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EVALUATION OF ANTI-OXIDANT ACTIVITY OF TENELIGLIPTIN BY DPPH ASSAY- AN IN-VITRO STUDY

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ABSTRACT

To assess the antioxidant potential of the new anti-diabetic drug Teneligliptin using DPPH assay. DPPH radical scavenging activity was done using the method of Naznin Araand HasanNur (2009)- . Here butylated hydroxy toluene (BHT) was used as the standard compound in this study. The free radical scavenging property of Teneligliptin was found to be 71.87±0.12%,65.62±0.25%,60.10±0.33%, %inhibition while that of BHT was found to be 93.91±0.76%, 82.4±0.34%, 74.12±0.16%, % inhibition at the following concentrations 1000, 750, 500 µg/ml respectively.From the study, it was found that the study drug teneligliptin has good antioxidant property which could act as a cytoprotective agent in conditions associated with oxidative stress along with its anti-diabetic action.

KEYWORDS: *Teneligliptin; anti-oxidant assay; DPPH (-2,2-diphenyl-1-picrylhydrazyl) Anti-diabetic drug.*

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INTRODUCTION

Diabetes mellitus (DM), commonly referred to as diabetes, is a group of metabolic diseases where there are high blood sugar levels over a prolonged period.[1] Symptoms of high blood sugar are vast and range from frequent urination, increased thirst, and increased hunger todiabetic ketoacidosis, and non-ketotic hyperosmolar coma, if left untreated and the disease would ultimately progress towards death.[2] Apart from the above-mentioned complications, there are also serious long-term complications associated with diabetes that include heart disease, stroke, chronic kidney failure, foot ulcers, and damage to the eyes. The incidence of diabetes is increasing at an alarming rate. According to the 'Global report on diabetes' by WHO, as of 2015, an estimated 415 million people had diabetes worldwide;with type 2 DM making up about 90% of the cases.This represents 8.3% of the adult population, with equal rates in both women and men. Diabetes doubles a person's overall risk of early death.From 2012 to 2015, approximately 1.5 to 5.0 million deaths each year resulted from diabetes.[23] All forms of diabetes increase the risk of long-term complications. These long term complications typically develop many years (10–20) after the onset of the disease, but may be the first symptom in those who have otherwise not received a diagnosis before that time. The blood vessels in the eyes, kidneys, and nerves are very vulnerable to the high blood sugar level seen in diabetes. In the eyes, the high blood sugar damages the microvasculature in the retina leading to diabetic retinopathy. Diabetic nephropathy is another serious complication in diabetes which causes fibrosis in the kidney, proteinuria, and these complications eventually add up and lead to chronic kidney disease (CKD)[3]. The above-mentioned complications are only the tip of the iceberg, as the damage caused by high blood sugar levels penetrates almost every cell and every organ in the body, sparing almost nothing.

Diabetes and Oxidative stress

It is interesting to note that all these major complications of diabetes have different pathologies in disease progression. But the Venn diagram of all these path physiologies and these complications intersects at one point called oxidative stress. Oxidative stress is defined as the measure of the steady state level of reactive oxygen or oxygen radicals in our body. Oxidative stress acts as a common bridge linking the diverse mechanisms for the pathogenesis of complications ofdiabetes[4]. Increased nonenzymatic glycosylation (glycation), auto oxidative glycosylation, changes in energy metabolism that alters sorbitol pathway, hypoxia, and ischemic reperfusion injury are some of the common biochemical pathways that have been deranged and which contributes to increased stress which in turn leads to increase in the level of inflammatory mediators[5]. Apart from these derangements in lipid metabolism like lipid peroxidation, oxidation of lipids in plasma lipoproteins, deranged metabolisms of lipids that contribute significantly to the complications of diabetes, most notably the vascular complication like diabetic retinopathy. The current groups of oral hypoglycemic drugs available for therapy are Sulfonylureas (eg. glimepiride, glipizide, glyburide), Biguanides (eg. metformin), Thiazolidinediones (eg. Pioglitazone), Alpha-glucosidase inhibitors (Acarbose), Meglitinides (eg.nateglinide), peptide analogues like Glucagon-like peptide (GLP) agonists, Di peptidyl peptidase 4 inhibitors (eg.Vildagliptin and teneligliptin). Apart from these, there are different formulations of injectable insulin that are available.

Teneligliptin

TeneligliptinIt was developed by Mitsubishi Tanabe Pharma and launched in September 2012 by both Mitsubishi Tanabe Pharma and Daiichi Sankyopharmaceutical company in Japan. It is approved for use in Japan, Korea, and India. Teneligliptin, a DPP-4 inhibitor, is a novel anti-diabetic drug. Teneligliptin is prescribed in cases where there is an insufficient improvement in glycemic control, even after drug, diet and oral hypoglycemic drugs. Teneligliptin is administered orally in the dose of 20 mg per day, the dose can be increased up to 40 mg per day. Teneligliptin is metabolized by the CYP3A4 pathway and the elimination is via both renal and hepatic excretion, no dose adjustment is needed for patients suffering from renal impairment. All these facts stress the need for a good anti oxidative system for the diabetic patients to prevent long term complications. It would be really great if the oral hypoglycemic drugs have a good anti-oxidant property along with the hypoglycemic activity within them. Since teneligliptin, a recently introduced antidiabetic drug had a very good glycemic control. The study was performed to evaluate the anti oxidant activity of this drug.Since there is a strong co relation between oxidative stress and diabetes, and Teneligliptin being a good anti-diabetic drug with least side effects, we would like to prove the antioxidant effect of this drug using DPPH assay.This study aims to estimate the anti-oxidant level of the anti-diabetic drug teneligliptin.

MATERIALS AND METHODOLOGY

The ability of the extract to scavenge DPPH radical was determined by the method described by Naznin Araand HasanNur (2009).[21]

Principle of the assay

Antioxidant activity assay is based on the reduction of 1, 1-diphenyl-2-picrylhydrazyl (DPPH).Due to the presence of an odd electron it gives a strong absorption maximum at 517nm.As the electron becomes paired off in the presence of a hydrogen donor, i.e., free radical scavenging antioxidant, the absorption strength is decreased, and the resulting decolourization is stoichiometric with respect to the number of electrons captured. Antioxidant activity is defined as decrease in absorbance value from initial to final at 517nm at standard conditions.

Reagents used

DPPH-1mg in methanol
BHT(Butylatedhydroxytoluene -standard)-1.6mg/ml in methanol
Teneligliptin samples-desired concentration from 1mg/ml –max of 5mg/ml (in methanol/DMSO)

Table 1
Amount of solution- Reagent, Blank and Test to be taken

S.no	Reagent	Blank	Standard	Test
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	_	100 µl	_
3	Sample	_	_	100 µl
4	DPPH	200µl	200µl	
Incubation at dark for 30 mins				
OD at 517 nm				

Procedure

3.7 ml of absolute methanol was allocated in all test tubes along with blank. Then 100µl of absolute methanol was added to blank. Then 100µl of BHT was added to the tube marked as standard and 100µl of teneligliptin was added to all other tubes marked as tests. Then, finally 200µl of DPPH reagent was added to all the test tubes at room temperature and the test tubes were incubated for minimum of 30minutes.After incubation absorbance of all samples was checked at 517nm.

Data acquisition

Table 2
Readings obtained- Optical Density and % Antioxidant activity

S.no	Sample	Concentration (µg/ml)	O.D				DPPH activity (%)
			I	II	III	Average	
1	Sample	1000	0.27	0.27	0.27	0.27	71.87
		750	0.32	0.33	0.34	0.33	65.62
		500	0.38	0.39	0.38	0.383	60.10
2	Standard-BHT	1000	0.02	0.02	0.02	0.02	93.91
		750	0.13	0.13	0.13	0.13	82.4
		500	0.21	0.21	0.21	0.21	74.12

The same procedure is carried out at 1000, 750 and 500 (µg/ml) concentrations for both sample and test.

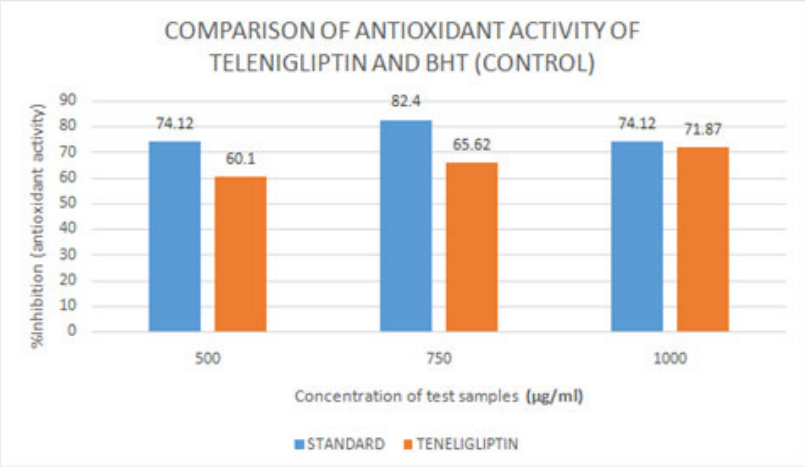
Calculation

Blank O.D: 0.96

All values obtained are acquired from UV spectrophotometer/colorimeter for assays. The antioxidant activity is calculated by using the formula

%Antioxidant activity.
= (absorbance at blank)-(absorbance at test)/ (absorbance at blank)× 100

RESULTS AND DISCUSSION



The free radical scavenging property (percentage inhibition) of Teneligliptin was found to be 71.87±0.12%, 65.62±0.25%, 60.10±0.33%,at the concentrations 1000, 750, 500 µg/ ml respectively. The% inhibition of BHT was found to be 93.91±0.76%, 82.4±0.34%, 74.12±0.16%, % inhibition at the concentrations 1000, 750, 500 µg/ ml respectively.

Figure 1
The Antioxidant activity of given samples using DPPH Assay method.

STATISTICAL ANALYSIS OF THE DATA

The statistics for the obtained data was performed on SPSS software version 15.0. The mean and standard deviation, standard error, t-value and the level of significance for the test drug teneligliptin and standard (BHT) was calculated. The Mean obtained was 65.86 and 83.47 for teneligliptin and standard respectively; standard deviation of teneligliptin and standard(BHT) was found to be 5.88 and 9.93. Standard error of teneligliptin and standard (BHT) are 3.39 and 5.73; the t-value for the data is 7.487; the p value is 0.01 which is statistically significant. The mean of teneligliptin and standard (BHT) are 65.86 and 83.47; standard deviation of teneligliptin and the standard are 5.88 and 9.93; Standard error of teneligliptin and standard BHT are 3.39 and 5.73; the t value is 7.487 and the level of significance is 0.01. The statistics was performed on SPSS software version 15.0. From the above results we can infer that teneligliptin has very good antioxidant property, almost approaching that of our control molecule BHT. The structure of teneligliptin, which is {(2S,4S)-4-[4-(3-methyl-1-phenyl-1H-pyrazol-5-yl)piperazin-1-yl]pyrrolidin-2-yl}(1,3-thiazolidin-3-yl) methanonehemipentahydrobromide hydrate is very unique structure in that itis characterized by five consecutive ringsand is peptidomimetic. This structure makes teneligliptin more potent as well as selective and confers teneligliptin a good anti-oxidant property at the same anti-diabetic dose.

CONCLUSION

From this study we can infer that teneligliptin not only serves as a good anti diabetic agent, having good effect in bringing down the blood sugar levels in resistant cases of post prandial hyperglycemia but also acts as a good anti-oxidant agent, scavenging the generated free radicals to an appreciable level, at the dose way below the ant diabetic dose and preventing the long term complications of diabetes mellitus.

CONFLICT OF INTREST

Conflict of interest declared none.

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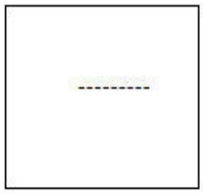

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