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EXTRACT OF *HYPERICUM PERFORATUM* BLOCKS NICOTINE-INDUCED LOCOMOTOR ACTIVITY IN MICE

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Abstract

In the present study, the effects of Hypericum perforatum on nicotine-induced locomotor activity have been investigated in mice. Adult male Swiss-Webster mice were subjects. Nicotine (0.5, 1 and 2 mg/kg) and saline were given to mice intraperitoneally. Locomotor activities of the mice were recorded for 30 min immediately following nicotine or saline administration. Hypericum perforatum extract (HPE) (6, 12, 24 and 48 mg/kg) and saline were injected to another five independent groups of the mice and 20 min later, locomotor activity was recorded for 30 min. In further study, HPE (6-24 mg/kg) was administered to other independent groups of mice 20 min before nicotine (1 mg/kg) injections and locomotor activity was recorded for 30 min immediately after nicotine administration. Nicotine (1 mg/kg) produced a significant increase in locomotor activity of the mice. HPE depressed significantly locomotor activity of the mice at dose of 48 mg/kg. Other doses of HPE (6-24 mg/kg) did not produce any significant change in the locomotor activities. HPE (24 mg/kg) blocked significantly nicotine (1 mg/kg)-induced locomotor. Our results suggest that HPE blocks nicotine-induced locomotor hyperactivity in mice. Thus, it may be a useful agent for treatment of nicotine-type dependence.

Key Words: *St. John's wort, Hypericum perforatum, Nicotine, Locomotor activity, Mice*

Hiperikum perforatum Ekstresi Farelerde Nikotin İle İndüklenen Lokomotor Aktiviteyi Bloke Ediyor

Sunulan çalışmada, farelerde nikotin ile indüklenen lokomotor aktivite üzerine Hiperikum perforatum ekstresinin etkileri incelenmiştir. Çalışmada denek olarak erişkin erkek Swiss-Webster türü fareler kullanıldı. Nikotin (0.5, 1 ve 2 mg/kg) ve serum fizyolojik farelere intraperitoneal yoldan enjekte edildi. Nikotin veya salin enjeksiyonlarından hemen sonra 30 dakika süre ile farelerin lokomotor aktiviteleri kaydedildi. Hiperikum perforatum ekstresi (HPE) (6, 12, 24 ve 48 mg/kg) ve salin diğer 5 farklı grup fareye enjekte edildi ve enjeksiyonlardan 20 dakika sonra farelerin 30 dakika süre ile lokomotor aktiviteleri kaydedildi. Daha ileri bir çalışma olarak, HPE (6-24 mg/kg) başka bağımsız gruplarda yer alan farelere nikotin (1 mg/kg) enjeksiyonundan 20 dakika önce verildi. Bu gruplarda da nikotin (1 mg/kg) enjeksiyonlarının hemen sonrasında farelerin 30 dakika süre ile lokomotor aktiviteleri kaydedildi. Nikotin (1 mg/kg) farelerin lokomotor aktivitelerinde anlamlı ölçüde artışa neden oldu. HPE 48 mg/kg'lık dozunda farelerin lokomotor aktivitesini anlamlı ölçüde deprese etti. HPE'nin çalışmada kullanılan diğer dozları (6-24 mg/kg) lokomotor aktivitelerde anlamlı bir değişiklik oluşturmadı. HPE (24 mg/kg) nikotin ile indüklenen lokomotor aktivite artışını anlamlı ölçüde bloke etti. Sonuçlarımız HPE'nin farelerde nikotin ile indüklenen lokomotor aktivite artışını bloke ettiğine ve bu ekstrenin nikotin tipi bağımlılığın tedavisinde faydalı olabieceğine işaret etmektedir.

Anahtar Kelimeler: *St. John otu, Hiperikum perforatum, nikotin, lokomotor aktivite, fareler*

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INTRODUCTION

Tobacco addiction has a considerable health and economic impact on society. It has become one of the largest health problems worldwide. Nicotine plays a key role in maintaining the smoking of tobacco and is the major component responsible for addiction (1).

Hypericum perforatum L. is the common plant usually called St John's wort. Extract of *Hypericum perforatum* L. (HPE) has been known as an herbal remedy since Greek and Roman times. It has been used for treatment of lots of disorders in folk medicine (2). Various experimental and clinical studies indicate that HPE may be useful for treatment of disorders originated from central nervous system, especially in depression. Thus, the antidepressant-like action of HPE was reported in rodents (3,4) and, it has been used for the treatment of mild to moderate depression in humans (5-7).

In some studies, the effects of HPE on some type of dependences have been subjected. It has been suggested that HPE attenuates alcohol intake in some strains of alcohol-preferring rats (8,9). In a couple of recent preliminary study from our laboratory, we also observed that HPE inhibited some signs of ethanol withdrawal syndrome in rats (10) and blocked caffeine-induced locomotor activity in mice (11), respectively. On the other hand, some antidepressants have been marketed to aid in smoking cessation (12). Furthermore, in a recent study, Catania et al. (13) showed that HPE attenuates nicotine withdrawal signs in mice.

Psychostimulant properties of drugs such as caffeine, ethanol, cocaine, amphetamine and nicotine can be assessed by locomotor activity, and these psychostimulant effects have been linked to their addictive properties (14). However, any study investigating the effects of HPE on locomotor stimulant effects of nicotine has not been published yet. Thus, the present study was designed to evaluate the effects of HPE on nicotine-induced locomotor activity in mice.

EXPERIMENTAL

Animals and Laboratory

Adult male Swiss-Webster mice (26-32 g) were used in the study (n= 8 for each group). They were housed in a quiet and temperature-and humidity-controlled room (22 ± 2°C and 60 ± 5 %, respectively) in which a 12-h (light/dark) cycle was maintained (07:00 – 19:00 h light). All experiments were performed at the same time of the day and in the light period (09:00 – 11:30 am).

All procedures in the present study are in accordance with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health (Washington, D.C. USA, 1996) and the Declaration of Helsinki.

Drugs used and preparation of HPE

Nicotine was purchased from Sigma Chemical (USA). Alcohol (96.5% v/v) using in the extraction processes was also purchased from Merck (USA). HPE was prepared using aerial parts of St. John's wort in Anadolu University, Department of Pharmacognosy as previously described

(15). St. John's wort were collected in Domaniç – İnegöl area of Turkey in June 2003. After collection, the plant materials were dried at room temparature and their voucher specimens are kept at the Herbarium of the Faculty of Pharmacy, Anadolu University, Eskişehir, Turkey. Fifty g of semi-crushed plant materials was macerated in 50% alcohol for 24 h at 37 °C. Following filtration and removal their alcohol by Rotavapor (Buchi, Flavil, Switzerland) and water contents by lyophilization (Leybold-Heraeus, Köln, Germany), dry extracts were obtained. Yields of HPE were 27.4% (w/v). Doses were expressed as dried extract (mg/kg body weight).

HPE was dissolved in saline with helping a vortex. HPE or saline were injected to mice intraperitoneally at a volume of 10 ml / kg. Drug solutions were prepared just prior to application to mice.

Procedure

Nicotine (0.5, 1.0 and 2.0 mg/kg) and saline were given to mice intraperitoneally (ip). Locomotor activity was measured for 30 min immediately following nicotine or saline administration. HPE (6, 12, 24 and 48 mg/kg) and saline were injected ip to another five independent groups of the mice and 20 min later, locomotor activity was measured for 30 min.

In further study, HPE (6-24 mg/kg) was administered to another group of mice 20 min before nicotine (1 mg/kg) injections and locomotor activity was recorded for 30 min immediately after nicotine administration.

Locomotor activity system (MAY 9908 model – Activity Monitoring System – Commat Ltd., TR) had eight Plexiglas cages (42 x 42 x 30 cm) equipped with infrared photocells. Locomotor activity was recorded as a total of horizontal, vertical and ambulatory activities of the mice.

Statistics

Data were evaluated by analysis of variance (one-way ANOVA) followed by Dunnett's test. The level of statistical significance was set at $p< 0.05$.

RESULTS

Nicotine (1 mg/kg) produced a significant increase in locomotor activity of the mice [$F(3,28)= 6,207$; $p= 0.002$, one-way ANOVA; $p= 0.04$, Dunnett's test for 1 mg/kg)] (Figure 1).

HPE depressed locomotor activity of the mice significantly at a dose of 48 mg/kg [$F(3,28)= 3,996$; $p=0.009$, one-way ANOVA; $p= 0.016$, Dunnett's test for 48 mg/kg). Dunnett's test following ANOVA revealed no significant change in the locomotor activities by the other doses of HPE (6, 12 and 24 mg/kg) ($p= 0.975$, $p= 0.982$ and $p= 0.250$, respectively) (Figure 2).

HPE (6-24 mg/kg) produces some inhibitory effects on nicotine (1 mg/kg)-induced locomotor hyperactivity [$F(3,28)= 3.519$; $p= 0.028$, one-way ANOVA]. Dunnett's test following ANOVA revealed a significant blockage in nicotine-induced locomotor hyperactivity by HPE (24 mg/kg) ($p= 0.017$) (Figure 3).

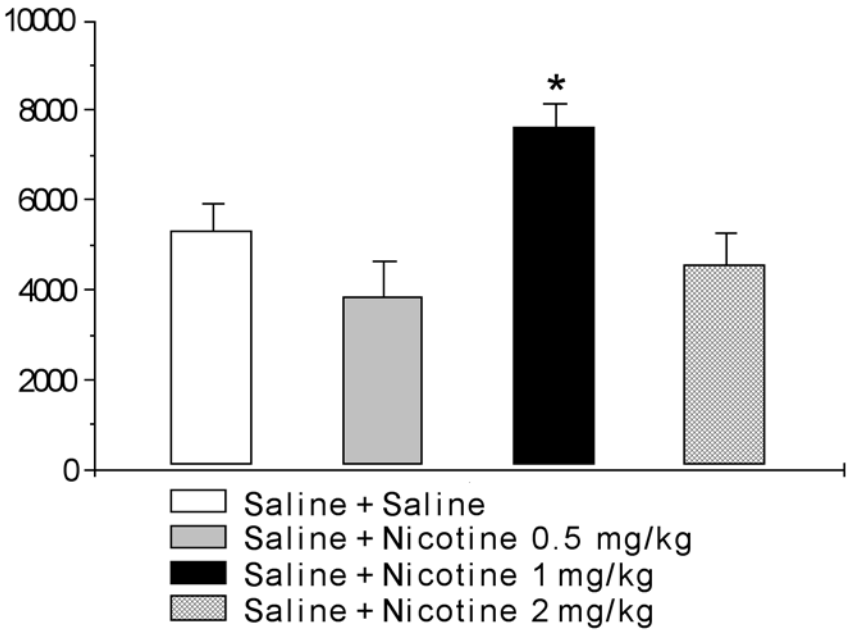

Figure 1- Effects of saline and nicotine on locomotor activities of the mice (* significantly different from the saline group; p= 0.04, Dunnett's test, n= 8 for each group).

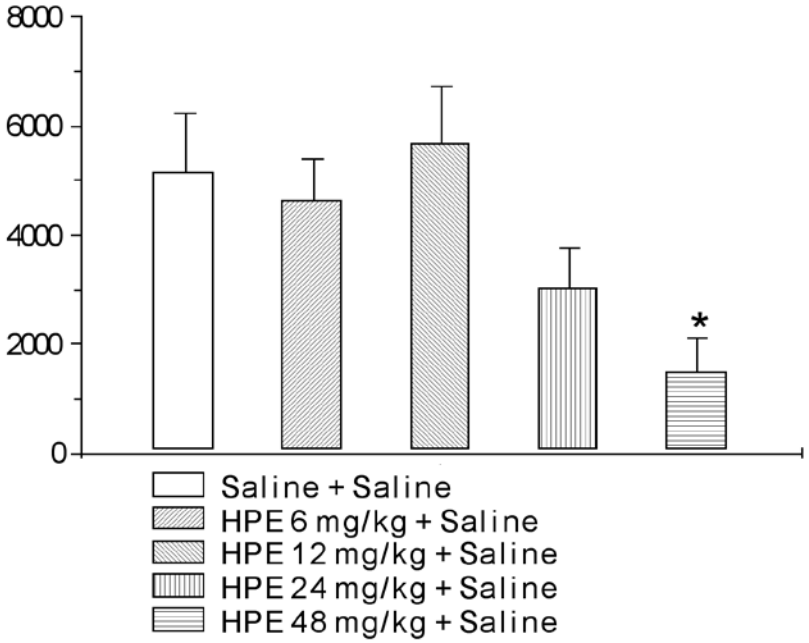

Figure 2- Effects of saline and *Hypericum perforatum* extract (HPE) on locomotor activities of the mice (* Significantly different from the saline group; p=0.016, Dunnett's test, n=8 for each group).

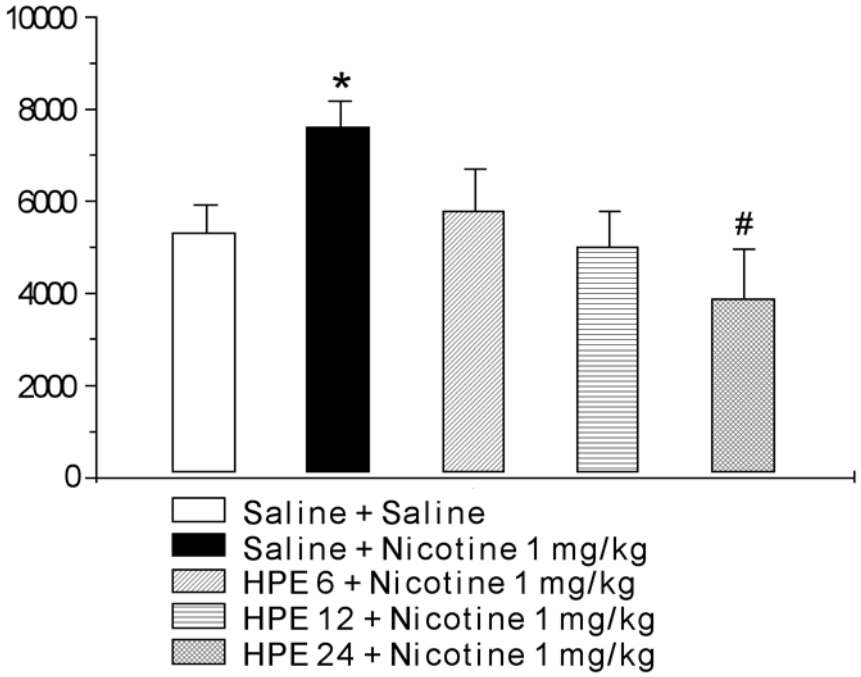

Figure 3- Effects of *Hypericum perforatum* extract (HPE) (6-24 mg/kg) on nicotine-induced locomotor activity in mice (* Significantly different from the saline group, p= 0.011, Student's t test; # significantly different from nicotine 1 mg/kg treated group, p= 0.017, Dunnett's test, n=8 for each group).

DISCUSSION

The results of the present study indicate that nicotine-induced locomotor activity was blocked by HPE in mice. In addition, HPE did not cause any significant effect on locomotor activity at used doses when they were administered alone. Thus, these findings imply that HPE acts through its effect rather than some non-specific, or unrelated effects such as sedation or muscle relaxation.

Consistent with our previous studies (16,17), we observed significant increases in locomotor activity in mice with nicotine (1 mg/kg, ip) treatment. Higher doses of nicotine was not selected and tested since they caused sedation. The doses of HPE were selected from our preliminary experiments and other studies. As it was shown previously, at doses between 6 and 24 mg/kg, HPE blocked caffeine-induced locomotor activity in mice similarly (11). A cross-locomotor sensitization between caffeine and nicotine was also detected in our laboratory (18). In the present study, higher doses than 24 mg/kg of HPE were not preferred in testing since the doses caused a significant inhibition in locomotor activity of the mice.

Evidence regarding the association between depression and tobacco smoking is emerging (19). In particular, it has been postulated that nicotine withdrawal in smokers may elicit a state in which they are more sensitive to the adverse effects of stress (20). Morever, bupropion, an antidepressant agent, has been recently marketed as an aid for smoking cessation (12). Some recent studies also

indicated that HPE, an antidepressant agent, have some beneficial effects on ethanol intake (8,9) and withdrawal syndrome (10), and caffeine-induced locomotor activity (11) in rodents. As more important, in a recent study, Catania et al. (13) showed that HPE attenuates nicotine withdrawal signs in mice. Overall the data implies that HPE may be useful for treatment of drug abuse or dependence and our data indicating that HPE inhibits nicotine-induced locomotor hyperactivity is in line with the results of these previous studies. Our finding may be important since there is a direct relationship between locomotor stimulant properties of drugs and their addictive potential (14).

The inhibitory effects of HPE on nicotine-induced locomotor hypreactivity can be explained by two different mechanisms. First, HPE are widely used for therapy of depression and the mechanism of its antidepressant action seems to imply, as with bupropion, an increase in deficient neurotransmitter activity associated with the pathogenesis of this disorder. Acute administration of HPE produces an increase in brain content of neurotransmitters such as noradrenaline, dopamine and serotonin in rodents (21), and deficits in these neurochemicals have been all considered to play a role in the expression of tobacco dependence (22). Thus it is possible to speculate that HPE attenuate the nicotine-induced hyperlocomotion in mice by enhancing the functional tone of these neurotransmitters.

A second explanation may be a central inhibition of nitric oxide synthase (NOS), an enzyme that produces nitric oxide (NO) from precursor L-arginine. It has been hypothesized that there is a marked relationship between NO and substance abuse and dependence (23). Some studies have been shown that NOS inhibitors cause a prominent attenuation signs of nicotine withdrawal syndrome in rats (24) and blocks nicotine-induced locomotor sensitization in mice (17). In addition, in a recent preliminary study from our laboratory indicated that HPE blocks caffeine-induced locomotor hyperactivity in mice via a NO related mechanism (11). On the other hand, Wegener et al. (25) suggested that local administration of serotonergic antidepressants significantly decreased hippocampal NOS activity in rat brain. Furthermore, Luo et al. (26) showed that HPE had some NOS inhibitory effects in rats. Thus, NOS inhibitory property of HPE may be responsible its inhibitory effects on nicotine-induced hyperlocomotion in mice.

A point needs to be explained is that a possible pharmacokinetic interaction between nicotine and HPE. Both nicotine and HPE interact with hepatic cytochrome P-450 (CYP) microsomal oxidase enzyme system and a pharmacokinetic interaction with nicotine via CYP system may due to the inhibitory effects of HPE on nicotine-induced hyperlocomotion. Ernst (27) reported that *Hypericum* extracts may roughly double the metabolic activity of CYP. Previous reports on some HPE products have also shown remarkably decreased plasma concentrations of certain co-medicated drugs (28,29). Nicotine or cigarette smoking also acts CYP enzyme system (30). However, no study investigated a pharmacokinetic or pharmacologic interaction between HPE and nicotine or tobacco smoking has been reported yet. Thus, the data on nicotine and HPE interaction is very limited and further studies are necessary for clarification of this situation.

CONCLUSION

In conclusion, this is the first time that a beneficial effect of HPE on nicotine-induced locomotor activity was shown. Our results suggest that HPE blocked nicotine-induced locomotor hyperactivity in mice and it may be a useful agent for treatment of nicotine-type dependence. However, further studies should be performed to understand which specific mechanism is responsible for the effects of HPE on nicotine-induced hyperlocomotion in mice.

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