Regular Article

Ephedrine Alkaloids-Free Ephedra Herb Extract, EFE, Has No Adverse Effects Such as Excitation, Insomnia, and Arrhythmias

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Ephedrine alkaloids-free Ephedra Herb extract (EFE) has been developed to eliminate the adverse effects caused by ephedrine alkaloid-induced sympathetic hyperactivation. Previously, we reported that EFE possesses analgesic, anti-influenza, and cancer metastatic inhibitory effects at comparable levels to that of Ephedra Herb extract (EHE). However, it has not yet been demonstrated that EFE is free from the known side effects of EHE, such as excitation, insomnia, and arrhythmias. In this study, the incidence of these adverse effects was compared between mice administered EHE and those administered EFE. Increased locomotor activity in an open-field test, reduced immobility times in a forced swim test, and reduced sleep times in a pentobarbital-induced sleep test were observed in EHE-treated mice, when compared to the corresponding values in vehicle-treated mice. In contrast, EFE had no obvious effects in these tests. In electrocardiograms, atrial fibrillation (*i.e.*, irregular heart rhythm, absence of P waves, and appearance of f waves) was observed in the EHE-treated mice. It was suggested that this atrial fibrillation was induced by stimulation of adrenaline β_1 receptors, but not by hypokalemia. However, EFE did not affect cardiac electrophysiology. These results suggest that the abovementioned side effects are caused by ephedrine alkaloids in EHE, and that EFE is free from these adverse effects, such as excitation, insomnia, and arrhythmias. Thus, EFE is a promising new botanical drug with few adverse effects.

Key words   Ephedra Herb; ephedrine alkaloid; adverse effect; ephedrine alkaloid-free

Ephedra Herb is defined in the 17th edition of the Japanese Pharmacopoeia (JP) as the terrestrial stem of *Ephedra sinica* STAPF., *Ephedra intermedia* SCHRENK *et* C. A. MEYER, or *Ephedra equisetina* BUNGE (Ephedraceae), with ephedrine alkaloid (ephedrine and pseudoephedrine) content greater than 0.7%.[1] Ephedra Herb is an important component in many Kampo prescriptions, such as kakkonto, maoto, shoseiryuto, eppikajutsuto, and makyoyokukanto. For instance, kakkonto is frequently used to treat the common cold at the early stage,[2] and maoto has been traditionally prescribed to patients with influenza in Japan.[3] Nabeshima *et al.* reported that maoto affects the early phase of influenza virus infection, and its anti-influenza activity is comparable with that of Oseltamivir.[4] Furthermore, eppikajutsuto and makyoyokukanto have been frequently used to treat arthralgia and rheumatism in Japan (http://mpdb.nibiohn.go.jp/stork/). The pharmacological actions of these medicines are thought to be derived from their main components, ephedrine alkaloids (*l*-ephedrine, *d*-pseudoephedrine, *l*-methylephedrine, *l*-norephedrine), in the Ephedra Herb.[5] Ephedrine alkaloids are also used as chemical drugs for bronchial asthma and the common cold in Western modern medicine.[6]

Ephedrine alkaloids can activate the central and autonomic nervous system through adrenaline- and dopamine-like activities,[7] which are thought to be responsible for the adverse effects of Ephedra Herb, such as excitation, insomnia, and arrhythmia.[8,9] When coadministered with Kampo medicines containing Ephedra Herb, cholinergic agents, monoamine oxidase inhibitors, thyroid gland preparation, and xanthine preparation should be administered with care, because enhanced sympathetic nerve stimulation has been suggested.[10–12] In addition, ephedrine alkaloid-containing drugs should be administered with care in patients with cardiovascular-related diseases.[13]

In the United States, dietary supplements containing Ephedra Herb or ephedrine alkaloids were once widely used for weight loss, but in April 2004, the use of Ephedra Herb at concentrations >10 mg in supplements was banned by the Food and Drug Administration (FDA) because of the adverse effects of ephedrine alkaloids. Many case reports regarding the adverse events due to dietary supplements were validated by the FDA. Thirty-one percent of cases were considered to be definitely or probably related to the use of supplements containing Ephedra Herb or ephedrine alkaloids, and 31% were deemed to be possibly related.[14] Among the adverse events that were deemed definitely, probably, or possibly related to the use of supplements containing Ephedra Herb, 47% involved cardiovascular symptoms and 18% involved the central nervous system. Hypertension was the most frequent adverse effect (17 cases), followed by palpitations, tachycardia,

or both (13 cases); stroke (10 cases); and seizures (7 cases). Ten events resulted in death, and 13 events produced permanent disability, representing 26% of the definite, probable, and possible cases. Therefore, the FDA judged that supplements containing Ephedra Herb induce adverse events.

In order to eliminate the severe adverse effects that are predicted to be caused by ephedrine alkaloids, we have developed an ephedrine alkaloids-free Ephedra Herb extract (EFE), which possesses analgesic, anti-influenza, and anti-cancer effects at comparable levels to those of Ephedra Herb extract (EHE).[15] However, it has not yet been demonstrated whether EFE is free from the frequent side effects of EHE, such as excitation, insomnia, and arrhythmia. In this study, we investigated the effects of 700 mg/kg EFE and 700 mg/kg EHE on the central and autonomic nervous systems, using an open-field test, forced swim test, pentobarbital-induced sleep test (using caffeine as a reference), and electrocardiogram test. Because EHE and EFE showed significant analgesic effects *in vivo* study at a dose of 700 mg/kg, respectively.[15] We used both the open-field and forced swim tests to examine the induction of excitatory behavior in mice by EFE and EHE, because central nervous system stimulants induce more locomotor activity in open-field tests and shorter immobility time in forced swim tests.[16,17] The pentobarbital-induced sleep test was used to assess the possible risk of insomnia by EHE and EFE. The barbiturate, pentobarbital, induces sedation and sleep by increased binding affinity between gamma-aminobutyric acid (GABA) and the GABA receptor and by direct activation of the chloride channel.[18] A delay of pentobarbital-induced sleep onset implies stimulation of the central nervous system,[19] and excessive excitation of the central nervous system causes insomnia.[20] In the electrocardiogram test, we studied the influence of EHE and EFE on cardiac electrophysiology using a radiotelemetry system in mice.

MATERIALS AND METHODS

Materials Caffeine (purity >98.5%, TLC) was purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan), and pentobarbital sodium salt (purity >98%, N) was obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan) EHE and EFE were provided from TOKIWA Phytochemical Co., Ltd. (Japan). The preparation method of these two extracts was described in our previous report.[21] EHE and EFE displayed similar chromatographic profiles, but several peaks were absent in that of EFE. The retention times and UV spectra of the EHE standard revealed the presence of ephedrine alkaloids (ephedrine, pseudoephedrine, norephedrine, methylephedrine), catechin, syringin, kaempferol 3-*O*-rhamnoside 7-*O*-glucoside, isovitexin 2″-*O*-rhamnoside, and cinnamic acid. However, ephedrine alkaloids were not present in the EFE chromatogram (Supplementary Fig. 1). Caffeine, EHE, and EFE were dissolved in purified water, and pentobarbital was dissolved in physiological saline. In a previous study, EHE and EFE showed significant analgesic effects in an *in vivo* paw-licking test at a dose of 700 mg/kg, respectively.[15] In the current study, we used 700 mg/kg of EHE or EFE as a maximum dose.

Animals Four-week-old male ddY mice were purchased from Japan SLC, Inc. (Japan). Prior to experimentation, mice were acclimatized for 1 week to a temperature of 25±2°C,
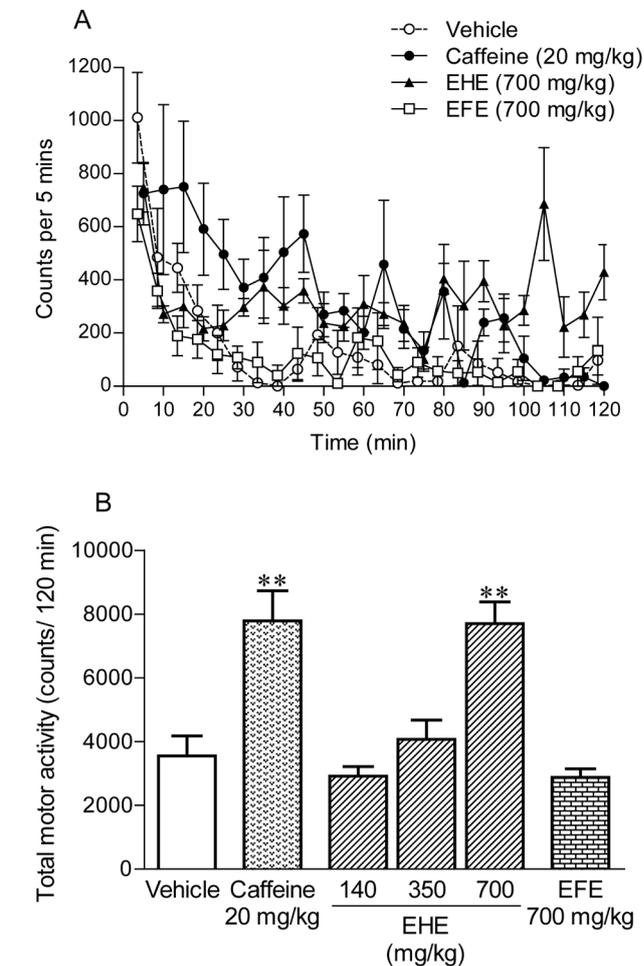

Fig. 1. Time-Dependent Changes in the Locomotor Activity (A) and Total Motor Activity (B) of Mice in an Open-Field Test

Locomotor activity was measured for 120 min after oral administration of caffeine (20 mg/kg), EHE (140, 350, 700 mg/kg) or EFE (700 mg/kg). Data represent the means±standard errors of the mean (S.E.M.) (*n*=8). Statistical significance was determined with Dunnett's test; ** *p*<0.01 *vs.* vehicle group. EHE, Ephedra Herb extract; EFE, ephedrine alkaloids-free Ephedra Herb extract.

humidity of 50±10%, and a 12-h light/12-h dark cycle. All behavioral observations were conducted between 10:00 and 15:00 h. The open-field, forced swim, and pentobarbital sleep tests were performed with 8 mice per group. Heart rate variability in the electrocardiogram test was measured in 4 mice per group. The measurement of serum potassium level was performed with 3 mice in each group.

Compliance with Ethical Standards The protocol for animal experiments was approved by the Institutional Animal Care and Use Committee of Kitasato University, and was performed in accordance with the Kitasato University guidelines for animal care, handling, and termination, which are consistent with the International and Japanese guidelines for animal care and welfare (approval number: FR07-2, date: May 1, 2013).

Open-Field Test Each mouse was placed in a cage just after oral sample administration, and the locomotor activity was measured for 120 min using an open-field test. The test was performed in cylindrical cages (22 cm in height, 25 cm in diameter) equipped with a passive IR sensor (PYS-001; Muromachi Kikai Co., Ltd., Japan). Spontaneous motor activity was recorded using a passive IR sensor detection system

(SUPERMEX; Muromachi Kikai Co., Ltd.) and analyzed using CompACT AMS software (Muromachi Kikai Co., Ltd.).[17)]

Forced Swim Test The same cylindrical cages used in the open-field test were used as water tanks for swimming (water depth 15 cm, water temperature 25°C). Twenty-four hours before experiments, mice were individually placed in the water tank for 15 min, and were grouped according to measurements of immobility times. Thirteen minutes after oral sample administration, mice were individually placed into the water tank and immobility times were measured over 6 min, using the SUPERMEX detection system (Muromachi Kikai Co., Ltd.). Data were analyzed using a personal computer with CompACT FSS software (Muromachi Kikai Co., Ltd.).[20)]

Pentobarbital Sleep Test Mice were given intraperitoneal injections of 30 mg/kg pentobarbital 30 min after oral sample administration, and were then placed in a cage. The sleep duration was defined as the difference in time between the loss and recovery of the righting reflex.[17)]

Radiotelemetry System The radiotelemetry system used in this study enabled recordings of electrocardiographic signals in freely moving animals. It consisted of transmitters (TA10ETA-F20, Data Sciences International, St. Paul, MN, U.S.A.) and platform receivers (RPC-1, Data Sciences International).

Transmitter Implantation Transmitter implantation was performed under isoflurane (Escain®) anesthesia (2% in 100% oxygen). The transmitter was placed in the abdominal cavity and the two electrodes (wire loops) were fixed, respectively, to the dorsal surface of the xiphoid process and in the anterior mediastinum close to the right atrium. After surgery, mice were individually housed and allowed one week for recovery of body weight and circadian rhythmicity of heart rate before the beginning of the experimental recordings.

Electrocardiographic Data Acquisition and Processing This test was performed by a crossover study, and the schedule is described in Table 1. Continuous electrocardiogram recordings were performed in three recording periods: 1st (baseline), electrocardiogram recording for 60 min with the last 15 min used as baseline; 2nd (pretest), purified water

as vehicle was administered orally to all groups and the electrocardiogram was immediately recorded for 60 min; 3rd (test): each sample solution was administered and the electrocardiogram was immediately recorded for 60 min. Electrocardiographic signals were fed to a personal computer containing the ART-Silver 1.10 data acquisition system (Data Sciences International) for monitoring and acquisition of electrocardiographic waves. The following electrocardiographic parameters were calculated every 20 min after administration of vehicle, EHE, or EFE: 1) mean of R-R interval duration (RR, ms), and 2) standard deviation of RR (SD$_{RR}$, ms) (Table 2).

Measurement of Serum Potassium Level Mice were administered vehicle, EHE (700 mg/kg) or EFE (700 mg/kg). Ten, 30, and 60 min after administration, whole blood was collected from the femoral artery of each mouse under isoflurane anesthesia, and was centrifuged at 1500×*g* for 10 min at 4°C to separate serum. Serum potassium levels were measured using an ion selective electrode method (Oriental Yeast Co., Ltd., Japan).

Statistical Analysis All results are expressed as the means±standard errors of the mean (S.E.M.). The data were compared using ANOVA followed by Dunnett's test, with $p<0.05$ considered statistically significant. All statistical analyses were performed using Prism 5 (GraphPad Software Inc.).

RESULTS

Comparison of the Excitatory Action between EFE and EHE Using Open-Field and Forced Swim Tests The overexcitatory action of EHE is considered to be caused by stimulation of the central nervous system with the ephedrine alkaloids that it contains. The excitatory action of EHE and EFE was compared in open-field and forced swim tests, using caffeine as a reference. Figure 1 shows the results of the open-field test. Locomotor activities of the mice administered 700 mg/kg EHE were as high as those of 20 mg/kg caffeine from 30 to 120 min (Figs. 1A, B). In contrast, the activities of the 700 mg/kg EFE-treated mice were as low as those of the vehicle-treated mice (Figs. 1A, B). In the forced swim test, EHE decreased the immobility time of mice in a dose-dependent manner. Both 700 mg/kg EHE and 20 mg/kg caffeine significantly reduced the immobility time when compared with vehicle (Fig. 2). However, 700 mg/kg EFE had almost no effect on immobility time in the forced swim test (Fig. 2). These results indicate that EHE had excitatory action, but EFE did not.

Effects of EFE and EHE on Sleep In the pentobarbital-induced sleep test, EHE prolonged sleep onset latency (Fig. 3A) and decreased sleep duration (Fig. 3B) in a dose-dependent manner, and the sleep duration of mice administered

Table 1. The Schedule of the Crossover Study by Electrocardiography

	Mouse-1	Mouse-2	Mouse-3	Mouse-4
Test drug-1	Vehicle	Vehicle	EHE	EFE
Washout period	4–6 d	4–6 d	4–6 d	4–6 d
Test drug-2	EHE	EHE	EFE	Vehicle
Washout period	4–6 d	4–6 d	4–6 d	4–6 d
Test drug-3	EFE	EFE	Vehicle	EHE

EHE: Ephedra Herb extract; EFE: ephedrine alkaloids-free Ephedra Herb extract.

Table 2. Fluctuation of RR Interval, SD$_{RR}$, and Heart Rhythm after Vehicle, EHE (700 mg/kg), or EFE (700 mg/kg) Administration as Determined by Electrocardiography

Time	60–80 min			80–100 min			100–120 min		
Group	Vehicle	EHE	EFE	Vehicle	EHE	EFE	Vehicle	EHE	EFE
RR	110.0±8.7	148.1±5.2*	121.4±5.6	125.1±13	149.1±8.8	131.0±7.2	130.2±9.1	154.2±5.9	127.8±7.8
SD$_{RR}$	17.7±7.9	40.0±3.4*	16.5±3.0	20.7±9.1	27.8±1.7	15.1±1.7	15.7±4.7	28.9±3.1*	20.3±2.9
Rhythm	Sinus	Af	Sinus	Sinus	Af	Sinus	Sinus	Af	Sinus

Data represent the means±S.E.M. ($n=4$). Statistical significance was determined with Dunnett's; *$p<0.05$ *vs.* vehicle group. Sinus: sinus rhythm; Af: atrial fibrillation; EHE: Ephedra Herb extract; EFE: ephedrine alkaloids-free Ephedra Herb extract.
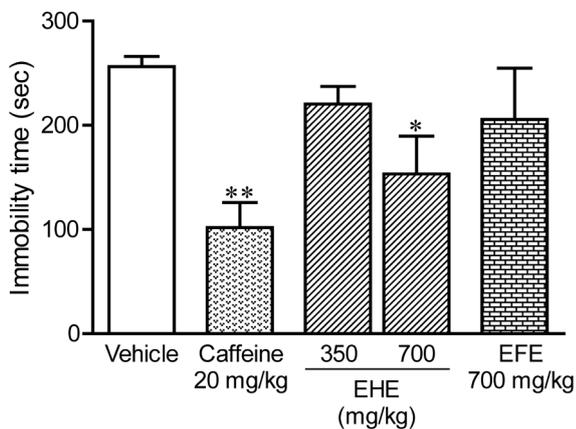
Fig. 2. Effects of Caffeine, EHE, and EFE on the Immobility Time in a Forced Swim Test

Caffeine (20 mg/kg), EHE (350, 700 mg/kg), or EFE (700 mg/kg) was administered orally 30 min before the forced swim test. Total immobility time was measured during 6 min of the forced swim test. Data represent the means±S.E.M. ($n=8$). Statistical significance was determined with Dunnett's test; $*p<0.05$ and $**p<0.01$ *vs.* vehicle group. EHE, Ephedra Herb extract; EFE, ephedrine alkaloids-free Ephedra Herb extract.
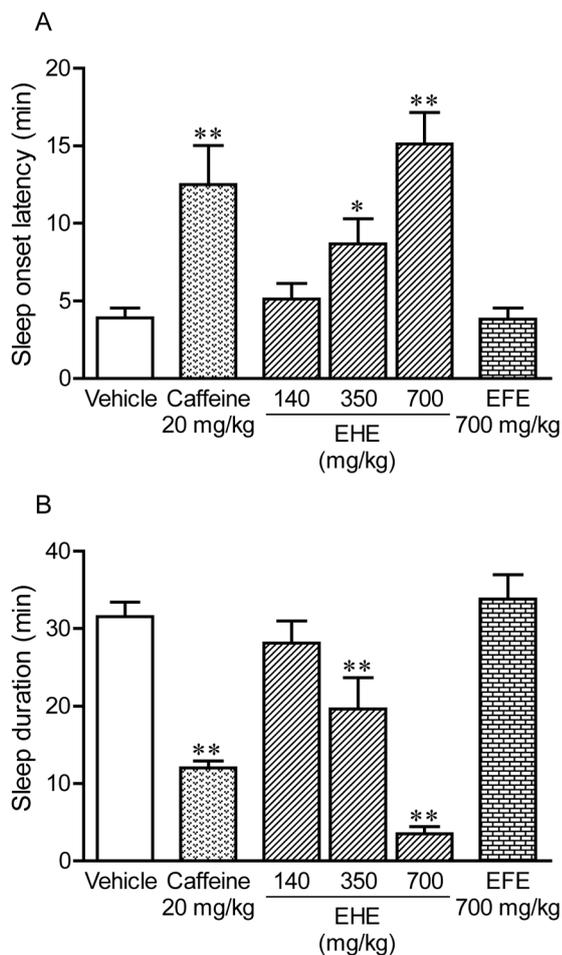

Fig. 3. Effects of Caffeine, EHE, and EFE on Pentobarbital-Induced Sleep Onset Latency and Sleep Duration in Mice

Caffeine (20 mg/kg), EHE (140, 350, 700 mg/kg), or EFE (700 mg/kg) was administered orally 30 min before intraperitoneal injection of pentobarbital. After intraperitoneal injection of pentobarbital, the sleep onset latency (A) and sleep duration (B) were recorded. Data represent the means±S.E.M. ($n=8$). Statistical significance was determined with Dunnett's test; $*p<0.05$ and $**p<0.01$ *vs.* vehicle group. EHE, Ephedra Herb extract; EFE, ephedrine alkaloids-free Ephedra Herb extract.
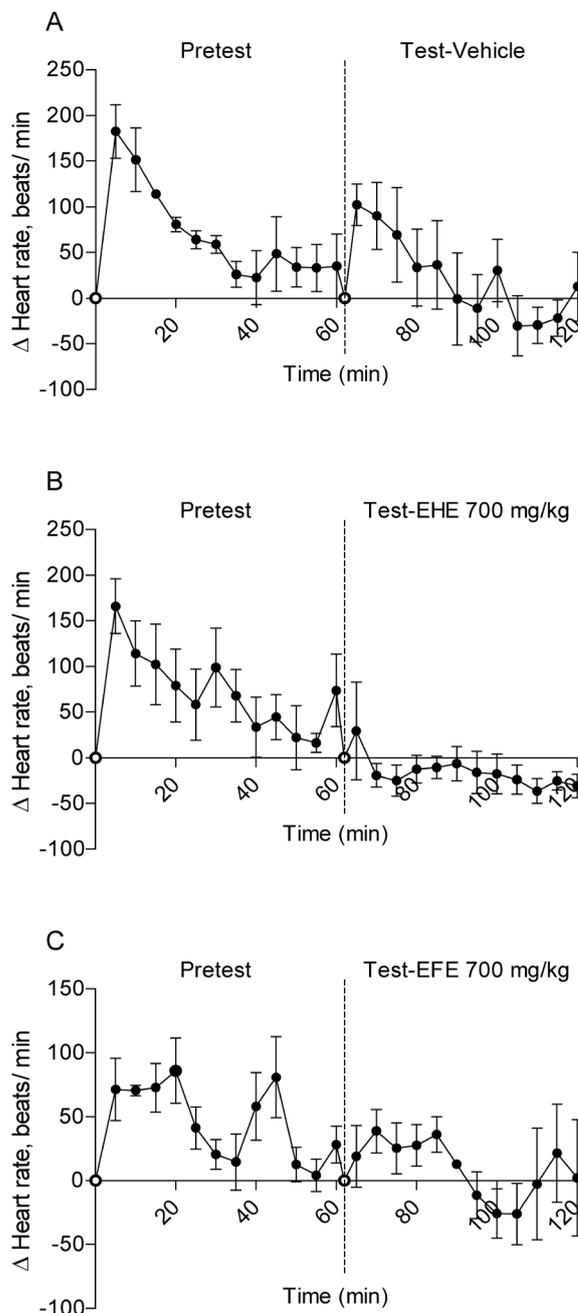

Fig. 4. Time-Dependent Changes in Heart Rate after Oral Administration of Vehicle, EHE, or EFE

First, all groups were orally administered vehicle, and their heart rate was recorded for 60 min (pretest period). Next, each group was administered vehicle (A), 700 mg/kg EHE (B), or 700 mg/kg EFE (C), and the heart rate was recorded for 60 min (test period). Data represent the means±S.E.M. ($n=4$). EHE, Ephedra Herb extract; EFE, ephedrine alkaloids-free Ephedra Herb extract.

700 mg/kg EHE was remarkably shorter than that of mice administered 20 mg/kg caffeine. However, 700 mg/kg EFE had no effect on sleep onset and sleep duration (Figs. 3A, B).

Effects of EHE and EFE on Cardiac Electrophysiology The effects of EHE and EFE on cardiac electrophysiology were evaluated. Figure 4 shows the time course change in heart rate after administration of EHE or EFE. The influence of oral administration itself on heart rate was examined by administration of purified water. The heart rate was observed for 60 min after the oral administration of water (0–60 min, pretest period). An acute elevation of heart rate was observed just
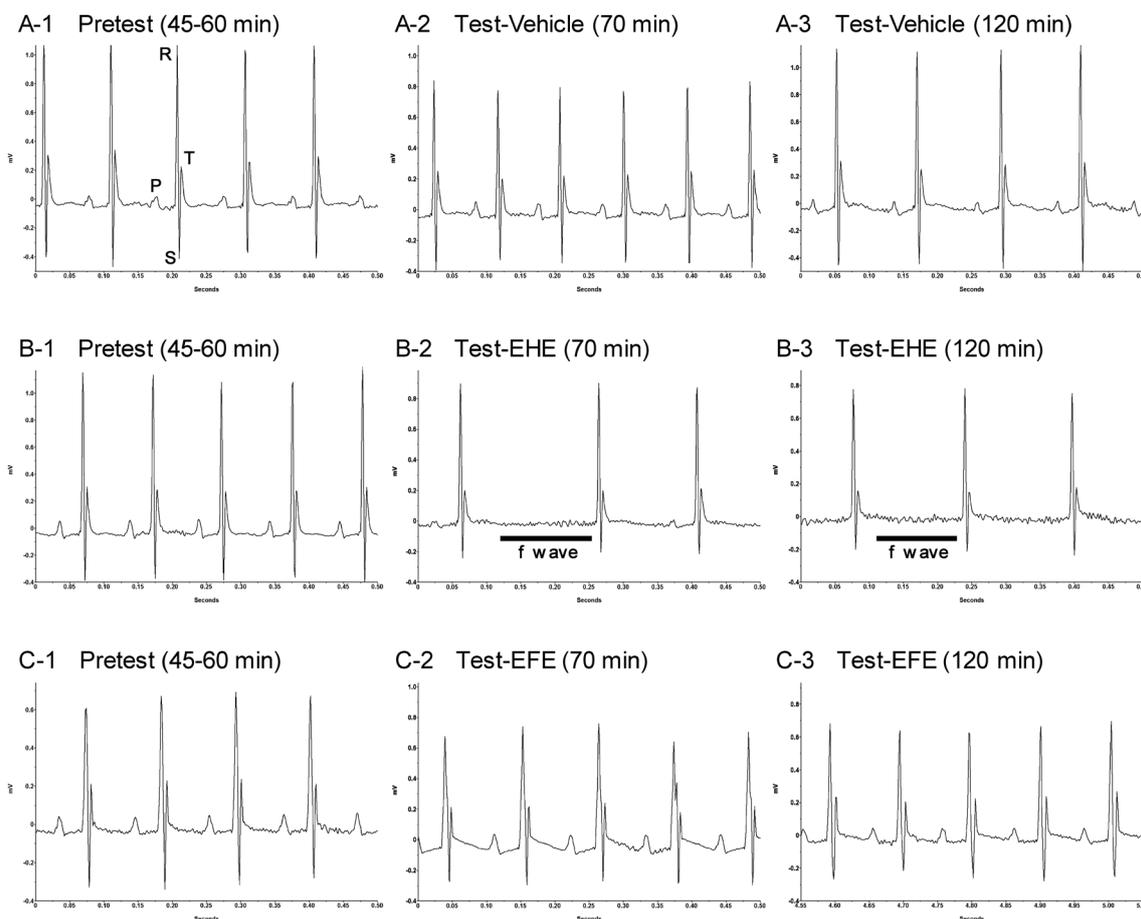
Fig. 5.　Electrocardiogram Recordings for Mice Administered Vehicle, EHE, or EFE

　Electrocardiograms were recorded in the pretest period (A-1, B-1, and C-1), and the test period from 10 and 60 min after administration of vehicle (A-2 and A-3, respectively), 700 mg/kg EHE (B-2 and B-3, respectively), and 700 mg/kg EFE (C-2 and C-3, respectively). Representative 0.5-s tracings are shown. EHE, Ephedra Herb extract; EFE, ephedrine alkaloids-free Ephedra Herb extract.

Table 3.　Serum Potassium Levels 10, 30, and 60 min after Vehicle, EHE (700 mg/kg), or EFE (700 mg/kg) Administration

Time	10 min			30 min			60 min		
Group	Vehicle	EHE	EFE	Vehicle	EHE	EFE	Vehicle	EHE	EFE
K^+ (mEq/L)	5.33 ± 0.45	5.60 ± 0.61	4.60 ± 0.10	4.83 ± 0.20	4.60 ± 0.15	5.47 ± 0.93	5.00 ± 0.17	5.13 ± 0.13	5.00 ± 0.41

　Data represent the means $\pm$ S.E.M. ($n = 3$).

after the oral administration, but it returned to baseline within 60 min (Fig. 4). For the next 60-min period, vehicle, EHE, or EFE was administered to the mice and the heart rate of each group was recorded for another 60 min (60–120 min, test period). In the vehicle group and the EFE group, acute elevation of heart rate was induced by the stress of oral administration, but the heart rate returned to baseline within 60 min (Figs. 4A, C). Unlike that of the vehicle and EFE groups, the heart rate of EHE group dropped lower than baseline value during the 60-min period (Fig. 4B). The measured RR interval (ms) was longer in the group administered EHE than in the other groups, and a significant extension was observed, especially during the first 20 min after EHE administration. Furthermore, the value of SD_{RR} increased (Table 2, *i.e.*, irregular heart rhythm was observed in the EHE-administered mice). Figure 5 shows the electrocardiograms for the last 15 min of the pretest period (A-1, B-1, and C-1), and for 0.5 s at 10 min (A-2, B-2, and C-2) and 60 min (A-3, B-3, and C-3) after ad-

ministration of vehicle (A-2 and A-3), EHE (B-2 and B-3), or EFE (C-2 and C-3). In the last 15 min of the pretest period, P, R, S, and T waves were detected clearly in all groups of mice (Figs. 5A-1, B-1 and C-1). In the test period, P, R, S, and T waves were also detected clearly in the vehicle group and the EFE group (Figs. 5A-2 and C-2). However, in the EHE group, 10 min after administration, the P wave disappeared and f waves were apparent (Fig. 5B-2), suggesting that atrial fibrillation was occurring after EHE administration. The occurrence of atrial fibrillation was still observed 60 min after EHE administration (Table 2 and Fig. 5B-3).

Serum Potassium Levels after Administration of EFE and EHE　In order to determine whether the effect of EHE on cardiac rhythm was caused by hypokalemia, serum potassium levels were measured. Table 3 shows the serum potassium levels 10, 30, and 60 min after administration of vehicle, EFE, and EHE. There were no significant differences in serum potassium levels among all groups (Table 3).

DISCUSSION

In this study, the incidence of adverse effects such as excitation, insomnia, and arrhythmia was compared among mice administered vehicle, EHE, or EFE. First, we examined the excitatory action of EHE and EFE in open-field and forced swim tests. Caffeine was used as a reference drug because it is a well-known central stimulant.[22] Anti-depressants decrease immobility in the forced swim test and decrease locomotor activity in the open-field test, while central stimulants, such as caffeine, decrease immobility and induce a pronounced increase in locomotor activity.[16] The administration of EHE (700 mg/kg) increased locomotor activity and also shortened immobility time, suggesting that EHE has central stimulation effects. Miller and Segert reported that an acute intraperitoneal injection of 10–30 mg/kg ephedrine produced dose-dependent hyperactivity in the open-field test.[7] Therefore, it is possible that the central stimulation of EHE is derived from ephedrine. In contrast, the total motor activity and the immobility time of the EFE-administered mice were unchanged compared with those of the vehicle-administered mice, indicating that EFE did not stimulate the central nervous system.

Next, we investigated the effects of EHE and EFE in the pentobarbital sleep test, again using caffeine as a reference drug.[20] The sleep time of mice administered EHE (700 mg/kg) was significantly shortened, and was much shorter than that of mice administered 20 mg/kg caffeine. The action mechanisms of caffeine and EHE on sleep may be different. Caffeine blocks the binding of endogenous adenosine to both adenosine receptors A_1 and A_{2A}, resulting in an increase in murine locomotor behavior.[23] It also promotes wakefulness by blocking adenosine A_{2A} receptor.[24] Dopamine D_2 receptor, which is co-expressed with the adenosine A_{2A} receptor, was also reported to play a role in the maintenance of wakefulness after caffeine administration.[24,25] In contrast, ephedrine alkaloids are known to express their pharmacological actions *via* the α- and β-adrenergic receptors, and are reported to induce release of endogenous catecholamines, such as noradrenaline and dopamine, indirectly and to prevent their neuronal reuptake.[26] Noradrenaline is one of the most abundant neurotransmitters in the central and peripheral nervous systems, and is implicated in many aspects of physiology and behavior, including cognition, attention, reward, locomotion, and arousal. Small-molecule activators of noradrenaline signaling have been shown to increase wakefulness, whereas inhibitors promote sleep.[27] Therefore, the inhibition of pentobarbital-induced sleep by EHE may be attributable to the adrenergic action of ephedrine alkaloids. Although 700 mg/kg of EHE prevented pentobarbital-induced sleep, 700 mg/kg of EFE had no effect on sleep time. These results indicate that the strong awakening effect of EHE was eliminated by the removal of ephedrine alkaloids.

Finally, the effect of EHE and EFE on cardiac electrophysiology was evaluated using electrocardiograms. Because an elevated heart rate was caused by the stress of oral administration, the effects of EHE and EFE were compared with those of purified water. In the EFE administration group, the elevation of heart rate was weak after water administration during pre-test period (Fig. 4C). In the crossover test used in this study, the stress response of mouse to oral administration with a sonde was gradually alleviated by habituation. Since Mouse-1 and Mouse-2 were given the same administration schedule, and the order of EFE administration was the very last in this schedule (Table 1). Therefore, it is thought that the weak elevations of heart rate after water or EFE administration resulted from habituation in EFE group. However, since the fluctuation pattern of heart rate after administration of EFE or vehicle was similar, we thought that EFE showed the same response as vehicle. In the EHE group, there was no increase in heart rate immediately after administration, unlike the vehicle or EFE group. Prolongation of the RR interval and an increase in SD_{RR} were observed after EHE administration (Table 2, *i.e.*, an irregular heart rhythm was observed in the EHE group). We analyzed the electrocardiogram in more detail to elucidate the effects. We noted that the P wave disappeared and was replaced with f waves, beginning 10 min after EHE administration (Fig. 5B-2), which indicates atrial fibrillation. Atrial fibrillation is characterized by irregular atrial depolarizations with a discrete lack of P waves on an electrocardiogram.[28] The f waves still occurred 60 min after EHE administration (Fig. 5B-3), and atrial fibrillation was still apparent at this time. In this study, it was revealed that administration of EHE to mice causes atrial fibrillation, one type of cardiac arrhythmia. Atrial fibrillation had been reported to be induced by $β_1$ adrenergic receptor agonists such as dobutamine and arbutamine,[29] or hypokalemia.[30] Hypokalemia was reported to be induced by ephedrine administration,[31] so we measured serum potassium levels. However, the serum potassium level was not affected by EHE administration in this study. On the other hand, Kawasuji *et al.* reported that ephedrine isomers increased beating rate of rat right atrium isolated from rat and that this positive chronotropic effects of ephedrine isomers were attenuated by pretreatment with atenolol, a selective $β_1$-adrenoceptor antagonist.[32] This report suggests that atrial fibrillation is evoked by abnormal excitation of atrial myocardium through stimulation of $β_1$ adrenergic receptors by ephedrine alkaloids in EHE. Furthermore, the bradycardia observed with EHE treatment in this study may have been caused by a low response of the atrioventricular node.

Kampo medicines containing Ephedra Herb are well known to cause palpitations, which may result from any cardiac arrhythmia producing changes in heart rate, rhythm, or contraction patterns.[33] However, EFE did not affect cardiac rhythm in mice. There was no abnormality in cardiac electrophysiology in the EFE group. These results suggest that EFE has no adverse effects on the autonomic nervous system.

This is the first *in vivo* study demonstrating the adverse effects of Ephedra Herb on the central and autonomic nervous systems. We have previously reported that both EHE and EFE had significant analgesic effects at a dose of 700 mg/kg.[15] In this study, we demonstrated that 700 mg/kg of EHE induced excitation, insomnia, and cardiac arrhythmia in mice, but 700 mg/kg EFE did not induce these adverse effects. Thus, EFE is a useful analgesic drug without the adverse effects caused by ephedrine alkaloids.

Supplementary Materials The online version of this article contains supplementary materials.

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