Anti-proliferative and antioxidant properties of rosemary *Rosmarinus officinalis*

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Abstract. Constituents in rosemary have shown a variety of pharmacological activities for cancer chemoprevention and therapy in *in vitro* and *in vivo* models. In order to further explore the chemopreventive properties of crude extracts of rosemary (*Rosmarinus officinalis* L), we studied its anti-proliferative property on several human cancer cell lines and its antioxidant and anti-inflammatory properties *in vitro* in a mouse RAW 264.7 macrophage/monocyte cell line. Our study shows that crude ethanolic rosemary extract (RO) has differential anti-proliferative effects on human leukemia and breast carcinoma cells. The 50% inhibitory concentration (IC50) was estimated at 1/700, 1/400, 1/150 and 1/500 dilutions, for the HL60, K562, MCF7 and MDA-MB-468 cells, respectively. Non-cytotoxic concentrations of RO at 1/1000 dilution minimally induced HL60 cell differentiation into granulocyte lineage at 9.5±2.2% compared to 2.8±0.8% in the untreated control ($p<0.001$), and did not induce HL60 cell differentiation into monocyte/macrophage lineage. The 6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) equivalent antioxidant capacity assay showed that RO has substantial antioxidant activity with RO at 1/10 and 1/5 dilutions having 8.1 and 12.6 μM Trolox equivalents, respectively. RO at non-cytotoxic 1/2000 and 1/1000 dilutions did not affect nitric oxide (NO) production by non-stimulated RAW 264.7 cells. However, at the same dilutions RO significantly reduced NO production by lipopolysaccharide (LPS)-activated cells in a dose-dependent manner from 32.6±2.3 μM in the LPS-activated cells to 19.2±2.2 μM ($p<0.01$), and 7.7±1.2 μM ($p<0.001$), respectively. RT-PCR analyses showed that RAW 264.7 cells treated with 1/1000 and 1/500 dilutions for 5 h did not affect TNFα, IL-1ß, iNOS and COX-2 mRNA expression in these cells when compared to the untreated controls, nor did the 1/1000 dilution of RO affect TNFα, IL-1ß, iNOS and COX-2 mRNA expression in the LPS-activated cells. At 1/500 dilution, RO significantly reduced IL-1ß ($p<0.01$) and COX-2 ($p<0.05$) mRNA expression and non-significantly reduced TNFα and iNOS mRNA expression in the LPS-activated cells. In view of the chemopreventive potentials, further studies are needed to explore other biological properties of this popular spice used by many cultures in the world.

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Introduction

Dietary phytochemicals are a rich source of flavonoids shown to have chemopreventive properties. Rosemary carnosol, green tea epigallocatechin gallate, tumeric, and curcumin are among the well-studied dietary phytochemicals in literature. The chemical constituents of rosemary (*Rosmarinus officinalis* L, family Lamiaceae) include flavonoids such as carnosol, carnosic and rosmarinic acid, and volatile oils (1,2). Carnosol and carnosic acid constitute 3.8-4.6% of the material extracted from rosemary, and they are among the many constituents in rosemary that show pharmacological properties (3). Carnosol has shown a number of *in vitro* and *in vivo* biological activities including strong antioxidant activity by nitric oxide inhibition assay (4,5), anti-mutagenic effects in the Ames assay (6), inhibition of DNA adduct formation in human bronchial cells (7), *in vitro* anti-metastasis in B16/F10 mouse melanoma cells (8), and *in vivo* inhibition of tumorigenesis in rodent skin (3), mammary (9), and gut (10) models. Constituents in the methanol extract of rosemary other than carnosol, and carnosic and rosmarinic acid are thought to be responsible for the inhibition of P-glycoprotein activity in a multidrug-resistant MCF7 cell line (11). In addition, the water insoluble fraction of rosemary extract increased nerve growth factor production in T98G human glioblastoma cells. This activity could be attributed to carnosic acid (12). These results suggest that constituents of rosemary other than carnosol may have pharmacological effects for cancer chemoprevention and therapy. In order to further explore the chemopreventive properties of crude extracts of rosemary, we studied its antioxidant and anti-inflammatory properties in an *in vitro* mouse RAW 264.7 macrophages system, and its anti-proliferative activity on several human tumor cell lines.

Materials and methods

Plant material and preparation of the extract. Two different lots of *Rosmarinus officinalis* dry leaves were purchased from the herbs and spice section in a grocery store in Vancouver. Voucher samples are stored in the authors' laboratory. The dry leaves were pulverized into fine powder in a blender. For cell culture studies, 1 g of the powder was extracted with 10 ml 70% ethanol for 2 h at 55˚C. The suspension was centrifuged at 5,000 x g for 10 min and the supernatant [rosemary extract (RO)] yielded a stock with an estimated 13.4 mg dry material in 1 ml of the RO extract. Varying dilutions of this extract were used for cell culture and other assay studies.

Chemicals and reagents. Unless specified, all chemicals were of reagent grade purchased from Sigma-Aldrich of Canada (Oakville, Ontario, Canada). Camptothecin (CAM, cat. no. C9911) and paclitaxel (PTX, cat. no. T7402) were dissolved in dimethyl sulfoxide (DMSO) as 1 mM stock and further diluted to the desired concentrations with a culture medium. 6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox, cat. no. 39,192-1), 2,2'-azinobis[3-ethylbenzothiazoline-6-sulfonic acid] (ABTS, cat. no. A1888), lipopolysaccharide (LPS cat. no. L2630), 4-beta-phorbol-12-myristate-13-acetate (PMA, cat. no. P8139), dimethyl formamide (DMF), nitro-blue tetrazolium (NBT, cat. no. N6876).

High performance liquid chromatography (HPLC) profile. A 70% ethanol extract of rosemary was prepared according to the prescribed method. British Columbia's Institute of Technology Forensic Science Center performed the HPLC fingerprint. An injection of 5 μl of this extract was used for chromatographic analysis. HPLC analysis was carried out using an Agilent 1100 HPLC equipped with a binary pump, autosampler, thermostatted column compartment, and a diode array detector. The mobile phase used was: Solvent A (H_2O; 0.1% formic acid) and Solvent B (Acetonitrile; 0.1% formic acid). The flow rate was 1 ml/min with a column temperature of 20˚C for analysis in a Zorbax SB-C18 4.6x150 mm with a 3.5-um particle size column (Agilent Technologies Inc. pn 863953-902). It was found that 210 nm provided the best representation of compounds present in the extract. The chromatograms for the ethanol extracts were background subtracted using the ethanol water mixture used for the extraction process.

Antioxidant activity - Trolox equivalent antioxidant capacity (TEAC). The test is based on the reduction of the ABTS radical cation by antioxidants (13,14). Trolox (25 mM) was prepared in ethanol for use as a stock standard. Fresh working standards of 0.5, 1 and 2 mM were prepared by dilution with distilled water. The stock solution of the ABTS radical cation was prepared by mixing ABTS (7 mM) with 2.45 mM potassium persulfate in water. The mixture was kept for 12-24 h at ambient temperature in the dark until the reaction was complete and the absorbance became stable. In order to prepare an $ABTS^{\bullet+}$ working solution, the $ABTS^{\bullet+}$ stock solution was diluted with water to an absorbance of 0.700±0.02 at 734 nm. For the photometric assay, 1 ml $ABTS^{\bullet+}$ working solution and 10 μl Trolox standards or test solution were mixed for 45 sec and measured immediately after 1 min at 734 nm. The antioxidant activity of the test substances was calculated by determining the decrease in absorbance using the following equation: % antioxidant activity = {(E [$ABTS^{\bullet+}$ - E [standard])/ E [$ABTS^{\bullet+}$]} x 100. The Trolox equivalents were estimated by linear interpolation of the antioxidant activity from the Trolox standards.

Cell lines and culture conditions. Human mammary adenocarcinoma MCF7 and MDA-MB-468, human promyelomonocytic HL60 cells, and the murine RAW264.7 macrophage/monocyte cell line were purchased from the American Type Culture Collection (Rockville, MD, USA). The human leukemia cell line, K562, was a gift from Dr A.J. Tingle, at British Columbia's Research Institute for Children's and Women's Health. The MDA-MB-468 cells were cultured in L15 medium, the HL60 cells were cultured in Iscove's medium, and the MCF7, K562 and RAW264.7 cells were cultured in Dulbecco's minimal essential (DMEM) medium supplemented with 10% fetal bovine serum, 2 mM L-glutamine, and 50 μg/ml gentamycin. The cultures were maintained in a humidified 5% CO_2 incubator at 37˚C except for MDA-MB-468, which was maintained in an incubator with air. The cells were sub-cultured every 3-4 days to maintain logarithmic growth and were allowed to grow for 24 h before use.

Cell proliferation assay. For testing, the tumor cells were cultured in 96-well plates. The starting cell numbers were $2.5x10^4$ cells per well for K562, and 10^4 cells for MCF7 and MDA-MB-468. For the HL60 cells, the starting cell number was 10^5 cells per well in 24-well plates in order to coordinate with the cell differentiation assay. After the cells stabilized overnight, triplicate (duplicate for HL60) samples of cells were treated with a culture medium containing different dilutions of RO extract. The chemotherapeutic agents, CAM or PTX, were included in the assays as the positive controls. Cell counting was set at 2 days for K562, 3 for HL60, 4 for MCF7 and 5 for MDA-MB-468. Cell numbers were determined by hemocytometer counting and viability was assessed by the trypan blue dye exclusion test. Cell counts in the samples treated with the test compounds were normalized to a percentage of the control, and the means and standard errors (SEM) were calculated from at least three independent experiments (15). The fifty percent inhibitory concentration (IC50) of the anti-proliferative effects was determined by linear regression estimation.

Cell differentiation assay.

Differentiation into granulocyte lineage by NBT reduction assay. Duplicate 50 μl aliquots of HL60 cells treated with the different dilutions of RO were incubated with an equal volume of phenol red free RPMI medium containing 200 ng/ml PMA and 0.2% NBT at 37˚C for 45 min in a flat bottomed 96-well tissue culture plate. For each preparation, 200 cells were examined microscopically to determine the proportion of cells containing blue-black formazan granules, indicative of the ability of HL60 to generate superoxide anion during an induced respiratory burst. In addition, cytocentrifuge smears of the control and treated cells were stained with Giemsa and

analyzed by microscopy to allow the observation of granulocytic features i.e. a multilobular nucleus, and prominent cellular indentation. The positive control of differentiation to the granulocytes was achieved by the addition of 100 mM DMF. The results are expressed as a percentage of the NBT-positive cells (16).

Differentiation into macrophage/monocyte lineage by non-specific esterase (NSE) activity assay. Standard NSE staining (17) was performed on duplicate microscope slides from samples prepared by a cytocentrifuge. Two hundred cells were examined under a microscope in order to determine the proportion of NSE-positive cells (a brownish red cytoplasm). Cells treated with 25 or 50 ng/ml PMA were used as the positive control.

Inhibition of LPS-activated nitric oxide (NO) production by RAW264.7 cells. RAW 264.7 cells were cultured in 24-well plates ($1x10^6$ cells/ml/well, NUNC culture plates) with phenol red free RPMI medium supplemented with 5% FBS. On the following day varying dilutions of RO extract were added to the RAW 264.7 cells alone, or in combination with 1 μg/ml LPS, in a fresh medium. After 48-h incubation at 37˚C, the amount of NO released into the culture supernatant was determined with Griess reagent and compared to a sodium nitrite standard of known concentration by absorbance at 550 nm. Cell proliferation and viability at the end of the experiment were determined by staining the cells with neutral red dye at 50 μg/ml for 1 h (18,19).

RT-PCR. RAW 264.7 cells (3 ml) at $2x10^6$ cells/ml, DMEM supplemented with 10% FBS, 2 mM L-glutamine and 50 μg/ml gentamycin, were cultured in each of the 6-well culture plates in a 37˚C, 5% CO_2 humidified incubator. After 48 h, the cells were treated with 1/1000 and 1/500 dilutions of RO alone or in combination with 1 μg/ml LPS in a fresh culture medium. Five hours later, RNA was isolated with TRIzol reagent (Invitrogen, Burlington, Ontario, Canada). Total RNA (1 μg) was reverse transcribed into cDNA using Superscript II reverse transcriptase according to the manufacturer's protocol (no. 18064, Invitrogen) at 42˚C for 50 min. PCR was performed with 100 ng of the cDNA template in 25 μl volumes containing 1x reaction buffer, 1.5 mM $MgCl^2$, 200 μM dNTPs, 0.5 U Platinum Tag DNA polymerase (no. 10966, Invitrogen), and 0.4 μM of the following primer pairs: IL1-ß, 5'- TTG ACG GAC CCC AAA AGA TG -3' and 5'- AGA AGG TGC TCA TGT CCT CA -3'; TNF-α, 5'- TCT CAT CAG TTC TAT GGC CC -3' and 5'- GGG AGT AGA CAA GGT ACA AC -3'; iNOS, 5'- CCC TTC CGA AGT TTC TGG CAG CAG -3' and 5'- GGC TGT CAG AGA GCC TCG TGG CTT TGG -3'; COX-2, 5'- CCC CCA CAG TCA AAG ACA CT -3' and 5'- CCC CAA AGA TAG CAT CTG GA -3' and G3PDH, 5'- TGA AGG TCG GTG TGA ACG GAT TTG GC -3' and 5'- CAT GTA GGC CAT GAG GTC CAC CAC -3 (20-22).

After an initial denaturation for 2 min at 94˚C, 28-35 cycles of amplifications (55˚C for 40 sec, 72˚C for 1 min, and 94˚C for 1 min) were performed followed by a 7-min extension at 68˚C in a DNA Thermal Cycler 480 (Perkin-Elmer, Norwalk, CT, USA). A 10 μl aliquot from each PCR reaction was electrophoresed in 2% agarose gels and visualized by ethidium bromide staining. The PCR products of mRNA expression were semi-quantitatively measured using the GelDoc system with a CCD camera (BioRad, Molecular Analyst), and the relative IL-1ß, TNF-α, iNOS and COX-2 signals were normalized against the housekeeping gene G3PDH. Data were expressed as the ratio of G3PDH.

Statistical analysis. Data are presented as the mean ± SEM from at least three independent experiments. The Student's two-tailed unpaired t-test was used in a statistical comparison of the two groups. Differences were considered significant when $p<0.05$.

Results

HPLC profile. HPLC spectra at 210, 250, 280 and 300 nm showed that numerous compounds are present in the RO extract. Fig. 1 shows a HPLC spectrum at 210 nm with the most spectral peaks.

Anti-proliferative activity. Fig. 2 shows that the RO extract caused differential anti-proliferative activity on the breast and leukemia cell lines tested, with HL60 being the most susceptible, and MCF7 the most resistant. IC50 was estimated at 1/700, 1/400, 1/150 and 1/500, for the HL60, K562, MCF7 and MDA-MB-468 cells, respectively.

HL60 cell differentiation. Fig. 3 shows that the RO extract at 1/1000 dilution induced 9.5±2.2% of HL60 cell differentiation along granulocyte lineage with $p<0.01$ compared to the untreated control at 2.8±0.8%. Non-cytotoxic concentrations of RO had no effect on the monocyte/macrophage differentiation of the HL60 cells.

Antioxidant activities. Cell free ABTS OH radical scavenging assay showed that the RO extract has substantial antioxidant activity with 1/10 and 1/5 dilutions of the RO showing 8.1 and 12.6 μM of the Trolox equivalents, respectively (Fig. 4A). Fig. 4B shows the effects of RO on NO production by the LPS-activated RAW 264.7 cells. The basal level of NO represented by the nitrite concentration in the culture medium was <2 μM. The addition of non-cytotoxic concentrations of RO at 1/2000 and 1/1000 dilutions to non-stimulated RAW 264.7 cells did not affect the NO production by these cells. However, RO at these concentrations significantly inhibited NO production by the LPS-activated RAW 264.7 cells in a dose-dependent manner. NO in the culture supernatant was reduced from 32.6±2.3 μM in the LPS-activated cells to 19.2±2.2 μM ($p<0.01$) and 7.7±1.2 μM ($p<0.001$), with the addition of 1/2000 and 1/1000 dilutions of RO, respectively. The RO extract at these dilutions was not cytotoxic to the RAW 264.7 cells as the cell viability assay by neutral red incorporation was >80% when compared to the untreated control.

RT-PCR. Fig. 5 shows that treatment with 1/1000 and 1/500 dilutions of RO extract for 5 h did not affect TNFα, IL-1ß, iNOS and COX-2 mRNA expression in RAW 264.7 cells when compared to the untreated controls, nor did treatment

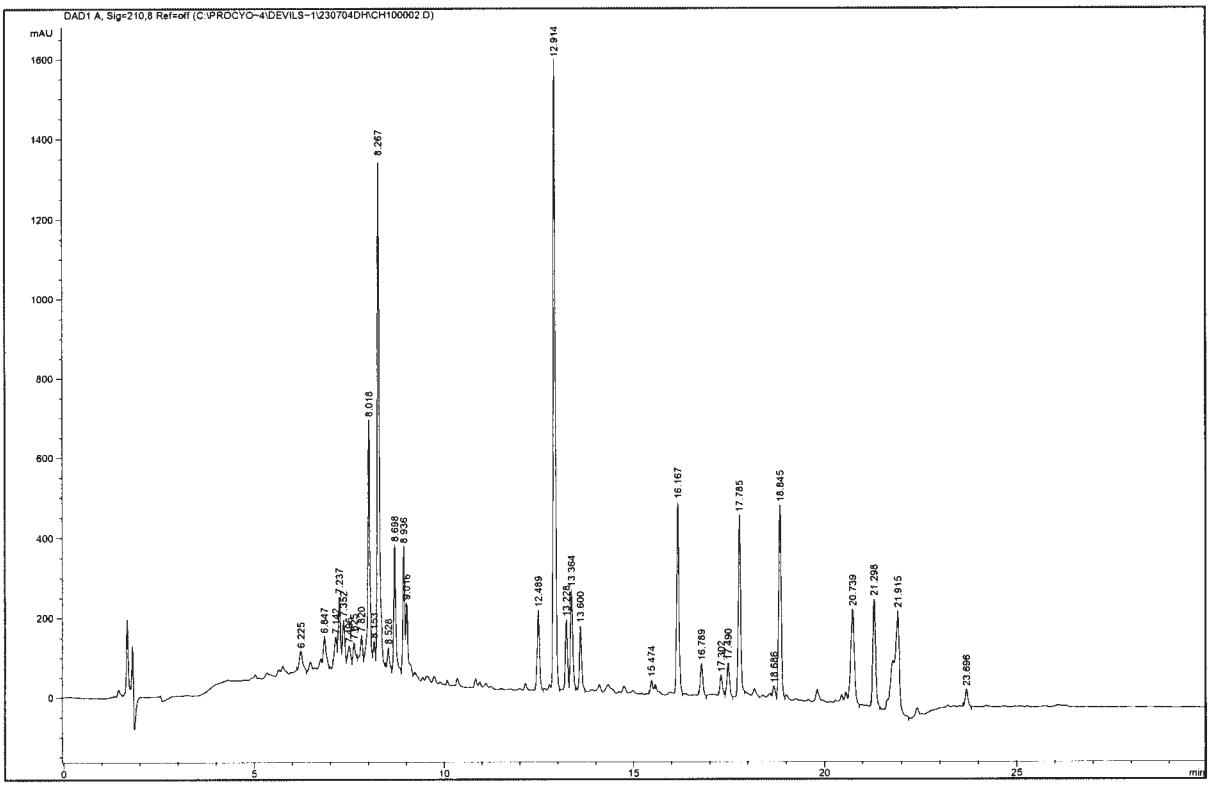

Figure 1. High performance liquid chromatography profile of 70% ethanol extract of *Rosmarinus officinalis* at 210 nm.

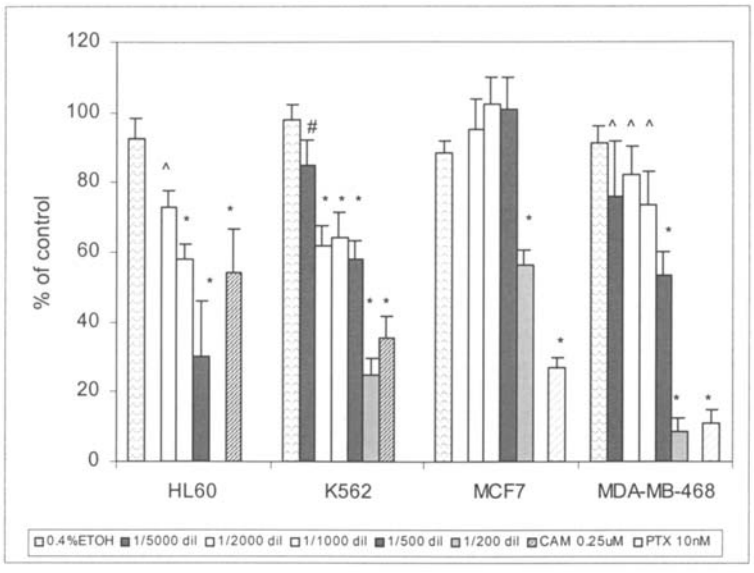

Figure 2. Effects of the rosemary ethanol extract on the proliferation of HL60, K562, MCF7 and MDA-MB-468 cells. The conditions for cell culture are described in 'Materials and methods'. Cell proliferation was assessed by the hemocytometer counting of viable cells with the trypan blue exclusion method. The results are presented as the means ± SEM of at least three independent experiments normalized to the control. Dilutions of rosemary ethanol crude extract (1/5000, 1/2000, 1/1000, 1/500 and 1/200 dil) were added to the cell cultures. ETOH (ethanol) (0.4%), CAM (camptothecin) 0.25 μM and PTX (paclitaxel) 10 nM were the negative and positive controls for the cell proliferation assay. t-test: $^{\wedge}p<0.05$, $^{\#}p<0.01$, $^{*}p<0.001$ compared to the untreated control.

with 1/1000 dilution of RO affect TNFα, IL-1ß, iNOS and COX-2 mRNA expression in the LPS-activated cells. At 1/500 dilution, RO significantly reduced IL-1ß ($p<0.01$) and COX-2 ($p<0.05$) mRNA expression, but non-significantly reduced TNFα and iNOS mRNA expression in the LPS-activated cells.

Discussion

Rosemary crude extract and its constituents carnosol and carnosic acid have been reported to show chemopreventive benefits in *in vivo* anti-tumorigenesis studies, with the activation of phase I and phase II detoxifying enzymes being

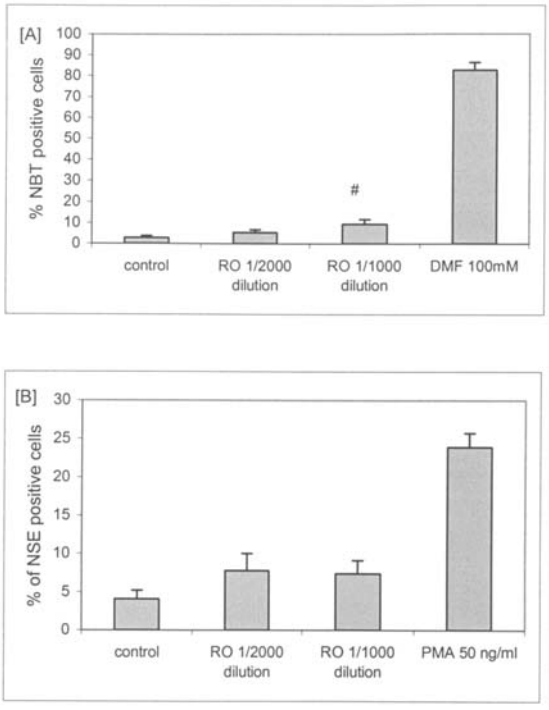

Figure 3. Effects of rosemary extracts (RO) on the differentiation of HL60 leukemia cells. HL60 cells (1 ml) at $1x10^5$/ml were transferred to each 24-well culture plate. After overnight culture, fresh media containing RO extracts at 1/500, 1/1000 or 1/2000 dilutions were added, and the cells were cultured for an additional 3 days for nitro-blue tetrazolium (NBT), and non-specific esterase (NSE) assay. Panel (A) shows the effects of RO on HL60 cell differentiation along the granulocyte lineage by the presence of NBT-positive cells. Panel (B) shows the effects of RO on HL60 cell differentiation along the macrophage/monocyte lineage by the presence of NSE-positive cells. ETOH (0.4%) is the vehicle control. 4-Beta-phorbol-12-myristate-13-acetate (PMA) (50 ng/ml) and dimethyl formamide (DMF) (100 mM) are inducers of macrophage/monocyte, and granulocyte differentiation, respectively. #p<0.05 when compared to the untreated control.

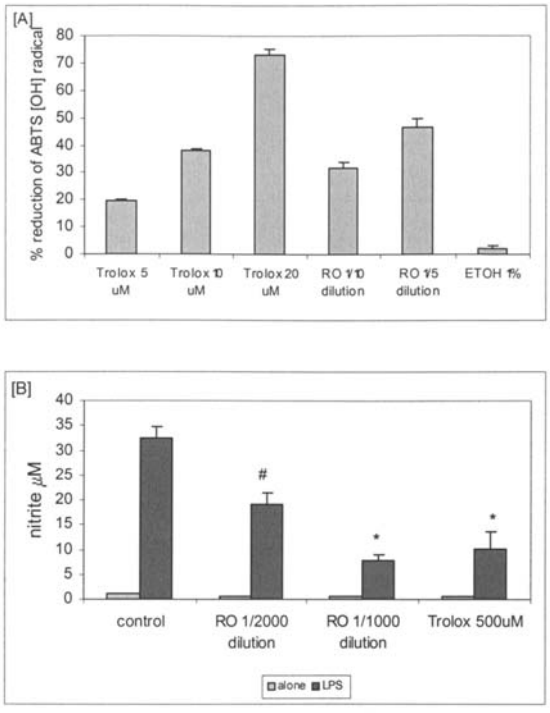

Figure 4. Antioxidant properties of the rosemary ethanol extract (RO). Panel (A) shows the antioxidant activity of RO measured by 6-Hydoxy-2,5,7,8-tetramethyl-chromasn-2-carboxilic acid (Trolox) equivalent antioxidant capacity assay. The reduction of the ABTS OH radical by RO was compared to Trolox, a chemical with potent antioxidant activity by this assay. Panel (B) shows the effects of RO on nitric oxide (NO) production in the mouse macrophage RAW 264.7 cell line. RAW264.7 cells were treated with 1/2000 or 1/1000 dilutions of RO alone or in combination with lipopolysaccharide (LPS) (1 μg/ml) for 48 h. The culture supernatants were subsequently collected and analyzed for NO content represented as nitrite levels with Griess reagent. Data represent the means ± SEM of at least six independent experiments. RO significantly attenuated NO production in a concentration-dependent manner in the LPS-activated RAW 264.7 cells. Student's t-test: #p<0.01 and *p<0.001 compared to the LPS-stimulated samples.

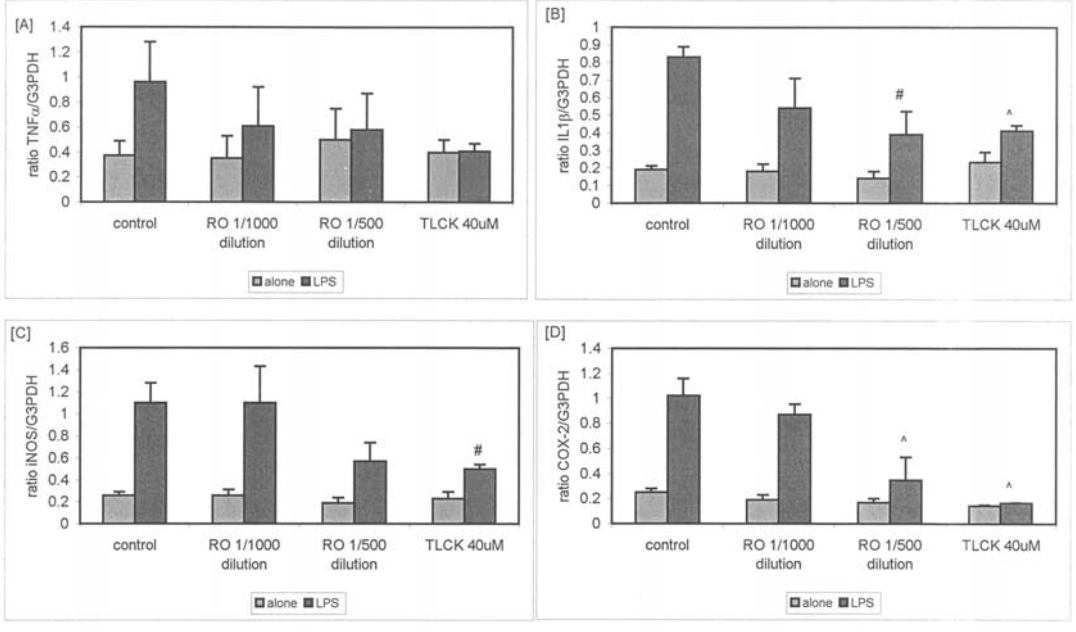

Figure 5. RT-PCR analyses of the effects of rosemary ethanol extract (RO) on the mRNA expression of (A) TNF-α, (B) IL1-ß, (C) iNOS, and (D) COX-2 in RAW 264.7 cells stimulated with lipopolysaccharide (LPS) (1 mg/ml) for 5 h. Data represent the densitometric analyses from gel photographs of the gene product normalized to that of the housekeeping gene, G3PDH. Results are the means ± SEM of more than three independent experiments. TLCK (40 μM) is an inhibitor of iNOS mRNA transcription via the NF-κB pathway. ^p<0.05 and #p<0.01, compared to the LPS-stimulated samples by the t-test.

implicated as the mechanisms of action (7,9,23). *In vitro* studies have shown that purified carnosol can interfere with tumor cell metastasis, chemtotaxis and attachment (8). There are several reported studies in the literature on the anti-proliferative activities of rosemary or its constituents, including one on the effects of carnosic acid on HL60 and U937 cells (24), and another, on the effects of rosmarinic acid on murine mesangial cells (25). Our study here shows that crude ethanolic rosemary extract has differential anti-proliferative effects on human leukemia and breast carcinoma cells, consistent with the reported growth inhibition by rosmarinic acid from rosemary on murine mesangial cells stimulated with the platelet-derived growth factor (PDGF) or TNFα (25). An earlier report showed that carnosic acid, when used alone, is an ineffective inducer of HL60 cell monocyte/macrophage differentiation. Although a direct comparison is not possible, our study here shows that rosemary extract at a non-cytotoxic 1/1000 dilution induced only a small percentage (9%) of HL60 cell differentiation along the granulocyte lineage compared to the >85% induction by the positive control DMF at 100 mM, and is thus similar to the marginal induction of HL60 cell differentiation into granulocyte lineage by carnosic acid at 6-7 μM, or 2-3 μg/ml, as well as the minor effects of the monocyte/macrophage lineage differentiation by carnosic acid alone (24,26,27).

The antioxidant properties of rosemary crude extract and its constituent compounds have been well studied. Most of these reported antioxidant activities are based on cell-free biochemical tests of either crude extract or chemically fractionated constituents of rosemary. In this study, we used the 70% ethanol extract of rosemary and employed a biochemical assay and a cell-based *in vitro* assay for testing the antioxidant activities. Data from our biochemical TEAC assay were consistent with the reported hydroxyl radical scavenging antioxidant activity of rosemary and its purified constituents, including carnosic acid, carnosol, rosmarinic acid and ursolic acid, by the DPPH assay (22). The findings are not unexpected since both the TEAC assay and the DPPH assay estimated the hydroxyl radical scavenging effect of the chemicals. Of the two reported studies utilizing cell-based *in vitro* antioxidant assay, both reports utilized purified constituents of rosemary for the studies. They showed that carnosol, the purified constituent of rosemary, inhibited NO production in LPS-activated murine peritoneal macrophages and RAW 264.7 cells. In contrast, the other constituents of rosemary, carnosic, rosmarinic and ursolic acid, were ineffective (4,22). Lo *et al* (22) estimated that the IC50 of carnosol was 9.4 μM (3 μg/ml) for the inhibition of NO production in the LPS-activated RAW 264.7 cells, while in our assay a 1/1000 dilution of the rosemary ethanol crude extract, containing ~13.4 μg/ml dry material extracted from rosemary dry leaves, effectively inhibited NO production (76%) in these cells. Although we cannot make an exact comparison with the reported studies, our data on the rosemary crude extract are in general agreement with the study showing that carnosol inhibited NO production by LPS-activated RAW 264.7 cells.

The inhibition of NO production by LPS-activated RAW 264.7 cells is modulated in part through the inhibition of the iNOS gene expression. The inhibition of iNOS mRNA and protein expression by carnosol at 20 μM was effected through the inhibition of the NF-κB activity in these cells (22). RT-PCR data in this study showed that a 1/500 dilution of the rosemary crude extract suppressed, though non-significantly, the iNOS mRNA expression in LPS-activated RAW 264.7 cells. This discrepancy could be due to the higher carnosol dosage of 20 μM (6.7 μg/ml), and the longer exposure time of 12 h in Lo *et al*'s study, compared to the 5-h treatment time in our study.

Many natural polyphenolic compounds with antioxidant activities also have anti-inflammatory activities. Our RT-PCR data on the mRNA expression of the pro-inflammatory cytokines, TNFα and IL-1ß, and the COX-2 enzyme represent additional information on the many biological activities of rosemary.

Despite the many beneficial anti-proliferative, anti-tumorigenic, anti-metastatic, antioxidant and anti-inflammatory activities of rosemary being reported in literature, information is still lacking on the absorption and bioavailability of the active constituents of rosemary *in vivo*. With its suggested chemopreventive potentials, further studies are needed in order to explore other biological properties of this common spice used by many cultures in the world. It is unknown whether meaningful amounts of the biological active ingredients are present in the daily consumption with the common practice of food preparation. In conclusion, the rosemary ethanol crude extract shows differential anti-proliferative activity in several human tumor cell lines and demonstrates strong antioxidant activity by the TEAC hydroxyl radical scavenging assay. The crude extract also shows antioxidant and anti-inflammatory activities in the inhibition of NO production, and reduction of proinflammatory IL-1ß cytokine and COX-2 mRNA expression in LPS-activated RAW 264.7 cells which substantiates its chemopreventive potential.

Acknowledgements

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