IJPSR (2014), Vol. 5, Issue 5 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 02 December, 2013; received in revised form, 14 January, 2014; accepted, 09 March, 2014; published 01 May, 2014

EVALUATION OF α – AMYLASE INHIBITORY ACTIVITY OF SOME CLINICALLY USED DRUGS

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Keywords:

Alternative uses of drugs, α-amylase inhibitory activity, Add – on therapy

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ABSTRACT: Finding out the alternative uses of the clinically proven and time tested drugs can be one of the best options for drug discovery research. In view of this the present study was carried out to evaluate α-amylase inhibitory activity of the some of the commonly used drugs. The drugs were procured as gift samples from pharmaceutical companies and were evaluated for in-vitro α-amylase inhibitory activity using a colorimetric assay. The results revealed that Buspirone, Amlodipine, Diltiazem, Verapamil, Chlorpheniramine maleate and Cetirizine had prominent α - amylase inhibitory potential in comparison to Acarbose. The drugs exhibiting prominent *in-vitro* α- amylase inhibitory activities are to be further evaluated in the *in-vivo* models so that can be used as add - on therapy in diabetes patients.

INTRODUCTION: The current patent regulations necessitate the discovery of new drugs for treating diseases. The latest data reveal that there are 27,000 clinically active agents which are already approved and available worldwide [1].

Safety of these drugs is already known and if an old drug is repurposed to a new indication, it can directly enter clinical trials. In the past two decades some of the clinically used old drugs have been repurposed for the new indications as shown in **Table 1.**

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(5).1843-46

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(5).1843-46

Currently there is a need to slip out of the view that a single drug can be used for treating only one disease. Hence, the assessment of the other possible uses of the currently used drugs in clinical practice can provide new therapeutic utilities of these drugs. If old drugs exhibit new activity then they can be used alone or as add on therapy to many chronic diseases. Finding out the new use of an already existing drug can also reduce the overall expenditure of drug discovery [17]. Currently FDA is also stressing on the need for repurposing of the clinically used drugs [18]. The approach of high throughput chemical screening, transcriptome matching or simple in silico ligand docking can be used for finding out the new uses of the drugs [19]. In addition to the above techniques the basic pharmacological studies and *in-vitro* testing approaches can also be used for finding the new applicability of old drugs. Enzyme inhibition

studies are currently being used in various facets of drug discovery research and the use of enzyme inhibitors in diabetes is considered as one of the prominent tools in the therapeutic management of the disease [20]. α- Amylase is one of the main enzymes responsible for the breakdown of starch to simple sugars [21]. These enzyme inhibitors can delay the carbohydrate digestion and reduce the rate of glucose absorption. Consequently postprandial rise in blood glucose is decreased, which in turn can improve glucose tolerance in diabetic patients [22]. Some of the clinically used drugs such as Naphazoline, Fluconazole, Astemizole, Fluoxetine, Clarithromycin and ampicillin have been proven to have prominent α-amylase inhibitory activity [23]. In similar lines, the present study was carried out to evaluate the α-amylase inhibitory activity of the commonly used drugs in clinical practice such as Amlodipine, Buspirone, Chlorzoxazone maleate, Cetirizine, Chlorpheniramine maleate, Clomiphene citrate, Diliazem, Emtricitabine, Sulphamethoxazole, Trimethoprim and Verapamil

TABLE 1: LIST OF DRUGS WITH EARLIER USE AND CURRENT/REPOSITIONED USE

Drug	Earlier Use	Current/Repositioned Use
Thalidomide	Nausea and vomiting (Pregnancy)	Leprosy [2] Multiple Myelom[3]
Raloxifene	Osteoporosis	Invasive Breast Cancer in post-menopausal women[4]
Tamoxifen	Metastatic breast cancer	Bipolar disorder[5]
Rapamycin	Prevent Organ transplant rejection	Autoimmune Lymphoproliferative Syndrome (ALPS)[6] Lymphangioleiomyomatosis[7]
Viagra	Heart disease	Erectile Dysfunction[8]
Minoxidil	High Blood Pressure	Hair loss therapy[9]
Cymbalta	Antidepressant	Fibromyalgia[10]
Gemzar	Antiviral	Anticancer[11]
Ibuprofen	NSAID	Protection against Parkinson's disease[12]
Finasteride	Prostate cancer	Hair loss[13]
Hydroxychloroquine	Antiparasitic drug	Anti-arthritic agent[14]
Doxepin	Antidepressant	Antipruritic[15]
Naltrexone	Opioid addiction	Alcohol withdrawal[16]

MATERIALS AND METHODS:

Materials: Starch, 3, 5 - Dinitrosalicylic acid, Sodium Potassium tartarate, Sodium hydroxide, Sodium Dihydrogen phosphate, Sodium chloride and α-Amylase were purchased from Hi Media Mumbai. Acarbose was purchased from Sigma Aldrich, Bangalore. All the drugs were procured as gift samples from various Pharmaceutical firms. Chlorzoxazone, Buspirone, Amlodipine, Diltiazem, Verapamil, Cetirizine and Ciprofloxacin were kind gift of Dr.Reddy's Laboratories, Hyderabad.

Naproxen (Natco, Hyderabad), Granisetron (Orchid Pharmaceuticals, Chennai), Sulphamethoxazole (Biochem, New Delhi), Trimethoprim (Biochem - New Delhi), Chlorpheniramine Maleate (Chemi Pharm, Mumbai), Clomiphene Citrate (Wockhardt, Mumbai).

Methodology: Different concentrations (1µg/ml, 3µg/ml, 5µg/ml, 10 µg/ml, 30 µg/ml and 50 µg/ml) of test drug samples were prepared with Phosphate buffer. To 0.2ml of the test sample, 0.4ml of enzyme solution containing 10mg of α-amylase in 100ml of phosphate buffer pH 6.9 (20Mm sodium di hydrogen phosphate containing 6.7mM of sodium chloride) was added.

Then to the above solution, 0.2ml of buffer was added and the solution was incubated for 20 min. Then 0.2ml of starch solution (1% W/W) was prepared and boiled for 15 min and added to the mixture. It was then incubated for 5 min. The samples were prepared in triplicate. To the above solution 1ml of DNS reagent [Dinitrosalicylic acid (1.5%), sodium potassium tartarate (12%) and sodium hydroxide (0.4M) in 100ml distilled water was added.

The solution was boiled for 5min and cooled in running tap water. Absorbance was measured at 540nm (Schimadzu Spectrophotometer UV-1800).

Control was considered 100% enzyme activity and was conducted in similar way by replacing test drug with vehicle. The results were expressed as % inhibition calculated using the formula;

$$\%\ Inhibition = \frac{(\text{Absorbance of control} - \text{Absorbance of test})}{\text{Absorbance of control}} X\ 100$$

The IC50 values (inhibitory concentration at which 50% inhibition of the enzyme activity occurs) of the test samples were determined by performing the assays as above with varying concentration of the test samples ranging from 1µg to 50µg/ml. The IC50 values were determined from plots of percentage inhibition Vs concentration. The total experiment was done in triplicate [24].

RESULTS: The IC_{50} values of the present investigation reveal that Amlodipine, Buspirone, Cetrizine, Chlorpheniramine maleate, Diltiazem, Naproxen and Paracetamol have prominent α - amylase inhibitory potential when compared to acarbose as shown in **Table 2.**

TABLE 2: IC_{50} VALUES OF THE VARIOUS DRUGS (n=3)

Drug	IC_{50} Value (µg/ml)
Acarbose	59.63
Amlodipine	28.00
Buspirone	20.50
Chlorpheniramine maleate	49.70
Cetrizine	17.8
Diltiazem	43.0
Verapamil	13.7

Of the tested drugs the lowest IC_{50} was found to be 9.10µg/ml for paracetamol while it was 59.63µg/ml for the standard Acarbose. The drugs like Clomiphene citrate, Emtricitabine, Sulphamethoxazole and Trimethoprim and Chlorzoxazone failed to exhibit α - amylase inhibitory. The order of lowest IC50 values was found to be Verapamil < Cetrrizine < Buspirone < Amlodipine < Diltiazem < Chlorpheniramine maleate.

CONCLUSION: The study data suggests that these drugs may be used as add-on drugs to diabetic patients according to clinical requirements whenever polypharmacy is essential. However, the data needs further evaluation in the animal models and human volunteers.

ACKNOWLEDGEMENTS: The authors wish to acknowledge the management of The Vaagdevi College of Pharmacy for providing the necessary facilities.

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How to cite this article:
Rodda HC, Sandhya T and Swetha P: Evaluation of α – amylase inhibitory activity of some clinically used drugs. *Int J Pharm Sci Res* 2014; 5(5): 1843-46.doi: 10.13040/IJPSR.0975-8232.5 (5).1843-46