Research Article

Clinical Journal of Cardiology

Supramolecular Complexes Of Etacizine With Glycosides As Promising New Antiarrhythmic Drugs

Yuliya I. Oshchepkova[1*], KhabibullaA. Yuldashev[1], RakhmatS. Esanov[1], Vyacheslav V. Uzbekov[1], KhulkarT. Saydullaeva[1], MakhmudjanB. Gafurov[1], InoyatZ. Jumayev[2], Shavkat Yu. Rustamov[2], PulatB. Usmanov[2] and Shavkat I. Salikhov[1]

[1]Institute of Bioorganic Chemistry named after A.S. Sadykov of the Academy of Sciences of Uzbekistan, Republic of Uzbekistan
[2]Institute of Biophysics and Biochemistry, National University of Uzbekistan, Republic of Uzbekistan

*Corresponding author:
Yuliya I. Oshchepkova ,
Institute of Bioorganic Chemistry named after A.S. Sadykov of the Academy of Sciences of Uzbekistan, Republic of Uzbekistan,
Email: oshepkova_ju@biochem.uz

Received: 23 July 2023
Accepted : 11 Aug 2023
Published: 21 Aug 2023

1. Abstract

The problem of effective and safe therapy of cardiac arrhythmias requires the creation of new antiarrhythmic drugs with low toxicity. Clathration of medicinal substances with cyclodextrins or plant glycosides is a promising method for reducing their side effects and increasing their solubility. Complexes of glycyrrhizic acid (GA) and its monoammonium salt (MASGA) with ethacizine hydrochloride (EtHC) in various molar ratios (2:1, 4:1, 8:1) characterized by certain physicochemical parameters have been obtained. The resulting inclusion compounds were studied by UV and IR spectroscopy. It has been determined that molecular complexes of GA and MASGA with EtHC are formed through weak intermolecular interactions, such as hydrogen bonding, electrostatic and hydrophobic interactions.

It was shown that GA-EtHC (2:1) and MASGA-EtHC (4:1) have a stronger negative inotropic effect than ethacizin and other complexes. Analysis of the obtained data showed that Na+-channels of cardiomyocytes play an important role in ensuring the negative inotropic effect (NID) of GA-EtHC (2:1) and MASGA-EtHC (4:1). At the same time, by blocking Na+ channels and reducing the content of Na+ ions in cardiomyocytes, these complexes can enhance the excretion of Ca2+ ions through the Na+/Ca2+ exchanger and decrease the level of [Ca2+]i, which results in a decrease in the contractile activity of the heart muscle. As a result of the studies, it was determined that the studied complexes, in comparison with ethacizine, have a pronounced antiarrhythmic effect, which is provided due to the blockade of Na+ channels, accompanied by a decrease in the [Ca2+]i level in cardiomyocytes and a decrease in the contractile activity of the heart muscle. In this case, the most pronounced effect has a complex MASGA-EtHC (4:1). The obtained experimental data can serve and/or supplement scientific data for the creation of new promising drugs with a broad therapeutic effect, targeted delivery, synergistic effect and low toxicity.

2. Keywords:
Cardiovascular Diseases, Antiarrhythmic Drugs, Ethacizine Hydrochloride, Inotropic Effect, Antiarrhythmic Effect

3. Introduction

In the 21st century arrhythmias began to occupy a special place in the structure of cardiovascular pathology due to the progressive increase in their prevalence among the population and, accordingly, the more frequent development of cardiovascular complications and sudden death. In the general population, the most common cardiac arrhythmia is sinus arrhythmia - it is recorded in 33.9–34.5% of cases. Among supraventricular arrhythmias, supraventricular extrasystole ranks first in terms of prevalence, which occurs in the general population in 34.9–56.7% of cases, and among middle-aged and elderly people in 88–99% of cases [1, 2]. Atrial fibrillation in terms of prevalence ranks second after extrasystole and is detected in the general population in 1.4–1.5% of cases, and among the elderly, its frequency reaches 3–17.8% [3, 4, 5]. Of the conduction disorders, the blockade of the right leg of the bundle of His is most often recorded in the population; complete blockade is recorded in 0.5-1.4% of the population, and incomplete - in 0.6-4.7% [6]. According to many authors, an increase in the prevalence of rhythm and conduction disorders is expected in the near future, which is associated with the aging of the population [7,8], as well as with an increase in the number of comorbid patients.

Ethacizine (3-diethylaminopropionyl-2-(ethoxycarbonylamino)phenothiosino hydrochloride) (EtHC) is a diethylamine derivative of ethmosine. Ethacizine has a wide spectrum of antiarrhythmic action. It is used not only for ventricular, but also for atrial extrasystoles and tachyarrhythmias, atrial fibrillation paroxysms, early ventricular repolarization syndrome. The effectiveness of the drug has been shown in clinical trials and with many years of use in wide medical practice [9,10]. Electrophysiological studies have shown that ethacizin effectively blocks not only the rapid penetration of sodium, but also the slow intake of calcium [11, 12]. Ethacizine is effectively used for the treatment of supraventricular and ventricular arrhythmias in the clinic. Most class I antiarrhythmic drugs, which specifically modify the function of Na+ channels, act by a similar

mechanism [13]. By blocking Na+ channels, all of them cause a decrease in the intracellular concentration of Na+ ions, which enhances the excretion of Ca2+ ions from cardiomyocytes through the Na+/Ca2+ exchanger. Increased excretion of Ca2+ ions, in turn, leads to a decrease in their intracellular concentration and, as a result, to the suppression of myocardial contractile activity [14]. The main disadvantage of this drug is its high toxicity, which manifests itself when it is used by side effects from the central nervous system (dizziness, headache, staggering when walking or turning the head, slight drowsiness; in some cases, diplopia, paresis of accommodation), from the gastrointestinal tract: nausea. Clathration of medicinal substances with cyclodextrins or plant glycosides is a promising method for reducing their side effects and increasing solubility [15, 16, 17]. Thus, works on the study of surface tension and surface-active properties of aqueous solutions of glycyrrhizic acid (GA) and its salts were first studied in [18]. The solubilization of water-insoluble corticosteroids, hydrocortisone and prednisolone, in a solution of the monoammonium salt of glycyrrhizic acid (MASGA) was studied in [19]. The authors have shown that the solubility of steroids increases by 100-120 times, and MASGA has a synergistic effect with respect to the latter.

These data suggest the possibility of controlled introduction of the required amount and even different classes of guest molecules into the formed micellar nanostructured associates, as well as the selection of the most effective supramolecular complexes of a certain composition by studying their biological activity. The aim of this study is to create new dosage forms of ethacizin and to study the specific activity in arrhythmia models in vitro on the papillary muscle of the rat heart.

4. Results And Discussion

4.1. Physico-Chemical Investigations Of Supramolecular Complexes

Synthesis of complexes of GA, MASGA with EtHC was carried out in a medium of 50% aqueous alcohol according to the following scheme: Figure:1

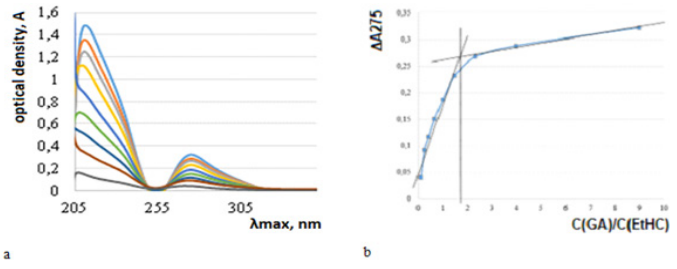


Fig 1: a) Absorption curve of UV spectra in an isomolar series of solutions (S_{EtHC} = 10^{-4}M, S_{GA} = 10^{-4}M, pH 7.2); b) plot of optical density (ΔA) against the ratio of the isomolar system of components [λ=275 nm].

The resulting complexes were characterized by some physicochemical parameters (S1 Table), the structures were studied by UV and IR spectroscopy.

Supplementary

S1 Table: Some physicochemical parameters of supramolecular complexes of GA, MASGA with EtHC

R	R'	n	Melting point, °C	Output, %	UV spectrum, λ_{max}, nm (lgε)
H	EtHC	2	190±2	88	209 (3,76); 244 (3,66)
H	EtHC	4	168±2	90	253 (3,87)
H	EtHC	8	195±2	87	253 (3,52)
NH_4^+	EtHC	2	170±3	87	209 (3,5); 246 (3,39)
NH_4^+	EtHC	4	175±3	90	252 (3,61)
NH_4^+	EtHC	8	160±3	88	252 (3,52)

In the electronic spectra of host-guest molecules in the UV region, a hypsochromic shift is observed, corresponding to electronic transitions (n → π*) of unshared electron pairs in comparison with the initial components (254 nm) [13]. In the IR spectrum of GA in the region of 3389 cm^{-1}, stretching vibrations of hydroxyl groups (OH) are observed in the form of a wide absorption band, and the absorption of the CH3 and CH2 groups is observed in the regions at 2973, 2936, 2875 cm^{-1}. In the region of 1713 cm^{-1}, absorption bands of stretching vibrations of the carbonyl (C=O) of the carboxyl group of GA are observed. The frequency of stretching vibrations of the carbonyl group at the C11 atom of the HA molecule is observed at 1651 cm^{-1} in the form of intense bands. Stretching vibrations of carboxylate groups (COO–) are observed with an average intensity in the region of 1615 cm^{-1}, and bending vibrations of CH3, CH2 are observed at 1453, 1431 cm^{-1}. Intense bands at 1039 cm^{-1} refer to the stretching vibrations of C–O–C and C–OH bonds; in the region of 981 cm^{-1}, bending vibrations of the =CH group are observed. The main difference in the spectra of MASGA and GA is in the bending vibrations at (NH4+) =1387 cm^{-1} (S2 Table).

S2 Table: Characteristic frequencies of functional groups in the IR spectra of GA, MASGA, EtHC and their complexes GA:EtHC (2:1; 4:1; 8:1) and MASGA:EtHC (2:1; 4:1; 8: 1)

Substance	Basic vibration frequencies in the IR spectrum, cm^{-1}
GA	ν(OH)=3389, ν(CH, CH_2, CH_3)=2973, 2936, 2875, ν(C=O)=1713, ν(C_{11}=O, C=C)=1651, ν(COO^-)=1615, δ(CH_2, CH_3)=1453, 1431, δ(CH)= 1361, 1325, 1256, 1212, δ(C–O–C, C–OH)=1166, ν(C–O–C)=1039, δ(=CH)=981

Clinical Journal of Cardiology

EtHC	ν(NH) =3304, ν (CH, CH_2, CH_3) =2979, 2933, ν(C=O) =1717, ν (C=O, C=C) =1671, ν(C=C, Ar) =1529, δ (CH_2, CH_3) =1464, 1407, δ(NH)=1364, δ(CH)=1351, 1319, 1294, 1222, δ(C–O–C) =1171, ν(C–O–C) =1053, δ(=CH) =990, δ(CH)=759, 692 (Ar), ν(C–S–C) =661
GA:EtHC2:1	ν(OH, NH)=3342, ν (CH, CH_2, CH_3) =2931, 2874, ν(C=O) =1716, ν (C_{11}=O, C=C) =1651, ν(COO^-) =1603, ν(C=C, Ar) =1532, δ (CH_2, CH_3) =1465, 1455, δ(NH) =1386, δ(CH)=1362, 1326, 1215, δ (C–O–C, C–OH, C–S–C) =1171, ν (C–O–C, C–S–C) =1041, δ(=CH) =982, δ(CH)=756, 684 (Ar), ν(C–S–C) =666
GA:EtHC4:1	ν(OH, NH)=3373, ν (CH, CH_2, CH_3) =2930, 2875, ν(C=O) =1722, 1698, ν (C_{11}=O, C=C) =1640, ν(COO^-) =1603, ν(C=C, Ar) =1532, δ (CH_2, CH_3) =1465, 1455, δ(NH) =1386, δ(CH)=1362, 1328, 1214, δ (C–O–C, C–OH, C–S–C) =1170, ν (C–O–C, C–S–C) =1039, δ(=CH) =981, δ(CH)=753, 684 (Ar), ν(C–S–C) =666
GA:EtHC8:1	ν(OH, NH)=3372, ν (CH, CH_2, CH_3) =2929, 2873, ν(C=O) =1716, ν (C_{11}=O, C=C) =1640, ν(COO^-) =1603, ν(C=C, Ar) =1540, δ (CH_2, CH_3) =1465, 1455, δ(NH) =1387, δ(CH)=1362, 1328, 1213, δ (C–O–C, C–OH, C–S–C) =1170, ν (C–O–C, C–S–C) =1039, δ(=CH) =981, δ(CH)=751, 684 (Ar), ν(C–S–C) =666
MASGA	ν(OH, NH_4^+)=3209, ν(CH, CH_2, CH_3)=2929, 2936, 2876, ν(C=O)=1702, ν(C_{11}=O, C=C)=1652, ν(COO^-)=1590, δ(CH_2, CH_3)=1452, 1417, δ(NH_4^+)=1387, δ(CH)=1363, 1328, 1259, 1213, δ(C–O–C, C–OH)=1167, ν(C–O–C)=1041, δ(=CH)=981
MASGA:EtHC2:1	ν(OH, NH)=3220, ν (CH, CH_2, CH_3) =2929, 2873, ν(C=O) =1718, ν (C_{11}=O, C=C) =1654, ν(COO^-) =1594, ν(C=C, Ar) =1533, δ (CH_2, CH_3) =1464, 1413, δ(NH) =1387, δ(CH)=1364, 1325, 1214, δ (C–O–C, C–OH, C–S–C) =1169, ν (C–O–C, C–S–C) =1040, δ(=CH) =981, δ(CH)=754, 697 (Ar), ν(C–S–C) =665
MASGA:EtHC4:1	ν(OH, NH)=3215, ν (CH, CH_2, CH_3) =2927, 2871, ν(C=O) =1717, ν (C_{11}=O, C=C) =1654, ν(COO^-) =1594, ν(C=C, Ar) =1530, δ (CH_2, CH_3) =1452, 1417, δ(NH) =1387, δ(CH)=1363, 1327, 1214, δ (C–O–C, C–OH, C–S–C) =1169, ν (C–O–C, C–S–C) =1039, δ(=CH) =981, δ(CH)=752, 698 (Ar), ν(C–S–C) =665
MASGA:EtHC8:1	ν(OH, NH)=3215, ν (CH, CH_2, CH_3) =2927, 2873, ν(C=O) =1701, ν (C_{11}=O, C=C) =1654, ν(COO^-) =1593, ν(C=C, Ar) =1531, δ (CH_2, CH_3) =1464, 1420, δ(NH) =1388, δ(CH)=1363, 1327, 1213, δ (C–O–C, C–OH, C–S–C) =1168, ν (C–O–C, C–S–C) =1039, δ(=CH) =980, δ(CH)=750, 697 (Ar), ν(C–S–C) =665

In the IR spectrum of EtHC, the vibrational frequencies of the NH group appear as a broad shoulder in the region of 3304 cm^{-1}. At 2979, 2933 cm^{-1}, the frequencies of stretching vibrations of CH, CH_2, CH_3 groups are observed, and in the region of 1717, 1671 cm^{-1} - the frequencies of stretching vibrations of the carbonyl group. At 1364 cm^{-1}, the bending vibration frequencies of the NH group appear. The frequencies of the stretching vibrations of the C–S–C group appear at 661 cm^{-1}. In addition, at 759, 692 cm^{-1}, bending vibrations of the CH groups of the aromatic ring are observed. By changing the vibration frequencies of the main functional groups and their shift in the IR spectra of the starting compounds, one can judge what are the interactions between molecules during the formation of molecular complexes [26]. In particular, the stretching vibrations of the OH groups of GA in the complex are shifted to the high frequency region by 45 cm^{-1} and were observed at 3343 cm^{-1}. In addition, the shape of the absorption band in the form of a wide shoulder indicates the presence of ion-dipole (–NH3+ O–H, N+···OH) interactions between molecules (S2 Table).

The frequencies of deformation vibrations of the quaternary ammonium salt in the EtHC molecule are observed at 1364 cm^{-1}, in the complex - at 1387 cm^{-1}, which indicates the presence, in addition to the characteristic NH4+ signals, of additional electrostatic (-COO- + N-) interactions in the complex. Stretching vibrations of C-S-C bonds in EtHC are observed at 661 cm^{-1}, and the frequency of these vibrations in the complex is partially shifted (666 cm^{-1}) to the low-frequency region. In addition, the fact that some frequencies of the bending vibrations of the CH groups of the aromatic ring in the EtHC molecule are shifted to a higher frequency region by 3–9 cm^{-1} in the spectrum of the complex gives grounds for assuming the presence of a hydrophobic interaction between the nonpolar parts of the molecules.

A comparative study of the IR spectra of the complexes GA:EtHC (4:1, 8:1) and MASGA:EtHC (2:1, 4:1, 8:1) with the spectra of GA:EtHC (2:1) showed a slight difference in the intensity absorption bands of the main

functional groups involved in the formation of complexes depending on the ratio of the complexing components. No significant differences were found in the spectra of supramolecular complexes of GA and MASGA with EtHQ, but taking into account the above literature data, it can be assumed with a high degree of probability that such a difference will be revealed when studying their biological activity. The stoichiometric composition was studied by the method of isomolar series, and the values of the stability constant and Gibbs free energy of the complexes of GA and MASGA with EtHC were determined (Ostromyslensky-Job method) [27]. It is known from the literature data that the presence of an isosbestic point on the absorption curve indicates the formation of only one type of complex between the initial components [28]. Figure 1a shows curves of the isomolar series of the supramolecular complex of GA with EtHC, with three isobestic points at 248, 262, 314 nm. It can be seen from Fig.1b that the index of the isomolar system of the complex of GA with EtHC in terms of the change in optical density in accordance with the ratio of components is ≈ 2.0, on the basis of which it is assumed that the ratio of components in the complex is 2:1.

The stability constant and Gibbs free energy of this complex were K_S= (5.032±0.698)x10^6 M^{-1} and ΔG = (3.824±0.839)x10^{-4}, J/mol, respectively(Fig 1).

Presumably, in the buffer solution, the following equilibrium is established between the components (GA and EtHC) and the complex:

$$[2GA] + [EtHC] \longleftrightarrow [2GA : EtHC]$$

$$K_S = \frac{[2GA : EtHC]}{[2GA][EtHC]}$$

Curves of isomolar series of UV spectra of MASGA with EtHC were obtained with one isosbestic point at 311 nm (Fig.2a). As in the case of GA, the index of the dependence of the change in optical density on the ratio of the components of the isomolar series of the MASGA complex with EtHC was equal to ≈ 2.0, and the stability constant and the Gibbs free energy of the complex were KS = (5.637±0.544) × 10^6 M^{-1}and ΔG = (3.832±0.516) × 10^{-4} J/mol, respectively (Fig.2b).

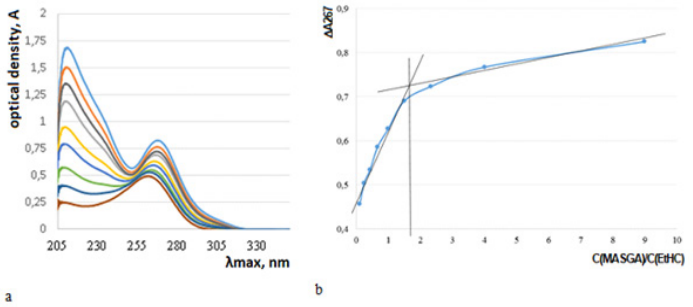
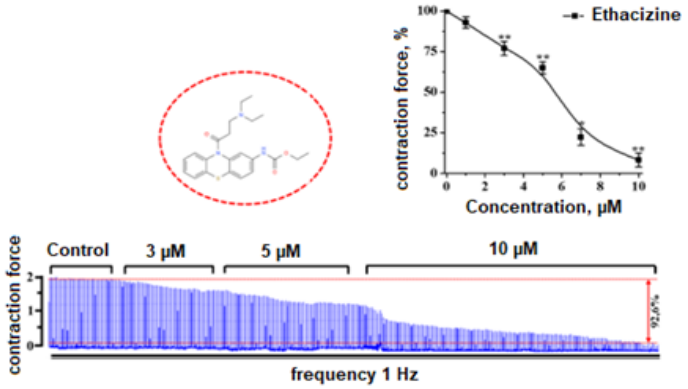


Fig 2: a) Absorption curve of UV spectra in an isomolar series of solutions (S_{EtHC} = 10^{-4}M, S_{MASGA} = 10^{-4}M, pH 7.2); b) plot of optical density (ΔA) versus ratio of isomolar system of components [λ=267 nm].

Complexes of GA and MASGA with EtHC are formed through weak intermolecular interactions, such as hydrogen bonding, electrostatic and hydrophobic interaction, etc. Supramolecular complexes of GA and MASGA with EtHC have the same stoichiometric composition and approximately equal stability constant, the negative value of the Gibbs free energy confirms the formation of a molecular complex due to auto association. The obtained experimental data can serve and/or supplement scientific data for the creation of new promising drugs with a broad therapeutic effect, targeted delivery, synergistic effect and low toxicity.

4.2. Specific Activity In Arrhythmia Models In Vitro

In preliminary experiments, ethacizin significantly suppressed the amplitude of rat heart papillary muscle contractions induced by stimulation with rectangular pulses with a frequency of 0.1–1 Hz. This effect of ethacizin was dose-dependent and, starting from a concentration of 1 μM, it suppressed the force of contraction by 7.4±4.2%, and with an increase in its concentration to 10 μM, it decreased by 92.6±3.3% relative to the control (S1 Fig).



S1 Fig: Effect of ethacizin on the contractile activity of the papillary muscle of the rat heart.
a) Suppression of the force of papillary muscle contraction with etacizin (original recording). b) Dose-dependent suppression of papillary muscle contraction force by ethacizins. On the y-axis - the force of contraction of the papillary muscle, expressed as a percentage of the control, taken as 100%, on the abscissa - the concentration of etacizin (μM), In all cases (*-p<0.05; **-p<0.01); n=6; (t=+ 36 ± 0.5°C).

In subsequent experiments on the papillary muscle of the rat heart, GA-EtHC(2:1), GA-EtHC (4:1), GA-EtHC (8:1), MASGA-EtHC(2:1), MASGA-EtHC (4:1) and MASGA-EtHC(8:1) caused a dose-dependent suppression of the contractile activity of the heart muscle, which indicates their negative inotropic effect (Fig 3). Dose-dependent suppression of the force of contraction of the papillary muscle complexes. On the ordinate axis - the force of contraction of the papillary muscle, expressed as a percentage of the control, taken as 100%, on the abscissa axis - the concentration of complexes (μM), In all cases (*-p<0.05; **-p<0.01). Stimulation frequency 1 Hz (t=+36±0.5°C; n=6). From the data presented in Fig.3, it can be seen that in the presence of the complexes GA-EtHC (2:1) (7 μM), GA-EtHC (4:1) (10 μM), GA-EtHC (8:1)

Clinical Journal of Cardiology

(20 μM), MASGA-EtHC (2:1) (20 μM), MASGA-EtHC (4:1) (5 μM) and MASGA-EtHC (8:1) (20 μM) papillary contraction force muscle decreases to 6.4±3.1%, 14.6±4.2%, 8.6±4.5%, 24.7±2.8%, 3.5±3.2% and 11, 6±3.9%, respectively, from the control level.

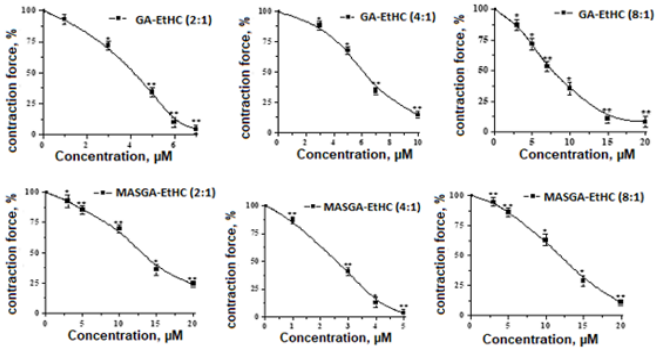


Fig 3: Influence of the complexes GA-EtHC (2:1), GA-EtHC (4:1), GA-EtHC (8:1), MASGA-EtHC (2:1), MASGA-EtHC (4:1) and MASGA-EtHC (8:1) on the contractile activity of the papillary muscle of the rat heart

Based on the above experiments, it can be said that GA-EtHC (2:1) and MASGA-EtHC (4:1) have a stronger negative inotropic effect than etacizin and other complexes. Thus, the analysis of the obtained data showed that Na+ channels of cardiomyocytes play an important role in ensuring the negative inotropic effect (NID) of GA-EtHC (2:1) and MASGA-EtHC (4:1). At the same time, by blocking Na+ channels and reducing the content of Na+ ions in cardiomyocytes, these complexes can enhance the excretion of Ca2+ ions through the Na+/Ca2+ exchanger and decrease the level of [Ca2+]i, which results in a decrease in the contractile activity of the heart muscle.

As noted above, class I antiarrhythmic drugs are also characterized by OID, which are based on the blockade of voltage-dependent Na+ channels of cardiomyocytes [29]. To assess the features of the antiarrhythmic action of the studied complexes, their effects on in vitro models of aconitine arrhythmia were studied. In these experiments, aconitine (1 μM) caused an increase in the force of contraction of the papillary muscle, which was accompanied by the manifestation of spontaneous contractions. The development of aconitine arrhythmia is due to an increase in the level of Na+ ions in cardiomyocytes, accompanied by the activation of the reversed mode of operation of the Na+/Ca2+ exchanger and the accumulation of Ca2+ ions in cardiomyocytes, which contribute to heart rhythm disturbance. In our experiments, the addition of EtHC, GA-EtHC (2:1) and MASGA-EtHC(4:1) against the background of arrhythmia caused by aconitine, led to a decrease in the frequency of spontaneous contractions from 254±11 to 41±6, 37±5 and 32±6 beats (Fig.5) per minute, respectively. On fig.4 is the original recording of the effect of the MASGA-EtHC(4:1) complex on spontaneous contractions of the papillary heart muscle induced by aconitine (1 μM) (Fig 4, 5). In this case, the frequency of muscle contraction under conditions of aconitine-induced arrhythmia is expressed in beats/minutes. Basal stimulation of the drug was performed at a frequency of 1 Hz (n=4).

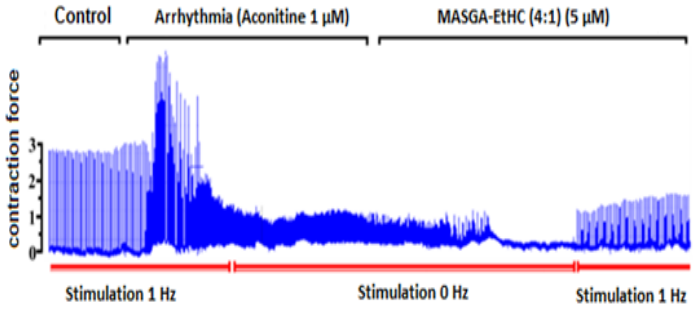


Fig 4: Antiarrhythmic activity of MASGA-EtHC(4:1) (5 μM) on aconitine (1 μM)-induced arrhythmia in the rat myocardium.

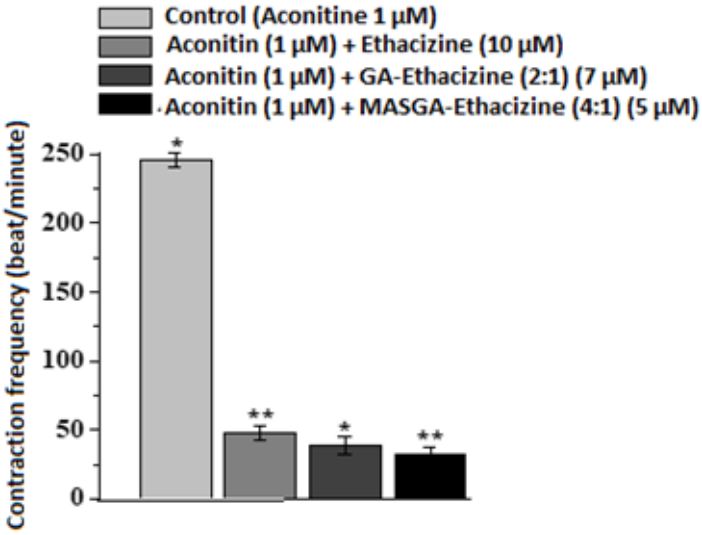


Fig 5: Antiarrhythmic activity of the drug EtHC (10 μM), GA-EtHC (2:1) (7 μM) and MASGA-EtHC (4:1) (5 μM) on aconitine (1 μM) - induced arrhythmia in the rat myocardium.

From the presented results, it can be seen that the studied preparations effectively suppress the development of spontaneous contractions of the papillary muscle of the rat heart, caused by aconitine. At the same time, the effect of the MASGA-EtHC complex (4:1) was more pronounced compared to the effects of ethacizin and GA-EtHC (2:1) preparations. It is known that the effects of class I antiarrhythmic drugs are due to the blockade of voltage-dependent Na+ channels of the sarcolemma, which is accompanied by a decrease in the contractile activity of the heart muscle [30]. The decrease in the contractile activity of the heart muscle caused by these antiarrhythmic drugs occurs as a result of a decrease in the [Ca2+]i level in cardiomyocytes, which is associated with the blockade of voltage-dependent Na+ channels [31]. In this regard, and in order to elucidate the role of Na + channels in providing the effects of the complexes MASGA-EtHC (4:1) and GA-EtHC (2:1), their effect on muscle contractions was studied in the presence of lidocaine, a specific channel blocker. In these preliminary studies, we found that lidocaine causes AIE and dose-dependently suppresses the contractile activity of rat papillary muscle

with an IC50 of 15 μM (Fig.6).

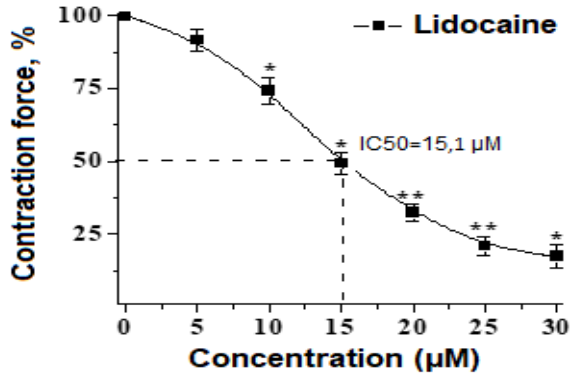


Fig 6: Effect of lidocaine on the contractile activity of the papillary muscle of the rat heart.

The y-axis shows the force of contraction of the papillary muscle, expressed as a percentage of the control, taken as 100%, the abscissa shows the concentration of lidocaine (μM).In all cases (*-p<0.05; **-p<0.01); n=4; (t =+ 36±0.5°C). In these experiments, it was found that in the presence of 15 μM lidocaine, a concentration corresponding to its IC50 value, the complexes GA-EtHC (2:1) (4.1 μM) and MASGA-EtHC (4:1) (2.6 μM) reduce the force of muscle contraction by 24.4±3.8% and 13.6±3.2%, respectively, compared with the control (Fig. 7, 8).

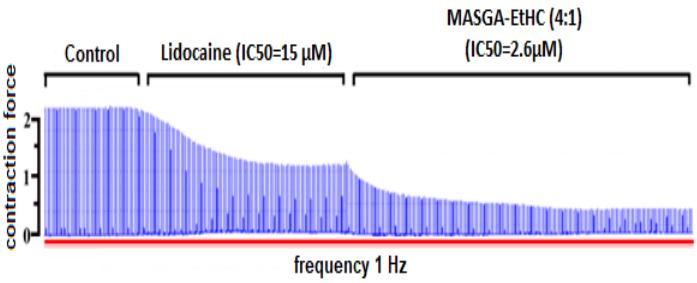


Fig 7: Original recording of the effect of the MASGA-EtHC (4:1) complex on the force of contraction of the papillary muscle of the rat heart in the presence of lidocaine. Stimulation frequency: 1 Hz, t=+36±0.5°C.

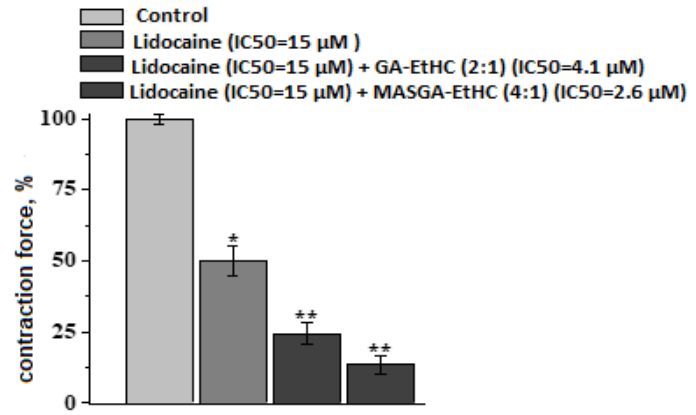


Fig 8: The role of voltage-dependent Na+-channels in ensuring the negative inotropic effect of the complexes GA-EtHC (2:1) and MASGA-EtHC (4:1).

The y-axis shows the papillary muscle contraction force, expressed as a percentage of the control, taken as 100%. Stimulation frequency 1 Hz. In all cases *-p<0.05 (n=5). These results, along with the results obtained in experiments with lidocaine, indicate that the Na+ channels of cardiomyocytes play an important role in providing OID for the GA-EtHC(2:1) and MASGA-EtHC(4:1) complexes. At the same time, by blocking Na+ channels and reducing the content of Na+ ions in cardiomyocytes, these complexes seem to enhance the excretion of Ca2+ ions through the Na+/Ca2+ exchanger, which may contribute to a decrease in the level of [Ca2+]i and their reserves in the SR.

From the presented results, it can be seen that the studied complexes effectively suppress the development of spontaneous contractions of the papillary muscle of the rat heart, caused by aconitine. At the same time, the effect of the MASGA-EtHC complex (4:1) was more pronounced, in comparison with the effects of the complexes of etacizin with GA and MASGA in other ratios. Thus, the results obtained during the implementation of this work indicate that the studied complexes have a pronounced antiarrhythmic effect, which is provided due to the blockade of Na+ channels, accompanied by a decrease in the [Ca2+]i level in cardiomyocytes and a decrease in the contractile activity of the heart muscle. The observed differences in the effectiveness of the negative inotropic and antiarrhythmic effects of the studied complexes, and possibly in the mechanisms underlying them, seem to be due to the peculiarities of their chemical structure. The obtained experimental data can serve and/or supplement scientific data for the creation of new promising drugs with a broad therapeutic effect, targeted delivery, synergistic effect and low toxicity.

5. Materials and Methods

Glycyrrhizic acid (GA) and its monoammonium salt (MASGA) were obtained from commercial glycyrrhizic acid by a known method [21]. Ethacizine (3-diethylaminopropionyl-2-(ethoxycarbonylamino) phenothiosino hydrochloride) (EtHC) is a pharmaceutical substance-powder (manufactured by NIOPIK State Research Center, Russia).

5.1. Obtaining A Supramolecular Complex GA:Ethc 2:1

0.421 g (0.5 mmol) of HA was dissolved in 50 ml of 50% ethanol, and 0.113 g (0.25 mmol) of EtHC was gradually added with stirring. The reaction mixture was intensively stirred for 6-8 hours at room temperature. The alcohol was distilled off on a rotary evaporator, the aqueous part was freeze-dried.

Output: 0.47 g (88%). Melting point 190±2°C.
Similarly, complexes of GA and MASGA with EtHC were obtained in various host-guest molar ratios of 2:1, 4:1, and 8:1.

5.2. IR Spectroscopy

The IR spectra of the resulting complexes were recorded in the vibrational frequency range of 400–4000 cm^{-1} on a Nicolet iS50 IR Fourier spectrometer from Thermo Scientific. Samples were taken in ATR (ATR) mode without sample preparation in the form of a powder.

5.3. UV Spectroscopy

UV spectra were obtained on a Shimadzu 1280 instrument (Japan), in the wavelength range from 190 to 1100 nm, cuvette thickness 1x1 cm (quartz), solvent: 50% aqueous ethanol solution.

5.4. Melting Point Determination

The melting point was determined on a PTP TU 25-11-1144 instrument.

5.5. Determination of Stoichiometric Composition

Isomolar series of components were prepared in phosphate buffer Na_2HPO_4–NaH_2PO_4 at a concentration of 10^{-4} or 5×10^{-4} M at pH 7.2, which were mixed in antibatic ratios (from 1:9 to 9:1) with the total volume unchanged. The mixture was kept for 40 min at constant temperature and stirring. To determine the stability constants of the complexes (K_s), 5 independent measurements were carried out (data are presented as mean value and mean error). Calculation of K_s was carried out according to the formula based on isomolar curves according to the method [22]. For complexes of composition 2:1, the calculation of K_s was performed on the basis of considering the ratio of the solution of the complex to the dilution, using formula (1):

$$K_s = \frac{(c_1 \sqrt[3]{\Delta A_2}) - c_2 \sqrt[3]{\Delta A_1})(\Delta A_1 \sqrt[3]{\Delta A_2} - \Delta A_2 \sqrt[3]{\Delta A_1})^2}{4(\Delta A_1 c_1 - \Delta A_2 c_1)^3}$$

where c1 is the total concentration of substances, M;

c2 is the total concentration after dilution, M;

ΔA1 and ΔA2 are the corresponding changes in optical density before and after dilution.

The Gibbs free energy ΔG for complex formation processes was calculated using formula (2):

$$\Delta G = -2{,}3RT \lg K_s, (2)$$

5.6. Obtaining Preparations Of Papillary Muscle Of The Rat Heart And Registration Of Their Functional Activity

In the experiments, outbred male rats (150–200 g) were used, grown in vivarium conditions of the Cell Biophysics Laboratory of the Institute of Biophysics and Biochemistry at the National University of Uzbekistan with standard access to food and water. Experiments on animals were carried out in accordance with the rules of the European Convention for the Protection of Animals Used in Experiments and Other Scientific Purposes [23]. The protocol was approved by Animal ethical committee based on Institute of Bioorganic chemistry, AS RUz (Protocol Number: 133/1a/h, dated August 4, 2014). Before the experiment, the animals were immobilized under light ether anesthesia (~20 mg/kg of weight intraperitoneally), after decapitation of the animals and opening the chest, the heart was quickly removed and papillary muscle preparations were placed in aerated physiological Krebs-Henseleit solutions of the following composition (in millimoles): NaCl - 118 ; KCl - 4.7; $CaCl_2$ - 2.5; $MgSO_4$ - 1.2; KH_2PO_4 - 1.1; glucose - 5.5; $NaHCO_3$ - 25, while maintaining a temperature of +36±0.5^{0}C using a thermostat U1 (Bulgaria) (pH=7.4).

The solutions were oxygenated with carbogen (O_2–95%, CO_2–5%), and constant perfusion of saline was provided using a LKB Bromma peristaltic pump (Sweden). The study of the effect of the studied preparations on the contractile and functional activity of the papillary muscle was carried out mechanographically using a system for recording the force of muscle contraction of the SI-BAM21-LC type (World Precision Instruments Inc. (WPI); USA). The force of muscle contraction was recorded in the isometric mode using an SI-OHO_2F type device (it allows performing ~ 16 (n = 16) experiments in 4 parallel experimental chambers for ~ 6–10 hours). The mechanical signal after conversion by SI - piezoelectric sensor KG20 SI - BAM21 - LCB is transmitted to the amplifier and recorded in digital format (WLabScribe2*iwxdata) using a special program iWorx LabScribe2 (iWorx Systems, Inc.; USA) on an IBM PC for further mathematical and statistical processing. The muscle was irritated using platinum electrodes and an ESL-2 stimulator (Russia) with rectangular pulses with a frequency of 0.1–1 Hz, a duration of 10 ms, and an amplitude exceeding the threshold by ~20% for ~45–60 min. until stable electromechanical characteristics of muscle preparation contraction are established. The base frequency of stimulating impulses was 0.1 Hz. After the stabilization period, the length of the preparation was found at which the muscle develops the maximum isometric tension (Lmax), and all experiments were performed under these conditions [24, 25].

5.7. Statistical Processing Of Results. The Contractile Activity Of The Heart Muscle Was Assessed In Isometric Mode

Statistical data processing was carried out using the Origin Lab Origin Pro v. 6.1 (USA). The inotropic activity of the chemical reagents used was calculated as a percentage (%) of the amplitude of the contraction force from the control value with a confidence interval (±). In this case, the data are presented as M±m, where M is the mean, m is the standard error. To identify the significance of changes before and after the influence of the drug, a paired Student's t-test was used. Differences were considered statistically significant at $p<0.01$ and $p<0.05$.

References

1. Conen D, Adam M, Roche F, Barthelemy JC, Dietrich DF, Imboden M, et al. Premature atrial contractions in the general population: frequency and risk factors. Circulation. 2012; 126(19): 2302-2308.
2. Zatonskaya EV, Matyushin GV, Gogolashvili NG, Novgorodtseva NYa. Epidemiology of arrhythmias (review of literature data). Siberian Medical Review. 2016; 3: 5-16.
3. Pelinovskaya LI, Demko IV, Mandrikova OM, Glizer RN. Frequency and nature of coronary artery lesions in patients with atrial fibrillation. Siberian Medical Review. 2013; 4: S. 60-62.
4. Murakoshi N, Aonuma K. Epidemiology of arrhythmias and sudden cardiac death in Asia. Circ J. 2013; (77): 2419-2431.
5. Zatonskaya EV, Matyushin GV, Gogolashvili NG. Prevalence

and clinical significance of cardiac arrhythmias. Rational pharmacotherapy in cardiology. 2017; 13(3): 403-408.

6. Bussink E, Holst AG, Jespersen L, Deckers JW, Jensen G.B, Prescott E. Right bundle branch block prevalence, risk factors, and outcome in the general population results from the Copenhagen City Heart Study Eur Heart J. 2013; (34): 138-146.
7. Jensen PN, Gronroos NN, Chen LY, Folsom AR, de Filippi C, Heckbert SR, Alonso A. Incidence of and risk factors for sick sinus syndrome in the general population. J Am Coll Cardiol. 2014; (64): 5315-5338.
8. Zatonskaya EV, Matyushin GV, Gogolashvili NG, NovgorodtsevaN Ya, Shulmin AV. The frequency of heterogeneous arrhythmias in the adult population of the Arctic. Siberian Medical Review. 2015; (4): 52-56.
9. Zatonskaya EV, Matyushin GV, Gogolashvili NG, NovgorodtsevaN Ya, Shulmin AV. The frequency of cardiac conduction disorders in the population of the adult population of the Arctic. Siberian Medical Review. 2015; (3): 74-78.
10. Malakhov VI, Golitsyn SP, Sokolov SF, Smetnev AS. Electrophysiological mechanisms of antiarrhythmic action of ethacizine. Bul VKNC. 1987; (3): 112-116.
11. Tsaregorodtsev DA. The problem of drug-resistant arrhythmias. Ros. cardiol. J. 2001; No. 2.
12. Cahalan MD. Local anesthetic block of sodium channels in normal and pronase-treated squid giant axons.Biophys J. 1978; 23(2): 285-311. doi: 10.1016/S0006-3495(78)85449-6.
13. Campbell TJ. Kinetics of onset of rate-dependent effects of Class I antiarrhythmic drugs are important in determining their effects on refractoriness in guinea-pig ventricle. and provide a theoretical basis for their subclassification. Cardiovascular Research. 1983; 17(6): 344–352.
14. Roden DM. Pharmacology and Toxicology of Nav1.5-Class 1 anti-arrhythmic drugs. Card Electrophysiol Clin. 2014; 16(4): 695-704. doi: 10.1016/j.ccep.2014.07.003.
15. Kim MJ, Park SR, Suh CK. Effects of Na+ and Ca2+ concentration in cardioplegic and reperfusion solutions on the intracellular Ca2+ of cardiac muscle cells. Yonsei Med J. 1993; 34(2): 133-44. doi: 10.3349/ymj.1993.34.2.133.
16. Tolstikova TG, Tolstikov AG, Tolstikov GA. On the way to low-dose drugs. Bulletin of the Russian Academy of Sciences. 2007; 77(10): 867–874.
17. Polyakov NE., Leshina TV. Glycyrrhizic acid as a novel drug delivery vector: synergy of drug transport and efficacy. Open Conf. Proc J. 2011; (2): 64–72.
18. Hostettmann K., Marston A. Saponins.Cambrige: Cambrige University Press, 1995. 548 p. https://doi.org/10.1017/CBO9780511565113
19. RJ Gilbert and KC. James. Preparation and surface-active properties of glycyrrhizic acid and its salts. J Pharm. Pharmacol. 1964; (16): 394-399.
20. Krasova TG, Bashura GS, Muravev IA. Studies of the solubilization of hydrocortisone and prednisolone in aqueous solutions of glycyram. Pharmacy. 1978; 27(5): 32-35.
21. Baltina LA, Kondratenko RM, Mustafina SR, V Danilov, TM Gabbasov, Yu I Murinov et al. Method for obtaining glycyrrhizic acid from Glyciram. Pharmacological properties. Chemical-pharmaceutical journal. 2001; 35(1): 38-41.
22. Babko AK. Physical and chemical analysis of complex compounds in solutions. K Publishing House of the Academy of Sciences of the Ukrainian SSR. 1955; (328).
23. Directive 2010/63/EU on the protection of animals used for scientific purposes. September 22 2010. Official Journal of the European Union. L 276/33- L276/79.
24. I.Z. Jumayev, PB Usmanov, ShYu Rustamov, Sh. Zhurakulov Comparative Inotropic Effects of the Some IsoquinolineAlkaloids. Biomedical& Pharmacology Journal, 2020; 13(1): 325-333.
25. PB Usmanov, IZ Jumayev, ShYu Rustamov et al. Biomedical & Pharmacology Journal. 2021; 14(2): 651-661.
26. Roy B, Saha A, Esterrani A, Arun K Nand. Time sensitive. temperature and pH responsive photoluminescence behavior of a melamine containing bicomponent hydrogel. Soft Matter. 2010; (6): 3337-3345.
27. Bulatov MI, Kalinkin IP. A practical guide to photometric methods of analysis. L. Chemistry. 1986; (432) .
28. Yakovishin LA. Molecular complexes of triterpene glycosides with biologically active substances preparation. chemical and pharmaceutical properties and biological activity. Diss on sois uch Art doc chem Sciences Sevastopol. 2018; (351) .
29. Ko T, Otani H, Imamura H, Omori K, Inagaki C. Role of sodium pump activity in warm induction of cardioplegia combined with reperfusion of oxygenated cardioplegic solution. J Thorac Cardiovasc Surg. 1995;110(1):103-10. doi: 10.1016/S0022-5223(05)80015-7.
30. Moreno JD, Zhu ZI, Yang PC et al. A computational model to predict the effects of class I anti-arrhythmic drugs on ventricular rhythms. Sci Transl Med. 2011; 3(98): 98ra83. doi: 10.1126/scitranslmed.3002588.
31. Antzelevitch C, Nesterenko V, Shryock JC, Rajamani S, Song Y, Belardinelli L. The role of late I Na in development of cardiac arrhythmias.Handb Exp Pharmacol. 2014; (221): 137-68. doi: 10.1007/978-3-642-41588-37.