



Effects of *Chelidonium majus* extracts and major alkaloids on hERG potassium channels and on dog cardiac action potential – A safety approach

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ABSTRACT

Chelidonium majus or greater celandine is spread throughout the world, and it is a very common and frequent component of modern phytotherapy. Although *C. majus* contains alkaloids with remarkable physiological effect, moreover, safety pharmacology properties of this plant are not widely clarified, medications prepared from this plant are often used internally. In our study the inhibitory effects of *C. majus* herb extracts and alkaloids on hERG potassium current as well as on cardiac action potential were investigated. Our data show that hydroalcoholic extracts of greater celandine and its alkaloids, especially berberine, chelidonine and sanguinarine have a significant hERG potassium channel blocking effect. These extracts and alkaloids also prolong the cardiac action potential in dog ventricular muscle. Therefore these compounds may consequently delay cardiac repolarization, which may result in the prolongation of the QT interval and increase the risk of potentially fatal ventricular arrhythmias.

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1. Introduction

Chelidonium majus L. or greater celandine (Papaveraceae) is spread throughout the world, including Europe, Asia, North-west Africa and North America. It is an important plant of the modern phytotherapy, used both externally and internally either in combination or in monotherapy in the form of different preparations (tincture, dry and liquid extracts, herbal

tea, gel, ointment, eye-drops). Greater celandine has been used historically in Europe for the treatment of several diseases, mainly against bile and liver disorders. Furthermore, its yellow–orange fresh latex has traditionally been applied for the treatment of warts and corns, and also for other skin complaints including tinea infections, eczema and different tumors of the skin [1]. *C. majus* derived extracts and purified compounds exhibit a broad spectrum of biological activities, such as anti-inflammatory, choleric, antimicrobial, antiviral, antitumoral, analgesic, anti-spasmodic and hepatoprotective effects [2–5]. It is an official drug in the European Pharmacopoeia

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(01/2008:1861), but also widely utilized in the traditional Chinese medicine [2,3].

Phytochemical profile of aerial parts and root of *C. majus* is well documented. Over 20 different *Chelidonium* alkaloids were identified. Its main alkaloids are benzyloisoquinolines (0.01–1%) with at least three subgroups: benzophenanthridines (chelerythrine, chelidonine, isochelidonine, sanguinarine), protoberberines (berberine, coptisine, dihydrocoptisine, stylopine) and protopine (protopine) [5].

In general these alkaloids are considered to be the active principles of the plant [5], since their pharmacological properties, including antispasmodic, anti-inflammatory, antitumor, antiviral and antimicrobial activities are well established [1].

However, several studies have demonstrated that berberine [6–9], chelerythrine [10], chelidonine [11], sanguinarine [11], and protopine [11] have hERG ion channel activities. The human ether-à-go-go-related gene (hERG, KCNH2, Kv11.1) encodes a cardiac voltage-gated potassium channel that provides the major repolarizing current (rapidly activating delayed rectifier potassium current, I_{Kr}) in phase 3 of the cardiac action potential. Blockade of the hERG potassium channel may lead to action potential prolongation at the cellular level, a prolongation of the QT interval on the electrocardiogram and could enhance the risk of cardiac arrhythmia and sudden cardiac death. If the hERG potassium current inhibiting effect of *Chelidonium* alkaloids is not counteracted by other constituents of the plant, the internal use of celandine extracts may increase the risk of potentially fatal cardiac arrhythmias.

It is also important to note that the alkaloid-mediated inhibition of the hERG channel may not necessarily lead to the lengthening of the action potential. Among these alkaloids, berberine was reported to inhibit hERG potassium current in transfected HEK cells expressing hERG channels [7–9], to block delayed rectifier potassium (I_{Kr}) current in cardiac myocytes [6], and to increase action potential duration on cardiac preparations [6,7]. Chelerythrine was also described to inhibit hERG current [10] and prolong action potential [12]. Although protopine also inhibits the hERG potassium current [11], in contrast with chelerythrine and berberine it shortens the action potential duration. This phenomenon is due to the multichannel effect of protopine, since protopine is not a selective ion channel antagonist. It acts as a promiscuous inhibitor of other ion channel currents including L-type Ca^{2+} ($I_{Ca,L}$) and sodium (I_{Na}) currents [13]. Effects of chelidonine or sanguinarine on cardiac action potential have not been studied so far.

The aim of the present study was to assess the potential cardiac risks of internally applied *C. majus* containing phytotherapeutics. Inhibitory effects of greater celandine herb extracts and alkaloids on hERG potassium current were investigated in HEK293 (human embryonic kidney) cells stably expressing the hERG potassium channel with automated patch-clamp system. Effects of *C. majus* herb extracts and alkaloids on cardiac action potential were also investigated in dog ventricular muscle with conventional microelectrode technique.

2. Materials and methods

2.1. Plant materials

The plant material was originated from a commercial source. A voucher (No. 824) was deposited in the Institute of

Pharmacognosy, University of Szeged. The extracts were prepared from 5 g grounded herb with 50 ml 25% aqueous ethanol and 50 ml 45% aqueous ethanol respectively, at room temperature using ultrasonic bath for 15 min. After filtration, the solvents were evaporated in vacuum until dryness. For electrophysiological assays 50 mg of the dried extract dissolved in 1 ml dimethyl sulfoxide was used. Stock solutions were stored at $-20\text{ }^{\circ}\text{C}$ until use.

2.2. Solvents and chemicals

Berberine chloride, chelerythrine chloride, chelidonine and sanguinarine nitrate were purchased from Sigma-Aldrich. Coptisine was isolated from 300 g powdered air dry herbs with 2000 ml 2% hydrochloric acid at room temperature. The pH of the filtered extract was adjusted to 12 with 2% aqueous sodium hydroxide solution. This step was followed by liquid-liquid extraction with 3×700 ml chloroform. The further purification of the concentrated chloroform fractions was performed by preparative TLC, and on silica using n-propanol–formic acid–water 90:1:9 as eluent affording pure coptisine. The identity and purity (>98%) of coptisine were controlled by ^1H NMR spectroscopy. For electrophysiological assay the compounds were dissolved in DMSO (10 μM stock solution) and stored at $-20\text{ }^{\circ}\text{C}$.

2.3. HPLC–MS apparatus and measurement conditions

Acetonitrile and methanol used for chromatographic analysis were of HPLC grade and formic acid and ammonia used for making a buffer solution were of analytical purity. Direct-Q UV3 clarifier was used to produce purified water for HPLC–MS measurements.

Chromatographic separation was performed using a Shimadzu liquid chromatographic system (2 pumps (LC-20AD); PDA detector (SPD-M20A); autosampler (SIL-20A); controller (CBM-20A); degasser (DGU-20A3); column thermostat (CTO-20AC)) equipped with a Phenomenex Gemini C18 (100 \times 4.60 mm, 5 μm) column. Isocratic elution was applied. The mobile phase was acetonitrile–methanol–30 mmol ammonium-formate (pH 3.5) 14.7:18:67.3, respectively. The flow rate was 0.8 ml/min. The HPLC was coupled to an API 2000 MS/MS with an electrospray (ESI) interface. The source temperature was 350 $^{\circ}\text{C}$. The measurements were carried out in positive ionization mode and the quantification was accomplished by using multiple reaction monitoring (MRM) with transitions of m/z 336 \rightarrow 320 for berberine, 320 \rightarrow 292 for coptisine, 348 \rightarrow 322 for chelerythrine, 354 \rightarrow 275 for chelidonine and 332 \rightarrow 317 for sanguinarine. Data acquisition and evaluation were performed using Analyst 1.5.2 software.

Calibration was performed using the standard stock solution (STD) and one working solution made from the STD with its dilution. These solutions contained all five measured analytes dissolved in methanol. The concentrations of the analytes in the STD were the following: 0.0165 mg/ml for berberine, 0.0186 mg/ml for coptisine, 0.167 mg/ml for chelerythrine, 0.0210 mg/ml for chelidonine and 0.0254 mg/ml for sanguinarine. Different volumes of the standard solutions were injected and the calibration curve was made by plotting the peak area of the analyte in function of the injected mass of the analyte. The correlation coefficients (R^2) of the calibration curves were between 0.9977 and 0.9992.

For HPLC–MS measurements, the dried extracts prepared from 5.00 g plant material with 50 ml solvents were filtered, evaporated in vacuum and dissolved in 2.50 ml methanol.

2.4. Automated patch-clamp experiments

The hERG channel current was measured by using planar patch-clamp technology in the whole-cell configuration with a 4 channel semi-high-throughput automated patch clamp system (Patchliner, Nanion Technologies GmbH) [14]. Current recordings were performed with an EPC-10 Quadro patch-clamp amplifier (HEKA Elektronik Dr. Schulze GmbH), using PatchMaster 2.43 software (HEKA Elektronik Dr. Schulze GmbH). The pipetting protocols were controlled by PatchControlHT 1.07.50 software (Nanion Technologies GmbH).

Experiments were carried out at room temperature, on HEK293 (human embryonic kidney) cells stably expressing

the hERG (Kv11.1) potassium channel. The cell line originated from Cell Culture Service. Cells were maintained at 37 °C, in 5% CO₂ in IMDM (PAA Laboratories GmbH) medium supplemented with 10% FBS (PAA Laboratories GmbH), 2 mM L-glutamine (Life Technologies Corporation), 1 mM Na-pyruvate (PAA Laboratories GmbH) and 500 µg/ml G418 (PAA Laboratories GmbH).

Suspension of cells was used for measurements from running cell culture. Cells were washed twice with PBS (Life Technologies Corporation) and then detached with trypsin–EDTA (PAA Laboratories GmbH) for 30–60 s before the measurement. Trypsin was blocked with MEM (PAA Laboratories GmbH) + 10% FBS. The cell suspension was next centrifuged (2 min, 100 g), resuspended in IMDM medium at a final density of 1×10^6 – 5×10^6 cells/ml, and kept in the cell hotel of the Patchliner. Cells were recovered after 15–30 min and remained suitable for automated patch clamp recordings for up to 4 h.

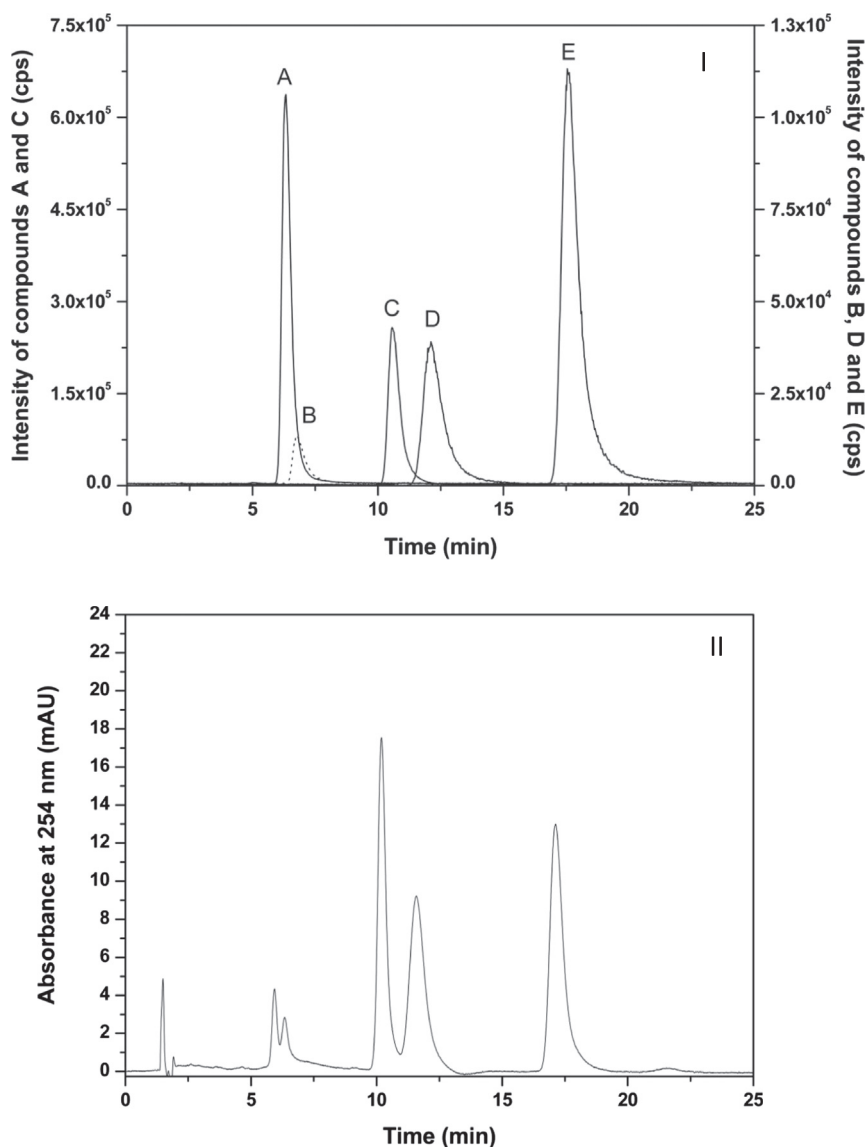


Fig. 1. MRM spectra (I) and HPLC chromatogram (II) of a STD solution (A: chelidonium, B: coptisine, C: berberine, D: sanguinarine, E: chelerythrine).

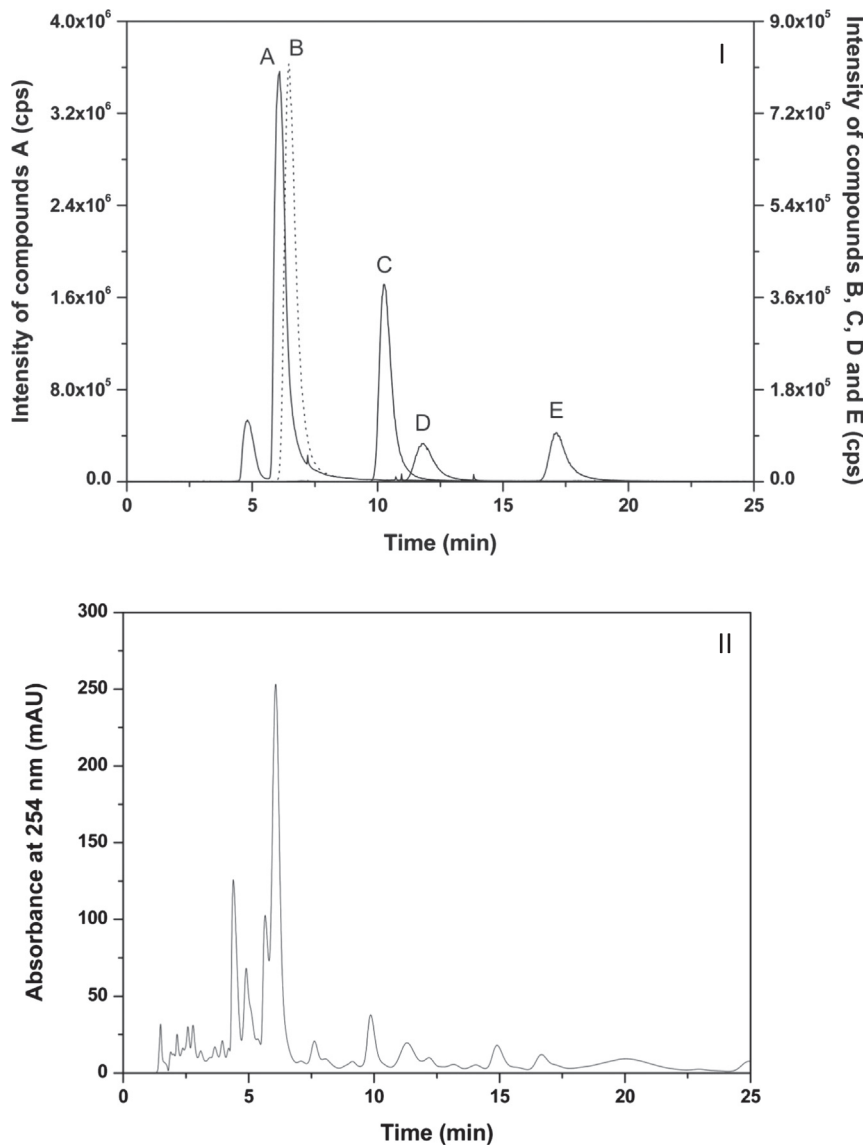


Fig. 2. MRM spectra (I) and HPLC chromatogram (II) of the 25% ethanol extract of *Chelidonium majus* (A: chelonine, B: coptisine, C: berberine, D: sanguinarine, E: chelerythrine).

The following solutions were used during patch-clamp recording (compositions in mM): internal solution: KCl 50, NaCl 10, KF 60, EGTA 20, and HEPES 10, pH 7.2 (KOH); and external solution: NaCl 140, KCl 4, glucose-monohydrate 5, $MgCl_2$ 1, $CaCl_2$ 3, and HEPES 10, pH 7.4 (NaOH). Chemicals were purchased from Sigma-Aldrich Corporation. All solutions were sterile filtered. Aliquots were stored at $-20^\circ C$ and warmed up to room temperature before use.

Effects of *C. majus* extracts and its alkaloids, berberine, coptisine, chelonine, and sanguinarine were tested. Stock solutions were further diluted with external solution, to give appropriate concentrations for the patch-clamp measurements. The final DMSO concentrations in the tested samples were 1% or less.

The voltage protocol for hERG ion channel started with a short -40 mV (100 ms) prepulse step, as a reference. A 20 mV

depolarizing step was applied for 3 s, and then the potential was -40 mV for 1 s to evoke outward tail current. Holding potential was -80 mV and pulse frequency was approximately 10 s. The peak tail current corrected the leak current defined during the first period to -40 mV.

Recording started in external solution. After this control period, the increasing concentrations of the test compound were applied, each for approximately 3 min. $10 \mu M$ amitriptyline was applied as a reference inhibitor then a wash-out step terminated the protocol.

2.5. Conventional microelectrode technique

All experiments were carried out in compliance with the *Guide for the Care and Use of Laboratory Animals* (USA NIH publication NO 85-23, revised 1996) and conformed to the

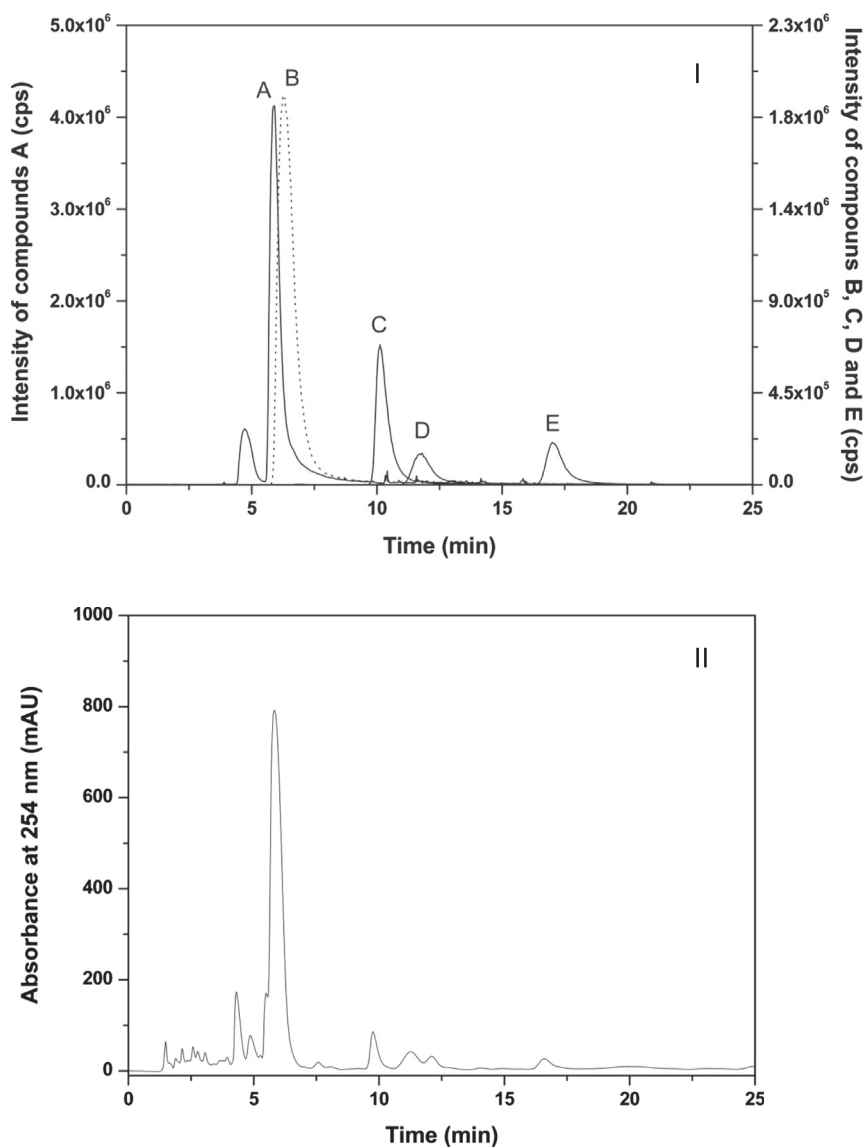


Fig. 3. MRM spectra (I) and HPLC chromatogram (II) of the 45% ethanol extract of *Chelidonium majus* (A: chelidionine, B: coptisine, C: berberine, D: sanguinarine, E: chelerythrine).

Directive 2010/63/EU of the European Parliament. The protocols have been approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged, Szeged, Hungary (approval number: I-74-5-2012) and by the Department of Animal Health and Food Control of the Ministry of Agriculture and Rural Development (authority approval number XIII/1211/2012).

Adult mongrel dogs (8–14 kg) of either sex were used. Following sedation (xylazine, 1 mg/kg, *i.v.*) and anesthesia (thiopental, 30 mg/kg, *i.v.*), the heart was rapidly removed through right lateral thoracotomy. The hearts were immediately rinsed in oxygenated modified Locke's solution containing (in mM): NaCl 120, KCl 4, CaCl₂ 1, MgCl₂ 1, NaHCO₃ 22, and glucose 11. The pH of this solution was set between 7.35 and 7.4 when saturated with the mixture of

95% O₂ and 5% CO₂ at 37 °C. Isolated muscle preparations obtained from the right ventricle were individually mounted in a tissue chamber with a volume of 50 ml. These preparations were stimulated through a pair of platinum electrodes in contact with the preparation using rectangular current pulses of 2 ms duration. These stimuli were delivered at a constant cycle length of 1000 ms for at least 60 min allowing the preparation to equilibrate before the measurements were initiated. Transmembrane potentials were recorded using conventional glass microelectrodes, filled with 3 M KCl and having tip resistances of 5–20 MΩ, connected to the input of a high impedance electrometer (Experimetria, type 309, Budapest, Hungary) which was coupled to a dual beam oscilloscope. The resting potential (RP), action potential amplitude (APA), maximum upstroke velocity (V_{max}) and action potential duration

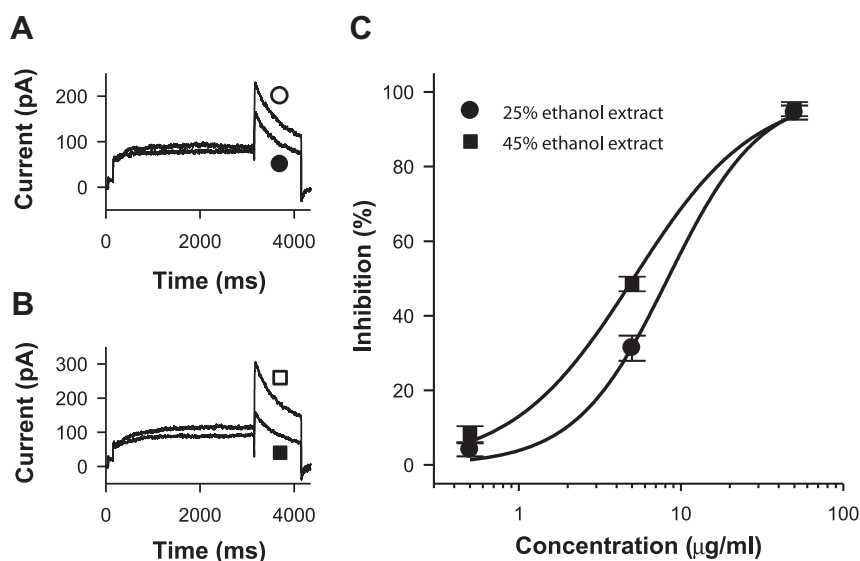


Fig. 4. Concentration-dependent effects of *Chelidonium majus* extracts on hERG current. Panels A and B depicted the representative hERG current curves obtained from cells treated with extracts. Empty symbols show the control curves, while full symbols show the effects of 25% ethanol extract (A) and 45% ethanol extract (B) at 5 µg/ml concentration. Panel C shows the dose–response curves of the 25% ethanol extract and the 45% ethanol extract on hERG current. Estimated IC_{50} values are 8.30 ± 0.69 µg/ml and 5.09 ± 0.49 µg/ml, respectively ($n = 5$).

measured at 50% and 90% of repolarization (APD_{50} and APD_{90} , respectively) were off-line determined using a home-made software (APES) running on an IBM compatible computer equipped with an ADA 3300 analogue-to-digital data acquisition board (Real Time Devices Inc., State College, PA, USA) having a maximum sampling frequency of 40 kHz. The following types of stimulation were applied in the course of the experiments: stimulation with a constant cycle length of 1000 ms; and stimulation with different constant cycle lengths ranging from 300 to 5000 ms. Attempts were made to maintain the same impalement throughout each experiment. In case an impalement became dislodged, adjustment was attempted, and if the action potential characteristics of the re-established impalement deviated by less than 5% from the previous measurement, the experiment continued.

2.6. Statistics

Data are expressed as arithmetic mean \pm SEM values. Results were compared using Student's t-tests for paired data. Differences were considered significant when P value was less than 0.05.

3. Results

3.1. The chemical composition of *C. majus* extracts

The chemical composition of the extracts was characterized by RP-HPLC. The main peaks were identified with the aid of on-line (LC–PDA–MS) spectroscopic data and external standards of berberine, coptisine, chelerythrine, chelidone and sanguinarine (Fig. 1). The berberine, coptisine, chelerythrine, chelidone and sanguinarine contents of the dried 25% ethanol extracts were 0.11, 6.42, 0.06, 1.75 and 0.22 mg/g, respectively (Fig. 2 and Table 1). The alkaloid contents of the dried 45% ethanol extract were the following: 0.23, 34.77, 0.13, 3.19 and 0.41 mg/g of berberine, coptisine, chelerythrine, chelidone and sanguinarine, respectively (Fig. 3 and Table 1).

3.2. Effects of extracts and alkaloids of *C. majus* on hERG current

The hERG potassium channel-blocking effects of *C. majus* extracts were investigated on HEK293 cells stably expressing the hERG channel. For this purpose 25% and 45% ethanol (V/V) extracts were prepared with regard to the Assessment report EMA/HMPC/369801/2009 on *C. majus* herb [1], which refers

Table 1

The alkaloid contents of *Chelidonium majus* extracts.

	Chelidone	Coptisine	Berberine	Sanguinarine	Chelerythrine
<i>25% ethanol extract</i>					
mg/g	1.7469	6.4194	0.1110	0.2164	0.0612
SEM	4.1569E–03	2.0756E–01	6.9282E–04	1.2124E–03	5.7735E–05
<i>45% ethanol extract</i>					
mg/g	3.1935	34.7687	0.2306	0.4064	0.1253
SEM	1.9457E–02	5.7793E–02	6.6973E–03	6.9859E–03	3.4641E–04

Table 2
IC₅₀ values of *Chelidonium majus* extracts and alkaloids on hERG K⁺ channel.

Sample	IC ₅₀ ± SEM (µg/ml)	IC ₅₀ ± SEM (µM)
25% ethanol extract n = 5	8.30 ± 0.69	
45% ethanol extract n = 5	5.09 ± 0.49	
Berberine n = 5	2.17 ± 0.18	6.46 ± 0.54
Coptisine n = 4	28.86 ± 0.92	90.08 ± 2.88
Chelidonine n = 4	0.35 ± 0.04	1.00 ± 0.10
Sanguinarine n = 5	0.29 ± 0.03	0.88 ± 0.08

these extracts for internal use. Both extracts revealed significant hERG potassium ion channel inhibitory activity with estimated IC₅₀ values of 8.30 ± 0.69 µg/ml and 5.09 ± 0.49 µg/ml (Fig. 4 and Table 2).

The hERG blocking potencies of the major alkaloids of the plant (berberine, chelidonine, coptisine and sanguinarine) were also evaluated and found that all alkaloids exhibited a substantial ion channel inhibitory effect apart from coptisine. Chelidonine and sanguinarine were to have the highest inhibitory effect with the IC₅₀ values of 1.00 ± 0.10 µM and 0.88 ± 0.08 µM, respectively, whereas coptisine exhibited only a marginal effect (IC₅₀ = 90.08 ± 2.88 µM). The hERG channel modulatory activity of berberine could be characterized by the IC₅₀ value of 6.46 ± 0.54 µM (Fig. 5 and Table 2).

3.3. Effects of extracts and alkaloids of *C. majus* on ventricular action potential

The effects of *C. majus* extracts on action potential configuration were studied in canine right ventricular muscle preparations. The results are shown in Fig. 6. Both extracts moderately prolonged the action potential duration at 5 µg/ml concentration in a statistically significant manner at a stimulation cycle length of 1000 ms. The prolongation of APD₉₀ (action

potential duration at 90% of repolarization) was 10.5% in the case of the 25% ethanol extract while the 45% ethanol extract lengthened the action potential duration with 6.7% at a stimulation cycle length of 1000 ms. The maximum rate of depolarization (V_{max}) was not influenced significantly by the extracts. To study the rate-dependent effect of the extracts on APD₉₀, the preparations were stimulated at cycle lengths ranging from 300 to 5000 ms. Under these circumstances the extracts produced a slight reverse rate-dependent APD prolongation, especially in the case of 45% extract (Fig. 6 and Table 3).

The effects of berberine, chelidonine and sanguinarine on the action potential parameters at concentrations of 1 µM and 10 µM were also investigated in dog ventricular muscle at a stimulation cycle length of 1000 ms. All drugs showed mild but statistically significant increase of APD₉₀ (4.6%, 6.1% and 6.3% prolongation, respectively) at 1 µM concentration. At 10 µM concentration the action potential lengthening was more remarkable (18.4%, 18.3% and 16.0%). All these compounds prolonged the action potential duration in a reverse-rate dependent manner (Fig. 7 and Table 3).

4. Discussion

In our electrophysiological study we investigated *C. majus* herb hydroalcoholic extracts and its four benzylisoquinoline alkaloids, berberine and coptisine from the protoberberine subgroup, and chelidonine and sanguinarine from the benzophenanthridine subgroup using patch-clamp method and conventional microelectrode technique. 25% and 45% ethanol extracts were prepared from the herb, because these extracts have a long tradition of internal application against a wide scale of different diseases according to the Assessment report EMA/HMPC/369801/2009 on *C. majus* herb. It was found

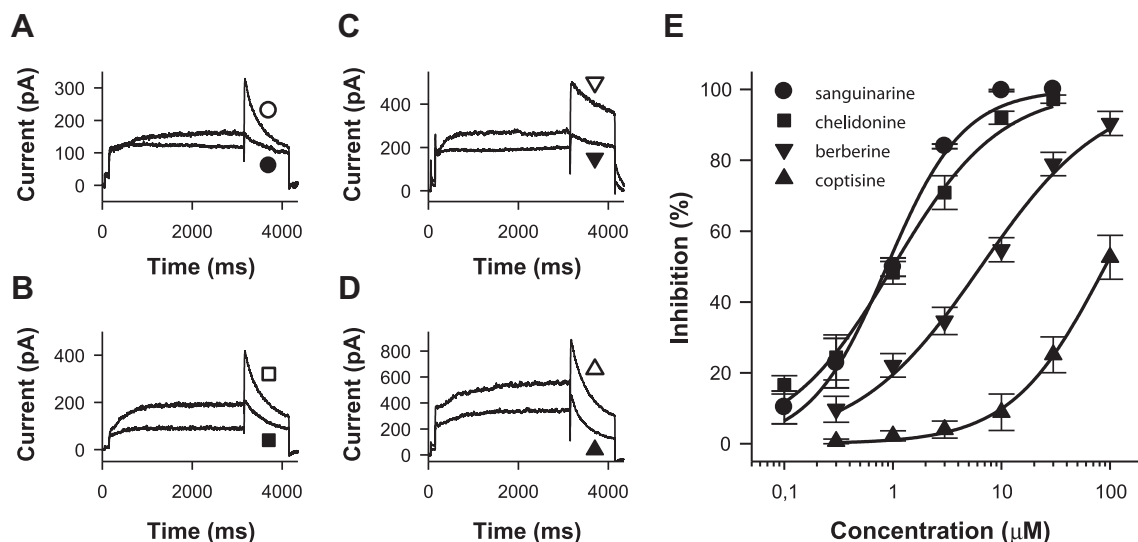


Fig. 5. Concentration-dependent inhibitory effects of *Chelidonium majus* derived alkaloids on hERG current. Panels A–D show representative hERG current curves obtained from cells treated with alkaloids. Empty symbols show the control curves, while full symbols show the effects of 1 µM sanguinarine (A), 1 µM chelidonine (B), 10 µM berberine (C) and 100 µM coptisine (D). Panel E shows the dose–response curves of the examined alkaloids. Sanguinarine and chelidonine were the most effective with IC₅₀ = 0.88 ± 0.08 µM and 1.00 ± 0.10 µM. Berberine IC₅₀ was 6.46 ± 0.54 µM, while coptisine exhibited only a marginal effect with IC₅₀ = 90.08 ± 2.88 µM (n = 4–5).

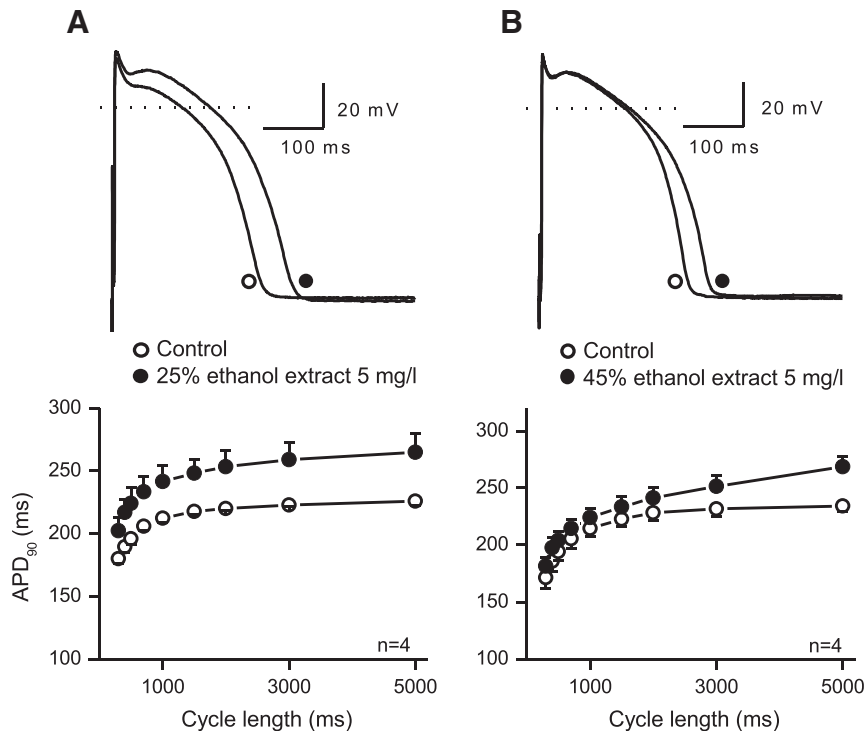


Fig. 6. Effects of *Chelidonium majus* extracts (25% ethanol extract and 45% ethanol extract on Panels A and B, respectively) on action potential waveform of dog ventricular muscle at stimulation cycle length of 1000 ms and rate-dependent effects on action potential duration (APD₉₀).

that both extracts possess hERG channel inhibition and prolong the duration of cardiac action potential for the usage of this herb. The European Medicines Agency published a public statement on *C. majus* stating that the benefit–risk assessment of oral use of the plant is considered negative. However, the reason for this negative opinion was the high number of spontaneously reported liver–biliary adverse drug reactions [15]. The cardiovascular risks of greater celandine were not considered as a reason for the restriction of therapeutic application of this plant.

Many of the alkaloids of greater celandine may be the reason for cardiovascular risk. Berberine was earlier described to inhibit hERG potassium current in HEK cells expressing hERG channels [8], to block I_{Kr} current in cardiac myocytes [6], and to increase action potential duration on cardiac preparations [6,7], which are well in line with our findings. Furthermore from this subgroup, we have also studied coptisine, the main alkaloid of *C. majus*. Coptisine showed only a marginal effect on hERG ion channel, therefore its effect on the action potential was not investigated. These data

Table 3

The electrophysiological effects of *Chelidonium majus* extracts in dog ventricular muscle preparations at basic cycle length of 1000 ms. RP, resting potential; APA, action potential amplitude; V_{max}, maximum rate of depolarization; APD₉₀ and APD₅₀, action potential durations at 50% and 90% of repolarization. Results are mean ± SEM, n = 5–7.

	RP (mV)	APA (mV)	V _{max} (V/s)	APD ₉₀ (ms)	APD ₅₀ (ms)
Control	−85.4 ± 0.5	106.5 ± 2.3	258.0 ± 39.2	222.4 ± 6.4	178.9 ± 9.2
25% ethanol extract 5 µg/ml	−85.0 ± 0.8	106.0 ± 2.7	244.1 ± 39.9	246.5 ± 13.5	200.8 ± 14.9
Control	−87.3 ± 0.5	106.3 ± 1.8	209.3 ± 25.6	226.0 ± 5.9	179.8 ± 7.2
45% ethanol extract 5 µg/ml	−85.8 ± 0.8	104.8 ± 1.2	245.3 ± 22.4	241.3 ± 8.8	186.2 ± 11.5
Control	−86.8 ± 1.2	105.4 ± 3.0	250.6 ± 46.5	219.4 ± 6.6	181.6 ± 7.3
Berberine 1 µM	−86.1 ± 0.9	107.6 ± 4.0	226.5 ± 44.6	233.1 ± 7.3	196.2 ± 6.6
Control	−86.8 ± 1.0	107.3 ± 3.2	247.2 ± 39.4	223.1 ± 6.7	183.0 ± 6.4
Berberine 10 µM	−86.1 ± 0.8	107.0 ± 2.5	247.9 ± 20.9	258.4 ± 8.5	215.5 ± 8.3
Control	−88.8 ± 3.8	106.8 ± 2.8	174.4 ± 18.0	230.4 ± 17.6	180.6 ± 13.6
Chelidonium 1 µM	−85.4 ± 1.4	108.7 ± 3.3	151.5 ± 9.0	240.1 ± 16.2	189.0 ± 11.4
Control	−89.0 ± 3.7	106.9 ± 2.7	172.9 ± 18.7	219.6 ± 15.9	174.6 ± 14.0
Chelidonium 10 µM	−85.9 ± 1.6	112.9 ± 3.2	197.8 ± 36.7	259.7 ± 17.7	204.8 ± 14.1
Control	−89.0 ± 2.1	104.0 ± 2.6	230.1 ± 43.5	200.4 ± 9.3	156.4 ± 8.5
Sanguinarine 1 µM	−87.6 ± 2.5	105.5 ± 4.3	242.8 ± 59.5	212.9 ± 11.9	170.9 ± 11.4
Control	−86.8 ± 0.9	102.1 ± 3.0	169.2 ± 23.7	208.0 ± 12.2	172.2 ± 13.0
Sanguinarine 10 µM	−85.8 ± 1.7	104.3 ± 1.7	142.3 ± 24.3	244.2 ± 6.2	202.5 ± 5.7

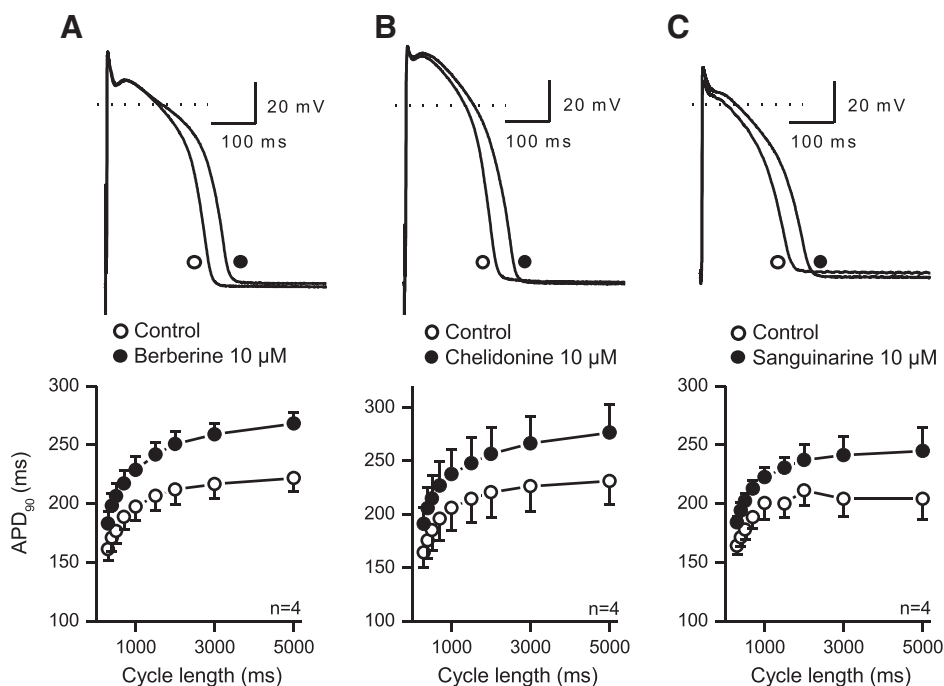


Fig. 7. Effects of *Chelidonium majus* alkaloids on action potential waveform of dog ventricular muscle at stimulation cycle length of 1000 ms and rate-dependent effects on action potential duration (APD₉₀). Results of treatment with berberine, chelidonine and sanguinarine are shown on Panels A, B and C, respectively.

suggest that protoberberines possess a mild effect on hERG channel. However, berberine prolonged the action potential to almost the same extent as benzophenanthridines. A potential explanation to this phenomenon is that berberine also blocks the slow delayed rectifier potassium channel (I_{Ks}) [8] and the inwardly rectifying potassium current (I_{K1}) [7] during cardiac action potential repolarization.

The two examined benzophenanthridines, chelidonine and sanguinarine showed an even stronger inhibitory effect on hERG channel, having IC_{50} values ≤ 1.0 μM, and prolonged the action potential in a dose-dependent manner. From these results we can conclude that benzophenanthridines are highly potent hERG inhibitors (with micromolar or submicromolar IC_{50} values).

Interestingly, the third subgroup of benzyloquinolines, represented by protopine [13], abbreviates the action potential duration, suggesting a very complex cardiac action of this herb with multiple ion channel effects, which are not completely clear yet.

To assess the significance of the safety concerns related to the benzyloquinoline alkaloids of greater celandine, the alkaloid content of the analyzed extracts was determined. Remarkable difference was found in the alkaloid content of the extracts because of the different polarities of the solvents (Table 1). Coptisine was found to be the major alkaloid of both the 25% and the 45% ethanol extracts. The chelidonine content is also considerable, while sanguinarine and berberine are presented in minor proportions. Chelerythrine was present only in comparatively lower quantity in either of the two extracts, thus we have not further studied this alkaloid.

In the present work, to the best of our knowledge, we report the first experimental evidence of hERG potassium channel blocking and action potential prolonging effects

of *C. majus* herb 25% and 45% ethanol extracts. Moreover, the effect of chelidonine and sanguinarine on the action potential was investigated for the first time. The examined substances not only inhibit the hERG current, but also extend the duration of the action potential. These findings are likely to contribute significantly to the clarification of the complex cardiotoxic effects of *C. majus*.

To estimate the actual risk of the internal usage of *C. majus* hydroalcoholic extracts, should know the plasma level of the alkaloids after oral administration of the herb. Appropriate cardiac risk estimation of hydroalcoholic extracts of the high aqueous solubility, poor permeability, and absorption of berberine result in its low plasma level. It was reported that the maximum concentration (C_{max}) of berberine in the plasma was 11 ng/ml after a single intragastric administration of 50 mg/kg [16], and 26 ng/ml after a single dose of 200 mg/kg [17] to rats. In humans, the value of C_{max} was only 0.4 ng/ml after a single oral dose of 400 mg berberine [18], indicating limited gastrointestinal berberine absorption.

Presently we have rather limited information on the pharmacokinetic properties of chelidonine and sanguinarine. The maximum level of sanguinarine in rat plasma after a single intragastric administration of 10 mg/kg was 192 ng/ml [19]. The C_{max} of chelidonine was 5 ng/ml and 41 ng/ml for sanguinarine in rats after the intragastric administration of *C. majus* extract; in this case the doses of the pure alkaloids were 4 mg/kg and 5.5 mg/kg, respectively [20].

These maximum plasma concentrations seem to be low and in the case of berberine and chelidonine are several orders of magnitude smaller than their active concentrations in this study. Conversely in the case of sanguinarine the literary C_{max} values are much closer to its IC_{50} on hERG and its effective concentration on cardiac action potential.

Therefore sanguinarine itself due to its relatively high bioavailability may represent some so far not well recognized risk. Furthermore and even more importantly use of all these alkaloids may have additive effects further increasing the possibility of proarrhythmic risk and cardiac sudden death during administration of drugs effecting the cardiac repolarization or in certain pathophysiological conditions where repolarization reserve is impaired (including cardiac hypertrophy, hypertrophic cardiomyopathy, increased sympathetic tone, genetic defects, diabetes mellitus, renal failure, hypokalemia, hypothyroidism, etc.) [21,22]. In conclusion, the internal use of *C. majus* extracts or its alkaloids, berberine, chelidonine and especially sanguinarine in some situations where cardiac repolarization reserve is weak may increase the risk of potentially fatal ventricular arrhythmias. Our results highlight the safety concerns regarding the internal use of *C. majus*: orally applied greater celandine products need cautious evaluation from the view of both hepatic and cardiovascular side effects. With regard to similar hERG blocking activity of related natural alkaloids [11], further studies are warranted to evaluate potential hERG-related safety aspects of medicinal plant extracts and to assess the clinical relevance of these findings.

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