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LEMBAR
HASIL PENILAIAN SEJAWAT SEBIDANG ATAU *PEER REVIEW*
KARYA ILMIAH : JURNAL ILMIAH

Judul Karya Ilmiah (Artikel)	:	The Risk Factor Analysis of Femorotibial Joint Morphometrics Associated with Severity of Anterior Cruciate Ligament Tear Using MRI Examination: Study in Indonesia
Penulis Artikel	:	Dwi Windi Juniarti , **Hermina Sukmaningtyas** , Robin Novriansyah
Jumlah Penulis	:	3 Orang
Status Pengusul	:	Corresponding Author
Identitas Jurnal Ilmiah	:	a. Nama Jurnal : Open Access Macedonian Journal of Medical Sciences (OAMJMS)
		b. Nomor ISSN : 1857-9655
		c. Volume/nomor/bulan/tahu : 11(B):361-366
		d. Penerbit : Scientific Foundation SPIROSKI
		e. DOI artikel (Jika ada) : https://doi.org/10.3889/oamjms.2023.11473
		f. Alamat web Jurnal : https://oamjms.eu/index.php/mjms/article/view/11473/8431
		g. Terindeks di : SQOPUS Q3 SJR 0,257
		h. Link Turnitin : https://doc-pak.undip.ac.id/18107/2/TURNITIN-The-Risk-Factor-Analysis.pdf
		i. Link korespondensi :

Kategori Publikasi Jurnal Ilmiah (beri ✓ pada kategori yang tepat) :
- [√] Jurnal Ilmiah Internasional / Internasional Bereputasi
- [ ] Jurnal Ilmiah Nasional Terakreditasi
- [ ] Jurnal Ilmiah Nasional tidak Terakreditasi / Nasional terindeks di DOAJ, CABI, COPERNICUS

Hasil Penilaian *Peer Review* :

Komponen Yang Dinilai	Nilai Maksimal Jurnal Ilmiah			Nilai Akhir Yang Diperoleh
	Internasional / Internasional Bereputasi **(20)**	Nasional Terakreditasi	Nasional	
a. Kelengkapan unsur isi artikel (10%)	2			1,6
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d. Kelengkapan unsur dan kualitas terbitan/jurnal (30%)	6			6,0
Nilai Total = (100%)	**20**			**18,6**
Nilai pengusul = 8			**(40%x18,6/2) =**	**3,72**

KOMENTAR/ULASAN PEER REVIEW

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Ruang Lingkup dan Kedalaman Pembahasan	:	Ruang lingkup sesuai dengan bidang ilmu pengusul
Kecukupan & Kemutakhiran Data/ informasi dan Metodologi	:	Data dianaliss sesuai dengan metode
Kelengkapan Unsur dan Kualitas Penerbit	:	Macedonia journal nasional tidak terindex scopus

Semarang, 16 Mei 2023
Penilai

Dra. Ani Margawati, M.Kes., Ph.D.
NIP 196505251993032001

Unit kerja : Fakultas Kedokteran
Bidang Ilmu : Ilmu Kedokteran
Jabatan/Pangkat : Lektor Kepala / Pembina Tk. I

OPEN ACCESS MACEDONIAN JOURNAL OF MEDICAL SCIENCES

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Vol. 11 No. B (2023): B - Clinical Sciences

Published: 2023-01-04

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Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Feb 23; 11(B):252-258.
https://doi.org/10.3889/oamjms.2023.10094
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Cardiology*

Past Myocardial Infarctions and Gender Predict the LVEF Regardless of the Status of Coronary Collaterals: An AI-Informed Research

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Edited by: Mirko Spiroski
Citation: Singh S, Al-Imam A, Singh S, Tirpude AP, Chaudhary N, Al-Alwany A, Konuri VK. Past Myocardial Infarctions and Gender Predict the LVEF Regardless of the Status of Coronary Collaterals: An AI-Informed Research. Open Access Maced J Med Sci. 2023 Feb 23; 11(B):252-258. https://doi.org/10.3889/oamjms.2023.10094
Keywords: Cardiovascular diseases; Coronary angiograms; Coronary collaterals; Ejection fraction; Frequentist statistics; Ischemic heart disease; Machine learning; Narrow artificial intelligence
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Received: 12-May-2022
Revised: 27-Aug-2022
Accepted: 13-Sep-2022

Funding: This research did not receive any financial support
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: The degree of the development of coronary collaterals is long considered an alternate – that is, a collateral – source of blood supply to an area of the myocardium threatened with vascular ischemia or insufficiency. Hence, the coronary collaterals are beneficial but can also promote harmful (adverse) effects. For instance, the coronary steal effect during the myocardial hyperemia phase and that of restenosis following coronary angioplasty.

AIM: Our study explores the contribution of coronary collaterals – if any exist – while considering other potential predictors, including demographics and medical history, toward the left ventricular (LV) dysfunction measured through the LV ejection fraction (LVEF).

METHODS: Our cross-sectional design study used convenience sampling of 100 patients (n = 100; a male-to-female ratio of 4:1). We conducted frequentist inference statistics using IBM-SPSS version 24 and Microsoft Office Excel 2016 with the analysis ToolPak plugin; we ran parallel neural networks (supervised machine learning (ML)) and a two-step clustering (non-supervised ML) for robust conjoint inference with frequentist statistics.

RESULTS: The past incidents of myocardial infarction (p = 0.036) and gender (p = 0.072) influenced the LVEF; both are significant predictors at a 90% confidence interval. We found that gender and past incidents of MI influenced the LVEF regardless of the status of coronary collaterals. Our study did not yield any positive or significant findings concerning the status of coronary collaterals or the coronary circulation dominance patterns.

CONCLUSION: Regardless of the status of coronary collaterals, we verified that the female gender is protective of the LV function, contrary to the past infarction incidents that predispose to a deteriorated LV function. Our study's innovation relates to its status as the first study from India to explore the coronary collaterals and the ejection fraction while incorporating frequentist statistics and narrow artificial intelligence to infer reliable results.

Introduction

In 1669, Richard Lower of Amsterdam first described the coronary collaterals [1]. Nearly one century later, in 1757, the Swiss Anatomist Albert von Haller was the next to describe them [2]. During the mid-20th century, researchers could resolve controversies regarding the existence or otherwise of these anastomoses by utilizing different imaging techniques in patients with coronary artery diseases; it took even longer till the 1960s to identify and examine these unique vessels in living patients [3,4]. The coronary collateral circulation and its extent are long considered an alternate – that is, a collateral – source of blood supply to an area of the myocardium that is in jeopardy of blood supply deprivation due to ominous cardiovascular accidents [5]. A well-developed collateral blood supply can benefit the infarct by minimizing the ischemic effects; however, a study conducted by Schaper and coworkers (1979) among patients with a more extensive disease confirmed that angiographic identification of collaterals might also signify an unfavorable prognosis [6].

Reimer (1981) gave a clinicopathological description concerning the survival from a sudden episode of coronary occlusion, which could relate to the status of coronary collaterals [7]. Researchers from the 1970s and 1980s could only document the functional importance of coronary collateral flow measurement [8]. It was not until 2003 did notable achievements were recorded in the *in vivo* measurement of collateral flow in patients diagnosed with coronary atherosclerosis [9]. The established role of the precipitating factors for the formation and dislodgement of atherosclerotic plaques and the protective role of well-developed coronary collaterals in determining infarct size is noted [10]. However, well-developed coronary collaterals could also pose some adverse effects; these are still under study

[10]. For example, the possible adverse effects of a well-developed collateral architecture would be the coronary steal effect during the phase of myocardial hyperemia and that of restenosis after coronary angioplasty [11]. The development of restenosis in the presence of well-developed collaterals relates to the competition between the anterograde flow and the collateral flow [12].

Two recent studies have documented that the incidence of cardiovascular events was less in patients with angiographically demonstrated coronary collaterals provided that they were suffering from a chronic stable disease; however, they could not uniformly demonstrate the same advantage in those suffering from a more severe or an acute form of coronary disability [13]. In 2007, Meier and coworkers had a controversy over the beneficial effects of coronary collaterals resulting from the blunt method they used to gauge the collaterals by coronary angiography; limitations of the study included those related to the sample size and the study duration [14]. Meier and colleagues aimed to study a larger population of 845 patients (mean of age=62 ± 11 years) for 10 years using the method of assessing survival – that is, a 10-year survival analysis – after quantitatively obtained recruitable coronary collaterals; they measured the collateral flow index after a 1 min occlusion of the coronary artery by balloon inflation. Further, Konuri *et al*. theorized that the evolution of the cardiac muscles results from a phase transition from smooth muscles due to the altered pressure loads within the vertebrate circulation [15]. Konuri also explained that the coronary vessels developed from a network of capillaries in early embryology and gradually transformed into the branching type of coronary arteries, which goes in parallel with the conversion of the peristaltic movement of the early embryonic heart into an apex-to-base contraction in the mature heart [16].

The present study aims to determine the coronary collaterals' role in influencing the LV ejection fraction (LVEF). We explored other variables, including patients' demographics and medical history; we analyzed potential predictors, including sex, history of previous episodes of myocardial infarction, diabetes mellitus (DM), hypertension (HPT), and dyslipidemia. We conducted data analysis for robust conjoint inference – based on frequentist statistics and machine learning (ML) – to test our hypothesis.

Materials and Methods

The researchers conducted the study following the standard protocol of the ethics and scientific committee at the department of anatomy at the All India Institute of Medical Sciences in Raipur – India. The institute ethics committee at the All India Institute of Medical Sciences (AIIMS, Raipur – Chhattisgarh, India) approved the study per the registration number ECR/714/Inst/CT/2015/RR-18 on the 22nd of September 2018. The researchers obtained informed consent from each study participant.

The researchers measured the LVEF based on echocardiography while exploring the status of coronary arterial dominance and the coronary collaterals through three-dimensional (3D) echocardiography and transthoracic echocardiography, respectively (Vivid E9, GE Healthcare). Researchers conducted data analytics, including frequentist statistics and ML models, using IBM-SPSS version 24 and Microsoft Office Excel 2016 with the analysis ToolPak plugin. Our study is cross-sectional; it deployed a convenience sampling of 100 patients (n = 100, male-to-female ratio of 4:1).

We categorized the age of participants into three groups, including young adults (<40 years of age), middle-aged adults (40–59 years), and senior adults (60 years and older). Further, age did not differ significantly between individuals with normal and dysfunctional LVEF (57.69 vs. 58.33, p = 0.794). We also referred to the categorization of the magnitude of the LV dysfunction into four groups, including standard or normal (LVEF 50–70%), mild dysfunction (40–49%), moderate dysfunction (30–39%), and severe dysfunction (below 30%).

Our neural network deployed a multilayer perceptron neural, a scaled conjugate gradient optimization algorithm, and a default SPSS allocation of the training set and testing set at 70% and 30% of the whole dataset. The neural networks yielded synaptic weights and independent variables importance analysis as an equivalent measure of the effect size in classical statistics. Two-step clustering utilized Schwarz's Bayesian criterion, and the log-likelihood distance measure.

Results

Our study deployed a convenience sampling of 100 patients (n = 100, male-to-female ratio of 4:1). According to descriptive statistics, we calculated the mean, standard error of the mean, skewness, and kurtosis for age (57.79, 0.961, −0.349, and 0.456), and the LVEF (60.26, 1.110, −0.213, and 0.077). Study participants allocated to males (n = 80, 80%) and females (n = 20, 20%), diabetic (n = 56, 56%) and non-diabetic (n = 44, 44%), hypertensive (n = 60, 60%) and non-hypertensive (n = 40, 40%), and participants with (n = 29, 29%) and without dyslipidemia (n = 71, 71%). Further, patients included those with (n = 56, 56%) and without a history of myocardial infarction (n = 44, 44%). Concerning coronary arterial dominance, patients represented four categories; Right coronary artery (RCA) (83%), Left circumflex (LCX) (9%), RCA+ Left coronary artery (5%), and RCA+LCX (3%), while

patients with coronary arterial collaterals accounted for 40% of the study participants.

Data analysis detected extreme values concerning age and LVEF. However, normality testing using the Shapiro–Wilk test confirmed that age (test statistic=0.987, df=100, p = 0.409) and LVEF (0.985, 100, p = 0.308) followed a normal distribution. Hence, we conducted parametric inferential statistics for hypothesis testing. We also carried descriptive statistics for the scale (continuous) variable, LVEF, while stratifying the sample based on the categorical variables (Table 1). Provisionally, LVEF varied based on three variables, including gender, past myocardial infarction incidents, and coronary arterial dominance. Accordingly, we shall test these assumptions using frequentist statistics.

Table 1: Stratification of left ventricular ejection fraction by independent variables and covariates

Independent variable	Mean	SEM
Gender		
Female	64.9550	2.65826
Male	59.0900	1.19008
Total	60.2630	1.10949
DM		
No	60.8864	1.41448
Yes	59.7732	1.64856
Total	60.2630	1.10949
HPT		
No	59.3725	1.68682
Yes	60.8567	1.47435
Total	60.2630	1.10949
Dyslipidemia		
No	60.6225	1.41336
Yes	59.3828	1.65607
Total	60.2630	1.10949
Coronary arterial dominance		
LCX	60.1556	3.89740
RCA	59.9843	1.24805
RCA+LCA	64.2400	2.92790
RCA+LCX	61.6667	6.17342
Total	60.2630	1.10949
Status of collaterals		
No	60.4733	1.64556
Yes	59.9475	1.28952
Total	60.2630	1.10949
History of MI		
No	63.2295	1.70010
Yes	57.9321	1.39882
Total	60.2630	1.10949

†LCX: Left circumflex, LCA: Left coronary artery, RCA: Right coronary artery, DM: Diabetes mellitus, HPT: Hypertension, MI: Myocardial infarction, SEM: Standard error of mean, LVEF: Left ventricular ejection fraction.

According to our pre-study hypothesis, principles of causality of the Bradford Hill criteria, and the preliminary descriptive statistics, we are assuming that the dependent variable (outcome) LVEF is affected by three independent variables (predictors); coronary arterial dominance, the status of collaterals, and history of MI, as well as covariates, including age, gender, DM, HPT, and dyslipidemia. Therefore, LVEF modulates in correspondence with the heart's inherent properties and biomechanical architecture, past incidents of infarction, and other demographic factors. We shall test this hypothesis while attempting to reconcile frequentist statistics with ML (supervised and non-supervised) to explore predictors that significantly affect the LVEF, which might be valuable to prognosticate which individuals can develop LVEF dysfunction in the future when running predictive models based on our analytics.

Using Pearson's bivariate correlations, age did not correlate significantly with LVEF (Pearson's correlation coefficient=0.017, p = 0.869). Pearson's Chi-square of independence and Fisher's exact test did not yield conclusive results concerning the categorical variables' association. Using SPSS, we ran linear modeling as a function of regression analysis to explore the existence of potential significant predictors concerning LVEF and the status of the LV dysfunction. Linear modeling implemented forward stepwise regression (information criterion = 477.345, accuracy = 6.9%). We realize that potentially hundreds of variables interact to manifest the full variance within the outcome LVEF. Thus, our model is simplistic, while more complex models mandate studies with extensive (larger) samples based on high-dimensional and big data using more advanced statistical packages, probably running on more powerful computers. Our regression model trimmed outliers and transformed the age variable to conduct multiple linear regression properly. The model assigned the predictor importance for two variables only, history of MI (coefficient = 4.657, predictor importance = 0.578, p = 0.036) and gender (−4.394, 0.422, and 0.072) (Figure 1); thus, both predictors are significant at an alpha value of 0.10 (90% confidence interval) and can serve as a predictive model to anticipate LV dysfunction. Based on the results from the regression analysis, we shall further explore the existence of significant between-groups differences based on the former two variables (history of MI and gender) and other variables concerning the LVEF, using the independent t-test, univariate analysis of variance (ANOVA), neural networks (supervised ML), and two-step cluster analysis (non-supervised ML).

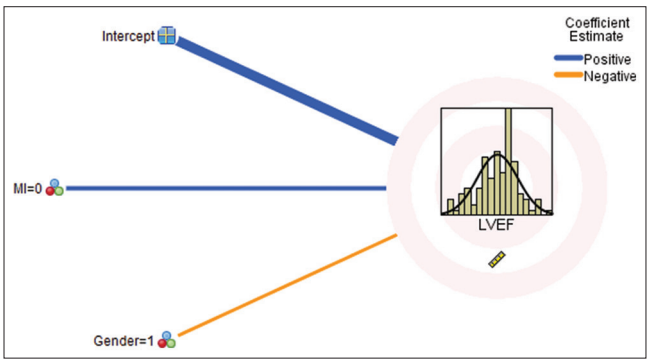

Figure 1: Linear modeling: Summary of the interaction of predictors and outcome. †MI=1 and 0 represent patients with and without a history of MI, respectively. Gender=1 and 2 represent male and female patients, respectively.

Independent t-testing confirmed the existence of significant differences between males and females in connection with LVEF (t =−2.153, df = 98, mean difference =−5.865, p = 0.034) and also based on history of MI (t = 2.428, df = 98, mean difference = 5.297, p = 0.017). There were no statistically significant differences among groups concerning diabetes, HPT, dyslipidemia, or the status of coronary collaterals; these results reconciled with those from the earlier linear regression model. We also evaluated age and coronary arterial dominance as potential predictors of LVEF using one-way ANOVA. Nevertheless, none has a significant

effect on LVEF (age: adjusted R^2 =−0.011, df = 2, mean square = 56.863, F = 0.457, p = 0.635; coronary arterial dominance: adjusted R^2 = −0.024, df=3, mean square = 30.514, F = 0.242, p = 0.867). Further, we ran a summative multifactorial ANOVA by incorporating both variables (age and arterial dominance) to assess the interaction effect that may significantly influence LVEF. However, this model also did not generate any significant predictors (adjusted R^2=-0.017, df = 8, mean square = 99.144, F = 0.792, p = 0.611).

We conducted three neural network analyses; the first deployed the three predictors (coronary arterial dominance, status of collaterals, and history of MI) without covariates; it assigned the highest predictor importance to the history of MI (importance = 0.456, normalized importance = 100%), coronary arterial dominance (0.430, 94.2%), and the status of collaterals (0.114, 25.1%). The second network considered covariates, including age, gender, DM, HPT, and dyslipidemia. Incorporating the covariates conveyed other results while assigning the highest predictors' importance to coronary arterial dominance (Table 2), which mandates running a third summative model. The results of the third network reconciled with the former regression analysis, t-test, ANOVA, and the previous neural networks; it integrated MI history (importance = 0.186, normalized importance = 35.5%), gender (0.290, 55.4%), and age (0.524, 100.0%).

Table 2: The second neural networks model: Independent variable importance analysis

Independent variable	Importance	Normalized importance (%)
Coronary arterial dominance	0.228	100.00
Age	0.211	92.40
Hypertension	0.165	72.20
History of MI	0.112	49.20
Dyslipidemia	0.110	48.50
DM	0.076	33.50
Status of collaterals	0.076	33.20
Gender	0.023	9.90

DM: Diabetes mellitus, MI: Myocardial infarction.

To complement analytics based on artificial intelligence, we ran a non-supervised ML model, using a two-step clustering algorithm by considering two variables: The history of MI and gender. The choice of the independent variables corresponds to the results from the earlier frequentist statistical analysis and the supervised ML models. The clustering model has a good quality (silhouette measure of cohesion and separation = 1). It generated four clusters (size of clusters: 48%, 32%, 12%, and 8%; the ratio of largest to smallest cluster = 6) while assigning the independent variable importance equally to gender (predictor importance = 1) and history of MI (predictor importance = 1). The first and second clusters had males strictly, while the third and fourth clusters had only females. The first cluster has the vast majority of cases with the past incidents of MI; the second cluster has the vast majority of patients without a history of MI; the third cluster has no contribution of cases with a history of MI, while the fourth cluster has a minimal contribution from participants with the past incidents of infarction. In summary, the history of MI and gender influences the LVEF, and it appears that the female gender is protective of the LV function contrary to the past incidents of infarction that predisposes to a deteriorated LV function which corresponds with lower LVEF. Most importantly, our analyses did not show any significant predictor effect of the status of coronary collaterals that might influence the LVEF.

Finally, we conducted a binary logistic regression in which we fed the model with a transformed LVEF scale (continuous) variable into a dichotomous categorical variable (normal versus dysfunctional) as the outcome (dependent) variable, while the model's predictors included all other variables; all were categorical variables, including a transformed age variable. The regression indicated a significant effect – but with an overall weak effect size (Nagelkerke pseudo r-squared = 0.233, exp(B) = 0.176, p < 0.001). Binary logistic regression analysis confirmed somewhat variegated results; it verified a statistically significant effect of two predictors; history of MI (exp(B) = 0.275, p = 0.084) and DM (exp(B) = 0.168, p = 0.035); these were significant at 90% and 95% CI, respectively.

Logistic regression conveyed some novel results concerning significant predictors, specifically DM, which can relate to data reduction by feeding the model with a categorical variable for age rather than a scale measurement and a dichotomous categorical variable (normal versus dysfunctional LV), rather than an ordinal one (normal function, mild dysfunctional, moderate dysfunctional, and severe dysfunctional). We also reinstate that the analytics in this study represent an oversimplification of hundreds of explanatory variables, including cofactors and covariates that interact elaborately and multidirectionally to manifest the complete variance within the observed LVEF.

Discussion

We successfully reconciled frequentist statistics and narrow artificial intelligence models concerning the study objectives; we established potential causal relations between the outcome (LV dysfunction) and the predisposing factors. Predictors included the history of myocardial infarction, DM, gender, and age. Future studies should incorporate a more extensive sample while considering more variables from a high-dimensional dataset.

Hoole *et al*. have noticed the scaffolding effect of coronary collaterals in remodeling the ischemic myocardium [17]. One year earlier (2011), Choi and collaborators demonstrated that well-developed angiographic collaterals distal to the occlusion were associated with a lower frequency and transmurality of the previous infarction; this suggests a protective

role for the demonstrable collaterals distal to the thrombotic occlusion venue [18]. Canto and colleagues (2012) studied the association of age and sex on the presentation of symptomatology; they found a positive correlation with in-hospital mortality among MI patients [19]. Researchers concluded that chronic stable coronary artery disease is a benign disease and to estimate the prognostic significance of the well-developed angiographically documented collaterals, they require the follow-up study of sizable populations of patients [20], [21]. Brugaletta *et al*. have studied the relationship between the presence of collaterals and the incidence of restenosis after coronary angioplasty [22].

The innovative aspects of our study relate to its status as the first observational study conducted in India to explore the coronary collaterals, the ejection fraction, and the LV function based on several risk factors and predictors while incorporating frequentist statistics and ML. The composite of classical statistics and narrow artificial intelligence (nAI) models is novel because it can yield superior results for inferential purposes. Al-Imam introduced the former methodology in a thesis in which he explored pterygomaxillary morphometrics [23,24]. The rationale for a hybrid analytic corresponds to and addresses several elements; for instance, it provides (a) Collateral evidence based on ML algorithms. (b) An alternative method to classical data analytics. (c) Reconciliation of non-Bayesian statistical models, including the univariate and multivariate models, with nAI models. (4) A convergent thinking approach that deals with the research question from alternative standpoints. (5) A novel problem-solving approach. (6) An innovative research method that can serve as a blueprint for future research within the discipline of cardiology, vascular surgery, and precision medicine.

Nonetheless, our research does have limitations other than those inherent to observational studies, including the sample size, which is relatively small. In addition, unique parameters for the sample cannot be fully known, for example, other demographic variables including socioeconomic status and underlying pathologies affecting other body systems. Statistical analyses also possess limitations, for instance, the augmented type-1 (α) statistical error due to multiple data analytics. Besides, the interpretation of causality that we implemented may accept different perspectives, including arguing the basis of the Bradford Hill criteria when classifying specific variables into independent (predictors) and dependent (outcomes) [25].

Future research should explore different populations while accessing representative samples with sufficient participants to detect the hypothesized effect per the pre-study hypotheses. Researchers can conduct more robust study designs of superior level of evidence, including prospective cohorts, quasi-experimental, and experimental designs; the latter include the randomized controlled trials of the supreme level of evidence [26]. Interdisciplinary scholars can deploy our methodology for replicable data analysis within other medical disciplines interlinked with cardiovascular diseases, relevant anatomy, and pathophysiology.

Scholars can explore the adverse effect of psychoactive substances – such as captagon, octodrine, and NBOMe – on the coronary vessels and coronary collaterals while monitoring the adverse effect on the entire cardiovascular system [27-32]. Psychedelics represent a subset of psychoactive and novel psychoactive substances (NPSs), also known as hallucinogens and entheogens, such as LSD, DMT, psilocybin ayahuasca, and cannabis, among others. Psychoactive substances, NPS, and psychedelics can affect the heart and its vasculature, including coronary collaterals and cardiac functioning [33-36]. Researchers can also explore the effect of pathogens – including viral infections – on coronary circulation and its collaterals, for instance, the novel coronavirus 2019 and its variants, responsible for the current SARS-CoV-2 pandemic [37]. These anticipated research attempts – in parallel with the exploitation of big data in medicine – will foster the march from classical medicine toward personalized and precision medicine; Ashley (2016) described precision medicine as “the definition of disease at a higher resolution by genomic and other technologies to enable more precise targeting of disease subgroups, or even individuals, with new therapies. Prominent examples include cystic fibrosis and cancer” [38-40].

Finally, our results indicated that the history of myocardial infarction and gender influenced the LVEF. The female gender is protective of the LV function, contrary to the past incidents of infarction that predispose to a deteriorated LV function and correspond with lower LVEF. Further, the female gender demonstrated a better LV function, irrespective of the past incidents of myocardial infarction. In the present study, we succeeded in reconciling non-Bayesian statistics and techniques of narrow artificial intelligence (ML) to evaluate the ejection fraction of the left ventricle and study its potential predictors – while emphasizing the status of the coronary collaterals. To conclude, our research succeeded in reconciling frequentist statistics and narrow AI models to evaluate the ejection fraction of the left ventricle and study its potential predictors. However, it did not to validate any positive or significant findings concerning the status of coronary collaterals versus the LVEF. Positive and significant findings existed concerning two parameters only: Gender and MI history.

Conclusion

In the present research, we succeeded in reconciling non-Bayesian (frequentist) statistics and narrow AI models to evaluate the ejection fraction of the left ventricle while examining other potential risk factors, including coronary dominance, coronary

collaterals, patients' demographics, and medical history. Nonetheless, our study did not yield any positive or significant findings concerning the status of coronary collaterals versus the LVEF. Positive findings existed concerning two parameters: Gender and history of MI. The history of MI and gender influenced the LVEF; the female gender is protective of the LV function, contrary to the past incidents of infarction that predisposes to a deteriorated LV function and corresponds with lower LVEF. Our results could be of importance for precision and personalized medicine.

Availability of data

The authors abide by an open-access policy concerning medical research data. All data will be available on request from the corresponding author for 3 years following the publication, pending justifiable requests.

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Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2023 Feb 24; 11(B):412-420.
https://doi.org/10.3889/oamjms.2023.11114
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Oncology*

Cytotoxicity and Antitumor Activity of Arglabin and its Derivatives

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Edited by: Ksenija Bogoeva-Kostovska
Citation: Adekenov S, Spiwok V, Beutler J, Maslova O, Rakhimov K. Cytotoxicity and Antitumor Activity of Arglabin and its Derivatives. Open Access Maced J Med Sci. 2023 Feb 24; 11(B):412-420. https://doi.org/10.3889/oamjms.2023.11114
Keywords: Sesquiterpene lactones; Arglabin; Molecular docking; Cytotoxicity; Antitumor activity; Farnesyl protein transferase; Topoisomerases -I and -II
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Received: 13-Oct-2022
Revised: 12-Dec-2022
Accepted: 14-Feb-2023

Funding: The work was carried out under the grant project AP09259740 "Study of the "structure-activity" relationship and pharmacological study of new plant metabolites and their derivatives" funded by the Science Committee of the Ministry of Education and Science of the Republic of Kazakhstan
Competing Interests: The authors have declared that no competing interests exist

Abstract

BACKGROUND: At present, more than 8000 sesquiterpene lactones have been isolated and described from natural sources, a significant part of which has cytotoxicity and antitumor activity. One of the practically available sesquiterpene lactones is arglabin, which, as a renewable material, is used for the synthesis of new compounds. The article presents data on the study of cytotoxicity and antitumor activity of the arglabin and its derivatives using molecular modeling methods and, in the experiment *in vitro* and *in vivo*.

AIM: The aim of this work is to study the cytotoxicity and antitumor activity of new compounds based on the sesquiterpene lactone arglabin using molecular modeling and experimental pharmacology.

METHODS: ChemDraw programs and a set of AutoDock programs were used for computer simulation. Molecular docking was carried out using the Maestro graphical interface of the Schrödinger Suite software package (Schrödinger, LLC, New York, NY, 2017). Docking modes standard precision and XP (extra precision) were used. In *in vitro* experiments, the antitumor activity of compound samples was studied in models of 60 human tumor cell lines, and clonogenic C6 rat glioma cells. The antitumor activity of the samples was studied in experiments *in vivo* on white outbred rats with transplanted tumors and was evaluated by the inhibition of tumor growth and the magnitude of the increase in average life expectancy.

CONCLUSION: When studying the antitumor activity on 60 cell lines of tumor cells (NCI60), clonogenic cells of C6 rat glioma, a high antitumor activity of some arglabin derivatives was established. The connection between the structure of arglabin derivatives and their inhibitory effect on farnesyl protein transferase, topoisomerases -I and -II was studied.

Introduction

In recent years, effective cytostatics based on natural compounds have been increasingly used in modern medicine. High expectations are placed on natural compounds of plants, the action of which is aimed at new cellular targets for solving one of the main problems of modern clinical oncology-multidrug resistance of tumors. A targeted search for new cytostatic agents involves, first of all, the development of molecules that can actively influence the pathological cells of the body and their growth.

One of the promising classes of natural compounds in this field is sesquiterpene lactones, many of which are cytotoxic *in vitro*, and some demonstrate an antitumor effect *in vivo* [1].

This is a large group of natural terpenoids with a wide range of biological activities: anti-inflammatory, cytotoxic, cardiotonic, analgesic, antispasmodic, hypoglycemic, hypotensive, antibacterial, antifungal, etc. [2]. To date, the structures of over 6000 sesquiterpene lactones have been established, and the largest number of them has been isolated from flowering plants - representatives of *the Asteraceae (Compositae)* family.

Cytotoxic sesquiterpene lactones include costunolide 1, eupatolid 2, parthenolide 3, dehydrocostuslactone 4, thapsigargin 5, and arglabin 6 [3]. On the basis of the sesquiterpene lactone derivative arglabin, JSC "IRPH "Phytochemistry" developed the antitumor drug "Arglabin" [4].

Sesquiterpene α-methylene-γ-lactones exhibit cytotoxicity, antiviral, antimicrobial, and antitumor properties. One of the important features of sesquiterpene lactones is the presence in their structure of an α-methylene-γ-lactone fragment, which is responsible for biological activity, especially antitumor activity. An exocyclic double bond conjugated to a carbonyl function is an alkylating agent and can act on transcription factors and enzymes in the human body. And it is assumed that the exomethylene group conjugated to the carbonyl group in the lactone ring is responsible for the cytotoxicity of sesquiterpene lactones [5-7].

The aim of this research is to study the cytotoxicity and antitumor activity of new derivatives

of the sesquiterpene lactone arglabin using computer modeling and experimental pharmacology methods.

Materials and Methods

The following derivatives of the sesquiterpene lactone arglabin were presented for the study: α-epoxyarglabin 7, β-epoxyarglabin 8, dioxyarglabin 9, dimethylaminodioxyarglabin 10, and dimethylaminodioxyarglabin hydrochloride 11.

In vitro antitumor activity of the compounds was studied on a model of 60 human cell lines of tumor origin. At the first stage of screening, three highly sensitive human cell lines MCF-7 (breast carcinoma), NCI-H460 (lung carcinoma), and SF-268 (glioma) were supplemented with the test substance at a standard concentration and incubated for 48 h. NCI60 is based on the SRB method for determining the viability of cell cultures using the pink anionic dye sulforodamine B [8]. If the test substance inhibits the growth of at least one cell line, it proceeds to the next stage of testing on a full panel of 60 cell lines. The test substance was then added to the cells at five different concentrations.

Furthermore, *in vitro* antitumor activity was studied on the model of clonogenic C6 rat glioma cells, i.e. experimental *in vitro* model of human glioblastoma multiforme, rat C6 glioma cell line 8–14 passages *in vitro*) and on the primary culture of *in vitro* cells isolated from biopsy material of children with brain gliomas (medulloblastoma) [9], [10].

In vivo antitumor activity of the samples was studied on white outbred rats with transplanted tumors of mice and rats. The antitumor effect of the studied compounds was determined by daily intraperitoneal injection in a 2% solution of dimethyl sulfoxide (DMSO) for 5 days at the maximum tolerated dose [11], [12]. To evaluate the antitumor activity of the compounds, we used the percentage inhibition of tumor growth and the value of the increase in life expectancy (IILE), determined immediately after the end of treatment.

Cytotoxicity was studied under *in vitro* conditions in the survival test of larvae of marine crustaceans *Artemia salina (Leach)* (Brine shrimp toxicity bioassay method) [13], [14]. The analysis was carried out according to the method proposed by Meyer and McLaughlin with minor modification.

The test was carried out using ready-made samples at a concentration of 100 μg/mL, 10 μg/mL and 1 μg/mL, as well as positive and negative controls, which were 13-dimethylamino-1,10β-epoxy-5,7α,6 hydrochloride, 11β(H)-guai-3,4-en-6,12-olide (substance of the Arglabin drug), which has antitumor (cytotoxic) activity, and DMSO, used to dilute the test compounds, in three parallel experiments. Each test sample and control sample were transferred to different labeled tubes into which about 10 actively swimming shrimp were released. The tubes were stored at room temperature at about 22°C. Lethality was observed within its range of activity and was assessed after 24 h of exposure. The analysis was carried out in triplicate and the values were recorded in vials containing 5 ml of solution and 10 shrimp. To determine IC_{50} values, eight different concentrations were tested [15].

Three-dimensional structures of receptors were taken from the RCSB PDB database (http://www.rcsb.org/): Human DNA topoisomerase I - PDB ID: 1SEU; human DNA topoisomerase II - PDB ID: 3QX3 and human farnesyl transferase - PDB ID: 1S63 which are intracellular targets of cytotoxic and antitumor drugs [16], [17].

Molecular docking was carried out using the Glide program of the Schrödinger Suite (Schrödinger, LLC, New York, NY, 2017). Docking modes standard precision and XP (extra precision) were used.

As the final results, the value of the scoring function *G-score* was used, showing the energy and strength of binding of the ligand to the target molecule.

Statistical analysis

Statistical processing of the results was carried out using “The GraphPad Prism v. 6.0.” The obtained results are presented as “mean value ± standard error of mean value.” Differences were considered significant at the achievement significance level $p<0.05$ using the Kruskal–Wallis H test.

Results and Discussion

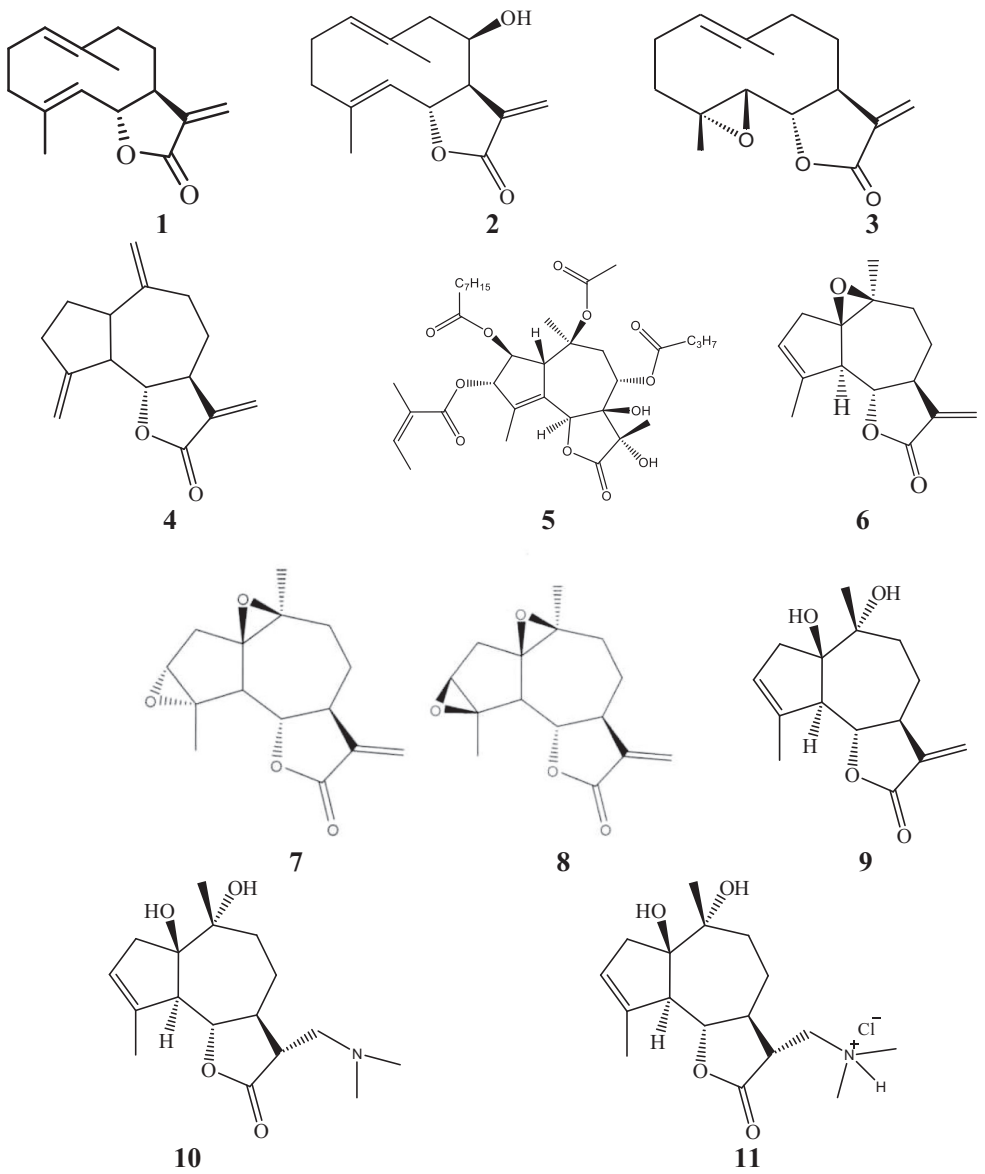
Molecular docking of arglabin and its derivatives

According to the results of the docking, it was found that the presented molecules of sesquiterpene lactones showed interaction with the receptors of DNA topoisomerase I, DNA topoisomerase II, and farnesyl transferase (Table 1).

Dioxyarglabin 9 and dimethylaminodioxyarglabin hydrochloride 11 showed relatively high binding energies to DNA topoisomerase II (−9.0 and −9.0 kcal/mol, respectively) and to DNA topoisomerase I (−8.4 and −7.1 kcal/mol, respectively).

α-Epoxyarglabin 7, β-epoxyarglabin 8, and dimethylaminodioxyarglabin 10 showed relatively strong binding to DNA topoisomerase II (−7.8, −7.6, and −7.6 kcal/mol, respectively) and to DNA topoisomerase I (−7.0, −6.0, and −6.8 kcal/mol, respectively).

Dioxyarglabin 9, dimethylaminodioxyarglabin 10, and dimethylaminodioxyarglabin hydrochloride



11 showed relatively high binding energies to farnesyl transferase (−7.4, −7.3, and −7.0 kcal/mol, respectively). A relatively strong bond with farnesyl transferase was shown by α-epoxyarglabin 7 and β-epoxyarglabin 8 (−6.6 and −5.5 kcal/mol, respectively).

Table 1: Binding energies of complexes of sesquiterpene lactone compounds with receptors of DNA topoisomerase I, DNA topoisomerase II, and farnesyl transferase

Compound	Receptor	Binding energy, kcal/mol
α-epoxyarglabin 7	DNA topoisomerase I	−6.0
	DNA topoisomerase II	−7.6
	Farnesyl transferase	−6.6
β-epoxyarglabin 8	DNA topoisomerase I	−6.8
	DNA topoisomerase II	−7.6
	Farnesyl transferase	−5.5
1β,10α-dioxyarglabin 9	DNA topoisomerase I	−7.1
	DNA topoisomerase II	−9.0
	Farnesyl transferase	−7.0
Dimethylamino-1β,10α-dioxyarglabin 10	DNA topoisomerase I	−7.0
	DNA topoisomerase II	−7.8
	Farnesyl transferase	−7.3
Dimethylamino-1β,10α-dioxyarglabin hydrochloride 11	DNA topoisomerase I	−8.4
	DNA topoisomerase II	−9.0
	Farnesyl transferase	−7.5

Dimethylaminodioxyarglabin hydrochloride 11 showed the best values of binding energy with all three studied biological targets for cytotoxic activity: DNA topoisomerase I, DNA topoisomerase II, and farnesyltransferase (−-8.4, −9.0, and −7.5 kcal/mol, respectively).

The obtained high binding energies of the studied arglabin derivatives with biological intracellular anticancer drug targets and DNA-processing enzymes suggest a mechanism of their cytotoxic action, which prevents the repair of breaks and causes the accumulation of damaged DNA molecules, thereby forcing the death of the tumor cell (Figures 1-3).

Antitumor activity on rats with transplanted tumors

Antitumor activity in relation to transplanted strains indicates the prospects of searching among

them for new agents for the chemotherapy of malignant tumors (Table 2).

It was found that the presence in the sesquiterpene lactone molecule of such alkylating centers as α-methylene-γ-lactone, α, β-unsaturated keto group, epoxy cycle, as well as functional groups such as the hydroxyl function, halogen atoms contribute to the inhibition of the growth of tumor strains [18].

In the series of sesquiterpene lactones with a guayanic structure, arglabin 6 and its modified derivatives exhibited high antitumor activity.

The study of the antitumor activity of arglabin 6 and its derivatives characterizes the different effect of their action on strains of 8 transplanted tumors [19]. Considering the dependence of the antitumor activity of arglabin 6 and its derivatives on the structural features of their molecules, the following should be noted:

- The introduction of halogen atoms (bromine and chlorine) enhances the antitumor effect, so 3,4-dibromide arglabin 15 inhibits the growth of strains of Pliss lymphosarcoma (LPS), alveolar liver cancer PG-I, sarcoma M-I and sarcoma-45 by 70–90%, and dichlorodihydroxyarglabin 16 inhibits the growth of sarcoma-45, sarcoma M-I and LSP resistant to prospidin, up to 71%.
- Epoxidation of arglabin at the C3-C4 skeletal double bond also increases its antitumor activity. Thus, β-epoxyarglabin 8 in MPD (30 mg/kg) significantly inhibits the growth of sarcoma-45, sarcoma M-I (78–88%), alveolar liver cancer PC-I, Pliss LPS, and breast cancer (59–88%). 72%. This compound 8 in MPD has a pronounced antitumor activity against Pliss LPS, resistant to leukoephdin (80% inhibition), rubomycin (78.0%) and sarcoma-45, resistant to 5-fluorouracil, prospidin and rubomycin (66.0–78%) [20].
- Among the amino derivatives of arglabin, dimethylaminoarglabin 17 and its hydrochloride 18 show pronounced antitumor activity.

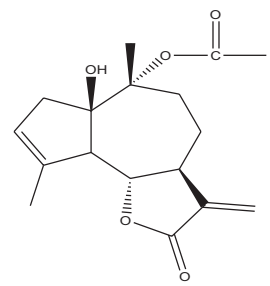
Of interest is dimethylaminoarglabin hydrochloride 18, highly soluble in water, which turned out to be practically important in the preparation of a rational prescription for the dosage form of the drug.

Dimethylaminoarglabin hydrochloride 18, when intraperitoneal and intratumor injection, inhibits the growth of LL lung carcinoma, Ehrlich solid tumor, Ca-755 breast adenocarcinoma, and sarcoma 37 by 86–90%; lymphocytic leukemia P-388 (IILE-84%), sarcomas-180 (70%), Pliss lymphosarcoses, M-I sarcomas, Walker's carcinosarcomas (72–79%), and alveolar mucosal liver cancer PC-I (up to 89%), causes more than 80% inhibition of the growth of Pliss LPS, resistant to prospidin, and sarcoma 45, resistant to sarcolysin [4].

Compound 16 effectively affects the initial stages of the formation of metastases in the lungs,

Table 2: Antitumor activity and toxicity of arglabin and its derivatives

Name of sesquiterpene lactone	Dose mg/kg	Growth inhibition of tumor strains, %										Resistant Options				
		Lymphosarcoma Pliss	Walker's carcinosarcoma	Guerin's carcinoma	Sarcoma 45	Sarcoma M-1	Breast cancer RMK-1	Solid Ehrlich tumor	Alveolar liver cancer PC-1	Leukemia R-388	Leukemia L-1210	Sarcomas 45			Lymphosarcoma Pliss	
												to 5-foruracil	to sarcolysin	to prospidin	to rubomycin	to leucoefdin
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	19
Arglabin 6	30	57.6	41.1	48	23.0	55.6			32.1	43.0	34.0		59.7			
3,4β-epoxy-arglabin 8	30	72.1	36.4		88.8	78.4	59.6		70.4			66.0		70.4	78.6	79.8
Dimethylamino arglabin 10	50	56.0	30.0	85.1	79.0				42.0	80.1		52.1				
3,4-dibromarglabin 15	50	51.0	17.1	90.0	74.2				69.0	46.9		46.3				
Dimethylaminoarglabin hydrochloride 16	50	52.0	76.1	86.5	83.1				80.0	109.0		62.3				
Dimethylamino-3, 4β-epoxy-arglabin 19	50	64.6	43.1	31.4	58.1				38.0	51.0		11.2				
1β,4β-dioxy-3β,10α- dichlorarglabin 20	50	29.0	63.2	71.4	70.9				51.0	92.1		70.6				
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	19
Dimethylamino-3, 4β-epoxyarglabin hydrochloride 21	50	47.0	51.4	15.6	32.4				29.1	31.2		13.2				
Chlorhydrinarglabin 22	50	49.1	38.4	43.1	21.0				31.0	20.4		15.2				
Kolhamin	2	54.4	30.1		20.4							19.6				



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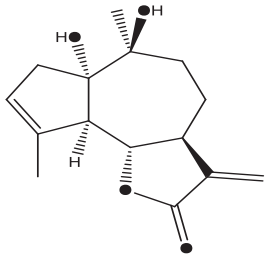

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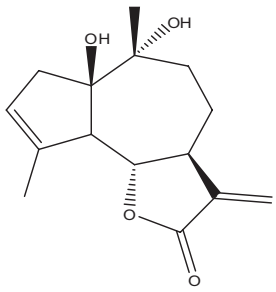

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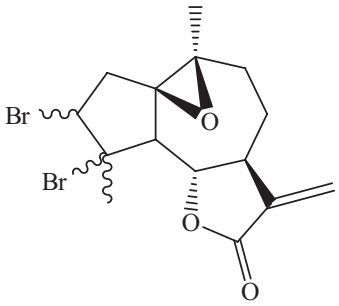

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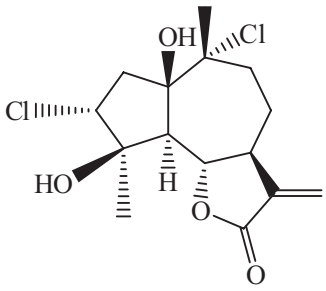

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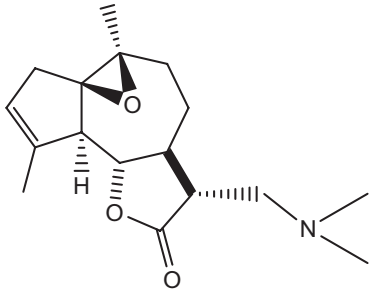

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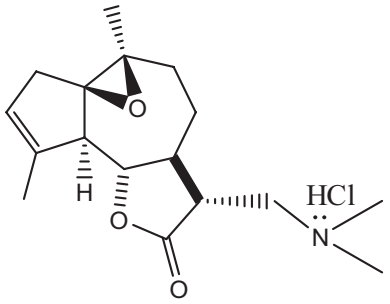

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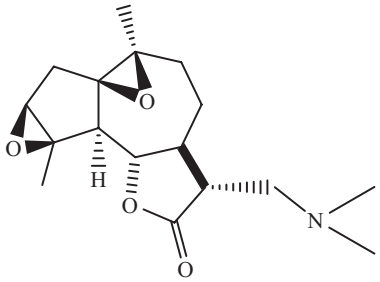

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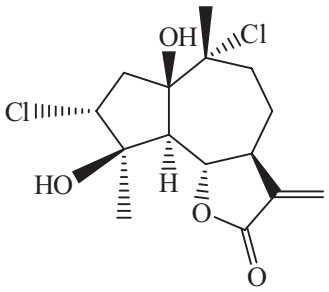

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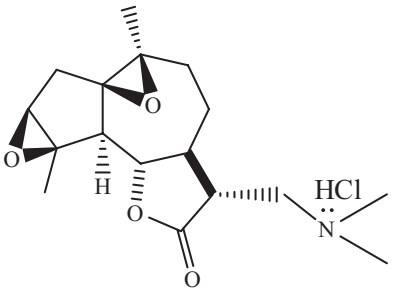

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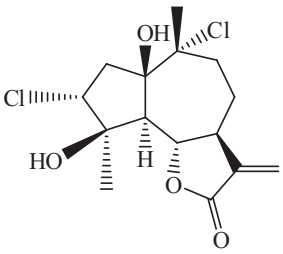

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that is, at the moment of attachment of tumor cells to the lung tissue, and by its activity exceeds the effect of the approved drug Vinblastine by 2 times. At a dose of 100 μg/mL, it inhibited by 70–80% the growth of tumor cells from the ascitic fluid of patients with ovarian cancer.

It has been proven that sesquiterpene lactones helenalin, its derivatives, parthenolide 3 modulate various cellular signaling pathways due to their high reactivity. Therefore, natural sesquiterpene lactones can be considered as specifically acting agents. On the other hand, due to the high reactivity of the thiol group in cysteine, as well as the amino group in lysine, they actively interact with the exomethylene group conjugated with the carbonyl of γ-lactone in the Michael type [21].

According to the results of the study of arglabin 6, it was determined that the drug has a pronounced cytotoxic effect against lung tumors and human ovarian cancer. At the same time, it does not have a depressant effect on hematopoiesis; it does not exhibit immunosuppressive activity, which distinguishes it favorably from the drugs currently used in the clinic, cyclophosphamide, and etoposide.

The sesquiterpene lactone parthenolide 3 inhibits nuclear factor kB (NF-kB), the STAT3 signaling protein, and NFAT-mediated transcription of anti-apoptotic genes. Thus, parthenolide 3 is a metabolic inhibitor to slow oncogenesis and inhibit tumor growth. Parthenolide 3 also inhibits gene expression induced by IL-6-type cytokines by blocking phosphorylation of

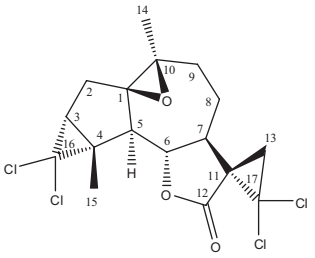
the STAT3 signaling protein on tyrosine 705 (Tyr705), which explains its anti-inflammatory activity [22,23].

In addition to the antitumor effect of parthenolide 3, it has been found that this pseudoguayanolide inhibits 5-lipoxygenase and cyclooxygenase in leukocytes, as well as the release of 14C-serotonin from platelets and platelet aggregation induced by adrenaline, adenosine diphosphate, sodium arachidonate, and collagen ionophore [24].

A problem with sesquiterpene lactones, as with many other natural compounds in the field of chemotherapy, is their poor aqueous solubility and high toxicity, which often preclude their clinical use. One of the approaches to solving this problem is the synthesis of their water-soluble derivatives based on sesquiterpene lactones. Thus, using the example of thapsigargin 5, which induces apoptosis in prostate cancer cells, its interaction with a peptide carrier synthesized a water-soluble form of guayanolide, which is selectively activated by the protease of the specific antigen of metastatic prostate cancer. At the same time, a complete cessation of tumor growth was observed without significant toxicity in the prostate cancer xenograft model [25,26].

Overall, this “pre-patient research” approach presents a major challenge that can only be solved with an interdisciplinary approach from chemists, molecular biologists, pharmacists, pharmacologists, and clinicians.

Antitumor activity for arglabin 6 and its derivatives: dimethylamino arglabin hydrochloride 18, tetrachlorcarbenarglabin 23, and dimethylamino arglabin methyl iodide 24 was confirmed experimentally in *in vitro* tests on a culture of cancer cells NCI60 (Table 3).



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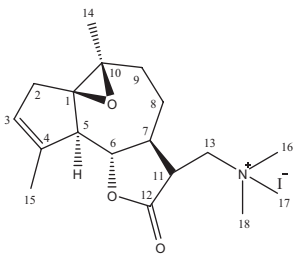

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Based on the data in Table 4, it was found that arglabin 6, with a single injection into the culture of NCI60 tumor cells at a dose of 8.3 μM, exhibited antitumor activity, inhibiting the growth of the following cell lines:

- Inhibition of leukemia cells of the line: SR–97.8%; HL-60(TB) - 97.4%; K-562 - 73.8%;
- Colon cancer cells line: HCT-116-82.6%; HCT-15-75.4%; SW-620 - 70.9%; HT29 - 59.3%; COLO 205 - 57.6%;
- Melanoma cell line: UACC-62-74.2%; MDA-MB-435 - 65.8%; UACC-257 - 55.9%;
- Ovarian cancer cells line OVCAR-3-suppression of tumor cell growth by 69.6%;
- Kidney cancer cells of the line: TK-10-94.3%; CAKI-1-73.4%; RXF 393-67.3%;
- Prostate cancer cells of the DU-145 line by 88.5%;
- Breast cancer cell line: T-47D-73.5%; BT-549-72.1%; MCF7-71.1%.

Table 3: Antitumor activity of arglabin and its derivatives in the NCI60 human cancer cell culture model

Group/cell line	Arglabin 6	Dimethylamino Arglabin Hydrochloride 18	Tetrachlorocarbene -arglabin 23	Dimethylamino -arglabin methyl iodide 24
1	2	3	4	5
	Growth of tumor cells, %			
Leukemia				
HL-60(TB)	2.63	98.28	103.12	19.66
K-562	26.23	83.71	87.93	31.81
MOLT-4	59.14	86.51	76.74	69.29
RPMI-8226	−5.73	60.46	49.35	8.51
SR	2.25	58.94	74.24	10.43
Non-small cell lung cancer				
A549/ATCC	83.94	99.60	93.42	103.60
HOP-62	67.33	85.20	97.97	58.41
NCI-H226	103.37	104.06	94.95	104.85
NCI-H23	62.36	102.45	96.42	78.22
NCI-H322M	114.06	110.90	96.02	105.72
NCI-H460	80.10	106.26	103.19	82.59
NCI-H522	−68.86	71.11	79.48	−68.41
Colon cancer				
COLO 205	42.37	101.68	110.34	54.14
HCC-2998	102.64	111.89	108.60	102.58
HCT-116	17.39	63.93	94.06	18.24
HCT-15	24.60	78.57	95.95	40.69
HT29	40.70	94.43	98.74	45.10
KM12	95.54	105.15	95.51	92.57
SW-620	29.05	61.20	109.32	26.60
Tumors of the central nervous system				
SF-268	78.99	104.09	96.56	91.78
SF-295	89.81	104.37	78.88	
SF-539	54.28	99.36	89.12	61.68
SNB-19	101.01	100.34	82.07	100.58
SNB-75	62.66		59.07	80.73
U251	81.31	99.23	97.82	92.74
Melanoma				
MALME-3M	67.65	98.30	101.72	72.81
M14	67.86	96.66	99.15	78.16
MDA-MB-435	34.20	87.19	97.89	39.82
SK-MEL-2	84.20	108.75	111.65	86.38
SK-MEL-28	88.53	89.92	95.00	91.98
SK-MEL-5	76.31	94.99	89.68	83.87
UACC-257	44.07	80.63	108.75	61.32
UACC-62	25.79	85.96	72.39	36.21
Ovarian cancer				
IGROV1	98.42	114.40	94.88	103.08
1	2	3	4	5
OVCAR-3	30.37	102.61	106.26	45.67
OVCAR-4	86.04	97.49	90.07	101.83
OVCAR-5	82.60	104.46	88.42	76.68
OVCAR-8	72.01	99.89	99.21	98.38
NCI/ADR-RES	77.13	109.25	93.18	79.00
SK-OV-3	101.08	103.25	91.59	96.75
Kidney cancer				
786-0	73.74	100.79	95.25	82.38
A498	71.05	85.96	92.28	77.05
ACHN	53.47	88.70	100.42	59.54
CAKI-1	26.61	90.74	69.62	67.38
RXF 393	32.69	88.55	97.09	53.62
SN12C	64.30	114.38	96.51	74.15
TK-10	5.66	86.38	111.91	18.59
UO-31	81.56	100.55	77.41	87.22
Prostate cancer				
PC-3	56.38	82.27	82.86	71.67
DU-145	11.52	42.70	99.59	13.74
Mammary cancer				
MCF7	28.88	38.33	81.70	29.45
MDA-MB-231/ ATCC	58.15	111.09	101.37	72.61
HS 578T	74.60	93.66	87.71	78,58
BT-549	27.88	63.40	86.00	41.64
T-47D	26.55	41.05	83.20	23.32
MDA-MB-468	−44.01	−22.99	82.29	−34.46
Mean tumor cell growth, %	54.15	89.30	91.96	61.54

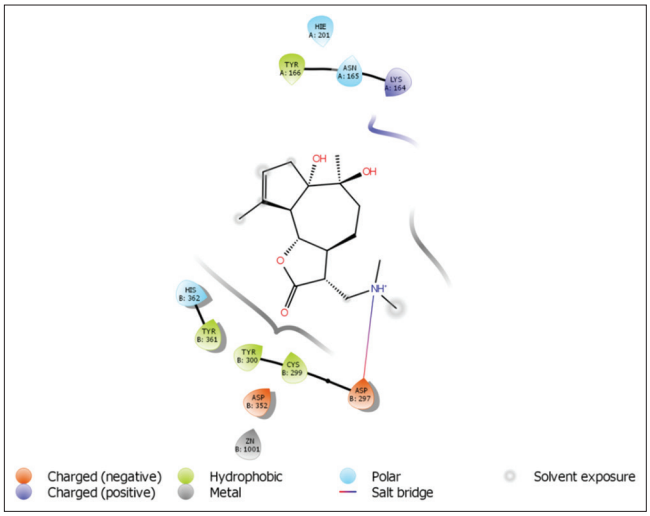

Figure 1: Interaction of farnesyl transferase with dimethylaminodioxyarglabin hydrochloride 11

At the same time, the average value of cell growth inhibition by the studied compound 6 for all 60 NCI60 human tumor cell lines is 45.9%.

When studying the culture of tumor cells NCI60 dimethylamino arglabin hydrochloride 18 with a single injection in dose 8.3μM, it was found that 18 inhibits the growth of cells of the following cell lines:

- Inhibits the growth of prostate cancer cells of the DU-145 line by 57.3%;
- Breast cancer cells of the MCF7 line-61.7%; T-47D - 58.9%.

On the culture of tumor cells NCI60 derivative of arglabin tetrachlorocarbene derivative 23 with a single injection at a dose of 8.3μM, exhibits antitumor activity, inhibiting the growth of RPMI-8226 tumor leukemia cells by 50.7%.

Dimethylamino arglabin methyl iodide 24 with a single injection at a dose of 8.3μM into a culture of NCI60 tumor cells inhibits the growth of the following cell lines:

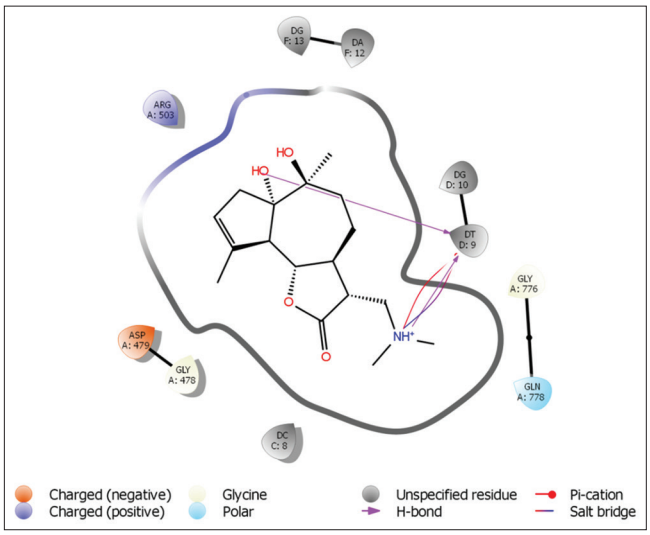

Figure 2: Interaction of DNA topoisomerase II with dimethylaminodioxyarglabin hydrochloride 11

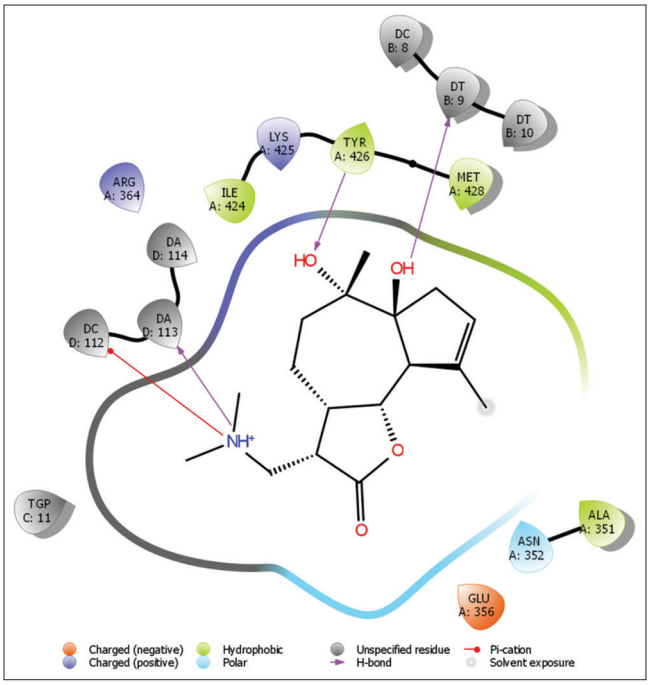

Figure 3: Interaction of DNA topoisomerase I with dimethylaminodioxyarglabin hydrochloride 11

- Leukemia cell line: RPMI-8226 – 91.5%; SR - 89.6%; HL-60(TB) - 80.3%; K-562 - 68.2%;
- Colon cancer cells line: HCT-116 - 81.8%; SW-620 - 73.4%; HCT-15 - 59.3%; HT29 - 54.9%;
- UACC-62 melanoma cells by 63.8%;
- Ovarian cancer cells of the OVCAR-3 line by 54.3%;
- Kidney cancer cells of the TK-10 line by 81.4%;
- Prostate cancer cells of the DU-145 line by 86.26%;
- Breast cancer cells of the T-47D line - 76.7%; MCF7 - 70.6%; BT-549 - 58.4%.

The model of glioblastoma in rats was experimental C6 glioma. In terms of morphology, the nature of invasive growth, and the pattern of expressed proteins, C6 glioma almost completely corresponds to human glioblastoma multiforme, which is the most invasive type of glioma, leading to human death within a year.

The antitumor activity of the arglabin derivative dimethylamino arglabin hydrochloride 18 was confirmed experimentally in *in vitro* tests on clonogenic

Table 4: Cytotoxicity of arglabin derivatives in the survival test of larvae of marine crustaceans *Artemia salina (Leach)*

Substance	LD500, mg/mL
1	2
α-epoxyarglabin 7	89.4
β-epoxyarglabin 8	92.9
dimethylaminodioxyarglabin 10	104.2
hydroxyarglabin acetate 12	77.8
1α, 10β dioxyarglabin 13	72.6
1β,10α dioxyarglabin 14	64.2
1	2
Control: dimethyl sulfoxide	930.27
Reference drug: 13-dimethylamino-1,10β-epoxy-5,7α,6, 11β(H)-guai-3,4-ene-6,12-olide hydrochloride	20.6

rat C6 glioma cells, manifesting itself in the effective suppression of the proliferation of clonogenic cells.

It has been established that under conditions of administration of dimethylamino arglabin hydrochloride 18 at doses of 1 mg/ml (140 mg/m2 for humans) and 0.1 mg/ml (10 times lower than the therapeutic dose for humans), the proliferation of clonogenic C6 cells is significantly reduced by 17 and 5 times (2.95 ± 0.58% and 11.17 ± 2.7%, respectively) in relation to the control (51.47 ± 3.54%).

As the dose of dimethylamino arglabin hydrochloride 18 is reduced by several orders of magnitude (0.01 mg/mL, 0.001 mg/mL, and 0.0001 mg/mL), the proliferation of clonogenic cells is suppressed. When exposed to compound 18 at a dose of 2.5 mg/mL (300 mg/m^2 for humans) on C6 clones, the proliferation of clonogenic cells significantly decreased by 2 times in relation to the control (4.19 ± 1.43% and 51, 47 ± 3.54%, respectively).

In this series of experiments, the effectiveness of suppressing the proliferation of clonogenic cells in C6 clones is observed with the injection of the studied compound 18 at doses of 1 mg/mL, 0.1 mg/mL, and 2.5 mg/mL, where a lethal dose (LD) was noted for clonogenic cells LD_{94}, LD_{79}, and LD_{92}, respectively.

It was shown that dimethylamino arglabin hydrochloride 18 at a concentration of 20 mg/mL, 2 mg/mL, 0.2 mg/mL inhibits the proliferation of clonogenic cells by 10.4 times (LD_{90}), 3 times (LD_{67}), and 5 times (LD_{80}) (4.96 ± 2.17%, 17.07 ± 2.06%, 10.3 ± 2.64%, respectively) compared with the control (51.47 ± 3.54%).

Thus, dimethylamino arglabin hydrochloride 18 at doses of 20; 2; 0.2 mg/mL, 1 mg/mL, 0.1 and 2.5 mg/mL effectively inhibited the proliferation of C6 clonogenic cells compared to controls in an *in vitro* experimental model of human glioblastoma multiforme. Probably, the pharmacological action 18 is associated with the inhibitory effect of farnesyl protein transferase.

Cytotoxicity in the survival test of larvae of marine crustaceans A. salina (Leach).

For arglabin derivatives, their cytotoxicity was determined, which was evaluated in the survival test of larvae of marine crustaceans *A. salina (Leach)* under *in vitro* cultivation conditions (Brine shrimp toxicity bioassay method).

In the experiment, the effect of the studied substances at concentrations of 100 μg/ml, 10 μg/ml, and 1 μg/ml was studied on the survival rate of marine crustaceans *A. salina (Leach)* in Table 4.

It has been experimentally established that the presented samples exhibit cytotoxicity against the larvae of marine crustaceans *A. salina (Leach).* At the same time, the IC50 of dimethylaminodioxyarglabin 10 is 104.2 μg/mL, while the IC50 of α-epoxyarglabin 7 and β-epoxyarglabin 8 are 89.4 and 92.9 μg/ml, respectively.

Compound 14 with an IC value of 64.2 μg/mL was the most active against the larvae of the marine crustaceans *A. salina (Leach)* (Table 4). Approximately the same effect was shown by compounds 12, 13, and 7. The IC50 values for these compounds are 77.8 μg/mL, 72.6 μg/mL, and 89.4, respectively (Table 4). IC50 compounds 8 and 10 are at and above the level of 100 μg/mL (Table 4), which indicates their low activity in this experiment relative to the reference drug.

Thus, samples of sesquiterpene lactones α-epoxyarglabin 7, β-epoxyarglabin 8, dimethylaminodioxyarglabin 10, hydroxyarglabin acetate 12, 1α,10β-dioxiarglabin 13, and 1β,10α-dioxiarglabin 14 exhibit cytotoxicity against larvae of marine crustaceans *A. salina (Leach).*

Conclusion

The conducted molecular docking shows that arglabin 6 and its derivatives predicted strong bonding on the receptors of DNA topoisomerase I, DNA topoisomerase II and farnesyl transferase, which suggests their destructive effect on the structure and functions of tumor cells, subsequently inducing their death, thereby exhibit cytotoxic and antitumor effects. Other anticancer drug targets cannot be ruled out. Molecular modeling data were supported by experimental studies.

According to the results of the experiments, it was found that the introduction of 6 atoms of bromine and chlorine into the molecule increases the antitumor activity of the synthesized samples 12, 13 against Pliss LPS, alveolar liver cancer RS-1, sarcoma M-1, and sarcoma 45.

Thus, the analysis of the results of computer modeling of the “ligand-target” complex and the pharmacological study of natural sesquiterpene lactones and their derivatives indicate the existence of a relationship between the chemical structure of their molecules, in particular, the presence of certain functional groups that can determine the final effect of molecules with the manifestation of the corresponding biological activity, in particular, cytotoxic and antitumor.

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