Li X, Lee BH. Hepatoprotective and free radical scavenging effects of *Nelumbo nucifera*. Phytomedicine 2003; 10: 165-169.
11. Purvis OW, Coppins BJ, Hawksworth DL, James PW, Moore DM. The lichen flora of Great Britain and Ireland. London: Natural History Museum Publications in association with The British Lichen Society; 1992.
12. Wirth V. Die Flechten Baden-Württembergs. Stuttgart; Ulmer 1992.
13. Aslan A. Lichens from the region of Artvin, Erzurum and Kars (Turkey). Israel Journal of Plant Sciences 2000; 48: 143-155.
14. Aslan A, Yazici K, Karagöz Y. Lichen flora of the Murgul district, Artvin, Turkey. Israel Journal of Plant Sciences 2002; 50: 77-81.
15 Lin J, Opoku AR, Geheeb-Keller M, Hutchings AD, Terblanche SE, Jager AK et al. Preliminary screening of some traditional zulu medicinal plants for anti-inflammatory and anti-microbial activities. J Ethnopharmacol 1999; 68: 267-274.
16. Perry P, Evans HJ. Cytological detection of mutagen-carcinogen exposure by sister chromatid exchange. Nature 1975; 258: 121-125.
17. Countryman PI, Heddle JA. The production of micronuclei from chromosome aberrations in irradiated cultures of human lymphocytes. Mutat Res 1976; 41: 321-332.
18. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.
19. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med 1967; 70: 158-169.
20. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979; 95: 351-358.
21. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 1976; 72: 248-54.
22. Geyikoglu F, Turkez H, Aslan A. The protective roles of some lichen species on colloidal bismuth subcitrate genotoxicity. Toxicol Ind Health 2007; 23: 487-492.
23. Türkez H, Geyikoglu F, Aslan A, Karagöz Y, Türkez O, Anar M. Antimutagenic effects of lichen *Pseudovernia furfuracea* (L.) Zoph. extracts against the mutagenicity of aflatoxin B_1 in vitro. Toxicol Ind Health 2010; 26: 625-631.
24. Zeytinoglu H, Incesu Z, Tuylu BA, Turk AO, Barutca B. Determination of genotoxic, antigenotoxic and cytotoxic potential of the extract from lichen Cetraria aculeata (Schreb.) Fr. in vitro. Phytother Res 2008; 22: 118-123.

25. Uysal H, Altun D, Askin H, Aslan A. Effects of water extract of *Usnea longissima* Ach. on some development stages of *Drosophila melanogaster*. Fresenius Environmental Bulletin 2009; 18: 450-455.

26. Cardarelli M, Serino G, Campanella L, Ercole P, De Cicco Nardone F, Alesiani O et al. Antimitotic effects of usnic acid on different biological systems. Cell Mol Life Sci 1997; 53: 667-672.

27. Yamamoto Y, Kinoshita Y, Matsubara H, Kinoshita K, Koyama K, Takahashi K et al. Screening of biological activities and isolation of biological active compounds from lichens. Phytochemistry 1998; 2: 23-34.

28. Halici M, Odabasoglu F, Suleyman H, Cakir A, Aslan A, Bayir Y. Effects of water extract of *Usnea longissima* on antioxidant enzyme activity and mucosal damage caused by indomethacin in rats. Phytomedicine 2005; 12: 656-662.

29. Odabasoglu F, Aslan A, Cakir A, Suleyman H, Karagoz Y, Halici M et al. Comparison of antioxidant activity and phenolic content of three lichen species. Phytother Res 2004; 18: 938-941.

30. Gülçin İ, Oktay M, Küfrevioğlu Öİ, Aslan A. Determination of antioxidant activity of lichen *Cetraria islandica* (L) Ach. J Ethnopharmacol 2002; 79: 325-329.

31. Russo A, Bonina F, Acquaviva R, Campisi A, Galvano F, Ragusa N et al. Red orange extract: effect on DNA cleavage. Journal of Food Sciences 2002; 67: 2814-2818.

32. Cardador-Martinez A, Loarca-Pina G, Oomah BD. Antioxidant activity in common beans (*Phaseolus vulgaris* L.). J Agric Food Chem 2002; 50: 6975-6980.

33. Yen GC, Chen HY. Relationship between antimutagenic activity and major components of various teas. Mutagenesis 1996; 11: 37-41.