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Research Article

Toxicity Evaluation of Statin Group Drugs Using in Silico Methods

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ABSTRACT

Cardiovascular diseases (CVDs) remain a leading cause of mortality in modern society, with factors such as sedentary lifestyles, unhealthy diets, and obesity contributing to their increasing prevalence. The widespread use of Statins for lipid-lowering therapy in both primary and secondary cardiovascular prevention is anticipated to rise in response to this trend. Given the rapid escalation in the prevalence of Statin usage, it is imperative to understand their toxicological effects on public health. While previous studies have explored various pharmacological effects of statins, comprehensive investigations into their genotoxic and Mutagenic potential are lacking. In this study, we conducted a comprehensive In silico evaluation of Statins using four different toxicological assessment programs, focusing on various genotoxicity, carcinogenicity, Mutagenicity, and Micronucleus formation endpoints. By comparing program outputs with experimental data, we assessed the reliability of In silico Toxicity predictions and discussed the consistency among different platforms. Our findings suggest discrepancies among the predictions of different programs, highlighting the importance of integrating multiple sources of data and methodologies in Toxicity evaluations. Despite inconsistencies, integrating in silico predictions with future in vitro and in vivo studies can contribute to a better understanding of the toxicological properties of statins and ensure their safe usage. This study underscores the necessity of careful evaluation and utilization of multiple data sources in decision-making regarding the toxicological profile of statins. Ultimately, leveraging in silico methods to guide future comprehensive toxicological studies will enhance our understanding of Statins' safety profiles and contribute to public health research.

1. Introduction

Cardiovascular diseases have been the leading cause of death worldwide. According to the World Health Organization's report, in 2021, 20.5 million people died from cardiovascular diseases [1]. Additionally, it is estimated that by the year 2030, 23 million people will die from cardiovascular diseases [2]. Cholesterol-lowering drugs known as Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) inhibitors, are the preferred choice in combating cardiovascular diseases [3].

The substance named mevastatin, isolated from the fungus *Penicillium citrinum* by Akira Endo in 1971, was the first statin. However, it was not released to the market due to adverse effects observed in dog animal models [4, 5].

Following the discovery of the cholesterol-lowering effects of mevastatin, research efforts were directed towards the discovery of other cholesterol lowering substances similar to mevastatin. As a result of these studies, lovastatin (mevinolin) was obtained from the strain of *Aspergillus terreus* (ATCC 20542) [6].

Lovastatin became the first statin to be approved by the United States Food and Drug Administration (FDA) as a hypercholesterolemic drug and was released to the market in August

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1987 [7]. Although atorvastatin, simvastatin, lovastatin, fluvastatin, pravastatin, pitavastatin, and rosuvastatin have been commercially available for sale, cerivastatin was withdrawn from the market in 2001 due to 52 deaths attributed to drug-induced rhabdomyolysis associated with renal failure [8].

According to product information for cholesterol-lowering drugs, statins have been observed to induce cancer in rodents. In most cases, the carcinogenic effect observed in rodents occurs at doses equivalent to the maximum recommended dose for humans [9]. A clinical study published in 2008 investigated the relationship between the use of lipophilic statins and cancer formation. The study, conducted on patients using atorvastatin, simvastatin, lovastatin, and fluvastatin, concluded that the sufficiently high doses of lipophilic statins may be associated with a clinically significant reduction in cancer cases [10].

Apart from non-clinical toxicology reports, there is a lack of comprehensive genotoxicity studies on statin drugs. In 2007, Gajski et al. published a study aiming to determine the potential genotoxic effects of atorvastatin. DNA damage was assessed using comet assay, micronucleus, and chromosomal abnormality tests. It was noted that human lymphocytes exposed to atorvastatin exhibited higher levels of genotoxic damage compared to the control group [11].

Mutagenicity studies similarly present diverse findings. Robison et al. examined gene mutations in *Salmonella typhimurium*, *Escherichia coli* strains and, hamster cells to determine the mutagenicity of fluvastatin but did not encounter any mutagenic phenomenon [12]. On the other hand, Orsolin et al.'s study revealed that atorvastatin and rosuvastatin did not exhibit a mutagenic effect on *D. melanogaster*, but these synthetic statins demonstrated a suppressive effect on the genotoxicity of DXR (mutagen) in somatic cells of *D. melanogaster* [13].

Increasing the number of clinical, in silico, and in vitro studies on the toxic effects of statins will play an important role in elucidating their toxicological mechanisms of action. Most of these studies have focused on the anticancer properties of statins. Recently, alongside these studies, in silico methods that support or predict toxic endpoints, such as developmental toxicity, genotoxicity, and in vitro mutagenicity, have gained increasing significance.

In silico toxicity studies, conducted using computer-based modeling and computational methods, are used to analyze or predict the toxicities of chemical substances [14]. These tools utilize data from various sources such as chemical structures, physicochemical properties, and biological pathways (Figure 1). By reducing the duration, cost, and use of experimental animals in toxicity tests, these methods provide a significant advantage. Additionally, they are effective in rapidly evaluating large datasets [15].

In recent years, numerous in silico toxicology prediction programs and methods have been developed. Some of the developed tools include Vega Hub, Toxtree, Lazar, and T.E.S.T. All of them are free and utilize Quantitative Structure-Activity Relationship (QSAR) methodologies to predict toxicity profiles of chemical compounds and assess potential risks.

The concept of "Structure-Activity Relationship" (SAR) suggests that the biological activity of a chemical can be correlated with its molecular structure. When this relationship is quantified, it is referred to as "Quantitative Structure-Activity Relationship" (QSAR). A QSAR model correlates the toxicity of chemicals with molecular properties using available experimental toxicity data and predicts the toxicity of new chemicals [16].

Vega-QSAR is a program that utilizes over 100 QSAR models and in silico approaches for the prediction and assessment of chemical properties, mutagenicity, and carcinogenicity of substances [17]. Toxtree is used to determine the Cramer class and potential toxicity of a chemical based on its similarity to specific structural alerts associated with certain toxicity classes, applying decision tree approaches [18]. Lazar (Lazy Structure-Activity Relationships) is a tool for predicting toxic properties such as mutagenicity or carcinogenicity of chemical structures based on a research database that includes both chemical structures and experimental data,

identifying compounds sharing similarities with the given query structure [19]. T.E.S.T. (Toxicity Estimation Software Tool) calculates various toxicological endpoints using molecular descriptors and QSAR methodologies [20].

This study aims to evaluate statin group drugs in silico for various toxicological endpoints such as mutagenicity, carcinogenicity, and micronucleus formation using Lazar, Vega, T.E.S.T, and Toxtree tools. The findings are compared with existing experimental data to assess the reliability and consistency of the developed in silico toxicology prediction tools. Additionally, the study aims to contribute to the literature regarding future risk assessments of the potential genotoxic, mutagenic, and carcinogenic effects of statins and the use of in silico toxicity tools in toxicity studies.

2. General Methods

This study aimed to assess the potential genotoxic, mutagenic, and carcinogenic properties of seven different statin drugs in silico. To achieve this goal, four different programs were utilized: VEGA-QSAR, TOXTREE, T.E.S.T, and LAZAR. These programs were preferred due to their open access, user-friendly interface, free availability, and ability to provide toxicity predictions based on chemical structure

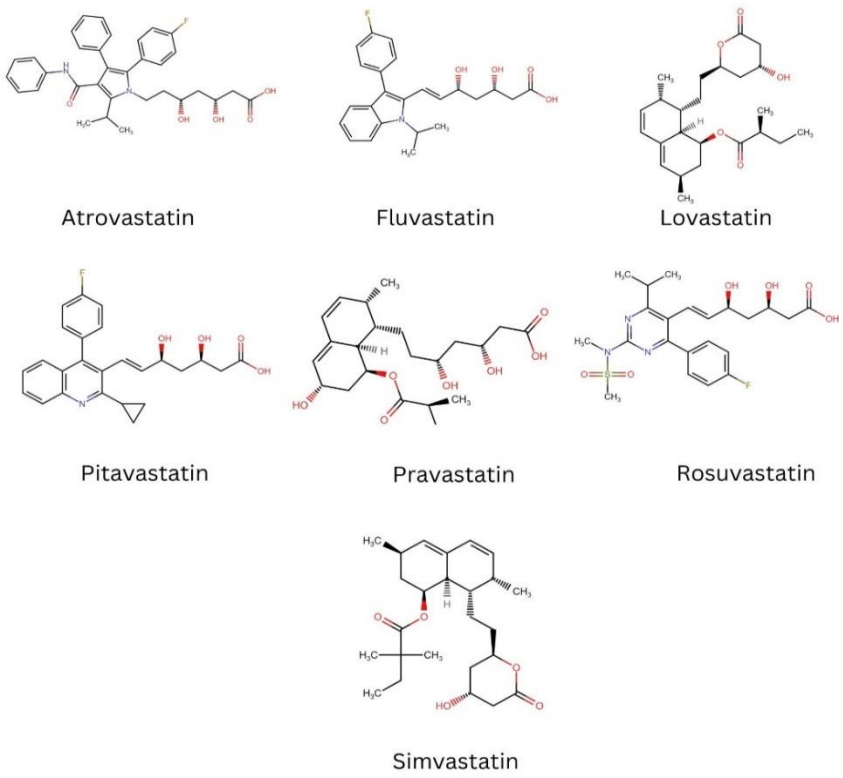

Figure 1. 2-dimensional chemical structures of statins

2.1. Chemical structures of molecules

The SMILES (Simplified Molecular Input Line Entry System) information for the chemicals was obtained from the CAS Common Chemistry (https://commonchemistry.cas.org/) source. CAS common Chemistry is an open community source providing access to various information of chemicals. SMILES is a standardized and concise representation of the three-dimensional structure of a chemical substance, which can be easily understood by computer software, transforming it into a series of symbols [21]. The obtained SMILES information of the chemicals (Table 1) has been entered into each software tool, and the desired endpoints have been selected.

2.2. VEGA QSAR

VEGA (Virtual models for property Evaluation of chemicals within a Global Architecture, www.vegahub.eu) is a free in silico toxicity program that integrates artificial intelligence (AI)

based prediction into toxicology. It utilizes computer simulations and prediction models to estimate biological endpoints such as BCF, carcinogenicity, mutagenicity, genotoxicity, and skin sensitivity. The program's logic relies on "Structure-Activity Relationship" (SAR) and "Quantitative Structure-Activity Relationship" (QSAR) models, which correlate the biological activity of a chemical with its molecular structure using existing experimental toxicity data to predict toxicity. It also employs various artificial intelligence methods, including rule-based expert systems, data mining, regression models, ensemble methods, and hybrid models [22]. Applicability domain is an important concept in quantitative structure-activity relationships (QSAR) and allows for predicting the uncertainty of a specific molecule's prediction based on how similar it is to the compounds used to build the model [23]. The VEGA program evaluates chemical structures and other properties related to the toxicological endpoints under assessment, is effective in measuring the applicability domain, and enables the user to eliminate unreliable predictions [17]. In this study, version 1.2.3 of the VEGA program was utilized.

Table 1. SMILES (Simplified Molecular Input Line Entry System) information of the tested chemicals

Name	SMILES
Atorvastatin	C(NC1=CC=CC=C1)(=O)C=2C(=C(N(CC[C@H](C[C@H](CC(O)=O)O)O)C2C(C)C)C3=CC=C(F)C=C3)C4=CC=CC=C4
Fluvastatin	C(=C/[C@@H](C[C@@H](CC(O)=O)O)O)\C1=C(C=2C(N1C(C)C)=CC=CC2)C3=CC=C(F)C=C3
Lovastatin	O(C([C@H](CC)C)=O)[C@@H]1[C@]2(C(C=C[C@H](C)[C@@H]2CC[C@@H]3C[C@@H](O)CC(=O)O3)=C[C@H](C)C1)[H]
Pitavastatin	C(=C/[C@H](C[C@H](CC(O)=O)O)O)\C1=C(C2=C(N=C1C3CC3)C=CC=C2)C4=CC=C(F)C=C4
Pravastatin	C(C[C@H](C[C@H](CC(O)=O)O)O)[C@@H]1[C@]2([C@@H](OC([C@H](CC)C)=O)C[C@H](O)C=C2C=C[C@@H]1C)[H]
Rosuvastatin	C(=C/[C@H](C[C@H](CC(O)=O)O)O)\C=1C(=NC(N(S(C)(=O)=O)C)=NC1C(C)C)C2=CC=C(F)C=C2
Simvastatin	O(C(C(CC)(C)C)=O)[C@@H]1[C@]2(C(C=C[C@H](C)[C@@H]2CC[C@@H]3C[C@@H](O)CC(=O)O3)=C[C@H](C)C1)[H]

Mutagenicity assessment provided by the VEGA software was determined through the combination of five different models (CAESAR version 1.0.4, ISS version 1.0.3, SarPy-IRFMN version 1.0.8, KNN version 1.0.1, and Consensus version 1.0.4). In the Consensus model, predictions from the CAESAR, ISS, KNN/Read-Across, and SarPy/IRFMN models are combined using the applicability domains of each model to determine a consensus score ranging from 0 to 1, providing a reliability indicator. Higher scores indicate more reliable predictions [24].

In the carcinogenicity analysis, the following models are used: CAESAR 2.1.10, ISS 1.0.3, IRFMN-ISSCAN-CGX 1.0.2, and IRFMN-Antares 1.0.2. For in vitro micronucleus activity prediction, the IRFMN-VERMEER 1.0.1 model is utilized, while for in vivo micronucleus activity prediction, the IRFMN 1.0.2 model is used.

2.3. Toxtree

TOXTREE program is a freely accessible tool developed to determine the toxicity of chemicals. The program categorizes substances into three different classes, namely Class I, Class II, and Class III, based on the Cramer classification scheme. These classes are determined considering the structural properties of each substance and known toxicity data. TOXTREE operates using a decision tree approach consisting of 33 questions for each substance. The answers to these questions enable classification of the substance and assess its potential toxic threat [25].

Class I substances have structures and data suggesting low oral toxicity levels. Class II substances may be potentially harmful to a less certain degree compared to Class I, but they are not as hazardous as those in Class III. Class III substances imply high toxicity [18].

The program relies on chemistry and biochemistry knowledge and classifies substances based on chemical structure properties along with metabolism or toxicity data. Additionally, TOXTREE features a module based on the Benigni and Bossa rules aimed at predicting characteristics such as carcinogenicity

and mutagenicity, allowing the program to conduct a more comprehensive toxicological assessment [26].

2.4. T.E.S.T.

T.E.S.T (Toxicity Estimation Software Tool), a Java software developed by the U.S. Environmental Protection Agency (EPA), allows users to easily predict toxicity using various QSAR (Quantitative Structure-Activity Relationships) methodologies without the need for any external programs. The TEST program enables the evaluation of various toxicity endpoints such as bioaccumulation factor, developmental toxicity, mutagenicity (Ames test), Daphnia magna LC50, among others [27].

2.5. Lazar

Lazar (Lazy Structure-Activity Relationships), various properties of chemical substances such as carcinogenicity, long-term toxicity, reproductive toxicity, and mutagenicity are predicted using a free program. It generates local QSAR models for the evaluation of each compound. These models include descriptor calculations, chemical similarity indices, and various algorithms for model generation. It obtains these predictions with data mining algorithms, providing a flexible prediction algorithm for different biological endpoints. It identifies similar compounds in the training data (the dataset used in machine learning) and creates local prediction models based on the experimental activities of these similar compounds, thus predicting the toxicological properties of the chemical of interest [28].
Lazar, rather than using a global (Q)SAR model, obtains a specialized prediction using a modified *k-nearest-neighbour* (KNN) algorithm. It searches a database containing chemical structures and experimental data for compounds similar to the query structure and makes a prediction based on the experimental measurements of similar compounds. Unlike traditional KNN techniques, it categorizes chemical similarities not as absolute values but as values that need to be determined based on a specific biological activity [19].

3. Results

Cardiovascular diseases are among the leading causes of mortality in today's world. Factors such as changing lifestyles, unhealthy diet, sedentary behavior, and obesity contribute to the increasing incidence of cardiovascular diseases. It is predicted that the prevalence and importance of cholesterol-lowering medications will rise as a result. Statin drugs are commonly used for both primary and secondary cardiovascular protection through lipid-lowering therapy.

Considering the widespread and rapidly increasing usage of statins, determining their toxicological effects has significant implications for public health. Various studies have investigated the carcinogenic, genotoxic, anticancer, antitumor, and antimutagenic effects of statin drugs, but there hasn't been a comprehensive study specifically focusing on their genotoxic and mutagenic effects. In our study, we utilized four different in silico toxicology assessment programs to investigate various toxicological endpoints such as genotoxicity, carcinogenicity, mutagenicity, and micronucleus formation activity of statin drugs. The program outputs were compared with each other and with experimental data to make predictions about the toxicity of statins and to discuss the reliability of the in silico toxicity programs used (Table 2).

3.1. Toxtree

According to the results of the Toxtree program, all 7 compounds evaluated are predicted to be of Class III, indicating high toxicity.

Table 2. Comparison of programs regarding the toxicological endpoints of statin drugs

Chemicals	Structural Alert for Carcinogenecity				Structural Alert for Mutagenicity					Structural Alert for Micronucleus			Cramer Classification
	TOXTREE		VEGA	LAZAR	TOXTREE	VEGA		LAZAR	T.E.S.T	TOXTREE	VEGA		TOXTREE
	GSA	NGSA					CS				In vitro	In vivo	
Atorvastatin	NO	YES	NO	YES	NO	NO	1	NO	YES	YES	NO	NO	CLASS 3
Fluvastatin	NO	YES	YES	N/A	NO	NO	1	NO	YES	YES	NO	NO	CLASS 3
Lovastatin	NO	YES	NO	N/A	NO	NO	0.825	NO	NO	YES	NO	NO	CLASS 3
Pitavastatin	NO	YES	YES	N/A	NO	YES	0.3	NO	NO	YES	YES	NO	CLASS 3
Pravastatin	NO	YES	YES	YES	NO	NO	0.35	NO	NO	YES	NO	NO	CLASS 3
Rosuvastatin	NO	YES	YES	N/A	NO	NO	0.3	NO	YES	YES	NO	NO	CLASS 3
Simvastatin	NO	YES	YES	N/A	NO	NO	0.825	NO	NO	YES	NO	NO	CLASS 3

*GSA: genotoxic structural warning, *NGSA: non-genotoxic structural warning, *N/A: Estimate could not be generated *CS: consensus score

Atorvastatin, Fluvastatin, Pitavastatin, and Rosuvastatin are classified as Class III because they contain elements other than carbon, hydrogen, oxygen, nitrogen, and divalent sulfur. Additionally, the answer to the question regarding whether the non-listed elements are found only as sodium, potassium, calcium, magnesium, nitrogen salts, sulphamate, sulphonate, sulphate, or hydrochloride is "no." According to the Cramer decision tree approach, the substances lovastatin and simvastatin are queried to determine whether they contain a lactone or cyclic diester in a heterocyclic structure. Since both substances contain a lactone ring, they are directed to question 9 in the decision tree. These substances have a heterocyclic lactone structure that does not contain an open ring, and this structure is not a three-membered heterocycle.

The heterocyclic ring found in lovastatin and simvastatin does not contain complex compounds, is not heteroaromatic, and is not commonly found as a component in foods. In light of all this information, the program directs us to the final rule, rule 33. Since the answer to the question, "Are there sufficient sulphonate or sulphamate groups?" is "no" for these two substances, they are classified as high toxicity class III by the program.

The substance pravastatin is classified as Class III because it answered "no" to question 33 of the decision tree approach, which asks, "Are there sufficient sulphonate or sulphamate groups?" If the answer had been "yes," it would have been classified as Class I, indicating low toxicity.

Additionally, it has been concluded that the chemicals contain at least one micronucleus and structural alerts for non-genotoxic carcinogenicity according to the Benigni/Bossa carcinogenicity and mutagenicity rules, but no structural alerts were found for genotoxic carcinogenicity and potential in vitro mutagenicity (Ames Test) based on ISS.

3.2. VEGA QSAR

3.2.1. Mutagenicity estimation

Atorvastatin is predicted to be non-mutagenic, with a consensus score of 1 based on 1 experimental value according to the Consensus model. While the Caesar model provides a moderately reliable prediction that the compound is non-mutagenic, the ISS and SarPy-IRFMN models suggest with low reliability that it is non-mutagenic. The experimental data obtained from the KNN model also indicates that the compound is non-mutagenic. All models, despite having different reliability scores, evaluated this compound as non-mutagenic.

Fluvastatin is predicted to be non-mutagenic, with a consensus score of 1 based on 2 experimental values according to the Consensus model. The Caesar and SarPy-IRFMN models provide high reliability evidence that the compound is non-mutagenic, while the ISS and KNN models suggest with low reliability that it is non-mutagenic.

Lovastatin is predicted to be non-mutagenic, with a consensus score of 0.825 based on 4 models

according to the Consensus model. The Caesar, SarPy-IRFMN, and KNN results indicate with high reliability that the compound is non-mutagenic, while the ISS result suggests with moderate reliability that it is non-mutagenic.

Pitavastatin is predicted to be mutagenic according to the Consensus model, with a consensus score of 0.3, considering four models together. While the Caesar and ISS models provide moderate and low reliability respectively that it is non-mutagenic, the SarPy-IRFMN and KNN results suggest with moderate reliability that it is mutagenic.

Pravastatin is predicted to be non-mutagenic, with a consensus score of 0.35 based on 4 models according to the Consensus model. The Caesar and SarPy-IRFMN models suggest with moderate reliability that it is non-mutagenic, while the ISS suggests with low reliability that it is non-mutagenic, and finally, the KNN suggests with moderate reliability that it is mutagenic.

Pitavastatin is predicted to be mutagenic according to the Consensus model, with a consensus score of 0.3, considering four models together. While the Caesar and ISS models provide moderate and low reliability respectively that it is non-mutagenic, the SarPy-IRFMN and KNN results suggest with moderate reliability that it is mutagenic.

Pravastatin is predicted to be non-mutagenic, with a consensus score of 0.35 based on 4 models according to the Consensus model. The Caesar and SarPy-IRFMN models suggest with moderate reliability that it is non-mutagenic, while the ISS suggests with low reliability that it is non-mutagenic, and finally, the KNN suggests with moderate reliability that it is mutagenic.

Rosuvastatin is predicted to be non-mutagenic, with a consensus score of 0.3 based on 4 models according to the Consensus model. The Caesar, ISS, and SarPy models suggest with low reliability that it is non-mutagenic, while the KNN suggests with moderate reliability that it is non-mutagenic.

Simvastatin is predicted to be non-mutagenic, with a consensus score of 0.825 based on 4 models according to the Consensus model. The Caesar, SarPy, and KNN models suggest with high reliability that it is non-mutagenic, while the ISS suggests with moderate reliability that it is non-mutagenic.

3.2.2. Carcinogenicity assessment

For Atorvastatin, the Caesar model predicts with moderate reliability that it is not carcinogenic, while the ISS, IRFMN-ISSCAN-CGX, and IRFMN-Antares models predict with low reliability that it is carcinogenic.

For Fluvastatin, experimental data suggests carcinogenicity. The Caesar model predicts with moderate reliability that the compound is carcinogenic. The ISS model's prediction is carcinogenic, with a low reliability score. The IRFMN-ISSCAN-CGX model predicts the compound as non-carcinogenic with low reliability, while the IRFMN-Antares model evaluates it as carcinogenic with high reliability.

Lovastatin's experimental data indicates non-carcinogenicity. The Caesar model evaluates the compound as non-carcinogenic with high reliability. The ISS model considers the compound as carcinogenic, but the program says that the result may not be reliable. The IRFMN-ISSCAN-CGX and IRFMN-Antares models evaluate the compound as carcinogenic, with high and low reliability respectively, which contradicts the experimental data.

Pitavastatin is classified as non-carcinogenic according to the Caesar model, but the result contains some critical points that need to be evaluated. The ISS model assesses the compound as carcinogenic with low reliability. The IRFMN-ISSCAN-CGX and IRFMN-Antares models evaluate the compound as carcinogenic, but the results also contain some critical points that need to be checked.

Pravastatin, according to the evaluations of the Caesar and ISS models, is considered carcinogenic, but the result has low reliability degree. The IRFMN-ISSCAN-CGX model evaluates the compound as carcinogenic with high reliability. The IRFMN-Antares model assesses the compound as carcinogenic, but the

result also indicates some critical points that need to be verified.

Rosuvastatin is evaluated as non-carcinogenic by the Caesar model, but the result has low realibility score. The ISS prediction suggests carcinogenicity, but the reliability is low. The IRFMN-ISSCAN-CGX and IRFMN-Antares models evaluate the compound as carcinogenic, but the results have low realibility value.

Although the Caesar model considers Simvastatin as carcinogenic, the result indicates some critical points that need to be verified. The ISS model assesses the compound as carcinogenic, but the result has low reliability score. The IRFMN-ISSCAN-CGX model evaluates the compound as carcinogenic with high reliability. The IRFMN-Antares model assesses the compound as carcinogenic with low reliability.

3.2.3. Micronucleus assessment

According to the in vitro IRFMN-VERMEER model, Atorvastatin and Pravastatin are evaluated as inactive in terms of micronucleus formation, but the results highlight some critical points that need to be checked. Fluvastatin, Lovastatin, Rosuvastatin, and Simvastatin are assessed as inactive in terms of micronucleus formation, while Pitavastatin is considered active.

According to the in vivo Micronucleus activity (IRFMN) model, Fluvastatin's experimental data is provided as non-genotoxic, and the model prediction, with high reliability, indicates that the compound is non-genotoxic. Lovastatin, Pitavastatin, and Simvastatin are evaluated as non-genotoxic compounds with high reliability. Atorvastatin and Pravastatin are considered non-genotoxic compounds, highlighting the presence of critical points that need to be evaluated. Rosuvastatin is evaluated as a non-genotoxic compound with low reliability.

3.3. Lazar framework

According to the Lazar software, Atorvastatin's likelihood of being carcinogenic is estimated at 0.3. However, its mutagenicity prediction indicates a probability of 0.73 that Atorvastatin is not mutagenic. The reliability of both assessments falls below that of biological assay results. Furthermore, the software warns that the similarity threshold for application is 0.2, which is below the standard threshold of 0.5. This suggests that the prediction may fall outside the applicability domain, indicating a certain level of unreliability. It cautions that reliability may decrease when applying the result to data beyond the model's learning dataset. Hence, it advises users that the model cannot assure a specific level of reliability for this prediction, urging careful evaluation of the results.

The carcinogenicity evaluation of Fluvastatin could not be conducted due to insufficient similar data points to meet the required similarity threshold for predictions. However, for mutagenicity prediction, there is a conclusive probability of 1.0 that the substance is not mutagenic, with reliability similar to biological assay results.

Similarly, the carcinogenicity assessment of Lovastatin was hindered by inadequate similar data points for prediction. Nevertheless, the mutagenicity prediction yields a probability of 0.216 that Lovastatin is not mutagenic, although with lower reliability compared to biological assay results, and potentially outside the applicability domain due to the threshold being below the standard.

For Pitavastatin, the carcinogenicity assessment could not proceed due to insufficient similar data points. However, the mutagenicity prediction suggests a probability of 0.531 that Pitavastatin is not mutagenic, with reliability comparable to biological assay results.

In the case of Pravastatin, the carcinogenicity assessment yields probabilities of 0.576 in mice, 0.413 in rats, and 0.576 in rodents that it is not carcinogenic. For mutagenicity prediction, there is a probability of 0.238 that it is not mutagenic, with lower reliability compared to biological assay results and potential applicability domain issues due to a threshold below the standard.

In the case of Pravastatin, the carcinogenicity assessment yields probabilities of 0.576 in mice,

0.413 in rats, and 0.576 in rodents that it is not carcinogenic. For mutagenicity prediction, there is a probability of 0.238 that it is not mutagenic, with lower reliability compared to biological assay results and potential applicability domain issues due to a threshold below the standard.

In the case of Pravastatin, the carcinogenicity assessment yields probabilities of 0.576 in mice, 0.413 in rats, and 0.576 in rodents that it is not carcinogenic. For mutagenicity prediction, there is a probability of 0.238 that it is not mutagenic, with lower reliability compared to biological assay results and potential applicability domain issues due to a threshold below the standard.

The carcinogenicity assessment of Rosuvastatin could not be conducted due to insufficient similar data points. However, for mutagenicity prediction, there is a probability of 0.333 that it is not mutagenic, although with reliability lower than biological assay results and potential applicability domain issues due to a threshold below the standard.

Similarly, the carcinogenicity assessment of Simvastatin faced limitations due to inadequate similar data points. Yet, for mutagenicity prediction, there is a probability of 0.212 that it is not mutagenic, although with reliability lower than biological assay results and potential applicability domain issues due to a threshold below the standard.

3.4. T.E.S.T.

In the evaluation using the T.E.S.T. software, it was determined that Atorvastatin, Fluvastatin, and Rosuvastatin have consensus scores of 0.61, 0.59, and 0.63, respectively, indicating they are mutagenic. On the other hand, Lovastatin, Pitavastatin, Pravastatin, and Simvastatin have consensus scores of 0.08, 0.27, 0.11, and 0.23, respectively, indicating they are not mutagenic.

4. Discussion

The Toxtree program, which uses the Cramer classification scheme, classified the tested 7 substances as Class 3, indicating high toxicity. This classification is based on the structural characteristics of chemicals and the presence of at least one structural alert for micronucleus formation and non-genotoxic carcinogenicity.

According to the results of the Toxtree program, none of the test substances showed a structural alert for genotoxic-based carcinogenicity. Compared to Vega and Lazar, the data provided by the Toxtree program are consistent when considering non-genotoxic-based carcinogenicity, except for Atorvastatin and Lovastatin. These findings may lead us to conclude that there is some level of uncertainty regarding the specific genotoxic end-points sensitivity of Toxtree.

It is observed that the Lazar program could not obtain data on the carcinogenicity of statin group substances except for Atorvastatin and Pravastatin. The program could not generate predictions because it did not have a sufficient amount of similar data points to meet the similarity threshold used for carcinogenicity assessment. This issue could be resolved by increasing the similar data points in the training dataset of the model.

The evaluation of carcinogenicity for Pravastatin is consistent between the Lazar and Vega programs, but contradictory results are observed for Atorvastatin. While the Caesar model used in the Vega program emphasizes with moderate reliability that Atorvastatin is not carcinogenic, other models used suggest with low reliability that the substance might be carcinogenic. The Lazar program evaluated Atorvastatin as carcinogenic with a value of 0.3, indicating low reliability of the obtained result. When comparing the reliability of the data, it can be concluded based on the data provided by the Vega program that Atorvastatin is not carcinogenic.

When comparing the mutagenicity predictions of the statin group chemicals by various programs, it is observed that the results obtained from the Toxtree and Lazar programs are consistent with each other, while Vega's predictions, except for Pitavastatin, are consistent with the predictions of other substances and contradict with the TEST program. According to the Consensus model of the Vega program, Pitavastatin is predicted to be mutagenic with a consensus score calculated

based on four models to be 0.3. The results of SarPy-IRFMN and KNN are consistent with this prediction, but the Caesar and ISS models predict with moderate and low reliability, respectively, that the substance is not mutagenic. Lazar, on the other hand, predicts with a probability of 0.531 in mutagenicity assessment that the substance is not mutagenic, and the reliability of this data is similar to bioanalysis results. This implies that the reliability of the data provided by Lazar is higher than that provided by Vega. Considering all the models and reliability scores, it can be inferred that Pitavastatin is not mutagenic.

The T.E.S.T program's assessment of Atorvastatin, Fluvastatin, and Rosuvastatin with consensus scores of 0.61, 0.59, and 0.63, respectively, as mutagenic contradicts the data provided by other programs. This discrepancy highlights the importance of using multiple programs and models when assessing whether specific substances are mutagenic or not in toxicological evaluations.

The Toxtree program provided data indicating that all 7 tested substances contain at least one structural alert for micronucleus formation and can potentially form micronuclei. These data contradict the reliability of the Vega program's data. The limited performance and low accuracy rate of the Toxtree program's micronucleus test indicate that many structural alerts are present in experimentally non-toxic compounds in its predictions, suggesting that the Toxtree model may be misleading in some cases [29].

The Vega program is consistent among its models for all substances except for pitavastatin. The In vivo Micronucleus activity (IRFMN) model has classified all substances as non-genotoxic agents. However, the In vitro IRFMN-VERMEER model has evaluated pitavastatin as active in micronucleus formation with low reliability. This data is consistent with the data provided by Toxtree, but as mentioned earlier, the reliability of the data is low.

In 1995, the genotoxic potential of atorvastatin was investigated using bacterial mutagenicity and micronucleus tests. This research found no biologically significant increase in the frequency of micronucleated polychromatic erythrocytes in the bone marrow, and the bacterial mutagenicity tests yielded negative results [30]. The result of the micronucleus test contradicts our findings from Toxtree but is consistent with the results from Vega. However, the outcome of the mutagenicity test aligns with the outputs of the other three programs except for T.E.S.T.

In a study conducted in 1994, the genotoxic and mutagenic potential of Fluvastatin was evaluated in vitro using Salmonella typhimurium, Escherichia coli (gene mutations), V79 Chinese hamster cells (HGPRT gene mutations, chromosomal abnormalities), primary cultures of rat hepatocytes (DNA repair), and BALB/3T3 cell malignant transformations. Additionally, in vivo testing was performed using the mouse bone marrow micronucleus test. The results of these tests did not reveal any evidence of carcinogenic, mutagenic, or genotoxic effects [12]. These data contradict our study findings. The empirical data and model prediction provided by the Vega program indicate that the substance is carcinogenic. The result of the micronucleus test contradicts with the Toxtree program, while the result of the mutagenicity test contradicts with the T.E.S.T program.

In a clinical study published in 2007 aimed at understanding the cancer risk among individuals using statins, people using statin drugs were followed for 9.4 years, and no significant evidence was found to suggest that statins contribute to causing cancer or preventing it [31]. A study conducted in 2010 using a rat model concluded that lovastatin has a significant tumor-preventive effect [32]. Various hypotheses have been proposed and the effects of statin drugs on site-specific cancer types have been investigated. Some studies have highlighted the anti-cancer properties of statin drugs, while clinical trials have emphasized that statin drugs do not cause cancer [33-35]. Although the in silico toxicity prediction programs we used provided various structural alerts for carcinogenicity, the development of cancer is influenced by many factors such as age, gender, diet, lifestyle, and others.

In the study conducted by Berber et al. in 2013, it was emphasized that Rosuvastatin may have in vitro genotoxic potential in human lymphocytes,

and micronucleus formation was observed along with chromosomal abnormalities [36]. Another study found that the total number of micronuclei in cells exposed to Atorvastatin was significantly higher compared to those in the control group [11]. The results of these studies are consistent with our micronucleus evaluation in Toxtree but contradict with Vega.

5. Conclusion

In this study, we conducted in silico toxicological assessments using four different tools to investigate various toxicological endpoints of statin medications. Through comparisons with experimental data and among the different in silico tools, we aimed to assess the reliability and consistency of these predictions. Our findings revealed inconsistencies across different models and programs, highlighting the importance of careful consideration and comparison of multiple sources of data and methodologies in toxicity assessments.

While some in silico models indicated potential genotoxic or carcinogenic properties for certain statins, these predictions were not always consistent across all models. Furthermore, discrepancies were observed when comparing in silico predictions with experimental data. These discrepancies may stem from fundamental classification schemes, training data sets, or the specific focus of each program and model. As each program has its own strengths and weaknesses, careful evaluation of the results regarding potential toxicological endpoints of statin drugs and the use of multiple data sources in decision-making are necessary. This underscores the necessity of incorporating multiple lines of evidence and utilizing a variety of methodologies in toxicity assessments to ensure robust and reliable conclusions.

Despite the limitations and inconsistencies in in silico predictions, our study contributes to the understanding of the potential toxicological effects of statins. Future research should focus on conducting comprehensive in vitro and in vivo genotoxicity studies to validate and further explore the findings derived from in silico assessments. Ultimately, such efforts will enhance our understanding of the safety profile of statin medications and facilitate informed decision-making regarding their clinical use.

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The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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