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Anti-diabetic and Anti-energy harvesting properties of common traditional herbs, spices and medicinal plants from India

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Abstract

Excessive caloric intake, hyperglycemia, irregular blood lipid levels and resulting oxidative stress are accepted etiologies of Diabetes mellitus (DM) and associated co-morbidities including cardiovascular disease (CVD) and obesity (OB). These diseases are fast becoming leading causes of mortality in the developed and developing world. One important strategy for managing DM is managing postprandial hyperglycemia by reducing the digestion of carbohydrates by α-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 18 different herbs, spices and medicinal plants (HSMP) commonly used in 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 α-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 different traditional medicines that can be used as an alternative and complimentary strategy to manage risk factors of DM and associated co-morbidities.

Keywords: *Diabetes mellitus, cardiovascular disease, obesity, energy harvest, a-glucosidase, pancreatic amylase, disaccharidases, maltase, sucrase, lipase, enzyme inhibition, antioxidant activity, herbs, spices, medicinal plants, traditional medicine.*

1. Introduction

Type 2 diabetes mellitus (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 cardiovascular disease (CVD), obesity (OB), microvascular damage, and eventual failure of the eyes, kidneys and nerves [1, 2]. DM is a serious public health concern

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for governments across the world, and, the number of people diagnosed with this disease is projected to increase to 380 million by 2025 [3]. Postprandial hyperglycemia, following a rapid increase in blood glucose, is one of the earliest risk factors associated with the development of type 2 DM [2]. Digestion of dietary starch by α-glucosidases, including glucoamylase and pancreatic α-amylase, contributes to this sharp increase in blood glucose [7-9]. Inhibition of these enzymes has therefore long been considered a tool for the management of hyperglycemia, and type 2 DM [1, 2, 7, 8]. In fact, several pharmaceutical drugs used for the treatment of DM, have relied on decreasing the digestion and absorption of starches and sugars which contribute to postprandial hyperglycemia [1, 2]. These drugs primarily work by reducing the metabolic activity of α-glucosidases, including pancreatic α-amylase, and intestinal dissacharidases, such as sucrase and maltase [7-9]. The currently available anti-diabetic 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 [1, 2, 7, 8]. Clearly, complementary and alternative solutions to pharmacological treatment of diabetes are warranted.

Cardiovascular disease, associated with vascular damage and atherosclerosis, is one the primary causes of mortality in the adult population [9, 10]. 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 by the enterocytes into the blood [11-13]. Another drug, sibutramine, is an amphetamine-like substance that is taken to promote satiety by increasing levels of serotonin and norepinephrine [11-13]. 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 [11-13]. Therefore, there is an urgent need for new and safer alternatives for prevention and treatment of overweight and CVD.

Herbs, spices and medicinal plants (HSMP) have been cherished by many ancient cultures for their ability to cure common ailments and promote good health [14, 15]. Recent research indicates that populations incorporating HSMP into their diets have a lower incidence of chronic disease [14]. 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 antioxidants in cellular systems, which impairs normal biological functions [16]. 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. Since antiquity, DM, CVD and OB have been managed and treated with medicinal plants by many cultures. These HSMP used in traditional medicines from the Indian subcontinent, China and Central/South Americas continue to serve as an abundant resource for discovery of new drugs [17-20] and offer an abundant repertoire for the

discovery of natural inhibitors of carbohydrate and lipid digestion [17-20]. We believe these HSMP have the potential to be incorporated into therapeutic strategies to control postprandial hyperglycemia, assist in weight management, and manage CVD. In this manuscript we report the in vitro ability of 18 commonly used HSMP on reducing oxidative stress and inhibiting carbohydrate and lipid digestion in the gastrointestinal tract.

2. Materials and Methods

2.1. Sample preparation and extraction

HSMP were obtained directly from Taj Imports (Austin, TX) and are listed in Table 1.

2.1.1. Extraction of Low Molecular Weight Fraction (LMWF)

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 minutes at 250 rpm. The samples were then vaccum filtered using a Buchner funnel equipped with Whatman No. 1 filter paper. The filtrate was called water extract and labeled as *LMWF*.

2.1.2. Extraction of High Molecular Weight Fraction (HMWF) [21, 22]

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 4N NaOH was added, and the mixture was stirred and allowed to digest for 30 minutes at 250 rpm. The samples were then vaccum filtered using a Buchner funnel and Whatman No. 1 filter paper. The pH of the filtrate was adjusted immediately to 7.0 and labeled as *HMWF*. The total phenolics was determined by an assay described previously [8]. All extracts were standardized to a phenolic content of 25 µg/ml gallic acid equivalents and used in all biochemical and enzymatic assays.

2.2. Antioxidant Assays

2.2.1. 2, 22'-azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) Assay: Total phenolics assay.

The ABTS assay was conducted by modifying a previously described method [23]. Briefly, to 1 mL of 7 mM ABTS (in water, activated overnight with 140 mM potassium persulfate) was added 50 µL of standardized extracts (*LMWF* or *HMWF*), 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:

$$\%\ \text{inhibition} = \left(\left[\frac{A_{734}^{Control} - A_{734}^{Extract}}{\left[A_{734}^{Control} \right]} \right] \right) x100$$

2.2.2. Thiobarburic acid reactive substances (TBARS) Assay

TBARS were measured by modifying a method previously described [24]. 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 standardized 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 (e) 156 $\mu mol^{-1} cm^{-1}$ and expressed as mmol/g FW. Inhibition of TBAR formation by the extracts was calculated by comparing with the control, which did not contain the extracts.

$$\%\ \text{inhibition} = \left(\left[\frac{A_{532}^{Control} - A_{532}^{Extract}}{\left[A_{532}^{Control}\right]} \right] \right) x100$$

2.3. Amylase assay

The amylase inhibition assay was carried out by a method described previously [11]. Briefly, a total of 500 µl of standardized extract and 500 µl of 0.02 M sodium phosphate buffer (pH 6.9 with 0.006 M sodium chloride) containing porcine pancreatic α-amylase (0.5 mg/ml; Sigma Chemical Co., St. Louis, MO) were incubated at 25°C for 10 minutes. 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 minutes. 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 minutes 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 a-amylase was calculated as follows:

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

2.3.1. Inhibitory activity on rat intestinal disaccharidases

The disaccharidases in rat intestinal extracts were prepared by modifying the method described by Dahlqvist, (1968) [25]. Briefly, 0.5 g of rat intestinal acetone powder (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.

2.4. Maltase assay

Maltase activity was assayed using a modification of the procedure described previously [26]. Briefly, samples were prepared by mixing 0.5 ml of 0.1 M Phosphate buffer, pH 7.0 (or standardized extract) (at 25°C) with 0.25 ml of 20 mM p-Nitrophenyl-α-D-glucopyranoside (PNPG). 0.3 ml of enzyme solution was added and mixed. The reaction mixture was incubated for 15 minutes 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 % inhibition was calculated by comparing to the control which did not have the extract.

$$\%\ \text{inhibition} = \left(\left[\frac{A_{400}^{Control} - A_{400}^{Extract}}{\left[A_{400}^{Control}\right]} \right] \right) x100$$

2.5. Sucrase Assay

Sucrase inhibition activity was determined by modifying an assay described by Nishioka (1998) [27]. Briefly, 500 µl of standardized extract (or 0.1 M phosphate buffer, pH 7.0) was mixed with and 500 µl enzyme solution in a test tube and pre-incubated at 28°C for 10 minutes. To this, 500 µl 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 minutes. The tubes were allowed to cool to room temperature and absorbance was read at 540 nm.

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

2.5.1. Inhibition of lipase

Inhibition of lipase by HSMP extract was determined by modifying the assay described by Smeltzer *et al.*, (1992) [28]. 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 standardized 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}. $\Delta A_{450} = [A_{450}(T_0) - A_{450}(T_{30})]$ was calculated for both control and the treatment and the % inhibition was calculated by:

$$\% \text{ inhibition} = \left(\left[\frac{\Delta A_{450}^{Control} - \Delta A_{450}^{Extract}}{\left[\Delta A_{450}^{Control}\right]} \right] \right) x100$$

2.6. Statistical Analysis

All experiments were performed at least in triplicates. Analysis at each data point from each experiment was carried out in duplicate or triplicate. Means, standard errors and standard deviations were calculated from replicates within the experiments and analyses using Microsoft Excel XP.

3. Results

3.1. ABTS Radical Formation

We measured the effectiveness of both the low molecular weight fraction (*LMWF*) and the high molecular weight fraction (*HMWF*) on neutralizing ABTS radicals (Fig. 1). Extracts were standardized to a phenolic content of 25 µg/ml gallic acid equivalents (data not shown). 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.

Table 1. List of different herbs, spices and medicinal plants from India used for the study.

Scientific Names	Abbreviation
Acacia concinna	AC
Ailanthus excelsa	AE
Anacyclus pyrethrum	AP
Pterocarpus marsupium	BF
Centratherum anthelminticum	CAN
Centella asiatica	CAS
Curcuma Longa	CL
Commiphora myrrha	CM
Evolvulus alsinoides	EA
Enicostemma littorale	EL
Emblica officinalis	EO
Embelia ribes	ER
Mesua ferrea	MF
Swertia chirayata	SC
Sapindus laurifolius	SL
Terminalia arjuna	TA
Terminalia chebula	TC
Withania somnifera	WS

Table 2. The herbs spices and medicinal plants from India with the highest bio-activity as measured by their ability to reduce TBAR formation, and inhibit amylase, maltase, sucrase and lipase activity along with their corresponding IC_{50} values (µg/ml) on dry weight (d.w) basis. (A) Low Molecular Weight Fractions (LMWFs) (B) High Molecular Weight Fractions (HMWFs). Extracts in bold are with multiple activities.

TBARS	Amylase	Maltase	Sucrase	Lipase					
LMWF	IC_{50} (µg/ml)	LMWF	IC_{50} (µg/ml)		LMWF	IC_{50} (µg/ml)		LMWF	IC_{50} (µg/ml)
LMWF	IC_{50} (µg/ml)								
SC	1.8	BF	13.4	SL	12.5	AE	5.7	CM	4.3
EA	2.2	SL	11.8	AE	14.9	AC	9.1	BF	5.0
CAS	2.2	TA	14.3	TC	18.0	SL	9.6	TA	5.4
CM	3.0	CAN	14.4	BF	22.9	TC	11.6	EA	6.9
TA	7.4	CL	14.7	TA	24.8	CAS	21.4	TC	7.3

TBARS	Amylase	Maltase	Sucrase	Lipase					
HMWF	IC_{50} (µg/ml)	HMWF	IC_{50} (µg/ml)		HMWF	IC_{50} (µg/ml)		HMWF	IC_{50} (µg/ml)
HMWF	IC_{50} (µg/ml)								
CL	3.4	EL	29.0	EO	17.7	SL	27.6	AE	20.7
CM	4.8	SL	29.5	AC	18.0	AE	38.5	SC	22.2
CAN	10.5	TA	38.7	AE	18.4	MF	41.5	BF	22.5
BF	13.0	AP	39.1	TA	18.6	****	****	WS	23.7
EA	17.9	EA	55.1	SC	71.4	****	****	CM	29.1

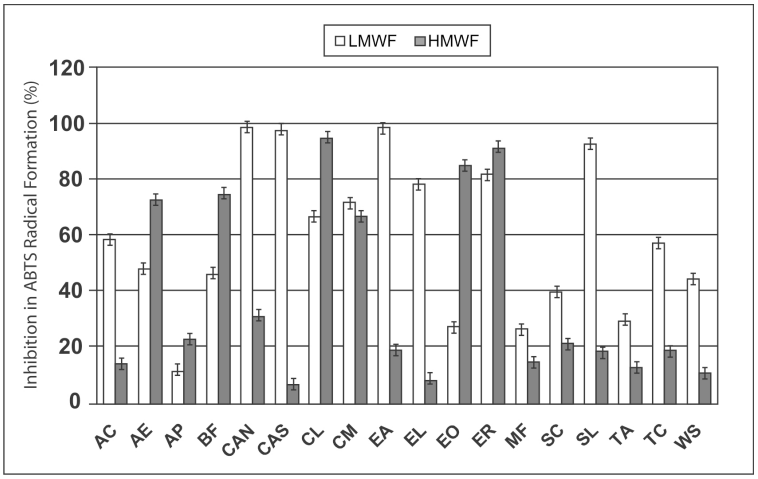

Fig. 1. ABTS free radical reducing activity of Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India. Disappearance of 2,22 -azinobis(3-ethyl-benzothiazoline-6-sulfonic acid) radical was monitored at 734 nm. Data expressed as Mean ± SEM [$LMWF_{(n=6)}$; $HMWF_{(n=6)}$]. All extracts standardized to a phenolic content of 25 µg/ml gallic acid equivalents before testing.

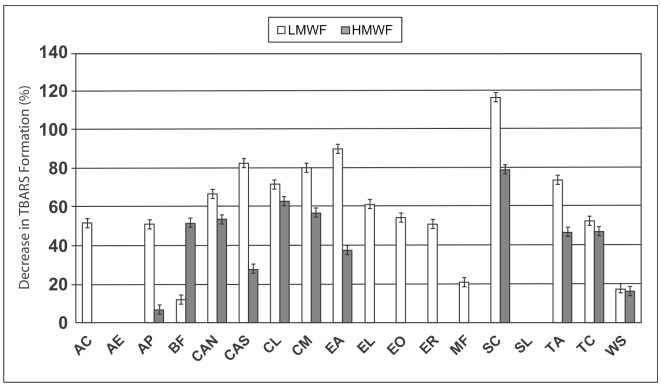

Fig. 2. Inhibition in TBARS formation by Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India. TBARS formation was measured at 532 nm in an accelerated lipid oxidation model. Data expressed as Mean ± SEM [LMWF $_{(n=6)}$; HMWF $_{(n=6)}$]. All extracts standardized to a phenolic content of 25 µg/ml gallic acid equivalents before testing.

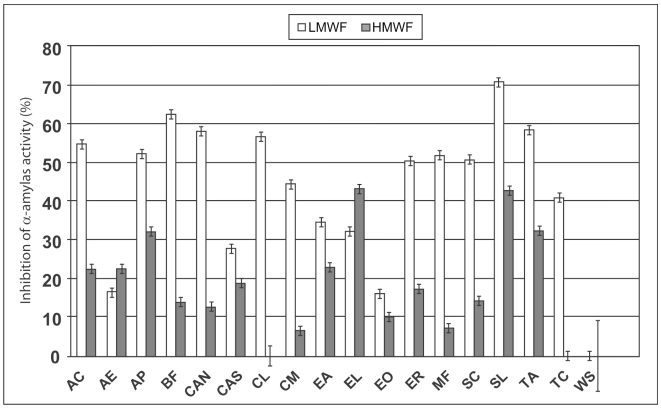

Fig. 3. Modulation of porcine a-amylase activity by Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India. α-amylase activity was assayed by measuring the formation of reducing sugars from starch measured using the DNS assay at 540 nm. Data expressed as Mean ± SEM [LMWF $_{(n=8)}$; HMWF $_{(n=8)}$]. All extracts standardized to a phenolic content of 25 µg/ml gallic acid equivalents before testing.

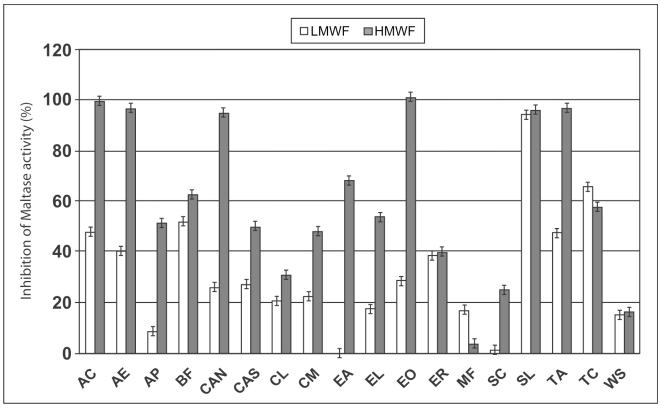

Fig. 4. Changes in rat intestinal maltase activity by Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India. Maltase activity was assayed by measuring the release of PNP from its substrate PNPG at 400 nm. Data expressed as Mean ± SEM [LMWF $_{(n=8)}$; HMWF $_{(n=8)}$]. All extracts standardized to a phenolic content of 25 µg/ml gallic acid equivalents before testing.

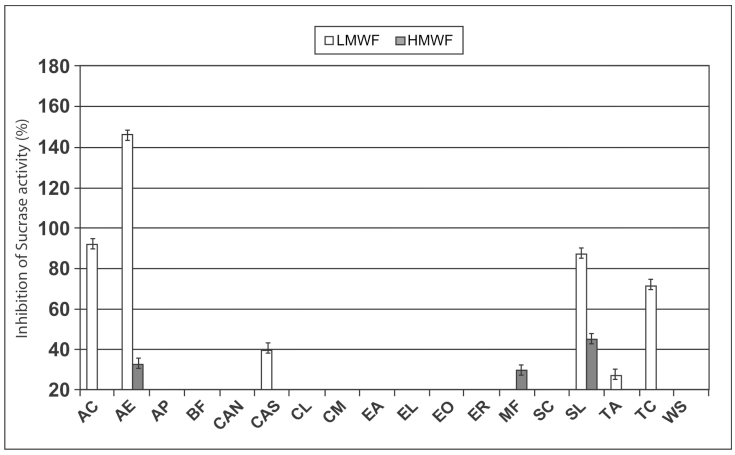

Fig. 5. Inhibitory activity of Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India on Rat intestinal sucrase activity. Sucrase activity was assayed by measuring the formation of reducing sugars from sucrose measured using the DNS assay at 540 nm. Data expressed as Mean ± SEM [LMWF $_{(n=8)}$; HMWF$_{(n=8)}$]. All extracts standardized to a phenolic content of 25 µg/ml gallic acid equivalents before testing.

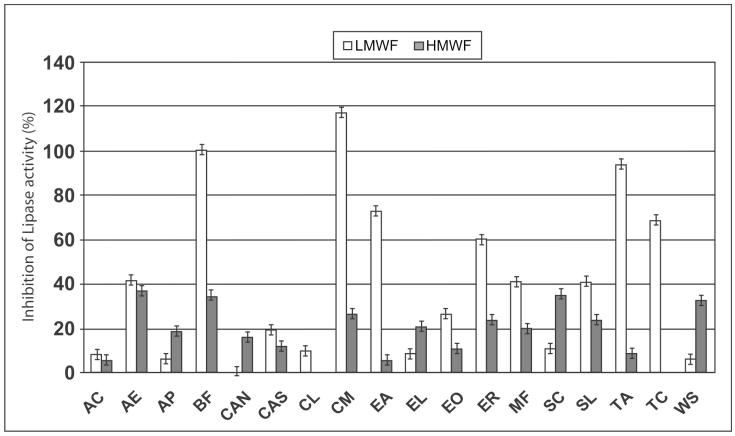

Fig. 6. Effect of Low Molecular Weight Fractions (*LMWFs*) and High Molecular Weight Fractions (*HMWFs*) of different herbs spices and medicinal plants from India on Lipase activity. The Lipase activity was assayed by measuring the release of oleic acid from its substrate triolein for 5 min at 450 nm. Data expressed as Mean ± SEM [LMWF $_{(n=8)}$; HMWF $_{(n=8)}$]. All extracts standardized to a phenolic content of 25µg/ml gallic acid equivalents before testing.

Our results indicate that *LMWF Terminalia chebula* (TC) had the most powerful ABTS neutralizing effect. Even at 1/100th dilution, the TC extract neutralized 59% of the ABTS radicals (Fig. 1). This was followed by *Terminalia arjuna* (TA) and *Emblica officinalis* (EO) which at 1/100th dilution had 30% and 26% inhibition respectively. *Acacia concinna* (AC) *Ailanthus excelsa* (AE), *Swertia chirayata* (SC), *Anacyclus pyrethrum*(AP) all had to be diluted 1/10th had an inhibition of 59%, 46%, 15% respectively. *Evolvulus alsinoides* (EA), *Centratherum anthelminticum* (CAN), *Centella asiatica* (CAS) almost completely inhibited the formation of ABTS radicals and their inhibition ranged from 99-100% (Fig. 1). Among the other undiluted extracts *Sapindus laurifolius* (SL), *Embelia ribes* (ER), *Enicostemma littorale* (EL),

Commiphora myrrha (CM) and *Curcuma Longa* (CL) also inhibited the formation of ABTS radicals significantly by 82%, 79%, 75% and 70% respectively (Fig. 1). *Pterocarpus marsupium* (BF), *Withania somnifera* (WS) and *Evolvulus alsinoides* (MF) (Fig. 1).

The antioxidant capacities of the *HMWFs* were not as powerful as the *LMWF*. EO, TC and TA had to be diluted 100 fold and inhibited the ABTS radical formation by 34%, 19% and 15% respectively. AE, AP, SC and AC had to be diluted 10 fold to obtain an accurate reading. Among these extracts, AE had the highest activity which decreased the ABTS radicals by 76% (Fig. 1). This was followed by AP, SC and AC which neutralized the ABTS radical formation by 22%, 21%, and 16%, respectively (Fig. 1). Among the samples that did not have to be diluted CL, ER, BF and CM had the highest antioxidant activity and reduced the ABTS radical formation by 97%, 95%, 77% and 68% respectively (Fig. 1). This was followed by CAN, which had 36% activity (Fig. 1). All remaining extracts were not very effective in reducing the formation of ABTS radicals; they were able to neutralize the ABTS radicals by less than 20% (Fig. 1).

3.2. TBARS Formation

The potential of the *LMWF* and *HMWF* 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. In addition, fewer number of the *HMWF*'s exhibited capacity to reduce TBAR formation than compared to the *LMWF*'s (Fig. 2). Among the *LMWFs*, SC, EA, CAS and CM had the highest antioxidant capacity and reduced the formation of TBARS by 118%, 85%, 82%, and 798%, respectively (Fig. 2). This was followed by TA, CL and CN which inhibited TBAR formation by 72%, 65% and 63% respectively. EL, EO and TC reduced TBAR formation by 60%, 56% and 55% respectively (Figure 2). AC, AP and ER were also effective in reducing lipid oxidation and did so by 50%, 49% and 46 % respectively (Figure 2 A). MF, WS and BF decreased TBAR formations by less than 20% (Fig. 2). AE and SL did not have any capacity to prevent lipid oxidation (Fig. 2).

When we tested *HMWFs*, our results indicated that, SC had the highest ability to reduce lipid oxidation. It reduced the formation of TBARS by 78%. This was followed by CL, CM, CAN and BF which decreased the formation of TBARS by 61%, 57%, 53% and 51%, respectively (Fig. 2). TA and TC also reduced lipid oxidation by 45% (Fig. 2); this was followed by EA and CAS which reduced the formation of TBARS by 37% and 27% respectively (Fig. 2). WS and AP did decrease TBAR formation in the emulsions but did so only by <15% (Fig. 2). The remaining extracts AC, AE, EL, EO ER, MF and SL were not effective in protecting linoleic acid from accelerated oxidation at high temperatures (Fig. 2).

3.3. α-amylase Inhibition

The effect of HSMP on the modulating the activity of porcine α-amylase are shown in Figures 3. In general, *LMWFs* were more effective in inhibiting digestion of starch than were the *HMWFs*. Among the *LMWFs*, BF which was measured at 10 fold dilution had the highest ability to inhibit the formation of maltose from starch by 62%. SL, TA, CAN and CL were also effective in inhibiting activity of amylase by 72%, 58%, 58% and 56% respectively. This was followed by AC, MF, AP, SC and ER whose inhibition activity ranged from 55% to 50% respectively (Fig. 3). CM, TC, EA, EL CAS, AE and EO were also effective in inhibiting the activity of porcine amylase and their inhibitory activity ranged between 15-45% respectively (Fig. 3). Among all the *LMWFs*, WS did not exhibit any amylase inhibition activity. *HMWFs*

was less potent in inhibiting the activity of the enzyme compared to *LMWFs*. EL and SL were most effective in inhibiting the enzyme activity and decreased the digestion of starch to maltose by 43% and 42% respectively (Fig 3). This was followed by TA and AP which inhibited the activity of the enzyme by 33% and 32% respectively. EA, AC and AE were also effective in reducing the activity of porcine α-amylase and inhibited the activity by 23%. All other extracts also inhibited the activity of the enzyme but did so only by less than 20%. CL, TC and WS were completely ineffective in reducing the activity of the enzyme.

3.4. Maltase activity

Ability of the HSMP to inhibit disaccharidases was measured in rat intestinal extracts. All the *HMWFs* were more effective in reducing the activity of the enzyme than the water extracts (Fig. 4). Among the *LMWFs*, our results indicated that SL was the most powerful inhibitor of the maltase enzyme and reduced its activity by 96%. This was followed by AE which was diluted 10 fold, TC and BF which reduced the hydrolysis of PNPG by 40%, 63% and 56% respectively (Fig 4). TA, AC and ER were also effective in inhibiting the activity of the enzyme by 48%, 46% and 39% respectively (Fig 4). The maltase inhibitory activity of all other extracts was less than 30% with EA showing not inhibition (Fig 4).

Among the *HMWFs*, EO, AC, AE, TA, SC and CAN demonstrated the strongest maltase activity, decreasing the activity of the enzyme by 105%, 100%, 97%, 96%, 95% and 94% respectively (Fig 4). This was followed by CL (diluted 10 fold), EA, BF, TC and EL which inhibited the hydrolysis of PNPG by 35%, 70%, 63%, 58% and 55% respectively (Fig 4). AP, CAS and CAM all had activity ranging between 47-50%. All the remaining extracts did inhibited the enzyme activity by less than 25%.

3.5. Sucrase activity

Among all the enzymes tested, sucrase was the most resistant to inhibition, with only six *LMWFs* and three *HMWFs* showing any sucrase inhibition activity (Fig. 5). AE, AC and SL were the most powerful *LMWFs*, they reduced the activity of the intestinal sucrase by 145%, 92% and 86%, respectively (Fig. 5). TC, CAS, and TA were also effective and decreased the activity of the enzyme by 75%, 39%, and 31%, respectively (Fig. 5). Among *HMWFs*, only SL, AE and MF were effective and inhibited the activity of the enzyme by 46%, 34% and 30% respectively (Fig. 5). All the other 24 extracts were completely ineffective in reducing the activity of this sucrose digesting intestinal enzyme (Fig. 5).

3.6. 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 *LMWFs* were more effective in inhibiting lipase than *HMWFs* (Fig. 6). Among all the *LMWFs*, CM and BF were the most potent extracts and decreased triglyceride hydrolysis by 118% and 100% respectively (Fig. 6). TA, EA, TC and ER were also effective in decreasing lipid hydrolysis and inhibited the activity of the enzyme by 95%, 76%, 73% and 60%, respectively (Fig. 6). These were followed by MF, SL and AE decreased the hydrolysis of the lipids by triglycerides by 40%. All other extracts were not very effective in inhibiting the activity of lipase and decreased the enzyme activity by <20% (Fig. 6). Among the *HMWFs*, AE, SC and BF which were able to inhibit the enzyme activity by 36%, 34% this was followed by WS, CM, ER, SL and EL which decreased the activity of lipase by 32%, 27%, 23%, 22% and 20% respectively (Fig. 6). All other extracts did not prove to be very effective in inhibiting triolein

digestion and decreased the lipase activity by <20%, with CL and TC proving to be completely ineffective (Fig. 6).

4. Discussion

Holistic management of diabetes and related co-morbidities should include therapeutic strategies that reduce postprandial hyperglycemia, hypertriglyceridemia and ideally, to decrease body weight or at least control weight gain. We investigated the potential of herbs spices and medicinal plants (HSMP) from India to reduce oxidative stress and energy harvest from carbohydrates and lipids in the gastrointestinal tract as a possible solution to a more efficient management of diabetes and thus reduce the risk for the development of co-morbidities [29]. To more fully characterize the enzyme-inhibitory properties of these herbs, spices and medicinal plants we tested two types of extracts - Low Molecular Weight Fraction (*LMWF*) which includes free phenolics, phenolic acids and flavonoids and the High Molecular Weight Fraction (*HMWF*), which includes insoluble polymerized phenolics, tannins, lignins and lignans from proteins and carbohydrates [21, 22]. Not surprisingly, the two types of HSMP extracts demonstrated different activities, and their potential use could therefore be also quite different. For example, bioactive 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. However, these substances can be isolated for 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 [30, 31]. 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 [30, 31]. We assessed this property of the HSMP using the TBARS assay, which utilizes an emulsion system which is more representative of a physiological scenario. Based on our results, we identified the five most effective *LMWFs* and *HMWFs* (Table 2A and 2B). The *LMWFs* of SC, EA, CAS, CM and TA were the most powerful TBAR inhibitors, whereas CL, CM, CAN, BF and EA were the most potent *HMWFs*. Only EA demonstrated lipid oxidation inhibition in both *LMWFs* and *HMWFs*.

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 *LMWFs* of BF, SL, TA and TC contained bioactive ingredients capable not only of reducing TBARS, but of inhibiting amylase, maltase, sucrase and lipase activity (Table 2A). Thus, aqueous extracts of these HSMP can potentially be offered in teas, foods and/or dietary supplements. The *HMWFs* of these HSMP did not appear to exhibit multiple functionalities (Table 2B). AE was the only HSMP that were found to have more than 3 types of functionality, it inhibited maltase, sucrase and lipase (Table 2B). All other extracts had two or less than two types of functionalities. In addition, only the *HMWFs* of WS, EL, EO, MF and AP were effective (Table 2B) and 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, AE, BF, CAN, CL, CM, EA, SC, SL, TA represented the most versatile extracts which had bioactive ingredients in both *LMWFs* and *HMWFs* their water extracts (Table 2A, 2B). Our results suggest that, the best combination extracts which would prove to be most effective in reducing energy harvest in the gut as well as manage oxidative stress were *LMWFs* of BF, SL, TA and TC (Table 2A) and the *HMWFs* of AE, BF, CM, EA and SC (Table 2B). We also tried to identify the HSMP which had bioactive ingredients capable of multiple functionalities, our results indicate that among all *LMWFs* TA was not only a powerful inhibitor of lipid oxidation but was also an effective inhibitor of amylase maltase and lipase (Table 2A). SL on the other hand, was a potent inhibitor of amylase, maltase and sucrase. BF was a potent inhibitor of amylase, maltase and lipase, whereas, TC was an effective inhibitor of Maltase sucrose and lipase (Table 2A). Among the *HMWF*'s, EA was found to be the most versatile bioactive ingredients, and was effective in inhibiting maltase, sucrase and lipase (Table 2B). CM and BF inhibited lipid oxidation as well as lipase activity. EA also inhibited lipid oxidation but was not effective in inhibiting lipase activity, it was however a powerful inhibitor of amylase. AE on the other hand inhibited the activity of maltase and sucrase (Table 2B).

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.

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