Int J Pharma Bio Sci 2017 October; 8(4): (P) 232-236

Original Research Article **Pharmacology**

International Journal of Pharma and Bio Sciences

ISSN 0975-6299

COMPARATIVE EFFICACY OF CANAGLIFLOZIN VERSUS EMPAGLIFLOZIN ON OXIDATIVE STRESS – IN-VITRO METHOD

DR. M.SINDHUJA[1], DR N.S MUTHIAH[2], DR. R. RAJESWARI[3]

[1,2]Department of Pharmacology, Sree Balaji Medical College & Hospital, Bharath University, Chrompet, Chennai, Tamil Nadu, India.
[3]Department of Biochemistry, Thanjavur Medical College, Thanjavur, India.

ABSTRACT

Diabetes is characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Hyperglycemia causes auto-oxidation of glucose to form free radical $^{\cdot}O_2^-$. The production of free radicals beyond the limit of scavenging activity of endogenous antioxidant defenses result in micro vascular and macro vascular complications. In this study the antioxidant efficacy of Canagliflozin and Empagliflozin was compared by 1, 1-Diphenyl-2-Picrylhydrazyl radical scavenging activity (DPPH) assay and nitric oxide scavenging activity. Sodium-glucose co-transporter-2 inhibitors shows pleotrophic therapeutic potential in the treatment of type II Diabetes. On comparison with ascorbic acid both Canagliflozin and Empagliflozin shows antioxidant property. However empagliflozin has higher efficacy for reducing oxidative stress when compared with canagliflozin.

KEYWORDS: ***Diabetes Mellitus, DPPH Assay, Nitric oxide scavenging activity, Empagliflozin, canagliflozin***

DR. M.SINDHUJA*

Department of Pharmacology, Sree Balaji Medical College & Hospital, Bharath University, Chrompet, Chennai, Tamil Nadu, India.

Received on: 10-03-2017
Revised and Accepted on: 11-10-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.p232-236

INTRODUCTION

Diabetes Mellitus is a metabolic disorder with increasing prevalence recently. Although obesity and physical inactivity accounts for the major risk, increased production of free radicals leading to oxidative stress may also contribute to the pathogenesis of diabetes and its complications[1]. Oxidative stress is defines as excess formation and/or insufficient removal of highly reactive molecules such as reactive oxygen species (ROS) and reactive nitrogen species (RNS)[1-2]. Oxidative stress causes damage to DNA, proteins, and other macromolecules which may lead to cellular death. To prevent oxidative stress, the body has a defense system of antioxidants. Antioxidants are chemical or biological agents that neutralize the potentially damaging free radicals. It scavenges the species that initiate peroxidation, quenching singlet oxygen, chelating metals, breaking free radical chain reactions, and reducing the concentration of $\cdot O_2^-$.[3-4] 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 vascular dysfunction[5]. Hence anti-diabetic drug along with antioxidant activity is more effective in the treatment of diabetes. Recent studies showed that many drugs have antioxidant action which may contribute to their pharmacological activity. Some examples are Pioglitazone, Metformin, Glibenclamide, Repaglinide, the drugs which are used in the treatment of Diabetes [6-7]. Sodium-glucose co-transporter-2 (SGLT2) inhibitors – Canagliflozin, Empagliflozin are a new group of oral medications used for treating type II DM[8]. In this study antioxidant potential of these drugs were compared.

METHODS AND MATERIALS

Canagliflozin (100mg) and Empagliflozin (25mg) were purchased from Sigma Aldrich; Newdelhi. L-Ascorbic acid, sodium nitroprusside (solvent), Griess reagent, 1,1 – diphenyl -2- picrylhydrazyl (DPPH), Dimethylsilphoxide (DMSO) were of analytical grade and purchased from Sd fine Chemicals; Mumbai.

Nitric oxide scavenging activity

Nitric oxide scavenging activity was estimated based on Griess Ilosvog reaction (Garrat, 1964) [9]. The drug was dissolved in distilled water for this quantification. Sodium Nitroprusside (5mM) in standard phosphate buffer saline (0.025M, pH 7.4) was incubated with 100 mg/ml of sample and tubes were incubated at 29ºC 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 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 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to sample.

Calculations

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

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 with the loss of this violet colour. 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. DPPH assay was done according to the procedure of Blois (1958)[10]. Aliquot 3.7 ml of absolute methanol in all test tubes and 3.8ml of absolute methanol was added to blank. 100µl of BHT was added to tube marked as standard. 100µl of respective samples were added 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.

Calculation

$$\% \text{ Antioxidant activity} = \frac{\text{(Absorbance at blank) - (Absorbance at test) x100}}{\text{(Absorbance at blank)}}$$

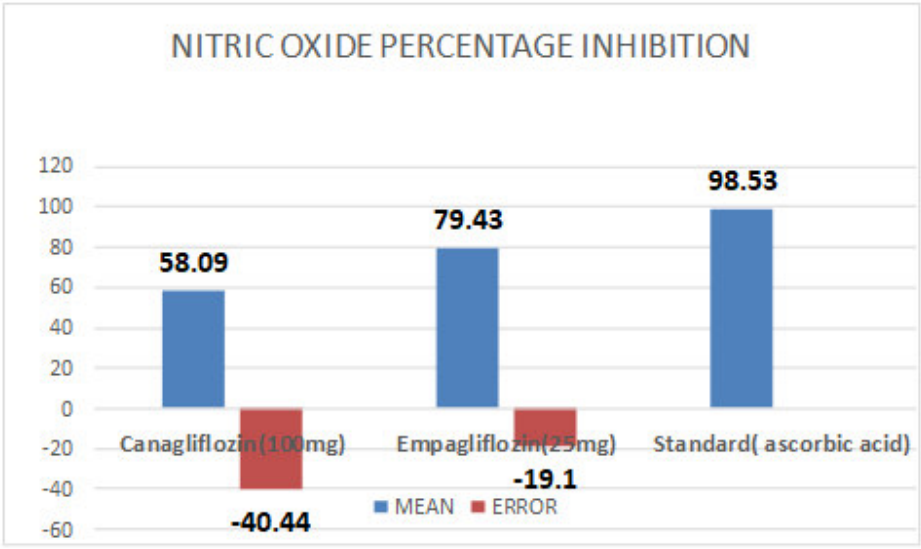
RESULTS

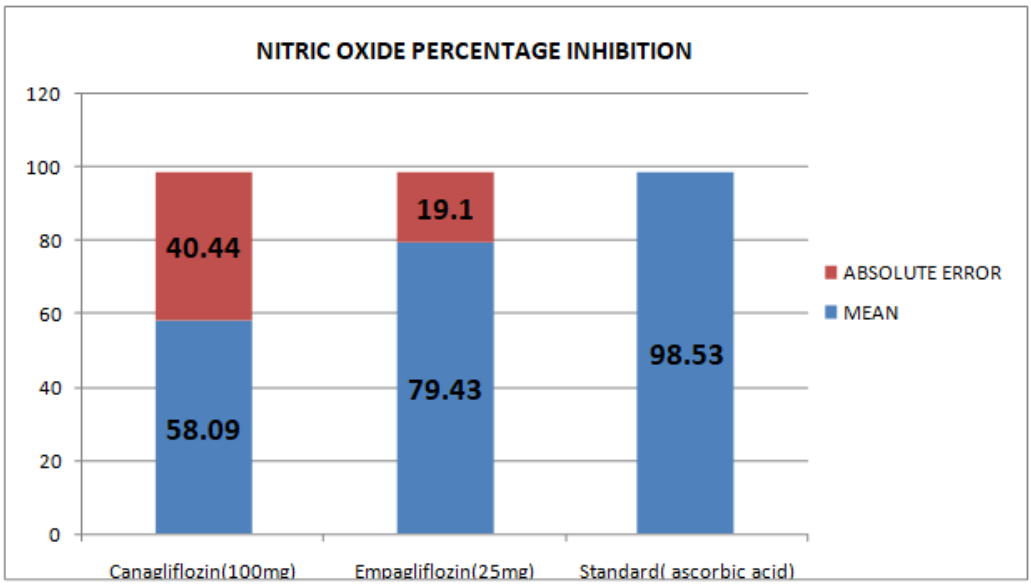
Table 1
Comparison of Percentage inhibition of Canagliflozin and Empagliflozin with Ascorbic acid (standard) by Nitric oxide scavenging activity

S.NO.	Sample	Inhibition %
1.	Canagliflozin (100mg)	58.09
2.	Empagliflozin (25mg)	79.43
3.	Standard	98.5

The nitrogen oxide scavenging activity was recorded in terms of percentage inhibition. It was observed that both canagliflozin (58.09%) and empagliflozin (79.43%) has shown nitrogen oxide scavenging activity (Table 1). The Results obtained were comparative to Ascorbic acid as standard.

Figure 1 & 2
Percentage Inhibition of Canagliflozin and Empagliflozin with Ascorbic Acid



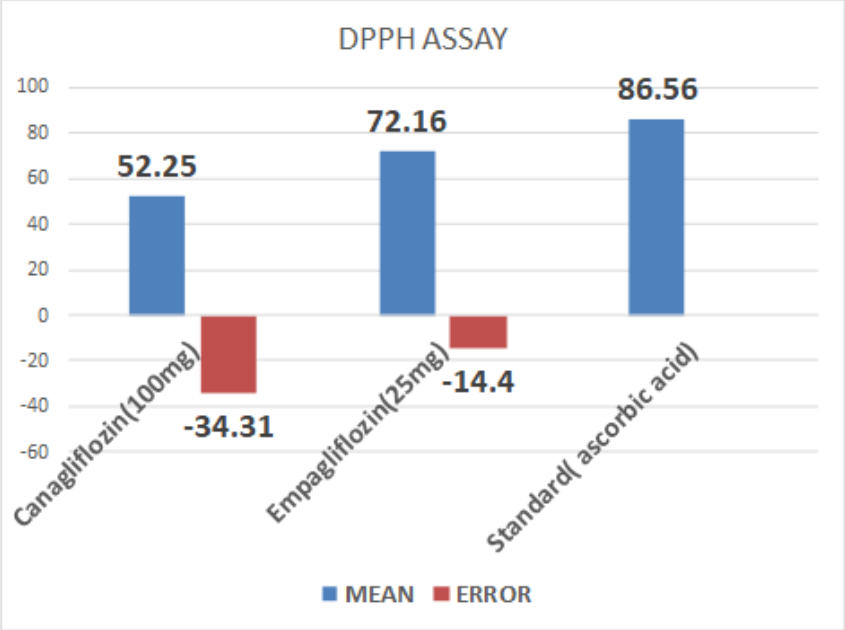


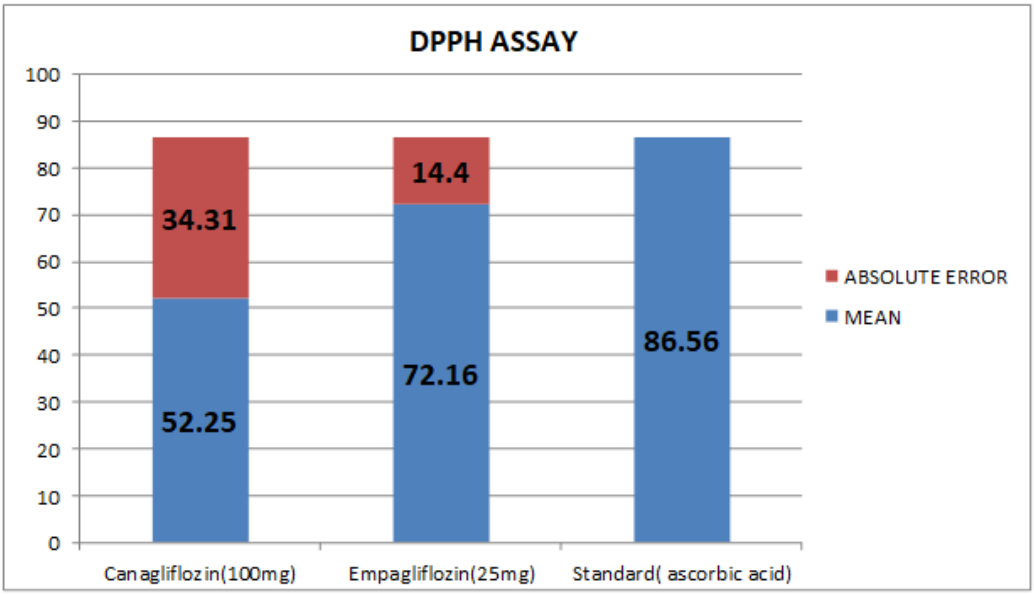
Higher Percentage Inhibition indicates better scavenging activity or antioxidant potential. Empagliflozin shows higher efficacy in inhibiting oxidative stress compared with Canagliflozin (Figure.1 and 2).

Table 2
Antioxidant activity of given samples using DPPH Assay method.

S.NO	Sample	DPPH activity (%)
1	Canagliflozin (100mg)	52.25
2	Empagliflozin (25mg)	72.16
3	Ascorbic Acid (standard)	86.56

Figure 3 & 4
Antioxidant activity of Canagliflozin and Empagliflozin by DPPH Assay.





DPPH Assay shows both Canagliflozin and Empagliflozin have antioxidant property (Table.2). Empagliflozin(72.16%) has higher DPPH activity percentage than Canagliflozin (52.25%) (Figure.3 & 4).

DISCUSSION

SGLT2 inhibitors reduce the renal reabsorption of urinary glucose by inhibiting the SGLUT2 present in proximal renal tubules thereby excreting the glucose in urine. The reduction in plasma glucose is independent of insulin secretion and insulin peripheral resistance.[11] In addition the glycosuria causes a caloric loss, which has been associated with an average weight loss of 2–3 kg over 6–12 months in clinical trials.[12] Apart from its antidiabetic, antimicrobial, anticalorigenic activity, SGLT2 inhibitors shows potent antioxidant property. Supplementation with an antidiabetic drug having antioxidant property and/or factors essential to nitric oxide (NO) production may potentially improve endothelial dysfunction in T2DM by re-coupling eNOS

and mitochondrial function, as well as decreasing vascular NAD(P)H oxidase activity[11-12]. Therefore inhibition of intracellular free radical formation provides a therapeutic strategy to prevent oxidative stress and the related diabetic vascular complications.

CONCLUSION

In this study both the SGLT inhibitors Canagliflozin and Empagliflozin possess antioxidant activity. Empagliflozin shows higher efficacy in reducing oxidative stress compared with Canagliflozin. Thus the inhibitors of sodium-glucose co transporters type 2 (SGLT2) possess additional antioxidant activity and acts as novel therapy for the management of type 2 diabetes mellitus.

ACKNOWLEDGEMENTS

My immense thanks to Sree Balaji Medical College for providing me with laboratory assistance in conducting my experiment and I would also like to express my gratitude to all my professors, Department of Pharmacology for their guidance and encouragement in executing my project.

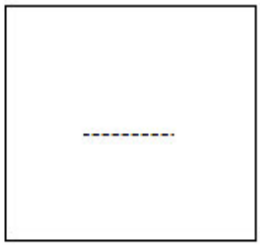
CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Turko IV, Marcondes S, Murad F. Diabetes-associated nitration of tyrosine and inactivation of succinyl-CoA:3-oxoacid CoA-transferase. Am J Physiol Heart Circ Physiol. 2001 Dec 1; 281(6): H2289-94.
2. Evans JL, Goldfine ID, Maddux BA, Grodsky GM: Oxidative stress and stress-activated signaling pathways: a unifying hypothesis of type 2 diabetes. Endocr Rev. 2002 Oct 1; 23(5): 599-622.
3. Giles GI, Jacob C. Reactive sulfur species: an emerging concept in oxidative stress. Biol. Chem. 2002 -Apr 12; 383(3-4):375–88.
4. Darmanyan AP, Gregory DD, Guo Y, Jenks WS, Burel L, Eloy D, Jardon P. Quenching of singlet oxygen by oxygen-and sulfur-centered radicals: evidence for energy transfer to peroxyl radicals in solution. J.Am.Chem.Soc. 1998 Jan 21; 120 (2):396-403.
5. Evans JL, Goldfine ID, Maddux BA, Grodsky GM. Are oxidative stress− activated signaling pathways mediators of insulin resistance and β-cell dysfunction? Diabetes. 2003 Jan 1; 52(1):1-8.
6. Chandrasekar.T, Muthiah N.S, Sandiya R, Sanitha and Aparna. Evaluation of antioxidant activity of pioglitazone: Nitric oxide scavenging activity (In-vitro method). J. chem. 2015; 7(8):833-6.
7. Vinoth kumar P, Ramesh N, Amala Bricey A and Veera Thamarai Selvi V. Evaluation of Lipid Peroxidation and Antioxidants activity of Metformin in high fructose fed diet induced type II Diabetic rat. Int J Pharm Technol. 2010 June; 2(3): 456-64
8. Scheen AJ. Pharmacokinetic and pharmacodynamic profile of empagliflozin, a sodium glucose co-transporter 2 inhibitor. Clin Pharmacokinet. 2014 Mar 1;53(3):213-25.
9. Garrat DC. The Quantitative Analysis of Drugs. 3rd ed. Japan: Chapman and Hall Ltd; 1964. p. 456-8.
10. Blois MS. Antioxidant determinations by the use of a stable free radical. Nature. 1958 Apr 26; 181(4617):1199-200.
11. Nauck MA. Update on developments with SGLT2 inhibitors in the management of type 2 diabetes. Drug Des Devel Ther. 2014 Jan 1; 8:1335-80.
12. Rosenstock J, Jelaska A, Wang F, Kim G, Broedl U, Woerle HJ, Bajaj HS. Empagliflozin as add on to basal insulin for 78 weeks improves glycemic control with weight loss in insulin-treated type 2 diabetes (T2DM). Canadian journal of diabetes. 2013 Oct 1; 37:S32.

Reviewers of this article



Akila .L, MD

Associate Prof,Pharmacology,Sree Balaji Medical College,Chrompet ,Chennai,India

Prof. Y. Prapurna Chandra Rao

Assistant Proffessor, KLE University, Belgaum, Karnataka, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript