RESEARCH ARTICLE

Screening for Alcohol Dehydrogenase Inhibitors from *Dendrobium* Using the *In-silico* Method

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ABSTRACT

The incidence of acute kidney failure has become a concern and needs preventive action as soon as possible. Fomepizole and ethanol are the treatment options for acute kidney injury (AKI). The development approach is carried out through structural approaches to fomepizole or ethanol, anti-inflammatory activity, and chelate formation. *Dendrobium* is a potential plant with various activities and many secondary metabolites such as bibenzyl, alkaloids, sesquiterpenes, and phenanthrenes. This study aims to find compounds that have the potential to be ADH inhibitors from *Dendrobium* using the *in-silico* method. To identify potential natural compounds, 94 compounds from *Dendrobium* were taken from the KNApSAcK family database. Files from the receptor were prepared with YASARA software. Protox-II used to predict compound toxicity. Three compounds were obtained: o-succinyl benzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl, and gigantol. All of them interact stably with ADH based on energy binding values. O-succinyl benzoic acid as the strongest, followed by gigantol, and 3,4'-Dihydroxy-5-methoxybibenzyl.

Keywords: Acute Kidney Injury, *Dendrobium,* Fomepizole, ADH Inhibitor.

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INTRODUCTION

Acute kidney failure has become a concern in recent months, especially in children. The percentage of mortality that continues to increase causes the need for preventive action as soon as possible.[1] Generally, these disorders are associated with sepsis, impaired cardiac function, and nephrotoxicity of drug.[2] The administration of treatment to patients bounded by patofisiologi complexity and the diagnosis.[3] At the same time, management and supportive interventions can prevent the condition from worsening.[4] Intervention can be done by maintaining fluid and electrolyte balance and reducing exposures that trigger nephrotoxicity.[5] Based on data from the National Kidney Foundation (NKF), patients with acute kidney failure are estimated to reach 10% of the human population. Based on data from the Indonesian Ministry of Health, from 2022 until October, acute kidney failure in children in Indonesia reached 304 cases with a mortality rate of 52%.

The exact cause of the increase in cases is unknown until now, but it is suspected to be caused by the ethylene glycol (EG) content in children's syrup. Ethylene glycol is one of the substances that causes many hazardous exposures. The lethal dose of EG is 1 to 2 mL/kg in 95% solution, which is equivalent to 1500 mg/kg.[6] EG be metabolized into glycolic acid and then an oxalic acid, which combines with calcium to form the water-insoluble oxalic acid monohydrate. In large quantities, they are stored in the tubules and trigger inflammation of the kidney.[7] Nephrotoxic substances can attach due to kidney function as a site of drug transport, metabolism, and excretion, resulting in acute tubular injury.[8]

Renal excretion mediates drug clearance via glomerular filtration or tubular secretion. Therefore, tubular cells can potentially be exposed to nephrotoxic substances through apical contact and cellular absorption.[8] Acute injury induced by drugs or their metabolites can occur up to 7 days after drug use, especially if the drug metabolism is abnormal.[9] Since 1997, Food and Drug Administration (FDA) has approved fomepizole (4-methylpyrazole) for EG poisoning. However, several countries use other alternatives because of the high cost of fomepizole.[10] Thiamin, pyridoxine, and folate can be used for adjuvant therapy, which can reduce toxic metabolites.[11]

Alternative therapy continues to be developed to solve this problem, especially for stopping AKI with strong pharmacodynamic potential. Required an understanding of pathology AKI as apoptosis, necrosis, and pyroptosis.[12]

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The development approach is carried out through structural approaches to fomepizole or ethanol, anti-inflammatory activity, and chelate formation.[13] *Dendrobium* is a potential plant with many secondary metabolites, such as bibenzyl, alkaloids, sesquiterpenes, and phenantrenes. Various activities that have been studied include antioxidants, anticancer agents, and anti-inflammatories.[14] *Dendrobium* alkaloids (Dendrocarbidamine) have strong anti-inflammatory potential.[15]

An approach that might be used to overcome ADH metabolism in the liver so that it does not produce oxalic acid, which is harmful to the kidneys, is to use plants that can repair liver damage. *D. huoshanense* has a higher ability than *D. officinale*, *D. henanense*, and *D. moniliforme* to repair liver damage caused by CCl_4, as indicated by decreased activity of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in serum, accompanied by decreased malondialdehyde (MDA) content and increased superoxide dismutase (SOD) activity in the liver.[16] The mechanism is through Nrf2 signaling so that Nrf2 is released from the Nrf2 and KEAP1 complexes.[17,18] This study aims to find compounds that have the potential to be ADH inhibitors from *Dendrobium* using the *in-silico* method.

MATERIAL AND METHODS

Molecular Preparation

This study used various molecular models: biological activity, ADME-Tox (Absorption, Distribution, Metabolism, Excretion, and Toxicology), docking, dynamic simulations, and compound calculation of the binding energy contained in *Dendrobium* from the KNapSAcK database. Samples were obtained from the KNApSAcK family database, which contains compounds in various types of *Dendrobium*. *Dendrobium* compounds have a variety of activities and can be used as natural medicines. This study targeted the alcohol dehydrogenase (ADH) enzyme because this enzyme, when activated, increases the risk of kidney failure. The ADH chosen for virtual screening as the receptor is the one in complex with NAD+ (PDB: 1HLD; resolution 2.10).[19] To identify potential natural compounds, 94 *Dendrobium* compounds from the KNApSAcK Family Database were used.[20] Preparation of ADH receptors by removing water molecules and ligands that are not involved in the interaction. The next step is to optimize the geometry of the steepest gradient approach (100 iterations).

Lapinski's Rule, Biological Activity, and ADMET Prediction

SwissADME (http://www.swissadme.ch/) and Molinspiration Web MEEditor 1.16 (https://www.molinspiration.com/) were used for computational screening of Lapinski's rule of 5. To predict biological activity against ADH, PASS Online (http://www.way2drug.com/passonline/) was used with a lower limit of 0.3 using the keyword "Alcohol dehydrogenase [NAD(P)+] inhibitor." Protox-II used to predict compound toxicity. (https://tox-new.charite.de/protox_II/). PreADMET (https://preadmet.webservice.bmdrc.org/) is used to predict which compounds will be absorbed in the intestine.

Molecular Docking

Virtual screening docking of compounds obtained from previous screening using Auto Dock Vina in the YASARA (Yet Another Scientific Artificial Reality Application) program with the academic version. To analyze the interaction between the ligand and the YASARA receptor, implement the AMBER03 force field and Auto Dock 4 Vina as a pose docking algorithm.[21] Of concern in the docking method used is the absence of a native ligand that binds to the alcohol dehydrogenase enzyme with code 1HLD, so the enzyme's active site is a matter of concern. An approach that can be done using Prank Web (https://prankweb.cz/). Binding energy from Auto Dock 4 Vina at YASARA identifies the best pose; the more positive the value, the better the bonding energy.[22] The docking bond energy equation is as follows.

$$\Delta G = \Delta G_{vdW} + \Delta G_{Hbond} + \Delta G_{elec} + \Delta G_{tor} + \Delta Gd_{esol}$$

Information: ΔGvdW = van der Waals; ΔGHbond = H bonding; ΔGelec = electrostatic; ΔGtor= torsional free energy when a compound transit from the unbound state to the bound state; ΔGdesolv = desolvasi

Molecular Dynamics

Molecular dynamics were conducted using YASARA software. The selected pH is the physiological pH of the body, so the program settings include optimizing hydrogen bonds to increase the stability of dissolved ligands and predicting pKa to improve the protonation of protein residues. NaCl ions are needed to stimulate the body's physiology, namely 0.9%, and Na and Cl ions need to be added to neutralize cell conditions. The density of the water used is 0.997 g/mL. Temperature simulation using 310 K and 1 atm pressure (NPT ensemble). the simulation was run for 20 ns using the AMBER14. Data analysis was done using md analyze. The energy binding uses the Poisson–Boltzmann method (PBS) without entropy (normal mode analysis) in accordance with YASARA guidelines; the more positive the binding energy value, the better the interaction, the equation used is as follows.

$$\text{Binding energy (I)} = [\text{epotrec(I)} + \text{esolrec (I)} + \text{epotlig} + \text{esollig}] - [\text{epotcom(I)} + \text{esolcomp(I)}]$$

or

$$\text{BEG(i)} = [\text{epotrec(I)} + \text{epotlig}] - \text{epotcmp(I)}$$

Where I is the position number, epot is the potential energy for the complex (epotcmp), free protein (epotrec), or free ligand (epotlig), and esolv is the solvation energy for the complex (esolcmp), free protein (esolvrec), or free ligand (esollig).[23] Apart from using YASARA, the interaction of the ligand with the protein was analyzed using the Discovery Studio Visualizer.

RESULTS

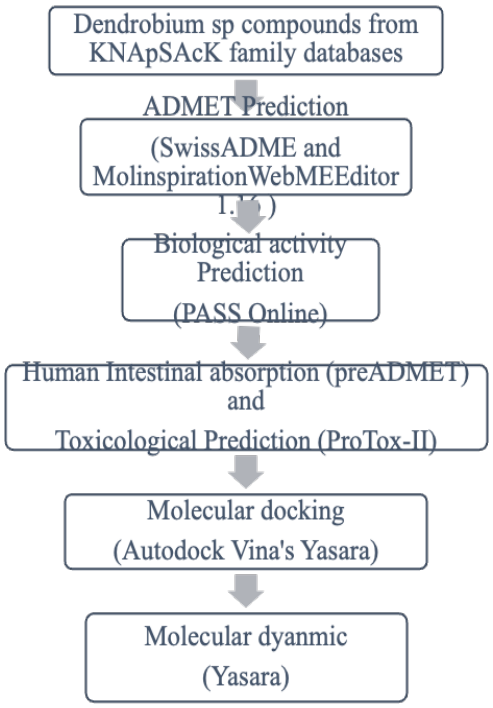


Figure 1: Flowchart screening inhibitor ADH

Virtual screening

Virtual screening of compounds from *Dendrobium* that have the potential to inhibit the action of ADH by downloading from the KNapSAcK database and obtained 94 compounds. The compounds that were successfully obtained were then screened in the first stage following the drug-likeness model of the Lapinski rule of five using Swiss ADME and MolinspirationWebMEEditor 1.16. In this process, 89 compounds were produced. The second stage involves testing ADH inhibitors for biological activity, particularly alcohol dehydrogenase [NAD(P)+] inhibitors. At this stage, using a threshold of 0.3 and obtaining 18 compounds, the basis of the threshold of 0.3 is a compound with computational potential, but no laboratory evidence yet. The third step was screening for toxicity using ProTox-II, and three compounds were obtained, namely o-succinyl benzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl, and Gigantol. Moniliformin was excluded because it is included in the class II category, so it is dangerous if swallowed. The three compounds were continued with a docking test for ADH.

Molecular Docking

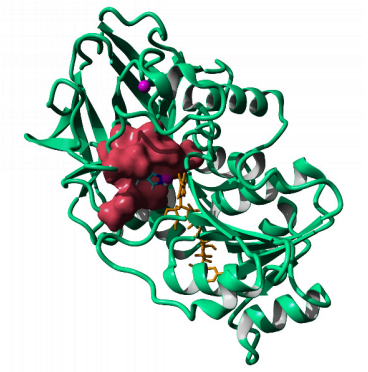


Figure 2: The active site of ADH is colored red, NAD is colored brown, Zinc is colored purple, and fomepizole is colored green

ADH doesn't have a Binding cite yet, therefore, the first docking step is to look for the binding cite. Prankweb (https://prankweb.cz/) were used to predict the active side (Figure 2). The results obtained for the highest active side have a score of 44.14, namely residues No. 46; 47; 48; 57; 58; 93; 116; 117; 119; 140; 141, which are adjacent to NAD and Zn with coordinates X: 1.3828; Y: 62.3674; and Z: 9.0418. Hackey predicted the active side of the ADH enzyme, the active side is related to hydrophobic bonds. The residue that became the active site was 52; 53; 58; 63; 55; 66; 57; 61; 60; 62; 64; 65 when the ADH enzyme at residue 141 is not methionine[19,24]. Docking was performed on fomepizole as a control and three compounds as the selected compound: o-succinyl benzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl, and Gigantol (Table 1 and Figure 3).

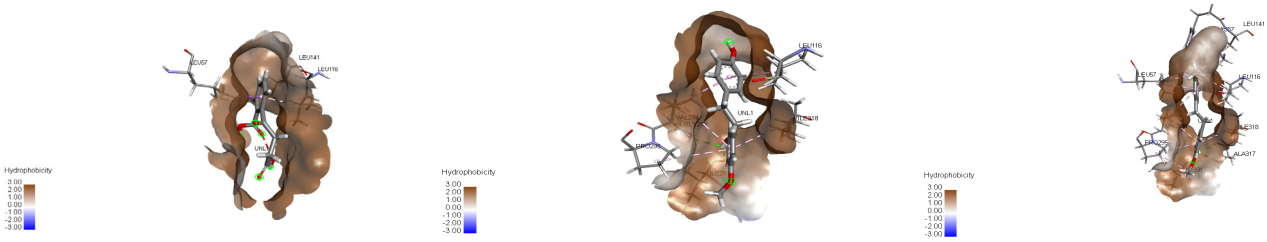
Residues that become the target of binding sites SER 48; LEU 57; HIS 67; PHE 93; LEU 116; PHE 140; LEU 141; and CYS 174.

Molecular Dynamics

Molecular dynamics aims to see the stability of the interaction between the ligand and the receptor from the RMSD backbone, protein-ligand contact, secondary structural changes, and RMSF in each bond. The first thing a dynamic molecular analyst looks at is the RMSD backbone of the protein. Simulations carried out for 20 ns showed only 8.9 ns above 3 Ångström and an average of 2.365 Ångström, so this simulation shows the stability of alcohol dehydrogenase during the simulation (Figure 4A). This figure can be simulated molecular dynamic as a form of fomepizole binding model and 3 selected ligands. Simulations carried out on fomepizole and the 3 selected ligands also show RMSD below 3 Ångström (Figure 4A),

Table 1: Human intestinal absorption (HIA) probability and toxicological prediction

Compound	*HIA (%)*	*Hepatotoxicity*	*Immunotoxicity*	*Carcinogenicity*	*Cytotoxicity*	*Mutagenicity*
Fomepizole	87,54	0,59 active	0,99 inactive	0,67 active	0,83 inactive	0,71 inactive
o-succinyl benzoic acid	77,98	0,67 inactive	0,99 inactive	0,77 inactive	0,86 inactive	0,9 inactive
3,4'-Dihydroxy-5-methoxybibenzyl	92,15	0,74 inactive	0,95 inactive	0,67 inactive	0,84 inactive	0,76 inactive
Gigantol	92,39	0,71 inactive	0,64 inactive	0,7 inactive	0,94 inactive	0,74 inactive



o-succinyl benzoic acid 3,4'-Dihydroxy-5-methoxybibenzyl Gigantol

Figure 3: 3D Interactions between ligands and ADH

Table 2: Docking results and interaction between ligand - ADH

Compound	*Binding Energy [kcal/mol]*	*Dissociation Constant [pM]*	*Donor → Acceptor*	*Distance*	*Close Contact*
Fomepizole	4.2620	751,471,616	CYS174:SG → Lig Lig → PHE93 Lig:C → LEU57 Lig:C → LEU116 Lig:C → LEU141 PHE140 → Lig1:C Lig → VAL294	5.0753 4.77047 4.78616 4.29499 4.32993 5.29246 5.22189	SER 48 ; HIS 67; ZN 375; NAD
o-succinyl benzoic acid	6.7960	10,434,363	LEU57:HD11 → Lig Lig → LEU116 Lig → LEU141	2.43826 3.9143 5.31136	SER 48; VAL 58; PHE 93; ASP 115; PRO 119; PHE 140; VAL 292; GLY 293; VAL 294; PRO 295; ALA 317; ILE 318; PHE 319; ZN; NAD
3,4'-Dihydroxy-5-methoxybibenzy l	7.6870	2,319,286	Lig:H → ILE291:O Lig:H → GLY293:O Lig → VAL294 Lig → PRO295 Lig → ILE318 Lig → LEU116	2.19007 2.76741 5.42548 5.45845 4.99565 4.88965	SER 48; LEU 57; HIS 67; PHE 93; PHE 140; LEU 141; VAL 292; GLY 316; ALA 317; ZN; NAD
Gigantol	7.6640	2,411,090.5	Lig:H → ILE291:O Lig:H → ALA317:O Lig:H → HIS67 Lig:C → LEU57 Lig:C → LEU116 Lig:C → LEU141 Lig:C → PRO295 PHE140 → Lig:C Lig → VAL294 Lig → ILE318	2.16057 2.77647 3.18607 4.31817 4.64224 4.72013 4.88005 5.03525 4.9147 4.99288	SER 48; VAL 58;THR 59; ASP 115; PRO 119; VAL 292; GLY 293; GLY 316; ZN; NAD

even lower than ADH in the unbound state, this indicates that the bond between fomepizole and three selected ligands is stable.[25] Solvent-accessible surface area (SASA) is the outer surface of the protein ligand that is accessible to solvents. Changes in SASA are affected by the ligand occupying the binding site. The higher the SASA value, the easier it is for the solvent to interact with the ligand-protein complex, but if there is a fluctuation in the SASA value; this indicates instability between the ligand complex and the protein.[26] The dynamic simulation results show that the surface area that the highest solvent can access is ADH in an unbound state (Figure 4B). The highest fluctuation occurs in ADH-Gigantol 403.345 Ångström 2 at 2.1 ns, this occurs below 5 ns. It can be ignored because it is still in the complex equilibrium period between ligand and protein.[27] In other complexes, fluctuations in SASA values were lower than those in ADH without binding, so it can be said that the complex between protein and ligand is stable.

The radius of gyration is an analysis to see the compactness of protein, or vice versa; the higher the fluctuation value, the looser the protein.[28] The fluctuation of the ADH protein Rg value was higher than 21.567 Ångström at 15.7 ns, and the lowest was 20.704 Ångström at 0.6 ns. The Rg value of the ligand complex had various fluctuations. The highest ADH-fomepizole was 21.304 Ångström at 7.2 ns, and the lowest was 20.64 Ångström at 0 ns, the highest ADH-o-succinyl benzoic acid was 21.017 Ångström at 16.8 ns, and the lowest was 20.657 Ångström at 19.6 ns, ADH-3,4'-Dihydroxy-5-methoxybibenzyl has the highest Rg value of 21.187 Ångström at 14.9 ns, and the lowest is 20.575 Ångström at 7.3 ns; The highest ADH-3,4'-Dihydroxy-5-methoxybibenzyl was 21.221 Ångström at 1.2 ns,

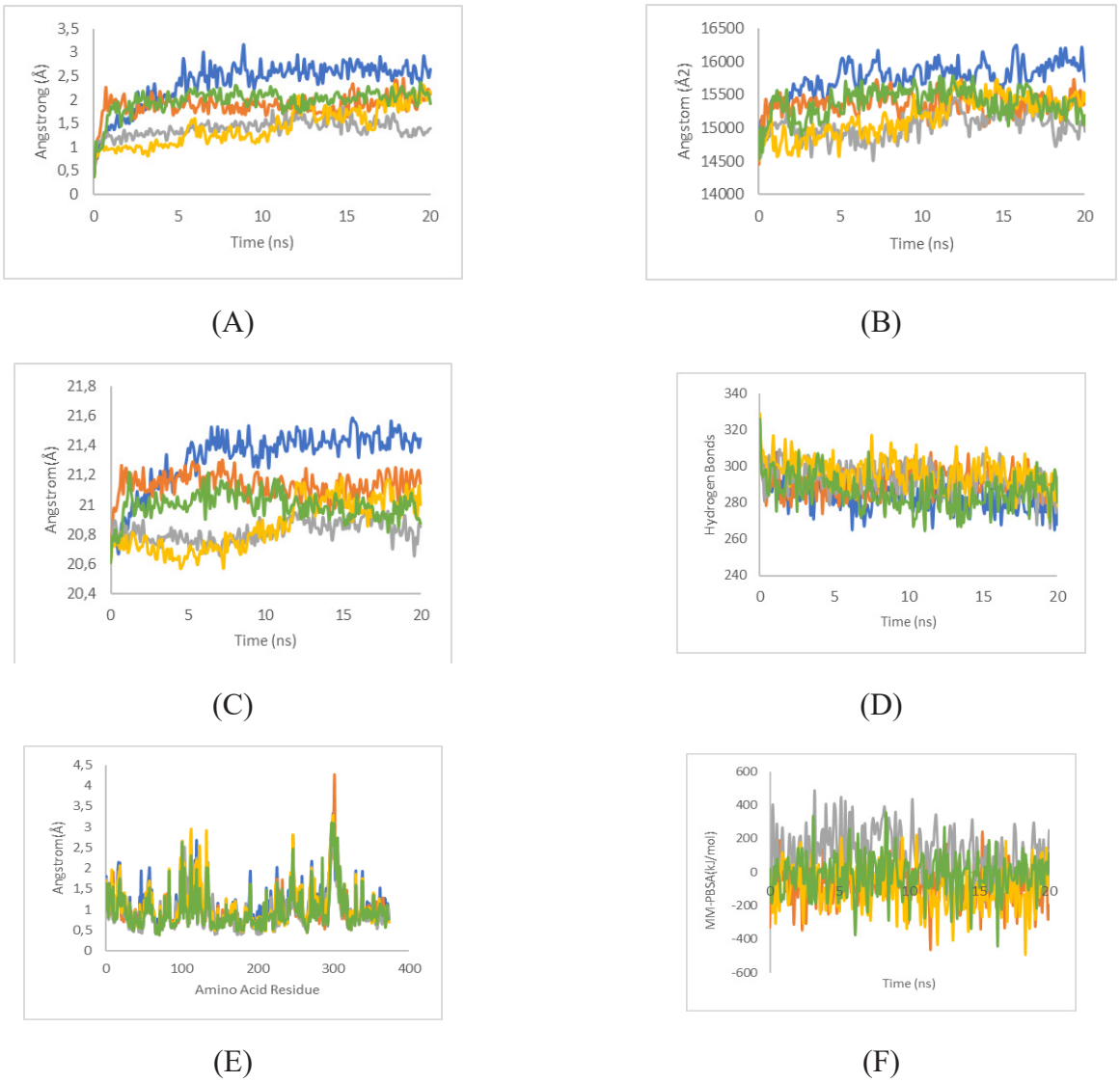


Figure 4: Molecular dynamic analysis A: RMSD backbone; B: SASA; C: Radius of gyration; D: Hydrogen bond; E: RMSF; F: MM-PBSA. Blue: ADH; orange: ADH-fomepizole; gray: ADH-o-succinyl benzoic acid; gold: ADH-3,4'-Dihydroxy-5-methoxybibenzyl; green: ADH-Gigantol.

and the lowest was 20.609 at "o" ns (Figure 4C). Overall the delta Rg is still below 1 Ångström, so that the fluctuations in the radius of gyration between simulation times are normal. Overall the presence of fomepizole and the 3 selected ligands stabilizes ADH. This is indicated by lower fluctuations when compared to ADH in the non-ligand bound state.

Hydrogen bonding will affect the protein's conformation, affecting the stability of the ligand-protein complex (Figure 4D). ADH's hydrogen bond value was 283.90 ± 8.58, and the fomepizole complex and 3 ligands had bigger hydrogen bonds than ADH. The highest hydrogen bond is ADH-3,4'-Dihydroxy-5-methoxybibenzyl with a value of 296.54 ± 8.37 so this ligand complex is stable during the simulation process, in line with research conducted by Rahman. A study on Mpro from SARS-CoV-2 reveals that protein hydrogen bonds are of lower value than protein-ligand complexes.[29]

Figure 3E illustrates RMSF, namely the stability of the alpha carbon of each residue; the lower the value, the more stable the interactions that occur between the ligand and ADH.[30] The figure shows that the interaction between residues that become active sites (SER 48; LEU 57; HIS 67; PHE 93; LEU 116; PHE 140; LEU 141; and CYS 174) has a low value, which is below 3 Å so that the interaction between the ligand and the ADH protein in the active side is stable.[31] Fluctuate RMSF values of more than 3 Å occur in ADH at ASN 300; ADH-fomepizole on LEU 301; ADH-3,4'-Dihydroxy-5-methoxybibenzyl at SER 298 and ASN 300; and ADH-Gigantol at SER 298 and LEU 301. The last analysis is the binding energy of each complex. The calculation uses the YASARA algorithm; the more positive the value, the stronger the interaction.[32,33]

The average binding energy value of the ADH-fomepizole complex is -65.407 KJ/mol, with the lowest value occurring

at 11.5 ns, namely -462.141 kJ/mol, and the best occurring at 0.7 ns, namely 185.731 kJ/mol. The ADH-o-succinyl benzoic acid complex has an average binding energy of 118.578 kJ/mol. The lowest occurs at 18.6 ns with an energy of -201.083 kJ/mol, and the best occurs at 18.9 ns with a 296.755 kJ/mol value. The ADH-3,4'-Dihydroxy-5-methoxybibenzyl complex has an average binding energy value of -98,611 kJ/mol. The lowest occurs at 13.1 ns at -406.091 kJ/mol, and the best occurs at 5.1 ns with a value of 197.688 kJ/mol. The ADH-Gigantol complex has an average binding energy of -15.03 kJ/mol, with the lowest value at 16.3 ns at -442.907 kJ/mol and the highest at 8.3 ns at 356.055 kJ/mol. Overall the complex bond of ADH-o-succinyl benzoic acid has the strongest bond, while ADH-3,4'-Dihydroxy-5-methoxybibenzyl has the weakest bond. However, ADH-o-succinyl benzoic acid and ADH-Gigantol have stronger bonds than ADH-Fomepizole.

DISCUSSION

Alcohol dehydrogenase is an enzyme that works in the liver. This enzyme plays a role in alcohol, aldehydes, and ketones by reducing NAD+ to NADH. In cases of acute kidney failure, the enzyme substrate is ethylene glycol (EG). EG is broken down into glycolic acid and oxalic acid, which react with calcium to form oxalic acid monohydrate, which is stored in the tubules and causes kidney inflammation.[7] The currently approved drug as an ADH inhibitor is fomepizole, but the price is relatively high, so another alternative is needed.[10]

One of ADH inhibitor is formamide. Formamide is an aldehyde analog compound that is non-toxic. Derivatives of this compound are N-heptylformamide, N-benzylformamide, N-1-methylheptylformamide and N-cyclopentyl-N-cyclobutylformamide which are non-competitive enzymes.[33] Binding efficiency molecular docking studies (kcal/mol) gallic acid (−5.85), hypophyllanthin (−3.23) and phyllanthin (−2.37) were compared with 4-methyl pyrazole (−4.18) using the Auto Dock 4 method, and screening site bindings used CASTp.[34] Chloroquine, as a malaria drug, has the ability to inhibit ADH in the retinol-retinal interconversion of the eye because ADH contains a thiol group.[35] Furfural from the hydrolysis of pentoses and hexoses has a competitive inhibitory ability against ADH with a K(m) value of 1.2 mM more than acetaldehyde of 0.4 mM[36]. Cimetidine acts as a competitive or non-competitive inhibitor with adjacent constant values, namely reducing less than 5% at 10 μM ethanol and acetaldehyde concentrations.[37] In tests on apple slices, nitric oxide (NO) also had the ability to inhibit ADH.[38] The use of aspirin and salicylates together can inhibit ADH either competitively or non-competitively on ADH1A/ADH2 and ADH1B2/ADH1B3, with a decrease of 75 to 86% and 31 to 52%, respectively. This difference occurs based on the docking approach due to the substitution of residue number 93.[39] Residue number 93 is a binding site for docking, and from the research, a bond was formed between residue number 93 of ADH and fomepizole, o-succinyl benzoic acid, and 3,4'-Dihydroxy-5-methoxybibenzyl using the vina docking method. This docking screening resulted in a positive binding energy value with the algorithm in YASARA. This value indicated a strong bond, so molecular dynamics research continued to observe the stability of the interaction between the ligand and the receptor and see the bond energy. A plant screening has been carried out that has the potential to improve kidney disorders, namely *Dendrobium*. These species are *D. huoshanense*, *D. officinale*, and *D. nobile*.[40] Therefore, screening compounds from *Dendrobium* that can potentially treat acute kidney failure is necessary.

The cause of acute kidney failure is oxalate crystals. These oxalate crystals are formed by ADH enzymes that work in the liver, so plants are needed to help overcome hepatotoxicity. The four *Dendrobium* species, namely *D. huoshanense*, *D. officinale*, *D. henanense*, and *D. moniliforme*, have the ability to prevent hepatotoxicity.[41] The mechanism of this species is through the Nrf2 signaling pathway. Nrf2 plays a role in overcoming toxins that cause hepatotoxicity. *Dendrobium* significantly induces the dissociation of Nrf2 from the Nrf2-Keap1 complex and promotes the nuclear translocation of Nrf2. Furthermore, Nrf2 activation causes expression of the catalytic GCLM, GCLR, HO-1 and NQO1, indicating that *Dendrobium* plays a hepatoprotective role through the Nrf2 signaling pathway, -Keap1 and suppress oxidative stress.[17] *D. officinale* is also able to protect the liver due to ethanol induction by increasing LO2 cell viability; preventing LDH release; reducing the secretion of TNF-α, IL-6, IL-1β; and reversing the expression of IL-1β, TLR4, caspase 1, TNF-α, IL-6 and p-NF-κB.[42]

Three potential compounds were obtained from the screening carried out: O-succinyl benzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl, and Gigantol. O-succinyl benzoic acid has never been tested on mammals either *in-vitro* or *in-silico* in relation to pharmacological effects; the existing research is antibacterial. O-Succinylbenzoic acid is an important biosynthesis in maintaining the life of bacteria, so the formation of this compound is an important target for antibacterials. O-Succinylbenzoic acid plays a role in the formation of vitamin K and bacterial energy but is not present in humans.[43,44] ADH is an enzyme that works in the liver, the related research in this regard is 3,4'-Dihydroxy-5-methoxybibenzyl isolated from Arundina graminifolia (D. Don) Hochr which has anti-hepatic fibrosis ability in an in vitro model with 61.9μg/ mL of HSC-T6 cells.[45] This compound also has the ability to increase cell survival signals (PI3K, Akt, and p70S6K) and induce antioxidant (HO-1, NQO-1, and TRX-1)[46] when oxidative stress occurs when ADH binds to ethylene or diethylene glycol. Gigantol plays a role in preventing CCl4-induced liver damage through the MAPK/JNK inhibition pathway, the cPLA2 pathway, 12-lipoxxygenase in platelets, liver leukocytes on 12-hydroxyeicosetraenoate activation, as well as the pan-LOX pathway by inhibiting nordihydroguaiaretic acid.[47] Gigantol also inhibits the development of liver cancer cells via the PI3K/Akt/NF-B pathway.[47] Gigantol can help prevent liver disease by acting as an antioxidant, anti-inflammatory, and inhibiting the formation of C5b-9 (liver).[48]

Gigantol has the ability to inhibit the growth of Hepatocellular carcinoma through the mechanism of the

HSP90/Akt/CDK1 pathway.[49] Based on the molecular dynamics carried out, O-succinyl benzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl, and gigantol have good interaction stability when binding to ADH based on RMSD, Rg, SASA, RMSF, and hydrogen bond analysis. This research is only a model, but this research can be a reference for further laboratory research both *in-vitro* and in vivo for O-Succinylbenzoic acid, 3,4'-Dihydroxy-5-methoxybibenzyl and gigantol in inhibiting ADH by competitive or non-competitive inhibition models.

CONCLUSION

In *in-silico* screening for ADH, inhibitors are needed to look for substitute drug candidates for fomepizole, where fomepizole is the only drug approved as an ADH inhibitor. Screening for the *Dendrobium* family produces three selected ligands, and based on molecular dynamics, the three ligands interact stably with ADH, but based on energy binding values, starting from the strongest, they are o-succinyl benzoic acid, gigantol, and 3,4'-Dihydroxy-5-methoxybibenzyl.

AUTHOR CONTRIBUTIONS

Conceptualization: Samsul Hadi, Deni Setiawan, and Kunti Nastiti, validation and investigation: Muhammad R. Ridha. and Yustinus Maladan; Supervision; All authors writing and review.

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