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Original Article

Inhibitory effect of thymoquinone from *Nigella sativa* against SARS-CoV-2 main protease. An in-silico study

Efeito inibitório da timoquinona de *Nigella sativa* contra a principal protease do SARS-CoV-2. Um estudo in silico

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Abstract

Nigella sativa is known for the safety profile, containing a wealth of useful antiviral compounds. The main protease (Mpro, 3CLpro) of severe acute respiratory syndrome 2 (SARS-CoV-2) is being considered as one of the most attractive viral target, processing the polyproteins during viral pathogenesis and replication. In the current investigation we analyzed the potency of active component, thymoquinone (TQ) of Nigella sativa against SARS-CoV-2 Mpro. The structures of TQ and Mpro was retrieved from PubChem (CID10281) and Protein Data Bank (PDB ID 6MO3) respectively. The Mpro and TQ were docked and the complex was subjected to molecular dynamic (MD) simulations for a period 50ns. Protein folding effect was analyzed using radius of gyration (Rg) while stability and flexibility was measured, using root means square deviations (RMSD) and root means square fluctuation (RMSF) respectively. The simulation results shows that TQ is exhibiting good binding activity against SARS-CoV-2 Mpro, interacting many residues, present in the active site (His41, Cys145) and also the Glu166, facilitating the pocket shape. Further, experimental approaches are needed to validate the role of TQ against virus infection. The TQ is interfering with pocket maintaining residues as well as active site of virus Mpro which may be used as a potential inhibitor against SARS-CoV-2 for better management of COVID-19.

Keywords: *Nigella sativa*; main protease; thymoquinone; SARS-CoV-2.

Resumo

Nigella sativa é conhecida pelo perfil de segurança, contendo uma grande variedade de compostos antivirais úteis. A principal protease (Mpro, 3CLpro) da síndrome respiratória aguda grave 2 (SARS-CoV-2) está sendo considerada como um dos alvos virais mais atraentes, processando as poliproteínas durante a patogênese e replicação viral. Na presente investigação analisamos a potência do componente ativo, timoquinona (TQ) de Nigella sativa contra SARS-CoV-2 Mpro. As estruturas de TQ e Mpro foram recuperadas de PubChem (CID10281) e Protein Data Bank (PDB ID 6MO3), respectivamente. O Mpro e o TQ foram acoplados e o complexo foi submetido a simulações de dinâmica molecular (MD) por um período de 50ns. O efeito de dobramento de proteínas foi analisado usando o raio de giração (Rg) enquanto a estabilidade e a flexibilidade foram medidas usando a raiz quadrada média dos desvios (RMSD) e a raiz média quadrada da flutuação (RMSF), respectivamente. Os resultados da simulação mostram que o TQ está exibindo boa atividade de ligação contra o SARS-CoV-2 Mpro, interagindo em muitos resíduos presentes no sítio ativo (His41, Cys145) e também o Glu166, facilitando o formato da bolsa. Além disso, são necessárias abordagens experimentais para validar o papel do TQ contra a infecção pelo vírus. O TQ está interferindo nos resíduos de manutenção do bolso, bem como no sítio ativo do vírus Mpro, que pode ser usado como um potencial inibidor contra o SARS-CoV-2 para um melhor gerenciamento da Covid-19.

Palavras-chave: *Nigella sativa*; protease principal; timoquinona; SARS-CoV-2.

1. Introduction

The main protease (Mpro, 3CLpro) is an important drug targets of sever acute respiratory syndrome 2 (SARS-CoV-2) proteome, processing the polyproteins. A number of studies has well characterized the Mpro (Anand et al.,

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2005; Liang, 2006; Gan et al., 2006; Xue et al., 2008; Pillaiyar et al., 2016) and its potential role. This viral protein acts on 11 positions along polyproteins. Human proteases do not share the cleavage specificity with NSP5 of SARS-COV-2 (Lee et al., 2020, p. 2).

The substrate-binding sites residues 10-99 (Domains I) and 100-182 (Domain II) in picornavirus, are six-stranded antiparallel β-barrels while domain III (198-303) forming five helices, regulating the dimerization of Mpro (Shi and Song, 2006). Residues Cys145 and His41 form the catalytic site. The catalytic activity depends on the dimerization of the enzyme, as the N-finger interacts with Glu166 to facilitate the S1 pocket shape of the substrate-binding site (Anand et al., 2002). The residue T285 and I286in CoV-2 Mpro is substituted by A285 and L286 respectively (Zhang et al., 2020) leading to a threefold upsurge Mpro (Lim et al., 2014). Inhibitors may be useful to reduce the catalytic degree against these locations.

Phytocompounds have been found, affective against many viral targets (Raj and Varadwaj, 2016; Setlur et al., 2017; Ismail and Jusoh, 2017; Li et al., 2020; Khare et al., 2020). Among the plants, Nigella sativa (Black cumin) is an annual flowering plant under the family Ranunculaceae (Amin and Hosseinzadeh, 2016). Its fruit is in inflated capsule of seeds, native to North Africa, Southeast Asia, Southern Europe, Mediterranean and Middle Eastern region (Ahlatci et al., 2014). N. Sativa seed is composed of some major components including 35.6-41.5% of fatty oil, fat (28.5%), proteins (26.7%), carbohydrates (24.9%) and several vitamins (A, B1, B2, B3, C) and minerals (Ca, K, Se, Cu, P, Zn, Fe) (Ahlatci et al., 2014; Islam, 2016). The volatile oil of N. sativa seeds has saturated fatty acids including thymohydroquinnone (THQ), dithymohydroquinone, carvacrol, thymoquinone (TQ), nigellone, thymol, α and β-pinene, d-citronellol, d-limonene, p-cymene volatile oil, t-anethole, longifoline and 4-terpineol (Enomoto et al., 2001). The medicinal characteristics focusing on various pharmacological efficacies of N. sativa seeds like: gastroprotective (El-Abhar et al., 2003), anti-oxidant (Hosseinzadeh et al., 2013), anti-cancer (Khan et al., 2011), anti-viral activity against cytomegalovirus have been reported in recent years. In some studies, the TQ was effective against avian influenza virus (H9N2 AIV) and cytomegalovirus infection in murine model l (Salem and Hossain, 2000; Umar et al., 2016). Nigella sativa extract prior decreases the coronavirus replication and significant reduction in coronavirus survival virus load inside cells. Recently, a am insilico study also proposed that thymoquinone (TQ) may interfere with ACE2 binding receptors, preventing virus entry.

In the drug discovery and their mechanism of action are important for better understanding the insight of the molecules. The molecular biologist desire to know that how a protein and small molecules works. An atomic level information is typically generating significant insight information of biomolecular interactions. The intermolecular interactions could be explored through the dynamic's studies. Unfortunately, such kind of information is difficult to obtained through experimental approaches. An alternative to such approaches is computational molecular dynamics simulation (MD) of proteins and natural compounds to understand the atomic level mechanism underlined. The MD simulations are time efficient and may accurately predict how interact (Liu et al., 2018; Hollingsworth and Dror, 2018). These MD studies are useful to capture a variety of biomolecular interactions, including ligand binding, protein folding, and changes in proteins behavior over time.

Knowing the strength of MD simulations and the importance of pharmacological characteristics of N. sativa, we performed the current study on thymoquinone (TQ) against Mpro to analyze the behavior of target protein at molecular level and their molecular effect on the SARS-CoV-2 target proteins.

2. Methods

2.1. Protein preparation

The worldwide biomolecular structural information is being archived at Brookhaven National Laboratories, called Protein Data Bank (PDB) (Bernstein et al., 1977; Berman et al., 2000). The researchers around the world can easily retrieve the crystal structures of biomolecules. The crystal structure of COVID-19 Mpro (PDB ID: 6M03) was retrieved from PDB. Prior to further analysis, the structure was subjected to Preparation, using MOE (molecular operating environment) (Vilar et al., 2008). The partial charges and missing hydrogen were assigned. The thymoquinone (CID:10281) was also prepared. Protein and ligand were docked and the complex was subjected to dynamics study.

2.2. Molecular Dynamics (MD) simulation

The molecular dynamics (MD) simulations in drug discovery capture the behavior of proteins in full molecular and atomic detail to an extent of fine temporal resolution (Liu et al., 2018; Hollingsworth and Dror, 2018). The Mpro and TQ docking complex were subjected to molecular dynamics (MD) simulation as described in our previous study using Amber package (Berendsen et al., 1995; Khan et al., 2020). Briefly, MD simulation was performed on docking complex with the ff14SB force field through Amber14 package (Salomon-Ferrer et al., 2013; Sun et al., 2014b, a). To solvate each system the TIP3P water model was applied while system was neutralized with counterions (Jorgensen et al., 1983). The system was energy minimized and conjugate gradient followed by heating upto 300K and 1atm pressure to equilibrate the system. Temperature regulations was achieved with the Langevin thermostat while Particle Mesh Ewald algorithm was applied for long-range electrostatic interactions (Essmann et al., 1995; Darden et al., 1993). The MD simulation production step was carried with pmemd code 30 (Götz et al., 2012).

3. Results and Discussion

The current study shows that TQ may be effective against Mpro of SARS-CoV-2. The calculation of drug-likeness may help to understand the pharmacokinetic of a novel compound as well the pharmaceutical properties before its clinical application. The TQ drug likeness properties,

calculated through Swiss ADME, is also in accordance the drug likeness rule (Walters and Murcko, 2002; Daina et al., 2017). The drug likeness properties and absorption has been given in Table 1. The pharmacokinetics of TQ seems in accordance with desire drug like compound. Similarly, drug likeness properties also favor its clinical application (Brüstle et al., 2002; Vistoli et al., 2008; Ursu et al., 2011). The TQ docked against SARS-CoV-2 Mpro seems potent, altering the stability of protein. The RMSD of TQ and Mpro in Figure 1 shows stability. The Mpro is not stable in the whole simulation period which might be useful for effective inhibition of viral activity.

The RMSD graph during the 50ns simulation period shows that the Mpro exhibited an unstable fluctuation. The RMSD at the start (1.06Å) is rising to 2.9Å at 18ns simulations. A downfall fall in fluctuation was again observed to 1.4Å at the end of 50ns MD simulation. The RMSD of Mpro seems highly unstable due to TQ which may alter the stability of target, assisting in the inhibition of viral proteins. Flexibility is also one of the important thermodynamic properties, maintaining the optimal functions of proteins (Nagasundaram et al., 2015). A large change in this property may alter the biomolecules optimal function. The TQ may cause an increase in the flexibility of Mpro (Figure 2) which might be useful for better management of SARS-CoV-2 infections. The Mpro exhibited the RMSF among 5Å and 25Å at residues position 48 and 310 respectively. Residues at location 145 to 160 also attained a high RMSF (20.4Å) which contains the active site residue Cys145. MD simulations may explore the insight mechanisms of changes at the molecular level (Liu and Yao, 2010; Liu et al., 2018; He et al., 2018) which might be difficult through experimental work. Several studies reported that any change in protein function might be due the change in RMSF (Berhanu and Masunov, 2011; Chong et al., 2011; Bavi et al., 2016).

The degree of folding stability could be measured through Rg. Fluctuations in Rg with respect to time shows unstable folding while a straight value reveals stability in folding (Lobanov et al., 2008; Smilgies and Folta-Stogniew, 2015; Khan et al., 2019, 2021). A protein with misfolding shows variations in Rg over time (Figure 3). The plot shows large variations between 22Å and 22.8Å. Majority of the variations have been found from 811ps to 2026ps.

Table 1. Chemical properties of thymoquinone.

Thymoquinone	Pharmacokinetics
*permeant to BBB	Yes
*Absorption (GI)	High
Inhibitor of CYP2C19	No
Inhibitor of CYP1A2	No
Inhibitor of CYP2C9	No
Inhibitor of CYP2D6	No
CYP3A4 inhibitor	No
	Drug likeness
Lipinski	Yes (0 violations)
Veber	Yes
	Physiochemical Properties
Formula	C10H12O2
Molecular weight	164.20 g/mol
Heavy atoms	12
*Rot: bonds	1
*HB acceptors	2
HBdonors	0

*BBB = blood brain barrier; GI = gastrointestinal; Rot = rotatable; HB = hydrogen bond.

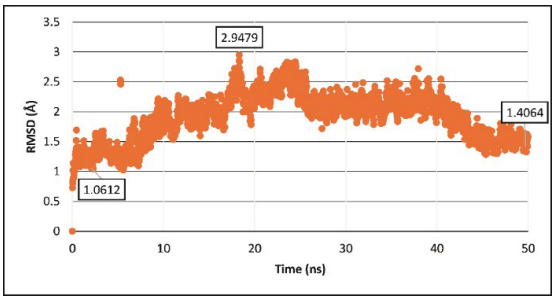

Figure 1. RMSD of TQ and Mpro during the 50ns MD simulation period. Stability seems fluctuating from 1.0612Å at 2ns and 2.94 Å at 18ns. Target stability seems unstable even at the end of simulation period.

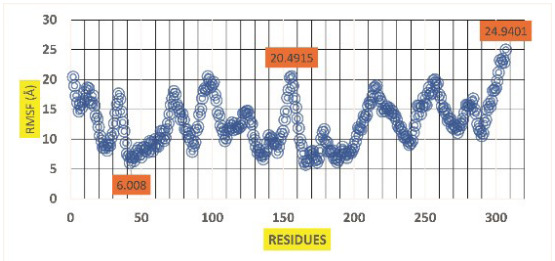

Figure 2. Residue's flexibility of TQ and Mpro complex during simulation. Flexibility is very high in last amino acid residues. This high flexibility may change the protein function, required for virus activity.

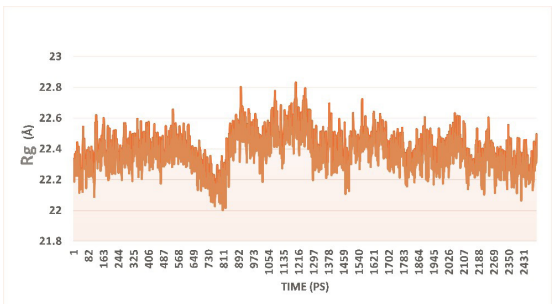

Figure 3. Radius of gyration TQ and SARS-CoV-2 Mpro complex. A constant Rg value is a measure of correct folding. Fluctuations in Rg shows that protein folding is not stable.

The lowest Rg was detected at 811ps (22Å) while the highest at 1216ps (22.8Å). This shows that the TQ may affect the folding of Mpro which might be important to inhibit the protein activity. The Rg plot of Mpro is not stable during the simulation peried which shows the potential activity of TQ. Change in protein stability may be due to the alteration of thermodynamic property (Chen and Shen, 2009). This includes protein RMSD, fluctuations and also the protein folding. Destabilization in folding and thermodynamic stability may affect biomolecules function.

The TQ is fitting in the pocket, interacting with active site (C145, H41) (Figure 4), altering the catalytic activity of viral protein. The residues located in the binding pocket and its surrounding (T24, L27, H41, F140, C145, H163, M165, P168, and His172) are imported for a natural compound to interact with. The phytocompound TQ form a hydrogen bond with Glu166, facilitating the pocket shape of the substrate-binding site (Anand et al., 2002) and many hydrophobic interactions with active site (His41, Cys145) and its surrounding residues (Figure 5).

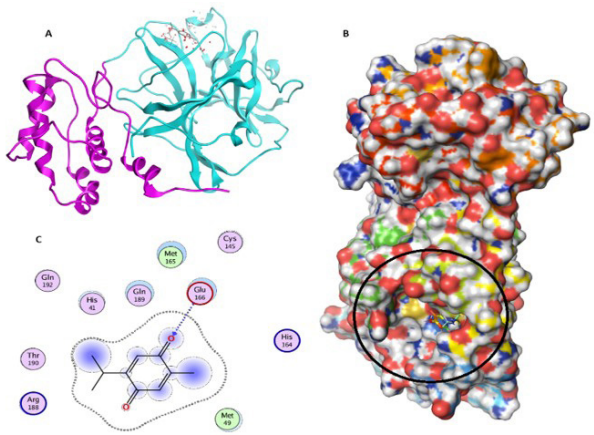

Figure 4. Thymoquinone and SARS-CoV-2 main protease interaction. (A) Docked thymoquinone. (B) Thymoquinone in binding Pocket. (C) Residues in the surrounding thymoquinone.

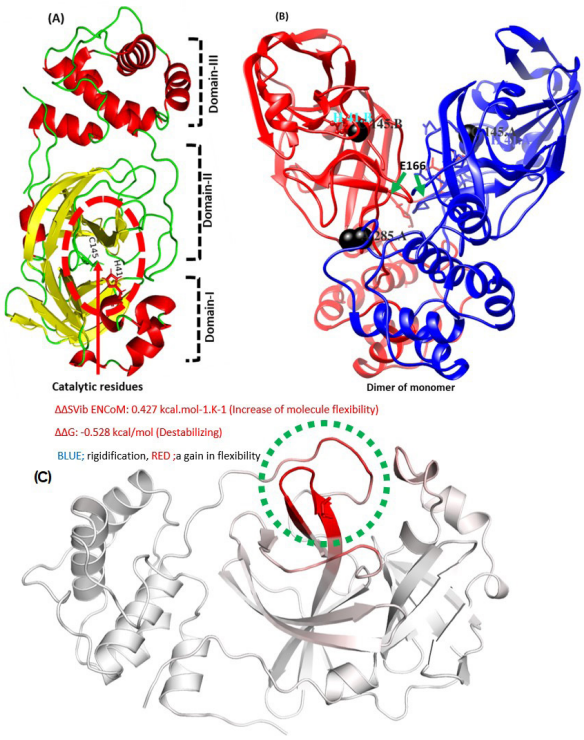

Figure 5. Mpro of SARS-CoV-2. (A) Domain organization. Active site residues have been shown. (B) Dimerization of two Mpro monomers and location of E166. (C) Impact of E166A mutation on the dynamics of Mpro. The mutant gain flexibility and show destabilizing effect (Rodrigues et al., 2018).

4. Conclusion

TQ shows good binding affinity with SARS-CoV-2 NSP5, interacting with active site residues and also with Glue166, maintaining the pocket shape for viral enzymatic activity. This phytomedicine alters the overall thermodynamics properties of SARS-CoV-2 Mpro which may useful for better management of COVID-19 in future. Further experimental validation is required to observe the TQ effect in vivo. The TQ may be used as therapeutic compound against SARS-CoV-2 after experimental confirmation.

Abbreviations

AIV: Avian influenza virus
COVID-19: Coronavirus disease-19
MOE: Molecular operating environment
Mpro: Main protease
MD: Molecular dynamics
NSP5: Non-structural protein 5
NPT: Number of moles, pressure, temperature
NVT: Number of moles, volume, temperature
RMSD: Root means square deviation
RMSF: Root means square fluctuation
Rg: Radius of gyration
SARS-CoV-2: Severe acute respiratory syndrome 2
TQ: Thymoquinone
PDB: Protein data bank
SPC: Simple point charge

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