DNA damage which was demonstrated by DNA tail formation, lipid peroxidation which was demonstrated by the formation of thiobarbituric acid reactive substance, and protein oxidation which was demonstrated by protein carbonyl formation. Based on these results, oryzadine protected H_2O_2-induced cell damage. Our results show that the cytoprotective effects of oryzadine stem from its ability to inhibit H_2O_2-induced apoptosis, as demonstrated by a decrease in apoptotic body formation and the inhibition of mitochondrial membrane potential ($\Delta\psi$) loss. **References:** [1] Kim, E.S. et al. (2009) Bull. Korean Chem. Soc. 30:739 – 741. [2] Shin, J.S. et al (2008) Cell Biol. Int. 32:1099-1107.

PA71 Wheatgrass extract increases proliferation of RAW 264.7 macrophages induced by hydrogen peroxide (H_2O_2) or lipopolysaccharide (LPS)

Özkan T[1], Karabay ZA[3], Koç A[3], Karadag A[2], Aydos S[2], Çalıskan E[1], Öztürk G[2], Ilgaz SN[2], Yükselen I[2], Sunguroglu A[2]
[1]Ankara University, Institute of Biotechnology, Ankara, Turkey; [2]Ankara University, Faculty of Medicine, Department of Medical Biology, Ankara, Turkey; [3]Ankara University, Faculty of Pharmacy, Department of Biochemistry Ankara, Turkey

Wheatgrass, the young grass of *Triticum aestivum* L. contains chlorophyll, amino acids, minerals, vitamins, and enzymes and acclaimed to have antioxidant properties. In RAW 264.7 macrophages, a high level of NO production accompanied by cell apoptosis was achieved with LPS treatment (1). Direct treatment of cells with oxidants such as hydrogenperoxide(H_2O_2) was thought to exclusively cause necrosis and apoptosis (2). Therapies aimed to inhibit NO-dependent cell apoptosis and oxidative stress mediated cell toxicity may contribute to improving the outcome of various diseases. In this study, the effect of wheatgrass extract on proliferation of RAW 264.7 macrophages induced with H_2O_2 or LPS was tested. RAW 264.7 cells seeded in 96 well plates were incubated with (positive controls) or without (negative controls) different concentrations of wheatgrass extracts dissolved in water, LPS(1 µg/ml and 10 µg/ml) or H_2O_2 (500 µM) for 24 h. To test the effect of wheatgrass extract on proliferation, cells were pre-treated with different concentrations of wheatgrass extract for 1 h and then induced with LPS or H_2O_2 for 24 hours. At the end of the incubation period cell proliferation was estimated by MTT test and the statistical significance of differences was evaluated using one-way ANOVA. After 24 hours of incubation with LPS(1 µg/ml and 10 µg/ml) and H_2O_2 (500 µM) cell proliferation decreased significantly ($p < 0{,}0001$) and wheatgrass extract increased cell proliferation in both LPS and H_2O_2 induced cells. The effective proliferative doses of wheatgrass extract in H_2O_2 and LPS induced cells were found to be 0,5%; 1,5%; 2,5%; 3,5%, 5%, 7,5%, 10%v/v with p values of < 0,0001 and < 0,001 respectively. Our previous research has demonstrated that wheatgrass extract induced apoptosis and decreased proliferation in various cancer cell lines (3). While wheatgrass has an antiproliferative effect on leukaemia cells, it protects macrophages which are one of the immune system cells against death. **References:** [1] Slomiany, B.L. et al. (1998) Mol. Biol. Int. 46:1063 – 1070. [2] Zhang, Y. et. al. (2005) Apoptosis 10:545 – 556. [3] Karadag, A. et al. (2007) Planta Med. 73:991 – 992.

PA72 Topical anti-inflammatory effects of *Ocimum basilicum* leaf extract in the phorbol-12,13-dibutyrate model of mouse ear inflammation

Yadav NP[1], Khatri R[2], Bawankule DU[1], Pal A[1], Shanker K[1], Srivastava P[1], Gupta AK[1], Chanda D[1]
[1]Central Institute of Medicinal and Aromatic Plants, P.O. CIMAP, Lucknow-226015, India; [2]Department of Pharmaceutical Sciences, Dr. H.S. Gour University, Sagar (M.P.)-470003, India

Indian basil *(Ocimum basilicum* L. Family Lamiaceae) is a well-known traditional medicinal plant in the Indian subcontinent. The wound healing activity of the leaves has been reported in our earlier work [1]. In search of possible mode of action for wound healing activity, the topical anti-inflammatory property of the ethanol-water (25%) extract of leaves in mice has been carried out. Swiss albino mice of 22 – 25 g of weight were used and approved by the ethical committee of the institute. Mice were divided into four groups (n = 5) viz. Normal, toxin control, positive control and treated group. Animals of each group except normal group were applied phorbol-12,13-dibutyrate (1 µg daily) on the right ear for 5 days as toxin. After 30 min of toxin application, the animals of Group III were applied 20 mg of cream formulation of indomethacin and Group IV animals were applied OB extract (4 mg) daily. On the 5th day, all the animals were sacrificed and their ears were separated for the estimation of various parameters viz. ear weight, lipid peroxidation, interleukin1-β, interleukin-6 and tumor necrosis factor-α. OB extract significantly ($p \leq 0.05$) reduced the ear weight variation (difference in the weight of right and left ear of animals), levels of LPO (malonaldehyde), IL-1β, IL-6 and TNF-α when compared with toxin group using ANOVA test and as shown in table.

Parameters	Group I Normal (mean ± SEM)	Group II Toxin control (mean ± SEM)	Group III Positive control (mean ± SEM)	Group IV OB extract (mean ± SEM)
Ear weight variation (mg)	3.47 ± 0.187	34.54 ± 0.39 [a]	19.34 ± 4.56 [b]	17.43 ± 1.54[b]
LPO (pg/ml)	11.05 ± 2.38	63.84 ± 3.196 [a]	19.65 ± 2.09 [b]	20.72 ± 2.10 [b]
IL-1β (pg/ml)	57.04 ± 4.10	606.02 ± 7.37 [a]	292.03 ± 10.67 [b]	362.01 ± 12.11 [b]
IL-6 (pg/ml)	118.79 ± 7.50	1431.84 ± 34.72 [a]	701.88 ± 18.78	644.07 ± 14.59 [b]
TNF-α (pg/ml)	60.14 ± 2.54	221.89 ± 8.05 [a]	166.29 ± 7.18	95.76 ± 3.68 [b]

Values with 'a' exhibit significant difference ($p \leq 0.5$) when compared to normal group and value with 'b' exhibit significant difference ($p \leq 0.5$) from toxin group. Therefore we can conclude that ethanol-water extract of OB has shown significant anti-inflammatory activity against phorbol-12,13-dibutyrate induced topical inflammation in mouse ear. **Reference:** [1] Yadav, N. et al. (2008) J. Pharm. Pharmacol. 60(Sup.1):A-31.

PA73 Phytochemical characterization of *Juniperus* spp. leaves

Tavares L[1], Pimpão RC[1], Santos C[1], McDougall GJ[2], Stewart D[2], Ferreira RB[1,3]
[1]Disease & Stress Biology Laboratory, Instituto de Tecnologia Química e Biológica, New University of Lisbon, Portugal; [2]Plant Products and Food Quality Programme, Scottish Crop Research Institute, Dundee, DD2 5DA, Scotland, UK; [3]Departamento de Botânica e Engenharia Biológica, Instituto Superior de Agronomia, Technical University of Lisbon, Lisbon, Portugal

Juniperus is the second most abundant genus among the conifers. Numerous folk medicinal uses have been reported for *Juniperus* leaves and fruits, such as their application as antirheumatic, blood cleansing, digestive, diuretic and febrifuge agents; they have also been used in the treatment of arteriosclerosis, bronchitis, colic, common cold, cough, inflammation, tuberculosis, cancer, psoriasis and wounds [1]. The aim of this work was to evaluate the potential application of *Juniperus* leaves from species naturally occurring in Portugal (*J. phoenicea* subsp. *phoenicea*, *J. turbinata*, *J. oxycedrus* subsp. *oxycedrus*, *J. oxycedrus* subsp. *badia* and *J. navicularis*) against some diseases in which oxidative reactions play a crucial role. To this end, the seasonal evolution of total polyphenols [2], total flavonoids [3] and antioxidant activity for peroxyl radical [4] was determined. All species exhibited minimum polyphenol and flavonoid contents in March/April and July and therefore a reduced antioxidant activity. Maximum concentrations of these compounds were detected in November/December, with the levels of antioxidant activity peaking three times a year, May/June, August/September and November/December. *J. phoenicea* subsp. *phoenicea*, the most widespread species, showed the lowest levels of polyphenols, flavonoids and antioxidant activity. To compare their metabolite composition by HPLC-MS, leaves from all *Juniperus* under study were collected in November/December. The polyphenolic profiles obtained for *J. phoenicea* subsp. *phoenicea* and *J. turbinata* are very similar. Analogous HPLC profiles were also obtained for both *J. oxycedrus* subspecies and for *J. navicularis*. *Acknowledgements: To FCT for financial support of C. Santos (SFRH/BPD/26562/2006) and L. Tavares (SFRH/BD/37382/2007).* **References:** [1] Johnson, T. (1999) CRC Ethnobotany Desk Reference. CRC Press. Boca Raton. [2] Singleton, V.L. et al. (1965) Am. J. Enol. Vitic. 16:144 – 158. [3] Michalska, A. et al. (2007) Eur. Food Res. Technol. 225:545 – 551. [4] Cao, G. et al. (1993) Free Radic. Biol. Med. 14:303 – 311.

■ Korrekturexemplar: Veröffentlichung (auch online), Vervielfältigung oder Weitergabe nicht erlaubt! ■