WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 5, Issue 2, 284-293. Research Article ISSN 2277– 7105

THROMBOLYTIC EFFECT OF SOME ANTIDIABETIC DRUGS: *IN VITRO* AND *IN SILICO* APPROACH.

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Article Received on
14 Dec 2015,

Revised on 04 Jan 2016,
Accepted on 25 Jan 2016

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ABSTRACT

The present study aims to investigate the thrombolytic effect of some antidiabetic drugs by *in vitro* clot lysis method and *in silico* molecular docking used to identify whether these drugs interact with the responsible protein (tissue-type plasminogen activator). *In vitro* clot lysis model was used to observe the thrombolytic effect of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs, where they exhibited 46.61 ± 2.84%, 43.89 ± 3.41%, 24.45 ± 2.62 % and 35.89 ± 3.57 % clot lysis, respectively. Reference drug streptokinase exhibited 78.70±0.92% clot lysis. A wide range of docking score found during molecular docking by CPI server. Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs showed the docking score -8.6, -4.9, -3.8 and -7.3, respectively. Glibenclamide possessed highest clot lysis effect and also showed best docking score among the antidiabetic drugs, where Sitagliptin phosphate exhibited the opposite. But results of Metformin hydrochloride and Vildagliptin were different compare with the other drugs result. Though Metformin hydrochloride showed higher clot lysis (43.89 ± 3.41%) effect than Vildagliptin, but in *in silico* molecular docking method, Vildagliptin showed well docking score over Metformin hydrochloride. Further *in vivo* investigation need to identify the thrombolytic effect of these antidiabetic drugs and also require making out the mechanism of them as thrombolytic agents.

KEYWORDS: Antidiabetic drugs, PASS prediction, Molecular docking, Glibenclamide, Metformin hydrochloride.

1. INTRODUCTION

Blood coagulation creates in the circulatory system which consolidates a mechanism in human body to repair the injured blood vessel (Key *et al.*, 2009; Kader SMA, 2016). In the event that thrombus is framed when it is not required, this can deliver noteworthy results (Wedro ;Uddin *et al.*, 2016) like embolism, ischemia, heart attack, stroke, and so forth (Shapiro; Hasan M and MR, 2016). Embolism occurs when blood clot is formed inside a blood vessel or an artery and remains there which fully or partially block blood supply to a part of body resulting potentially severe consequences. For example, an aspiratory embolism leads illogical breathing trouble, hemoptysis, and mid-section torment when one or more supply routes in lung are obstructed by embolus (Ali *et al.*, 2014; Kabir *et al.*, 2015). Blood clot can block blood flow or oxygen to tissue which results in ischemia. Cardiac ischemia appears when blood flow to cardiac muscle becomes fully or partially restricted resulting shortness of breath, syncope, angina, myocardial infarction, cardiac arrhythmia, or even death (Maseri *et al.*, 1978; Chowdhury *et al.*, 2015; Tarek *et al.*, 2015). Blood clots may also disrupt the flow of blood to the brain, leading to an ischemic stroke (Shiber *et al.*, 2010). An ischemic stroke can happen as an aftereffect of a hindrance inside of a vein supplying blood to the cerebrum (thromblic stroke) or embolus created from cluster elsewhere in the body and goes to obstruct a little corridor in the mind (embolic stroke). Sometimes blood clot forms in the heart and get trapped in the brain's narrow arteries (cerebral stroke). These outcomes deny the cerebrum of fundamental oxygen which bring about lasting mind cell demise in and around the influenced range (Ohira *et al.*, 2006).

Our aim of this study to investigate the thrombolytic effect of some antidiabetic drugs like, Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin, which were studied by using *in vitro* clot lysis and *in silico* Molecular docking model. However, no earlier studies have been conducted experimentally to characterize the thrombolytic effect of oral antidiabetic drugs.

2. MATERIALS AND METHODS

2.1 *In vitro* thrombolytic effect

2.1.1 Drugs and chemicals

Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin were got from a renowned Pharmaceuticals company as gift, Bangladesh. To the commercially available lyophilized streptokinase (SK) vial (Square Pharmaceuticals Ltd.) of 1500000 I.U., 5mL sterile distilled water was added and mixed properly. This suspension was used as a stock from which 100 μL (30,000 I.U.) was used for *in vitro* thrombolysis. All chemicals and reagents were of reagent grade.

2.1.2 Drugs solution preparation

A 100 mg each of the drugs was suspended in 10 ml distilled water and the suspension was shaken vigorously on a vortex mixer. The suspension was kept overnight and decanted to remove the soluble supernatant, which was filtered through a 0.22-µm syringe filter. A 100 µl of this aqueous preparation was added to the Eppendorf tube tubes containing the clots to check thrombolytic activity.

2.1.3 *In vitro* Thrombolytic effect assay

Experiments for clot lysis were carried as reported earlier (Prasad *et al.*, 2006). Briefly, 3 ml venous blood drawn from the healthy volunteers was distributed in six different pre weighed sterile Eppendorf tube (0.5 ml/tube) and incubated at 37°C for 45 min. After clot formation, serum was completely removed without disturbing the clot and each tube having clot was again weighed to determine the clot weight (clot weight = weight of clot containing tube – weight of tube alone). To each Eppendorf tube containing pre-weighed clot, 100 µl of aqueous solution of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin were added separately. As a positive control, 100 µl of SK and as a negative non-thrombolytic control, 100 µl of distilled water were separately added to the control tubes numbered. All the tubes were then incubated at 37°C for 90 min and observed for clot lysis. After incubation, fluid released was removed and tubes were again weighed to observe the difference in weight after clot disruption. Difference obtained in weight taken before and after clot lysis was expressed as percentage of clot lysis. The experiment was repeated with the blood samples of the 12 volunteers.

2.2 *In silico* Molecular docking

All the antidiabetic drug structures need to upload in mol2 format with charges and hydrogens added. When a molecule submitted, The CPI server checks the format suitability and calculates the interaction profile of this drug towards all the targets in the database using DOCK6 (Ewing *et al.*, 2001; Luo *et al.*, 2011). Users can view the real-time progress online, and the page showing the current docking status of the uploaded drug will also be provided for bookmarking. It takes between 6 and 20 h to finish a one-molecule task and an email will be sent on completion. The outputs comprise the two following major elements:

(i) Library drugs which share similar (or opposite) interaction profile with the user's molecule, ranked by the similarity (or disparity) with known indications and ADR information, suggesting the underlying new indication and ADR of the user's molecule.

(ii) The candidate off-targets that tend to interact with the user's molecule. The server will visualize the drug-protein interactions, with amino acid residues around $6A^{o}$ of the molecule highlighted.

2.3 Statistical analysis

The significance between % clot lysis by SK and drugs tested by Tukey test using the software SPSS, version 22.0 (SPSS for Windows, Version 22.0, IBM Corporation, New York, USA). Data are expressed as mean ± SEM. The mean difference between positive and negative control was considered significant at *P* values < 0.05 and 0.0001.

3. RESULTS

3.1. *In Vitro* Thrombolytic effect: In thrombolytic effect assay, addition of 100µl streptokinase as positive control (30,000 I.U.) to the clots and subsequent incubation for 90 minutes at 37°C, showed 78.70±0.92 % lysis of clot. On the other hand, distilled water treated as negative control exhibited a negligible percentage of lysis of clot (5.30±1.65%). The mean difference in clot lysis percentage between positive and negative control was found statistically very significant (P<0.0001). Treatment of clots with Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs provided the clot lysis 46.61 ± 2.84%, 43.89 ± 3.41%, 24.45 ± 2.62 % and 35.89 ± 3.57 %, respectively. All the results presented in Table 1 and Figure 1. The thrombolytic effects of antidiabetic drugs are as follows,

Glibenclamide > Metformin hydrochloride > Vildagliptin > Sitagliptin phosphate

Table 1: Clot lysis effect of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs on human blood.

drugs	% of clot lysis (mean ± SEM)
Negative control (water)	5.30±1.65
Positive control (streptokinase)	78.70±0.92 [a]
Glibenclamide	46.61 ± 2.84 [a,b]
Metformin hydrochloride	43.89 ± 3.41 [a,b]
Sitagliptin phosphate	24.45 ± 2.62 [a,b]
Vildagliptin	35.89 ± 3.57 [a,b]

Values are mean ± SEM (n = 12); [a]P <0.0001, Tukey test as compared to negative control, [b]P < 0.001, compared to positive control. Statistical representation of the effective clot lysis percentage by drugs preparations, positive thrombolytic control (streptokinase), and negative control (sterile distilled water) processed by Tukey test by using SPSS for windows, version 22.0.

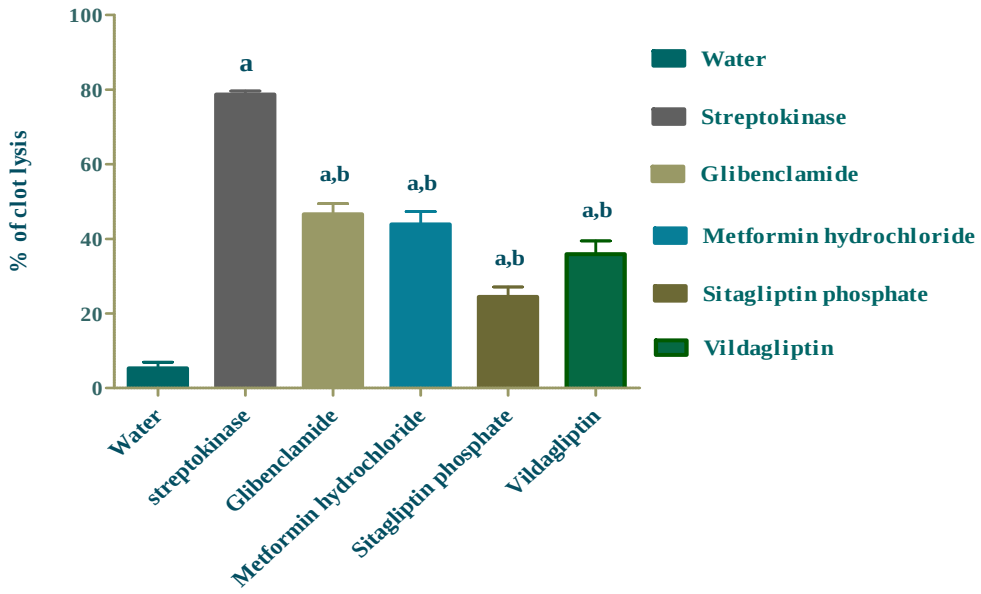

Figure 1: Clot lysis effect of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs on human blood.

Values are mean ± SEM (n = 12); [a]P <0.0001, Tukey test as compared to negative control (Water), [b]P < 0.001, compared to positive control (Streptokinase). Statistical representation of the effective clot lysis percentage by drugs preparations, positive thrombolytic control (streptokinase), and negative control (sterile distilled water) processed by Tukey test by using SPSS for windows, version 22.0.

3.2 *In silico* Molecular docking

In the present study, molecular docking performed to identify the docking score of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin towards tissue-type plasminogen activator (PDB code 1A5H), which is a protein involved in the breakdown of blood clots. A wide range of docking score found during molecular docking by CPI server. Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin drugs showed the docking score -8.6, -4.9, -3.8 and -7.3, respectively. All the results presented in Table 2 and Figure 2. The docking score of antidiabetic drugs are as follows, Glibenclamide > Vildagliptin > Metformin hydrochloride > Sitagliptin phosphate.

Table 2: Docking results with antidiabetic drugs in the tissue-type plasminogen activator.

Drug name	Docking Score
Glibenclamide	-8.6
Metformin hydrochloride	-4.9
Sitagliptin phosphate	-3.8
Vildagliptin	-7.3

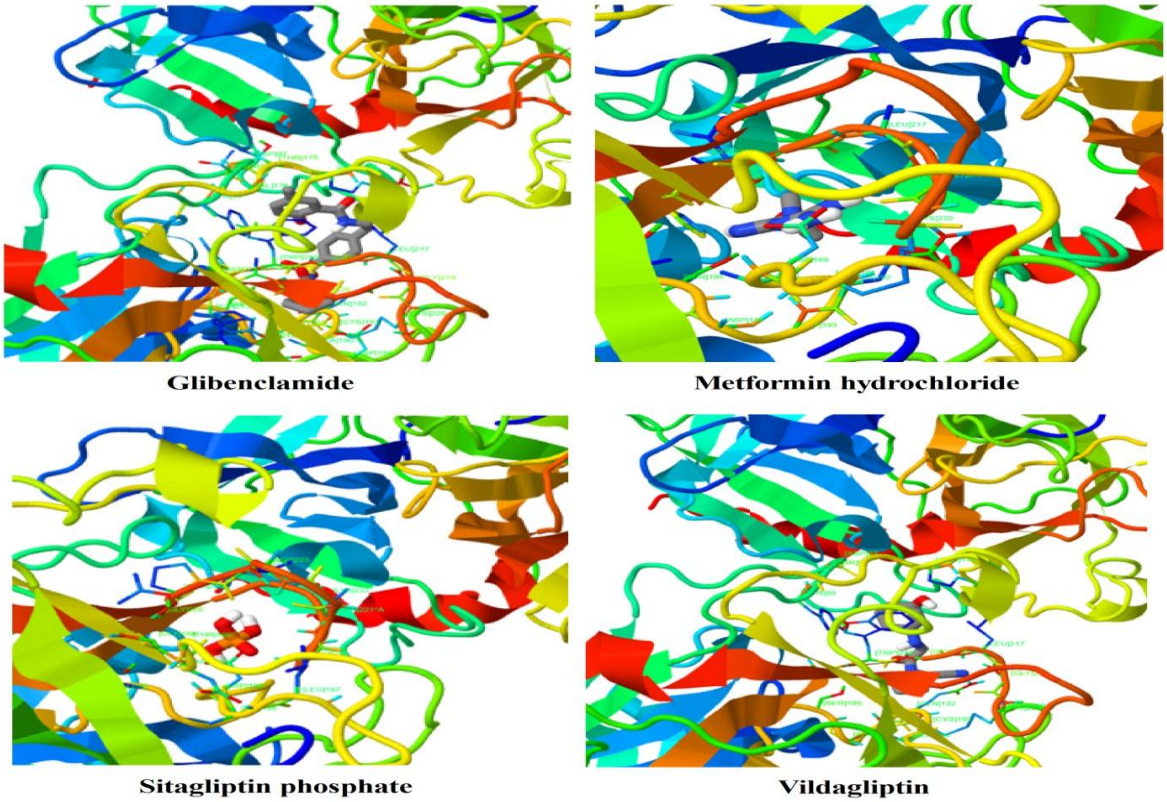

Figure 2: Molecular docking analysis of Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin with tissue-type plasminogen activator complex obtained from docking.

4. DISCUSSIONS

In our thrombolytic assay, the comparison of positive control with negative control clearly demonstrated that clot dissolution does not occur when water was added to the clot. When compared with the clot lysis percentage obtained through water, a well significant (*P* value < 0.001) thrombolytic activity was observed after treating the clots Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin. However, the clot lysis values for Sitagliptin phosphate were lower than other drugs.

In molecular docking study, Glibenclamide, Metformin hydrochloride, Sitagliptin phosphate and Vildagliptin showed the docking score -8.6, -4.9, -3.8 and -7.3, respectively towards tissue-type plasminogen activator. From all these antidiabetic drugs, Glibenclamide exhibited best docking score (-8.6), which also possessed maximum clot lysis (46.61 ± 2.84%) effect on human blood. After Glibenclamide, Vildagliptin showed well docking score (-7.3) but exhibited low clot lysis (35.89 ± 3.57%) effect compare to standard drugs (streptokinase).On the other hand, Metformin hydrochloride showed clot lysis (43.89 ± 3.41) effect, which was near to the thrombolytic effect of Glibenclamide. But, Metformin hydrochloride showed low docking score (-4.9).

From the present study, it was clear that antidiabetic drugs have moderate to well thrombolytic effect and it established that antihyperglycemic drugs improving endothelial function and markers of atherogenesis, with the potential to reduce cardiovascular morbidity and mortality (Macfarlane *et al.*, 2007). Though, previous study point out that antidiabetic can be used as cardiovascular drugs for particular reason and we also found the some of them also have good thrombolytic effect and also they showed well docking score for tissue-type plasminogen activator, so we can think the use of antidiabetic drugs for thrombosis management. However further investigation need to proof the thrombolytic effect of these antidiabetic drugs in *in vivo* model.

5. CONCLUSION

Glibenclamide possessed highest clot lysis effect and also showed best docking score among the antidiabetic drugs, where Sitagliptin phosphate exhibited the opposite. But results of Metformin hydrochloride and Vildagliptin were different compare with the other drugs result. Though Metformin hydrochloride showed higher clot lysis effect than Vildagliptin, but in *in silico* molecular docking method, Vildagliptin showed well docking score over Metformin hydrochloride. Further *in vivo* investigation need to identify the thrombolytic effect of these

antidiabetic drugs and also require making out the mechanism of them as thrombolytic agents.

Conflict of interest statement

The authors declare that they have no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to the authority of International Islamic University Chittagong, Bangladesh, for providing the facilities to conduct this research work. The authors thank GUSTO (A research group) for the financial support. The authors are thankful to all members of GUSTO (A research group) for their kind help in the experiments. The authors are also thankful to Raju Dash, Department of Pharmacy, BGC Trust University Bangladesh, Bangladesh, for intellectual help in the experiment.

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