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ORIGINAL RESEARCH ARTICLE- EXPERIMENTAL STUDY

ANTI-DIABETIC POTENTIAL OF YAVA MANTHA – AN IN VITRO STUDY

HARSHITHA K.J[1] M.B KAVITA[2] V.BASKARAN[3] GURUBASAVARAJ Y[4]

ABSTRACT

Background: Diabetes Mellitus [DM] is one among the group of metabolic diseases, in which there is high blood sugar levels over a prolonged period. Presently, more than 7.1% of India's adult population affected by this disease. Yava Mantha administered as a therapeutic diet is expected to decrease the glucose levels in DM. In the present study, the adopted samskara (processing) is manthana (churning). As per the verse "samskaro hi gunantaradhanam ucchayte (processing brings in the quality change/improvement)" , by means of churning, it is expected to improve the quality of yava (Barley). Because DM is a yapya vyadhi (disease not curable but can be managed), importance has been focused on the use of traditional food preparations to tackle this disease. **Objectives:** The main objective of this study was to study the inhibitory effect of yava mantha on alpha amylase and alpha glucosidase, two intestinal enzymes, and glucose lowering potential of yava mantha. **Materials and methods:** Work was conducted at CFTRI, Mysore. Barley was purchased from departmental store, cleaned, dry roasted, milled and stored in an air tight container. yava mantha was prepared by adding 48 grams (one Pala) of powdered yava (yava saktu), 12 grams (one Karsha) of ghee (Go Ghrita) and 192 ml of water (4 Pala) was added and churned using magnetic churners at the rate of 520 rpm till the homogenous mixture is obtained. i.e., 1:1:4 parts. **Results:** On comparison with different samples, sample churned for 20 minutes has higher inhibition activity. The inhibition concentration (IC-50, mg/ml) of yava mantha churned for 20 minutes (B1) on alpha glucosidase enzyme (methanol extract) was 1.40mg/ml , on alpha glucosidase enzyme (methanol extract) was 6.34 mg/ml, on alpha glucosidase enzyme (water extract) was 189.17 mg/ml. **Conclusion:** The inhibitory effect of yava mantha on alpha-amylase and alpha-glucosidase activities in vitro indicate that, there is hypoglycemic potential for yava mantha and can be considered as a very good anti-diabetic plant source.

Keywords: Ayurveda**,** Alpha amylase, Alpha glucosidase, Diabetes Mellitus, Inhibitory activity, Manthana, Yava mantha

[1]Final year PG scholar**,** [2]Associate professor [4]Assistant professor, Department of Swasthavritta and Yoga, Shri Dharmasthala Manjunatheshwara College of Ayurveda and Hospital, Hassan.

[3]Senior Principal Scientist, HOD, department of biochemistry,CSIR- CFTRI, Mysore

Corresponding Author Email id: harshithakjr22@gmail.com Access this article online: www.jahm.in

INTRODUCTION

Diabetes Mellitus [DM] is one among the group of metabolic diseases, in which there is high blood sugar levels over a prolonged period. The risk of diabetes is expected to rise to 592 million by 2035 globally [1]. Prevention, management and treatment of this disease involve a healthy diet, physical exercises and therapeutic approach. *pathya*, a dietary and behavioural regime, plays a major role to restore health.

Yava (barley – *Hordeum vulgare*) is an ancient cereal crop widely used in different parts of the country. Flour of *Hordeum vulgare* (*yava saktu*) is listed as one of the *kritanna* [2] that can be used in the preparation of *mantha* (*yava mantha*) and is indicated in *prameha* (DM) [3].

An attempt is made in this study to evaluate the inhibiting property of *yava* on intestinal enzymes by adopting *manthana samskara* in the form of *yava mantha.*

AIMS AND OBJECTIVES

1. To assess glucose lowering potential of *yava mantha*.
2. To study the inhibitory effect of *yava mantha* on alpha amylase and alpha glucosidase (intestinal enzymes)

MATERIALS AND METHODS

Study design

Work was conducted at Council of Scientific and Industrial Research- Central Food Technological Research Institute (CSIR- CFTRI), Mysore

Materials:

Barley and cow ghee was procured from a local super market, Hassan and Mysore, Karnataka. Filter water was used for preparation of *yava mantha. Yava* was pre-cleaned manually to remove stones and other foreign particles. The cleaned sample was roasted in medium flame till it attained reddish brown color and a fine aroma is perceived. The roasted *yava* was powdered using a pulverizer. The *yava saktu* (roasted yava powder) and *go-ghrita* (cow ghee) was used to prepare the *yava mantha* and analyzed for proximate principles (nutrient composition) as per AOAC (2010).

Chemical:

All the chemicals and standards used were of analytical grade, chemicals HPLC grade, and purchased from Sisco Laboratories, Mumbai.

Sample preparation:

Barley was purchased from departmental store, cleaned, roasted, milled and stored in an air tight container.

Method of preparation of *yava mantha*:

yava mantha was prepared by adding 48 grams (one Pala) of powdered *yava* (*yava saktu*), 12 grams (one *karsha*)[4] of ghee (Go Ghrita) and 192 ml of water (4 Pala) was added and churned using magnetic churners

at the rate of 520 rpm till the homogenous mixture is obtained. i.e., 1:1:4 parts [5].

1. Roasted *yava* powder , *go-ghrita* and water just mixed (A)
2. Roasted *yava* powder, *go-ghrita*, and water churned for 20 minutes (B1)
3. Roasted *yava* powder, *go-ghrita* and water churned for 30 minute (B2)

All the samples mentioned above were subjected to enzyme inhibition activities.

Enzyme assay:

Alpha amylase inhibition assay in vitro (Miller et al 1959)

Various concentration of sample (80 – 200 µl) was added with 0.01 M of $CaCl_2$ dissolved in 0.1 M sodium citrate buffer (ph-6.9) [maximum quantity of buffer used for assay was 450 µl] and 50 µl of α-amylase and pre-incubated for 10 minutes at 37^0C. 1% of pre gelatinized starch solution was added to the reaction mixture and incubated again at 37^0C for 30 minutes. The reaction was stopped with 1 ml of dinitrosalicyclic acid (DNS) reagent and then boiled for 5 min. After cooling, 8 ml of distilled water was added and the amount of reducing sugars released was determined spectrophotometrically at 540nm. The α-amylase activity was calculated relative to control without inhibitor added and expressed as percentage inhibition. Controls contained with only test compound with no enzyme, were used to determine any background absorbance. Each assay was performed in duplicates.

Alpha glucosidase inhibition assay *In vitro:* (Li et al 2009)

The inhibitory effect of prepared samples against rat intestinal α- glucosidase enzyme was determined according to the method of Li et al (2009) using 4- para nitrophenyl α-D glucopyranoside (PNPG as substrate. The reaction mixture contained 120 µl of 0.01M phosphate buffer (pH 6.8), 50 µl of 3mM PNPG.

The sample concentration ranged from 40 µl - 100 µl and 50 µl of α-glucosidase enzyme solution (25 mg/ml) in a 96 numbered well plate. The plate was incubated for 40 min at 37 ^{0}C and the reaction was stopped by adding 50 µl of 0.67M- Na_2CO_3. The enzyme activity was determined by measuring the release of p-nitrophenol from the PNPG substrate. The reaction was monitored by the change of absorbance at 405nm. α-Glucosidase activity was calculated relative to control without inhibitor added and expressed as percentage inhibition.

IC_{-50} values for enzyme inhibition:

Inhibitory activities of α-amylase and α- glucosidase were expressed as IC_{-50} values against the drug used. IC_{-50} is defined as the concentration of extract/ compound required to inhibit 50% of the enzyme activity. IC_{-50} values were obtained from the least squares

regression line of the plots of the logarithm of the sample concentration (log) versus the α-amylase or α-glucosidase activity (%).

Enzyme Inhibition Assay

RESULTS:

Table 1: Results of enzyme inhibition activity

Experiment enzyme inhibition (mg/ml)	Just mixed with water(A)	Churned for 20 min(B1)	Churned for 30 min(B2)
IC-50, inhibitory concentration on α-amylase (methanol extract)	2.863	**1.40**	2.185
IC-50, inhibitory concentration on α-glucosidase (methanol extract)	34.44	**6.34**	31.89
IC-50, inhibitory concentration on α-glucosidase (water extract)	206.06	**189.17**	204.756

IC-50, alpha amylase and alpha glucosidase enzyme inhibition activity Values are mean of duplicate analysis. Wherein, A- roasted barley + Ghee + water in proportion but without churning, B1 - *yava mantha* churned for 20 minutes, B2 -*yava mantha* churned for 30 minutes.

The IC50 concentrations of B1 {1.4 mg/ml, 6.34 mg/ml, 189.17 mg /ml) for both the enzymes (alpha amylase and alpha glucosidase) is very minimal than other samples, Which indicates the higher enzyme inhibition of sample B1(sample churned for 20 minutes)

DISCUSSION:

It is evident from the results that the methanol extract exhibited higher alpha-amylase and alpha-glucosidase inhibition whereas the inhibition by the water extract is comparatively less. Higher inhibition of the methanol extract may be due to the presence of lipid soluble components like phenols, flavonoids, carotenoids. Lipid soluble compounds are more potent in methanol extract when compared to water soluble components from *yava*. Interestingly, the IC50 concentrations of B1 for both the enzymes is very minimal than other samples which may be due to the effect of churning on destruction of starch. This is in agreement with the viscosity values of these samples. Further, the higher antioxidant potential of A, B1 and B2 also could have been due to higher methanol extractability of bio-actives from *yava*.

The results indicate that *yava mantha* prevented alpha- amylase and alpha-glucosidase activity on starch indicating that release of glucose from these products is

prevented and hence could be considered as hypoglycemic foods as mentioned in classical medicine. The inhibitory effect of *yava mantha* on alpha-amylase and alpha-glucosidase activities *in vitro* indicate that, there is hypoglycemic potential for *yava mantha* and can be considered as a very good anti-diabetic plant source. The results showed that the activity of these enzymes was significantly suppressed by *yava mantha*. However, the mechanism of inhibition or binding affinity of active compounds from the *yava* needs scientific validation along with drug (molecular biology studies). *Yava mantha* perhaps binds to the active sites of these enzymes, resulting in the inhibition of the enzyme activity.

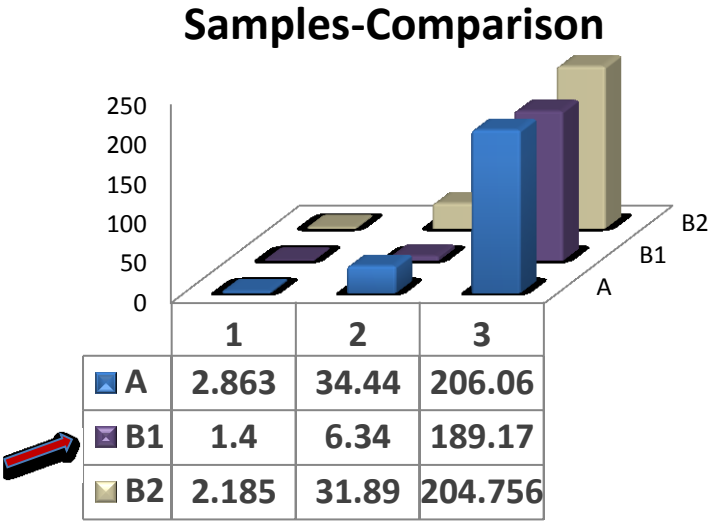

Figure 1: Bar diagram representing IC-50 value comparison between the samples.

Figure 1: Representing the IC-50 value comparison between the samples. Wherein 1, 2 ,3 – Indicates the IC- 50 values (experiments) A, B1 and B2 – samples.

The following studies also support the results:

The effect of barley on the blood glucose levels of normal and STZ induced diabetic rats was reported by Minaiyan et al (2014)[6] and they have showed that the hydro alcoholic extracts of barley seeds exhibited a role in diabetes control in long term consumption and the effect might be due to the high fibre content (beta glucan).

Zhou et al (2013[7] reported an enhanced antioxidant and antidiabetic activities of barley and wheat after soaking with tea catechin. Studies have also showed that barley as natural alpha glucosidase inhibitors and having potent inhibitory activity against the antidiabetic enzyme alpha glucosidase, maltase inhibitors and attributed to the phenolic content of barley.

In a study, Abe et al (1993) [8] has proved that, barley has the proteinaceous alpha amylase inhibitors**.** Rajesh kumara et al (2016) [9], described the glucose tolerance effect of Yava. Fiber administered orally to type 2 DM goto-kakizaki male rats for 9 months improved the area under the plasma glucose concentration time curves**,** lowered the fasting plasma glucose and glycosylated hemoglobin levels (Li et al (2003).

This study highlights the alpha-amylase and alpha-glucosidase inhibitory activity of Yava Mantha, acts as natural hyperglycemic inhibitor. Yava mantha may delay the absorption of dietary carbohydrates by inhibiting alpha- amylase and alpha-glucosidase activity in the intestine, resulting in the suppression of increased blood glucose levels. However, the mechanism of its action needs further study, thus the therapeutic use of commonly used plant derived inhibitors like Yava Mantha would be of medical and nutritional relevance in the treatment of diabetes mellitus.

The schematic representation showing the mechanism of inhibitory action of Yava Mantha on alpha-amylase and alpha-glucosidase is shown in Figure 2**.**

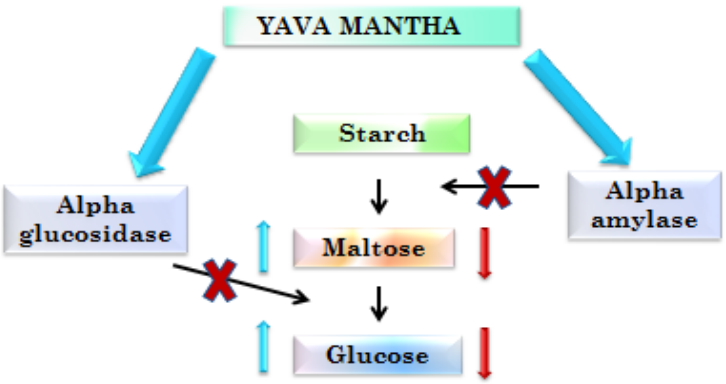

Figure 2. Flow-chart demonstrating the mechanism of action of Yava and Yava Mantha on α-amylase and α-glucosidase activity.

Blue arrow indicates normal action of enzymes and metabolism of starch to glucose and red arrow indicates inhibitory action of Yava and Yava Mantha on these enzymes and conversion of starch to glucose.

PROBABLE MODE OF ACTION OF YAVA MANTHA IN AYURVEDA

Gupta Sanjat et al, 2009 [10] in a review article states that the *gunantaradhana* (change in the gunas) is possible by *samskara* (processing). In the current study it is exhibited that, *manthana samaskara* has modified physical and chemical properties. *Yava mantha* is a *toya - agni sannikarsha* type of *samskara*

(processing with water and fire). Here, *jala* and *agni mahabhuta* are predominant and the changes in *guna* (properties) are acquired due to process of churning. At the level of *panchamahabhuta*, *prithvi* is *adhara* for all process and *akasha* provides space. *jala, agni, vayu* are responsible for transformation / changes that takes place in substance. *agni mahabhuta* is seen at its minute level during the process of churning. Rate of transformation is difficult to identify on a gross level. Even though *jala* is added to *mantha*, because of the influence of *agni* and *vayu mahabhuta* increased during the processing, *rukshata* is maintained in *mantha.*

Yava mantha is '*na ati sandra and na ati drava'*, (nor too thick nor too thin) this delays the digestion process in the *amashaya* and absorption in the *Pakwashaya*. The presence of *agni* and *vayu mahabhuta* clears the channels of obstruction (srotorodha). *vayu, jala and akasha mahabhuta* are dominant in *yava mantha* hence acts as *kaphahara*. Though ghee is added to *mantha* in little amount, based on the verse '*Samskarasya Anuvartanam'* (processing makes the material act as catalyst) [11], ghee has adopted the role of catalyst. Hence, though *sneha* is added, the preparation has not turned *snigdha* (unctuousness). Hence it can be ascertained that *yava mantha* acts as *pramehagna* (anti diabetic) by its *ruksha guna* (causing dryness), *kledahara* (removes moisture), *amahara* (scavenging), *srotoshodhana* (purifying channels of the body) property.

CONCLUSION:

Yava mantha prevented enzyme activity on starch indicating prevention of glucose release. Hence *yava mantha* acts as natural hyperglycemic inhibitor by delaying the absorption of dietary carbohydrates in the intestine.

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