Evaluation of the anti-inflammatory activity of a blackberry thick fruit extract using in vivo model and molecular docking

A. O. Marchenko[A,D], M. A. Komisarenko[B,E], O. Yu. Maslov[A,C], I. O. Lebedinets[B,C], T. K. Yudkevych[E], S. V. Kolisnyk[E,F], A. O. Koval[B,C]

National University of Pharmacy, Kharkiv, Ukraine

A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article

In recent years, increasing attention has been paid to natural compounds as potential agents due to their broad spectrum of biological activities. Plant-derived antioxidants with anti-inflammatory properties are of particular interest, as they may suppress pathological pathways. Therefore, the search for novel natural compounds capable of attenuating inflammation remains a promising area of biomedical research.

The aim of work was to evaluate the anti-inflammatory activity of a blackberry thick fruit extract using an *in vivo* model and molecular docking.

Materials and methods. The object of study was a blackberry thick fruit extract. Molecular docking was performed using AutoDockTools 1.5.6. Anti-inflammatory effect was assessed by the model of carrageenan-induced paw edema in rats.

Results. Theoretical assessment of the anti-inflammatory activity of the blackberry fruit extract showed that blackberry anthocyanins as cyanidin-3-glucoside, cyanidin-3-(3”-malonyl glycoside) and cyanidin-3-xyloside blocked highly selective three out four pro-inflammatory targets as cyclooxygenase-2 (COX-2), phospholipase A2 and 5-lipooxygenes (5-LOX), whereas, cyanidin-3,3’-diglucoside and cyanidin-3-rutinoside highly selective blocked two out of four targets as phospholipase A2 and 5-LOX. There was not found any high selective inhibitor among anthocyanins of nuclear factor kB (NF-kB), whereas cyanidin-3-xyloside showed a moderate selectivity. Experimental studies have shown the blackberry fruit thick extract at a dose of 60.0 mg/kg and 30.0 mg/kg significantly reduces edema after 1, 2, 3 and 4 hours compared to the control group.

Conclusions. Theoretical and experimental research of anti-inflammatory properties of blackberry fruit extract using molecular docking analysis and *in vivo* model of carrageenan-induced paw edema in rats, respectively, has been conducted. Theoretical results have shown blackberry anthocyanins possessed ability to inhibit all crucial pro-inflammatory targets as COX-2, phospholipase A2, 5-LOX and Nf-kB. Experimental results have demonstrated that blackberry thick fruit extract at doses of 60.0 mg/kg and 30.0 mg/kg possessed the ability to significantly inhibit inflammation at all stages of the carrageenan-induced paw edema model.

Keywords: blackberry, fruit, inflammation, in silico, extract, carrageenan model.

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Оцінювання протизапальної активності густого екстракту плодів ожини з використанням in vivo моделі та молекулярного докінгу

А. О. Марченко, М. А. Комісаренко, О. Ю. Маслов, І. О. Лебединець, Т. К. Юдкевич, С. В. Колісник, А. О. Коваль

Останніми роками все більшу увагу приділяють природним сполукам як потенційним агентам завдяки їх широкому спектру біологічної активності. Особливий інтерес становлять антиоксиданти рослинного походження з протизапальними властивостями, оскільки вони можуть пригнічувати патологічні процеси. Тому пошук нових природних сполук, що можуть послаблювати запалення, залишається перспективною галуззю біомедичних досліджень.

Мета роботи – оцінити протизапальну активність екстракту густих плодів ожини за допомогою моделі *in vivo* та молекулярного докінгу.

Матеріали і методи. Об’єкт дослідження – густий екстракт плодів ожини. Молекулярний докінг виконали за допомогою AutoDockTools 1.5.6. Протизапальний ефект оцінювали на моделі набряку лапи, індукованого карагенаном, у щурів.

Результати. Теоретичне оцінювання протизапальної активності екстракту плодів ожини дало змогу встановити, що антоціани ожини, як-от ціанідин-3-глюкозид, ціанідин-3-(3”-малонілглікозид) і ціанідин-3-ксилозид, високоселективно блокували три з чотирьох прозапальних мішеней (циклооксигеназа-2 (ЦОГ-2), фосфоліпаза А2 та 5-ліпооксигени (5-ЛОГ)), а ціанідин-3,3’-диглюкозид і ціанідин-3-рутинозид високоселективно блокували дві з чотирьох мішеней (фосфоліпаза А2 та 5-ЛОГ). Не виявлено жодного високоселективного інгібітора серед антоціанів ядерного фактора kB (NF-kB), а ціанідин-3-глюкозид характеризувався помірною

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селективністю. У результаті експериментальних досліджень встановлено, що густий екстракт плодів ожини в дозі 60,0 мг/кг та 30,0 мг/кг значно зменшує набряк через 1, 2, 3 та 4 години порівняно з контрольною групою.

Висновки. Здійснили теоретичне й експериментальне дослідження протизапальних властивостей екстракту плодів ожини з використанням молекулярного докінг-аналізу та *in vivo* моделі набряку лапи, індукованого карагенаном, у щурів відповідно. Теоретичні результати показали, що антоціани ожини мають здатність пригнічувати всі ключові прозапальні мішені, такі як ЦОГ-2, фосфоліпаза А2, 5-ЛОГ та Nf-kB. Експериментальні результати підтвердили, що екстракт густих плодів ожини в дозах 60,0 мг/кг та 30,0 мг/кг може значно пригнічувати запалення на всіх стадіях моделі набряку лапи, індукованого карагенаном.

Ключові слова: ожина, плоди, запалення, in silico, екстракт, карагенанова модель.

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Inflammation is one of the main reactions of the body to foreign invasion such as microorganisms, chemical substances and etc. The crucial role of the immune system during inflammation indicates foreign compounds, eventually activate several pro-inflammation pathways, which lead to the significant increasing of cytokines, activate immune cells as lymphocytes and macrophages [1]. The inflammation process is controlled with the variety of pathways as mitogen-activated protein kinase, NF-kB and prostaglandins synthesis. The main cascade of inflammation consists of several steps: above all, foreign substances attract neutrophils and macrophages, which activate prostaglandins synthesis using COX 1 and 2 enzymes, cytokines as interleukin-6, interleukin-1β, and tumor necrosis factor α [2].

Treatment of inflammation consists of the application of steroidal anti-inflammatory drugs and non-steroidal drugs. Both groups of anti-inflammatory drugs possessed the following side effects: steroidal drugs induce inhibition of the immune cells, downregulation of the production of calcium in bones, whereas non-steroidal caused an ulcer disease, and bronchospasm [3]. Therefore, the search and creation new anti-inflammation drugs based on natural products is the task number for medicine and pharmacy.

Today, medical plants that are a rich source of anthocyanins have a high level of attention from the scientific community [4]. Above all, it relates with fact that natural compounds have a potent antioxidant effect and moreover, the side effects rarely happened after the application of natural compounds than after synthetic drugs.

The perspective source of anthocyanins was chosen blackberry fruits. Blackberry is a shrub of the *Rosacea* family. The distribution area is Europe, North America, Asia [5]. The chemical composition of blackberry fruits is represented by anthocyanins, organic acids and hydroxycinnamic acids [6].

There is a lot of research about the investigation of the pharmacological activity of blackberry fruit. It is known that anthocyanins from blackberry fruit, possess: anti-inflammatory, antioxidant, antimicrobial, anti-hyperglycemic, immune-modulation, and anticancer effects [6,7,8]. Besides, in folk medicine blackberry are traditionally applied to treat fever, infections, diabetes, and liver diseases [9]. In our view, the anthocyanins are perspective for the development of new antimicrobial, and antioxidant pharmaceuticals.

There is a lot of scientific research about determination an anti-inflammatory activity of blackberry fresh fruit extracts [10,11]. However, there is no date about assessing anti-inflammatory activity on carrageenan model and conducting molecular docking blackberry anthocyanins against pro-inflammatory targets.

Aim

The aim of the work was to evaluate anti-inflammatory activity of a blackberry thick fruit extract using *in vivo* model and molecular docking.

Materials and methods

Blackberry fruits (*Rubus plicatus* Weihe & Nees) were collected in 2021 in July, near the village of Ternova in the Kharkiv region (50°19’31”N, 36°66’93”E).

Exactly 100.0 g of blackberry fruit was pressed and extracted with 96 % ethanol using a threefold solvent-to-material ratio. After filtration, the resulting filtrate was concentrated under reduced pressure using a vacuum evaporator at 50–60 °C to obtain an extract with a final extract-to-raw material mass ratio of 1:0.35.

Thirty male outbred white strain rats, each weighing about 180–220 grams, were used in the experiment. The rats used in the experiment were obtained from the National University of Pharmacy (NUPh) vivarium. The rats were kept in pairs in Macrolon boxes throughout the experiment. Food and water were available ad libitum, and were replenished daily. Bedding was changed every three days. The animals were kept in an environment that was monitored and maintained to have a temperature of 22 ± 2 °C, relative humidity of 60 ± 5 %, and a light / dark cycle of 12 hours.

All study procedures were aligned in accordance with the Order of the Ministry of Education and Science of Ukraine “On approval of the Procedure for conducting experiments on animals by scientific institutions” (01 March 2012, No. 2012) and the Ukrainian Law “On Protection of Animals from Cruel Treatment” (21 February 2006, No. 344). The study protocol was sanctioned by the Local Ethics Committee (Protocol No. 17, March 5, 2025).

The anti-exudative activity of the extract was studied on 25 white male outbred rats, aged 180–220 g. The model of acute inflammation was created by subplantar injection of 0.1 mL of 1 % carrageenan (Fluka, Switzerland) into the right hind paw of the rats. Edema was measured at 1, 2, 3, and 4 hours post-injection [12].

All animals were divided into 6 groups. The first group was control pathology (animals of the positive control groups were not treated.), the second group – sodium diclofenac at a dose 8 mg/kg, the third group – “Quertin” (Public Joint-Stock

Table 1. Molecular docking of the anthocyanins and anti-inflammatory drug standard diclofenac sodium and quercetin with the COX-2

Ligand	Binding energy, ΔGbind[a] (kcal/mol)	Ki[b], mmol	Binding site	Level of selectivity
Cyanidin-3-xyloside	-13.15	0.00000023042	A: ALA199, ALA202, TYR385, TRP387, HIS388, LEU390, LEU391	High selectivity
Cyanidin-3-(3"-malonyl glycoside)	-11.89	0.00000192	A:ALA199, LEU391, TRP387, TYR385, GLU203	High selectivity
Cyanidin-3-glycoside	-10.85	0.0000112	A:TYR385, HIS386, TRP387, HIS388, TRP387, LEU390, LEU391, VAL447	High selectivity
Diclofenac sodium	-5.76	0.05977	A:ALA199, ALA202, GLN203, TRP387, LEU390, LEU390, TYR385, HIS388	Low selectivity
Quercetin	-4.59	0.42855	A: CYS36, ASN39, CYS41, PRO154, ALA156	Low selectivity
Cyanidin-3-rutinoside	-1.32	107.87	A: CYS36, ASN39, CYS41, PRO154, ALA156	Low selectivity
Cyanidin-3,3'-diglucoside	78.0#	–	A: CYS36, ASN39, CYS41, PRO154, ALA156	Inactive

ΔGbind: free-binding energy; **Ki:** 50 % enzyme inhibition concentration.

Company "Scientific and Production Center "Borshchagov Chemical and Pharmaceutical Plant") at a dose of 50 mg/kg in terms of quercetin 3.5 mg/kg, the fourth group – blackberry extract at a dose 60 mg/kg in terms of polyphenolic compounds, the fifth group – blackberry extract at a dose 30 mg/kg in terms of polyphenolic compounds, and the six group – blackberry extract at a dose 10 mg/kg in terms of polyphenolic compounds.

Molecular docking studies were performed using AutoDockTools version 1.5.6 [13]. The structures of the investigated ligands were obtained from the PubChem database [14]. The three-dimensional structures of cyclooxygenase-2 (COX-2; PDB ID: 1DDX), phospholipase A2 (PLA2; PDB ID: 3HSW), 5-lipoxygenase (5-LOX; PDB ID: 2Q7M), and NF-κB (PDB ID: 1SVC) were retrieved from the Protein Data Bank (PDB) [15]. The active sites of the target proteins were identified using CASTp 3.0 [16]. The resolutions of the selected protein structures were 3.00 Å for 1DDX, 2.50 Å for 3HSW, 4.25 Å for 2Q7M, and 2.60 Å for 1SVC.

To obtain statistical results, the Statistica 10 program was used, and the results were analyzed using Mann–Whitney test. Differences were considered significant at $p < 0.05$.

Results

At the first stage of your research a molecular docking study was provided to understand the anti-inflammatory potential of blackberry fruit extract. There are a variety of pro-inflammatory pathways responsible to activate cytokines as TNF-α, IL-6, IL-1 and others. In our view, the most important key pro-inflammatory enzymes belong COX-2, 5-LOG, phospholipase A2, and NF-kB. In order to compere potential of blackberry fruit extract it was taken gold standards' such as sodium diclofenac. Since this drug is indicated in official protocols of treatment of acute and chronic diseases as well as among anti-inflammatory drugs based on natural compounds quercetin was chosen as on the market present drug "Quertin" applied in the treatment of cardiological, neurological and renal diseases.

In our previous research [7] it was estimated an anthocyanins composition of blackberry fruit extract by high performance liquid chromatography. According to this study the following anthocyanins were identified: cyanidin-3-glucoside (84.10 % out of total anthocyanins), cyanidin-3,3'-diglucoside (7.38 % out of total anthocyanins), cyanidin-3-(3"-malonyl glycoside) (6.30 % out of total anthocyanins), cyanidin-3-xyloside (1.50 % out of total anthocyanins) and cyanidin-3-rutinoside (0.60 % out of total anthocyanins). These identified compounds were evaluated with a molecular docking study to realize anti-inflammatory potential of blackberry fruit extract. Moreover, the level of selectivity of inhibition of compounds was classified according to the following requirements: $IC_{50} < 0.001$ mM (high selective); $0.05 > IC_{50} > 0.01$ (medium selective); $IC_{50} > 0.05$ mM (low selective) [17].

According to the presented results in *Table 1*, it was shown that highly selective inhibitors of the COX-2 enzyme include cyanidin-3-xyloside, cyanidin-3-(3"-malonyl glycoside), cyanidin-3-glycoside, while sodium diclofenac, quercetin, cyanidin-3-rutinoside were low-selective inhibitors and cyanidin-3,3'-diglucoside was inactive respectively to the COX-2 enzyme. The active center of the COX-2 enzyme structure is the following series of amino acids: ALA199, ALA202, THR206, TYR385, GLU203, HIS388, LEU391, LEU390, TRP387 *(Table 1)*.

Further, the compounds' ability of blackberry fruit extract to inhibit phospholipase A2 was assessed. According to the results it was found that cyanidin-3-xyloside, cyanidin-3,3'-diglucoside, cyanidin-3-glucoside, cyanidin-3-(3"-malonyl glycoside) and cyanidin-rutinoside were highly selective inhibitors, while sodium diclofenac, quercetin were moderately selective inhibitors. The active center for inhibiting phospholipase A2 are amino acids: LYS147, VAL145, HIS144, TYR 60, THR146, PHE22, TYR28 *(Table 2)*.

The following important pro-inflammatory enzyme is 5-LOX. According to the results of the study, it was shown that cyanidin-3-xyloside, cyanidin-3-(3"-malonyl glycoside), cyanidin-3-rutinoside, cyanidin-3,3'-diglucoside and cyanidin-3-glucoside were highly selective inhibitors. Quercetin and diclofenac sodium had medium selectivity to the pro-inflammatory enzyme is 5-LOX. Amino acids VAL81, ALA84,

Table 2. Molecular docking of the anthocyanins and anti-inflammatory drug standard diclofenac sodium and quercetin with the phospholipase A2

Ligand	Binding energy, ΔGbind[a] (kcal/mol)	Ki[b], mmol	Binding site	Level of selectivity
Cyanidin-3-xyloside	-13.63	0.00000010272	A: PRO18, PHE22, LEU31, CYS45, HIS48, ASP49, TYR69, HIS48, PHE22, ASN23	High selectivity
Cyanidin-3,3'-diglucoside	-11.52	0.0000036	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin-3-glucoside	-11.52	0.0000036	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin-3-(3''-malonyl glycoside)	-11.35	0.00000478	A: PHE5, ILE9, ASN23, TYR69, PRO18, TYR28, GLY30, GLY32, HIS48, ASP49, TYR69	High selectivity
Cyanidin-rutinoside	-9.73	0.00007347	A:PHE22, PHE106, GLY30, CYS45, HIS48, TYR69	High selectivity
Diclofenac sodium	-7.65	0.00248	A:PHE5, PHE22, HIS48, PHE106, TYR69	Moderate selectivity
Quercetin	-6.79	0.01062	A: PHE5, ILE9, PHE22, GLY30, CYS45, HIS48, ASP49	Moderate selectivity

ΔGbind: free-binding energy; **Ki:** 50 % enzyme inhibition concentration.

Table 3. Molecular docking of the anthocyanins and anti-inflammatory drug standard diclofenac sodium and quercetin with the 5-LOX

Ligand	Binding energy, ΔGbind[a] (kcal/mol)	Ki[b], mmol	Binding site	Level of selectivity
Cyanidin-3-xyloside	-11.36*	0.00000412	A:VAL70, ILE119, PHE123	High selectivity
Cyanidin-3-(3''-malonyl glycoside)	-9.57*	0.000009607	A:PHE123, VAL70, ILE119, THR66	High selectivity
Cyanidin-3-rutinoside	-9.45*	0.0001193	A:PHE123, ILE110, THR66	High selectivity
Cyanidin-3,3'-diglucoside	-8.97*	0.00026528	A:VAL70, ILE119, THR66	High selectivity
Cyanidin-3-glucoside	-8.65*	0.000458	A:VAL70, ILE119, THR66	High selectivity
Quercetin	-6.45[&]	0.01857	A:ILE119, THR66	Moderate selectivity
Diclofenac sodium	-6.00[&]	0.03982	A:VAL81, ALA84, LEU11, ILE14, VAL15, LEU88	Moderate selectivity

ΔGbind: free-binding energy, **Ki:** 50 % enzyme inhibition concentration.

LEU11, ILE14, VAL34, and LEU88 acted as an active center for binding the enzyme structure of 5-LOX *(Table 3)*.

The following significant enzyme, which has a high activity in chronic inflammation process is NF-kB. There was not indicated any of a high selective inhibitor, except one medium selective inhibitor, cyanidin-3-xyloside, was established. In turn, the remaining compounds showed low selectivity for inhibition of the active center of NF-kB, which may indicate the complexity, and particularly the importance of inhibition of this enzyme. The active center of NF-kB was represented by the following amino acids: LYS147, LYS148, THR146, TYR60, LEU210, HIS144 *(Table 4)*.

Furthermore, all obtained data were summarized, and the compounds were conditionally classified into three categories. The first category comprised compounds with high selectivity for the active site, the second included compounds with moderate selectivity, and the third consisted of compounds with low selectivity. This classification approach was applied to clearly identify compounds that interact most effectively with pro-inflammatory targets, as well as those exhibiting lower levels of interaction.

Table 5 shows the summarized results of molecular docking of pro-inflammatory enzyme inhibition of anthocyanins of blackberry fruit extract. Results demonstrate, that no compound among both anthocyanins and drug standards inhibit high selective all mentioned pro-inflammatory targets. However, cyanidin-3-glucoside, cyanidin-3-(3''-malonyl glycoside) and cyanidin-3-xyloside blocked three out four pro-inflammatory targets as COX-2, phospholipase A2 and 5-LOX. Whereas cyanidin-3,3'-diglucoside and cyanidin-3-rutinoside blocked two out of four targets as phospholipase A2 and 5-LOX. Although, it was determined that widespread applicable "gold standards" in medicine and science as sodium diclofenac and quercetin do not so effectively inhibit crucial targets of inflammation.

In an *in vivo* experimental study on a rat paw carrageenan edema model, blackberry fruit extract at a dose of 60.0 mg/kg significantly reduced paw edema by 100 % compared to the control group at the first and second hour. Subsequently, paw edema decreased by 82.0 % and 69.0 % after 3 and 4 hours, respectively, compared to the control group. The level of antiexudative activity of blackberry fruit extract at a dose of 30.0 mg/kg and 10.0 mg/kg was significantly ($p > 0.05$) lower compared to the administration of a dose of blackberry fruit extract of 50.0 mg/kg. The use

Table 4. Molecular docking of the anthocyanins and anti-inflammatory drug standard diclofenac sodium and quercetin with the Nf-kB

Ligand	Binding energy	Ki[b]	Binding site	Level of selectivity
	ΔGbind[a] (kcal/mol)	mmol		
Cyanidin-3-xyloside	-6.34	0.0198	A:LYS244, TYR60, HIS144, THR146, LYS147	Moderate selectivity
Cyanidin-3-(3''-malonyl glycoside)	-5.74	0.06224	A:LYS244, TYR60, HIS144, THR146, LYS147	Low selectivity
Cyanidin-3-glucoside	-5.38	0.1148	A:LYS244, PRO246, ALA245, TYR60, HIS144, SER211, LEU210, LYS147, ASP209, MET208	Low selectivity
Cyanidin-3-rutinoside	-4.44	0.55329	A:LYS147, LEU210, THR146, HIS144, TYR60	Low selectivity
Cyanidin-3,3'-diglucoside	-4.44	0.56509	A:LEU210, TYR60, HIS144, LYS147	Low selectivity
Diclofenac sodium	-3.90	1.38	A:TYR50, HIS144, LEU210, VAL145, THR146, LYS147	Low selectivity
Quercetin	-3.61	2.28	A:LYS145, LYS147, LEU210, THR146, THR60, HIS144	Low selectivity

ΔGbind: free-binding energy, **Ki:** 50 % enzyme inhibition concentration.

Table 5. Schematic overview of the classification of anti-inflammatory drug standards and principal compounds identified in blackberry fruit extract

Compound	COX-2	phospholipase A2	5-LOX	Nf-kB	Number of closed key enzyme of inflammation
Drug standard					
Diclofenac sodium	#	&	&	#	2
Quercetin	#	&	&	#	2
Compounds blackberry fruit extract					
Cyanidin-3-glucoside	✓	✓	✓	#	3
Cyanidin-3,3'-diglucoside	#	✓	✓	#	2
Cyanidin-3-(3''-malonyl glycoside)	✓	✓	✓	#	3
Cyanidin-3-xyloside	✓	✓	✓	&	4
Cyanidin-3-rutinoside	#	✓	✓	#	2

✓**:** high level of selectivity; **&:** medium level of selectivity; **#:** lower of selectivity.

Table 6. Anti-inflammatory activity of the blackberry extract on the carrageenan edema model, n = 5 (M ± m)

Experimental conditions	Dose, mg/kg	Parameter	Dynamics of inflammation development, hours			
			1	2	3	4
Control pathology	–	ΔV, mL	0.47 ± 0.03	0.84 ± 0.06	1.10 ± 0.09	1.16 ± 0.09
Diclofenac Sodium	8.0	ΔV, mL	0.20 ± 0.01*	0.52 ± 0.04*	0.71 ± 0.04*	0.73 ± 0.04*
		AA, %	58.0	38.0	35.0	37.0
Quertin	3.5[5]	ΔV, mL	0.27 ± 0.01*	0.51 ± 0.04*	0.84 ± 0.04*	0.81 ± 0.04*
		AA, %	43.0	39.0	24.0	30.0
Blackberry fruit extract	10.0	ΔV, mL	0.26 ± 0.02*,**	0.62 ± 0.04*,**,#	0.94 ± 0.06*,**,#	1.00 ± 0.08*,**,#
		AA, %	45.0	26.0	15.0	14.0
Blackberry fruit extract	30.0	ΔV, mL	0.0	0.46 ± 0.04*,#	0.73 ± 0.04*,#,&	0.81 ± 0.06*,&
		AA, %	100.0	45.0	34.0	30.0
Blackberry fruit extract	60.0	ΔV, mL	0.0	0.01	0.20 ± 0.04*,**,#,&,$	0.36 ± 0.04*,**,#,&,$
		AA, %	100.0	99.0	82.0	69.0

:** p < 0.05 – the level of statistical significance of the control pathology group; *:** p < 0.05 – reliable values for the drug diclofenac sodium; **#:** p < 0.05 – reliable values of the Quertin; **&:** p < 0.05 – reliable values of the blackberry fruit extract at a dose 10 mg/kg; **$:** p < 0.05 – reliable values of the blackberry fruit extract at a dose 30 mg/kg; Dose of Quertin expressed in term of quercetin, dose of dosage form – 50 mg/kg; **AA:** anti-inflammatory activity; **ΔV:** size of the edema; **n:** number of animals in group.

of blackberry fruit extract at a dose of 60.0 mg/kg showed a significant reduction in edema after 1, 2, 3 and 4 hours compared to the reference drug diclofenac sodium. When comparing the experimental groups of the drug "Quertin" and blackberry fruit extract at a dose of 60.0 mg/kg, it was found that the level of antiexudative activity of "Quertin" was significantly ($p > 0.05$) lower at all hours.

At a dose of 10.0 mg/kg, blackberry fruit extract was significantly inferior to the antiexudative effect of diclofenac sodium, and when compared with the reference drug "Quertin" at 1 hour the difference was not significant ($p > 0.05$), and at 2, 3 and 4 hours the effect of "Quertin" was significantly higher ($p > 0.05$).

The use of blackberry fruit extract at a dose of 30.0 mg/kg showed a significant reduction in edema at 1 hour, when compared with the drug diclofenac sodium, it was found that the level of antiexudative activity of diclofenac sodium was significantly ($p > 0.05$) lower at 1 and 2 hours, and at 3, 4 hours the difference was not significant ($p > 0.05$). In case of comparing the drug "Quertin" and blackberry fruit extract at a dose of 30 mg/kg, it was found that the level of antiexudative activity of "Quertin" was significantly ($p > 0.05$) lower at 1, 2 and 3 hours, and at 4 hours the difference was not significant ($p > 0.05$) *(Table 6)*.

Discussion

Inflammation represents a complex biological response to external or internal stimuli and is closely associated with the development of oxidative stress. To evaluate anti-inflammatory activity, the carrageenan-induced rat paw edema model was employed, as it reliably reflects the key mechanisms underlying the inflammatory process. According to the canonical progression of inflammation in this model, histamine and serotonin predominate during the first hour, pro-inflammatory cytokines during the second hour, and prostaglandins, particularly COX-2, from the third to the fifth hour. Based on the results obtained, the blackberry fruit extract demonstrated inhibitory activity across all phases of inflammation.

In a previous study, A. Rossi et al. [18] evaluated the protective effects of anthocyanins from blackberry at doses 10 mg/kg and 30 mg/kg in a rat model of acute lung inflammation. The blackberry fruit extract at a dose of 30 mg/kg showed a significant decreasing production of lipid peroxidation, prostaglandin E2 and nitrite/nitrate (NOx) as well as ameliorated the histopathological alteration compared to the control pathology group. Consistent with these findings, our study confirms the anti-inflammatory potential of blackberry-derived bioactive compounds; however, in contrast to the isolated anthocyanins used by A. Rossi et al., whether in our research we investigated a blackberry fruit extract at three doses 10 mg/kg, 30 mg/kg and 60 mg/kg. In our *in vivo* model of carrageenan-induced inflammation, the extract at doses 30 mg/kg and 60 mg/kg exhibited completely inflammation at all phases that are comparable with A. Rossi et al. research.

Conclusions

1. Theoretical and experimental research of anti-inflammatory properties of blackberry fruit extract has been conducted using molecular docking analysis and an *in vivo* model of carrageenan-induced paw edema in rats, respectively.

2. Theoretical results have shown that blackberry anthocyanins possessed ability to inhibit all crucial pro-inflammatory targets as COX-2, phospholipase A2, 5-LOX and Nf-kB.

3. Experimental results have demonstrated that blackberry thick fruit extract at doses 60.0 mg/kg and 30.0 mg/kg possessed ability significantly inhibited inflammation at all stages of the carrageenan-induced paw edema model.

Prospects for further research. Creating dosage form with blackberry fruit extract with pronounced antioxidant, anti-inflammatory and antimicrobial activity. Moreover, conduct research of dosage form with blackberry extract for adjuvant therapy with antimicrobial drugs against resistant strains.

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Information about the authors:
Marchenko A. O., PhD student of the Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0009-0002-8616-0410
Komisarenko M. A., PhD, Associated Professor of the Department of Pharmacognosy and Nutriciology, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0002-1161-8151
Maslov O. Yu., PhD, Assistant at the Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0001-9256-0934
Lebedinets I. O., Specialist of Research Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0001-6901-0045
Yudkevych T. K., Deputy Director of Research Educational and Scientific Institute of Applied Pharmacy, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0001-6173-2780
Kolisnyk S. V., PhD, DSc, Professor, Head of the Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0002-4920-6064
Koval A. O., PhD, Associated Professor of the Department of General Chemistry, National University of Pharmacy, Kharkiv, Ukraine.
ORCID ID: 0000-0001-9491-0459

Відомості про авторів:
Марченко А. О., аспірант каф. фармакогнозії і нутриціології, Національний фармацевтичний університет, м. Харків, Україна.
Комісаренко М. А., канд. фарм. наук, доцент каф. фармакогнозії і нутриціології, Національний фармацевтичний університет, м. Харків, Україна.
Маслов О. Ю., д-р філософії, асистент каф. загальної хімії, Національний фармацевтичний університет, м. Харків, Україна.
Лебединець І. О., фахівець Навчально-наукового інституту прикладної фармації, Національний фармацевтичний університет, м. Харків, Україна.
Юдкевич Т. К., заступник директора з наукової роботи, Навчально-науковий інститут прикладної фармації, Національний фармацевтичний університет, м. Харків, Україна.

Колісник С. В., д-р фарм. наук, професор, зав. каф. загальної хімії, Національний фармацевтичний університет, м. Харків, Україна.
Коваль А. О., канд. фарм. наук, доцент каф. загальної хімії, Національний фармацевтичний університет, м. Харків, Україна.

Oleksandr Maslov (Олександр Маслов)
alexmaslov392@gmail.com

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