University Journal of Medicine and Medical Specialities

ISSN 2455- 2852 2021, Vol. 7(5)

An in vitro study to compare the anti-oxidant properties of cardiovascular and anti-diabetic drugs

Yamuna Devi M S and Bhuvaneswari K

Department of Pharmacology, P S G Institute of Medical Sciences and Research, Coimbatore

ABSTRACT

Introduction: Oxidative stress plays an important role in various pathological conditions like Diabetes mellitus, Hypertension, Malignancies, Alzheimers disease, etc. Scientific evidence suggests that antioxidants reduce the risk of these chronic diseases.[2] There are numerous cardiovascular and anti-diabetic drugs available which have additional antioxidant properties. The aim of this study is to comparatively evaluate the antioxidant properties of cardiovascular drugs like Nebivolol, Carvedilol, Cilnidipine, Atorvastatin and that of anti-diabetic drugs like Sitagliptin, Vildagliptin and Tenegliptin by their free radical scavenging property using DPPH (2,2-diphenyl 1-picryl hydrazyl hydrate) assay. **Methodology:** Oral formulations of Nebivolol, Carvedilol, Cilnidipine, Atorvastatin, Sitagliptin, Vildagliptin, Teneligliptin were crushed separately into fine powders and stock solutions of 1000 and 500 µg/ml were prepared using ethanol. Ascorbic acid was used as the reference antioxidant. The percentage of antioxidant activity was assessed by DPPH assay method described by Brand Williams et al. **Results:** At concentrations of 1000 µg/ml and 500 µg/ml, the percentage of DPPH inhibition by the drugs were: Nebivolol - 46.38% and 35.28%, Carvedilol - 59.58% and 35.74%, Cilnidipine - 64.37% and 54.67%, Atorvastatin - 69.39% and 47.55%, Teneligliptin - 68.81% and 53.74%, Vildagliptin - 63.90% and 50.58% and Sitagliptin - 53.86% and 36.21% respectively. The percentage of DPPH inhibition for the same concentration of Ascorbic acid was 98.83% and 70.44% respectively**.** **Conclusion:** Our study has demonstrated the in vitro anti-oxidant activity of the commonly used cardiovascular drugs (Nebivolol, Carvedilol, Cilnidipine and Atorvastatin) and antidiabetic drugs (Teneligliptin, Vildagliptin and Sitagliptin) which could have a therapeutic advantage of preventing oxidative stress induced complications in patients who are on long term treatment for cardiovascular diseases and diabetes mellitus.

INTRODUCTION

Oxidative stress refers to the increased production of reactive oxygen species (ROS) in the cells and tissues and the antioxidant system will not be able to neutralize them. Imbalance in this protective mechanism can lead to the damage of cellular components such as DNA, proteins, and lipids. Reactive oxygen species are normally produced within the body in limited quantity and are important in the maintenance of cell homeostasis and functions such as signal transduction, gene expression, and activation of receptors.[1]

Oxidative stress plays an important role in various pathological conditions like Diabetes mellitus, Hypertension, Malignancies, Alzheimers disease, etc. Scientific evidence suggests that antioxidants reduce the risk of these chronic diseases.[2]. There are numerous cardiovascular and anti-diabetic drugs available which have additional antioxidant properties. The aim of this study is to comparatively evaluate the antioxidant properties of cardiovascular drugs like Nebivolol, Carvedilol, Cilnidipine, Atorvastatin and that of anti-diabetic drugs like Sitagliptin, Vildagliptin and Tenegliptin by their free radical scavenging property using DPPH (2,2-diphenyl 1-picrylhydrazyl hydrate) assay.

REVIEW OF LITERATURE

Oxidative stress and antioxidants

Oxidative stress is defined as production of reactive oxygen species (ROS) in excess, relative to the levels of antioxidants, thus creating an imbalance between pro-oxidant and antioxidant factors in favour of pro-oxidants, thereby potentiating cellular oxidative damage.[3]

Antioxidants can be defined as “synthetic or natural substances added to products to prevent or delay their deterioration by action of oxygen in air. In biochemistry and medicine, antioxidants are enzymes or other organic

substances, such as vitamin E or β-carotene that are capable of counteracting the damaging effects of oxidation in animal tissues."Antioxidants help neutralize or destroy "Reactive Oxygen Species" (ROS) or free radicals before they can damage cells.[4]

Antioxidant property of cardiovascular drugs

Hypertension is associated with reduced activity of antioxidant enzymes and increased production of reactive oxygen species (ROS: O2• and H2O2) leading to oxidative stress as measured by lipid and DNA oxidation. Indeed, in almost all experimental models of hypertension ROS are increased in multiple organs, including critical centers of the brain, the blood vessels and the kidneys.[5]

Not only Hypertension, but majority of cardiovascular diseases is accompanied by an imbalance between the formation of reactive oxygen species (ROS, including superoxide, hydrogen peroxide as well as precursor products peroxynitrite or hypochlorous acid) and antioxidant enzymes leading to a deviation from the steady state. More recent evidence suggests that adverse redox signaling and oxidative stress are not only the side effects of the progression of cardiovascular disease but are also potent triggers for their development and pathogenesis. Major focus of research in the cardiovascular field is on the "repair" of vascular damage, by improvement of the function of endothelial progenitor cells by drugs with antioxidant and other pleiotropic properties.[6]

Two beta adrenergic blockers have been used for the pharmacological treatment of hypertension and cardiac failure, Nebivolol and Carvedilol. Nebivolol is a third-generation beta adrenergic blocker with pronounced vasodilator properties. Carvedilol is a non-selective alpha adrenergic receptor antagonist with beta1 blocking properties. Clinical studies have shown that Carvedilol and Nebivolol reduce mortality and improve event-free survival in HF patients. These two agents have favourable properties such as vasodilatory, anti-proliferative, and anti-oxidant effects in addition to other agents. Furthermore, both drugs were found to be effective on oxidative stress with different pathophysiological mechanisms in hypertension patients.[7,8]

Cilnidipine, a long-acting, second-generation 1,4-dihydropyridine (DHP) is a calcium channel blocker found to block both Land N-type calcium channels. It has been found that Cilnidipine induced N-type calcium channel blockade mediates neuroprotective effect by scavenging free radicals.[9] Furthermore, the antioxidant property of Cilnidipine was found to be stronger than Amlodipine.[10] It also has renoprotective effects which are mediated through its antioxidant properties.[11]

Atherosclerosis is a chronic, multifactorial disease that develops in response to inflammation and oxidative stress triggered by immune responses to autoantigens or by cross reactions to foreign antigens, triggering the formation of lesions in arterial blood vessels. Several studies have shown that statins exert various effects beyond lowering cholesterol to ameliorate endothelial dysfunction, by increasing atherosclerotic plaque stability and activating anti-inflammatory and antioxidant mediators.[12]

Diabetes is associated with abnormalities in lipid profile and increased oxidative stress. Statins are preferred agents in diabetic patients due to their antioxidant and LDL-C lowering effects. A study in Diabetic patients by Koksal M et al, found that both Atorvastatin and Rosuvastatin are found to be equally effective in reducing increased oxidative stress in diabetic patients with hyperlipidemia.[13,14]

Thus all these cardiovascular drugs have been found to have antioxidant properties and operate on different mechanisms to reduce oxidative stress thereby reducing cellular damage.

Antioxidant property of Anti-diabetic drugs

Patients with Type 2 diabetes mellitus (T2DM) are at the increased risk of developing cardiovascular diseases. Substantial clinical evidences suggest that endothelial dysfunction is a crucial early step in the development of cardiovascular diseases. Continuous exposure to high levels of glucose has been accepted as the major factor implicated in the pathogenesis of diabetic vascular complications. Several studies indicate that hyperglycemia causes tissue damage mostly through excessive production of reactive oxygen species (ROS), increasing oxidative stress and fostering the development of endothelial dysfunction. However, lowering of blood glucose levels is not sufficient to switch off this intracellular pro-oxidant environment. Various in vitro experiments on cultured endothelial cells (ECs), as well as in vivo studies on diabetic animal models, shows that a ROS-mediated persistence of vascular stress after glucose normalization, along with long standing markers of oxidative stress are widely linked to the pathogenesis of endothelial dysfunction and other diabetic complications.[15]

Glucagon-like peptide 1 (GLP-1) improves endothelial function in diabetes, so therapeutic strategies for patients with T2DM are now focused in increasing the incretin response, either by inhibiting the enzyme dipeptidy peptidase-4 (DPP-4) activity or by using degradation-resistant GLP-1 analogues. DPP-4 is a ubiquitously expressed transmembrane glycoprotein that cleaves N-terminal dipeptides from a variety of substrates, including the incretin hormones, GLP-1 and gastric inhibitory polypeptide (GIP).[16]

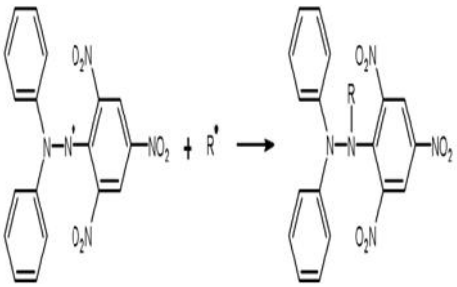
Studies suggest that DPP-4 inhibitors exert beneficial effects on emerging cardiovascular risk factors. Indeed, these agents appear to exert anti-inflammatory effects, mitigate oxidative stress, improve endothelial function and reduce urinary albumin excretion.[17]

Sitagliptin, Vildagliptin and Teneligliptin are the dipeptidyl peptidase-4 (DPP-4) inhibitors also known as gliptins used in the treatment of Type2 Diabetes mellitus to improve blood glucose levels. These gliptins were found to have antioxidant properties exerted through multiple mechanisms as evidenced by various studies.[15,18,19]

There are several studies confirming the antioxidant properties of these cardiovascular and antidiabetic drugs. However comparative evaluation of the antioxidant properties of these drugs has not been done so far. Hence this study was done to compare the in vitro antioxidant properties of cardiovascular drugs (Nebivolol, Carvedilol, Cilnidipine, Atorvastatin) and anti-diabetic drugs (Sitagliptin, Vildagliptin, Teneligliptin) by using DPPH assay.

DPPH assay principle

Figure.1DPPH• free radical conversion to DPPH by an anti-oxidant compound



A rapid, simple and inexpensive method to measure antioxidant property of compounds involves the use of the free radical, 2,2-Diphenyl,1-picrylhydrazyl (DPPH). DPPH is widely used to test the ability of compounds to act as free radical scavengers or hydrogen donors (Figure 1), and also to evaluate antioxidant activity of foods. It has also been used to quantify antioxidants in complex biological systems in the recent years.[20]

The DPPH assay is based on the reduction of DPPH, a stable free radical. The free radical DPPH with an odd electron gives a maximum absorption at 517 nm (purple colour). When Antioxidants react with DPPH, it becomes paired in the presence of a hydrogen donor (E.g., a free radical scavenging antioxidant) and is reduced to the DPPHH which results in decolorization (yellow colour) with respect to the number of electrons captured. More the decolorization, more is the reducing ability. This test has been the most accepted model for evaluating the free radical scavenging activity of any new drug. 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 (Diphenylpicrylhydrazine; non radical) with the loss of this violet colour.[2]

METHODOLOGY

Oral formulations of Nebivolol, Carvedilol, Cilnidipine, Atorvastatin, Sitagliptin, Vildagliptin, Teneligliptin were crushed separately into fine powders and stock solutions of 1000 and 500 µg/ml were prepared using ethanol.

Ascorbic acid was used as the reference antioxidant. Ascorbic acid and the reagents used in the study were of analytical standard procured from Sigma Aldrich.

The percentage of antioxidant activity was assessed by DPPH assay. The measurement of DPPH radical scavenging activity was performed according to the methodology described by Brand Williams et al.[21] The samples were reacted with stable DPPH radical in ethanol solution. The reaction mixture consisted of adding 0.5 ml of sample, 3ml of absolute ethanol and 0.3 ml of 0.5mM DPPH solution in ethanol.

When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The change in color (from deep violet to light yellow) was read at 517nm after 100 minutes using a Spectrophotometer. The mixture of ethanol (3.3 ml) and sample (0.5 ml) served as blank and the mixture of ethanol (3.5 ml) and DPPH solution (0.3 ml) served as control. The antioxidant activity percentage (AA%) as indicated by % DPPH inhibition was determined using the formula,[22]

$$AA\% = 100 - \left[\frac{(Abs_{sample} - Abs_{blank}) \times 100}{Abs_{control}} \right]$$

RESULTS

The experiment was carried out in triplicate for each drug. The results were expressed as percentage decrease with respect to control values and compared.

Table 1: % of DPPH inhibition of cardiovascular and antidiabetic drugs

	% of DPPH inhibition	
Drugs	**Concentration**	
	1000 µg/ml	**500 µg/ml**
Nebivolol	46.38	35.28
Carvedilol	59.58	35.74
Cilnidipine	64.37	54.67
Atorvastatin	69.39	47.55
Teneligliptin	68.81	53.74
Vildagliptin	63.9	50.58
Sitagliptin	53.86	36.21
Ascorbic acid	98.83	70.44

The change in colour of the reaction mixtures was appreciable in both test and standard mixtures after an incubation period of 100 minutes. At concentrations of 1000 µg/ml and 500 µg/ml, the percentage of DPPH inhibition by the drugs were: Nebivolol - 46.38% and 35.28%, Carvedilol - 59.58% and 35.74%, Cilnidipine - 64.37% and 54.67%, Atorvastatin - 69.39% and 47.55%, Teneligliptin - 68.81% and 53.74%, Vildagliptin - 63.90% and 50.58% and Sitagliptin - 53.86% and 36.21% respectively. The percentage of DPPH inhibition for the same concentration of Ascorbic acid was 98.83% and 70.44% respectively. (Table 1, Figure 2)

Figure 2: % DPPH inhibition of various drugs in comparison with standard (Ascorbic acid)

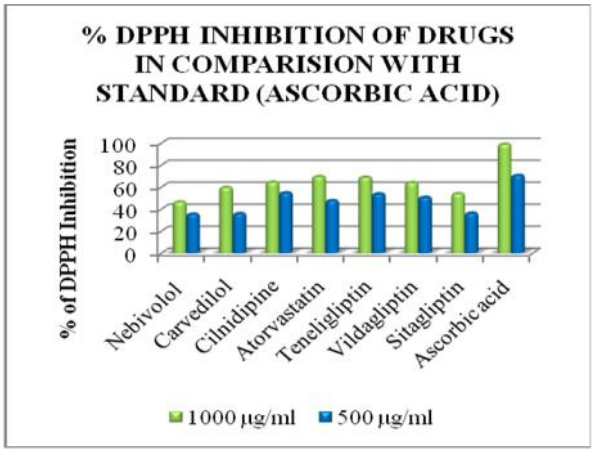
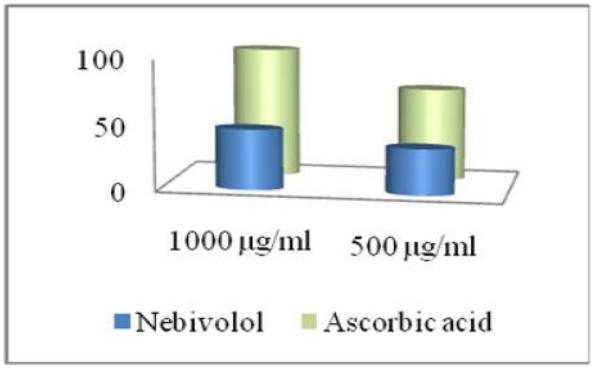
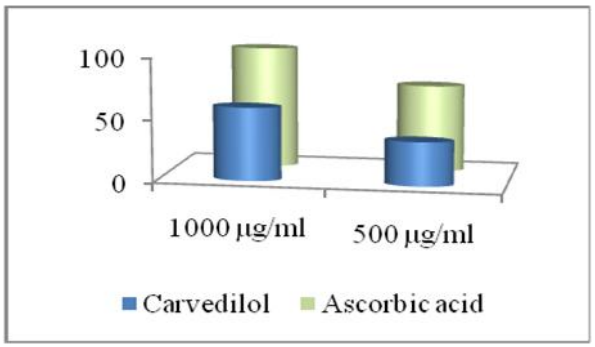
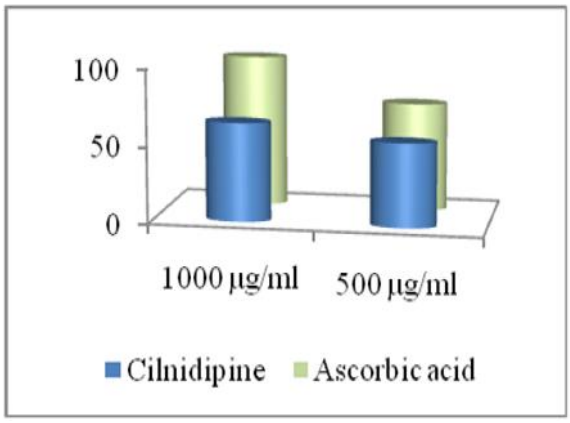
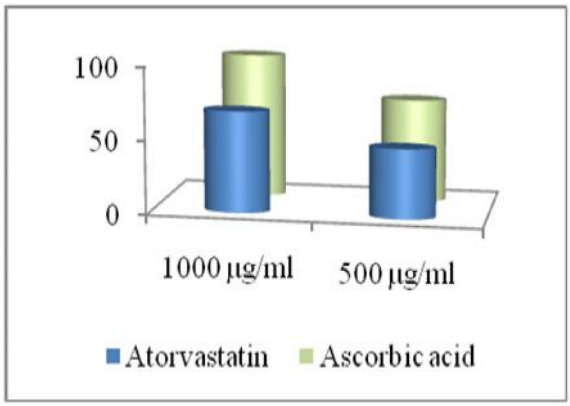


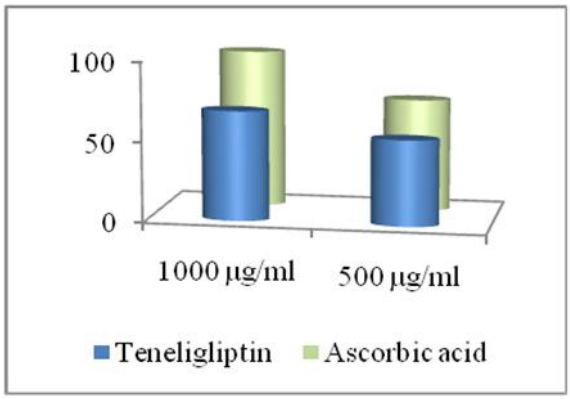
Figure 3: % DPPH inhibition of individual drugs vs Standard (Ascorbic acid)

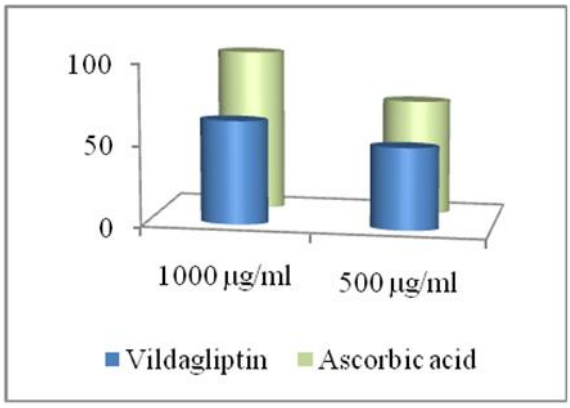


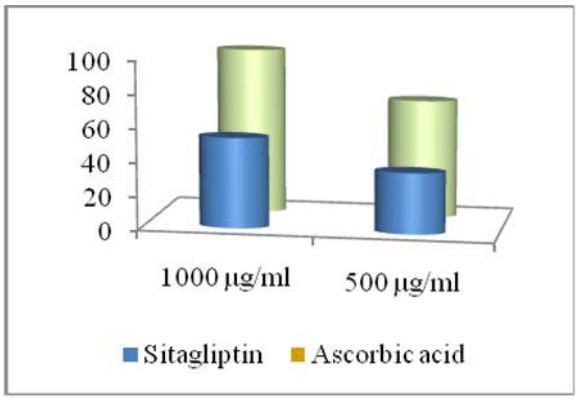












DISCUSSION

Our study has demonstrated that there was considerable free radical scavenging activity seen with all the seven drugs (cardiovascular and anti diabetic) studied as evidenced by their percentage of inhibition of DPPH. Furthermore the antioxidant activity of these drugs was dose dependent.

Among the cardiovascular drugs, at the concentration of 1000 mcg/ml, Atorvastatin showed the maximum in vitro antioxidant activity with 69.39 % of DPPH inhibition. Clinidipine had a slightly lower in vitro antioxidant activity than Atorvastatin with 64.37 % of DPPH inhibition. Nebivolol had the least in vitro antioxidant activity (46.38%) among the four cardiovascular drugs studied.

Among the antidiabetic drugs, the novel DPP-4 inhibitor, Teneligliptin showed a maximum in vitro antioxidant activity with 68.81% of DPPH inhibition as compared to other two gliptins (Vildagliptin – 63.90% and Sitagliptin 53.86%) which were only slightly lower than that of Teneligliptin. (Figure 3)

Though there are numerous studies confirming the antioxidant properties of these drugs, only a handful of studies have addressed the in vitro antioxidant activity of these cardiovascular and antidiabetic drugs. Furthermore, no study has compared the in vitro antioxidant properties of these drugs. Our study results were consistent with the fewer studies available on DPPH assay of gliptins, which showed dose dependent increase in antioxidant activity of Vildagliptin and Sitagliptin.[18,19]

CONCLUSION

Our study has demonstrated the in vitro antioxidant activity of the commonly used cardiovascular drugs (Nebivolol, Carvedilol, Cilnidipine and Atorvastatin) and antidiabetic drugs (Teneligliptin, Vildagliptin and Sitagliptin) which could have a therapeutic advantage of preventing oxidative stress induced complications in patients who are on long term treatment for cardiovascular diseases and diabetes mellitus.

Further quantification of the antioxidant activity of these drugs can be done in vivo using animal models or in patients with cardiovascular disease and diabetes mellitus.

REFERENCES

1. Hussain T, Tan B, Yin Y, Blachier F, Tossou MC, Rahu N. Oxidative stress and inflammation: what polyphenols can do for us?. Oxidative medicine and cellular longevity. 2016 Sep 22;2016.

2. Tailor CS, Goyal A. Antioxidant activity by DPPH radical scavenging method of Ageratum conyzoides Linn. leaves.

3. Sies H. Oxidative stress: oxidants and antioxidants. Experimental physiology. 1997 Mar 1;82(2):291-5.

4. Gupta D. Methods for determination of antioxidant capacity: a review. International Journal of Pharmaceutical Sciences and Research. 2015 Feb 1;6(2):546.

5. Dikalov SI, Dikalova AE. Contribution of mitochondrial oxidative stress to hypertension. Current opinion in nephrology and hypertension. 2016 Mar 1;25(2):73-80.

6. Steven S, Münzel T, Daiber A. Exploiting the pleiotropic antioxidant effects of established drugs in cardiovascular disease. International journal of molecular sciences. 2015 Aug 5;16(8):18185-223.

7. Karabacak M, Dogan A, Tayyar S, Bas HA. The effects of carvedilol and nebivolol on oxidative stress status in patients with non-ischaemic heart failure. Kardiologiapolska. 2015 Jan 1;73(3):201-6.

8. Zepeda RJ, Castillo R, Rodrigo R, Prieto JC, Aramburu I, Brugere S, Galdames K, Noriega V, Miranda HF. Effect of carvedilol and nebivolol on oxidative stress-related parameters and endothelial function in patients with essential hypertension. Basic & clinical pharmacology & toxicology. 2012 Nov 1;111(5):309-16.

9. Lee YJ, Park KH, Park HH, Kim YJ, Lee KY, Kim SH, Koh SH. Cilnidipine mediates a neuroprotective effect by scavenging free radicals and activating the phosphatidylinositol 3-kinase pathway. Journal of neurochemistry. 2009 Oct 1;111(1):90-100.

10. Hishikawa K, Takase O, Idei M, Fujito T. Comparison of antioxidant activity of cilnidipine and amlodipine. Kidney international. 2009 Jul 2;76(2):230-1.

11. Soeki T, Kitani M, Kusunose K, Yagi S, Taketani Y, Koshiba K, Wakatsuki T, Orino S, Kawano K, Sata M. Renoprotective and antioxidant effects of cilnidipine in hypertensive patients. Hypertension Research. 2012 Nov 1;35(11):1058-62.

12. Ma Y, Chen Z, Zou Y, Ge J. Atorvastatin represses the angiotensin 2-induced oxidative stress and inflammatory response in dendritic cells via the PI3K/Akt/Nrf 2 pathway. Oxidative medicine and cellular longevity. 2014 Jul 3;2014.

13. Zhang L, Cheng L, Wang Q, Zhou D, Wu Z, Shen L, Zhang L, Zhu J. Atorvastatin protects cardiomyocytes from oxidative stress by inhibiting LOX-1 expression and cardiomyocyte apoptosis. ActabiochimicaetbiophysicaSinica. 2015 Mar 1;47(3):174-82.

14. Koksal M, Eren MA, Turan MN, Sabuncu T. The effects of atorvastatin and rosuvastatin on oxidative stress in diabetic patients. European journal of internal medicine. 2011 Jun 30;22(3):249-53.

15. Pujadas G, De Nigris V, Prattichizzo F, La Sala L, Testa R, Ceriello A. The dipeptidyl peptidase-4 (DPP-4) inhibitor teneligliptin functions as antioxidant on human endothelial cells exposed to chronic hyperglycemia and metabolic high-glucose memory. Endocrine. 2016 Aug 16:1-2.

16. Baggio LL, Drucker DJ. Biology of incretins: GLP-1 and GIP. Gastroenterology. 2007 May 31;132(6):2131-57.

17. Papagianni M, Tziomalos K. Cardiovascular effects of dipeptidyl peptidase-4 inhibitors. Hippokratia. 2015 Jul;19 (3):195.

18. Cijoy Jose P, Vasanthi C, David DC, Stephen A. Evaluation of antioxidant property of vildagliptin.

19. Ravi A, Muthiah NS, Elizabeth AA, Muniappan, Philip S, et al. Evaluation of radical scavenging property of Sitagliptin. Journal of chemical and pharmaceutical research. 2015 Jul; 7(10):273-275.

20. Shalaby EA, Shanab SM. Antioxidant compounds, assays of determination and mode of action. African journal of pharmacy and pharmacology. 2013 Mar 15;7(10):528-39.

21. Brand-Williams W, Cuvelier ME, Berset CL. Use of a free radical method to evaluate antioxidant activity. LWT-Food science and Technology. 1995 Jan 1;28(1):25-30.

22. Garcia EJ, Oldoni TL, Alencar SM, Reis A, Loguercio AD, Grande RH. Antioxidant activity by DPPH assay of potential solutions to be applied on bleached teeth. Brazilian dental journal. 2012;23(1):22-7.