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The Effect of Aqueous Extract of *Crocus sativus* on the Electrophysiological Properties of Isolated Perfused Rabbit AV-Node

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Abstract: In the present study we used isolated perfused AV-node of rabbit as an experimental model to determine the effect of various concentrations of extract of *C. sativus* (A = 9×10^{-2}, B = 19×10^{-2}, C = 28×10^{-2}, D = 37×10^{-2} mg L^{-1}) on electrophysiological properties of isolated heart. Selective stimulation protocols were used to independently quantify AV nodal recovery, facilitation and fatigue in 12 rabbits. Our results showed concentration dependent depressant effects of aqueous extract of *C. sativus* on Wenchebach Cycle Length (WBCL), AV Conduction Time (AVCT), Functional Refractory Periods (FRP). *C. sativus* (28×10^{-2} mg L^{-1}) prolonged AVCT from 44.4±5 (control) to 52.2±4 m sec, Rate-dependent properties such as Facilitation and fatigue significantly increased by 19×10^{-2} mg L^{-1} of *C. sativus*. In control group magnitude of fatigue was 6.7±0.6 and increased by Saffron to 20±11.5 ($p< 0.05$). The effect of extract was prominent on fast pathway. The above results indicated differential effects of *C. sativus* on slow and fast pathways which has a dominant role on the fast pathway. This research for the first time has explained the role of saffron on the protective mechanism of artrioventricular node against supraventricular arrhythmia. These results showed the non-specific effect of saffron on the transitional cells of fast nodal pathway which was manifested as a rate-independent increase of basic and functional (facilitation and fatigue) parameters of artrioventricular node.

Key words: *C. sativus*, atrioventricular node, superfused AV node

INTRODUCTION

The Atrioventicular node (AV-Node) is the most significant determinant of the normal variability of the PR interval, essential for optimal ventricular pumping and serves as safeguard to slow or block premature atrial impulses that could trigger ventricular fibrillation (Billette and Shrier, 1995). The wide varieties of delay that the AV-Node can generate in response to an increased rate are explained by dynamic interactions between the three intrinsic properties of recovery, facilitation and fatigue (Billette and Nattle, 1994).

Plant originated biological substance considered as an important part of drug treatment of the diseases. Herbal medicines often are cheaper with less side effects than synthetic drugs . Herbal treatments is available for patients with arrhythmia (Nick *et al*., 1998). Digoxcin from *Digitalis purpura* has been used for more than 100 years and the value of quinidine for terminating AF first became apparent at the beginning of the twentieth century by lewis and Vico (Guerra *et al*., 1998). *C. sativus* (saffron) is a plant with pale violet flowers. Long stripped leaves, with plenty green color. The stigma lobes contain the bulb, which is the active constituent of the plant (Rios *et al*., 1996). This plant is cultivated in India and in some parts of Iran (Fatehi *et al*., 2003). Crocin, safranol and flavanol are among the chemical constituents of saffron (Rios *et al*., 1996). There are some reports about the antihypertensive and anti-ischemic properties of *C. sativus* (Fatehi *et al*., 2003; Abdullaev, 1993). Alcoholic and aqueous extract of saffron was able to reduce blood pressure in rat also aqueous extract of saffron could inhibit vasodephran muscle contraction, induced by 0.1 μmol epinephrine (Fatehi *et al*., 2003). Up till now there has not any report of saffron effects on the electrophysiological properties of isolated perfused rabbit artrioventricular node. The aims of this study are to assess the rate–dependent effect of *C. sativus* on nodal conduction time and refractoriness and particularly its effect on the nodal slow and fast pathways.

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MATERIALS AND METHODS

Saffron was purchased from famous cultivated commercial brand (Novin zaferan company) in Mashad. A voucher specimen (159-0319-02) was designated for the saffron used in this investigation by the Mashad University of Medical Sciences in Khorasan province in east of Iran.

The stigmas were collected in autumn and air-dried at 40°C. The saffron powder was dissolved in 60 cc water. The solution was kept in dark for four days. The prepared solution was filtered and was concentrated at 30-40°C temperature using a vacumed rotatory. The extraction rate was 5% and the final concentration of aqueous extract was 28%. The HPLC analysis of saffron extract using UV detector with wavelength of 308 nm showed the presence of safranal with concentration of 5.09%. The pure Safranal (FULKA. CO) was used as a standard to determine the safranal concentration in Saffron extract.

Animals: All experiments were performed *in vitro* on isolated perfused rabbit cardiac preparations obtained from hearts on Newsland, white male rabbits of weighing 1-1.3 kg. Anesthesia was induced with pentobarbital (35 mg kg^{-1}) injection in ear vein and heparin (200 IU kg^{-1}) was used as anticoagulant. Lateral thoracotomy was performed and the heart was excised. Ethical approval and animals care were in use accordance to the principles in the regulations at the University of Golestan.

Isolated AV-nodal preparation: Experiments were performed in isolated, perfused rabbit AV-nodal preparations. The preparation, perfusion system, stimulation technique and recording system were similar to those previously described in detail (Nayebpour *et al*., 2001).

The final preparation which included the right atrium, AV node area and upper part of the interventricular septum was mounted in a tissue bath superfused at 200 mL min^{-1} with a 6-L volume of oxygenated (95% O_2 -5% CO_2) Tyrods solution, maintained at 37°C (pH = 7.38).

The aorta was retrogradely perfused by a peristaltic pump, at a constant pressure equivalent to: 60-80mm. Hg. the composition of the Tyrods solution was (mmol L^{-1}): NaCl, 128.2; KCl, 4.7; $CaCl_2$, 2; $MgCl_2$, 1; 1; $NaHCO_3$, 25; NaH_2PO_4, 0.7 and dextrose, 11.1. A bipolar iridium-platinum stimulating electrode was positioned on the upper atrium near the sinus node and unipolar electrograms were recorded from the near of sinus node and His bundle. Stimulation protocols were executed by custom-made software running on a Pentium computer interfaced with a D/A converter and a stimulus isolator. Electrogram signals were filtered (30 HZ to 3 KHz) and amplified by the amplifier, afterward A/D conversion data was saved on the hard disk and was analyzed off-line.

Stimulation protocols used to quantify recovery, facilitation and fatigue: Specific stimulation protocols were used, to quantify the properties of AV nodal recovery, facilitation and fatigue, as previously described (Nayebpour *et al*., 2001).

To construct the basic recovery curve a single premature or delayed stimulus (S2) was introduced after every 10 basic stimuli (S1). The relation between the conduction time of the test beat (A2 H2) and the preceding recovery time (H1 A2) was established and fitted to an exponential function as previously described (Nayebpour *et al*., 2001). To study facilitation, the recovery curve was constructed following a facilitation inducing short cycle introduced after the last basic stimulus. To analyze AV nodal fatigue, two series of tachycardia with a constant AA interval were initiated and change in AH interval over 5 min at a given AA interval were observed. A recovery period of at least 5 min was allowed after each tachycardia for the dissipation of fatigue before the next tachycardia was initiated. The functional and effective refractory periods and Wenckebach of the AV node (AVFRP and AVERP, WBCL, respectively) were measured with extra stimulus technique, as previously described (Nayebpour *et al*., 2001).

Experimental procedure: All the stimulation protocols were carried out as follow: Control conditions (no intervention) and the presence of either of two groups of experiments (A-B) were performed as follow:

Experiments A: In the first series of experiments the effects of different concentration of saffren (9×10^{-2}, 19×10^{-2} and 28×10^{-2} mg L^{-1}) were studied separately and accumulatively on electrophysioligical properties of AV node. These set of experiments were used to determine optimum concentration of saffron. to produce desirable extract.

Experiments B: In a separate series of experiments we compared the relative potency of Saffron with a calcium channel blocker verapamil. The stimulation protocols were carried out during: Control conditions (no intervention). Addition of Verapamil (0.1 μM) after washing out of preparation from saffron extract.

The protocols were performed after perfusion period of 20 min (Verapamil). Our pilot studies showed that 45 min of perfusion with extract is enough to obtain steady state electrophysiological effects. Pilot studies also showed that the concentrations (A = 9×10^{-2} mg L^{-1}, B = 19×10^{-2} mg L^{-1} and C = 28×10^{-2} mg L^{-1}) of saffron extract is the best concentration to evaluate effects of the plant. Six rabbits in each set of experiment were used.

Statistical analysis: Results were reported as the mean±SEM and comparisons among multiple groups which were made by two-way analysis of variance (ANOVA) with Scheffe contrasts. Comparisons between two groups of experimental study were made with the Wilcoxon test. A probability of 5% was taken to indicate statistical significance.

RESULTS AND DISCUSSION

Aqueous extract of saffron in concentration dependent manner (A, B and C) caused the basic and rate-dependent depression of nodal parameters (Table 1, Fig. 1). The effect of saffron is appeared after 30 min and it reaches it's peak gradually in about 90 min, (Fig. 2). A, also the inhibitory rate-dependent behavior of saffron indicate its similar effect on the slow and fast heart beats. This effect is appeared as upward shift of conduction curve. The minimum conduction time (AH min) was significantly increased but the AH max had a non-significant increase in conduction time. The

Table 1: The effects of different concentrations of Saffron extract on various parameters of AV-Node. (n = 6, mean±SEM, *p<0.05, **p<0.01 compared to the control)

		WBCL (m sec)	FRP (m sec)	ERP (m sec)	AH (m sec)
9×10^{-2}	Control	147±8	156.8±7	118.2±14	43.3±4
(mg L^{-1})	Case	155.2±7	154±6	128.8±18	45.5±4
19×10^{-2}	Control	135.4±4	154.3±4.8	108±8	45±3.5
(mg L^{-1})	Case	142.2±5*	162.3±5.4**	112±9.8	48.8±4**
28×10^{-2}	Control	141.4±8	151.4±6	106.8±11	44.4±5
(mg L^{-1})	Case	172±15*	181.6±12*	137±24	52.2±4**
Δverapamil	(0.1 μm)	8%	15%	12%	15%

WBCL: Wenckebach, ERP: Effective refractory period, FRP: Functional Refractory Period, Δ Verapamil (%): Difference between effects of Verapamil and *C. sativus* (C = 29×10^{-2} mg L^{-1})

Table 2: Mathematical analysis of the effects of different concentrations of Saffron extract on the nodal recovery curve (n = 6, mean±SEM, *p<0.05 compared to the control)

S. crocus		τ_{rec}	AH max (m sec)	AH min (m sec)
9×10^{-2}	Control	48.6±11	96.7±10.9	49.5±3.4
	Case	54.5±17	103.5±15.6	51±3.5
19×10^{-2}	Control	48.6±21	99.2±9	48.4±3
	Case	52.5±13	107.6±14	53±3*
28×10^{-2}	Control	48.6±10	103.3±12.4	46.6±2.7
	Case	58.4±16	111.6±14.1	53.6±3.6*

AH min: Minimum nodal conduction time (m sec), AH max: Maximum Nodal conduction time (m sec), τ_{rec}: time constant of recovery curve

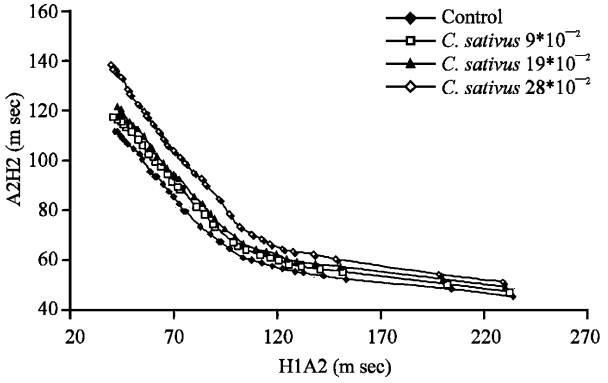

Fig. 1: The effects of different concentrations of Saffron extract on nodal recovery curve. There is a concentration–dependent upward shift of recovery curve

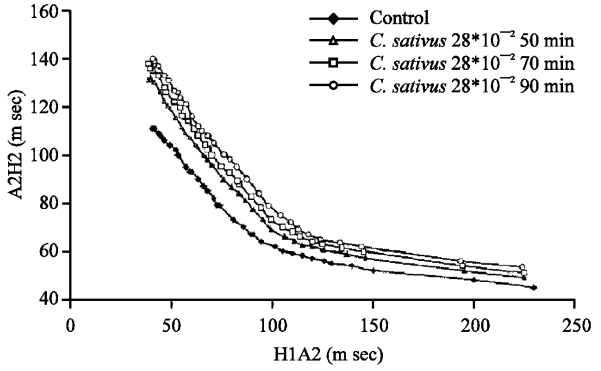

Fig. 2: The effect of aqueous extract of saffron (27.9×10^{-2} mg L^{-1}) on nodal recovery curve. There is a time-dependent upward shift of recovery curve

recovery curve analysis using mono-exponential model showed a non-significant prolongation of time constant of recovery (Fig. 3). The results of adding Verapamil (0.1 μmol) showed a significant increase in WBCL, ERP, FRP and AVCT (Table 2).

The aqueous extract of saffron in concentration-dependant manner was able to cause lower depressant effect in comparison to Verpamil, for example depressant effect of *C. sativus* (19×10^{-2} mg L^{-1}) on atrioventicular conduction time was 15% of Verapamil (Table 1). The comparison between saffron extract and Verapamil is indicative of that this plant in a concentration- dependant model affects artrioventicular conduction time, WBCL, FRP, EFP. However the depressant effect of saffron compared to 0.1 μmol concentration of Verapamil is negligible (Table 1).

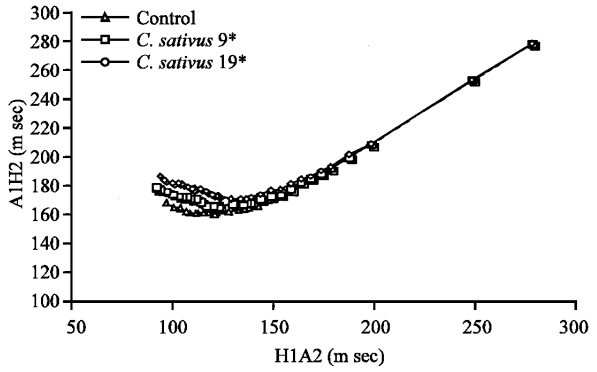

Fig. 3: The effects of different concentrations of Saffron extract on nodal Refractory curve. There is a concentration-dependent depression upward shift of refractory curve

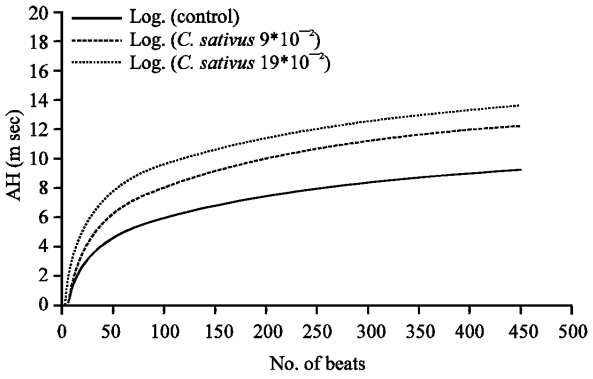

Fig. 4: The effects of different concentrations of Saffron extract on nodal fatigue curve. There is a concentration-dependent upward shift of fatigue curve. log: logarithmic trend line plotting of fatigue curve

C. sativus increased the amount of facilitation and magnitude of fatigue (Fig. 4). The amount of facilitation and degree of fatigue was 6.7±0.6 and 4.5±0.5 in control group and increased by saffron to 20±11.5 and 11.8±3, respectively ($p<0.05$). Saffron in concentration of 19×10^{-2} mg L^{-1} caused significant increase in WBCL from 135±4 to 154±9 and FRP from 156±7 to 185±20, respectively ($p<0.05$). It didn't has any significant effect on nodal ERP ($p>0.1$) (Table 2).

As a whole, the findings from this investigation showed the depressant effect of aqueous saffron extract on the nodal conduction time and refractoriness. These effects were demonstrated in concentration and time-dependent manner and rate-independent model. Recent studies indicate that the conduction curve of artrioventricular node consist of two different sections. The smooth part of the curve in long atrial stimulation is conducted through fast pathway and the section of curve with sharp slope represent conduction through slow pathway by fast beats (Reid *et al*., 2003). Inhibitory effect of saffron on the fast pathway is more than the slow pathway. The significant increase of minimum conduction time (AH min) reflecting the plant effect on fast pathway (the transitional cell of the posterior section of the compact node), on the other hand the non-significant increase of the maximum conduction time (AH max) and reduction of ERP are the indication of its effect on the compact node cells, also the inhibitory effect of aqueous extract of saffron in having a non-significant increase in time constant of recovery explaining rate-independent effect of saffron. The plant extract by affecting the compact node cells increase the WBCL and FRP. On the other hand, the unnoticeable decrease of ERP is an indication of lower effect of the extract on the slow pathway (transitional cells of posterior nodal extension).

Artrioventricular node acts as a control centre for the ventricular impulses during the tachiarrythmia (Billette and Shrier, 1995). The mechanism of this delay in nodal conduction in the AV node, still has not been understood (Billette and Shrier, 1995). In the functional model the reason for the delay in nodal conduction was due to the specific nodal intrinsic properties (Recovery, facilitation and fatigue) (Billette and Nattle, 1994). Therefore by understanding the mechanism of above phenomenon, we can explore the anti-arrhythmic effect of endogenous and exogenous substances.

The effect of saffron in fatigue represents the potential role of plant in preventing arrhythmia. In the fatigue protocol the tissue is stimulated by various stimuli similar to supraventricular tachyarrhythmia. The increase in fatigue index by saffron reflecting plant role in decreasing excitability of the distal node and an increase in the protective role of artrioventricular node. The previous studies demonstrate the role of transitional cells of proximal AV-node in facilitation mechanism (Mazgalev *et al*., 1997). Therefore, we can assume that Saffron probably exerts some of its effect on the transitional cells of the proximal AV-node. The fatigue mechanism is related to the prolonged refractoriness of compact nodal cells (Mazgalev, 2000; Billette *et al*., 1998). The prolongation of fatigue by saffron is due to the effect of this plant to increase effective and functional refractoriness of the node, this may be related to disperse depressant effects of Saffron on the posterior nodal extension and the compact nodal cells of distal AV- node.

This research for the first time has explained the role of saffron on the protective mechanism of artrioventricular node against supraventricular arrhythmia. The results from this research showed the non-specific effect of

saffron on the transitional cells of fast nodal pathway which was manifested as a rate-independent increase of basic and functional (facilitation and fatigue.) parameters of artrioventricular node. Further investigation is required to reveal the cellular mechanism of saffron on the AV node and its potential use in treating supraventricular tachyarrhythmia in human.

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ABBREVIATIONS

N	:	Normal
Cm	:	Centimeter
m sec	:	Mili seconds
Na	:	Natrium
Ca	:	Calcium
K	:	Potassium
W/V	:	Weight per volume percent
Hz	:	Hertz
g	:	Gram
°C	:	Degrees celecius
$g\ L^{-1}$	:	Gram per Liter
$mg\ L^{-1}$	:	mili garm per Liter
h	:	Hour
A/D	:	Analog to Digital
μ	:	Micro
M	:	Molar
AV-node	:	Atrioventricular-node
BCL	:	Basic cycle length
WBCL	:	Wenchebach cycle length
AVCT	:	Atrio- Ventricular conduction time
NCT	:	Nodal conduction time
FRP	:	Functional refractory period
ERP	:	Effective Refractory Period
τ	:	Time constant of recovery curve

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