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Effect of Central/South American medicinal plants on energy harvesting ability of the mammalian GI tract

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Diabetes mellitus (DM) and associated co-morbidities including cardiovascular disease (CVD) and obesity (OB) are fast becoming leading causes of mortality in the developed and developing world. While the etiologies of these diseases are not completely understood, they are often associated with excessive caloric intake, hyperglycemia, irregular blood lipid levels and resulting oxidative stress. One important strategy for managing DM is managing postprandial hyperglycemia by reducing the digestion of carbohydrates by -glucosidases. The risk factors for CVD and obesity can further be managed by regulating postprandial increases in blood triglyceride and fatty acid levels by modulating the activity of lipase. We investigated the potential of 26 different herbs, spices and medicinal plants (HSMP) commonly used in Central/South American traditional medicine to reduce the energy harvest capacity of the mammalian gut by α-glucosidases and lipases in different model systems. In addition, we also assessed their abilities to confer antioxidant protection in biological systems. Our results indicate that several HSMP had the ability to prevent the digestion of carbohydrates by inhibiting -amylase, maltase and sucrase. These HSMP were also effective in inhibiting the activity of lipase and therefore digestion of triglycerides in the mammalian gut. Based on our results, we have identified HSMP from Central/South American that can be used as an alternative and complimentary strategy to manage risk factors of DM and associated co-morbidities.

Key words: Diabetes mellitus, cardiovascular disease, obesity, energy harvest, α- glucosidase, pancreatic amylase, disaccharidases, maltase, sucrase, lipase, enzyme inhibition, antioxidant activity, herbs, spices, medicinal plants, Central/South American traditional medicine.

INTRODUCTION

Herbs, spices and medicinal plants (HSMP) have been cherished for their use in curing common ailments and promoting good health by many ancient cultures (Duthie et al., 2003; Lewis and Elvis-Lewis, 2003). Recent research indicates that populations incorporating HSMP into their diets have a lower incidence of chronic disease (Duthie et al., 2003). Studies have suggested that oxidative stress-related chronic diseases, including type 2 diabetes mellitus (DM), cardiovascular disease (CVD) and obesity (OB), are all linked to excessive intake of calories, causing an imbalance of prooxidants and antioxidant

in cellular systems, which impairs normal biological functions (Droge et al., 2002). One benefit of HSMP is that they contain bioactive ingredients called 'phytochemicals' that can reduce oxidative stress and modulate harmful biological pathways, therefore ameliorating these chronic diseases.

Type 2 DM is a chronic metabolic disease of epidemic proportions resulting from defects in insulin secretion and/or insulin action. The disease has been associated with CVD, OB, microvascular damage, and eventual failure of the eyes, kidneys and nerves (Haffner, 1998; Dicarli et al., 2003). DM is a serious public health concern for governments across the world, and, the number of people is projected to increase to 380 million by 2025 (IDF, 2007). Postprandial hyperglycemia, following a rapid increase in blood glucose, is one of the earliest risk

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factors associated with the development of type 2 DM (Dicarli et al., 2003). Digestion of dietary starch by -glucosidases, including glucoamylase and pancreatic -amylase, contributes to this sharp increase in blood glucose (Elsenhans and Caspary, 1987; Harris and Zimmer, 1992; Bischoff, 1994). Inhibition of these enzymes has therefore long been a tool for the management of hyperglycemia, and type 2 DM (Puls et al., 1977; Haffner, 1998; Dicarli et al., 2003; Kwon et al., 2007). In fact, several pharmaceutical drugs used for the treatment of DM, have relied on decreasing the digestion and absorpion of starches and sugars which contribute to postprandial hyperglycemia (Haffner, 1998; Dicarli et al., 2003). These drugs primarily work by reducing the action of -glucosidases, including pancreatic -amylase, and intestinal dissacharidases, such as sucrase and maltase (Elsenhans and Caspary, 1987; Harris and Zimmer, 1992; Bischoff, 1994). The currently available antidiabetic drugs, such as acarbose have many side effects, including weight gain, hypoglycemia, lactic acidosis and gastrointestinal irregularities, which decrease their compliance rates and therefore effectiveness (Haffner, 1998; Dicarli et al., 2003; Kwon et al., 2007). Clearly, an alternative solution to treatment of diabetes is warranted.

Cardiovascular disease, associated with vascular damage and atherosclerosis, is one the primary causes of mortality in the adult population (Goran et al., 2003; Steinberger and Daniels, 2003) . While modulating diet, increasing physical activity and making positive lifestyle changes are preferred therapeutic options, many patients prefer to take therapeutic drugs (in addition to diabetic medications) to reduce body weight and other symptoms associated with CVD. One such drug, tetrahydrolipstatin (Orlistat), inhibits pancreatic lipase, thus decreasing the hydrolysis of triglycerides (TG) to free fatty acids (FFA) and reducing the eventual absorption of lipids by the enterocytes into the blood (Armand, 2007; Chaput et al., 2007; Lee and Aronne, 2007). Another drug, sibutramine is an amphetamine-like substance that is taken to promote satiety by increasing levels of serotonin and norepinephrine (Armand, 2007; Chaput et al., 2007; Lee and Aronne, 2007). These drugs, though popular, have several medicinal and physiological side effects. For example, side effects of tetrahydrolipstatin include steatorrhea, increased flatulence and occasional fecal incontinence, whereas hypertension and arrhythmias are side effects associated with sibutramine (Armand, 2007; Chaput et al., 2007; Lee and Aronne, 2007). Therefore, there is an urgent need for new and safer alternatives for prevention and treatment of overweight and CVD.

Since antiquity, DM, CVD and OB have been treated with medicinal plants, which continue to serve as an abundant resource for discovery of new drugs (Ivorra et al., 1989; Grover et al., 2002; Andrade-Cetto and Heinrich, 2005; Jung et al., 2006). Specifically, HSMP used in traditional medicines from the Indian subcontinent, China and Central/South Americas offer an abundant repertoire for the discovery of natural inhibitors of carbohydrate and lipid digestion (Ivorra et al., 1989; Grover et al., 2002; Andrade-Cetto and Heinrich 2005; Jung et al., 2006). Potentially, these HSMP can be incorporated into lifestyles and thus offer an attractive strategy to control postprandial hyperglycemia, assist in weight management, and manage CVD, with minimal side effects. In this manuscript we report the *in vitro* ability of 26 commonly used HSMP from Central and South Americas on reducing oxidative stress and inhibiting carbohydrate and lipid digestion in the gastrointestinal tract.

MATERIALS AND METHODS

Sample preparation and extraction

HSMP from Central/South America were obtained directly from Cantu's Mexican Hierberia Imports (Austin, TX) and are listed in Table 1.

Water extraction: 1.5 g of sample was suspended in 30 ml of water in an Erlenmeyer flask at 80ºC with stir bar and allowed to mix for 30 min at 250 rpm. The samples were then filtered under vacuum using a Buchner funnel equipped with Whatman No. 1 filter paper. The filtrate was called water extract and labeled as Extract 1.

NaOH digestion: (Krygier et al., 1982; Sun et al., 2002). The residue remaining on the filter paper after water extraction was scraped with a spatula into an Erlenmeyer flask containing a stir bar. To this residue, 20 ml of 4 N NaOH was added, and the mixture was stirred and allowed to digest for 30 min at 250 rpm. The samples were then filtered under vacuum using a Buchner funnel and Whatman No. 1 filter paper. The pH of the filtrate was adjusted immediately to 7.0 and labeled as Extract 2.

Antioxidant Assays

2,2 - azinobis(3-ethyl-benzothiazoline- 6-sulfonic acid) (ABTS)Assay: The ABTS assay was conducted by modifying a previously described method (Re at al., 1999) . Briefly, to 1 mL of 7 mM ABTS (in water, activated overnight with 140 mM potassium persulfate) was added 50 µL of Extracts 1 or 2, and the mixture was incubated for 2.5 min (RT). The absorbance was measured at 734 nm and compared with control containing ethanol in place of the extract. The percentage inhibition in ABTS radical due to the extract was
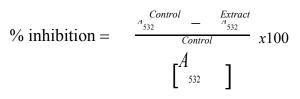
calculated by:
$$\% \text{ inhibition} = \frac{A^{Control}_{734} - A^{Extract}_{734}}{\left[A^{Control}_{734} \right]} \times 100$$

Thiobarburic acid reactive substances (TBARS) assay: TBARS were measured by modifying a method previously described (Moore et al., 1998). Briefly, an emulsion containing 1% linoleic acid and 1% Tween in 25 ml deionized water was sonicated for 3 min. 0.8 ml of emulsion was added to 0.2 ml of extracts to which 500 µL of 20% (w/v) trichloroacetic acid and 1 mL of 10 mM thiobarbutyric acid were added. Contents were vortexed and incubated for 30 min at 100°C. After incubation, tubes were centrifuged at 13,000 g for 10 min and the absorbance of the supernatant was measured at 532 nm. The concentration of malondialdehyde (MDA) was calculated from its molar extinction coefficient (ε) 156 μmol^{-1}cm^{-1} and expressed as μmol/g FW. Inhibition of TBAR formation by the extracts

Table 1. Different HSMP from Central/South America used for the study.

Name	Scientific Names	Abbreviation
Cuachalalate	*Amphipterygium adstringens*	AA
Texas/Mexican Olive Anacahuita	*Cordia boissieri*	AC
Yerba del Cancer (Foxtail Copper Leaf)	*Acalypha alopecuroidea* Jacq.	AJ
Manzanilla	*Anthemis nobilis*	AN
Artemsia	*Ambrosia artemisiaefolia*	AR
Bearberry Leaves	*Arctostaphylos uva-ursi*	AU
Mexican Heather (Cancerina)	*Calluna vulgaris*	CA
Golden Shower	*Cassia fistula*	CF
Mexican Hawthorn	*Crataegus mexicana*	CM
Mexican-hat-plant (Flor de manita)	*Chiranthodendron pentadactylon*	CP
Red Bark	*Cinchona succirubra*	CS
Castella	*Castela texana*	CT
Echium (Boraginaceae))	*Echium Sp.*	EB
Flor de Arnica	*Arnica montana*	FA
Hojase	*Cassia obovata* Collad	HJ
Lemon	*Citrus limon*	LE
Mugwort	*Artemisia vulgaris*	MH
Mexican/Cinnamon Basil	*Ocimum basilicum*	OB
Groundsel/Matarique	*Psacalium decompositum*	PD
Heal All (Toronguil Morado)	*Prunella vulgaris*	PV
Peppertree	*Schinus molle*	SM
Suelda	*Senecio formosus*	SU
Feverfew (Altamisa)	*Tanacetum parthenium*	TP
Mayan Mint Marigold (Yerbaniz)	*Tagetes lucida*	TL
Texas Sage	*Leucophyllum frutescens*	V1
Yerba Buena	*Satureja douglasii*	YB

was calculated by comparing with the control, which did not contain the extracts.

$$\% \text{ inhibition} = \frac{A^{Control}_{532} - A^{Extract}_{532}}{\left[A_{532}^{Control} \right]} \times 100$$

Amylase assay

The amylase inhibition assay was carried out by a method described previously (Kwon et al., 2006). Briefly, a total of 500 μl of extract and 500 μl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing porcine pancreatic -amylase (0.5 mg/ml; Sigma Chemical Company, St. Louis, MO) were incubated at 25°C for 10 min. After this pre-incubation, 500 μl of a 1% starch solution in 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added to each tube at timed intervals. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of dinitrosalicylic acid (DNS) color reagent. The test tubes were then incubated in a boiling water bath for 5 min and then cooled to room temperature. The reaction mixture was then diluted with 10 ml distilled water and absorbance was measured at 540 nm. The inhibition of -amylase was calculated as follows:

$$\% \text{ inhibition} = \frac{A^{Control}_{540} - A^{Extract}_{540}}{\left[A_{540} \right]} \times 100$$

Inhibitory activity on rat intestinal disaccharidases

The disaccharidases in rat intestinal extracts were prepared by modifying the method described by Dahlqvist (1968). Briefly, 0.5 g of rat intestinal acetone powder (Sigma, St. Louis, MO) was suspended in 15 mL of 0.1 M phosphate buffer (pH 7.0), sonicated (1 min × 3) and then centrifuged (3000 rpm, 30 min, 10°C). The supernatant was used in the maltase and sucrase assays.

Maltase assay: Maltase activity was assessed using a modification of the procedure described previously (Takii et al., 1996.). Briefly, samples were prepared by mixing 0.5 ml of 0.1M Phosphate buffer, pH 7.0 (or extract) (at 25°C) with 0.25 ml of 20 mM p-Nitrophenyl- -D-glucopyranoside (PNPG). 0.3 ml of enzyme solution was added and mixed. The reaction mixture was incubated for 15 min at 37°C after which 2.0 ml of 0.2 M Na_2CO_3 was added to stop the reaction, and then vortexed. Absorbance was measured at 400 nm and percentage inhibition was calculated by comparing to the control which did not have the extract.

$$\% \text{ inhibition} = \frac{A^{Control}_{400} - A^{Extract}_{400}}{\left[A_{400} \right]} \times 100$$

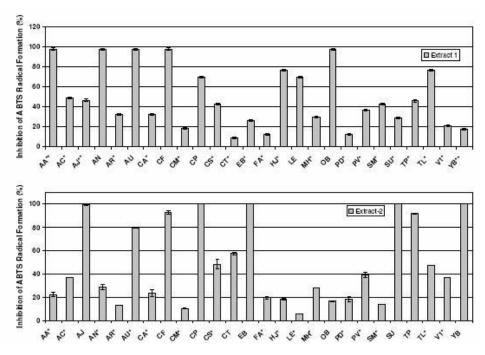**Figure 1.** Effect of different HSMP from Central/South America on reducing ABTS Free radicals (A) Extract 1 (B) Extract 2. * = 1/10th dilution of the extract used ** = 1/100th dilution of the extract used.

Sucrase assay: Sucrase inhibition activity was determined by modifying an assay described by Nishioka (1998). Briefly, 500 l of extract (or 0.1M phosphate buffer, pH 7.0) was mixed with and 500

l enzyme solutions in a test tube and pre-incubated at 28°C for 10 min. To this, 500 l of a 4% sucrose solution in 0.1 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) was added and the mixture incubated at 37°C for 30 min. The reaction was stopped by adding 1.0 ml of DNS color reagent and the tubes incubated in boiling-water bath for 5 min. The tubes were allowed to cool to room temperature and absorbance was read at 540 nm.

$$\% \text{ inhibition} = \frac{A^{Control}_{540} - A^{Extract}_{540}}{\left[A_{540} \right]^{Control}} \; x100$$

Inhibition of lipase

Inhibition of lipase by HSMP extract was determined by modifying the assay described by Smeltzer et al. (1992). Briefly, a suspension containing 1% of triolein, and 1% Tween 40 in 0.1 M Phosphate buffer (pH 8) was prepared. The suspensions were emulsified by sonication (40 W for 3 min). Assays were then initiated by adding 800 µl of this triolein emulsion to 200 µl of porcine pancreatin (0.5 g pancreatin in 15 ml 0.1 M Phosphate buffer at pH 8) and 200 µl of extract (or 0.1 M Phosphate buffer, pH 8). The contents were vortexed and absorbance measured immediately at 450 nm and designated as T_0. The test tubes were incubated at 37°C for 30 min and at the end of the incubation absorbance at 450 nm was again recorded and designated as T_{30}. $A_{450} = [A_{450} (T_0) - A_{450}(T_{30})]$ was calculated for both control and the treatment and the % inhibition was calculated by:

$$\% \text{ inhibition} = \frac{\Delta A^{Control}_{450} - \Delta A^{Extract}_{450}}{\left[\Delta A_{450} \right]}$$

RESULTS

ABTS radical formation: We measured the effectiveness of both the water extract (Extract 1) and the NaOH digested extract (Extract 2) on neutralizing ABTS radicals (Figure 1A). The extracts, which immediately decolorized the ABTS solution, were too powerful to be measured according to the protocol. Thus, they were diluted 10 fold or 100 fold to allow for more accurate calculation of their antioxidant activity. Our results indicate that extract-1 of *Amphipterygium adstringens* (AA) had the most powerful ABTS neutralizing effect. Even at 1/100th dilution, the AA extract neutralized 98% of the ABTS radicals (Figure 1A). This was followed by *Acalypha alopecuroidea* (AJ), which at 1/100th dilution had 48% inhibition. AN, *Cordia boissieri* (AC), *Cinchona succirubra* (CS), *Cassia obovata* (HJ), *Schinus molle* (SM), *Tanacetum parthenium* (TP) and *Tagetes lucida* (TL) all had to be diluted 1/10th to calculate their antioxidant activity. Among these extracts, TL and HJ had the highest antioxidant activity and reduced ABTS radicals by 78 and 77%, respectively. This was followed by AC, TP, SM and CS which reduced ABTS radical formation by 49, 44, 42 and 41%, respectively (Figure 1A). *Ambrosia artemisiaefolia* (AR), *Calluna vulgaris* (CA), *Echium Sp.*(EB), *Artemisia vulgaris* (MH), *Prunella vulgaris* (PV), *Senecio formosus* (SU) also had to be diluted 10 fold, and at that concentration, neutralized ABTS formation by 36, 35, 28, 29, 37, and 30% respectively (Figure 1A). *Crataegus mexicana* (CM), *Castela texana* (CT), *Arnica montana* (FA), *Psacalium decompositum* (PD), *Leucophyllum frut-*
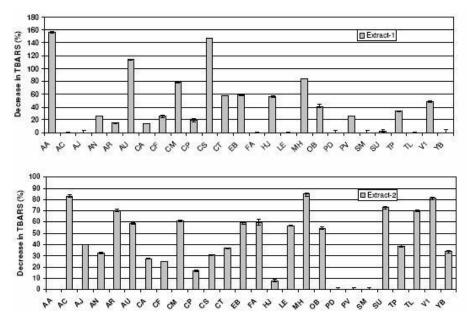
Figure 2. Effect of different HSMP from Central/South America on reducing the formations of TBARS (A) Extract 1 (B) Extract 2.

escens (V1) and *Satureja douglasii* (YB) all decreased the ABTS radicals by less than 25% at 10 fold dilutions (Figure 1A). Among the extracts that did not have to be diluted, *Anthemis nobilis* (AN), *Arctostaphylos uva-ursi* (AU), *Cassia fistula* (CF) and *Ocimum basilicum* (OB) had the highest ability to reduce ABTS radicals and they reduced them by 98% (Figure 1A). This was followed by *Chiranthodendron pentadactylon* (CP) and *Citrus limon* (LE), which decreased ABTS radicals by 70% when undiluted (Figure 1 A) . The antioxidant capacity of the NaOH digested extracts (Extract 2) was not as powerful as the water extracts (Extract 1). None of the NaOH extracts needed to be diluted 100 fold, but several had to be diluted 10 fold to obtain an accurate reading. Among these extracts, AU had the highest activity which decreased the ABTS radicals by 79% (Figure 1B). This was followed by CS, TL, PV, AC and V1, which neutralized the ABTS radical formation by 48, 44, 39, 36 and 35%, respectively (Figure 1 B). AN, MH, CA, AA, FA, PD, HJ, AR, SM, CM and LE all had to be diluted 10 fold, but had inhibitions less than 25% (Figure 1B). Among the samples that did not have to be diluted CP, EB, SU, YB had the highest antioxidant activity and completely neutralized the ABTS radicals (Figure 1 B) . This was followed by AJ, which had 98% activity (Figure 1B). CF and CT had the lowest activity among the undiluted samples and were 88 and 58%, respectively (Figure 1B).

TBARS formation: The potential of the water (Extract 1) and NaOH digested extracts (Extract 2) in reducing the formation of TBARS due to oxidation of linoleic acid was quite different. The water extracts, in general, had higher TBAR inhibition capacity compared to the NaOH digested

extracts. However, a greater number of the NaOH digested extracts exhibited capacity to reduce TBAR formation than compared to the water extracts (Figure 2 A and B).

Among the water extracts, AA, CS and AU had the highest antioxidant capacity and reduced the formation of TBARS by 158, 143 and 118%, respectively (Figure 2A). This was followed by MH and CM, which inhibited TBAR formation by 82 and 79% respectively. CT, EB and HJ reduced TBAR formation by 58, 59 and 56%, respectively (Figure 2A). V1, OB and TP were also effective in reducing lipid oxidation and did so by 50, 41 and 36% respectively (Figure 2A). AN, AR, CA, CF, CP and SU decreased TBAR formations by less than 25% (Figure 2A). AC, AJ, FA, LE, PD, SM, TL and YB did not have any capacity to prevent lipid oxidation (Figure 2A).

When we tested Extract 2, our results indicated that MH, AC and V1 decreased the formation of TBARS by 84, 82 and 81%, respectively (Figure 2B) . SU, TL and AR all prevented lipid oxidation by 70 - 72% (Figure 2B); this was followed by CM, FA, EB AU, LE and OB, which all reduced the formation of TBARS by 55 - 62% (Figure 2B). AJ, TP, CT, AN, YB CS, CA and CF were also effective in reducing formation of MDA by 40 - 26% Figure 2B). CP and HJ did decrease TBAR formation in the emulsions but did so only by <25% (Figure 2B). The (remaining four extracts AA, PD, PV and SM, were not effective in protecting linoleic acid from accelerated oxidation at high temperatures (Figure 2B).

-amylase inhibition: The effect of HSMP on the modulating activity of porcine -amylase are shown in Figures 3A and 3B. In general, water extracts were more effective in inhibiting digestion of starch than were the
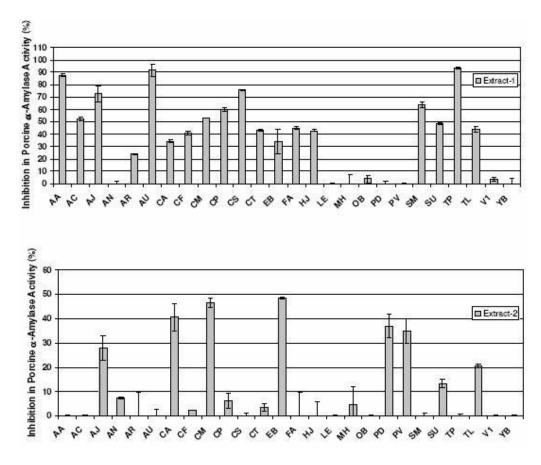
Figure 3. Effect of different HSMP from Central/South America on the activity of porcine α-amylase (A) Extract 1 (B) Extract 2.

NaOH digested extracts. Among the water extracts, AU, AA and TP had the strongest effect on inhibiting the ability of -amylase to digest starch, and decreased the enzyme activity by 93, 92 and 87%, respectively (Figure 3A). CS, AJ and SM decreased the enzyme activity by 76, 73 and 65%, respectively (Figure 3A). These were followed by CP, AC and CM which decreased the digestion of starch by the enzyme by 60, 53 and 52% respectively (Figure 3A). FA, CT, HJ, TL and CL were also effective in inhibiting the activity of the enzyme and decreased the release of maltose by 41 - 46% respectively (Figure 3A). EB, CA and AR also decreased the activity of the enzyme by 33, 32 and 23% respectively (Figure 3A). V1 and OB did display marginal capacity to inhibit the enzyme but the inhibition was <8%. AN, LE, PD, PV and YB did not show any effect on decreasing the activity of -amylase (Figure 3A). Extract 2 was less potent in inhibiting the activity of the enzyme compared to Extract 1. EB, CM and CA were most effective in inhibiting the enzyme activity and decreased the digestion of starch to maltose enzyme 36, 35 and 28%, respectively (Figure 3B). In addition to these extracts, none of the extracts had any significant effect on -amylase inhibition activity with several extracts showing no inhibitory activity (Figure 3B).

Maltase activity: Ability of the HSMP to inhibit disaccharidases was measured in rat intestinal extracts. All the HMSP extracts demonstrated very potent maltase inhibition activity and a majority of extracts completely inhibited the enzyme. In order to distinguish the differrences among the enzyme inhibition activities of the HMSP, we diluted the extracts 10- fold and then measured their maltase inhibition activity (Figure 4A and 4B). Among the water extracts, our results indicated that V1, AC and PV were the three best extracts and decreased the activity of the maltase enzyme by 32, 28 and 26%, respectively (Figure 4A). This was followed by CS, MH, HJ, TL and EB, which decreased the activity of the enzyme by 17, 14, 13, 12 and 11%, respectively (Figure 4A). The activity of each of the other extracts was below 10% at 10 fold dilution, with the YB extracts not exhibiting any maltase inhibition activity (Figure 4A).

Among the NaOH digested extracts, AN, CS and PV demonstrated the strongest maltase activity, decreasing the activity of the enzyme by 28, 27 and 25%, respectively (Figure 4B). This was followed by AC, SU and PD extracts which inhibited the maltase enzyme by 22, 14 and 11% respectively (Figure 4B). All other extracts had<10% activity at 10 fold dilution with CP showing no
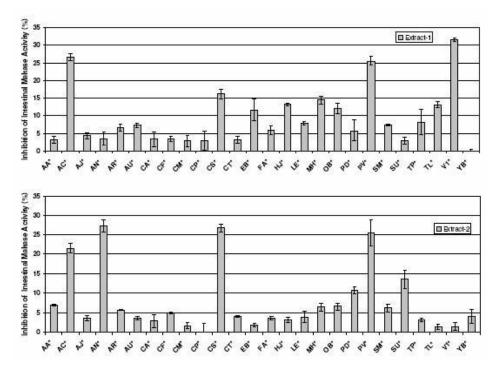
Figure 4. Effect of different HSMP from Central/South America on the activity of rat intestinal maltase (A) Extract 1 (B) Extract 2 * = 1/10th dilution of the extract used.

ability to inhibit the activity of the enzyme (Figure 4B).

Sucrase activity: Among all the enzymes tested, sucrase was the most resistant to inhibition, with only ten water extracts (Extract 1) and two NaOH digested extracts (Extract 2) showing any sucrase inhibition activety (Figure 5A and 5B). LE, CF and AU were the most powerful water extracts, the activity of the intestinal sucrase by 100, 89 and 76%, respectively (Figure 5A). EB, HJ, AA and AN were also effective and decreased the activity of the enzyme by 51, 43, 39 and 31%, respecttively (Figure 5A). CS, SM and SU also inhibited the digestion of sucrose by sucrase and did so by 24, 17 and 11%, respectively (Figure 5A) . Among NaOH digested extracts, only AN and SU were effective and inhibited the activity of the enzyme by 18 and 16% respectively (Figure 5B). All the other 24 extracts were completely ineffective in reducing the activity of this sucrose digest-ing intestinal enzyme (Figure 5B).

Lipase activity: We measured the activity of the HSMP to decrease the ability of pancreatic lipase to digest triolein and liberate oleic acid in an emulsion at alkaline pH. Our results suggested that NaOH digested extracts were more effective in inhibiting lipase than water extracts (Figure 6A and 6B). Among all the water extracts, AR was the most potent extract and decreased triglyceride hydrolysis by 38% (Figure 6A). CA, LE, CP and PD were also effective in decreasing lipid hydrolysis and inhibited the

activity of the enzyme by 33, 31, 18 and 17%, respectively (Figure 6A). All other extracts were not very effective in inhibiting the activity of lipase and decreased the enzyme activity by <15% with several extracts proving to be completely ineffective (Figure 6A). Among the NaOH digested extracts, AU proved to be the most powerful extract, which even at 10-fold dilution was much more effective than any other extract, decreasing the activity of lipase by 54% (Figure 6A). SU, SM, PV and TL were next in strength, and inhibited the hydrolysis by triolein 50, 46, 42 and 41%, respectively (Figure 6A) . CT, LE, CP and CF were also effective in inhibiting lipase activity, and decreased the activity of the enzyme by 32, 30, 29 and 25%, respectively (Figure 6A). All other extracts did not prove to be very effective in inhibiting triolein digestion and decreased the lipase activity by <20%, with several extracts proving to be completely ineffective (Figure 6A).

DISCUSSION

The etiologies of chronic diseases such as diabetes mellitus, obesity and cardiovascular disease are interrelated by the physiological occurrence of oxidative stress that is largely mediated by lipid peroxide derived reactive oxygen species (ROS) (Bierman, 1992; Keaney and Loscalzo, 1999; Jakus, 2000; Droge et al., 2002; IDF, 2007). As these diseases progress, the generation of excessively high levels of free radicals, coupled with impaired
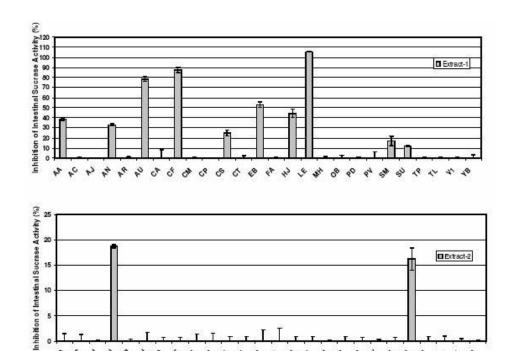
Figure 5. Effect of different HSMP from Central/South America on the activity of rat intestinal sucrase (A) Extract 1 (B) Extract 2.
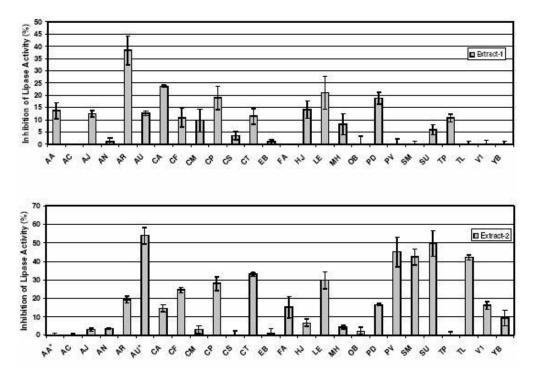

Figure 6. Effect of different HSMP from Central/South America on the activity of lipase (A) Extract 1 (B) Extract 2.

antioxidant defense systems, can result in damage to cellular proteins, membrane lipids, nucleic acids, and ultimately in cell death (Jakus, 2000; Droge et al., 2002).

While the exact cause of the increase in ROS is largely unknown, several theories revolve around diet, lifestyle and impaired metabolism. In the case of diabetes,

hyperglycemia may be the most significant contributor to elevated ROS (Maritim et al., 2003; Wiern-sperger, 2003a; 2003b). During hyperglycemia, excess glucose reaches the mitochondria, leading to overproduction and leakage of superoxide anions in the mitochondrial electron transport chain (Wiernsperger, 2003a; 2003b). These anions are normally scavenged by mitochondrial superoxide dismutase (SOD) (Nishikawa et al., 2000; Yamagishi et al., 2001; Recchioni et al., 2002). However, excess glucose can inactivate antioxidant enzyme systems such as SOD by glycating enzymes, further increaseing oxidative stress (Kawamura et al., 1992; Kaneto et al., 1994; Yan and Harding, 1997; Morgan et al., 2002). ROS may also be generated as a result of the elevated postprandial and fasting plasma triglycerides, free fatty acids and cholesterol levels characteristic of type 2 diabetes (Ohara et al., 1993; Inoguchi and Umeda, 2000) . These elevated lipids also increase insulin resistance and contribute to the development of cardiovascular disease by engaging in atherosclerotic and inflammatory processes. The insulin resistance itself contributes to weight gain (Steinberger and Daniel, 2003). In turn, weight gain, accompanied by growth and differenttiation of adipocytes, is associated with diabetes by increasing insulin resistance, and with cardiovascular disease via increased production of inflammatory factors (Goran et al., 2003; Steinberger and Daniels, 2003). Thus, the pathologies associated with these chronic diseases create a vicious cycle. It follows, then, that holistic management of diabetes should include therapeutic strategies to reduce postprandial hyperglycemia, hypertriglyceridemia and ideally, to decrease body weight or at least control weight gain. These alternative therapeutic strategies, in addition to dietary and lifestyle modifications such as decreasing calorie intake, moderating carbohydrate and fat intake, and increasing physical activity, can contribute to a more efficient management of diabetes and thus reduce the risk for the development of co-morbidities (American Diabetes Association, 2007) . To support the quest for alternative therapies to treat diabetes, we investigated the potential of HSMP from Central/South America to reduce oxidative stress and energy harvest from carbohydrates and lipids in the gastrointestinal tract.

To more fully characterize the enzyme-inhibitory properties of these herbs, we tested two types of extracts – one simple water extract, and another pre-digested with NaOH. Water extraction isolates bioactive compounds that are readily soluble in water and extracted relatively easily, including such substances as free phenolics, phenolic acids and flavonoids (Krygier et al., 1982; Sun et al., 2002). The digestion of a sample with NaOH releases insoluble polymerized phenolics, tannins, lignins and lignans from proteins and carbohydrates (Krygier et al., 1982; Sun et al., 2002). Not surprisingly, the two types of HSMP extracts demonstrated different activities, and their potential use is also quite different. For example, bio-

active ingredients that can be extracted in water can permit a more convenient and versatile usage, such as addition to teas or foods where the medium is predominantly aqueous. In contrast, bioactive ingredients present in plants that are released only upon digestion with NaOH will be relatively challenging to use in regular foods or beverages - they will have relatively low bioavailablity since they will not be released completely by the digestive processes taking place in the stomach and intestine (Karakaya et al., 2004; Spencer et al., 2004). However, these substances can potentially be isolated for potential use as therapeutic agents.

Our results suggest that almost all the HSMP that we investigated have free radical reducing capacity in a polar system, as indicated by their ability to neutralize ABTS free radicals. This feature is common to most natural products, due to the presence of hydroxylated phenolic groups (Vattem, 2004). However, the majority of oxidative stress in biological systems occurs at the lipid/water interface characteristic of plasma and organelle membranes. Thus, an extract capable of exhibiting antioxidant ability at these interfaces has more potential to prevent oxidative stress related damage in cellular systems (Vattem, 2004). We assessed this property of the HSMP using the TBARS assay, which utilizes an emulsion system. Based on our results, we identified the five most effective water and NaOH digested extracts (Table 2A and 2B). The water extracts of AA, CS, AU, MH and CM were the most powerful TBAR inhibitors, whereas MH, AC, V1, SU and AR were the most potent extracts in the NaOH digested system. Only MH demonstrated lipid oxidation inhibition in both water and NaOH digested extracts.

We also identified and ranked the HSMP based on their ability to inhibit amylase, maltase, sucrase and lipase activity in both water and NaOH digested extracts (Table 2A and 2B) . We determined that the AA, AJ, CF, CP, HJ, LE and TP water extracts contained bioactive ingredients capable not only of reducing TBARS, but of inhibiting amylase, maltase, sucrase and lipase activity (Table 2A). The NaOH digested extracts of these HSMP did not appear to contain any significant bioactivity in our investigation (Table 2B). Thus, aqueous extracts of these HSMP can potentially be offered in teas, foods and/or dietary supplements. AN, SM and SU were the only HSMP that were found to inhibit TBARS and amylase, maltase, sucrase and lipase activities only when extracted after NaOH digestion (Table 2B). Thus, extracts of these HSMP would not be effective if added to beverages or foods, but would be therapeutically useful only if the bioactive ingredients were released from their matrices after extensive processing (Table 2B). AC, AR, AU, CA, CM, CS, EB, MH, PD, PV and V1 represented the most versatile extracts which had bioactive ingredients in their water extracts and also when these HSMP were digested using NaOH (Table 2A, 2B). Our results suggest that, the best combination extracts which would prove to be most

Table 2. The HSMP with the highest activity as measured by their ability to reduce TBAR formation, and inhibit amylase, maltase, sucrase and lipase activity. (A) Water extracts (B) NaOH digested extracts. Extracts in red are with multiple activities.

TBARS	Amylase	Maltase	Sucrase	Lipase
Extract-1	Extract-1	Extract-1	Extract-1	Extract-1
AA	**AU**	V1	**LE**	AR
CS	TP	AC	CF	CA
AU	AA	PV	**AU**	**LE**
MH	**CS**	**CS**	EB	CP
CM	AJ	**MH**	HJ	PD
TBARS	Amylase	Maltase	Sucrase	Lipase
Extract-2	Extract-2	Extract-2	Extract-2	Extract-2
MH	EB	**AN**	**AN**	AU
AC	CM	CS	**SU**	**SU**
V1	CA	**PV**	****	**PV**
SU	PD	**AC**	****	SM
AR	**PV**	**SU**	****	TL

effective in reducing energy harvest in the gut as well as manage oxidative stress was AA, AU, V1, LE and AR for water extracts (Table 2A) and MH, EB, AN, AN and AU for NaOH digested extracts (Table 2B). These extracts are ranked in their ability to reduce TBARS, and inhibit amylase, maltase, sucrase and lipase activity respectively. We also tried to identify the HSMP which had bioactive ingredients capable of many different type of activities, our results indicate that among all water extracts CS was not only a powerful inhibitor of lipid oxidation but was also an effective inhibitor of amylase and maltase. AU on the other hand, was not only an effective lipid oxidation inhibitor but also a potent inhibitor of amylase and sucrase. Among the NaOH digested extracts, SU was found to be the most versatile bioactive ingredients, and was effective in reducing, TBARS, maltase, sucrase and lipase. This was followed by PV which was very effective in reducing the activity of amylase, maltase and lipase.

While the exact mechanism of the enzyme inhibition is still largely not well understood, the possible mode of action could be due to the interactions between secondary metabolites in these HSMP and the energy harvesting enzymes, as described by us previously (Vattem et al., 2004; Kwon et al., 2006). Phenolic phytochemicals are well known for their ability to bind to lysine side chains, at the indole ring of the tryptophan residues, at the carboxyl groups of aspartic and glutamic acids, and at the free thiol groups of cysteine side chains of the enzymes (Rohn et al., 2002; Gupta et al., 2007; Chauhan et al., 2007). Thus, it is possible that the enzymes are inhibited due to a non-active site allosteric interaction

between the phenolic phytochemicals and the enzymes (Mitaru et al., 1984). A non- covalent binding of the phenolic phytochemical to the enzyme at a site away from the active site could induce a conformational change in the tertiary structure of the enzyme molecule and allosterically inhibit the formation of a stable enzyme-substrate complex (Mitaru et al., 1984; Gupta et al., 2007). It is also possible that these phenolic phytochemicals, both in their glycosidic form or as aglycosides could be competing with the substrate to bind with the active site of the molecule either due to the phenolic group or with the sugar moiety of the phenolic- glycoside as discussed previously (Vattem, 2004; Kwon et al., 2006). Isolating the bioactive ingredients present in the HSMP that have the best biological activity and elucidating their mechanism of enzyme inhibition using enzyme kinetic studies will form the basis of our future research.

Conclusion

It is important to note that while historical records indicate that Central and South American populations have practised traditional medicine with dietary herbs since the time of Incan, Mayan and Aztech civilizations, the body of literature on health benefits of herbs used by the hispanic culture is very limited (Huxtable, 1983; Gomez-Beloz and Chavez, 2001; Lewis and Elvis-Lewis, 2003; Mikhail et al., 2004). A systematic, functional evaluation of herbs commonly used in traditional hispanic cuisines and traditional medicine has never been performed either *in vitro* or *in vivo* systems, and their complete health benefits

have not been elucidated. If this trend continues, valuable information available in this form traditional medicine might be lost forever (Gomez-Beloz and Chavez, 2001; Mikhail et al., 2004). We have demonstrated, for the first time that herbs, spices and medicinal plants from Central and South America have the potential to discourage excessive energy harvest from mammalian gut and offer protection from oxidative stress. This knowledge may serve as a foundation for the development of comprehensive therapeutic strategies to manage type 2 diabetes mellitus and thereby reduce the risk of associated morbidities such as cardiovascular disease.

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