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Antidiabetic Activity of Extracts from Needle, Bark, and Cone of *Picea glauca*: Organ-Specific Protection from Glucose Toxicity and Glucose Deprivation*

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Abstract

The incidence of type 2 diabetes mellitus has reached epidemic proportions worldwide. Canadian aboriginal communities, particularly the Cree Nation of Eeyou Istchee, have been identified as a high-risk population. Culturally acceptable treatment options are limited notably for diabetic complications resulting in peripheral neuropathy. Here, we describe results of an ongoing collaborative research project with Cree of Eeyou Istchee to identify botanicals capable of protecting peripheral neuronal precursors from glucose toxicity and glucose deprivation *in vitro*. Polar fractions of three plant organs (needles, cone, and bark) collected from *Picea glauca* (Moench) Voss (Pinaceae), were tested for toxicity under normoglucose, glucotoxicity, and glucose deprivation conditions. The profile of phenolic metabolites in each extract was first characterized by high-performance liquid chromatography-diode array detection-atmospheric pressure chemical ionisation mass spectrometry (HPLC-DAD-APCI/MS). We report here that these fractions are well-tolerated by PC12 neuronal precursors under normoglucose conditions. LD_{50} concentrations of needle extracts exceeded 100 μg/mL, whereas the LD_{50} of bark and cone extracts was 40 and 36.4 μg/mL, respectively. We further show that the cytoprotective properties of minhikw after glucose challenge are concentration-dependent and organ-specific. Needle extracts protected PC12 cells from both glucotoxicity and glucose deprivation. Bark extracts had negligible activity. Cone extracts further impaired PC12 cell glucose tolerance. This study provides the first validation of antidiabetic activity of minhikw organs at the cellular level relevant to the management of diabetic peripheral neuropathy.

Keywords: Glucose deprivation, glucose toxicity, *P. glauca*, natural health products, PC12 cells, peripheral neuropathy, plant extracts, Type 2 diabetes mellitus.

Introduction

The incidence of type 2 diabetes (T2D) has reached epidemic proportions afflicting more than 171 million persons worldwide (WHO, 2004). Prevalence rates are predicted to double over the next 30 years (WHO, 2004). Aboriginal populations are particularly susceptible with incidence 20–30% higher than dominant national cultures (for a review, see Yu & Zinman, 2007). In Canada, First Nations communities are recognized as among the highest at-risk populations in the world (Pollex et al., 2006). This increased disease incidence is influenced, in part, by specific genetic predispositions whose effects are emerging at alarming rates given changes in diet and lifestyle (Hegele, 2001). With the devastating health impact of "acculturation

*Dedicated to John Thor Arnason of the University of Ottawa, Department of Biology, on the occasion of his sixtieth birthday.

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and adoption of a 'Westernized' lifestyle" (Yu & Zinman, 2007), it is not surprising that poor disease prognosis is compounded by a low compliance rate with modern pharmaceuticals (Young et al., 2000). Arguably, most T2D treatment strategies are designed by professionals unfamiliar with the needs of the local community and without consideration of cultural awareness and accepted practice (Boston et al., 1997).

To address these problems, we have entered into a collaborative research program between basic scientists and elders and healers of the Cree of Eeyou Istchee (CEI). The Cree are the largest First Nations group in Canada with more than 72,000 persons (Statistics Canada, 2001). The Cree of Eeyou Istchee make up nine communities in the northern regions of the province of Quebec, Canada, represented by approximately 13,500 members. These communities are experiencing a dramatic increase in T2D incidence with few strategies designed to manage peripheral neuropathies associated with chronic pain, infection, paralysis, and loss of sensation (Kuzmina & Dannenebaum, 2004; Légaré, 2004). To evaluate alternative and complementary treatment strategies that promote culturally acceptable T2D management, we conducted ethnobotanical surveys targeting natural medicines used by CEI for the treatment of diabetic symptoms. Two CEI communities, representing inland and coastal communities, participated (Leduc et al., 2006). Multiple, potentially antidiabetic plants were identified in this survey and subsequently screened for a variety of *in vitro* antidiabetic activities, including the ability to protect peripheral neuronal precursors from glucose toxicity and glucose deprivation (Spoor et al., 2006). When the bioactivities of extracts made from separated organs of the same species (i.e., root, stem, leaf, and fruit extracts) were compared, certain plants exhibited organ-specific activities (Martineau et al., 2006), some of which could be attributed to differences in phenolic constituents within the plant organs (Harris et al., 2007). Here, we extend these findings to an analysis of minhikw, the Cree term for *Picea glauca*, a plant reported useful for controlling diverse diabetic complications among several First Nations groups (Arnason et al., 1981; Leduc et al., 2006), particularly the Cree of Whapmagoostui. We report that polar fractions of *Picea glauca* (minhikw) needle are more effective than bark and cone extracts at protecting PC12 peripheral neuronal precursors from both glucose toxicity and glucose deprivation. The profile of phenolic metabolites in each extract was characterized by HPLC-DAD-APCI/MS to identify marker compounds within the different organs to gain further insight into the identity of potential active metabolites.

Materials and Methods

Reagents

All cell culture reagents were obtained from Invitrogen (Burlington, ON, Canada) and all chemicals were purchased through Sigma-Aldrich (St. Louis, MD, USA) unless otherwise stated.

Plant material

Samples of glaura *Picea glauca* were harvested in Mistissini and Whapmagoostui, Quebec, Canada, as per the instructions of the elders and healers from these communities (Leduc et al., 2006). Plants were identified by Dr. A. Cuerrier (Plant Biology Institute, Montréal Botanical Garden), and voucher specimens were deposited in the herbarium of the Montréal Botanical Garden. Whole plants were air dried and transported to the University of Ottawa for separation into bark, cones, and needles. Plant parts were processed using a Wiley Mill with a 2-mm filter and were subsequently extracted twice with 10 mL of 80% ethanol per gram dry material on a mechanical shaker for 24 hours then vacuum-filtered using Whatman paper no 1. The supernatants of first and second extracts were pooled and dried by rotary evaporation and lyophilization. Prior to experimental use, the lyophilized extracts were prepared in DMSO at a concentration of 100 mg/mL and filtered through an 0.2-μm nylon membrane filter (Chromatographic Specialties Inc., Brockville, ON, Canada). Required serial dilutions were made in DMSO on the day of use to ensure all cultures were exposed to a final concentration of 0.1% DMSO (vehicle).

Assessment of total phenolic content

The total phenolic content of bark, cone, and needle extracts was determined using the Folin-Ciocaulteu method previously described (Singleton, 1965) and modified for reduced volumes as previously described (Harris et al., 2007). Each extract was dissolved in 80% methanol at 1 mg/mL and combined with freshly diluted Folin-Ciocaulteu reagent and incubated at room temperature for 5 min prior to mixing with 7.5% anhydrous $NaHCO_3$ solution. After an additional 2 h incubation, absorbances were measured at 725 nm with a SpectraMax Gemini XS microplate fluorometer (Molecular Devices, Sunnyvale, CA, USA). Total phenolic content of each plant extract was calculated relative to serial dilutions of concurrently analyzed quercetin and are expressed as quercetin equivalents. Each sample was tested in duplicate or triplicate on two separate occasions ($n = 5$).

Phytochemical characterization

Bark, cone, and needle extracts were characterized by HPLC-DAD-APCI/MS analysis using a validated method for the identification of phenolic metabolites (Harris et al., 2007). Analyses were performed on an Agilent 1100 LC MSD system (Palo Alto, CA, USA) comprising an autosampler, a quaternary pump, a column thermostat, a photodiode array detector (DAD), and an online APCI/MS. Separations were performed on a YMC ODS-AM column (100 × 2 mm i.d.; 3-μm particle size) (Waters, Mississauga, Canada) at 50°C and a flow rate of 0.3 mL/min. The elution conditions consisted of aqueous tribluoroacetic acid (TFA, 0.05%), pH

3.4 (solvent A), and methanol (solvent B), as mobile phase with initial conditions 92%:8% (A:B) maintained for 0–5 min, 8–13% B in 2 min, 13–30% B in 14 min, 30–60% B in 3 min, and 60–100% B in 2 min. After a 2-min wash at 100% B, solvent compositin was returned to 92%:8% in 2 min followed by 6 min of post-run equilibration, resulting in a total run time of 36 min. The ethanol extracts (1 μL) were filtered through 0.2-μm PTFE membrane filter (Chromatographic Specialities Inc., Brockville, ON, Canada) and 1 μL of extract individually injected through a 100-μL loop of the autosampler. The chromatographic separation was monitored on-line at 325 nm and at 280 nm by DAD. The mass spectrometric detection was performed in positive ionization mode with the following optimized conditions: drying gas flow rate at 6.0 L/min, nebulizer pressure at 40 psig, drying gas temperature at 300°C, vaporizer temperature at 400°C, capillary voltage at 3000 V, and corona current at 3.0 μA. The total ion chromatogram was obtained in scan mode within the mass range of 100 to 800 amu with a fragmentation voltage at 20 V.

Compound identifications were performed by matching the UV spectra of each eluted peak with those of standards. Further confirmation of identification was achieved by comparing the fragmentation patterns and relative retention time with those of standards and/or reported in literature. The identifications of marker compounds were established by performing a match with a Chemstation library comprising 120 spectra of reference spectra. The threshold for an authenticated match was set at 95% spectral similarity with in a specified UV absorbance range of 190–400 nm. Further confirmation of the identity of marker compounds was achieved by comparing the mass spectrometric fragmentation with those of standards and/or reported in the literature (Slimestad, 2003; Harris et al., 2007).

Cell culture, glucose toxicity, and glucose deprivation conditions

PC12-AC cells, a clonal derivate (Brewer et al., 2002) of the PC12 rat adrenal pheochromocytoma cell line (American Type Culture Collection), were maintained in complete media (RPMI 1640 containing 10% horse serum and 5% newborn calf serum) under normoglucose (11 mM) conditions. Cells were seeded in 96-well plates (1.25×10^4 cells/well) and allowed to adhere at 37°C in 5% CO_2 overnight. To establish LD50 concentrations, cultures were treated for 96 h in serum-free RPMI media at a glucose concentration of 11 mM with 0.1% DMSO (vehicle control) or varying concentrations of plant extract (0–100 μg/mL). Media was supplemented with 0.025% bovine serum albumin (BSA) to facilitate intracellular passage of hydrophobic compounds. To elicit glucose toxicity (hyperglycemia), complete medium was replaced with serum-free medium supplemented with 150 mM glucose and 0.025% BSA. Substitution of D-glucose for L-glucose abolishes toxicity indicating that PC12 cell death is glucose-specific and not the result of osmotic stress under these treatment conditions (Koshimura et al., 2002). To elicit glucose deprivation (hypoglycemia), glucose (1 mM) was added to glucose-free RPMI media lacking serum and supplemented with 0.025% BSA as we have described previously (Spoor et al., 2006). Cultures were exposed to various concentrations of plant extract below the relevant LD_{50} for each preparation.

Cell viability assay

After 96 h of treatment in normoglucose, high-glucose, or low-glucose conditions, the formazan dye WST (Roche Diagnostics, Laval, QC) was added to each well. Cultures were incubated for 60 min before spectrophotometric analysis at 420 nm (formazan) and at 620 nm (reference). Experimental samples were blanked against cell-free treatment media incubated for the same period. Cell number/well was calculated from standard curves of known cell density prepared for each assay. Compounds were tested in multiple wells over two or three independent experiments (n = 7 to 10 wells/condition). Data from control cultures in normoglucose, high glucose, and low glucose treated with 0.1% DMSO were combined across plates (n = 55 to 65 wells). Percent viability was calculated as follows:

$$\%\ \text{viability} = \text{cell number}_{(\text{treatment well})} / \text{mean cell number}_{(\text{normoglucose control})}.$$

Cell survival assay

Cell survival was directly assessed by Live/Dead viability/cytotoxicity assay (Invitrogen). Viable cells were identified by the enzymatic conversion by intracellular esterases of nonfluorescent calcein-AM to fluorescent calcein. Dead cells were identified by uptake of ethidium homodimer (ET) indicative of a loss of membrane integrity. Cells were imaged using a DMIR epifluorescent inverted microscope (Leica, Richmond Hill, Canada) equipped with a QICAM digital camera (Quorum Technologies, Guelph, Canada) and captured using OpenLab software v5.05 (Improvision, Waltham, MA, USA). Percent survival was calculated as follows:

$$\left(\text{Viable cell number}^{(\text{calcein}^+ - \text{calcein}^+/\text{ET}^+)} / \text{mean number of viable cells in vehicle control}^{(\text{calcein}^+ - \text{calcein}^+/\text{ET}^+)}\right) \times 100.$$

Statistical analyses

Data were analyzed using unpaired Student's *t*-tests or one-way factorial ANOVA tests followed by*post hoc* Dunnett's

Table 1. Extraction yield and total phenolic content of minhikw bark, cone, and needle extracts.

		Total phenolic content[b]	
Organ	Yield[a]	mg/g dry material	μg/per mg extract
Bark	10.4%	32.1 ± 0.4	308.7 ± 3.7
Cone	24.0%	49.8 ± 0.7	207.4 ± 2.8
Needle	13.1%	12.4 ± 1.2	109.8 ± 9.3

[a] Yield was calculated as (mass recovered extract/mass dry material) × 100%.
[b] Total phenolics expressed as quercetin equivalents.

t-tests on each organ as applicable. p values under 0.05 were considered statistically significant (shown as * or #); p values under 0.01 were considered highly significant (shown as ** or ##).

Results

We found that the ethanol extraction of minhikw bark, cones, and needles produced 10%, 24%, and 13% yields, respectively. Employing the Folin-Ciocalteu method to approximate the total content of phenolic compounds in each extract, the bark extract proved to be the richest in phenolics, containing 50% more than the cone extract and 200% more than needle extract (Table 1). On the basis of dry weight, however, the cones contain more phenolics than the bark (Table 1). To identify marker compounds and characterize the classes of phenolic compounds present in each organ, the extracts were analyzed by HPLC-DAD-APCI/MS. In total, 12 compounds were identified, representing three phenolic acids (PA_{1-3}), four stilbenes (S_{1-4}), and five flavonoids (F_{1-5}) (Table 2). In contrast with the bulk phenolic content of the three extracts, the HPLC chromatogram of the bark extract revealed relatively few major peaks, all of which were identified as hydroxylated stilbenes (Fig. 1A). After performing the spectral library match, it was found that the UV absorption spectra of these peaks, as well as the minor peaks eluting after S_3, are very similar to that of standard piceatannol. The UV and MS data obtained from both cone and needle extracts showed a higher diversity of phenolic compounds, containing phenolic acid, stilbene, and flavonoid derivatives (Fig. 1B, C; Table 2). While the cone and bark extracts appeared relatively distinct, sharing only the major stilbene peak (S_1) common to all organs, the observed phytochemical profile of needle extract was intermediate between bark and cone, containing three stilbenes found in the bark (S_{1-3}) and three flavonoids found in the cones (F_1, F_{4-5}) (Fig. 1).

PC12-AC cells are a clonal derivate of the established adrenal pheochromocytoma cell line that can be differentiated to a peripheral neuron phenotype by the combination of serum deprivation and treatment with nerve growth factor. As such, these cells represent an accessible model for assessment of antidiabetic activity with respect to peripheral neuropathies. To establish LD_{50} concentrations of *P. glauca* bark, cone, and needle extracts, cultures were treated with various concentrations of complex extract or vehicle (0.1% DMSO) in serum-free media for 96 h under normoglucose (11 mM) conditions. Viable cell number was established by mitochondrial dehydrogenase cleavage of the formazan dye WST relative to standardized controls of known cell densities. We found that cell viability was not compromised over the 96-h treatment period in vehicle-treated cultures (data not shown). LD_{50} concentrations were defined as the amount of extract in μg/mL eliciting death of 50% of cultured cells relative to control cultures. Needle extracts were not toxic at any of the concentrations tested exhibiting an $LD_{50} > 100$ μg/mL. LD_{50} concentrations of bark and cone extracts were comparable at 40 μg/mL and 36.4 μg/mL, respectively.

Table 2. List of phenolic metabolites detected by HPLC-PAD-APCI/MS in *P. glauca* extracts.

					Detected in:		
Identified metabolites	Peak	R_t (min)	λ_{max} (nm)	M^+ (m/z)	Needle	Cone	Bark
Benzoic acid derivative	PA_1	6.1	220, 280, 300sh	153	√		
Benzoic acid derivative	PA_2	9.9	220, 280	137	√		
Phenylpropanoid derivative	PA_3	13.5	210, 230sh, 320	179		√	
Tetrahydroxystilbene	S_1	15.3	200, 220, 300	245	√	√	√
Trihydroxystilbene	S_2	18.9	200, 220, 300	229			√
Methoxytrihydroxystilbene	S_3	20.9	200, 220, 300	259	√		√
Methoxytrihydroxystilbene	S_4	25.6	200, 220, 300	259			√
Catechin	F_1	5.8	210, 230sh, 280	139, 291	√	√	
Taxifolin	F_2	16.4	195, 230sh, 290	305	√		
Quercetin glycoside	F_3	24.5	255, 350	303, 465	√		
Kaempferol glycoside	F_4	26.4	200, 265, 350	287, 449	√	√	
Isorhamnetin glycoside	F_5	26.8	255, 360	317, 479	√	√	

PA, phenolic acid; S, stilbene; F, flavonoid.

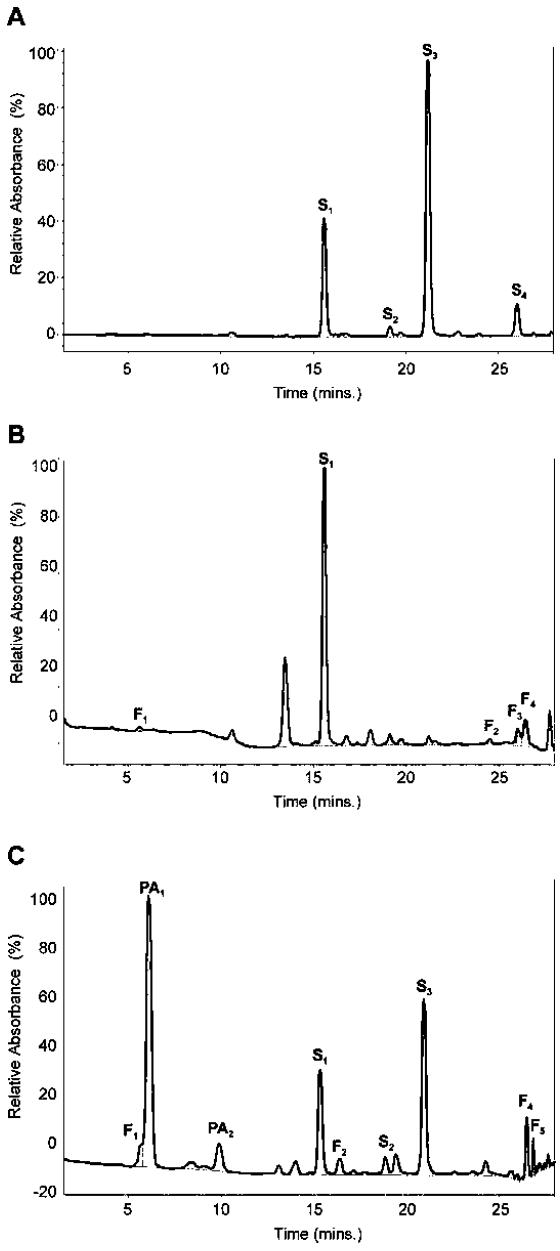

Figure 1. HPLC chromatograms of *P. glauca* extracts with photodiode array detection (PAD) at 325 nm. HPLC-PAD chromatograms of (A) bark, (B) cone, and (C) needle extracts. Labeled peaks represent identified metabolites as reported in Table 2. Three classes of phenolic compounds were detected, flavonoids (F), phenolic acids (PA), and stilbenes (S).

To screen for bioactivity relevant to glucose toxicity, PC12-AC cells were exposed to elevated glucose concentrations (150 mM) under conditions of serum deprivation (Fig. 2). We have previously demonstrated that this protocol elicits ~50% cell death relative to normoglucose concentrations (Spoor et al., 2006). Consistent with this earlier study, a statistically significant 43% loss in cell viability was detected in DMSO-treated control cultures

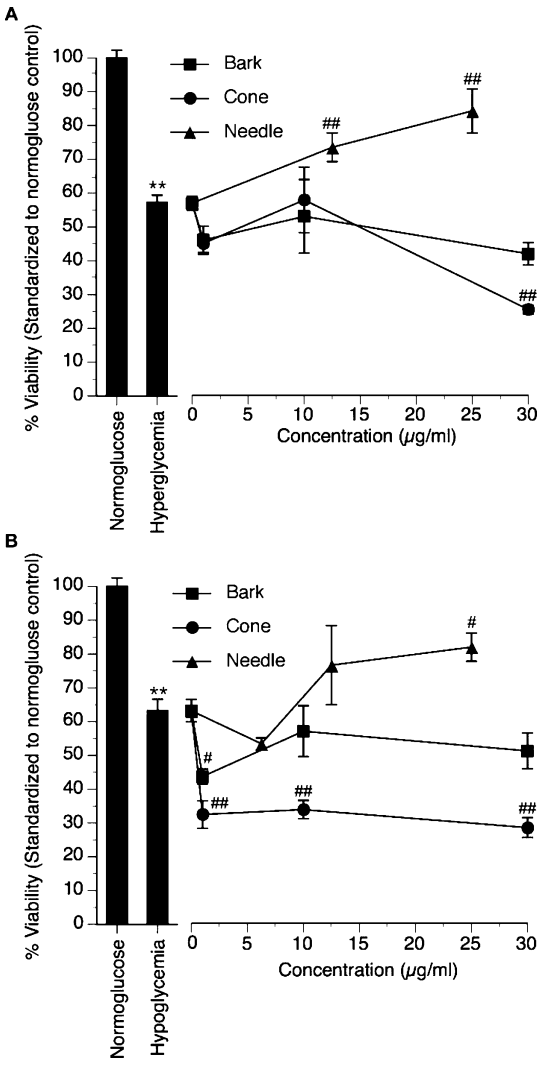

Figure 2. Dissociation of cytoprotection and cytotoxicity in high-glucose and low-glucose media between bark, cone, needle extracts of *P. glauca*. (A) Exposure to hyperglycemic media in the presence of 0.1% DMSO elicited loss of cell viability as assessed by mitochondrial dehydrogenase activity measured by cleave of the formazan dye WST (bar graph, **p < 0.01 Student's *t*-test, n = 55 to 65 wells/condition). A concentration-dependent increase in cell viability was detected in cultures treated with needle extracts. Bark extracts had no statistical effect on PC12 viability in high-glucose media. Cone extracts decreased cell viability in high-glucose media at 30 μg/mL [line graph, ##p < 0.01 versus DMSO control (0 h), ANOVA, *post hoc* Dunnett's *t*-test, n = 7 to 55 wells/condition]. (B) Culture in hypoglycemic media in the presence of vehicle (0.1% DMSO) significantly reduced cell viability (bar graph, **p < 0.01 Student's *t*-test, n = 55 to 65 wells/condition). Needle extracts protected PC12-AC cells in concentration-dependent manner. Bark extracts had no significant effect on cell viability in low-glucose media. Cone extracts decreased cell viability following glucose deprivation at all concentrations tested [line graph, #p < 0.05, ##p < 0.01 versus DMSO control (0 h), ANOVA, *post hoc* Dunnett's *t*-test, n = 7 to 55 wells/condition]. Data are reported as mean ± standard error of measurement (SEM). Extracts were tested at concentrations below their LD_{50} concentrations established under normoglucose conditions.

as a result of glucose toxicity (Fig. 2A, bar graph). We next tested the ability of *P. glauca* extracts to alter cell viability under hyperglycemic conditions. Extracts were tested at concentrations approaching but not exceeding their LD_{50} concentrations established under normoglucose conditions (i.e., 0–30 μg/mL). We found that bark extracts had no statistical effect on glucose toxicity, whereas cone extracts significantly exacerbated cell loss when concentrations approached the LD_{50} concentration of 36.4 μg/mL (Fig. 2A, bar graph). By contrast, significant concentration-dependent cytoprotection was observed when PC12-AC cells were exposed to high glucose in the presence of minhikw needle extracts (Fig. 2A, line graph).

To assess protection from glucose deprivation, PC12-AC cultures were exposed to low glucose (1 mM) and treated with 0.1% DMSO or *P. glauca* extracts (Fig. 2B). As previously reported (Spoor et al., 2006), glucose deprivation elicited ~40% loss of viable cell number (Fig. 2B, bar graph). We found bark extract did not confer cytoprotection under low-glucose conditions (Fig. 2B, line graph). Cone extracts elicited significant toxicity at all concentrations tested (Fig. 2B, line graph). Needle extracts were cytoprotective, inhibiting cell death associated with glucose deprivation, at the highest concentrations tested (Fig. 2B, line graph).

Because mitochondrial dehydrogenase activity can increase in cells undergoing mitochondrial-dependent apoptosis (Vogt et al., 2004), it was necessary to confirm cytoprotection observed in *P. glauca* needle extracts by directly assessing viable cell number. Serum-deprived cultures were treated for 96 h with DMSO (control) or increasing concentrations of minhikw needle extracts and cell survival quantified by Live/Dead assay. Viable cells were identified by cleavage of calcein AM by intracellular esterases to its fluorogenic product. Dead cells were identified by uptake of the membrane-impermeant ethidium bromide homodimer. As observed using the WST assay, 0–25 μg/mL *P. glauca* needle extract had no effect on cell viability in serum-deprived cultures under normoglucose conditions (data not shown). Culture in high glucose–containing (Fig. 3A, bar graph) and low glucose–containing media (Fig. 3B, bar graph) in the presence of vehicle (DMSO) significantly compromised cell survival. Needle extracts enhanced PC12-AC cell survival at concentrations of 12.5 and 25 μg/mL under hyperglycemic (Fig. 3A, line graph) and hypoglycemic (Fig. 3B, line graph) conditions.

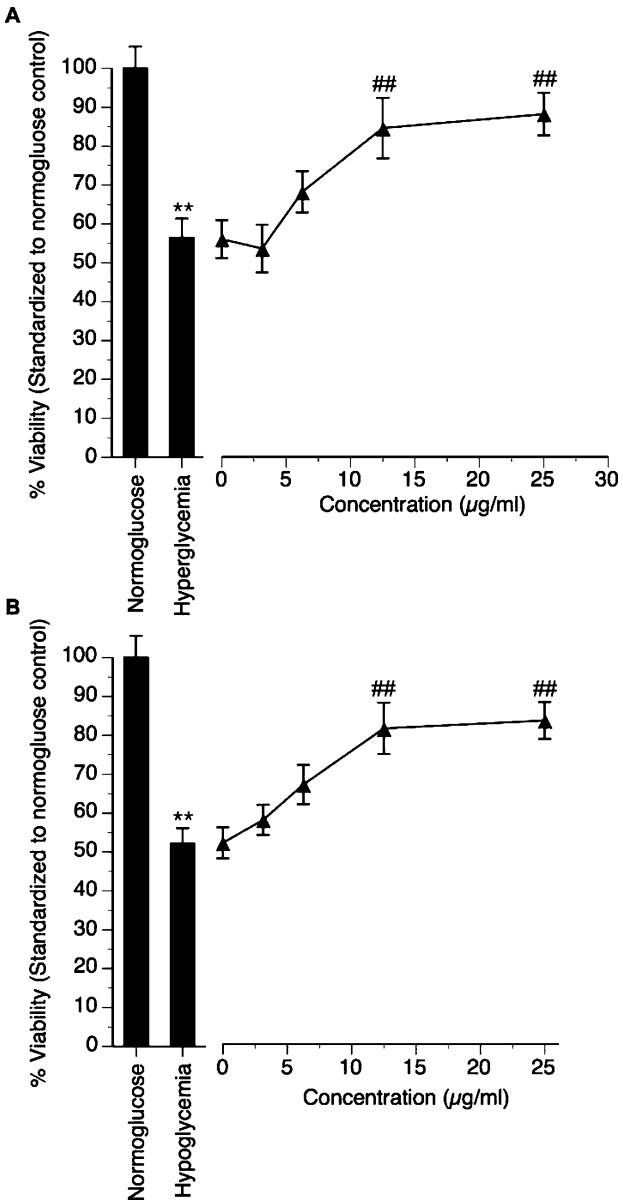

Figure 3. Concentration-dependent protection of PC12-AC cells from glucose toxicity and glucose deprivation by *P. glauca* needle extract. Cell survival was directly assessed by Live/Dead assay. (A) Culture in high-glucose media in the presence of DMSO (vehicle) significantly compromised cell survival (bar graph, **p < 0.01 Student's *t*-test, n = 6 to 11 wells/condition). Needle extracts enhanced cell survival [line graph, ##p < 0.01 versus DMSO control (0 h), ANOVA, *post hoc* Dunnett's *t*-test, n = 6 to 11 wells/condition]. (B) Culture in low-glucose media in the presence of DMSO (vehicle) significantly compromised cell survival (bar graph, **p <0.01 Student's *t*-test, n = 6 to 11 wells/condition). Needle extracts concentration-dependently enhanced cell survival [line graph, ##p < 0.01 versus DMSO control (0 h), ANOVA, *post hoc* Dunnett's *t*-test, n = 6 to 11 wells/condition]. Data are reported as mean ± SEM. Extracts were tested at concentrations below their LD_{50} concentrations established under normoglucose conditions.

Discussion

This study compared the activity of *P. glauca* bark, cone, and needle extracts administered to PC12 cells in two *in vitro* paradigms of diabetic neuropathy (glucotoxicity and glucose deprivation). This study was designed to model cellular effects of impaired glucose regulation associated with T2D. The observed effects of each extract were consistent in both models and revealed organ-specific antidiabetic

activity. Here, we show that the needle extract displayed concentration-dependent protection whereas the cone extract intensified cell loss and the bark extract failed to affect cell viability.

These findings are consistent with the ethnobotanical uses of P. glauca among Native Peoples of North America indicating that, where available, organs of this species are exploited for a wide variety of medicinal purposes. As reviewed by Arnason et al. (1981) as well as by Moerman (1988), the needles, cones, and bark are used separately by various First Nations groups of eastern Canada to treat different symptoms, including those associated with diabetes and its related complications (Arnason et al., 1981; Moerman, 1988; McCune & Johns, 2002). For instance, the cone and bark (gum) are prepared by the Abenaki for frequent urination whereas the Mi'kmaq and Atikamekw employ the bark to treat sores and wounds. Though the needles are made as a tea by the Algonquin to "heal the insides" (Black, 1980), until our recent work with the CEI, the bark, twigs, and cones appeared to be more commonly used than the needles (Leduc et al., 2006). But old statements stemming from anthropological/ethnological studies do not differentiate between twigs sensu stricto and twigs sensu lato, which also comprises the needles. Indeed, strong in vitro activities against a wide range of microbes have been demonstrated by *P. glauca* (Richardson et al., 1992; Ritch-Krc et al., 1996). Taken together, these results clearly validate the Traditional Knowledge shared by the Healers and Elders of CEI at a cellular level.

Although bark and cone extracts did not exhibit cytoprotective activity on peripheral neuronal precursors in the presence of high (150 mM) or low glucose (1 mM), these extracts have proved important in other studies to reduce other diabetic-associated symptoms. When collections of boreal plants used for treating diabetes were compared, the bark/twig extract proved effective as an antioxidant (McCune & Johns, 2002; McCune, 2003). Oxidative stress is implicated in the etiology of diabetes and many of its complications (Fridlyand & Philipson, 2005). Moreover, we have recently reported promising antidiabetic activity in the bark and cone extracts of other Pinaceae species [*Picca mariana* (mills.) B.S.P., *Pinus bamksiana* Lamb, and *Abies balsamea*(L.) (Spoor et al., 2006)]. The data presented here begin to dissociate the different bioactivities of boreal plant organs relevant to treatment of T2D-associated peripheral neuropathies versus other diabetic complications at the cellular level.

To provide further insight, and as expected by the distinct activities elicited by the three extracts, characterization of the phenolic metabolites detected considerable variation in the total phenolic content and the classes of identified compounds among plant parts. These differences in phytochemical composition likely underlie not only the distinct biological activities profiles of bark, cones, and needles in PC12 cells but also their varied traditional medicinal uses as well. Phenolic metabolites were targeted for analysis as these compounds have frequently been associated with both antidiabetic and neuroprotective abilities (Galli et al., 2002; Rodriguez de Sotillo & Hadley, 2002; Dajas et al., 2003; Coskun et al., 2005). Many identified phenolic compounds have been previously reported in closely related species. As the name implies, piceatannol derivatives and other hydroxylated stilbenes are found in multiple *Picea* species and Pinaceae members (Pan & Lundgren, 1995; Spoor et al., 2006). The common occurrence of this type of compound in all plant parts was therefore not surprising. The five detected flavonoids have been identified in *Picea abies* (L.) Karst. needles (taxifolin, kaempferol, and isorhamnetin glycosides) (Slimestad, 2003) or bark (catechin, taxifolin) (Lieutier et al., 2003) but were only detected in the needle and cone extracts of minhikw. Although the LC-MS data provided the identification of marker compounds allowing the extracts to be differentiated on a phytochemical basis, speculation on possible active metabolites is difficult at this stage considering the overlap in identified compounds between organs and requires further investigation. Furthermore, additional phytochemicals left unidentified and/or undetected using the current methods, such as terpenes or tannins, may equally possess the biological activity of interest. Biossay-guided fractionation of the needle extract is therefore currently being pursued to gain further insight into the active constituent(s) and their mechanism(s) of action.

In summary, this study identifies a novel cytoprotective activity promoting peripheral neuronal precursor survival under conditions of both glucotoxicity and glucose deprivation in needle extracts of *P. glauca*. Bark and cone extracts were not cytoprotective. As such, active principles present in needle extracts of minhikw represent ideal candidates for further investigation as adjuvant therapies in the treatment and prevention of diabetic neuropathy for the CEI and other communities. As a natural health product, *P. glauca* preparations may be accepted more readily than prescription drugs for some patient groups, particularly in First Nations communities afflicted with rising incidence of T2D and a paucity of culturally acceptable treatment options.

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