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Indian medicinal plants known to contain intestinal glucosidase inhibitors also inhibit pancreatic lipase activity—An ideal situation for obesity control by herbal drugs

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Dietary excess of lipids causes substantial health disorders like hyperlipemia, obesity and cardiovascular problems. Use of lipase and α-glucosidase inhibitors in combination is considered to be the ideal therapy for obesity control. Anti-obesity activity of natural products is usually assessed by inhibition of pancreatic lipase activity by the preparations. We have reported earlier that some known hypoglycemic medicinal plants, such as, *Eugenia jambolana* Lam., *Azadirachta indica* A. Juss., *Tinospora cordifolia* (Thunb.) Miers and *Trigonella foenum-graceum* L. contain α-glucosidase inhibitors. The present study reports that their plant extracts also inhibit pancreatic lipase activity. The methanolic extracts showed an IC_{50} value (µg/mL) of 230±20, 520±15, 360±25 and 564±12 for *E. jambolana* (stem-bark), *A. indica* (root), *T. cordifolia* (leaves) and *T. foenum-graceum* (seeds), respectively. Bioassay guided partial purification yielded active fractions with IC_{50} (µg/mL) of 23±5 (*E. jambolana)*, 14±3 (*A. indica*) and 11±2.5 (*T. cordifolia*). All the active fractions inhibited the enzyme in a mixed-competitive manner with Ki and Ki' values (µg/mL) as 1.26±0.22, 3.96±0.28 (*E. jambolana*); 0.95±0.46, 2.3±0.16 (*A. indica*); and 1.1±0.2, 4.2±0.26 (*T. cordifolia*).

Keywords: *Azadirachta indica*, glucosidase inhibitor, lipase inhibitor, medicinal plants, obesity

Introduction

Obesity is caused by an imbalance between energy intake and expenditure, and is widely recognized as a major public health problem. Obesity can lead to some serious diseases, including hypertension, hyperlipidaemia, arteriosclerosis, and type II diabetes[1]. Diets high in fat tend to promote obesity, and the pharmacologic inhibition of the digestion and absorption of dietary fat has been used as a strategy to treat obesity[2]. Basically, there are two types of anti-obesity agents, classified as inhibitors of lipid (lipase inhibitor) and carbohydrate (α-glucosidase inhibitor) absorption[3]. Pancreatic lipase plays a key role for triglyceride absorption in the small intestine. This enzyme, secreted from the pancreas into the intestine, hydrolyzes triglycerides into fatty acids[4] and, thus, pancreatic lipase inhibitors are considered to be a valuable therapeutic reagent for treating diet-induced obesity in humans. Tetrahydrolipstatin, isolated from actinomycetes (*Streptomyces toxytricini)*, is a potent inhibitor of pancreatic lipase and available commercially as an anti obesity drug in the brand name 'Orlistat'[5]. Lipase inhibitors (saponins, mangiferin, tea polyphenols, triterpene oligoglycosides, etc) have been isolated from various plants[6]. As for inhibitors of carbohydrate absorption, α-glucosidase inhibitors are now available as anti-diabetic drugs[3]. Inhibition or lowering of activities of some intestinal membrane bound carbohydrases like maltase-glucoamylase and sucrase-isomaltase by suitable inhibitors slows down the absorption of glucose into the blood stream. During the last forty years, different glucosidase inhibitors isolated from plants and microorganisms include diverse types of compounds like acarbose, isoacarbose, cyclodextrins, acarviosine-glucose and hibiscus acid[7-10].

Common Indian medicinal plants like *Eugenia jambolana* Lam. (jamun), *Azadirachta indica* A. Juss. (neem), *Tinospora cordifolia* (Thunb.) Miers (guduchi) and *Trigonella foenum-graceum* L. (methi) are used in Ayurdedic medicine system as anti-diabetics and hypolipidemics. Hypoglycaemic and hypolipidemic effects of *E. jambolana*, *T. cordifolia* and *T. foenum-graceum* have also been reported by several scientific workers[11-13]. Hypoglycaemic effect of *A. indica* leaf extracts and seed oil was observed in normal and alloxan induced diabetic rabbits[14]. However, the mechanism and mode of action was never very clear. In a patent, we reported earlier that medicinal plants like *E. jambolana*, *A. indica*, *T. cordifolia* and *T. foenum-graceum* contain

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α-glucosidase inhibitors and thus could be very beneficial for the treatment of diabetes type II[15-17]. α-Glucosidase inhibitors present in *T. cordifolia* and *A. indica* have been isolated and characterized as saponarin and nimbidiol, respectively[18-19]. The present study reports the presence of lipase inhibitors in these medicinal plants. The study also attempts in partial purification of the bioactive compound(s) present in the extract responsible for inhibition of pancreatic lipase. Thus, a combination of both pancreatic lipase and α-glucosidase inhibitors would be a potent herbal therapy for obesity control.

Materials and Methods

Chemicals

Olive oil was purchased from SRL Chemicals, India. Soluble starch (potato), maltose and sucrose were purchased from Sigma Chemicals, USA. Sodium deoxycholate was purchased from Himedia. HPLC grade isopropanol, sodium carbonate, dimethyl sulphoxide (DMSO), cupric acetate, gum arabica and silica gel (60-120 mesh) were purchased from Merck, India. Pancreatic acetone powder (porcine) and rat intestinal acetone powder (I-1630), used as a source of pancreatic lipase and intestinal glucosidases, respectively were purchased from Sigma Chemicals, US. All other chemicals used were of chemically pure grade.

Plant Material

Stem-bark and root of *A. indica*, leaves and stem of *T. cordifolia* and stem-bark of *E. jambolana* were collected from wild. *T. foenum-graceum* seeds were purchased from local market.

Preparation of Plant Extract

Aqueous Extract

Fresh and healthy stem-bark and roots (100 g each) of *A. indica* was collected, washed in tap water and crushed into small pieces. Stem-bark and roots were separately blended with 100 mL of water for 3-4 min to obtain a paste. The paste obtained was squeezed through a nylon cloth and filtered under suction to obtain 90 mL of clear liquid. The clear liquid was passed through PM10 kDa membrane filter. The filtrate (80 mL) collected was lyophilized to dryness (3.0 g). The dry powder was re-suspended in 10 mL of water and used as crude inhibitor extract. The same extraction methodology was followed using 100 g each of fresh leaves and stem of *T. cordifolia*, stem-bark of *E. jambolana* and seeds of *T. foenum-graceum.*

Methanolic Extract

Fresh root and stem-bark (100 g each) of *A. indica* was collected and dried in a hot air oven at 50-60°C for 4-5 h to obtain 23.75 g of dry mass. The dry mass was crushed, immersed in 1 L of methanol and kept for 48 h at room temperature. The methanolic extract was filtered and evaporated in a rotary evaporator to obtain approximately 1.1 g of dry solid. The dry solid was further suspended in 10 mL of 80% (v/v) methanol. The solution was used as a crude inhibitor sample. The same process was repeated using 100 g each of fresh leaves and stem of *T. cordifolia*, stem-bark of *E. jambolana* and seeds of *T. foenum-graceum*, which yielded 0.9, 1.4, 1.8 and 1.2 g of dry solid, respectively.

Determination of Enzyme Inhibition

The potency of crude inhibitor extract to inhibit pancreatic lipase, pancreatic amylase, intestinal glucosidases (maltase, sucrase) was assayed. Pancreatic lipase inhibition was determined by turbidimetric method [20], using olive oil-ethanol suspension. 1 mL of olive oil was added to 100 mL of ethanol and shaken vigorously. 1mL of this olive oil-ethanol suspension was added to 9 mL of 0.05 *M* Tris-HCl buffer, *p*H 8.0 containing 0.025 *M* of sodium deoxycholate. This emulsion was used as substrate. Reaction mixture containing enzyme and inhibitor (in requisite amount) was incubated at room temperature for 10 min. Reaction was started by addition of 1 mL of substrate. Incubation lasted for 10 min at 37°C. The decrease in turbidity was measured at 660 nm. Inhibitors present in the reaction prevented the decrease of turbidity of the mixture. Suitable 'control' tubes were run parallel. Optical density values obtained were co-related with an olive oil standard curve.

Pancreatic lipase inhibitory activity was also determined by copper-soap colorimetry according to the method of Lowry and Tinsley[21] using olive oil as substrate and cupric acetate-pyridine as a colour developing reagent. 0.5 mL each of olive oil and gum arabica were well homogenized in 100 mL of 0.1 *M* phosphate buffer, *p*H 8.0. This emulsion was used as a substrate. Reaction mixture (1 mL) containing requisite amount of enzyme and inhibitor sample were incubated at room temperature for 10 min. Reaction was started by addition of substrate (1 mg/mL) and incubation lasted for 15 min at 37°C. Reaction was terminated by addition of 2 mL of benzene. The tubes were vortexed and centrifuged. The organic layer (1 mL) was added to 1 mL of cupric acetate-pyridine

reagent and tubes were further centrifuged. The blue colour developed was measured at 715 nm. Suitable 'blank' and 'control' tubes were run parallel. Fatty acids formed in the reaction were correlated with an oleic acid standard curve using the same protocol.

Pancreatic amylase activity was determined using 0.1 *M* phosphate buffer, *p*H 7.0 at 37°C. The incubation mixtures (2 mL) containing 0.5 U of enzyme in buffer was pre-incubated with requisite amount of inhibitor extract for 10 min at room temperature. The reaction was initiated by adding 1 mg/mL of starch and incubation lasted for 20 min. Amount of reducing sugars formed was estimated by dinitrosalicylic acid reagent according the method of Sengupta *et al*[22]. One unit (U) of enzyme activity was taken as the amount of enzyme that could liberate one µmol of reducing sugar per min under the experimental conditions. Intestinal maltase and sucrase activities were determined in 0.1 *M* phosphate buffer *p*H 6.8 at 37°C by estimating the amount of glucose liberated from maltose and sucrose, respectively by glucose oxidase-peroxidase (GOD-POD) method[23]. One unit (U) of enzyme activity was taken as the amount of enzyme that could liberate one µmol of glucose per min from the substrate under the experimental conditions. The reaction mixture (0.5 mL) containing enzyme (10 mU) and requisite amount of inhibitor extract was pre-incubated for 10 min. The reaction was started by addition of substrate (1 mg/mL). The reaction continued for 20 min and was terminated by keeping the tubes in boiling water bath for 3-4 min. Liberated glucose was estimated by GOD-POD reagent (Span Diagnostic Ltd., India). The concentration of inhibitor required to inhibit 50% of enzyme activity (substrate conc. 1 mg/mL) under the above-mentioned conditions was taken as IC_{50} value.

Bioassay Guided Partial Purification of Bioactive Compounds

It has been observed that methanolic extracts of the plants were more potent against pancreatic lipase than aqueous extracts. Thus, bioassay-guided partial isolation and purification of the active compound(s) was carried out using methanolic extracts with respect to its inhibitory activity on pancreatic lipase.

A. indica

Air-dried and milled root bark (400 g) of *A. indica* was percolated with methanol (2.5 L) for 48 h at room temperature. The extract was concentrated to a small volume (100 mL) under reduced pressure at 50°C. The entire process was repeated twice more to ensure complete extraction. The combined concentrates (300 mL) was further concentrated to 50 mL, diluted with water (500 mL) with stirring and extracted with ethyl acetate (300 mL × 3). The organic extract was evaporated to dryness under reduced pressure at 50°C to get a brown residue (12.5 g). The residue was fractionated by chromatography over a column of silica gel using methanol:water (3:1 & 1:1) as solvent system. The process was repeated twice. The active fraction obtained was mixed together and dried to obtain 1.22 g of powder. IC_{50}, Ki and Ki' values of this active fraction were determined.

T. cordifolia

Air-dried and milled leaves (200 g) of *T. cordifolia* were percolated with methanol (3.5 L) for 48 h at room temperature. The extract was concentrated to a small volume (100 mL) under reduced pressure at 50°C. The entire process was repeated twice to ensure complete extraction. The combined concentrate was completely dried in a rotary evaporator. The residue (4.2 g) was fractionated by chromatography over a column of silica gel using butanol:water (1:1) as solvent system. The active fractions obtained were concentrated and IC_{50}, Ki and Ki' values were determined.

E. jambolana

Dried and milled stem bark (200 g) of *E. jambolana* was percolated with methanol (3 L) for 48 h at room temperature. The extract was concentrated to a small volume (100 mL) under reduced pressure at 50°C. The entire process was repeated twice to ensure complete extraction. The combined concentrates (300 mL) was further concentrated and was fractionated over a column of silica gel using ethyl acetate as the mobile phase. The active fractions obtained were pooled and dried (6.5 g). IC_{50}, Ki and Ki' values of the active fraction towards pancreatic lipase were determined.

Determination of Enzyme Inhibition Kinetics Using Active Fractions

Inhibitory kinetics of the active fractions on pancreatic lipase was studied using *p*-nitrophenyl palmitate as substrate[24]. *p*-Nitrophenyl palmitate was dissolved in HPLC grade isoproanaol. The reaction mixture (0.5 mL) contained 0.050 U of enzyme in 0.1 *M* phosphate buffer, *p*H 7.5 and varying concentration (0.5-8 µg/mL) of inhibitor samples (dissolved in DMSO). The reaction mixtures containing enzyme and inhibitor were pre-incubated for 10 min at 37°C and the reaction was started by addition of substrate (0.5-6 mM). The incubation lasted for 30 min and the

reaction was terminated by addition of 2 mL of 1 *M* sodium carbonate solution. Amount of *p*-nitrophenol liberated was measured at 420 nm. 1U of enzyme activity is defined as the amount of enzyme which can produce 1 µmol of *p*-nitrophenol/min under the assay conditions. The concentration of inhibitor required to inhibit 50% of enzyme activity at a substrate concentration of 1 mg/mL under the above-mentioned conditions was taken as IC_{50} value. Ki and Ki' values were determined from 1/V *vs* I (Dixon plot) and S/V *vs* I plots, respectively[25].

Results

Inhibitory Activity of Crude Extracts

Table 1 shows the inhibitory activities present in the aqueous and methanolic extract of the medicinal plants. The extracts inhibited glucosidases (mammalian intestinal) very strongly (Table 1), while there was little inhibition observed in case of pancreatic amylase (data not shown). The crude methanolic extract was more active and potent than the aqueous extract. The crude methanolic root extract of *A. indica* showed IC_{50} value of 7±1.5 µ*M* and 8.5±1.0 µ*M* for intestinal maltase and sucrose, respectively, while methanolic stem-bark extract of *E. jambolana* showed an IC_{50} value (µg/mL) of 12±2 and 5±2.5 for the same (Table 1). All the plant extracts were more potent inhibitors of mammalian intestinal sucrase than maltase. Methanolic extract of *E. jambolana* stem bark showed the most potent inhibitory activity towards pancreatic lipase (IC_{50}, 230±20 µg/mL), while methi (*T. foenum-graceum*) seed extract was the least potent (IC_{50}, 564±12 µg/mL) among the plants tested. *T. cordifolia* leaf extract and *A. indica* root extract also showed potent inhibitory activities (IC_{50}, 323±11 and 476±14 µg/mL, respectively) towards mammalian pancreatic lipase (Table 1).

Inhibitor Constants of Partially Purified Plant Extracts on Pancreatic Lipase

Table 2 and Figs 1-3 show the IC_{50} and inhibitory constants of the partially purified plant extracts

Table 2—Inhibitor constants of the active fractions

	Inhibitor constants of the bioassay guided active fractions		
	IC_{50} (µg/mL)	Ki (µg/mL)	Ki' (µg/mL)
Active fraction from *Eugenia jambolana* (stem-bark)	23±5.0	1.26±0.22	3.96±0.28
Active fraction from *Azadirachta indica* (root)	14±3.0	0.95±0.46	2.3±0.16
Active fraction from *Tinospora cordifolia* (leaves)	11±2.5	1.1±0.20	4.2±0.26

Active fractions were obtained by bioassay guided method as mentioned in the text. Enzyme inhibition was determined as mentioned in the text. Ki and Ki' values were calculated using 1/V *vs* I plot and S/V *vs* I plot, respectively. The data shown here represent the average value of triplicate similar sets of experiments.

Table 1—IC_{50} values of aqueous and methanolic plant extracts for mammalian pancreatic lipase and intestinal glucosidase inhibition

	IC_{50} values of dry extracts obtained from the plants(ug/ mL)					
	Pancreatic lipase		Intestinal maltase		Intestinal sucrase	
	Aqueous	Methanolic	Aqueous	Methanolic	Aqueous	Methanolic
Eugenia jambolana (stem bark)	285±15	230±20	22±3.0	12±2.0	11±3.5	5±2.5
Tinospora Cordifolia						
Leaves	404±13	323±11	86±5.5	75±5.0	47±2.0	36±2.0
Whole stem	435±12	360±14	92±4.0	88±4.0	52±3.5	45±3.0
Azadirachta indica						
Whole root	543±15	476±14	70±2.5	52±2.0	40±2.5	28±2.5
Stem bark	595±12	520±15	75±1.5	57±2.0	42±1.5	34±2.2
Trigonell foenum-graceum (seeds)	613±11	564±12	112±8.0	98±5.0	65±6.5	52±4.5

Aqueous and methanolic plant extracts were prepared as mentioned in the text. α-Glucosidase inhibition was determined as mentioned in the text. Lipase inhibitory activities were determined using both turbidimetric and copper-soap colorimetric methods. The data given here are the average of triplicate similar sets of experiments.

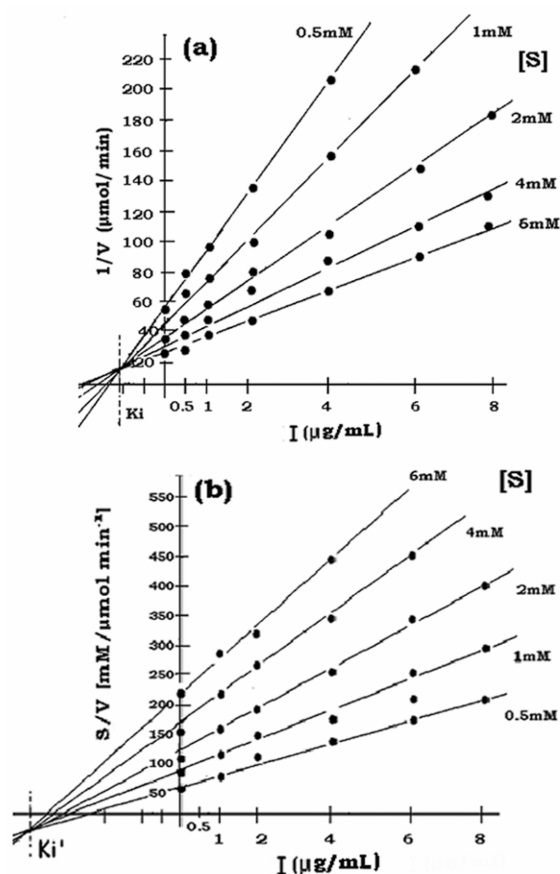

Fig. 1 (a & b)—Determination of Ki and Ki' of active fraction obtained from *T. cordifolia* leaves on pancreatic lipase: Dixon plot (1/V *vs* I) for determination of Ki (a); S/V *vs* I plot for determination of Ki' (b). [Active fraction (0.5-8 µg/mL) was added against different concentrations (0.5-6 m*M*) of *p*-nitrophenyl palmitate (S)]; Enzyme activities were assayed by the method as described in text]

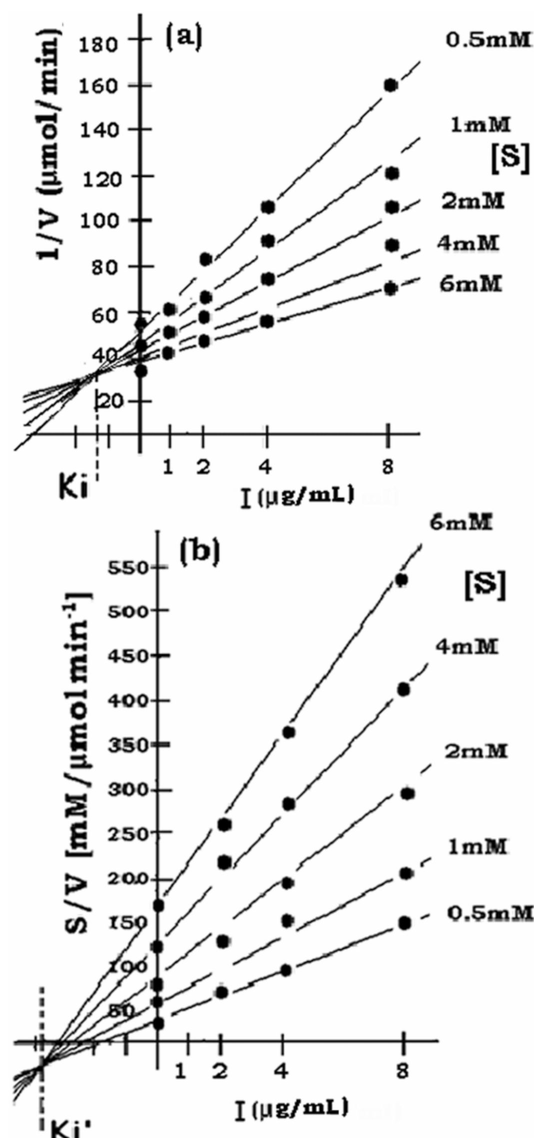

Fig. 2 (a & b)—Determination of Ki and Ki' of active fraction obtained from *E. jambolana* stem bark on pancreatic lipase: Dixon plot (1/V *vs* I) for determination of Ki (a); S/V *vs* I plot for determination of Ki' (b). [Active fraction (1-8 µg/mL) was added against different concentrations (0.5-6 m*M*) of *p*-nitrophenyl palmitate (S); Enzyme activities were assayed by the method as described in text]

on pancreatic lipase. The active fraction obtained from *T. cordifolia* leaves showed most potent lipase inhibitory activities with IC_{50}, Ki and Ki' values (µg/mL) of 11$\pm$2.5, 1.1$\pm$0.2 and 4.2$\pm$0.26, respectively. Partially purified root extract of *A. indica* also showed strong lipase inhibitory activities. Ki and Ki' values were calculated using Dixon plot and S/V *vs* I plots, respectively (Figs 1-3).

Discussion

Obesity is caused by excess caloric intake and this can be improved by inhibiting pancreatic lipase activity and by inhibiting or delaying lipid absorption. Pancreatic lipase, the key enzyme in the efficient digestion of triglycerides[26] is responsible for the hydrolysis of 50-70% of total dietary fats[27]. Orlistat (lipase inhibitor) is currently the only clinically approved drug for obesity management in Europe. Its long-term administration accompanying an energy restricted diet results in weight loss[28]. Reduction on intestinal lipid digestion has been related to a decrease in the intra-abdominal fat content[29]. Inhibition of α-glucosidase activity and inhibition of carbohydrate absorption also play an important role in the prevention and treatment of diabetes and obesity[30] by delaying and lowering the carbohydrate digestion and thereby the release of glucose into the blood

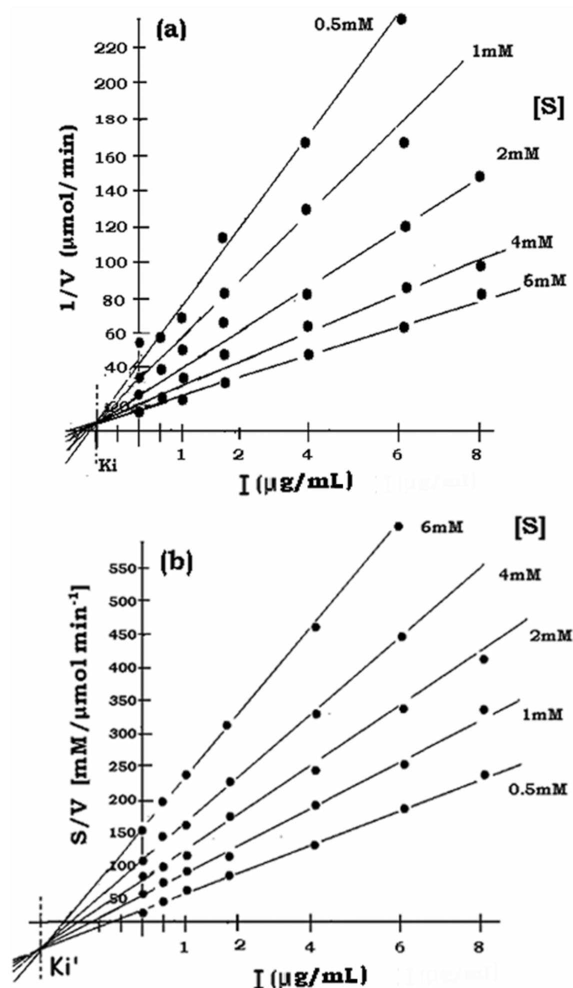

Fig. 3 (a & b)—Determination of Ki and Ki' of active fraction obtained from *A. indica* root on pancreatic lipase: Dixon plot (1/V *vs* I) for determination of Ki (a); S/V *vs* I plot for determination of Ki' (b). [Active fraction (0.5-8 µg/mL) was added against different concentrations (0.5-6 m*M*) of *p*-nitrophenyl palmitate (S); Enzyme activities were assayed by the method as described in text]

stream. Anti obesity effects of a potent α-glucosidase inhibitor, 1-deoxynojirimycin has been reported and studied in rates[31]. Studies have also been conducted testing the antiobesity effect of acarbose[32].

Salacia reticulate Wight is known for the presence of α-glucosidase inhibitors, salacinol and kotalanol[33]. Interestingly, the aqueous extract of the plant is also reported to possess lipase inhibitory activities with an IC_{50} value of 264 ug/mL[34]. Recently, it has been reported that polyphenols isolated from bark of *A. mearnsii* possess both α-glucosidase (IC_{50} 0.22 and 0.60 mg/mL for intestinal maltase and sucrose, respectively) and pancreatic lipase (IC_{50}, 0.95 mg/mL) inhibitory activities[35].

We earlier have reported the presence of α-glucosidase inhibitor in the aqueous and methanolic extract of *E. jambolana*, *T. cordifolia*, *A. india* and *T. foenum-graceum*. α-Glucosidase inhibitors from *T. cordifolia* and *A.indica* have been isolated, characterized and patented by us[15-17]. It must be mentioned here that IC_{50}, Ki and Ki' values (µ*M*) of saponarin (isolated from *T. cordifolia* leaves) were found to be 48±3.55, 8±0.15, 19.5± 0.20 (for intestinal maltase) and 35±1.95, 6±1.33, 13±0.13 (for intestinal sucrase), respectively; while that of nimbidiol isolated from stem-bark and root of *A. indica* were 12±1.23, 1.22±0.344, 3.65±0.48 (for intestinal maltase) and 6.75±0.80, 0.7±0.12, 1.44±0.65 (for intestinal sucrase), respectively[18-19]. Interestingly, the aqueous and methanolic extracts of these plants also possess potent pancreatic lipase inhibitory activities. The plant extracts were capable of inhibiting 85-88% of porcine pancreatic lipase activity (data not shown). Crude methanolic extract of *T. cordifolia* leaves inhibited pancreatic lipase with an IC_{50} of 323±13 µg/mL, while methanolic root extract of *A. indica* showed an IC_{50} of 476 ±14 µg/mL towards the same (Table 1). Thus, methanolic extract of *E. jambolana* stem-bark showed more potency (IC_{50}, 230±20 µg/mL) compared to *S. reticulate* extract[34] and acacia polyphenols[35] in terms of lipase inhibitory activities. Ethanolic extract of grape seed[2] could inhibit 80% of pancreatic lipase activity at a concentration of 1 mg/mL, which is much less potent compared to the above mentioned plant extracts.

An attempt was made to partially isolate and characterize the bioactive compound(s) present in the extract of neem, jamun and guduchi. Methi seed extract was a less potent inhibitor of pancreatic lipase activity compared to others and thus was not selected for further purification analysis. Partially purified butanol extract of *T. cordifolia* leaves showed a potent lipase inhibitory activity with IC_{50} of 11±2.5 µg/mL. The sample showed a mixed competitive mode of inhibition towards pancreatic lipase with Ki and Ki' values (µg/mL) of 1.1±0.20 and 4.2±0.26 (Figs 1a & b). Although Orlistat ($IC_{50,}$ 0.22 µg/mL)[1] appears to be me much more potent than *T. cordifolia* and *A. indica* extract, further purification and ultimate identification of the bioactive compound(s) would certainly increase the potency of the herbal drug. Further, *Taraxum officinale* F. H. Wigg extract was reported to inhibit pancreatic lipase with an IC_{50} value of 78.2 µg/mL[1], while alcoholic extract of

Rosmarinus officinalis L. showed an IC_{50} value of 13.8 µg/mL for pancreatic lipase inhibiton[36]. Thus, these two plants showed the potential to provide bioactive compound(s) for inhibiting pancreatic lipase. However, partial purification of these plant extracts or identification of the bioactive compound(s) have not been reported till date. On the other hand, alcoholic extract of mango stem-bark (*Mangifera indica* L.) fared poorly in inhibiting human pancreatic lipase with an IC_{50} of 1 mg/mL[37]. Interestingly, mangiferin isolated from *M. indica* showed α-glucosidase inhibitory activity with an IC_{50} of 87 µg/mL for intestinal sucrase[32], which is much less compared to both saponarin (from *T. cordifolia*) and nimbidiol (from *A. indica*). Partially purified active fraction from root-bark of *A. indica* root was also a potent inhibitor of pancreatic lipase (IC_{50}, 14$\pm$3.0; Ki, 0.95$\pm$0.46; Ki', 2.3$\pm$0.16 µg/mL). This fraction too inhibited the enzyme in a mixed competitive fashion (Figs 2a & b). It must be mentioned here that the methanol extract of *Dioscorea nipponica* Makino showed similar potent inhibitory activity against porcine pancreatic lipase, with an IC_{50} value of 5-10 µg/mL[38]. However, the total saponin fraction obtained from the fruits of *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim. exhibited very low and poor pancreatic lipase inhibitory activity (IC_{50}, 3.63 mg/mL)[39] compared to the partially purified active fractons obtained from *T. cordifolia* and *A. indica*.

Interestingly, crude methanolic extract of jamun showed strongest lipase inhibitory activity (Table 1), whereas its partially purified sample recorded a lower IC_{50} value in comparison to active fractions of neem and guduchi extract (Table 2). The possible reason may be that the percentage of purification achieved in case of jamun stem-bark is still quite low. Further isolation and purification of the active compound will help in proper assessment. Activity-guided fractionation of methanolic extract of the leaves of *Eremochloa ophiuroides* (Munro) Hack. (centipede grass) led to the isolation and identification of *C*-glycosidic flavones which showed potent inhibitory effects on pancreatic lipase, with IC_{50} values ranging from 18.5±2.6 to 50.5±3.9 µ*M*[40].

Conclusion

A combination of α-glucosidase and pancreatic lipase inhibitor would prove to be a very valuable therapy for treatment of obesity. This potent herbal therapy would also be beneficial for controlling postprandial hyperglycemia and hyperlipemia. The plants used in the study are common medicinal plants that have been used in our country in Ayurvedic and folk medicine since time immemorial and thus can be considered safe and non-toxic.

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