



## EFFICACY OF DAPAGLIFLOZIN ON OXIDATIVE STRESS – IN VITRO STUDY

## Pharmacology

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## ABSTRACT

**BACKGROUND:** Diabetes Mellitus is characterized by hyperglycemia resulting either from deficiency in insulin secretion, insulin action or both. There is increasing evidence that excess production of highly reactive free radicals resulting in microvascular and macrovascular complications in diabetes. In Type 2 Diabetes Mellitus, antioxidants play a vital role to avoid this complications. In recent past, Dapagliflozin, a novel agent, an inhibitor of renal sodium glucose co- transporter 2 is being used to attain glycemic control. To know its pleotropic effect, especially as an antioxidant to prevent long term complications of diabetes mellitus, the antioxidant property of Dapagliflozin is evaluated by DPPH assay and scavenging of Nitric oxide Radicals. In this study it is compared against standard (ascorbic acid) and found to have significant Antioxidant property.

## KEYWORDS

Diabetes Mellitus, DPPH Assay, Scavenging of Nitric Oxide Free Radicals, Antioxidant role of Dapagliflozin.

## INTRODUCTION

Diabetes Mellitus is a Metabolic disorder characterized by hyperglycemia and the late development of vascular and neuropathic complications. Increased glucose production causes oxidative stress due to increase in the production of mitochondrial reactive oxygen species [ROS], non enzymatic glycation of proteins, and glucose auto oxidation[1]. Elevated free radical production is highly related to the changes in the pathogenesis of diabetes and its complications [2]. . Damage of lipids, proteins, and DNA are also due to the elevated levels of ROS.

In uncontrolled Diabetes, superoxide radical can be inactivated by the enzyme superoxide dismutase. An Antioxidant is a stable molecule which neutralize the damaging free radicals. Antioxidant scavenges the reactive species that initiate the peroxidation, breaking of free radical reactions, chelating metals and there by reducing the o concentration[3,4]. Due to elevated glucose metabolism Reactive oxygen species generation occurs through the sorbitol pathway leading to vascular dysfunction[5]. Therefore antidiabetic with antioxidant activity to be preferred for type 2 Diabetes Mellitus patients. Many drugs have showed an antioxidant action along with their pharmacological activity. Few examples of Antidiabetic drugs are empagliflozin, metformin, canagliflozin, pioglitazone, etc. In this study antioxidant potential of Dapagliflozin, a sodium glucose co- transporter 2 (SGLT2) inhibitor is compared with standard (ascorbic acid)[6].

## DPPH ASSAY:

DPPH (1,1 diphenyl -2- picrylhydrazyl) is characterized as a stable free radical by virtue of the delocalization of the spare electron over the molecule as a whole, so that the molecule do not dimerise, as would be the case with most other free radicals. The delocalization also gives rise to the deep violet colour, characterized by an absorption band in ethanol solution centered at about 520nm. when a solution of DPPH is mixed with that of a substance that donate a hydrogen atom, then this give rise to the reduced form (Blois, 1958) with the loss of this violet colour (although there would be expected to be a residual pale yellow colour from the picryl group still present).

Representing the DPPH radical by Z. and the donor molecule by AH, the reaction is  $Z + AH = ZH + A$ .

Where ZH is the reduced form and A. is free radical produced in this first step. This latter radical will then undergo further reactions which control the overall stoichiometry, that is, the number of molecules of DPPH reduced (decolorized) by one molecule of the reductant.

## CHEMICALS:

- 1,1 - diphenyl -2- picrylhydrazyl (DPPH)
- Dimethylsulphoxide (DMSO)
- BHT (standard) - 1.6 mg/ml in methanol

4. Samples desired concentration from 1mg/ml – max of 5mg/ml (in / DMSO)

## PROCEDURE:

Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8 ml of absolute methanol was added to blank.

Add 100µl of BHT to tube marked as standard and 100µl of respective samples to all other tubes marked as tests. 200µl of DPPH reagent was added to all the test tubes including blank. Incubate all test tubes at room temperature in dark condition for 30 minutes. The absorbance of all samples was read at 517nm.

S.NO	REAGENTS	BLANK	STANDARD	TEST
1	Methanol	3.8ml	3.7ml	3.7ml
2	BHT	-	100µl	-
3	Sample	-	-	100µl
4	DPPH	200µl	200µl	200µl
Incubation at dark for 30 minutes				
O.D at 517 nm				

## CALCULATION :

$$\% \text{ Antioxidant activity} = \frac{(\text{Absorbance at blank}) - (\text{Absorbance at test})}{(\text{Absorbance at blank})} \times 100$$

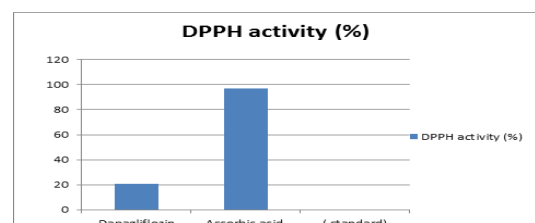
## RESULT:

**FIGURE 1:** The Antioxidant activity of given samples using DPPH assay method:

S.N	sample	Concentration (µg/ml)	O.D	DPPH activity (%)
1	Dapagliflozin	1000	0.768	21
2	Ascorbic acid (standard)	1000	0.971	97.07

Control OD: 0.971

**FIGURE 2:** bar Chart Comparing The Antioxidant Effect Of Dapagliflozin And Ascorbic Acid



## SCAVENGING OF NITRIC OXIDE RADICALS

### Principle

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 (Kumar S *et al.*, 2008).

### Reagents and chemicals

5mM Sodium Nitroprusside, Griess reagent (1 part of 1% sulphonil amide and 1 part of 0.1% N 1-naphthylethylenediamine in 2% orthophosphoric acid), Phosphate buffer (pH- 7.4).

### Procedure

Sample was dissolved in distilled water for this quantification. Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with different concentration (100-400µg/ml) of methanol extract and tubes were incubated at 29°C for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Naphthyl ethylene diamine hydrochloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. Calculated the % inhibition by formula and plot graph in compared to standard.

### CALCULATION:

$$\% \text{ Inhibition} = \frac{\text{OD of control} - \text{OD of test}}{\text{OD of control}} \times 100$$

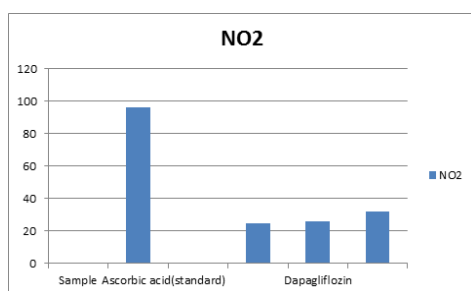
### RESULTS:

#### FIGURE 3: The Antioxidant activity of sample using NO<sub>2</sub> Assay method

S.NO	Sample	Concentration µg/ml	O.D	NO <sub>2</sub> Inhibiton %
1	Ascorbic acid (standard)	100	0.303	96.26
2	Dapagliflozin	200	0.228	24.75
		400	0.224	26.07
		600	0.206	32.01

Control: 0.303

#### Figure 4: Bar Chart Comparing The Antioxidant Effect Of Dapagliflozin With Ascorbic Acid



### DISCUSSION:

Dapagliflozin on DPPH assay at 1000 µg shows antioxidant property of 21% and in Scavenging of nitric oxide on increasing concentration 200µg, 400µg, 600µg shows antioxidant property of 24.75%, 26.07%, 32.01% respectively.

Sodium glucose transporter 2 inhibitor present in the renal tubules reduces the reabsorption of glucose by excreting the glucose in urine[ 7].

The caloric loss is caused by glycosuria, as a result weight loss is seen in clinical trials[8]. Dapagliflozin, SGLT2 inhibitors shows potent antioxidant property along with its antidiabetic activity. To improve endothelial dysfunction in Type 2DM, an antidiabetic drug with antioxidant property increase nitric oxide (NO) production re-coupling mitochondrial function and NOS[9,10]. Therefore free

radical formation inhibition helps to prevent oxidative stress and diabetic vascular complications.

### CONCLUSION:

In this study, Dapagliflozin SGLT2 Inhibitor possess antioxidant property when compared with standard (ascorbic acid). This additional antioxidant activity of Dapagliflozin makes it as a novel therapy for the management of Type 2 Diabetes Mellitus.

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### CONFLICTS OF INTEREST:

Conflict of interest declared none.

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