In Silico: γ-Oryzanol as Anti-inflammatory During Folliculogenesis in Rattus Novergicus Exposed to Pyrethroid Aerosol

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Abstract. The use of insect repellent is a disease preventive effort due to mosquito vectors as well as sources of pollutants that disrupt human health, especially reproductive health. This study aimed to determine the effect of γ-Oryzanol on the expression of Foxo3a, TNF-α, GDF-9 in the folliculogenesis of mice (Rattus Novergicus) exposed to pyrethroid aerosol mosquitoes. The research use bioinformatics technique with in silico approach. The result of study showing that γ-oryzanol has the potential as an anti-inflammatory and NO scavenger, the influence of the active compound γ-oryzanol on Foxo3 expression obtained pathway analysis with the results of the prediction of the target HITPICK able to bind to APP, AKT1 and Mtor expressions through modification of post-translational Foxo1, Effect of γ-oryzanol on Gdf9 expression, through bonding with APP, AKT1, AKT3, Pik3cg and modifying Smad 4 post-translation, The effect of the active compound γ-oryzanol on TNF-α expression via APP binds with AKT1, AKT3, AKT2 then activates IkbkB and bindings with Casp3, Fadd, Tradd, Tnfrsf1a, Tnfrsf1b, Traf3, Birc2, Ikbkg. Based on average part length analysis AKT1 is the fastest interact with protein target. The conclusion, γ-oryzanol has predicted to be used for the treatment of insecticide exposure, because it can act as an anti-inflammatory, and lipid peroxidase inhibitor. This condition influences normally folliculogenesis with stable expression of Foxo3a, GDF 9 and TNF-α.

Keywords: γ-Oryzanol, Folliculogenesis, Pyrethroid

1. Introduction

Indonesia as a tropical climate has the potential to become a hotbed for developing mosquitoes. The results of a survey by Sunaryo (2016) that most people use household insecticides (86.33%) with the highest intensity of use once a day (85.4%) for more than 5 years (74.51%) [1]. The types of use of mosquito repellent, spray or aerosol (12.2%) are widely used [2].
The insect repellent uses synthetic parathyroids [3,4]. Pyrethroids as xenoestrogens and act as hormone disruptors or known as Endocrine Disruptor Chemical (EDC) [5].

The use of pyrethroid active ingredients in aerosol mosquito repellents causes adverse effects on health by causing oxidative stress. This is indicated by a decrease in the level of Extracellular Superoxide (EC-SOD) to fight ROS [5].

Through the PI3K-Akt approach Exposure to parathyroid aerosol mosquitoes during cellular folliculogenesis can result in cell damage through increased ROS levels and indicates the occurrence of oxidative stress. PI3K pathway it can be seen how the expression of Foxo3a, GDF-9 and TNF-α so that in this condition there is a need for antioxidant and anti-inflammatory sources to repair damaged cells during the folliculogenesis process [6,7,8].

This condition needs to be followed by changes in healthy lifestyles including through healthy food supplements that contain antioxidants and anti-inflammatory. Brown rice bran has higher antioxidant activity than other bran variants, which is 68.66 ± 3.682 ppm and it is known that the bioactive composition of red rice is as follows; -oryzanol 24,201 ± 945 ppm, β-carotene 15,007 ± 482 ppm, tocopherol 3,706 ± 460 ppm [9]. This study use γ-Oryzanol an inflammatory and antioxidant source to suppressing complication insecticide exposes by parathyroid in folliculogenesis process.

2. Methods
2.1. Target selection
2.1.1. Pass Server. Pass server is a structural approach the server will compare the inputted compounds with compounds that have been shown to have certain activities. The higher the Pa value, the more similar the structure and function of the compound.

2.1.2. Way2drug. The analysis uses the way2drug web server to predict the bioinformatics potential of the γ-oryzanol compound.

2.1.3. SMILE. The target protein for γ-oryzanol is obtained by entering the PUBCHEM SMILES server, (http://pubchem.ncbi.nlm.nih.gov/ The server provides a list of predicted target proteins for γ-oryzanol; these are compared, and target proteins with the most potential as a ligand γ-oryzanol (PUBCHEM ID 5282164).

2.2. Intermolecular interaction
2.2.1. STRING. The interaction of protein using STRING from γ-oryzanol interacts with the target protein GDF9, FOXO3, TNF through the intermediary of several proteins.

2.3. Average analysis transport part length
Average transport path length analysis is an analysis to find the fastest and most effective path from a network pathway.

3. Results and Discussion
3.1. Pass server

| Bioactivity                  | Probability Active of Score (Pa) |
|------------------------------|----------------------------------|
| Antiinflammatory             | 0.66                             |
| Immunosuppressant            | 0.538                            |
| Antioxidant                  | 0.456                            |
| Lipid peroxidase inhibitor   | 0.457                            |
| Free radical scavenger       | 0.196                            |
| Apoptosis agonist            | 0.844                            |
| Caspase 3 stimulant          | 0.832                            |
Table 1 shows that the Pass Server analysis, the $\gamma$-oryzanol is predicted to be used for the treatment of inoxide exposure, because it can act as an antiinflamatory, and lipid perosidase inhibitor.

Insecticides can trigger oxidative stress. This oxidative stress can induce lipid peroxidase. Lipid peroxidase makes vasodilation change due to increased Selectin, ICAM, VCAM resulting in displacement of proinflammatory molecules such as cytokines, macrophages, LDL and immune cells. This molecule then undergoes oxidation and inflammation. As a result, endothelial cells become damaged and inflammation occurs, the appearance of plaque and other disorders because the barrier is dysfunctional.

The final product of lipid peroxidase is MDA (Reactive Aldehyde / Malodialdehyde) and 4-HNE. Both of these molecules can cause DNA adducts. DNA adduct is a DNA segment attached to a chemical, which can induce mutations. This mutation can cause the emergence of certain diseases such as cancer. While $\gamma$-oryzanol has a role that is predicted to neutralize oxidative stress because it can act as an antioxidant and lipid peroxidase inhibitor.

3.2. Way2drug

Figure 1. Probability active $\gamma$-Oryzanol.

Figure 1. show that The bioactive potential of $\gamma$-oryzanol through the in silico approach has the potential as an anti-inflammatory and NO scavenger.

3.3. SMILE

Objective: To find out the interactions of Cypemethrine, Transfluthrin, and Prallethrine on TNF, FOXO3, and GDF9 proteins

Sample: Gamma oryzanol (PUBCHEM ID 5282164)

SMILE: CC (CCC = C (C) C) C1CC2 (C1 (CCC34C2CCC5C3 (C4) CCC (C5 (C) C) OC (= O) C = CC6 = CC (= C (C = C6) O) OC) C) C

Figure 2. Intermediate protein target of $\gamma$-Oryzanol.
Table 2. Prediction signaling target.

| Compound               | Signaling target | Result                                                                 |
|------------------------|------------------|------------------------------------------------------------------------|
| Cypemethrine and Transfluthrin | Foxo3            | Cypemethrin dan Transfluthrin are predicted to target SCN1A protein. Protein SCN1A protein can bind with SCN8A dan SGK1,1 to influence FOXO3 expression |
|                        | GDF9             | Cypemethrin and Transfluthrin are predicted to target SCN1A protein. The SCN1A protein can bind with SCN8A and SGK1, to then influence FOXO1 expression. Foxo 1 can bind with SMAD2 and then SMAD2 can affect GDF9 expression either directly or through ACVR1B activation |
|                        | TNF              | Cypemethrin and Transfluthrin are predicted to target SCN1A protein. The SCN1A protein can bind with SCN8A and SGK1, to then affect MTOR expressions. MTOR is predicted to have interactions with TNF even though the interactions that occur do not yet know the type of interaction |
| Prallethrin            | Foxo3            | Prallethrine is predicted to target PRSS1 and F2 proteins. F2 itself has interactions with SGK1 even though the type of interaction is not yet known. SGK1 itself is predicted to affect the expression of FOXO3 |
|                        | GDF9             | Prallethrine is predicted to target PRSS1 and F2 proteins. F2 itself has interactions with SGK1 even though the type of interaction is not yet known. SGK1 itself is predicted to affect the expression of FOXO1. Foxo 1 can bind with SMAD2 and then SMAD2 can affect GDF9 expression either directly or through ACVR1B activation |
| γ-oryzanol             | Foxo3            | γ-oryzanol is predicted to target Elane, APP, and Prtn3 proteins. Elane and PRTN3 proteins can interact with PRSS1. PRSS1 interacts with F2R and F2. F2 itself has interactions with SGK1 even though the type of interaction is not yet known. SGK1 is predicted to affect the expression of FOXO3 |
|                        | GDF9             | γ-oryzanol is predicted to target Elane, APP, and Prtn3 proteins. The APP interacts with IKBKG and IKBKB to then interact with SMAD2 to go to GDF |
|                        | TNF              | γ-oryzanol is predicted to target Elane, APP, and Prtn3 proteins. APP proteins are predicted to be able to interact with TNF, although we cannot yet know the types of interactions that occur. |

3.4. STRING

Table 3. Prediction of bioactive target protein and biology process.

| No | Compound | Target | Interaction   | Biology Process       | Precision (%) | TC Similarity |
|----|----------|--------|---------------|-----------------------|---------------|---------------|
| 1  | γ-oryzanol | APP    | Direct-activation | Foxo signaling pathway | 26.4          | 0.35          |
|    |          |        | Direct-binding  | Foxo signaling pathway | 15.7          | 0.25          |
| 2  | Prallethrin | PRSS1  | Direct-binding  | Foxo signaling pathway | 15.4          | 0.25          |
|    |          |        |                | TNF signaling pathway  |               |               |
| 3  | Transflutrin | CYP2B6 | Direct         | Foxo signaling pathway | 43.5          | 0.49          |
|    |          | CYP3A4 |                |                        | 24.6          | 0.49          |
|    |          | SCN1A  |                |                        | 53.3          | 0.49          |
3.5. Average analysis transport part length

Figure 3 shows that in average transport path length AKT1 is predicted to be the fastest pathway intermediary protein to interact with the target protein (Foxo3a, GDF-9 and TNF-α).

4. Conclusion
The γ-oryzanol compound has a role that is predicted to neutralize oxidative stress from perithroid aerosol, because it can act as an inflammatory and lipid peroxidase inhibitor. This condition influences normally folliculogenesis with stable expression of Foxo3a, GDF 9 and TNF-α. Based on the analysis of betweeness centrality protein AKT1 is a mediator protein that is widely passed to interact with the target protein. The larger the size and the darker the color of the visualization of the protein, the more dominant the role of the protein in reacting.

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