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**Research Article**

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# Evaluation of Antioxidant Property of Empagliflozin-Dpph Assay and Nitric Oxide Scavenging Activity (*In Vitro* Method)

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**ABSTRACT**

*Background: Diabetes Mellitus is a group of metabolic disease with increasing prevalence recently. Although obesity and physical inactivity accounts for the major risk for type II DM, increased production of free radicals leading to oxidative stress may also contribute to the pathogenesis of type II DM. Hence it is important to discover a hypoglycemic drug that reduces oxidative stress in diabetic patients. Aim and Objective: This study, was performed to evaluate the antioxidant efficacy of Empagliflozin with Ascorbic acid by DPPH assay and nitric oxide scavenging activity, an in vitro method. Results: This study show that Empagliflozin on comparison with Ascorbic acid has antioxidant property and can be effectively used for reducing oxidative stress in patients with Diabetes.*

**Keywords:** Diabetes mellitus; Nitric oxide scavenging activity; DPPH assay; Empagliflozin; SGLT2 inhibitors

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## INTRODUCTION

Oxidative stress occurs due to an imbalance between free radical generation and radical scavenging activity which may lead to an increased radical production or decreased activity of antioxidant defenses or both [1,2]. Free radicals are molecules or ions with unpaired electrons, which readily react with other molecule to produce chemical reactions. The excessive free radical produced damage the bases in nucleic acids, amino acid side chains in proteins and double bonds in unsaturated fatty acids, and leads to oxidative stress, which causes damage to the DNA, RNA, proteins and lipids resulting in an increased risk for cancer, cardiovascular disease and other disorders [3]. To prevent the consequences of oxidative stress, the body has a defense system of antioxidants. Antioxidants are molecules which neutralize the free radical by eliminating its unpaired condition by either accepting or donating electron(s). The antioxidants exerts its effects through various ways: free radical oxidation reaction inhibitors, by inhibiting the formation of free lipid radicals, by disrupting the propagation of the auto oxidation chain reaction, as singlet oxygen quenchers, as metal chelators that convert metal pro-oxidants into stable products and pro-oxidative enzymes inhibitor. Diabetes is one of the major risk factor for cardiovascular disease with its micro and macro vascular complications resulting in Myocardial infarction, cerebrovascular disease, coronary artery disease leading to increased number of death in diabetes population [4]. When endothelial cells are exposed to hyperglycemia there is increased production of free radicals. Also there is increased metabolism of glucose through polyol (sorbitol) pathway which partly contributes to the generation of reactive oxygen species leading to micro and macrovascular dysfunction.

### Empagliflozin

Sodium-glucose co-transporter-2 (SGLT2) inhibitors-a new group of oral medications used for treating type II DM. SGLT2 inhibitors reduce the renal reabsorption of urinary glucose by inhibiting the SGLUT2 present in the

proximal renal tubules thereby excreting the glucose in urine [5]. The reduction in plasma glucose is independent of insulin secretion and insulin peripheral resistance [6].

## EXPERIMENTAL SECTION

### Nitric Oxide Scavenging Activity

1. Test sample: crude drug of Empagliflozin (25 mg)
2. Reference antioxidant: Ascorbic acid
3. Solvent: sodium nitroprusside in phosphate buffer
4. Reagent: Griess reagent
5. Spectrophotometer

### Procedure

The crude drug extract was dissolved in distilled water for this quantification. Sodium Nitroprusside (5 mM) in standard phosphate buffer saline (0.025 M, pH 7.4) was incubated with 100 mg/ml of sample 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 [7-10]. After 3 hours incubated samples were diluted with 1 ml of Griess reagent. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Napthyl ethylene diamine hydrochloride was observed at 550 nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to sample.

### Calculations

$$\% \text{ inhibition} = \frac{O.D.of\ control - O.D.of\ test}{O.D.of\ control}$$

**Table 1: Comparison of percentage of inhibition of empagliflozin with ascorbic acid (standard)**

| S.no | Name of the sample | Inhibition % |
|---|---|---|
| 2 | Empagliflozin (25mg) | 79.43 |
| 3 | Standard (Ascorbic Acid) | 98.5 |

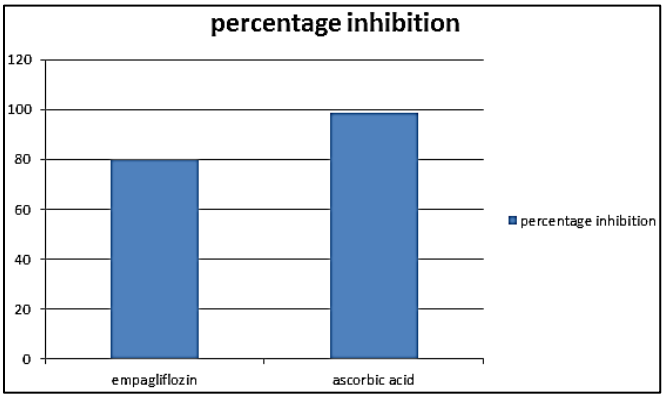


**Figure 1: Bar chart comparing percentage of inhibition of empagliflozin with ascorbic acid**

### DPPH Assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole, so that the molecules do not dimerise, as would be the case with most other free radicals. The delocalisation also gives rise to the deep violet colour, characterised by an absorption band in ethanol solution centered at about 520 nm. When a solution of DPPH is mixed with that of a substance that can donate a hydrogen atom, then this gives rise to the reduced form (Blois) with the loss of this violet colour [11-13].
Representing the DPPH radical by Z• and the donor molecule by AH, the primary reaction is:

$$Z\bullet + AH = ZH + A\bullet$$

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 (decolorised) by one molecule of the reductant.

**Chemicals**

1. 1,1 – diphenyl -2- picrylhydrazyl (DPPH)
2. Dimethylsilphoxide (DMSO)
3. BHT (standard)-1.6 mg/ml in methanol
4. Samples desired concentration from 1 mg /ml –max of 5 mg/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 100 µl of BHT to tube marked as standard and 100 µl of respective samples to all other tubes marked as tests. 200 µl 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 517 nm.

**Table 2: Incubation test results in all test tubes**

| S.no | Reagents | Blank | Standard | Test |
|---|---|---|---|---|
| 1 | Methanol | 3.8 ml | 3.7 ml | 3.7 ml |
| 2 | BHT | - | 100 µl | - |
| 3 | Sample | - | - | 100 µl |
| 4 | DPPH | 200 µl | 200 µl | 200 µl |
| Incubation at dark for 30 minutes | | | | |
| O.D at 517 nm | | | | |

**Calculation**

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

**Table 3: The antioxidant activity of given samples using DPPH assay method**

| S.no | Sample | Concentration (µg/ml) | O.D | DPPH activity (%) |
|---|---|---|---|---|
| 1 | Ascorbic Acid (Standard) | 1000 | 0.11 | 86.56 |
| 2 | Empagliflozin (25 mg) | 1000 | 0.228 | 72.16 |

Blank O.D: 0.819

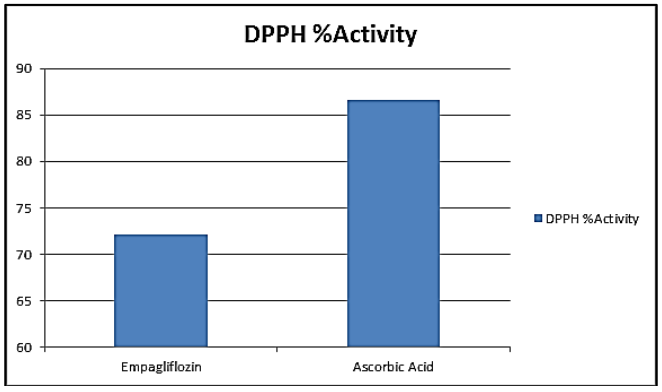


**Figure 2: Bar chart comparing DPPH activity percentage of empagliflozin with ascorbic acid**

## DISCUSSION

The nitrogen oxide scavenging activity was recorded in terms of percentage Inhibition. It was observed that Empagliflozin has shown 79.43% inhibition in nitrogen oxide scavenging activity (Table 1). The Results obtained were comparative to Ascorbic acid as standard. The antioxidant efficacy of empagliflozin is demonstrated in (Figure 1). DPPH Assay shows the efficacy of Empagliflozin to reduce the oxidative stress (Tables 2 and 3). Empagliflozin has 72.16% of DPPH % Activity in comparison with Ascorbic acid (86.56%) (Figure 2). SGLT2 Inhibitors are the new group of drug in the management of diabetes. It acts by increasing the renal glucose threshold by inhibiting the

SGLT2 present in proximal renal tubules and causes increased excretion of glucose in urine. Apart from its antidiabetic, antimicrobial, anticalorigenic activity, SGLT2 inhibitors shows potent antioxidant property.

## CONCLUSION

This study shows that Emapagliflozin is highly efficacious in reducing oxidative stress. Thus the inhibitors of sodium-glucose co transporters type 2 (SGLT2) also possess an additional antioxidant activity and can be used as an novel therapy for the management of type 2 diabetes mellitus.

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