Vol 4| Issue 2 | 2014 | 76-79.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

A COMPARATIVE STUDY OF *IN-VITRO* ANTI-OXIDANT POTENTIAL OF PHOSPHODIESTERASE -5 INHIBITORS

***Mrinal Borgohain, S.Seetha lakshmi, P.Duriarajan**

Department of Pharmacology, Sri Ramachandra Medical College & Research Institute, Porur, Chennai, Tamilnadu, India.

ABSTRACT

Phosphodiesterase 5 (PDE-5) Inhibitors are drugs that inhibit Phosphodiesterase 5 (PDE-5), the cGMP degrading isoenzyme causing rise in concentration of cGMP in smooth muscle cells thereby reducing muscular tone. They are used for treating Pulmonary artery hypertension, erectile dysfunction, reducing symptoms of Lower Urinary Tract secondary to Benign Prostatic Hyperplasia (BPH) and recently on Cardio-protective effect for which the exact mechanism is not known. To investigate and compare the anti-oxidant potential of currently available PDE-5 inhibitors: Tadalafil, Vardenafil and Sildenafil by in-vitro Nitric oxide (NO) radical scavenging assay. Method: The anti-oxidant activities of PDE-5 inhibitors were done using *In-vitro* Nitric Oxide (NO) radical scavenging assay. The nitric oxide radical scavenging activity was highest for Tadalafil followed by Sildenafil and Vardenafil showing topmost activity of 64.74%, 54.34% and 49.83% respectively at 1000 µg /ml whereas at the same concentration ascorbic acid (i.e. standard anti-oxidant) shows 90.16% inhibition. From our study it showed all PDE-5 inhibitors have good anti-oxidant property that may probably play a Cardio-protective role by improving Endothelial Dysfunction.

Key words: PDE-5 inhibitors, Sildenafil, Tadalafil, Vardenafil, Antioxidant, Nitric Oxide Scavenging activity.

INTRODUCTION

Phosphodiesterases (PDE) belongs to a group of enzyme system that causes hydrolysis of cyclic nucleotides, thereby regulating an important role in intracellular levels of second messengers cAMP & cGMP and consequently cell function. So far 11 isoenzyme families have been recognised and based upon the knowledge about their role at cellular and molecular level, an incitement is provided in developing newer selective inhibitors for various therapeutic applications [1-8].

The Selective PDE inhibitors that have been developed currently with its therapeutic applications are shown in Table1.

PDE-5 inhibitors

Phosphodiesterase 5 (PDE-5) Inhibitors are drugs that inhibits Phosphodiesterase 5 (PDE-5), the cGMP degrading isoenzyme causing rise in concentration of cGMP in smooth muscle cells thereby reducing muscular tone. PDE-5 inhibitors available currently are: Tadalafil, Vardenafil and Sildenafil. They are used for treating Pulmonary artery hypertension and erectile dysfunction. They are being explored for their role in reducing symptoms of Lower Urinary Tract Secondary to BPH and recently on Cardio-protective effect for which the exact mechanism is unclear.

As oxidative stress plays a major role in endothelial dysfunction leading to detrimental response on the myocardium. Therefore the aim of our study is to investigate and compare the anti-oxidant potentials of PDE-5 inhibitors using *In-vitro* Nitric oxide (NO) radical scavenging assay [10-16].

MATERIALS AND METHODS

Chemicals

Tadalafil, Vardenafil, and Sildenafil was

Corresponding Author :- **Mrinal Borgohain** Email:- mrinal19856@gmail.com

purchased from Glenmark Pharmaceuticals Ltd. Griess reagent, Sodium nitroprusside (SNP), phosphate buffer saline (PBS) and ascorbic acid was purchased from A to Z Lab Needs, Chennai, India.

Table 1. PDE inhibitors and its applications

PDE inhibitors	Therapeutic applications
2	Sepsis, Acute Respiratory Distress Syndrome(ARDS)
3	Airways disease, fertility
4	Dermatitis, Allergic rhinitis, Multiple sclerosis, Psoriasis, Cancer, Memory loss, Depression
5	Pulmonary hypertension, Erectile dysfunction, cardiovascular disease, premature ejaculation
7	Inflammation

***In-vitro* assay of Nitric oxide (NO) scavenging method**

Anti-oxidant activity is based on Nitric oxide (NO) radical scavenging assay. This method is based on the inhibition of NO radical produced from Sodium nitroprusside (SNP) in phosphate buffer saline (PBS) and estimation done by adding Griess reagent [17,18].

1 ml of SNP (5mM) in PBS was taken in 5 different test-tubes and 5 different concentrations (100, 200, 400, 800 & 1000µg/ml) of each test drugs (viz. Tadalafil, Vardenafil and Sildenafil) dissolved in 1ml of methanol (99.8%) was added to the test-tubes. The test tubes are then incubated at 29°C for 3 hrs. Similar concentrations of ascorbic acid was prepared and incubated in a similar manner which was taken as reference anti-oxidant in the study. For control a test tube filled with distilled water was taken and conducted in an identical manner [19,20].

After 3 hours incubated samples were diluted with 1 ml of Griess reagent. The absorbance that is formed as a result of diazotization of nitrite with sulphanilamide and consecutive coupling with naphthylethylenediamine dichloride was analysed on Spectrophotometer at 546 nm. Percentage of Inhibition of NO scavenging activity is given by the formula:

$$\text{Nitric Oxide scavenged (\%)} = \frac{A_{control} - A_{test}}{A_{control}} \times 100$$

Where, $A_{control}$= Absorbance of control and A_{test}= Absorbance of test sample IC50 values were obtained by probit analysis.

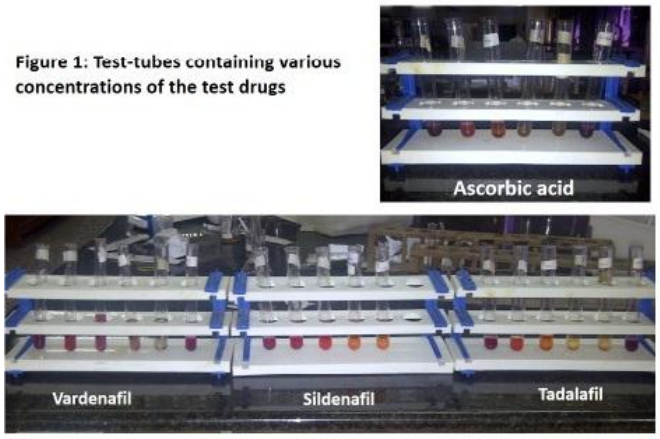
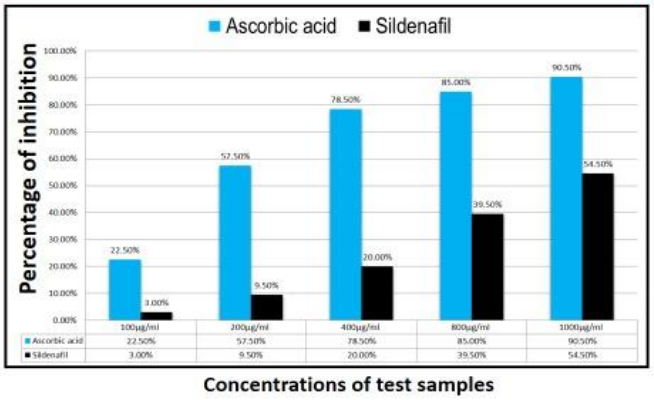
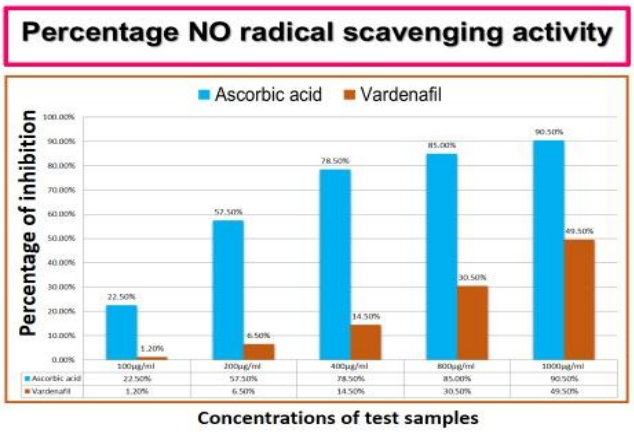
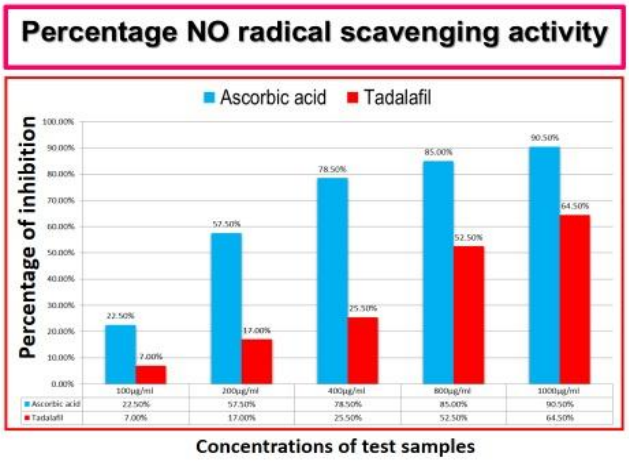
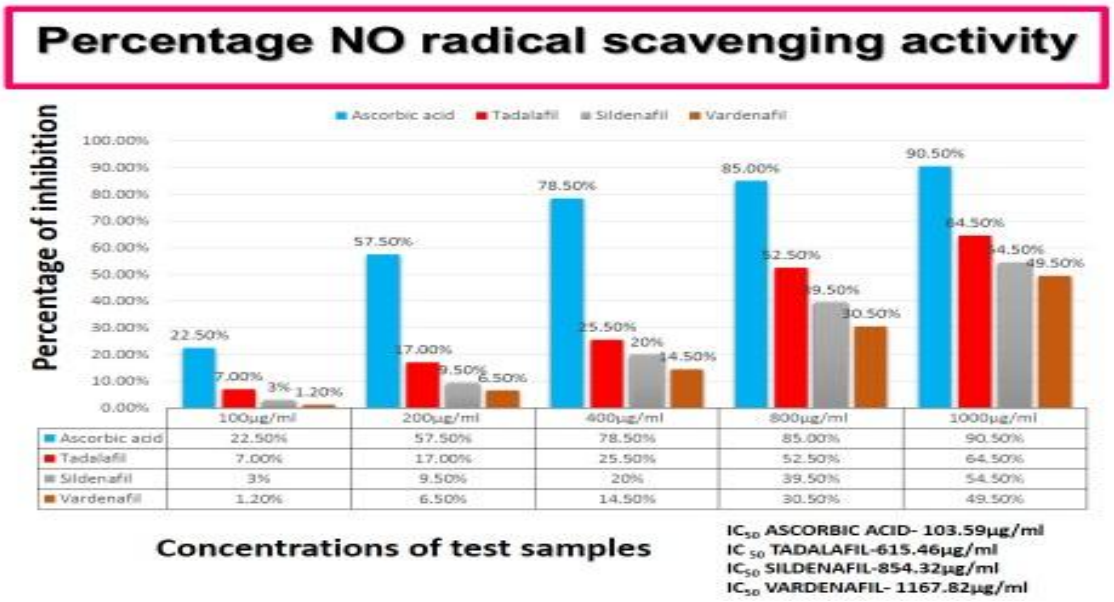


Figure 1: Test-tubes containing various concentrations of the test drugs









RESULTS & DISCUSSION

The nitric oxide radical scavenging activity was highest for Tadalafil followed by Sildenafil and Vardenafil showing topmost activity of 64.74%, 54.34% and 49.83% respectively at 1000 µg/ml whereas at the same concentration ascorbic acid (i.e. standard anti-oxidant) shows 90.16% inhibition. In the study we found the Ic 50 value for ascorbic acid to be 103.59µg/ml, Tadalafil to be 615.46µg/ml, Sildenafil to be 854.32µg/ml and Vardenafil to be 1167.82 µg/ml.

Nitric oxide (NO) is a free radical that plays a pivotal role in regulating many biological processes inside the body. It acts as a cell signalling molecule involved in neurotransmission, relaxation of smooth muscle in vascular endothelium leading to vasodilatation, suppress aggregation of platelets and also involved in modulating cell mediated toxicity. Excess of NO free radical is detrimental to the tissues and involved in many pathological processes. It can cause endothelial dysfunction leading to series of event that has detrimental response on the myocardium. Further it leads to myocardial ischaemia and disorder in myocardial reperfusion by promoting aggregation of platelets. Inhibiting the release of NO free radical by PDE-5 inhibitors ascribe to its potential anti-oxidant activity. The anti-oxidant activity of all the PDE-5 inhibitors increases with increase in dose.

CONCLUSION

Our study showed that all the PDE-5 inhibitors have significant anti-oxidant property. The nitric oxide radical scavenging activity was highest for Tadalafil followed by Sildenafil and Vardenafil. We can therefore conclude that, the observed NO radical scavenging activity of the PDE-5 inhibitors may contribute to improve endothelial dysfunction which plays a cardio-protective role in myocardial ischemia & reperfusion.

ACKNOWLEDGEMENTS

I gratefully acknowledge the Head of the Department of Pharmacology Dr. S.Seethalakshmi who helped me to conduct this Research.

REFERENCES

1. Saisudha Koka, Anindita Das, Shu-Guang Zhu, David Durrant, Lei Xi, and Rakesh C. Kukreja. Long-Acting Phosphodiesterase-5 Inhibitor Tadalafil Attenuates Doxorubicin-Induced Cardiomyopathy without Interfering with Chemotherapeutic Effect. *The Journal Of Pharmacology And Experimental Therapeutics, JPET,* 334, 2010, 1023–1030.
2. Thorsten Reffelmann and Robert A Kloner. Cardiovascular Effects of Phosphodiesterase 5 Inhibitors. *Current Pharmaceutical Design*, 12, 2006, 3485-3494
3. Stephen L. Archer and Evangelos D. Michelakis. Phosphodiesterase Type 5 Inhibitors for Pulmonary Arterial Hypertension. *The New England Journal Of Medicine*, 361, 2009, 1864-71.
4. Gresser U, Gleiter CH. Erectile dysfunction, Comparison of efficacy and side effect of PDE-5 inhibitors Sildenafil, Vardenafil and Tadalafil. *European Journal Of Medical Research*, 7, 2002, 435-446
5. Kevin T. McVary, Claus G. Roehrborn, Jed C. Kaminetsky, Stephen M. Auerbach, Barton Wachs, Jay M. Young, Anne Esler, Gregory D. Sides and Bela S. Denes. Tadalafil Relieves Lower Urinary Tract Symptoms Secondary to Benign Prostatic Hyperplasia. *The Journal Of Urology*, 177, 2007, 1401-1407.

6. Ferdinando Franzonia, Alfredo Quin˜ones-Galvanb, Francesco Regolic, Ele Ferranninia, Fabio Galetta. A comparative study of the *In-vitro* antioxidant activity of statins. *International Journal of Cardiology*, 90, 2003, 317–321
7. Dharmendra Singh, Manish Mishra, Monika Gupta, Poonam Singh1, Abhishek Gupta1, Rajeev Nema. Nitric Oxide radical scavenging assay of bioactive compounds present in methanol Extract of *Centella asiatica*. *International Journal of Pharmacy and Pharmaceutical Science Research*, 2(3), 2012, 42-44.
8. Feenstra J, Van Drie-Pierik RJ, Lacle CF, Stricker BH. Acute myocardial infarction associated with sildenafil. *Lancet*, 352, 1998, 957-8.
9. Kloner RA. Cardiovascular risk and Sildenafil. *Am J Cardiol*, 86, 2000, 57F-61F.
10. Reffelmann T, Kloner RA. Therapeutic potential of phosphodiesterase 5 inhibition for cardiovascular disease. *Circulation*, 108, 2003, 239-44.
11. Billups KL. Endothelial dysfunction as a common link between erectile dysfunction and cardiovascular disease. *Curr Sex Healthy Rep*, 2004, 1, 137-41
12. Kukreja RC, Ockaili R, Salloum F, Yin C, Hawkins J, Das A, et al. Cardioprotection with phosphodiesterase-5 inhibition – a novel preconditioning strategy. *J Mol Cell Cardiol*, 2004, 36,165-173.
13. Arruda-Olson AM, Mahoney DW, Nehra A, Lickel M, Pellika PA. Cardiovascular effects of Sildenafil during exercise in men with known or probable coronary artery disease. *JAMA*, 287, 2002, 719-25.
14. Jackson G, Kloner RA, Costigan TM. Update on clinical trials of Tadalafil demonstrates no increased risk of cardiovascular adverse events. *J Sex Med*, 1, 2004, 161-7.
15. Das S, Maulik N, Das DK, Kadowitz RJ, Bivalacqua TJ. Cardioprotection with sildenafil, selective inhibitor of cyclic 3', 5'-guanosine monophosphate specific phosphodiesterase 5. *Drug Exp Clin Res*, 28, 2002, 213-9.
16. Du Toit EF, Rossouw E, Sapie R, Opie LH, Lochner A. Effects of sildenafil on reperfusion function, infarct size,and cyclic nucleotide levels in the isolated rat heart model. *Cardiovasc Drugs Ther*, 19, 2005, 23-31
17. Van Ahlen H, Zumbe J, Stauch K, Landen H. The real-life safety and efficacy of vardenafil. An international post-marketing surveillance study – results from 29 358 German patients. *J Int Med. Res*, 33, 2005, 337-48.
18. Emmick JT, Stuewe SR, Mitchell M. Overview of the cardiovascular effects of tadalafil. *Eur Heart J*, 4(1), 2002, H32-H47.
19. Pomara G, Morelli G, Pomara S, Taddei S, Ghiadoni L, Dinelli, et al. Cardiovascular parameter changes in patients with erectile dysfunction using PDE-5 inhibitors, a study with Sildenafil and vardenafil. *J Androl*, 25, 2004, 625-629.
20. Halcox JPJ, Nour KRA, Zalos G, Mincemoyer RA, Waclawiw M, Rivera CE, et al. The effect of sildenafil on human vascular function, platelets activation, and myocardial ischemia. *J Am Coll Cardiol*, 40, 2002, 1232-40.