ORIGINAL ARTICLE

GMJ.2017;6(1):52-60
www.gmj.ir

Received 2017-02-13
Revised 2017-03-1
Accepted 2017-03-8

Effect of *Echinacea Purpurea* Extract on Anxiety-Like Behaviors in Neonatal Rats

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Abstract

Background: The use of herbal products is increased among people wordwide. Regarding lacking evidence on the use of herbal medication in children, this study was aimed to determine the effects of *Echinacea Purpurea* extract on anxiety-like behavior in neonatal rats. **Materials and Methods:** Forty male Wister strain pups in four groups received i.p injections of *E. Purpurea* extract (40, 80, and 250 mg/kg) or saline on 5th to 9th postnatal days. All pups were examined for anxiety test on the 22nd postnatal day in the elevated plus-maze test. During the test, parameters include percentage of open arm time (%OAT), percentage of open arm entry (%OAE), head dipping, rearing and locomotor activity have been measured. **Results:** In the present study, administration of 40, 80 and 250mg/kg doses of the *E. Purpurea* extract revealed a significant decrease in the %OAT compared to saline group ($P<0.01$). The *E. Purpurea* extract decreased head dipping parameters ($P<0.01$) and increased rearing parameters ($P<0.01$). *E. Purpurea* did not significantly change the locomotor activity.**Conclusions:** The results of present study showed that postnatal administration of *E. Purpurea* extract increases anxiety in neonatal rats comparing to control group. **[GMJ.2017;6(1):52-60]**

Keywords: *Echinacea Purpurea*; Anxiety; Elevated Plus-Maze

Introduction

The use of complementary and alternative medicine (CAM) has noticeably increased among people. The potential risk of using common medicine, and individual beliefs about leading a healthy lifestyle have led to the use of CAM among both physicians and patients [1, 2]. These types of alternative therapies will continue to be used if their safety and efficacy are proved. A recent study on the percentage of people using alternative therapies among 46,000 random respondents showed that 35 percent of the respondents take alternative therapies [3]. The CAM is not only used by adult consumers but also by children. Although the exact number of children using alternative therapies is not clear, it has been estimated that more than 10 percent of the human children population use them [4]. Studies that have been conducted on the neurological effects of CAM concentrated on adult consumers and showed that the side effects of using CAM on children and neonates are low [5].

Echinacea purpurea L. is one of the important medicinal plants in the world, belonging to the Asteraceae family. Native Americans used *E. Purpurea* to cure different kinds of inflammatory and immune system illnesses [6]. Current

GMJ
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extracts of *E. Purpurea* are made of its roots, leaves, flowers, and the seeds of different subtypes of the Echinacea family, such as *E. purpurea, E. pallidaand,* and *E. angustifolia* in the United States [7]. *E. Purpurea* preparations are used as anti-inflammatory agents and as therapies for upper respiratory tract and urinary tract infections [8-10]. *E. Purpurea* preparations are also used to prevent respiratory tract infections in children [11]. Despite the extensive use of *E. Purpurea* and all previous research studies, the neurologic effects of *E. Purpurea* are still unknown. Recent studies reported the effects of *E. Purpurea* on anxiety-like behaviors [1].

Of all of the mental disorders that exist, different types of anxiety disorders are the most frequent ones in the 21st century [12]. Anxiety disorders are chronic. These disorders can start from childhood and last until adolescence [13].

In this research, neonatal rats were used to evaluate the anxiety-related effects of E. *purpurea.* The reason for using animal models is that these models, in general, are more accurate and controllable than clinical trials are. For example, factors such as age, gender, environmental impact, sleep cycle, drugs, and doses are carefully under control in animal models. In addition, previous studies proved the age-neurodevelopment relationship between rat neonates and human infants using a model of cross-species neurodevelopment. This cross-species neurodevelopment model is based on estimating a human being's brain growth plate, periventricular germinal matrix composition, neurological expression, patterns, and synapse formation [14,15].

Additionally, giving to a human child a drug whose side effects we know little about is clearly unacceptable. Therefore, using an animal model is an appropriate way of evaluating possible side effects, such as anxiety, of Echinacea's oral administration in neonates.

In the current study, we used elevated plus maze (EPM) test for evaluating anxiety level. The EPM apparatus is designed based on the avoidance of open spaces and the preference for closed spaces in rodents. The numbers and results gained through the EPM will significantly change under the influence of anxiogenic and anxiolytic drugs [16]. The aim of this study was to determine the effects of *E. Purpurea* on the anxiety of neonatal rats by the EPM test.

Materials and Methods

Drugs and Reagents

Normal saline and hydro-ethanoic extract of *E. Purpurea* (product number: 16-529009) were purchased from (Zardband Co, Iran).

Animals

Male and female neonatal Wistar rats, weighing about 200gr, were purchased from Herbal Pharmacology Research Center, Tehran Medical Sciences Branch. The study was approved by Ethics Committee of Tehran Medical Sciences Branch, Islamic Azad University with (Code: IR.IAU.TMU.REC.1395.60). Rats were housed with free approachability to rat chow and tap water, in room temperature (25± 2˚C) and on a 12-hour light: dark cycle. All rules applying to animal safety and care were under control. Rats with signs of scars and skin problems were excluded.

Study Design

Female rats were mated using a trio mating system (2 females: 1 male). Assuming a 21-day-long pregnancy period, two female pregnant rats were housed in one cage until the 18th day of gestation. After 18 days, each pregnant rats was housed in a separate cage. They were checked twice a day until the pups were born. The day of birth was called 1st postnatal day (PD1). Neonatal rats in the treatment groups and the control group received intraperitoneal (i.p) injections of *E. Purpurea* (40, 80, and 250 mg/kg) or sterile normal saline in a 5-day period (PD5 – PD9). Neonatal rats were separated from their mothers on PD21, and they were housed in new cages in groups of four. All neonatal rats were tested on the EPM apparatus on PD22. The protocol of drug i.p. injection in neonatal rats has been used in other studies, such as Kamphuis *et al*. and Velisek *et al*. [17, 18]. Also, in the current study, programming the EPM test on PD22 was based on the experiments of Velisek *et al*. [18]

EPM Test

One of the most common tests for the study and evaluation of anxiety in rodents is the EPM [1]. This test is based on rodents' avoidance of height and open spaces and their tendency to prefer closed places. This wooden appliance is made of four arms in the shape of a plus sign (+). Two of the arms without any side or end walls are called open arms (50×10). To prevent neonatal rats from falling the open arm, we attached a 1cm high glass wall at the end of the open arm. Each of the other two arms has two side walls, an end wall, and an open top, which are called closed arms (50×10×40). These four arms are connected through a squared platform called the central area (10×10). The maze is elevated with metal bases to a height of 50 cm.

One 100-watt lamp provides appropriate light at the height of 120 cm over the central area of the maze. Rats are placed in one of the open arms, facing the central area of the maze. The rat has 5 minutes to move freely and investigate different parts of the maze. Special parameters, such as the percentage of open arm time (%OAT), the percentage of open arm entry (%OAE), rearing, head dipping, and so on, are observed and recorded. For each rat, %OAT and %OAE are calculated as follows:

%OAE = ratio of entries into open arms to total entries × 100

%OAT= ratio of time spent in the open arms of total time spent in any arms × 100

A significant increase in these two parameters represents a decrease in the anxiety levels of the rodents.

In comparing the importance of these two parameters in recording the anxiogenic and anxiolytic behaviors, the %OAE is less sensitive than the %OAT. Other behavioral parameters are evaluated and recorded, too.

All of the EPM tests were conducted in an area with sound insulation.

Statistical Analysis

Data were presented as the mean±S.E.M. All statistical analysis was made with SPSS version 23, using one-way analysis of variance (ANOVA).

The $P< 0.05$ was set as the significance level.

Results

Figure-1 shows the effects of *E. Purpurea* on %OAT in the EPM. A one-way ANOVA revealed that *E. Purpurea* (40, 80 and 250 mg/kg) decreased %OAT ($F (3,36) = 3.78$, $P=0.01$). No significant change in the %OAE ($F (3,36) = 0.52$, $P > 0.05$) and locomotor activity ($F (3,36) = 0.67$, $P > 0.05$) was observed following administration.

The data showed that the *E. Purpurea* induced

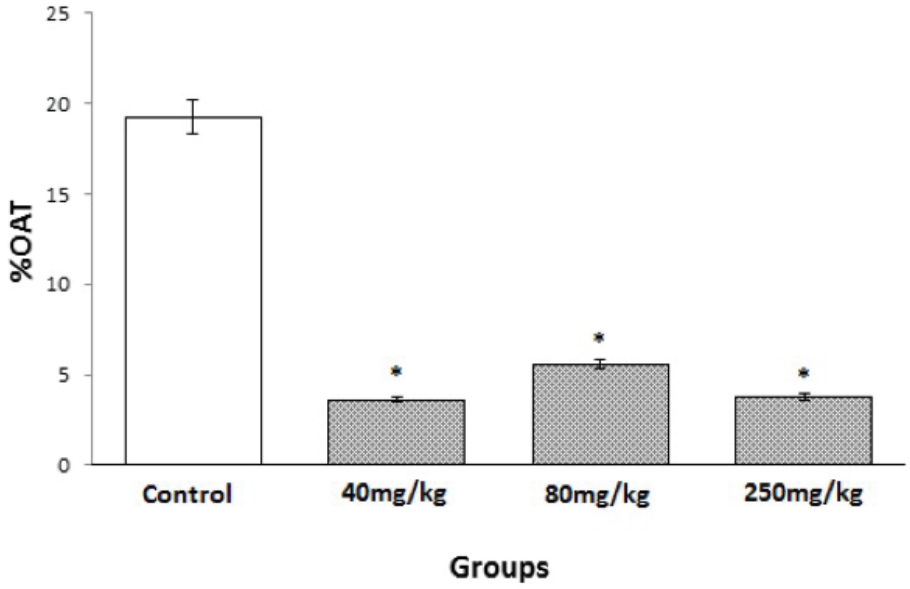

Figure1. Effects of i.p injection of *E. Purpurea* preparations on %OAT comparing to control group, which received saline i.p injection. Each bar represents mean± S.E.M. One-way ANOVA and post hot analysis showed *E. Purpurea* administration was significantly decreased %OAT. *P≤0.05 vs. control group.

the anxiolytic-like effect by different dose.
Figure-2 shows the effects of *E. Purpurea* on the head dipping number in the EPM. In the animals that received *E. purpurea*, one-way ANOVA revealed a significantly decreased head dipping count ($F(3, 36) = 6.12$, $P = 0.002$), indicating an anxiolytic response.
Rearing was assessed following the injection of E. purpurea. A one-way ANOVA revealed that *E. Purpurea* (40 mg/kg) increased in its rearing behavior ($F(3,36) = 21.98$, $P < 0.001$).

Discussion

In this research, the effects of *E. Purpurea* on anxiety in neonatal rats was evaluated. EPM is known as a standard model for studying anxiety in rodents [8]. In the present study, the i.p. administration of *E. Purpurea* extract at different doses significantly reduced %OAT compared to the saline group. Also, the rate of head dipping in the *E. Purpurea* group decreased significantly in comparison with the control group. Takeda *et al*. proved that the head dipping parameter could be used as an evaluating factor for anxiety, and a decrease in head dipping means an increase in anxiety [19]. In addition, rearing increased significantly in the *E. Purpurea* group (dose of 40 mg/kg). Different opinions exist on the relationship between rearing and anxiety. Some researchers, such as Broderick *et al*. showed the increase of rearing as a sign of a decrease in anxiety [20], whereas Wellberg *et al*. considered it to be a sign of increased anxiety [21]. Dielenberg *et al*. [22] and McGregor *et al*. [23] demonstrated that an increase in rearing is accompanied by enhanced blood pressure and decreased locomotor activity in rats. These findings support the relationship between increased rearing and increased anxiety. In our study, we evaluated the rearing parameter's result according to this conclusion. In the current investigation, decreased %OAT, head dipping, and increased rearing is pieces of evidence of increased anxiety in the *E. Purpurea* group compared to the control group.
Previous studies represented the anxiolytic effects of *E. Purpurea* on rats [1]. Haller *et al*. demonstrated that receiving low doses of Echinacea (3- 8 mg/kg) will decrease anxiety levels in rats [1]. However, most of the anxiolytic CAMs are used at higher doses compared to the dosage used in Haller *et al*. study [22]. The doses used in our experiment were 40, 80,

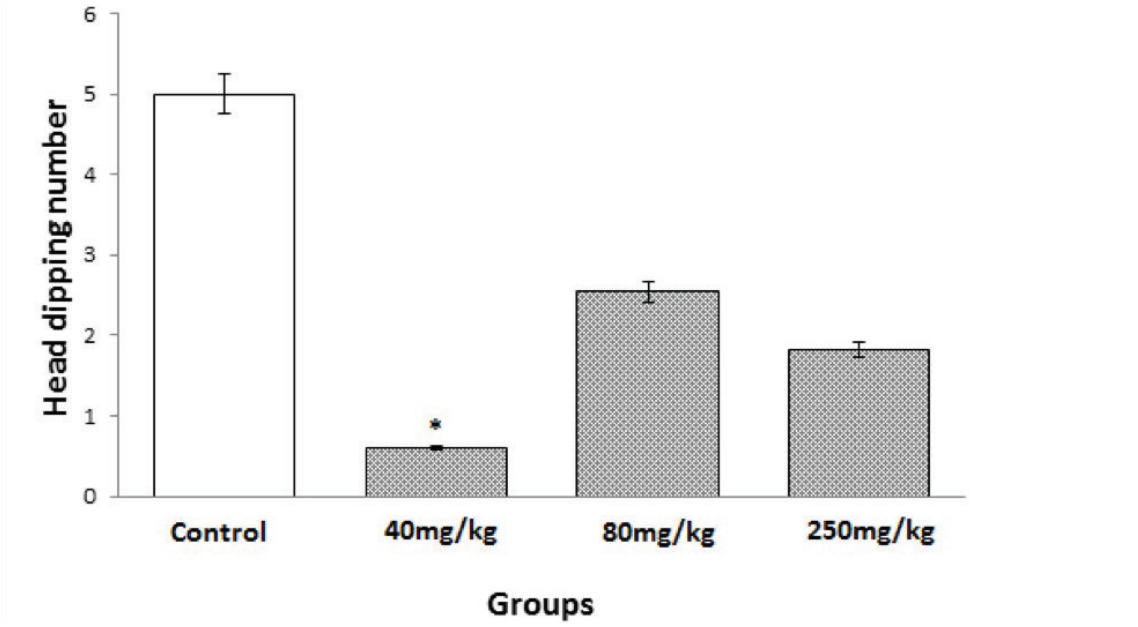

Figure 2. Effects of i.p injection of *E. Purpurea* preparations on head dipping number comparing to control group, which received saline i.p injection. Each bar represents mean± S.E.M. One-way ANOVA and post hot analysis showed *E. Purpurea* administration was significantly decreased head dipping number. *P≤0.05 vs. control group.

and 250 mg/kg. In fact, the safety and side effects of herbal medicine depend on the dosage and the frequency of using it [25].

In addition, as the most important difference, we examined the effects of *E. Purpurea* on neonatal rats in our study, whereas Haller *et al*. studied the effects of this herb on adult rats. It seems that, with the evolution of the neural system in the brain, the effects of neural mediators might change.

Most people believe that the *E. Purpurea* herb is an anxiolytic agent and a strong booster of the immune system. Therefore, a different preparation of *E. Purpurea* is used for the treatment of infections and inflammation of the upper respiratory tract [7, 8, 26].

Due to the effective products in the Echinacea plant, we can relate the effects of Echinacea to anxiety, to alkamides, and to the activation of cannabinoid (CB) receptors.

To explain the mechanism of strengthening the immune system in *E. Purpurea*, it is mentioned that specific alkamides in the *E. Purpurea* attach to CB type-2 (CB2) receptor, which mainly exists in the immune system. Some other alkamides in *E. Purpurea* bind to the CB1 receptor, which exists in neurons, and these alkamides affect the inhibition of the fatty acid amide hydrolase (FAAH) enzyme. The FAAH enzyme's role is to decrease the number of endocannabinoid anandamide [27]. Hence, the activation of CB1 receptors and the inhibition of FAAH both affect anxiety. It is hypothesized that *E. Purpurea* preparations can affect anxiety-like behaviors in laboratory animals [24].

According to the information mentioned above, it is assumed that part of the behavioral effects of *E. Purpurea* is related to the dosages and number of the medications used, and the other is associated with the different alkamides available in various preparations. Behavioral effects can appear according to the synergic and antagonistic features of different alkamides [1].

A mammal's tissues contain at least two types of CB receptors called that CB1 and CB2, and also G proteins. The CB1 receptors are mainly found in the synaptic ends of neurons and inhibit other neurotransmitters [28].

The CBs are one of the most important elements in the *E. purpurea*, which are involved in different behavioral processes, such as memory, cognition, anxiety, appetite, inflammation, vomiting, and immune response. The CB agonists have dual effects. As an example, they can cause hyperactivity in high doses and mobility disorders in low doses [29].

The CBs produce complex and different behavioral effects caused by various neural connections.

The interactions among CBs, dopamine, acetylcholine, opioids and the GABAergic system in the brain are studied [18]. Another important point about substances inside E. purpurea, such as rosmarinic acid, cafeic acid, and alkamide, is that these chemicals can easily pass through the blood-brain barrier [1, 26, 30, 31].

In our study, anxiety level's changes in neonatal rats were examined. Anxiety-like behaviors are all controlled in specific segments of the brain. Products such as *E. purpurea*, which contain alkamides, can easily pass through the blood-brain barrier and affect the anxiety related centers in the brain.

The CB agonists can cause complex and controversial effects on anxiety levels in both humans and laboratory animals. Information gained from animal studies shows the evidence of controversial effects and dose depending on the consequences of the CB system on anxiety, and it also reveals the effect of the environmental impact on the CB system. It seems that although the CB system affects anxiety-like behaviors, it involves CB1 and CB2 receptors too. Some parts of the brain that have a direct impact on anxiety levels, such as the amygdala, hippocampus, and brain cortex, have a large accumulation of CB1 receptors.

Mutated rats without CB1 receptors revealed signs of depression and significant changes in adrenocortical function, in different anxiety tests. The pharmacological blockage of the CB1 receptor can cause anxiety in rats, and the inhibition of anandamides' metabolism leads to anxiolytic behaviors.

In conclusion, endocannabinoid system plays a significant role in adjusting moods and feelings [32].

Generally, low doses of CBs have anxiolytic effects, and high doses of them mainly have

anxiogenic effects [33]. Although the available information clearly expresses that CB signals play a role in controlling anxiety, considering the precise function of these signals is difficult. Indeed, both the anxiogenic and anxiolytic effects of CB receptor agonists at low and high doses [33], and the CB1 receptor antagonist [34, 35] are reported. Controversial effects on anxiety levels are not just limited to *E. Purpurea* among all of the herbal medicines.

Kennedi *et al*. studied the effects of *Melissa officinalis* (Lemon balm) and *Valeriana officinalis* (Valerian) on a group of volunteers. These two herbs are known for their anxiolytic effects.

Although low doses of these two herbs caused anxiolytic results in volunteers, a high dose led to an increased level of anxiety compared to the placebo group [36].

These results regarding *M. officinalis* and *V. officinalis* showed the same dose-related controversial behavioral effect that we observed in *E. purpurea.*

Although the relation between consuming *E. Purpurea* preparations (containing alkamides that activate CB1 receptors) and behavioral reactions are not directly dose dependent, controversial effects are observed [37].

Most people using CAM in the study believed that herbal medicine is safer and has fewer side effects compared to chemical drugs in general.

This standpoint will affect the patient and physician's engagement and will increase intractably used drugs and medications among patients, without the consultations of physicians.

This type of unconscious use of medications without having information about the possible side effects has been observed in other statistical studies [38, 39].

The false belief can cause this information that herbal medicine is safe and healthy just because it is natural [40].

Certainly, parents who have these types of beliefs will tend to give herbal medicine to their children. In some cases of herbal medications, there is no support or recommendations from scientific, clinical evidence [7, 41].

Physicians and healthcare providers do not need to eliminate the use of herbal medicines completely.

Rather, they need to gain more information about the details of materials inside each plant to provide a more useful and complete consultation for using CAM.

It is especially important for those patients who are used to using herbal medications. Further research studies are needed for getting more information and making more accurate decisions about *E. Purpurea* as a CAM.

Conclusion

Our data showed that *E. Purpurea* i.p administration in neonatal rats could induce anxiety-kike behaviors.

Also, EPM test showed a decrease in %OAT and head dipping without any changes in locomotor activity and OAE%.

This result reveals the anxiogenic effects *E. Purpurea* in neonatal rats. Finally, further researches are needed for understanding unknown side effects of *E. Purpurea* as a CAM.

Acknowledgements

This study was supported by Herbal Pharmacology Research Center, Tehran Medical Sciences Branch, Islamic Azad University, Tehran, Iran

Conflict of Interest

The authors do not declare any conflict of interests.

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