Insulin-like Biological Activity of Culinary and Medicinal Plant Aqueous Extracts in Vitro

C. Leigh Broadhurst, Marilyn M. Polansky, and Richard A. Anderson*

Nutrient Requirements and Functions Laboratory, Beltsville Human Nutrition Research Center, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705-2350

To evaluate the possible effects on insulin function, 49 herb, spice, and medicinal plant extracts were tested in the insulin-dependent utilization of glucose using a rat epididymal adipocyte assay. Cinnamon was the most bioactive product followed by witch hazel, green and black teas, allspice, bay leaves, nutmeg, cloves, mushrooms, and brewer's yeast. The glucose oxidation enhancing bioactivity was lost from cinnamon, tea, witch hazel, cloves, bay leaf and allspice by poly(vinylpyrrolidone) (PVP) treatment, indicating that the active phytochemicals are likely to be phenolic in nature. The activity of sage, mushrooms, and brewers's yeast was not removed by PVP. Some products such as Korean ginseng, flaxseed meal, and basil have been reported to be effective antidiabetic agents; however, they were only marginally active in our assay. Our technique measures direct stimulation of cellular glucose metabolism, so it may be that the active phytochemicals in these plants improve glucose metabolism via other mechanisms or that this in vitro screening is not a reliable predictor of hypoglycemic effects in vivo for some products. In summary, the positive effects of specific plant extracts on insulin activity suggest a possible role of these plants in improving glucose and insulin metabolism.

Keywords: *Glucose; insulin; diabetes; antidiabetic plants; hypoglycemic plants*

INTRODUCTION

Previously Khan et al. (1990) reported that extracts of common culinary herbs and spices such as cinnamon, cloves, and bay leaf demonstrated insulin-like or insulin-potentiating action in vitro. The increases in glucose metabolism were apparently due to intrinsic phytochemical actions of the extracts. Over 200 pure phytochemicals are known to be hypoglycemic. Marles and Farnsworth (1994) caution that one- to two-thirds of the 1123 plants that affect blood glucose may be dangerous, and many of the phytochemicals are hypoglycemic due to metabolic or hepatic toxicity. However, cultures around the world have long utilized medicinal plants for diabetes safely and with reasonable success (Bailey and Day, 1989; Ivorra et al., 1989; Marles and Farnsworth, 1994; Duke et al., 1998). Botanical products can improve glucose metabolism and the overall condition of persons with diabetes not only by direct hypoglycemic effects but also by improving lipid metabolism, antioxidant status, and capillary function (Broadhurst, 1997).

A number of medicinal/culinary herbs have been reported to yield hypoglycemic effects in subjects with diabetes. These include bitter melon, *Momordica charantia* (Srivastava et al., 1993; Raman and Lau, 1996); gurmar, *Gymnena sylvestre* (Basakaran et al., 1990; Shanmugasundaram et al., 1990; Bishayee and Chatterjee, 1994); Korean ginseng, *Panax ginseng* (Sotaniemi et al., 1995); onions and garlic, *Allium cepa, A. sativum* (Koch and Lawson, 1996); holy basil, *Ocimum sanctum* (Rai et al., 1997); and flaxseed meal, *Linum usitatissimum* (Cunane et al., 1993).

* Address correspondence to this author at BHNRC, ARS, USDA, Bldg. 307, Room 224, Beltsville, MD 20705-2350 [telephone (301) 504-8091; fax (301) 504-9062; e-mail anderson@307.bhnrc.usda.gov].

Some common botanicals demonstrating in vivo hypoglycemic activity in animals include juniper berries (Sanchez de Medina et al., 1994); Siberian ginseng and reishi mushroom (Kimura et al., 1988; Bailey and Day, 1989); cumin, cucumber, and bottle gourd (Roman-Ramos et al., 1995); java plum (Bailey and Day, 1989; Ivorra et al., 1989); and alfalfa (Gray and Flatt, 1997). Bearing these observations in mind, we have extracted 49 botanical products and assayed them for their in vitro effects on insulin-dependent glucose metabolism in adipocytes. Some of these are medicinal plants that have been traditionally used for diabetes, and others are culinary plants that are nearly ubiquitous in Western diets.

MATERIALS AND METHODS

In all cases, commercially available spices, culinary herbs, foods, and medicinal plants were used. Herbs, spices, and mushrooms were in dry form, and large pieces of bark, leaves, roots, or whole seeds were ground to coarse powder prior to extraction. The approach of using commercially available material ensures that we are testing botanical products in forms identical to those which individuals would purchase for their own use.

Plant products were extracted, using reagent grade chemicals, with a 20-fold excess (wt/vol) of 0.1 N NH_4OH by shaking at room temperature at 200 rpm for 1 h (Controlled Environment Incubator Shaker, New Brunswick, NJ). For some assays, plant products were also extracted with a 20-fold excess of water or 0.1 N acetic acid by heating for 20 min in an autoclave at 15 psi. All samples were centrifuged at 12000*g* for 25 min. Glucose metabolism was assayed in rat epididymal adipocytes according to the method of Anderson et al. (1978). Briefly, 0.43 μCi of [U-^{14}C]glucose, 72 μg of glucose, and adipocytes were incubated with insulin or plant extract in a final reaction volume of 2 mL of Krebs−Ringer phosphate buffer, pH 7.1. After quantitation of $^{14}CO_2$ release by the cells

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in response to insulin or plant extract, an insulin activity ratio was determined. The insulin activity ratio was calculated by dividing the basal counts per minute of $^{14}CO_2$ released by the cells into those of the activity of the cells plus plant extracts. Biologically active extracts were incubated with soluble poly-(vinylpyrrolidone) (PVP) (Sigma Chemical Co., St. Louis, MO) to determine if the biological activity was associated with tannins and/or other polyphenols (Wall et al., 1996). One milliliter of biologically active sample was incubated with 0.1 g of PVP for 30 min. Samples were mixed three times during incubation and then centrifuged at 14000*g* for 20 min and assayed as indicated.

RESULTS

In Table 1 are shown the botanical products tested following extraction with 0.1 N ammonium hydroxide. All of the materials were assayed in duplicate in three or more dilutions in at least two assays. Values in Table 1 indicate the increase in insulin-dependent activity measured from the cells exposed to the plant extracts divided by the control value. Significant effects were defined as an activity >2 in extracts diluted 2-fold and an ability to sustain values >1 upon a 5-fold dilution. In some cases initial values were less than values on subsequent dilutions, probably due to inhibitory effects at high concentrations. Various insulin levels (500 to 2 μU/mL) and brewer's yeast extract were used as reference standards.

In Table 2 are shown the percentages of glucose oxidation enhancing bioactivity that were lost by treating the botanical extracts with soluble PVP. PVP binds with compounds containing aromatic hydroxyl groups (Wall et al., 1996). The results in Table 2 indicate that the majority of the biologically active compounds bind to PVP and thus are likely to be phenolic in nature. The activity of sage, mushrooms, and brewers's yeast was not removed by PVP, and the activity of nutmeg and oregano appeared to be due partially to compounds with aromatic hydroxyl groups.

Table 1. Summary of Botanical Products Tested[a]

common name	botanical name	dilution 1:2	1:10	1:50	dry wt, mg/mL
allspice	*Pimenta officinalis*	16.1	1.4	1.0	8.8
almond	*Prunus dulcis*	1.4	1.0	1.0	
anise seed	*Pimpinella anisum*	0.8	1.1	1.1	
astragalus	*Astragalus membranaceus*	1.5	1.2	1.1	
ashwagandha	*Withania somnifera*	0.7	0.9	0.9	
basil, Italian	*Ocimum basilicum*	2.7	1.4	1.1	
basil, French	*Ocimum basilicum*	2.4	1.4	1.1	
bay leaf	*Laurus nobilis*	16.0	4.2	1.0	14.2
bee pollen	n/a	1.3	1.2	1.1	
cardamom	*Elettaria cardamomum*	0.9	0.9	1.0	
catnip	*Nepeta cataria*	1.4	0.9	0.9	
celery seed	*Apium graveolens*	0.8	1.0	1.0	
cinnamon, cassia	*Cinnamomum cassia*	5.6	20.2	15.2	11.5
cinnamon, Ceylon	*Cinnamomum verum*	5.9	32.9	10.0	8.5
cloves	*Syzigium aromaticum*	12.4	3.7	0.8	18.4
coffee, roasted	*Coffea arabica*	1.1	1.0	1.0	
curcumin[b]	*Curcuma longa*	0.2	0.9	1.0	
dill seed	*Anethum graveolens*	0.9	1.0	1.0	
echinacea, root	*Echinacea purpurea*	1.4	1.0	1.0	
flaxmeal, defatted	*Linum usitatissimum*	1.5	1.1	1.0	
garlic, granulated	*Allium sativum*	1.1	1.1	1.0	
ginger, dried	*Zingiber officinale*	1.8	1.4	1.1	
ginseng, American	*Panax quinquefolius*	1.8	1.4	1.0	
ginseng, Korean	*Panax ginseng*	1.8	1.3	1.0	
ginseng, Siberian	*Eleutherococcus senticosus*	1.3	0.9	1.0	
gurmar	*Gymnema sylvestre*	0.2	0.6	1.0	
mace	*Myristica fragrans*	0.6	0.9	1.0	
mushroom, shiitake, dried	*Lentinus edodes*	2.6	2.0	1.0	24.0
mushroom, white, dried	*Agaricus bisporus*	7.0	3.2	1.2	29.5
mustard, dry yellow	*Brassica nigra*	0.5	0.8	1.0	
nutmeg	*Myristica fragrans*	14.7	2.4	0.9	8.1
oatmeal, dry	*Avena sativa*	1.3	1.0	1.0	
oregano, Italian[c]	*Origanum vulgare*	2.7	0.9	1.0	12.5
parsley, dried	*Petroselinum crispum*	0.8	1.1	1.0	
peanut butter	*Arachis hypogaea*	1.5	1.2	1.1	
peanut, roasted	*Arachis hypogaea*	1.5	1.2	1.1	
pecan	*Carya illinoensis*	1.5	1.3	1.1	
pepper, black	*Piper nigrum*	0.9	1.0	1.0	
pepper, red chile pequin	*Capsicum annum*	0.8	1.0	1.0	
rosemary	*Rosmarinus officinalis*	1.1	1.0	1.0	
soybean sprout, raw dried	*Glycine max*	1.9	1.5	1.1	
tea, black	*Camellia sinensis*	25.3	1.8	1.3	19.0
tea, green	*Camellia sinensis*	23.5	1.6	1.1	18.1
sage[c]	*Salvia officinalis*	1.9	1.2	0.9	13.2
salt bush	*Atriplex halimus*	1.0	0.9	0.9	
turmeric	*Curcuma longa*	1.4	1.3	1.1	
vanilla bean	*Vanilla planifolia*	1.5	1.1	1.0	
witch hazel, dried bark	*Hamamelis virginiana*	26.4	3.1	1.0	8.0
yeast, brewer's	*Saccharomyces cerevisiae*	5.1	1.6	1.0	15.5

[a] Values represent increase or decrease in insulin-dependent glucose metabolism induced by the botanical extract versus control. One gram samples were extracted in 20 mL of 0.1 N NH_4OH by shaking at 200 rpm for 1 h. Insulin reference values: 500 μU/mL (22.1); 100 μU/mL (18.4); 25 μU/mL (6.9); 12.5 μU/mL (2.6), 2 μU/mL (1.2); values in parentheses denote the fold increase in glucose oxidation; a value of 1 denotes no effect. [b] Standardized medicinal extracts of turmeric with curcuminoids concentrated by a factor of 19. [c] Oregano and sage gave consistently positive results in other assays when extracted in water but minimal activity in base. Dry weights were determined on the active extracts to ascertain the relative activity per milligram of material.

DISCUSSION

The flowering plant (angiospermata) botanical products that were the most effective come from a relatively small number of botanical families: Hamamelidaceae (witch hazel); Labiatae (oregano, sage); Lauraceae (cinnamon, bay leaf); Myrtaceae (clove, allspice); and Theaceae (green and black teas). In addition, the plant parts represented in these products are barks, leaves, or dried woody buds (clove) and berries (allspice).

Cinnamon was the most effective botanical product, and the effectiveness was maintained even at high dilution. The compounds in cinnamon that are biologically active in this assay have recently been shown to be chalcone polymers (unpublished data). Cinnamon extracts have also been shown to improve insulin receptor function by activating insulin receptor kinase and inhibiting insulin receptor phosphatase, leading to increased insulin sensitivity (Imparl-Radosevich et al., 1998).

Witch hazel, also a tannic bark like cinnamon, and both green and black teas also exhibited high bioactivity with respect to the remaining plant products tested. Aqueous extracts of black and green teas given intragastrically lowered blood glucose in rats with diabetes induced by streptozotocin (STZ) injection (Gomes et al., 1995). However, the teas were thought to have acted mainly to prevent and/or reverse severe STZ damage to pancreatic β-cells, so this study may not be an appropriate model for investigating the effect of chronic tea consumption on Type 2 diabetes. Hale et al. (1989) found that a traditional Chinese antidiabetic tea lowered blood glucose in STZ diabetic rats, but patients with Type 2 diabetes failed to show clinical improvements when given the tea. Both polyphenolic and polysaccharide components in tea are considered to be

Table 2. Percentage of Insulin-like or Insulin-Potentiating Bioactivity Removed from Selected Botanical Extracts by PVP Treatment

botanical product	% activity removed	botanical product	% activity removed
allspice	100	nutmeg	77
bay leaf	86	oregano	71
cinnamon, cassia	98	sage	0
cinnamon, Ceylon	98	tea, black	97
cloves	98	tea, green	95
mushroom, shiitake	0	witch hazel	95
mushroom, white	0	yeast, brewer's	0

[a] One milliliter of biologically active sample was incubated with 0.1 g of PVP for 30 min. Samples were mixed three times during incubation followed by centrifugation at 14000*g* for 20 min. Supernatant was assayed for insulin-like biological activity (Anderson et al., 1978).

hypoglycemic (Gomes et al., 1995), and our PVP results indicate that polyphenolics were responsible for the insulin-like activity exhibited in this study. At this point, daily consumption of tea has not been shown to provide clinical benefits to persons with diabetes, but a possible preventive effect among high consumers of tea cannot be ruled out.

Sage, oregano, and basil are members of Labiatae (mint family) and are fairly similar to one another in their growth habits and known phytochemical constituents (Beckstrom-Sternberg and Duke, 1996; Duke et al., 1998). The positive results from sage and oregano correlate with the beneficial effects observed with holy basil whole leaf in humans (Rai et al., 1997) and aqueous sage extract in animals (Jimenez et al., 1986).

We note that insulin-like action was not a broad characteristic of Labiatae in this study because mint family members catnip and rosemary did not yield positive results. Al-Hader et al. (1994) report hyperglycemic and insulin release inhibitory effects from rosemary volatile (essential) oil in diabetic rabbits, but because the lipophilic fraction alone was investigated rather than an aqueous extract, these results may not be relevant. We found repeatedly that lipophilic fractions were not responsible for the activity.

Korean ginseng, flaxseed meal, and basil have been shown in human studies to be effective antidiabetic agents; however, they were marginally active under our test conditions. Our technique measures direct stimulation of cellular glucose metabolism, so it may be that the active phytochemicals in these plants improve glucose metabolism via additional mechanisms or that we did not extract the most active phytochemicals. Alternatively, it may be that for some botanical extracts, in vitro screening is not a reliable predictor of hypoglycemic effects in vivo. In vivo studies of the antidiabetic effects of the most biologically active extracts in this study are currently in progress.

With Korean ginseng, we tested aqueous acidic, basic, and neutral extracts of commercially prepared dried root powder, as well as a commercially prepared standardized hydroalcoholic extract, but none of these gave strong positive results. Because the major biologically active phytochemicals in Korean ginseng are the steroidal saponin ginsenosides, which are known to be mild adrenal cortex stimulants (Tang and Eisenbrand, 1992; Huang, 1993), this may be the primary action of ginseng with respect to decreasing plasma glucose, rather than a direct stimulation of cellular glucose metabolism. *Gymnema sylvestre* is considered to stimulate insulin secretion (Basakaran et al., 1990; Shanmugasundaram et al., 1990; Bishayee and Chatterjee, 1994), so it may be that the reported hypoglycemic effect of *G. sylvestre* is also not necessarily a direct effect on cellular glucose metabolism. In the case of flaxseed meal, if the biologically active fraction contains mainly lignans (Thompson et al., 1991), it is likely that we did not extract it efficiently because aqueous extraction is not optimum for these phytochemicals.

The fungi tested (white and shiitake mushrooms and brewer's yeast) gave positive results in the adipocyte assay, which is consistent with the beneficial effects observed with diabetic mice consuming white and reishi (*Ganoderma lucidum*) mushrooms (Kimura et al., 1988; Bailey and Day, 1989). However, previous research has identified bioactive constituents in fungi that are very different fromn those in the angiosperms discussed above. The peptidoglycans ganodera A and B have been identified as hypoglycemic factors in reishi mushroom (Tomoda et al., 1986). Similarly, a fraction from white mushroom was found to be hypoglycemic (Bailey and Day, 1989). This agrees with our results in Table 2, which show that PVP treatment did not affect the bioactivity of the mushroom extracts; therefore, the active phytochemicals are unlikely to be polyphenolic.

In summary, the effective plants in the 49 tested are safe, inexpensive culinary and/or medicinal species used throughout the world and thus have potential to be developed into alternatives or adjuncts to current antidiabetic medications. In vitro studies such as these demonstrate that botanical extracts can safely improve the utilization of glucose and function of insulin.

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