SHORT COMMUNICATION

Antituberculosis, antioxidant and cytotoxicity profiles of quercetin: a systematic and cost-effective in silico and in vitro approach

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ABSTRACT

The ineffectiveness and the slowdown of newer anti-TB drug approval rates directly indicate searching for potential alternative agents. However, validation of isolated phytochemicals through hit-and-trial experiments is more expensive and time-consuming. Simultaneously, cost-effective computational tools can recognize most potential candidates at an initial stage. The present study selected seven plant-derived polyphenols, then verified anti-TB and drug-ability profiles using advanced computational tools before the experimental study. Among all, the quercetin showed a potential docking-score within $-8$ to $-11$ kcal/mol than the standard isoniazid and ofloxacin, $-5$ to $-10$ kcal/mol. Additionally, quercetin exhibited a higher drug-ability score of 0.53 than isoniazid 0.19. Further, quercetin exhibited the minimum inhibitory concentration at 6 and 8 $\mu$g/mL, while ofloxacin showed at 2 $\mu$g/mL against InhA, and katG mutated Mtb-strains, respectively. Parallelly, quercetin showed promising free-radical-scavenging activity from nitric-oxide assay at IC$_{50}$ = 14.92 $\mu$g/mL, and lesser-cytotoxicity from cultured HepG2 cell lines at IC$_{50}$ = 159 $\mu$g/mL, respectively.

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1. Introduction

*Mycobacterium tuberculosis* (Mtb) is one of the most grievous infectious diseases with long-term morbidity and high mortality (Swain et al. 2020; WHO 2020). From the World Health Organization (WHO) records, approximately 8 to 10 million new cases and 1.4 million people died in 2019 were recorded globally (Swain et al. 2020; WHO 2020). In most cases, long-term anti-TB treatment with current medicines manifested from common nausea, vomiting to severe hepatotoxic and neurotoxic side effects (Mirlohi et al. 2016). Moreover, socio-economic factors such as illiteracy, poverty and malnutrition are significant barriers to completing the long-term anti-TB therapy and survival rate. As a result, a lack of awareness in early diagnosis and incomplete treatment courses directly added fuel to the rise of drug-resistant Mtb (Swain et al. 2020). Thus, there is an urgent requirement for newer anti-TB agents with high potency, less toxicity, fewer side effects with a short treatment course.

Alternatively, plant-crude extracts or individual phytochemicals bioactive and lesser toxicity profiles make them an ideal anti-TB agent (Amir et al. 2016; Sanusi et al. 2017; Mazlun et al. 2019). Comparatively, the multipotential polyphenolic class of phytoconstituents is safer and suitable for mainstream drug development with potential antioxidative and immune-modulating actions (Amir et al. 2016; Sytar et al. 2018; Wang et al. 2018; Mazlun et al. 2019). However, most active secondary metabolites are unable to fulfill the required drug-ability profiles during clinical validation. As a result, using such active candidates in mainstream medicine is a challenge (Shen et al. 2012; Ntie-Kang et al. 2019). Therefore, the present attempts to identify the most multi-potent polyphenol constituents on anti-TB, antioxidant, and cytotoxicity profiles by *in silico* and *in vitro* approaches (Figure S1).

2. Results and discussion

2.1. Ligand and target structures preparation for molecular docking

From docking analysis, quercetin (QUE) showed a higher docking score ranging from $-8$ to $-11$ kcal/mol than standard isoniazid (INH) ($-5$ to $-7$ kcal/mol), and ofloxacin (OFX) ($-7$ to $-10$ kcal/mol) against InhA and KatG enzymes of Mtb (Table S1). The molecular interactions also confirmed that the QUE strongly interacted by three H-bonds, ASP137, VAL230, and LEU265 (Figure S2) than the OFX by two H-bonds, LEU48 and LYS730 (Figure S3), with a mutated version of KatG (S315T). Thus, the computer-
aided drug design (CADD) could be a resource-saving protocol for identifying potential anti-TB phytochemicals before random experimental study with the traditional hit-and-trial method (Swain et al. 2018, 2019; Machado et al. 2018).

2.2. Physicochemical, pharmacokinetics and drug-likeness analyses

The physicochemical parameters of anti-TB drugs (INH and OFX) and selected eight polyphenols were recorded and analysed to observe the oral-drug suitability profiles (Table S2). Based on the prediction, the QUE exhibited a suitable druggable profile than INH but lesser than OFX (Table S2). Simultaneously, both anti-TB drugs and polyphenols showed comparatively similar ADME/T outlines. Overall, the QUE carries some suitable profiles for mainstream drug development (Table S2). Thus, the CADD tools are also helpful to assess physicochemical, possible activity, and drug-likeness profiles of any desired candidates before synthesis or experimental study.

2.3. Anti-TB potency of quercetin

The anti-TB potency of QUE with the positive control OFX and negative control dimethyl sulfoxide (DMSO) against INH-resistance strains (InhA and KatG mutated strains) were evaluated using the resazurin dye method. The MIC values of QUE, 6 and 8 µg/mL and OFX, 1 and 2 µg/mL respectively were recorded (Table S3). A natural product at this MIC value could be considered a productive result to use against INH-resistant strains individually or in combination with INH or OFX. The KatG mutated strain is more aggressive than the InhA mutated strain. The mutation at S315T in KatG is considered the universal mutation associated with 60–94% INH resistance, which was proved in silico in vitro herein, too (Aye et al. 2016).

2.4. Nitric oxide inhibition potency of quercetin

We have estimated the antioxidant potency of QUE using the pro-inflammatory mediator nitric oxide (NO) assay method. The IC50 at 20 and IC90 at 100 µg/mL from NO-assay were recorded (Figure S4). Mainly, NO-production influences tissue damage, chronic inflammation and plays a crucial role in the host-defence system during TB infection (Idh et al. 2012). Thus, the QUE proved as a potential anti-TB activity with antioxidant or free-radical scavenger activity.

2.5. Cytotoxicity profile of quercetin

The toxicity profile is directly proportional to the concentration of biological activity for a candidate or formulation (Machado et al. 2018). We have recorded the IC50 value of QUE at 159 µg/mL from cultured HepG2 cell lines (Figure S5). Mostly, INH and other front-line anti-TB drugs showed hepatotoxic activity; as a result, higher-dose treatment is not ideal (Li et al. 2016; Mirlohi et al. 2016). Thus, the immune-stimulant and lesser-toxic QUE could be an excellent alternative natural product-based drug candidate against Mtb.

Indian Ayurveda, Chinese traditional medicines, and Western regimens played a vital role in treating and controlling various human diseases, including TB from the primitive era
(Atanasov et al. 2015; Yuan et al. 2016). Polyphenolic secondary metabolites such as quercetin, fisetin, galangin, gossypetin, morin, myricetin, kaempferol, etc., are wildly distributed in the plant kingdom, and mostly from fruits, vegetables, herbs, cereals belong to Asteraceae, Rosaceae, and Lamiaceae plant family (USDA 2014; Anand David et al. 2016; Sytar et al. 2018). The advanced CADD with other multi-OMICS approaches are a suitable and cost-effective method to explore the anti-TB potency and other additive activity of phytochemicals and natural products (Mehralitabar et al. 2019; Goff et al. 2020). The WHO also motivates and supports the development of active natural/phytochemical-based anti-TB agents for the ‘END-TB’ mission. Thus, not only QUE, the selection and validation of most drug-able phytochemicals through combined computational and experimental approaches could be a cost-effective strategy in current anti-TB drug development.

3. Conclusions

The current anti-TB drugs are gradually becoming ineffective against emerging drug-resistant Mtb-strains and even showed several adverse or host-toxicity in long-term use. At this time, the systematic computational and experimental studies validated that QUE had a potential natural anti-TB regimen with MIC values of 6 and 8 μg/mL against InhA and KatG mutated strains. It also showed potential antioxidant activity from nitric oxide assay and lesser cytotoxicity in HepG2 cell lines. Thus, overall results concluded that the QUE could be considered as a potential alternative anti-TB agent for mainstream use.

Disclosure statement

The authors declare that they have no conflict of interests.

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