Jurnal Sains dan Kesehatan (J. Sains Kes.)

Journal homepage: https://jsk.farmasi.unmul.ac.id

Toxicity of Anti-Inflammatory Substances in Hemigraphis Alternata Leaves: In Silico Study Using ProTox-II

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Abstract

Hemigraphis alternata is empirically used to treat wounds. *Hemigraphis alternata* leaves ethyl acetate extract can assist in resolving the inflammatory process by inhibiting enzymes that play a role in the inflammatory cycle. Twenty-two substances found in the leaves of *Hemigraphis alternata* were predicted to have an anti-inflammatory effect by inhibiting cyclooxygenase-1 (COX-1) or 5-lipoxygenase (5-LOX) as an enzyme target. In-silico toxicology was carried out to acquire new anti-inflammatory drugs with low toxicity from 22 compounds. ProTox-II was utilized to measure the level of toxicity of these drugs at many endpoints. In this study, five compounds have LD_{50} > 5000 mg/kg body weight, toxicity class 5-6, and inactive for cytotoxicity, carcinogenicity, hepatotoxicity, mutagenicity and immunotoxicity parameters. They are *2-methyleneoctanenitrile*, *nerolidol, 2,7-dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9-diene, 9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione*, and *phytol*.

Keywords: in silico, toxicity, *Hemigraphis alternata*, anti-inflammatory, ProTox-II

Received: 16 August 2023 **Accepted**: 29 October 2023

DOI: https://doi.org/10.25026/jsk.v5i5.1975

How to Cite:

Yeni, Y., Rachmania, R. A., 2023. Toxicity of Anti-Inflammatory Substances in Hemigraphis Alternata Leaves: In Silico Study Using ProTox-II. *J. Sains Kes.*, **5**(5). 810-815. **DOI**: https://doi.org/10.25026/jsk.v5i5.1975

1 Introduction

Toxicity is a negative impact on health carried on by drug use. Medication side effects might be moderate or life-threatening. During the drug discovery process, toxicological effects are evaluated. Toxic substances are quickly identified and not prioritized as medication candidates. Toxicology influences the phases of lead selection, lead optimization, and drug candidate selection [1].

A compound's toxicity might appear at the cellular or systemic level. Cytotoxicity is the term for toxicity at the cellular level. It is frequently induced by direct biomaterial exposure (which can damage cells and result in cell death), an inflammatory reaction, or an immunological response [2], [3]. Systemic toxicity is when excessive or severe cytotoxicity can harm any organ or organ system. It is essential to note that the toxicity of biomaterials is typically dose-dependent [3].

The assessment of the toxicity of pharmacological substances is a crucial technique that is typically performed prior to their commercialization [4]. Conventional toxicity evaluation involves multiple processes (in vitro testing, preclinical and clinical evaluation), making these studies complicated, time-consuming, and costly. The results of preclinical studies using animals provide less insight into human toxic reactions due to variances in species and disease models. Meanwhile, human clinical trials are dangerous endeavors, and half of the new medications are toxic or ineffective at the final test [5].

The computational methods for finding novel medications have the potential to reduce the number of experimental investigations significantly. These strategies can make up for the deficiencies of conventional procedures [6]. In the current early phase of drug discovery, in silico technique is applied to analyze the toxicity of substances. High-throughput screening and combinatorial chemistry can provide many potential lead molecules. Promoting early evaluation of toxicity is becoming increasingly critical [7]–[9].

ProTox-II webserver estimates the toxicity of compounds. The toxicity parameters predicted by applying ProTox-II are acute toxicity, organ toxicity, toxicological endpoints, toxicological pathways (Tox21), and target toxicity. This application uses molecular similarity, fragment propensity, machine learning and pharmacophore approaches. Predictive models were developed from in vitro and in vivo experimental data that have been conducted. An independent data set verified the model's excellent performance [10]–[13].

Inflammation is the body's reaction to physical injuries, infections, or the answer of the body's tissues to antibody rejection [14], [15]. The inflammatory process is a biological process in protecting the body from harmful agents so that tissue homeostasis can be rebuilt [16], [17]. The appearance of inflammation is distinguished by swelling, flushing, soreness, high temperature, and loss of function [18]. In the inflammatory process, three enzymes have important roles, COX-1, COX-2, and 5-LOX. Inhibition of these enzymes has become a strategy for treating inflammation [19].

Hemigraphis alternata, known as red ivy, is native to tropical Asia. This plant has attractive purple leaves, so it is cultivated as an ornamental plant. This plant has a history of use as a traditional remedy for wound repair [20]. *Hemigraphis alternata* plants contain flavonoids which are thought to have anti-inflammatory effects [21]. By suppressing the COX-1, COX-2, and 5-LOX enzymes, an ethyl acetate extract of *Hemigraphis alternata* leaves can cure inflammation [22]. It has been calculated that the chemical present in the leaves of *Hemigraphis alternata* has an affinity for COX-1 and 5-LOX. COX-1 is anticipated to have a high affinity for the chemical *phytol*. 5-LOX possesses a strong affinity for the molecule *hexadecanoic acid* [23], [24].

The objective of this work is to predict the toxicity of 22 compounds found in the leaves of *Hemigraphis alternata* using in-silico modelling. Meanwhile, anticipating the toxicity of substances in *Hemigraphis alternata* leaves is advantageous to obtain potential lead compounds.

2 Methods

The 3D structures of 22 compounds discovered in *Hemigraphis alternata* leaves were prepared using PubChem (pubchem.ncbi.nlm.nih.gov). The Online SMILES Translator converted these structures into canonical SMILE form (https://cactus.nci.nih.gov/translate/).

A canonical SMILE of substances was submitted on the ProTox-II webpage (https://tox-new.charite.de/protoxII). The endpoints used in the prediction of toxicity using ProTox-II were the median lethal dose (LD_{50}) (mg/kg), toxicity class, cytotoxicity, carcinogenicity, hepatotoxicity, mutagenicity, and immunotoxicity. The results of the predicted endpoints are categorical (active and inactive) except for LD_{50} and toxicity class.

The LD_{50} and toxicity class are determined using chemical molecules comparable to dangerous chemicals. Based on the severity, the compounds can be grouped into six toxicity classes. ProTox-II focuses on the toxicity class of several measurements of toxic compounds. ProTox-II validation was carried out with LOO cross-validation. The prediction parameters of ProTox-II have been optimized to maximize the level of toxicity class and prediction of LD_{50} [10], [25], [26].

3 Result and Discussion

In silico toxicity prediction is a vital tool for lead selection in new drug discovery prior to in vitro and in vivo approaches which may be ethical, time, and cost-constrained. Several computational methods can be used to estimate the toxicity of chemical compounds [27]. In this study, the toxicity of 22 chemicals found in *Hemigraphis alternata* leaves was predicted using ProTox-II.

ProTox-II is a virtual laboratory for the prediction of the toxicity of small molecules. ProTox-II uses 33 models to predict toxicity endpoints [28]. The endpoints selected for the 22 test compounds were LD_{50}, toxicity class, cytotoxicity, carcinogenicity, hepatotoxicity, mutagenicity, and immunotoxicity. Table 1 displays the outcomes of 22 compounds' toxicity prediction analyses.

Table 1 Toxicity Prediction Result of Compounds in *Hemigraphis alternata* Leaves

Compounds	L	Tc	Cy	Ca	H	M	I
2-methyleneoctanenitrile	5300	6	-	-	-	-	-
nerolidol	5000	5	-	-	-	-	-
2,7-dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9-diene	5000	5	-	-	-	-	-
9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione	5000	5	-	-	-	-	-
phytol	5000	5	-	-	-	-	-
8a-methyl-3,4,4a,5,6,7-hexahydro-2H-naphthalene-1,8-dione	2400	5	-	-	-	-	-
cyclobutanol	1300	3	-	-	-	-	-
hexadec-1-yne	753	4	-	-	-	-	-
2-propylpropanedioic acid	343	4	-	-	-	-	-
hexadecanoic acid	900	4	-	-	-	-	-
4-(2-methoxyphenyl)piperidine	600	4	-	-	-	-	-
(2S)-2-aminopropanoic acid	2000	3	-	-	-	-	-
15-chloropentadec-4-yne	1840	4	-	+	-	-	-
1,2-dimethyl-4-nitroindol-5-ol	1000	4	-	+	+	+	-
(E)-3-(3-(2,2-dimethylcyclopropyl)-2,2-dimethylcyclopropyl)acrylonitrile	1720	4	-	-	+	-	+
2,5-dimethyl-3,5-dihydro-2H-1,4-dioxepine	2460	5	-	+	-	+	-
(Z)-dodec-2-en-1-ol	5000	5	-	+	-	-	-
(8R,9S,10S,13R,14S,17R)-10,13-dimethyl-2-methylene-17-((R)-6-methylheptan-2-yl)-hexadecahydro-1H-cyclopenta[a]phenanthren-3-ol	5000	5	-	-	-	-	+
(2S)-2-amino-3-(3,4-dihydroxyphenyl)propanoic acid	1460	4	-	-	-	+	-
2-[(2-aminoacetyl)-methylamino]acetic acid	5000	2	-	-	-	-	-
5-(hydroxymethyl)furan-2-carbaldehyde	2500	5	-	+	-	+	-
undec-10-yn-1-ol	520	4	-	-	-	-	-

Acute toxicity parameters include LD_{50}. The LD_{50} is the lethal dose at which 50% of the tested animal population perishes. Assessing acute toxicity is often the first screening step in analysing and evaluating the hazardous characteristics of drugs [29]–[31]. Many exposure modalities (such as oral, dermal, and inhalation) are employed in acute toxicity

studies, and rodents are the most common animal model used to estimate LD_{50} [32]. In most cases, the LD_{50} is stated as milligrams per kilogram of animal body weight (mg/kg BW). Compounds with an oral LD_{50} between 0 and 50 mg/kg BW are considered highly toxic, whilst those with an LD_{50} greater than 2,000 mg/kg BW are considered low toxicity. [33].

The Globally Harmonized System (GHS) defines toxicity groups based on the LD_{50} of the compound. There are six classes of toxicity in ProTox-II. They are classified as class 1 (fatal, LD50 ≤ 5), class 2 (fatal, 5 < LD50 ≤ 50), class 3 (toxic, 50 < LD50 ≤ 300), class 4 (harmful, 300 < LD50 ≤ 2000), class 5 (may be harmful, 2000 < LD50 ≤ 5000), and class 6 (non-toxic, LD50 > 5000). The higher the toxicity class of the compound, the lower the toxicity if ingested [28], [32], [34].

Hepatotoxicity is organ toxicity which refers to liver dysfunction or liver damage. The liver is often the target organ for injury from exposure to drugs or chemicals. Many diverse mechanisms contribute to the toxicity of substances in the liver. Several chemicals can cause hepatocellular degeneration and death via various potential processes [35], [36]. The trained machine learning model using the random forest classifier and discriminative features is the basis for predicting organ toxicity in ProTox-II [28].

ProTox-II forecasts four toxicological endpoints, including cytotoxicity, carcinogenicity, mutagenicity, and immunotoxicity [28]. The capacity of some chemicals or cell mediators to destroy live cells is referred to as cytotoxicity. The cytotoxicity of live cells near the wound might deleteriously influence the healing process [37]. Mutagenicity is the ability of a chemical agent or pharmaceutical product to damage DNA or chromosomes (mutation). Substances with this ability are known as mutagens. The terms mutagenicity and genotoxicity are frequently used interchangeably. Genotoxicity is a chemical characteristic that defines a temporary change in DNA or chromosomes. In other words, every substance that can cause mutations is also genotoxic. Nonetheless, not all genotoxic substances are mutagenic [38], [39]. Carcinogenicity is defined as the capacity or propensity to cause cancer. A substance is said to be a carcinogen if it has the potential to accelerate cell growth and development to become abnormal in experimental animals or humans [40], [41]. Immunotoxicity is substance-induced alterations in the local and systemic immune system function. Immunotoxic substances may be natural toxins, synthetic compounds, or pharmaceuticals. The alteration in the immune system can be in the form of suppression of the immune response. It resulted in reduced host resistance to infectious pathogens or cancer cells. In addition, the effect of changes in the immune system can be an increase in immune response which can exacerbate autoimmune disorders or cause hypersensitivity. Identifying an immunotoxicity is challenging because substances can have various complex impacts on immunological function [42]–[45].

The projected toxicity of 22 compounds was determined using ProTox-II. The five compounds have lower toxicity than the other tested compounds. They have LD_{50} ≥ 5000 mg/kg BW, toxicity class 5-6, and are inactive for cytotoxicity, carcinogenicity, hepatotoxicity, mutagenicity, and immunotoxicity. They are *2-methyleneoctanenitrile, nerolidol, 2,7-dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9-diene, 9,9-dimethoxybicyclo[3.3.1] nonane-2,4-dione,* and *phytol.*

4 Conclusion

The five chemicals in Hemigraphis alternata leaves were predicted to be less harmful than others and to be potential anti-inflammatory medication candidates, *2-methyleneoctanenitrile, nerolidol, 2,7-dioxa-tricyclo[4.4.0.0(3,8)]deca-4,9-diene, 9,9-dimethoxybicyclo[3.3.1]nonane-2,4-dione*, and *phytol*.

5 Acknowledgments

Special thanks to Research and Development Institute of Universitas Muhammadiyah Prof. DR. HAMKA for the support.

6 Declaration

6.1 Author Contribution

Conceptualization, Yeni Yeni; methodology, Yeni Yeni; software, Rizky

Arcinthya Rachmania; formal analysis, Yeni Yeni; investigation, Rizky Arcinthya Rachmania; resources, Yeni Yeni; data curation, Rizky Arcinthya Rachmania; writing-original draft preparation, Yeni Yeni; writing-review and editing, Rizky Arcinthya Rachmania.

6.2 Research Funding

This research was not supported by any funding sources.

6.3 Conflict of Interest

The authors state that the publication of this manuscript does not involve any conflicts of interest.

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