IJBPAS, June, 2021, 10(6): 1885-1894

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

EVALUATION OF ANTI-DIABETIC POTENTIAL OF MASOOR DAL & MASURA YUSHA

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Received 13th July 2020; Revised 11th Aug. 2020; Accepted 20th Sept. 2020; Available online 1st June 2021

https://doi.org/10.31032/IJBPAS/2021/10.6.5513

ABSTRACT

Background:

Masura Yusha has been stated as '**Pramehajith**' in the classical text '**Yogaratnakara**'. Hence Masura Yusha can be a potential diet in Diabetes Mellitus

Objectives:

- To evaluate the anti -diabetic potential of Masura (Lens culinaris)
- To evaluate the anti- diabetic potential of Masura Yusha

Methods:

A commercial sample of Masoor Dal was collected from a local vendor at Bengaluru. Three samples were extracted from this namely Raw Masoor Dal, Fresh Masura Yusha & Sundried Masura Yusha. Fresh Masura Yusha will be prepared by adding eighteen parts of potable water and cooked till it softens completely. Sundried Masura Yusha was prepared in the similar protocol & was dried in sun for 36 hours. These samples were tested for Anti-diabetic potential.

Results

Analysis	Raw Masoor Dal	Fresh Masura Yusha	Sundried masura Yusha
% Inhibition IC_{50}	7.8	7.6	6.5

Interpretation and Conclusion:

On comparison of IC_{50} values of different preparations it is observed that there is no significant difference between Raw Masoor Dal (7.8) & Fresh Masura Yusha (7.6). However, IC_{50} value of Sun-Dried Masura Yusha was found to be 6.5 µg.

An attempt was made to compare the α – amylase inhibitory potential of all three preparations with reference standard acarbose which is a known molecule α – amylose inhibitory potential of 2.154 µg/dl & found that crude masoor dal preparation have got comparable inhibitory action as that of pure reference standard.

Keywords: Masura (Lens culinaris), Masura Yusha, Anti-diabetic Potential

INTRODUCTION

Ayurveda is a way of life. It is one of our richest heritages gifted by the ancestors. Still playing a key role for better human health, in many incurable, chronic and degenerative diseases by its effectiveness it has proven its Eternity. In the today's scientific era, time has come to prove the Ayurvedic concepts and efficacy of the Ayurvedic drugs on the various diseases by the applying all the modern parameters.

Diabetes mellitus has emerged as an important public health problem globally. It has become an endemic disease affecting people irrespective of their age, sex, socio-economic status. More than 346 million people worldwide have diabetes. There is an emerging global epidemic of diabetes. Diabetes is a chronic disease that occurs either when the pancreas does not produce enough insulin or when the body cannot effectively use the insulin it produces. The incidence is expected to rise to 592 million by 2035 globally. According to the Indian Heart Association, India is Diabetes capital of the world with a projected 109 million individuals with diabetes by 2035 **[1]**.

In Ayurveda, Diabetes Mellitus is known to be a 'Rich man's disease' which can be understood by etiological factors of Prameha mentioned as enjoying the pleasures of life with reduced or no physical activity and sedentary life. Madhumeha is considered as a type of Vataja Prameha & it is the nearest resembling condition with Diabetes Mellitus. Prameha is a disease concerned with abnormalities manifested in the urine. Qualitative and quantitative disturbances of urine occur in Prameha. Madhumeha patient passes excessive sweet and astringent urine and exhibits sweetness all over the body. Hence Madhumeha can be entitled clinically with Diabetes Mellitus from the above description.

There are wide varieties of medicines available for the treatment of DM. in terms of efficiency, the main drawback of metformin, sulfonylureas,

gliptins & to a lesser extent glitazones is durability. The main adverse effect of metmorfin is gastro-intestinal discomfort. Major concerns related to sulfonylureas are hypoglycaemia & weight gain. The use of pioglitazone has been associated with the increased risk of bladder cancer, oedema, cardiac failure, weight gain & distal bone fractures in the post-menopausal women. The most common adverse reactions associated with glucagon like peptide-1 agonists are gastro-intestinal discomfort. Due to the chronic use & adverse effects as above there is a paradigm shift in in the food formulation regarding the drugs that delays the absorption & digestion of carbohydrates leading to the drugs like acarbose, miglitol, etc.

Ayurveda has emphasized diet as one essential part of healthy life. It is likely that no other science has described the unique effect of diet as thoroughly as Ayurveda described it thousands of years ago. It also describes a concept that the health and illness, both are the products of Ahara, where Hita and Ahita Ahara maintain the homeostasis and illness respectively. Attainment of Swaasthya is by following Hitakar Ahara in appropriate amount on appropriate time **[2]**. Wholesome diet ensures the proper growth and development of the body, on the contrary, unwholesome diet is the cause of several diseases **[3]**.

"Na cha aaharam samam kinchit bhaishajyam upalabhyate |

Shakyate api anna maatrena naraha kartum niramayaha ||"

No medicine is equivalent to food. It is possible to make a person disease free with mere diet **[4]**. Food is the best medicine so evaluating the anti-diabetic potential of commonly used food substances is essential.

Masura is said to be Pathya in Prameha. Masura Yusha has been referred to as "Pramehajit **[5]** in the Ayurveda classics. Hence, Masura in Prameha can be administered as Ahara.

In this study the anti-diabetic potential of masoor dal & Masura Yusha was evaluated as discussed in materials & methods.

SOURCE OF THE DRUG:

The commercial sample of Masura was procured from a local vendor at Bengaluru & was tested at Think & Ink Education & Research Foundation, Bengaluru.

CHEMICAL:

All the chemicals and standards used were of analytical grade and purchased

from Sisco Laboratories, Mumbai. Methanol, acetonitrile, dichloromethane, ethyl acetate, acetone and hexane were of HPLC grade and ammonium acetate were purchased from Sisco Laboratories, Mumbai

SAMPLING & SAMPLE PREPARATION:

Three samples were taken for the analysis.

1. Raw Masoor Dal (RMD)
2. Fresh Masura Yusha (FMY)
3. Sundried Masura Yusha (SDMY)

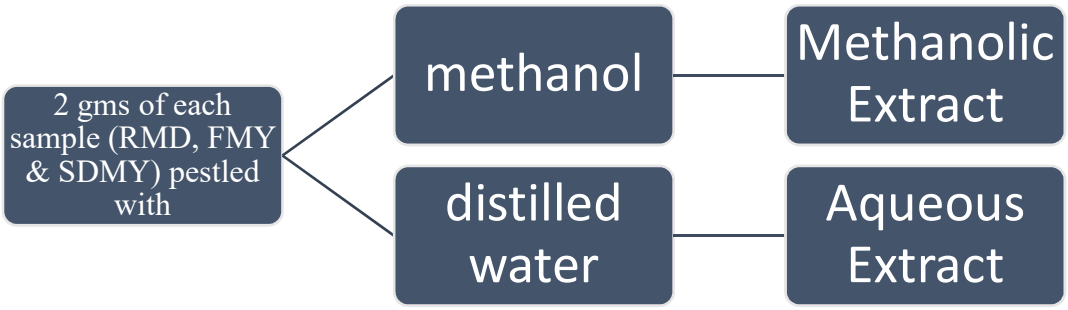

Figure 1: Flowchart Showing Preparation of Methanolic & Aqueous Extracts Of The Sample

Preparation of Masura Yusha:

- Masura Yusha was prepared as per the classical reference **[5]**.
- 10gms of Raw Masoor Dal was weighed.
- 180 ml of distilled water was measured.
- The weighed Masoor Dal was taken in a glass beaker & was cooked in the measured distilled water in moderate flame until the dal completely softens.
- This is the Fresh Masura yusha sample

Preparation of Sundried Masura Yusha:

- Masura Yusha was prepared as per classical reference.
- This was kept in the sun for 36 hours for drying.
- This was collected as Sundried Masura Yusha Sample after complete Drying.

ASSAY OF AMYLASE INHIBITION [6-14]

In vitro amylase inhibition was studied by the method of Bernfeld . In brief, 100 µLof the test extract (100,200,400,600,800 and 1000 µg/ml) was allowed to react with 200 µL of α-amylase enzyme (Hi media Rm 638) and 100 µL of 2 mM of phosphate buffer (pH-6.9). After 20-minute incubation, 100 µL of 1% starch solution was added. The same was performed for the controls where 200 µLof the enzyme was replaced by buffer. After incubation for 5 minutes, 500 µL of dinitrosalicylic acid reagent was added to both control and test.

They were kept in boiling water bath for 5 min. The absorbance was recorded at 540 nm using spectrophotometer and the percentage inhibition of α-amylase enzyme was calculated using the formula

Inhibition (%) = Abs 540 (control) – Abs 540 (extract) * 100/ Abs 540(control)

ALPHA AMYLASE INHIBITION ASSAY BY IODINE METHOD

Starch – Iodine Colour Assay screening of the given samples were carried out based on Starch Iodine test. The total assay mixture composed of samples of different concentrations (0.2, 0.4, 0.6. 0.8, 1ml respectively), 1.5 ml of amylose (5mg/ml concentration), 80 mM sodium phosphate buffer (pH-7) in different concentrations (to make the volume up to 4 ml along with the sample), 0.1 ml of enzyme of the concentration 125ml/ml. This assay mixture was incubated for 3 mins at 37° C. 2ml of Iodine reagent was added to each test tube to stop the enzyme reaction. The colour change was noted & the absorbance was read at 590 nm. The Inhibition was calculated by the formula:

Inhibition (%) = 100 – (100/ amount of amylose digested in blank * amount of amylose digested in sample)

ALPHA-GLUCOSIDASE INHIBITION ASSAY [6-14]

Principle:

Alpha-glucosidase activity can be measured in-vitro by determination of the reducing sugar (glucose) arising from hydrolysis of sucrose by α-glucosidase enzyme, isolated from small intestine of rat.

Materials

α-glucosidase enzyme- isolated from rat intestine (stored at -20^{0}C), Sucrose, Acarbose, Total protein estimation kit (Biuret method), Sodium dihydrogen orthophosphate,Disodium hydrogen phosphate, Glucose reagent.

Isolation of enzyme:

Rats were sacrificed, intestine removed and chilled with ice cold 80 mM phosphate buffer. The intestine was then cut open, the mucosa scraped off with a piece of glass rod and homogenized with four parts (v/v) of cold buffer. Nuclei and large cell debris were removed by centrifugation at 2000 to 4000 rpm for 10 mins and supernatant aliquoted into 1.5 ml vials and stored at -20^{O}C. [Protein content= 0.5g/dl by Biuret method]

Procedure:

In the test tube labeled them as control, standard and test samples in different concentration (100, 200, 400, 600, 800 and 1000µg/ml. first

pre-incubate the mixture for 30 min at 37^0C containing 225 µl of 80mM Phosphate buffer (pH 7.0) with 75 µl of α-glucosidase enzyme in all test tubes. After incubation Add 500 µl of 37 mM Sucrose and again Incubate at 37^0C for 20 mins. Above test tubes Kept in boiling water bath for 2 mins, then cooled and add 250 µl of glucose reagent. Finally incubate at RT for 10 mins and Measure OD at 510 nm.

% Inhibition = control OD - Test OD / Control OD x100

OBSERVATIONS & RESULTS

ALPHA AMYLASE INHIBITION ASSAY BY IODINE METHOD

1. Raw Masoor Dal

Table 1 : OD Values Of Raw Masoor Dal

S. No.	Sample	Amylose	RMD	Buffer	Enzyme	Iodine reagent	OD @ 590 nm
1	Blank	0	0	3.9	0.1	2	0
2	B6	1.5	0	1.5	0	2	0.711
3	T6	1.5	0	1.4	0.1	2	0.493
4	St_{6a3}	1.5	0.2	1.2	0.1	2	0.344
5	St_{6b3}	1.5	0.4	1	0.1	2	0.247
6	St_{6c3}	1.5	0.6	0.8	0.1	2	0.148
7	St_{6d3}	1.5	0.8	0.6	0.1	2	0.084
8	St_{6e3}	1.5	1.0	0.4	0.1	2	0.013
9	$St_{6a(b)3}$	1.5	0.2	1.3	-	2	0.575
10	$St_{6b(b)3}$	1.5	0.4	1.1	-	2	0.501
11	$St_{6c(b)3}$	1.5	0.6	0.9	-	2	0.412
12	$St_{6d(b)3}$	1.5	0.8	0.7	-	2	0.365
13	$St_{6e(b)3}$	1.5	1.0	0.5	-	2	0.321

2. Fresh Masura Yusha

Table 2 : OD Values Of Fresh Masura Yusha

S. No.	Sample	Amylose	RMD	Buffer	Enzyme	Iodine reagent	OD @ 590 nm
1	Blank	0	0	3.9	0.1	2	0
2	B6	1.5	0	1.5	0	2	0.711
3	T6	1.5	0	1.4	0.1	2	0.493
4	St_{6a1}	1.5	0.2	1.2	0.1	2	0.439
5	St_{6b1}	1.5	0.4	1	0.1	2	0.388
6	St_{6c1}	1.5	0.6	0.8	0.1	2	0.346
7	St_{6d1}	1.5	0.8	0.6	0.1	2	0.260
8	St_{6e1}	1.5	1.0	0.4	0.1	2	0.188
9	$St_{6a(b)1}$	1.5	0.2	1.3	-	2	0.652
10	$St_{6b(b)1}$	1.5	0.4	1.1	-	2	0.558
11	$St_{6c(b)1}$	1.5	0.6	0.9	-	2	0.872
12	$St_{6d(b)1}$	1.5	0.8	0.7	-	2	0.644
13	$St_{6e(b)1}$	1.5	1.0	0.5	-	2	0.615

3. Sundried Masura Yusha

Table 3: OD Values Of Sundried Masura Yusha

S No.	Sample	Amylose	RMD	Buffer	Enzyme	Iodine reagent	OD @ 590 nm
1	Blank	0	0	3.9	0.1	2	0
2	B6	1.5	0	1.5	0	2	0.711
3	T6	1.5	0	1.4	0.1	2	0.493
4	St_{6a2}	1.5	0.2	1.2	0.1	2	0.450
5	St_{6b2}	1.5	0.4	1	0.1	2	0.323
6	St_{6c2}	1.5	0.6	0.8	0.1	2	0.238
7	St_{6d2}	1.5	0.8	0.6	0.1	2	0.149
8	St_{6e2}	1.5	1.0	0.4	0.1	2	0.087
9	$St_{6a(b)2}$	1.5	0.2	1.3	-	2	0.631
10	$St_{6b(b)2}$	1.5	0.4	1.1	-	2	0.604
11	$St_{6c(b)2}$	1.5	0.6	0.9	-	2	0.605
12	$St_{6d(b)2}$	1.5	0.8	0.7	-	2	0.594
13	$St_{6e(b)2}$	1.5	1.0	0.5	-	2	0.591

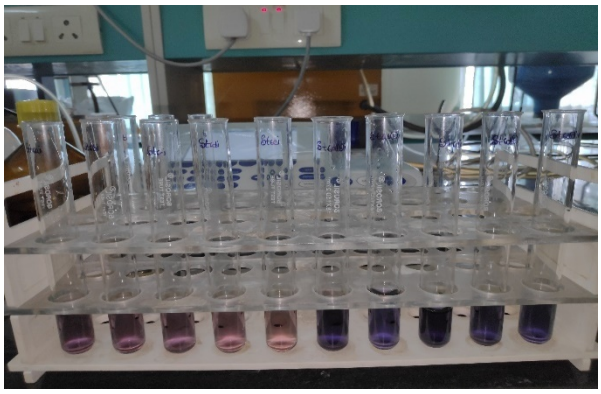

Figure 2: Final Reaction Alpha Amylase Inhibition

Alpha Amylase Inhibition Assay By DNS Method

Table 4: Results of Alpha Amylase Inhibition Assay By DNS Method

Concnetration (µg/ml)	OD at 540 nm	% inhibition activity
100	0.43	24.56
200	0.41	28.07
400	0.40	29.82
600	0.37	35.08
800	0.33	42.10
1000	0.31	45.61
Blank	0.57	
Standard acarbose	0.22	61.40

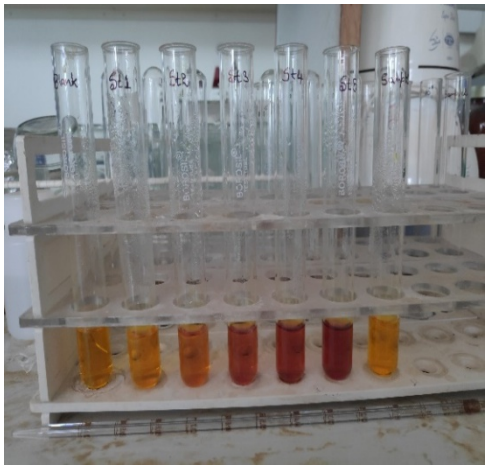

Figure 3: Final Reaction of Alpha Amylase Inhibition Assay

DISCUSSION

In this study the anti-diabetic potential of masoor dal was evaluated as discussed in materials & methods.

Amylase inhibitory potential of different preparations namely Raw Masoor Dal(RMD), Fresh Masura Yusha(FMY), Sundried Masura Yusha (SDMY) was performed under in-vitro conditions as detailed in **[12]**.

In all the preparations the enzyme inhibitory activity studies were performed in the concentration range of 4, 8, 12, 16, 20 µg/ml. though the dose response curves of all the samples showed a sigmoidal nature suiting Boltzmann's model, the initial & final rate of inhibitions were found to be different in different samples. Among the lowest concentrations studied here for inhibitory potential, RMD could achieve 46.72% inhibition at 4 µg/ml & at this concentration the percentage inhibition observed in FMY & SDMY were 36.6 & 34.31 respectively. Though RMD could achieve more than 45 % inhibition at 4 µg/ml, the rate of inhibitions were found to be less at higher concentration. At the highest concentration used in this study [20 µg/ml], maximum inhibition achieved was 62.4 %. In case of FMY & SDMY at this concentration 100% enzyme inhibition was observed.

As the rate of enzyme inhibition was different at the early, middle & late stage of enzyme kinetics study by different preparations of masoor dal, IC_{50} values were drawn for better comparison. IC_{50} values were drawn from dose response curve as per the Boltzmann's fit & by using interpolation tools [RMD-7.8, FMY- 7.6, SDMY- 6.5].

On comparison of IC_{50} values of different preparations it is observed that there is no significant difference between RMD (7.8) & FMY (7.6). However, IC_{50} value of SDMY was found to be 6.5 µg. This result shows that the active principles present in RMD have not been chemically modified much during the preparation of Yusha. A slightly higher activity observed in SDMY needs to be investigated further to understand the chemical modifications, if any.

An attempt was made to compare the α – amylase inhibitory potential of all three preparations with reference standard acarbose which is a known molecule α – amylose inhibitory potential of 2.154 µg/dl & found that crude masoor dal preparation have got comparable inhibitory action as that of pure reference standard. This sheds more light into the opportunities of food formulations that can delay carbohydrate digestion & absorption.

CONCLUSION

All the three samples showed similar increasing pattern of inhibition percentage with increasing concentration. But the

maximum inhibition was shown by Sundried Masura Yusha followed by Fresh Masura Yusha & Raw Masoor Dal.

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