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Effect of Shikoni on Phosphomonoesterase Activity of Mycobaterium Tuberculosis Protein Tyrosine Phosphotases (PTPs)

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Abstract— Several factors such as patient noncompliance and drug resistance affect the success rate of conventional treatments against tuberculosis and other pathogenic diseases; therefore there is need to improve the available therapeutic ways used in targeting tuberculosis treatment as well as design of new drugs that could act more rapidly on them. This research aimed to determine the effect of shikoni on phosphomonoesterase activity of mycobacterium tuberculosis using protein tyrosine phosphatases as enzyme inhibitor. Purified homogenous PTPs were obtained and shikoni phosphomonoesterase activity was determined by measuring the p-nitrophenol releases from the pNPP substrate. PTP acts as potential drug target which enables strategies to develop modern treatment regimen and regulate the levels of protein tyrosine phosphorylation under normal and pathological conditions.

Keywords— Shikoni, Phosphatases activity, Mycobacterium Tuberculosis, Protein Tyrosine Phosphatases (PTPs)

I. INTRODUCTION

Microorganisms are considered not to be pathogenic until they cover a certain population size larger enough to cause disease. Tuberculosis has been reported to be one of the greatest health challenge worldwide and which causes high prevalence of morbidity and mortality rate particularly in developing countries.[1] It is obvious that *M. tuberculosis* and other pathogenic species such as *E.coli, M. kansasii* and *V. cholerae*, which often cause severe infections in immune-depressed and AIDS patients, are internalized into phagosomes which remain refractory to fusion with lysosomes [2]. The World Health Organization (WHO) declared TB as a global health emergency, which highlights the importance of TB as a major threat to humans [3]. In the last decade, exoenzymes protein tyrosine phosphatase A (PtpA) and B (PtpB) have emerged as promising therapeutic targets to discover new anti-TB agents. These enzymes are secreted into the host cell by *Mycobacterium tuberculosis* (Mtb) and attenuate host immune defenses by interfering with the host signaling pathways has discussed by [4].

Currently, due to increasing drug resistance, antibiotics are losing efficacy in the treatment of tuberculosis infection.[5] Factors such irregular diagnosis, patient's noncompliance to prescriptions, insufficient availability of drugs, and genetic moderation contributes to drug resistance in the treatment of tuberculosis[6]. As recently reported by WHO, there are approximately 50 antibiotics designed [7]. However, these antibiotics are insufficient to alleviate the problem of associated with the increase in antibiotic resistance. In contribution to the available antibiotics, In 2017, eight new antibiotics were added to the available antibiotics, but many have limited clinical advantage compared to the existing drugs. [8]

The idea of analyzing protein tyrosine phosphatases (PTP) under different enzyme inhibitors is not only to understand their regulation but also to identify strategies for pharmacological utilization of PTP activity.[9] However, mycobacterium is mostly isolated using *M. Smegmatis* [10] which is harmful to work with pathogenic bacteria such as *M. tuberculosis*, hence, it is important to use a harmless inhibitor to compete and isolate the pathogenic-like organism in a more rapid and simple therapeutic strategy[11]. The presence of multi drug-resistant and extensively drug-resistant tuberculosis demands the development of therapeutic agents with novel mechanisms of action [12] which under this study is aimed to evaluate the effect of shikoni inhibitor on mycobacterium tuberculosis. The objectives of this study are to analyze the effectiveness of shikoni as a curative agent of tuberculosis and propose a drug-like inhibitor for the prevention of mycobacterium tuberculosis infection in human.

II. MATERIALS AND METHODS

Materials

The Purified homogenous *Protein Tyrosine Phosphatases* obtained from Institute of Molecular Cell and System Biology (Glasgow, Scotland). The substrate of the enzyme, p-nitrophenyle phosphate (pNPP), the ligand shikoni is a

product of Sigma Chemical Company, St. Loius, United State.

Method

Determination of Shikoni on Phosphomonoesterase activity of MTbPTP

Principle: PTPs catalyzes are hydrolysis of pNPP substrate to para-nitrophenol and inorganic phosphate, pNPP is reported to be colorless while para-nitrophenol is reported to be a yellow colored product with a strong absorbance of ≥ 405nm.

Procedure: Inhibition of PTP was determined by measuring the p-nitrophenol released from the pNPP substrate. The buffer is 25mM Tris-HCl (pH 7.5). The reaction was started with a mixture of 25mM of Tris buffer (62µL), the ligand (shikoni in solution in 1% DMSO) at a same concentration (8µL).

Data Analysis

All laboratory activities and enzyme assays were performed in double and results were presented using descriptive statistical method. All obtained values were computed at significant different of $P < 0.05$ while Graphpad prism version 7.0 was used to plot all the graphs.

III. RESULTS AND DISCUSSION

Results

Considering the concentration dependent inhibition of Protein Tyrosine Phosphotases (PTPs) by shikoni is presented in figure 1, the concentration investigated were at an interval of 25µM ranges from 25µM - 300µM, in which 25µM shows a high tendency of PTPs inhibition (Figure 1). The data gotten from the kinetics was used to obtain Michaelis-Menten curve (Figure 2) for Shikoni and (figure 3) for PDB and the Lineweaver-Burk plot for Shikoni (Figure 4), the value of Km and Vmax were obtained. It can be observed from the table presented below that the Vmax and Km value of Shikoni is 0.843 and 183.3 respectively while that of PDB is 0.366 and 81.04.

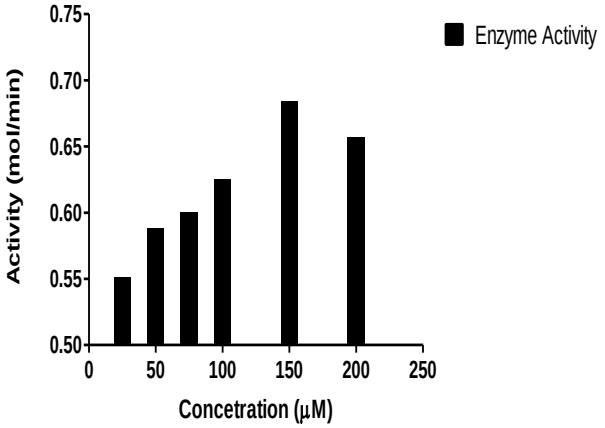

Fig. 1: Effect of shikoni on the activity of protein tyrosine phosphatases

Each bar represents the value in Mean + SEM of duplicate determinate of protein tyrosine phosphatases activity against six different concentrations of shikoni.

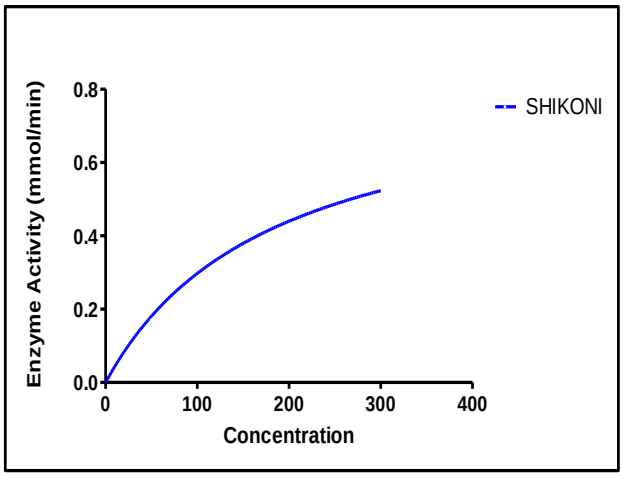

Fig. 2: Michaelis-Menten Kinetic study of Shikoni on PTPs activity

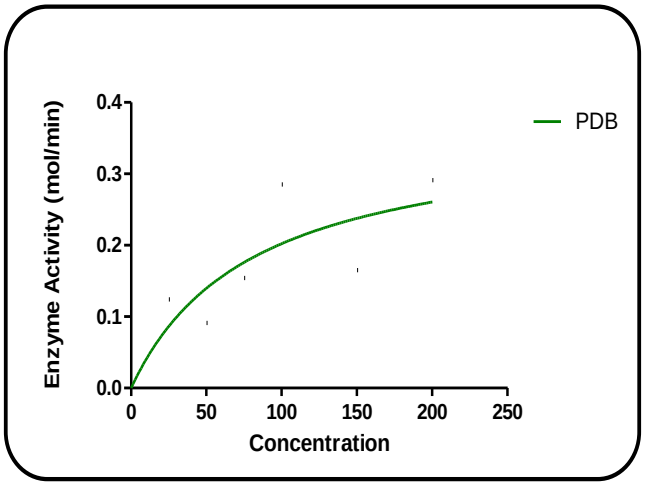

Fig. 3: Michaelis-Menten Kinetic study of PDB ligand on PTPs activity

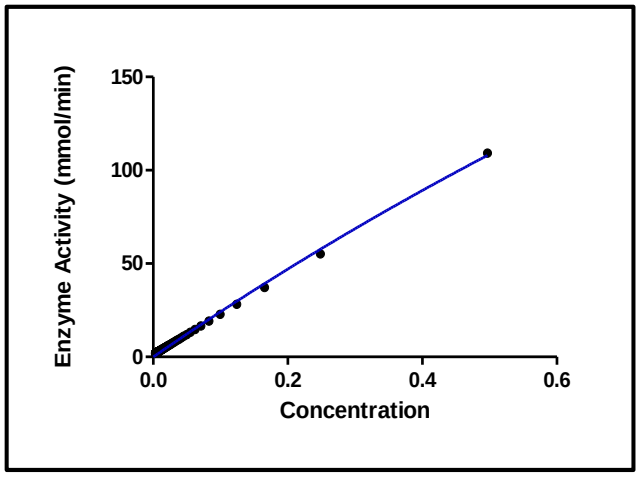

Fig. 4: Lineweaver-Burk plot of Shikoni eliciting a competitive inhibition on *protein tyrosine phosphatase* activity

Discussion

The secretary protein tyrosine phosphatases target multiple pathways within the host including the bacteria involved in tuberculosis pathogenic agent. Thus, identification of any conserved host proteins from mycobacteria should be considered.

Many attempts have been made to produce plant secondary metabolites by tissue or organ cultures of plants and due to this fact, study has advance to consider hairy root culture as an alternative to obviate challenges in the manufacture of the organ specific metabolites part of where shikoni inhibitor is derived.

The concentration of the effect of shikoni on the activity of the enzyme (protein tyrosine phosphotases) shows that 25µM of the concentration is the smallest at an enzyme activity value of 0.55mol/min; meaning that the substrate concentration (25µM) is mostly active at an absorbance level of 0.55mol/min.

Michaelis-Menten enzyme kinetic study graph presented in figure 2 reveals that the shikoni inhibitor on protein tyrosine phosphatases is competitive with a Km value of 183.3 higher than the Vmax value of 0.843 while in figure 3, the substrate concentration of PDB ligand reveals that the Vmax value (0.366) is lower than the Km value (81.04). Therefore, this result deduced that the effectiveness of shikoni on phosphomonoesterase activity of mycobacterium tuberculosis inhibit competitively using protein tyrosine phosphatases as enzyme.

The Lineweaver-Burk graph in figure 4 provided a Km value of 842.9 in the presence of inhibitor and Vmax value of 3.381 in the presence of inhibitor. Corresponding to the value of Km and Vmax obtained, it is deduced that shikoni inhibited mycobacterium tuberculosis in a competitive condition.

Since the enzyme (PTPs) is inhibited competitively, hence, shikoni inhibits with the substrate at the enzyme active site. The competition for binding site can be as a result of conformation in structure of the inhibitor and the substrate interaction. Also the inhibitor (shikoni) was able to bind with the enzyme leading to alteration of the enzyme activity.

IV. CONCLUSION AND FUTURE SCOPE

The protein tyrosine phosphatase enzymes deregulation is the major cause of several diseases including tuberculosis, diarrhea and typhoid in human. These diseases can be best prevented through the inhibition of the activity of the hosted PTP enzyme and exhibit remarkable role in pharmacological drug-like discovery and development.

Protein tyrosine phosphatase acts as potential drug target that enables scopes and initiative towards the development of modern treatment regimen. PTPs regulate the level of protein tyrosine phosphorylation under usual and pathological conditions which posses both positive and negative effects on cellular signal transduction; meanwhile abnormal activity of these enzymes is associated with numerous disorders including tuberculosis, therefore shikoni as an inhibitor in this study is effective for the development of drug – like inhibitor of mycobacterium tuberculosis.

AUTHORS' CONTRIBUTIONS

All laboratory work was carried out in the Biochemical laboratory of Kwara State Polytechnic. Formatting, review and edition was carried out by the author.

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STUDY LIMITATIONS

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Conflict of Interest

None

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