Research Article

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COMPARATIVE INHIBITORY POTENTIAL OF *ASHODHITA* (RAW) AND *SHODHITA CURCUMA LONGA* LINN ON α -AMYLASE AND α-GLUCOSIDASE

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ABSTRACT

Turmeric (Haridra) is one of the versatile herb for spices, condiment and anti- diabetic activity in Indian System of medicine and tropical country. To compare the in-vitro enzymatic inhibitory effect (α-amylase and α-glycosidase) for supporting purified (shodhita) and raw turmeric samples. Authenticate unprocessed and processed (ayurvedic classic) turmeric powder extracts prepared by maceration with methanol and subjected (in different level concentration) to the anti-hyperglycemic selective enzymatic activity and colour reaction was measured by UV-VIS spectroscopy. This study proves the alteration in the inhibitory effect of raw and shodhita samples of Haridra on α-amylase of IC_{50} in decreasing order TZ (113.72) < TW (119.22) < TR (121.861) < TT (161.35) < TGM (218.85) and α-glycosidase TZ (121.32) < TW (139.48) < TR (145.29) < TT (185.51) < TGM (276.12) respectively. Purified turmeric samples gives better effect than raw sample. In that way shodhan effect established indirectly synergistic and supra-additive effect of turmeric in context to antidiabetic.

Keywords: Turmeric, Shodhana, α-amylase, α-glycosidase, antidiabetic

INTRODUCTION

Indian medicinal plants used in the Ayurvedic traditional system to treat diabetes are more valuable source of novel anti-diabetic agents now a days due to less expensive when compared to synthetic anti-hyperglycemic agents and have very less side effect.[1,2] It will be assessed that more than 200 species about plants show hyperglycaemic properties.[3,4,5,6] Turmeric (*Curcuma longa* Linn) belongs to the family Zingiberaceae, is also referred to Ayurvedic system of medicine and also found good anti-diabetic effect as it is describe in various classical texts[7,8,9] as well as modern texts[10,11] and research paper[12,13,14]. Shodhana is not just a purification process, it is a process of enhancement of drug quality as per Ayurveda.[15] On the other hand the classical (Ayurvedic) principle drug act as a whole there is no need for isolation of primary and secondary plant metabolite due to belief in the holistic sense[16] which counteract modern science selecting one –two marker to ignore complexity of herbal medicine[17]. Accepting the extraction technique of selected samples to find comparative effect of natural α-amylase and α-glycosidase inhibitors in quest of finding the comparative anti-diabetic efficacy of raw and all shodhita. These α-amylase and α-glucosidase inhibitory activity was conducted due to therapeutic approach to prevent postprandial hyperglycemia is to retard the digestion and absorption of carbohydrates in the gastrointestinal tract through inhibition of enzymes such as α-amylase and α-glycosidase. The α-amylase (α-1, 4-glucan-4-glucano-hydrolases) is one of the major secretory products of the pancreas and salivary glands, playing a role in digestion of starch and glycogen and can be found in microorganisms, plants and higher organisms. α -glycosidase enzyme acted on maltose, maltriose and branched oligosaccharides of α-(1-6) and α-(1-4) oligoglucans to monosaccharaides and also degraded glucose absorption in the blood stream. On the other hand, α-amylase hydrolysed of starch of various type of low molecular weight saccharides[18]. Although, these activities for Turmeric have been done previously[19, 20, 21] but none of them have done shodhan of Haridra which make this work noval and significant. In this study, methanolic extracts of ashodhita and shodhita Haridra powders have been tested for α-amylase and α-glucosidase inhibitory activities.

MATERIALS AND METHODS

Samples description

Standard Sample (AC)

Acarbose tablets IP 50 mg of brand name "Glucobay 50" strip was purchased from Jamnagar which having batch no. P16141 and manufactured by Bayer Pharmaceutical Pvt. Ltd. At village Malpur, Baddi- 1732015.

Test Samples

Sample (TR): Methanolic extract of powder of raw turmeric rhizome without treated any media.
Sample (TT): Methanolic extract of powder of turmeric rhizome after treated with Takra (Nimajjana of Turmeric mother rhizome in takra for 10 days[22], it is prepared with 1/4th amount of water[23])
Sample (TW): Methanolic extract of powder of turmeric rhizome after treated water (Swedana/boiling of turmeric mother rhizome in drinking mineral water for 3 hours[24])
Sample (TGM): Methanolic extract of powder of turmeric rhizome after boiling in water for 3 hours followed by steaming with fresh Gomutra (Cow's urine)
Sample (TZ): Methanolic extract of powder of turmeric rhizome after boiling in fresh Gomutra (1hour) followed by boiling in Panchapallava Kwatha (1hour) followed by boiling in Mundi Kwatha (1hour), followed by steaming in fresh Gomutra (15 minutes) [25, 26]

Activities
α-Amylase inhibitory activity

The α-amylase inhibitory activity was determined according to the method described by Miller. A total of 500 µl of test samples and standard drug (50-200µg/ml) were added to 500 µl of 0.20 mM phosphate buffer (pH 6.9) containing α-amylase 500 µl (0.5mg/ml) solution and were incubated at 25°C for 10 min. After these, 500 µl of a 1% starch solution followed by 0.02 M sodium phosphate buffer 500 µl (pH 6.9) was added to each tube. The reaction mixtures were then incubated at 25°C for 10 min. The reaction was stopped with 1.0 ml of 3, 5 dinitrosalicylic acid colour reagent. The test tubes were then, incubated in a boiling water bath for 5 min and cooled at room temperature. The reaction mixture was then diluted after adding 10 ml distilled water and absorbance was measured at 540 nm. Control represent 100% enzyme activity and were conducted in similar way by replacing extract with vehicle.[27, 28]

α-Glucosidase inhibitory activity

The inhibitory activity was determined by incubating a solution of starch substrate (2 % w/v maltose or sucrose) 500 µl with 1000 µl (0.2 M) Tris buffer pH 8.0 and various concentration of plant extract (50µg to 200 µg), keep it for 5 min at 37°C. The reaction was initiated by adding 1 ml of α-glucosidase enzyme (1U/ml) to it followed by incubation for 10 min at 37°C. Then, the reaction mixture was heated for 2 min in boiling water bath to stop the reaction and then standard glucose reagent was added 250 µl in each test tube. The amount of liberated glucose is measured by glucose oxidase peroxidase method and absorbance was measured at 510 nm.[29, 30, 31]
The results of both were expressed as % inhibition calculated using the formula:

$$\text{Inhibitory activity of α-amylase enzyme} = \frac{(\text{Abs. of Test} - \text{Abs. of control}) \times 100}{\text{Abs. of Test}}$$

Table 1: α-Amylase and α-glucosidase inhibitory activity of methanolic extracts of Turmeric Test samples and Reference drug Acarbose

Samples	Concentration (in µg/ml)	Percentage of Inhibitions	
		α -Amylase	α - Glucosidase
AC (Standard)	50	47.53	49.81
	100	53.01	53.47
	150	60.2	57.59
	200	62.5	62.58
TR	50	40	42.73
	100	49.13	45.01
	150	54.29	50.55
	200	58.06	54.88
TT	50	36.4	31.02
	100	42.23	40.56
	150	49.65	45.37
	200	54.11	51.28
TW	50	40.57	42.72
	100	49.65	46.91
	150	54.11	50.78
	200	58.33	54.89
TGM	50	26.41	34.17
	100	33.89	37.34
	150	40.45	40.22
	200	47.29	45.11
TZ	50	41.35	43.78
	100	50.79	49.63
	150	54.54	52.51
	200	58.51	55.17

Table 2: IC_{50} of Reference drug Acarbose and Turmeric Test samples

Samples	IC_{50} (in µg)	
	In α -Amylase	**In α - Glucosidase**
AC (standard)	**69.24**	**48.66**
	y = 0.1042x + 42.785, R^2 = 0.9665	y = 0.0823x + 45.995, R^2 = 0.9905
TR	**121.86**	**145.297**
	y = 0.1187x + 35.535, R^2 = 0.9591	y = 0.084x + 37.795, R^2 = 0.9773
TT	**161.35**	**185.51**
	y=0.1211x+ 30.46, R^2 = 0.9919	y = 0.1312x + 25.66, R^2 = 0.9773
TW	**119.22**	**139.48**
	y = 0.1155x + 36.23, R^2 =0.9605	y = 0.0808x + 38.73, R^2 = 0.9998
TGM	**218.85**	**276.12**
	y = 0.1384x + 19.71, R^2 =0.9993	y = 0.0714x + 30.285, R^2 = 0.9845
TZ	**113.72**	**121.32**
	y = 0.110x + 37.49, R^2 =0.943	y = 0.0741x + 41.01, R^2 = 0.9592

Note: IC_{50} = Half maximal inhibitory concentration

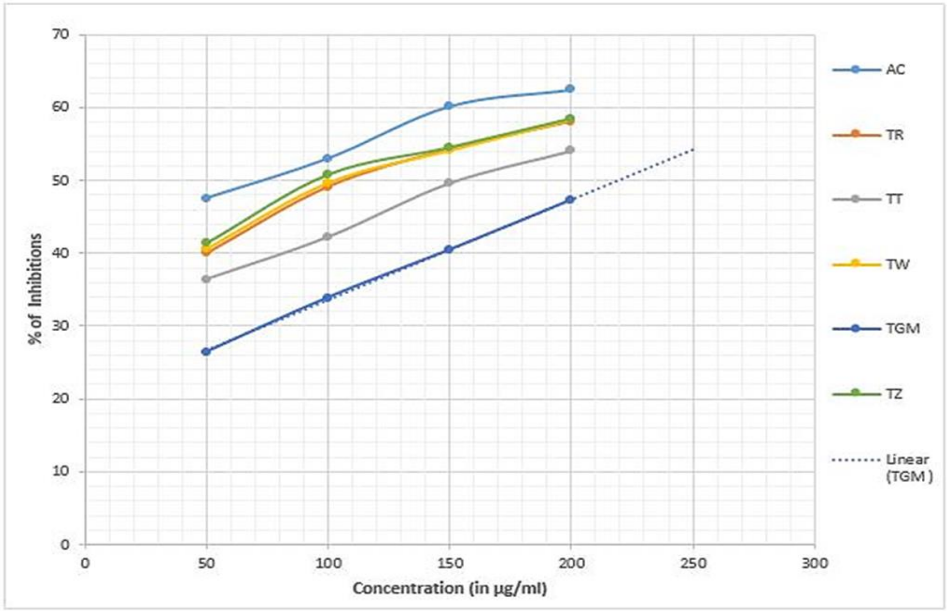

Figure 1: Graphical representation of Methanolic extracts of test samples, potential of inhibition of α-amylase enzyme.

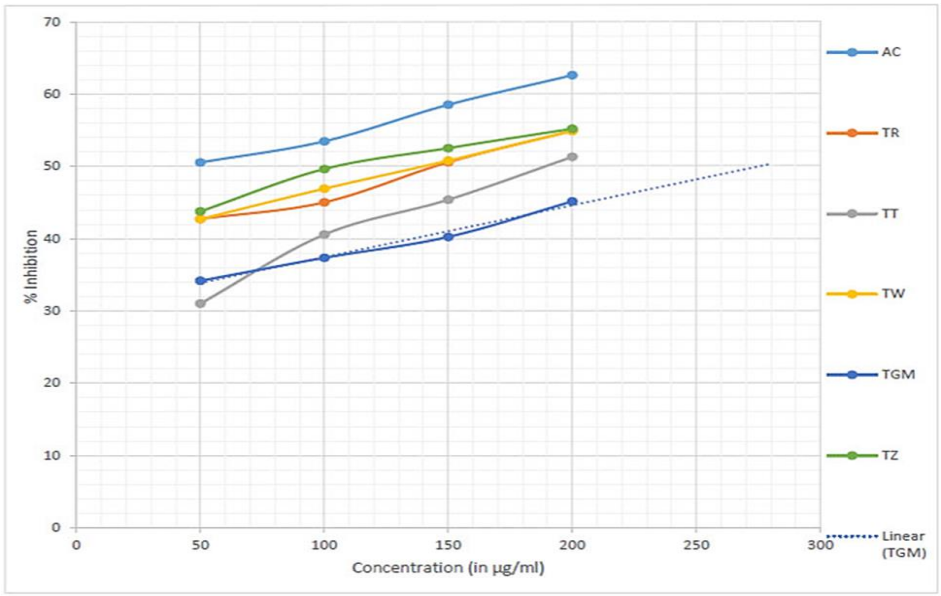

Figure 2: Graphical representation of Methanolic extracts of test samples' potential of inhibition of α-glucosidase enzyme.

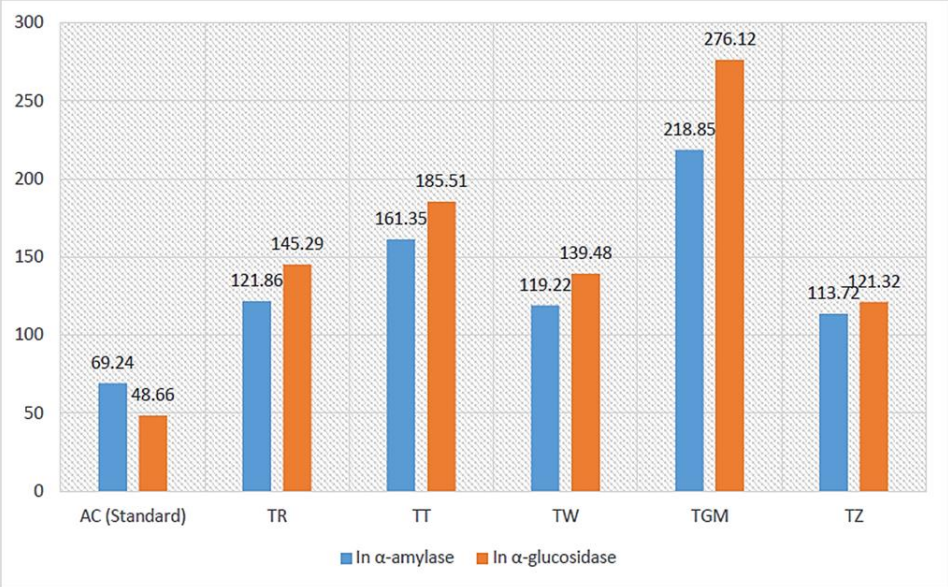

Figure 3: Comparison of IC_{50} dose of test samples for inhibition of α-amylase & α-glucosidase enzymatic activity

OBSERVATIONS AND RESULTS

There was a dose-dependent inhibitory effect of raw and Shodhita Haridra activity on α-amylase and α-glucosidase enzyme. It is evident from Table 3.1 that comparatively more inhibitory activity on α-amylase enzyme was demonstrated by standard drug Acarbose.

Test samples TR, TW and TZ have comparatively more inhibitory effect than other test samples like TT and TGM. Graphical representation shows similar pattern of inhibitory effect of TR, TW and TZ.

TGM showed lowest inhibition activity than other test samples, even it could not get IC_{50}. So, trendline in the graph was extended to 50 points, to get the IC_{50}. Same thing can be seen in case of α-glucosidase enzyme activity. Here, trendline of TGM was extended to 80 points to get the IC_{50}. From the above graphical representation of both α-amylase and α-glucosidase inhibition, TZ showed comparatively more inhibitory effect than others. Quantitatively, it's not a big value but comparatively, it is a significant difference.

IC_{50} of all samples and standard were calculated by using regression equation by linear curve drawn as trendline for both α-amylase and α-glucosidase. Although, IC_{50} of Acarbose in α-glucosidase could not be calculated because trendline has range 53.47% to 60.58 % and regression equation fail to give IC_{50}. Haridra samples show good effect in context to inhibition of α-amylase and α-glucosidase enzymes, however TZ show better inhibition than all other samples because it requires minimum to dose to get IC_{50}. (Table 2)

DISCUSSION

In treatment of type II diabetes mellitus treatment concern key role control the human pancreatic α-amylase which increases in post-prandial glucose levels. Inhibitory activity of such type of enzyme, α-amylase in the form of dealing carbohydrate digestion, reduction of absorption blood glucose level[32,33,34]. Recent research trend is focused on inhibition of carbohydrate metabolizing enzyme. Recently acarbose and miglitol such type of inhibitor are successfully used in clinically. To search naturally occurring herbal samples which treated traditional shodhan process in extract level inhibitory activity of α-amylase and α-glucosidase. This study proves the alteration in the inhibitory effect of raw and Shodhita samples of Haridra on α-amylase and α-glucosidase. TZ show best IC_{50} in among all the samples then, followed by raw Haridra; TR and then, TW > TT > TGM in both enzymatic inhibition activity. (see in below cluster chart)

CONCLUSION

Pharmacological in-vitro enzymatic study was done to check the anti-diabetic property of raw and Shodhita Turmeric, in context to inhibitory activity of the α-amylase and α-glucosidase. Both methods were very sensitive with respect to time interval. Variation in the properties of inhibition of both enzymes can be seen as their decreasing order of IC_{50} dose (µg) in α-amylase: TZ (113.72) < TW (119.22) < TR (121.861) < TT (161.35) < TGM (218.85) and in α-glucosidase: TZ (121.32) < TW (139.48) < TR (145.29) < TT (185.51) < TGM (276.12). So, TZ is effective at minimum dose levels than others, shows significant therapeutic effect of Shodhana. Although TW and TR is also close to TZ but TZ is consider more better due to it shows minimum curcumin level in analytical section, that proves some special properties of media has increase the therapeutic property of TZ, which proves the synergic approach of Shodhana.

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