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IN VITRO EVALUATION OF ANTIOXIDANT ACTIVITY OF TENELIGLIPTIN BY FRAP AND NITRIC OXIDE ASSAY

Pharmacology

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ABSTRACT

The aim of the study is to evaluate the antioxidant activity of the newer anti-diabetic drug by using FRAP and nitric oxide assay. Methodology: FRAP assay was done by using Benzie and Strain method (1999). The antioxidant activity is measured by the reduction of ferric complex (Fe3+ tripyridyltriazine) which is colourless to blue-colored ferrous complex ($Fe2_+$ tripyridyltriazine) at 593nm.This action is due to the electron donating antioxidants at low pH. FRAP values are calculated by comparing the absorbance change at 593 nm in test reaction mixtures with that of the standard (ascorbic acid).Nitric oxide assay was done by using Kumar S et al. method (2008). In this method Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitrite oxide which interacts with oxygen to produce Nitrite ions, which can be measured at 550nm by spectrophotometer in the presence of Griess reagent and was compared with that of the standard (ascorbic acid).Since Teneligliptin which is the novel DPP4 inhibitor plays a vital role in the management of type 2 diabetes mellitus we have evaluated the antioxidant potential of this drug by using FRAP and nitric oxide assay.

KEYWORDS

Teneligliptin, FRAP, Anti Oxidant Assay,nitric Oxide, Dpp4 Inhibitor, Ascorbic Acid

INTRODUCTION

Free radicals are generated in our body by means of daily metabolic activities.This leads to cellular damage.The anti oxidants helps to prevent the cellular damage by means of defence mechanism against free radicals.Diabetes mellitus is one of the major metabolic disorder in which there is overpooling of free radicals causing oxidative stress due to disruption of anti oxidant mechanism. This in turn may lead to microvascular and macrovascular complications[1].

Tenligliptin is the newer Dipeptidyl peptidase 4 (DPP4) inhibitor which is given as oral anti-hyperglycemic agent [2]. It is metabolized by cyp3A4 pathway and is eliminated by both renal and hepatic excretion[3].

It was found that Teneligliptin 20 mg once daily helps to improve the vascular endothelial function at 2 weeks of treatment [4]. Dose adjustments are not needed in renal impairment patients.There is also reduction in HbA1c of 0.8%-0.9% within 12 weeks of therapy [5].The dose of Teneligliptin can be increased to 40 mg once daily.Among the DPP4 inhibitors Teneligliptin has least side effects.Since there is strong connection between the oxidative stress and diabetes mellitus we need to prove the anti oxidant activity of this drug by using FRAP and Nitric oxide assay.

MATERIALS ANN METHODS:

FRAP ASSAY:

PRINCIPLE:

FRAP assay was done by using Benzie and Strain method (1999) [6]. The antioxidant activity is measured by the reduction of ferric complex ($Fe3_+$ tripyridyltriazine) which is colourless to blue-colored ferrous complex ($Fe2_+$ tripyridyltriazine) at 593nm.This action is due to the electron donating antioxidants at low pH. FRAP values are calculated by comparing the absorbance change at 593 nm in test reaction mixtures with that of the standard (ascorbic acid).

REAGENTS USED:

Reagent A: Acetate Buffer (300 mM, pH 3.6):

16 ml of glacial acetic acid was added to 3.1g of sodium acetate trihydrate. The solution was then made up to 1 litre using distilled water. The pH of the solution was checked using pH meter.

Reagent B: TPTZ solution:

0.031g of TPTZ was added to 10 ml of 40 mM HCl and dissolved at 50◦C.

Reagent C: Ferric chloride solution:

0.054g of ferric chloride was dissolved in 10 ml of distilled water

The working FRAP reagent was prepared by mixing A, B & C in the ratio of. 10:1:1 at the time of use.

PROCEDURE:

- 100 µl of sample was added to the tube marked test and 3 ml of FRAP reagent was added to it.
- 3 ml of FRAP reagent was taken as a blank.
- Absorbance is measured at 0 minutes after vortexing at 593 nm.
- Samples are then placed at 37ºC in water bath and absorption is again measured after 4 minutes.
- Ascorbic acid was used as the standard.

S.No.	Contents	FRAP ASSAY	
		Blank	Test
1.	Sample	-	100µl
2.	Working FRAP Solution	3ml	3ml

CALCULATION:

FRAP value of sample (µM) = (Change in absorbance of sample from 0 to 4 minute / change in absorbance of standard from 0 to 4 minutes) x FRAP value of standard (1000 µM)

Note: FRAP value of ascorbic acid is 2.

NITRIC OXIDE ASSAY:

PRINCIPLE:

Nitric oxide assay was done by using Kumar S et al. method (2008) [7]. In this method Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates Nitrite oxide which interacts with oxygen to produce Nitrite ions, which can be measured at 550nm by spectro hotometer in the presence of Griess reagent and was compared with that of the standard (ascorbic acid).

REAGENTS USED:

- Sodium nitroprusside
- Griess reagent

PROCEDURE:

100 mg/ml of sample was incubated with Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) at 29ºC for 3 hours. The same procedure was followed for control without the test compounds in the same amount of buffer.The samples were diluted with 1 ml of Griess reagent after 3 hours of incubation period. The absorbance at which the colour changed was noted down at 550nm on

spectrophotometer. This was due to the diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthyl ethylene diamine hydrochloride. The similar procedure was carried out with ascorbic acid which was used as the standard.

CALCULATIONS

$$\% \text{ inhibition} = \frac{\text{O.D.of control - O.D. of Test}}{\text{O.D. of control}} \times 100$$

RESULTS AND DISCUSSION:

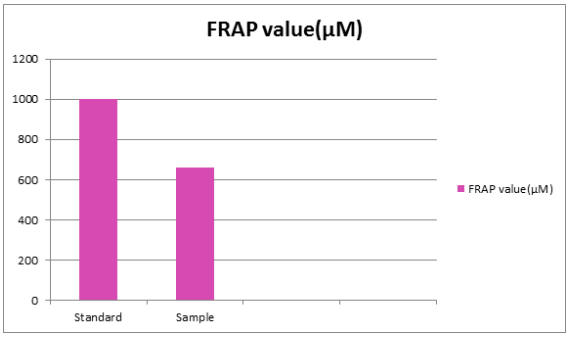
FRAP ASSAY:

TABLE 1:The Antioxidant activity of given samples using FRAP Assay method.

S.No	Name of the Solution	FRAP(µM)
1	Sample	660

O.D Value for Sample at 0 to 4 min = 0.132
O.D Value for Ascorbic acid at 0 to 4 min = 0.2

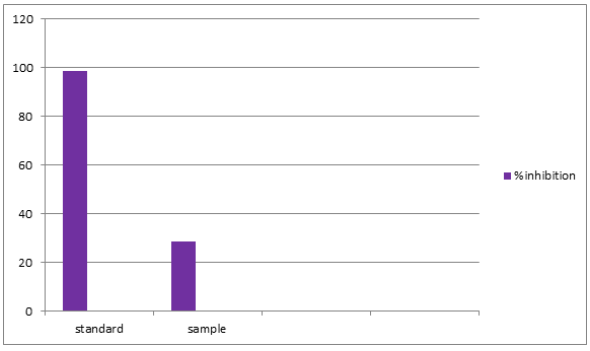
FIGURE 1:



NITRIC OXIDE ASSAY:

TABLE 2:

S.NO	NAME OF THE SOLUTION	O.D	INHIBITION %
1	Control	0.836	
2	Sample	0.598	28.46
3	Standard(ascorbic acid)	0.013	98.5



The antioxidant activity of Teneligliptin by FRAP assay was found to be 660 µM and the percentage inhibition of free radicals by nitric oxide assay was 28.46%. Although in previous studies, the antioxidant property of DPP4 inhibitors like sitagliptin,vildagliptin was evaluated by DPPH method, we have assessed the antioxidant activity of Teneligliptin by using simple methods such as FRAP and NO assay. These methods are also cost effective than DPPH method.

CONCLUSIONS:

From the above study it can be concluded that there is significant antioxidant activity shown by Teneligliptin evaluated using FRAP and NO assay.Since Teneligliptin is an emerging drug in the treatment of type 2 diabetes mellitus, it helps in preventing microvascular as well as macrovascular complications owing to its antioxidant property.

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