

Inhibitory effects of four active components in saffron on human ether-a-go-go-related gene (hERG) K⁺ currents

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Abstract. The main active components of saffron are crocin, crocetin, picrocrocin, and safranal. There are many studies on their cardioprotective effects, but their cardiotoxicities have not been reported. The human ether-a-go-go-related gene (hERG) K⁺ channels are of considerable pharmaceutical interest as the target responsible for acquired long QT syndromes. The aim of this study is to explore the effects of crocin, crocetin, picrocrocin, and safranal on the K⁺ channels encoded by hERG. The interaction of these components with the rapid delayed rectification of K⁺ currents (I_{Kr}) were studied using the perforated patch recording technique. Crocin and picrocrocin had no significant effects on I_{Kr}, but crocetin and safranal inhibited hERG K⁺ currents in a concentration-dependent manner, with IC₅₀ values of 36.35 μM and 37.86 μM, respectively. The maximum inhibitory effects were 37.74 ± 4.14% and 33.74 ± 4.81%, respectively, and the effects were reversible upon washout. The results demonstrate that crocetin and safranal significantly inhibit hERG K⁺ current, but crocin and picrocrocin do not. This suggests that crocetin and safranal may increase the risk of cardiac arrhythmias by inhibiting I_{Kr}.

Key words: hERG K⁺ currents — Saffron — Active components — Cardiac arrhythmia — Cardiotoxicity

Introduction

Crocus sativus L. (saffron) is a perennial herb and has been used for medicinal purposes for many centuries (Bathaie and Mousavi 2010; Bukhari et al. 2018). It is of great value for changing the color and flavor of a variety of foods and

drinks and has been widely used in medicine to treat several illnesses, including cardiovascular diseases (Razavi and Hosseinzadeh 2017; Hatziagiapiou and Lambrou 2018).

The medicinal part of saffron is its dry stigma, and its main active components are crocin, crocetin, picrocrocin and safranal (Fig. 1) (Li et al. 1999; Broadhead et al. 2016; Bagur et al. 2017; Mykhailenko et al. 2019). Many studies have shown various cardioprotective effects of the active components of saffron, including attenuating myocardial ischemia-reperfusion injury, inhibiting cardiac hypertrophy and improving myocardial ischemia (Bharti et al. 2012; Huang et al. 2016; Feidantsis et al. 2018). However, there have been few reports about their side effects on the cardiovascular system. Our previous studies have demonstrated that crocin blocks L-type calcium channels (LTCCs) (Liu et al. 2015). Considering that most calcium channel blockers can cause Q-interstitial syndrome (Huang et al. 2015; Pilote et al. 2017), it is necessary to study the influence of saffron's active

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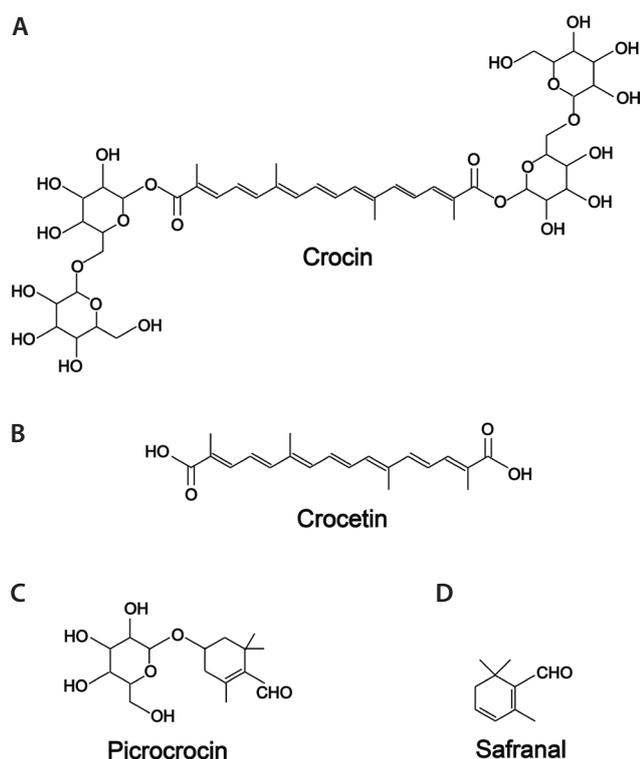


Figure 1. Structures of crocin (A), crocetin (B), picrocrocin (C), and safranal (D).

components on hERG K^+ currents to avoid potentially toxic cardiac-related side effects.

A variety of drugs, including antihistamines and anti-arrhythmics, have been shown to induce prolongation of the QT interval and lead to severe and potentially fatal ventricular tachyarrhythmias (Darpo et al. 2013; Cubeddu 2016). The side effects of drug-induced QT prolongation have become an important issue that threatens the safety of the currently approved drugs, and many of them have been withdrawn from the market. The cardiac K^+ channels' human ether-a-go-go-related gene (hERG) encodes the rapid delayed rectification of K^+ currents (I_{Kr}) in the heart. In the ventricular myocytes of the heart, I_{Kr} is crucial for repolarization of myocardial action potentials (Barhanin et al. 1996; Wu and Sanguinetti 2016). I_{Kr} inhibition can lead to the extension of the QT interval and cause arrhythmia (Witchel and Hancox 2000; Thomas et al. 2006).

Therefore, in this study, we used the perforated patch recording techniques to observe the effects of crocin, crocetin, picrocrocin and safranal on I_{Kr} . This technique is a classic and widely used experimental method for the measurement of hERG K^+ currents (Horn and Marty 1988; Lippiat 2008; Linley 2013). Our results show detailed insights into the biophysical mechanisms of hERG K^+ currents being blocked by crocetin and safranal.

Materials and Methods

Cell culture and transfection

To induce hERG K^+ currents in human embryonal kidney 293 (HEK 293) cells, Lipofectamine (Invitrogen) was used to stably transfect these cells with hERG cDNA. The transfected cells were cultured in Dulbecco's Eagle's medium (DMEM, Invitrogen) in an incubator at 37°C with saturated humidity and 5% CO_2 . The cultures were passaged every 2–3 days with trypsin-EDTA according to the cell density, followed by seeding onto a 12-mm glass microscope coverslip. Electrophysiological experiments were performed after 12–24 hours.

Reagents

Crocin (17304-1G) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Crocetin (PRF8042401) was obtained from Chengdu Purifa Technology Development Co., Ltd. (Sichuan, China). Picrocrocin (20180327) was purchased from Hubei Wande Chemical Co., Ltd. (Hubei, China). Safranal (Lot# 1-KOP-14-1) was purchased from Toronto Research Chemicals Inc. (Ontario, Canada). Purity levels were all more than 98%. The transfer pipette solution contained 150 mM KCl, 10 mM HEPES, 5 mM $MgCl_2$ (pH 7.2), and 250 $\mu g/ml$ amphotericin B (Song et al. 2017). Crocin, crocetin, picrocrocin and safranal were separately dissolved in dimethyl sulfoxide (DMSO) to obtain stock solutions of 100 mM and further diluted into working solutions. The external solution contained 160 mM NaCl, 2.5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 8.8 mM glucose, and 10 mM HEPES (adjusted to pH 7.4 with KOH). The final concentration of DMSO was 0.1%. This amount of DMSO was also added to the normal external solution as a control and had no effects on the potassium current.

Electrophysiological recordings

Experiments were performed at room temperature (20–25°C). hERG K^+ currents were recorded by the perforated patch-clamp technique (Song, et al. 2017) with an Axopatch 200B patch-clamp amplifier. Micropipettes were created from glass tubing (PG10165-4; World Precision Instruments) using a puller (PC-97; Narishige). Micropipettes producing resistances of 2–5 $M\Omega$ in the external solution were used. Voltage pulse generation and data acquisition were controlled with the software pClamp 10.0 (Axon Instruments, Union City, CA, USA).

Statistical analysis

The results are presented as the means \pm standard error of the mean (SEM). Intergroup differences were assessed for

significance using an analysis of variance (ANOVA) or an independent-sample t-test. A value $p < 0.05$ was deemed statistically significant.

Results

Different actions of crocetin, safranal, crocin and picrocrocin on hERG K⁺ currents

We studied the effects of saffron components on hERG K⁺ currents in detail, as shown in Figure 2. Extracellular application of crocetin (300 μM) and safranal (300 μM) potently inhibited the hERG K⁺ currents by 37.74 ± 4.14% and 33.74 ± 4.81%, respectively ($p < 0.01$, $n = 8$). However, crocin and picrocrocin had no significant inhibitory effect on I_{Kr}.

Figure 3 shows the effects of crocetin (100 μM) and safranal (100 μM) on hERG K⁺ currents during the drug wash-in and wash-out. Crocetin and safranal effectively reduced the hERG K⁺ currents by 31.48 ± 0.46% and 28.48 ± 0.23%, respectively, and this inhibition was reversible ($p < 0.01$, $n = 8$).

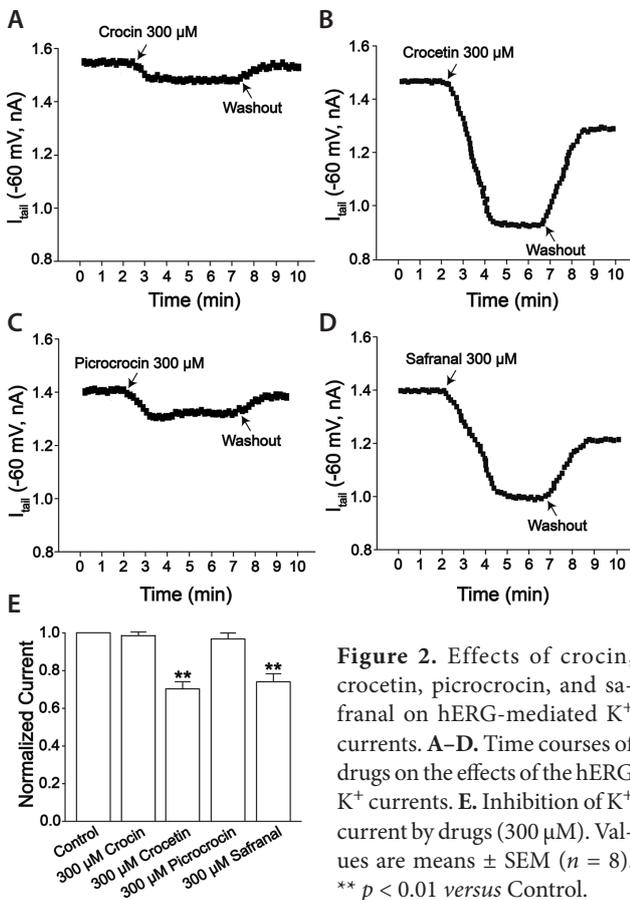


Figure 2. Effects of crocin, crocetin, picrocrocin, and safranal on hERG-mediated K⁺ currents. **A–D.** Time courses of drugs on the effects of the hERG K⁺ currents. **E.** Inhibition of K⁺ current by drugs (300 μM). Values are means ± SEM ($n = 8$). ** $p < 0.01$ versus Control.

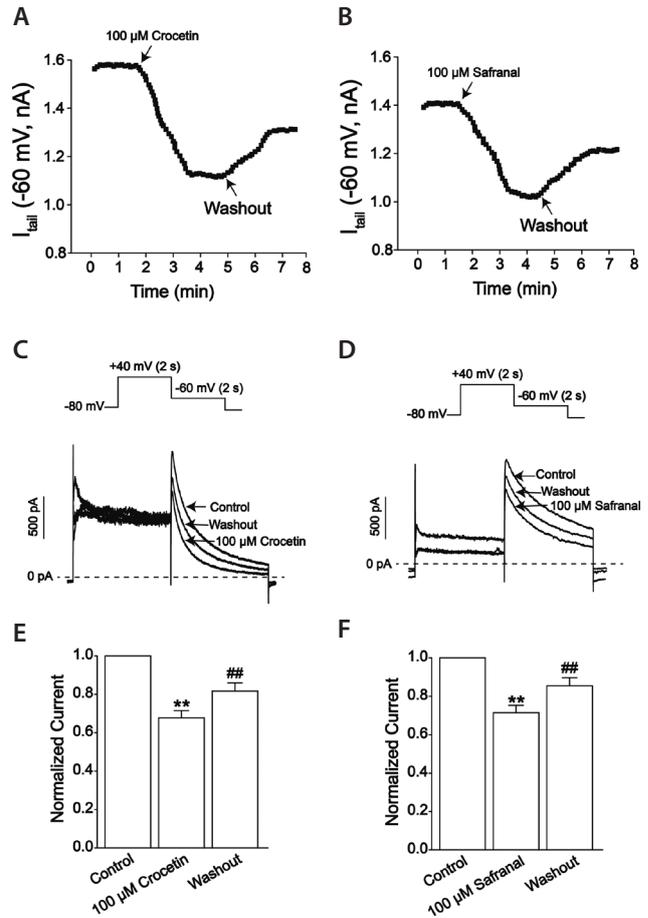


Figure 3. Effects of crocetin and safranal on hERG K⁺ currents. Time courses (**A, B**), exemplary traces (**C, D**) and pooled data (**E, F**) of hERG K⁺ currents recorded during the application of drugs and washout. Values are means ± SEM ($n = 8$). ** $p < 0.01$ versus Control; ## $p < 0.01$ versus drugs.

Effects of crocetin and safranal on activation curve of hERG K⁺ currents

Figure 4A and B shows an instance of a voltage-clamp recording of hERG K⁺ currents with representative current traces in the absence or presence of drugs (10 and 100 μM) or 100 nM terfenadine. Figure 4C–F shows the steady-state activation and current-voltage relationship curves of K⁺ currents ± drugs (10, 100 μM). The application of 100 nM terfenadine, a specific blocker of I_{Kr} (Tanaka et al. 2014; Chu et al. 2015), was used to compare the inhibitory efficiency of the drugs (10, 100 μM). Tail currents were normalized to the maximum currents in the absence (control) and presence of drugs (10 and 100 μM), as shown in Figure 5A and B. Crocetin at a concentration of 10 and 100 μM caused the mean half-maximum activation voltage (V_{1/2}) of the activation curve of the hERG tail currents to be shifted

by 7.3 ± 2.7 mV ($p < 0.05$) and 10.7 ± 2.3 mV ($p < 0.01$) toward negative potentials, respectively. Safranal at a concentration of 10 or 100 μM caused respective shifts of by 6.1 ± 1.8 mV ($p < 0.05$) or 16.7 ± 1.7 mV ($p < 0.01$) toward negative potentials.

The concentration-response relationship of hERG-mediated K^+ currents by crocetin and safranal

As shown in Figure 6A and B, the time courses of the hERG K^+ currents were recorded. The hERG K^+ currents were suppressed by crocetin and safranal in a concentration-dependent manner. Compared with the drugs (3, 30, 300 μM), terfenadine (100 nM) almost completely inhibited hERG K^+ currents (Fig. 6C and D). Figure 6E shows the concentration dose-response curves. The IC_{50} values of crocetin and safranal

were 36.35 μM and 37.86 μM for the hERG K^+ currents, respectively. The inhibition rates of 3, 10, 30, 100, and 300 μM crocetin on hERG K^+ currents were 7.21 ± 0.20 , 12.58 ± 0.62 , 21.50 ± 0.66 , 31.48 ± 0.38 , and $37.74 \pm 4.14\%$, respectively. The inhibition rates of 3, 10, 30, 100, and 300 μM safranal on hERG K^+ currents were 8.21 ± 0.18 , 13.67 ± 0.66 , 20.50 ± 0.65 , 28.48 ± 0.24 , and $33.74 \pm 4.81\%$, respectively.

Discussion

We investigated the effects of crocin, crocetin, picrocrocin, and safranal on hERG K^+ currents in HEK293 cells. The results demonstrate that crocetin and safranal effectively inhibit the expression of hERG K^+ channels in HEK293 cells in a concentration and state-dependent manner. The

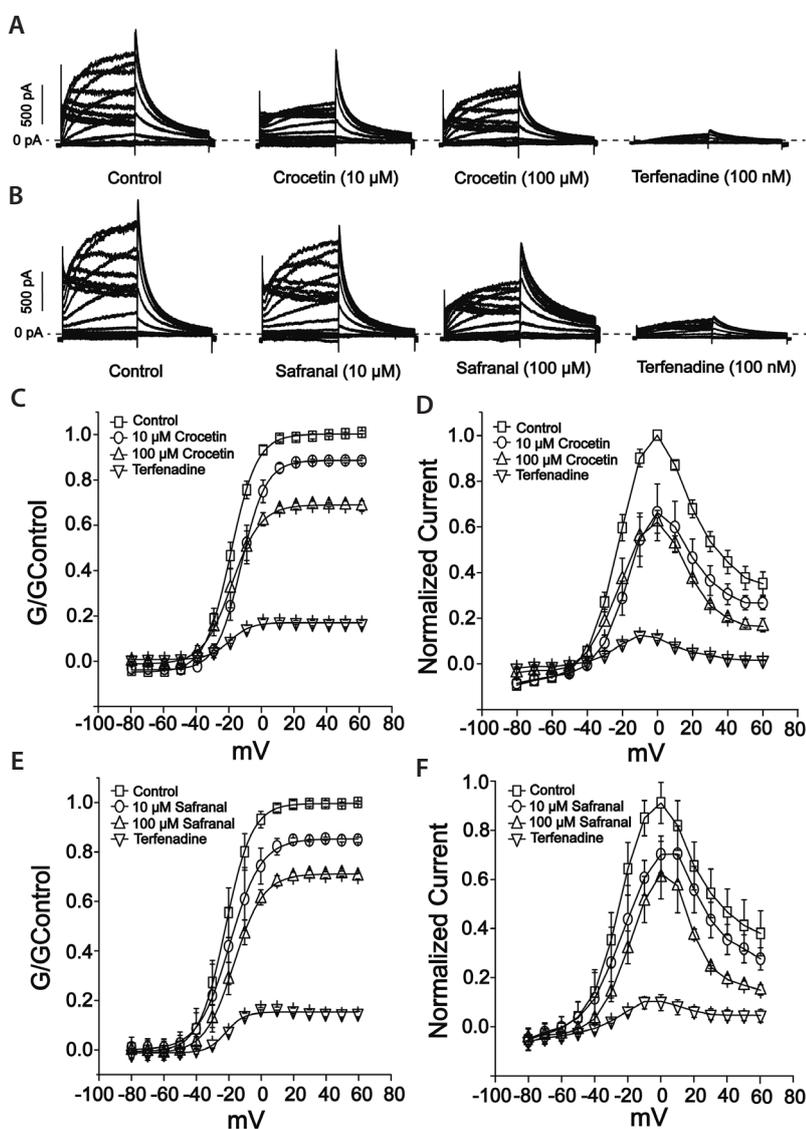


Figure 4. Effects of crocetin and safranal on the steady-state activation and current-voltage relationship of hERG K^+ currents. **A, B.** Exemplary hERG K^+ currents. **C–F.** Steady-state activation curves and current-voltage curves of hERG K^+ currents. Values are means \pm SEM ($n = 8$).

beginning of the block was fast, and the effects were partly reversed after washing-out the drug. However, crocin and picrocrocin had no significant effects on hERG K⁺ currents.

Long QT syndrome (LQTS) is associated with the torsade de pointes (TdP). This syndrome can slow down myocardial repolarization and can cause action potential extension (Towbin and Vatta 2001; Li and Ramos 2017). Inhibition of I_{Kr} is the main cause of drug-induced LQTS, which may induce arrhythmia and relative bradycardia in many circumstances (Fenichel et al. 2004; Ten-Tusscher and Panfilov 2006). Crocetin can provide protection against myocardial ischemia reperfusion injury (MIRI) in rats by inhibiting ROS production, blocking inflammation, and reducing myocardium apoptosis (Wang et al. 2014). Safranal can alleviate MIRI injury due to its strong antioxidant and anti-apoptotic potential (Bharti et al. 2012). However, to the best of our knowledge, there has been no report about the influences of these drugs on hERG K⁺ currents.

Acquired LQTS occurs frequently as a side effect of the blockade of cardiac hERG K⁺ channels by commonly used medications. A large number of structurally diverse compounds have been shown to inhibit K⁺ current through hERG, such as anti-arrhythmics, antibiotics, antihistamines, antipsychotics, gastrointestinal prokinetics, antifungals, and antimalarial drugs. These drugs have been reported to cause LQTS risk and are involved in inhibiting the hERG K⁺ channels (Rajamani et al. 2006; Chen et al. 2010). The assessment of a direct hERG channel block has proven useful for the evaluation of drugs suspected of causing delays in cardiac repolarization and TdP (Yap 2003). In the present experiments, terfenadine was used as the positive control drug and showed a stronger inhibitory effect on hERG K⁺ currents than the tested drugs crocetin and safranal. The inhibitory effect of 100 nM terfenadine was $82.98 \pm 0.32\%$, which is consistent with a previous study (Song, et al. 2017). However, crocetin and safranal inhibited hERG K⁺ currents in a concentration-dependent manner. The IC₅₀ values were $36.35 \mu\text{M}$ and $37.86 \mu\text{M}$, and the maximum inhibitory effects were $37.74 \pm 4.14\%$ and $33.74 \pm 4.81\%$, respectively. Compared with terfenadine, crocetin and safranal are weak hERG K⁺ channel inhibitors.

The risk of QT prolongation of ventricular repolarization or TdP is increased in patients with organic heart diseases, such as congenital LQTS, myocardial infarction, congestive heart failure, dilated cardiomyopathy, hypertrophic cardiomyopathy, bradycardia, hypokalemia, and hepatic impairment (Yap 2003). Therefore, clinicians must practice constant vigilance when using crocetin or safranal in patients with pre-existing heart disease, any of the risk factors listed above, previous ventricular arrhythmias, or electrolyte imbalances, such as hypokalemia.

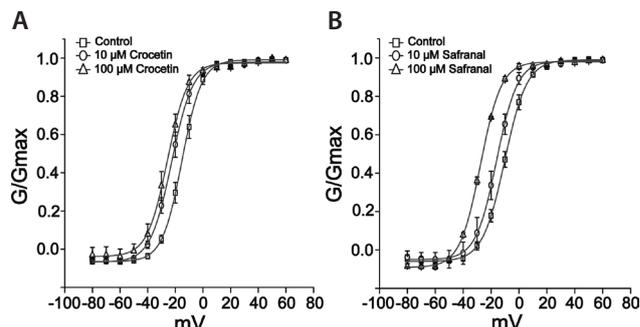


Figure 5. Effects of crocetin (A) and safranal (B) on the activation curves of hERG normalized tail currents. Values are means \pm SEM ($n = 8$).

Crocetin or safranal at $3 \mu\text{M}$ can suppress hERG K⁺ currents. The peaks of the hERG K⁺ currents were reduced after exposure to $100 \mu\text{M}$. After washing out the drugs, the hERG K⁺ currents partially recovered, indicating that the inhibitory effect was reversible (Fig. 3A–D). Crocetin or

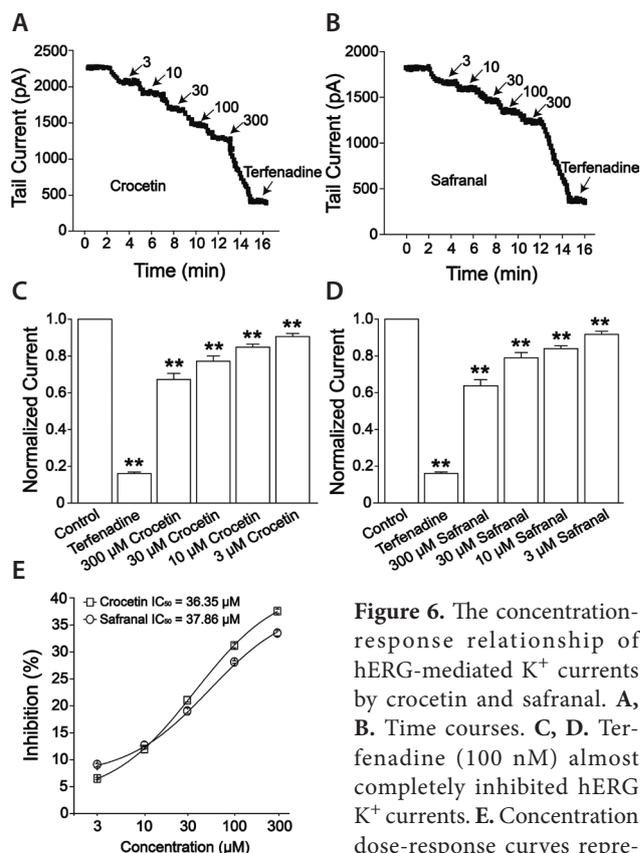


Figure 6. The concentration-response relationship of hERG-mediated K⁺ currents by crocetin and safranal. A, B. Time courses. C, D. Terfenadine (100 nM) almost completely inhibited hERG K⁺ currents. E. Concentration dose-response curves representing the inhibitory effects of crocetin and safranal in regard to hERG K⁺ currents. IC₅₀ values were $36.35 \mu\text{M}$ and $37.86 \mu\text{M}$. Values are means \pm SEM ($n = 8$). ** $p < 0.01$ versus Control.

safranal blocked hERG currents with a negative shift in voltage-dependent activation (Figs. 4 and 5). Exemplary traces of the hERG K⁺ currents were recorded at various concentrations of drugs (3–300 μM). Crocetin or safranal significantly reduced the current density of hERG in a concentration-dependent manner. Their IC₅₀ values were 36.35 μM and 37.86 μM, respectively, while their maximum inhibitory effects on hERG K⁺ currents were 37.74 ± 4.14% and 33.74 ± 4.81% (Fig. 6A–E). The doses of crocetin and safranal in experiments in vivo are 20–200 mg/kg (Liang et al. 2007; Zhang et al. 2017) and 0.25–725.7 mg/kg (Hosseinzadeh and Sadeghnia 2005; Samarghandian et al. 2017), respectively. The two drugs are commonly administrated by intraperitoneal injection. According to Diehl (Diehl et al. 2001), the blood concentrations of crocetin or safranal were higher than those in this study, which suggests that attention should be paid to the risk of arrhythmia caused by crocetin or safranal in the treatment of cardiovascular diseases.

Acquired LQTS has become an important liability for clinically available drugs and developmental compounds. The mechanism commonly proposed for drug-induced QT interval prolongation is the direct block of hERG (Kv11.1) K⁺ channels or its native current, which rapidly results in delayed rectifier potassium current (I_{Kr}) (Sanguinetti and Mitcheson 2005). Ketoconazole was reported to block hERG channels predominantly by binding to the closed state of hERG channels and causing tonic block, with lower affinity binding to the open state (Dumaine et al. 1998). In addition, it has been reported that the blockage of hERG channels by miconazole required channel activation in the closed state, and the blockage was not easily reversed by drug washout (Kikuchi et al. 2005).

In conclusion, the present results have shown that crocetin and safranal block the hERG K⁺ currents in a concentration-dependent manner. However, crocin and picrocrocin do not have this effect. Thus, the results suggest that crocetin and safranal may increase the risk of LQTS by inhibiting I_{Kr}.

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Conflict of interests. The authors declare that they have no competing interests.

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