

Computational Analysis For Revealing The Role of Thymoquinone (Active Compound From Ethanolic Extract of *Nigella sativa*) as Inhibitor of P65 NF-kb Activation in Preclampsia Treatment

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Available Online: 1st May, 2016

ABSTRACT

Thymoquinone is the main active compound from *Nigella sativa* and can be used as traditional medicine such as preclampsia treatment. Inflammation is one of the pathophysiologic process in Preeclampsia. Preeclampsia is a systemic inflammatory disease that induces endothelial dysfunction as the main disorder. The aim of this study is to evaluate the role of thymoquinone as antiinflammatory through computational study. The method for predicting the activity is Pass Server online that can predict activity of chemical compound based on structure activity relationship. Model of thymoquinone was retrieved from pubchem and model of NF-kB inhibitor was collected from protein data bank. The prediction of protein-protein interaction was conducted using string db and molecular docking analysis for evaluating the potential inhibition of thymoquinone was done using patchdock and firedock web service. Molecular interaction was done using ligplot and the visualization of biomolecules was used PyMol, Chimera and Ligand Scout. The result showed that thymoquinone have antiinflammatory activity indicated by probability score (Pa) 0.6. Docking result showed that thymoquinone can bind to NF-kB inhibitor with high binding affinity (-4.10 Kcal/mol) and it interact with threonin 39 using hydrogen bond and three hydrophobic interaction. The complex can inhibit the phosphorylation process of NF-kB inhibitor. It can be concluded that Thymoquinone is potential for inhibiting the p65 NF-kB activation through inhibit Ikb. Thymoquinone from *nigella sativa* extract can be a candidate for preclampsia treatment in the future.

Keywords: inflammation, p65 NF-kB, preclampsia, thymoquinone.

INTRODUCTION

Nigella sativa is well-known as traditional medicine in some countries such as Indonesia and Middle east countries. Black cumin seed from *nigella sativa* extract can be used as antihypertension, antiinflammation, immunomodulator and anticancer. Thymoquinone is identified as the most potential active compound from black cumin seed¹. Previous study explained that thymoquinone can act as antiinflammatory. It is predicted as NF-kB inhibitor, NF-kB has the main role as transcription factor in inflammatory condition. But there is no evidence data for supporting this information, especially the mechanism of p65 NF-kB inhibition²⁻⁴. Based on that fact, thymoquinone is potential for preclampsia treatment because it can inhibit NF-kB activation. Preeclampsia is disorder of pregnancy indicated by high blood pressure and a large amount of protein in the urine⁵. The disorder usually caused by genetics, inflammation and oxidative stress. Those factors can cause decreasing of Trophoblast invasion, So hypoxia occur in placenta. hypoxia condition can induce Trophoblast cell for producing proinflammatory cytokines that cause endothel dysfunction. this dysfunction initiate preclampsia symptom that are hipertention and proteinuria⁶⁻⁹. Therefore, the research aim is to evaluate

the role of thymoquinone for preclampsia treatment molecular docking approach was used for revealing the mechanism of p65 NF-kB inhibition.

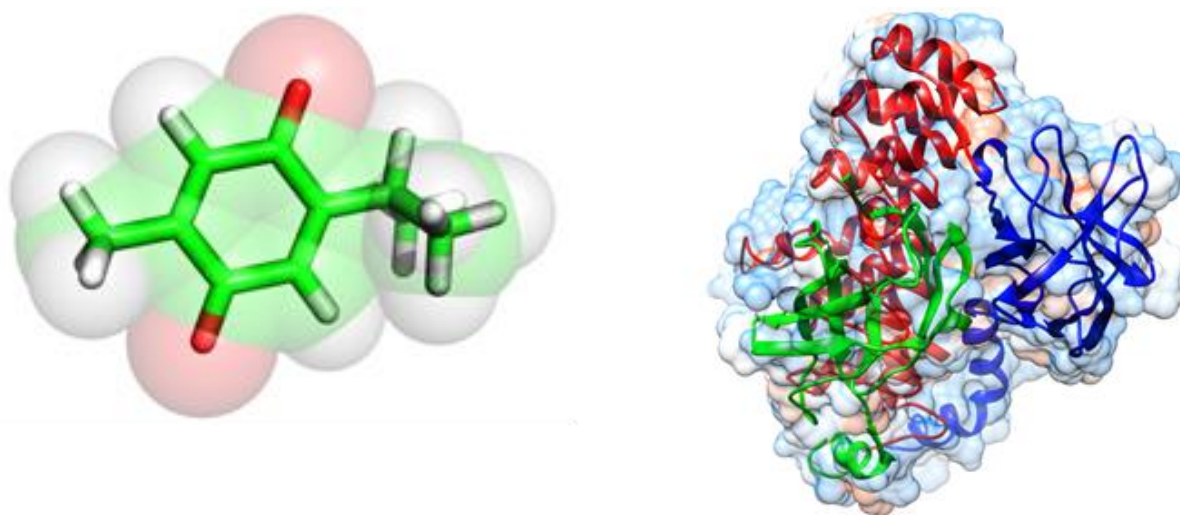
METHODS

Thymoquinone and protein structure

Thymoquinone as active compound was retrieved from PUBCHEM (CID 10281). 3D chemical structure was minimized using openbabel for eliminating bad contact. While protein NF-kappa-B p65-p50 complex was identified can be activated when the inhibitor (ikkb) was phosphorylated. So for inhibiting the translocation mechanism (NF-kB activation), ikkb must be inhibit by active compound so ikkb could not be phosphorylated. Model protein of ikkb bind with P65 was retrieved from Protein Data Bank (PDBID:1K3Z). It is experimental structure using X-Ray Diffraction with 2.5 Å resolution. 3D structure was prepared using VEGA ZZ Software for eliminating water molecules and other ligand in the complex. Action mechanism of NF-kB can be predicted by protein interaction network. The analysis was conducted using STRINGDB¹⁰.

Computational analysis for antiinflammatory

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A B
Figure 1: Structure of Thymoquinone (a) model of IKKB complex with NFKB-p65 (b)

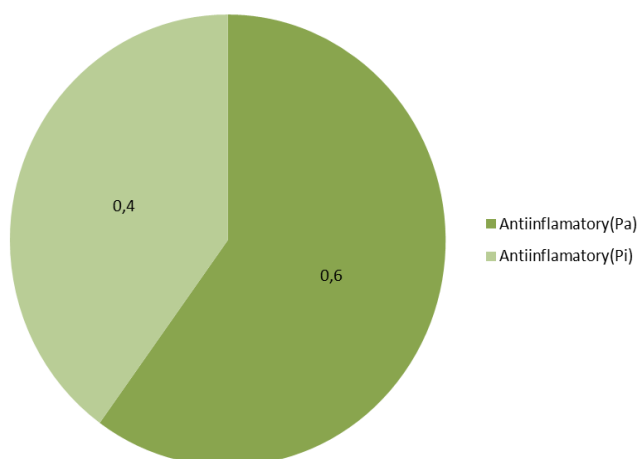


Figure 2: Probability activity of thymoquinone as antiinflammatory

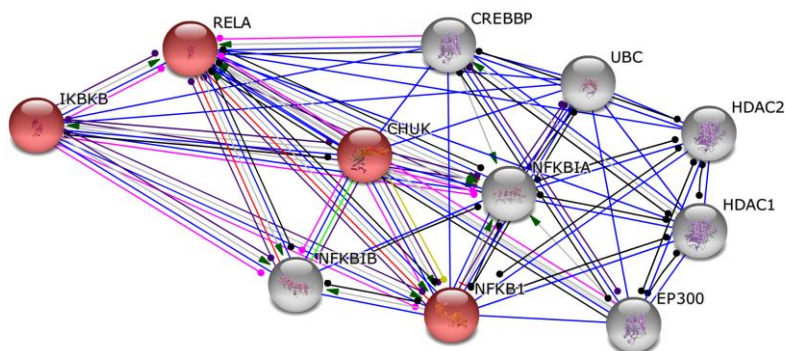


Figure 3: Protein-protein interaction of inflammatory response (red color)

Antiinflammatory activity of active compound can be predicted using computational study. The prediction is based on structure activity relationship (SAR). Program for prediction is PASS SERVER. The result was indicated by Probability activity score (Pa)¹¹.

Molecular Docking of thymoquinone with ikkb

Molecular docking process was done using patchdock and Firedock webservices. Docking process was conducted for predicting the binding affinity of

thymoquinone with ikkb protein. Specific docking approach was used in this experiment. We docked the active compound directly on the active site of ikkb. For revealing the mechanism of inhibition, we evaluated the type on interaction and amino acid that had role in the interaction. Molecular interaction analysis was done by LIGPLOT program¹².

Molecular visualization of biomolecules

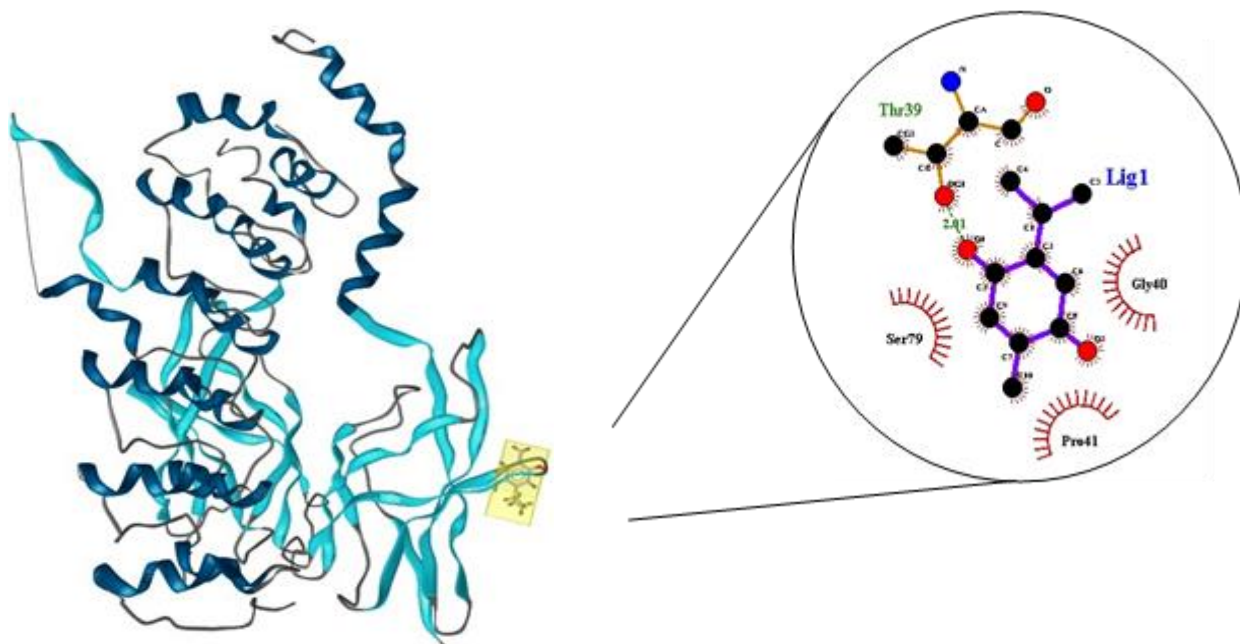


Figure 4: Molecular interaction of thymoquinone with Ikb, there are one hydrogen bond and three hydrophobic interaction.

Table 1: Molecular properties of thymoquinone

Molecular Weight	164.20108 g/mol
Molecular Formula	C ₁₀ H ₁₂ O ₂
XLogP3	2
Hydrogen Bond Donor Count	0
Hydrogen Bond Acceptor Count	2

Molecular visualization was done by several program that are, PyMol v1.3, Chimera 1.8.1 and Ligandscout v.2.0^{13,14}.

RESULT AND DISCUSSION

Thymoquinone is an potential active compound isolated from *nigella sativa*. For evaluating the antiinflammatory potential of this compound computationally, molecular properties of thymoquinone can give initial information about the activity (Table 1).

Compound is more likely to be membrane permeable and easily absorbed by the body if it matches the following criteria:

Its molecular weight is less than 500.

The compound's lipophilicity, expressed as a quantity known as logP (the logarithm of the partition coefficient between water and 1-octanol), is less than 5.

The number of groups in the molecule that can donate hydrogen atoms to hydrogen bonds (usually the sum of hydroxyl and amine groups in a drug molecule) is less than 5.

The number of groups that can accept hydrogen atoms to form hydrogen bonds (estimated by the sum of oxygen and nitrogen atoms) is less than 10.

The rules, based on the 90-percentile values of the drugs' property distributions, apply only to absorption by passive diffusion of compounds through cell membranes; compounds that are actively transported through cell

membranes by transporter proteins are exceptions to the rule. Due in no small part to their simplicity, the Lipinski criteria are widely used by medicinal chemists to predict not only the absorption of compounds, as Lipinski originally intended, but also overall drug-likeness. Thymoquinone meet the requirement for drug because it can pass through the permeable membrane. Thymoquinone can bind to target protein directly.

Antiinflammatory activity of thymoquinone is relative high. Probability activity (Pa) is 0.6 (Figure 1). this score showed that possible activity for antiinflammatory. If the Pa > 0.3 so the compound can active as antiinflammatory computationally. But it needed to test in-vitro for validating the activity¹¹.

Previous study stated that thymoquinone has antiinflammatory effect. The computational analysis support that result. But for molecular mechanism is still unclear. We try to evaluate the mechanism of inflammatory response that mediated by NF- κ B (Figure 2). We used protein-protein analysis network for revealing the mechanism. The result showed that NF- κ B (RELA) can activated by IKB. IKB has important role for controlling the activation of NF- κ B. So this protein can be a target for inhibiting the inflammatory response.

NF- κ B inhibitor beta (Ikb-B0) can inhibits NF- κ B by complexing with and trapping it in the cytoplasm. However, the unphosphorylated form resynthesized after cell stimulation is able to bind NF- κ B allowing its transport to the nucleus and protecting it to further NFKBIA-dependent inactivation. Association with inhibitor kappa B-interacting NKIRAS1 and NKIRAS2 prevent its phosphorylation rendering it more resistant to degradation.

Molecular docking analysis showed that the protein can interact with thymoquinone with high binding affinity.

Docking score was -4.10 Kcal/mol. It means the thymoquinone can bind directly with the IKB in the cytoplasm. Negative value indicated that the complex is favorable. For evaluating the inhibition mechanism. It can be evaluated by the binding site and the amino acid that interact with the thymoquinone. We found that there is one hydrogen bond that from threonine 39 that interact with thymoquinone (Figure 3). Amino acid threonine is the key for phosphorylation process. If this amino acid was bind with the thymoquinone so the Ikb cannot be phosphorylated. The effect of this binding is p65 NF- κ B can not be translocated to nucleus. P65 NF- κ B remains in cytoplasm and will be degraded soon^{4,15}.

CONCLUSION

Thymoquinone is potential for inhibiting the p65 NF- κ B activation through inhibit Ikb. If Ikb cannot be phosphorylated so there is no translocation of p65 NF- κ B to the nucleus to activates several genes that related to inflammatory responses. It can be concluded that thymoquinone from *nigella sativa* extract can be used as drug candidate for preeclampsia treatment.

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