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Anxiolytic-like Effects of Dichloromethane Extracts of Valerian (DEV) in Adult Male Wistar Rats

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Abstract**—**Anxiety is a common disorder that attacks many people in society and often accompanied by physiological sensations such as tachycardia, chest pain, shortness of breath, insensitivity and etc. The purpose of this study is to characterize the putative anxiolytic-like effects of DEV (dichloromethane extracts of valerian) using the elevated plus maze (EPM) in rats. DEV was dissolved in DMSO and orally administered at different doses to adult male wistar rats, 0.5, 1.5 and 3 hours before behavioral evaluation in an EPM respectively. Control rats were treated with an equal volume of DMSO. Single treatment of DEV (at 0.1,0.2. 0.3, and 0.4 g/kg) significantly increased time-spent and arm entries into open arms of EPM versus control groups ($p<0.05$).However , no changes in the locomotor activity ccured. This result suggests that DEV might prove to be an effective anxiolytic agent.

Keywords**—**Anxiety, Dichloromethane extracts, Valerian, Rat

I. Introduction

ANXIETY and depression are the most frequent mental disorders. More than 20% of adult population suffer from this condition at some time during their life [1]. Anxiety has became a very important area of research interest in psychopharmacology in this decade. This increased interest is the result of a rapid growth of scientific studies and the discovery of new drugs that alter anxiety in animal models [2]. Benzodiazepines have been the most widely used anxiolytics in general practice for many years [3] and are relatively safe drugs for short term treatment of anxiety despite their dependence potential and side effects [3], [4]. Nevertheless, there is considerable interest in development of new anxiolytics. New synthesized compounds as well as drugs derived from traditional herbs may have a possible therapeutic relevance in the treatment of anxiety [3], [5], [6].

Valerian (Valeriana Officinalis *L*.) is an herb that has long been used as a tranquilizer and sleep inducer [7].Multiple substance in the valerian extracts are held responsible for their effects and most important among them are the terponoid esters named valepotriates, their decomposition products, the baldirinals and various components of the essential oil, in particular, the valerenic acid derivatives. The valepotriates exhibit an activity between sedation and tranquilization and are called aequilans, which have a specific dampening effect on the central nervous system [4], [7], [8].

In the present study, effects of DEV administrated and their possible roles in the modulation of anxiety behaviors using elevated plus-maze in rats have been investigated.

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II. Material and Methods

A. Animals

Male Wistar rats from Pasteur Institute (Iran), weighing 180–230 g at the time treatment, were used. The animals were housed four per cage in a room with a 12:12 h light / dark cycle (lights on 07:00 hours) and controlled temperature (23 ± 1°C). They had access to food and water ad libitum and were allowed to adapt to the laboratory conditions for at least 1 week before treatment. Rats were handled about 3 minutes each day prior to behavioral testing. All experiments were performed between 12:00 and 15:00 hours and each rat was tested only once.

B. Elevated Plus-Maze (EPM)

This wooden, plus-shaped apparatus was elevated to a height of 50 cm, and consisted of two 50×10 cm open arms, and two 50×10×50 cm enclosed arms, each with an open roof. The maze was in the center of a quiet and dimly lit room. The rats' behaviour was observed using a mirror that was suspended at an angle above the maze. Behavioural data were collected by a "blind" observer who quietly sat 1 meter behind one of the closed arms of the maze. Five minutes following their respective drug treatment, rats were placed individually in the center of the plus-maze, facing one of the closed arms. The observer measured (1) time spent in the open arms, (2) time spent in the closed arms, (3) number of entries into the open arms, and (4) number of entries into the closed arms during the 5-min test period. An entry was defined as all four paws in the arm. The maze was cleaned with distilled water after each rat was tested. For the purpose of analysis (Degroot et al., 2001; Pellow, 1986; Pellow et al., 1985), open-arm activity was quantified as the amount of time that the rat spent in the open arms relative to the total amount of time spent in any arm (open/total×100), and the number of entries into the open arms was quantified relative to the total number of

entries into any arm (open/total×100). The total number of arms entered, as well as the total number of closed arms entered were used as indexes of general activity [9; 10; 11]

C. Preparation of Extract

Roots of valerian were collected from regional farms in Esfahan province of Iran and Taxonomic identification was performed by dr. Ghahremani Nejad is deposited in herbarium of Tarbiyat Moallem university of Tehran. Roots were dried under dark conditions at room temperature for 2 weeks and then grounded in an electrical mill to obtain particle lesser than 5 mm. 20 g of powdered plant material was homogenized and extracted with 300 mLit dichloromethane for 24 hours and then filtered. The filterate was evaporated to dryness under vacuum at 30 °C. The residue was weighed and dissolved in DMSO and used for treatment [7; 12; 13].

DEV was dissolved in 1 mLit DMSO and orally administrated (gavage) at doses of 0.025, 0.05, 0.1, 0.2, 0.3, 0.4 , 0.5 and 1 g/kg of body weight . Each dose administrated to 3 groups (N=8) of rats, 0.5, 1.5 and 3 hours before behavioral evaluation in an EPM respectively. Control rats were treated with an equal volume of DMSO.

D. Statistical Analysis

Since data displayed normality of distribution and homogeneity of variance, one-way ANOVA was used for comparison between the effects of different doses of drugs with vehicle.

III. RESULTS

Fig.1 shows the effect of DEV (0.1 g/kg), 0.5, 1.5 and 3 hours after oral administration in the elevated plus-maze in rats. One-way ANOVA revealed that DEV increased time spent in the open arms (%OAT)(0.5 and 1.5 hours after oral administration) and number of entries into the open arms (%OAE) at the doses of 0.1 g/kg, indicating the induction of anxiolytic response by DEV. No change in the locomotor activity was observed.

Oral administration of DEV (0.2 g/kg) produced clear anxiolytic effects in the elevated plus-maze. Rats treated with DEV (0.2 g/kg) showed a significantly greater percentage of %OAT and %OAE than control rats. No change in the locomotor activity was observed (fig. 2)

Fig.3 shows the anxiolytic-like effect of DEV (0.3 g/kg) in the elevated plus-maze in rats (0.5, 1.5 and 3 hours after oral administration). One-way ANOVA revealed that DEV increased time spent in the open arms (%OAT) and number of entries into the open arms (%OAE) at the dose of 0.3 g/kg, indicating the induction of anxiolytic response by DEV. No change in the locomotor activity was observed.

Fig.4 shows the behavioral effect of DEV (0.4 g/kg) in the elevated plus-maze in rats (0.5, 1.5 and 3 hours after oral administration). One-way ANOVA revealed that DEV at dose of 0.4 g/kg have no significant effect on time spent in the open arms (%OAT), This dose increase number of entries into the open arms (%OAE) 1.5 hours after oral administration. No change in the locomotor activity was observed.

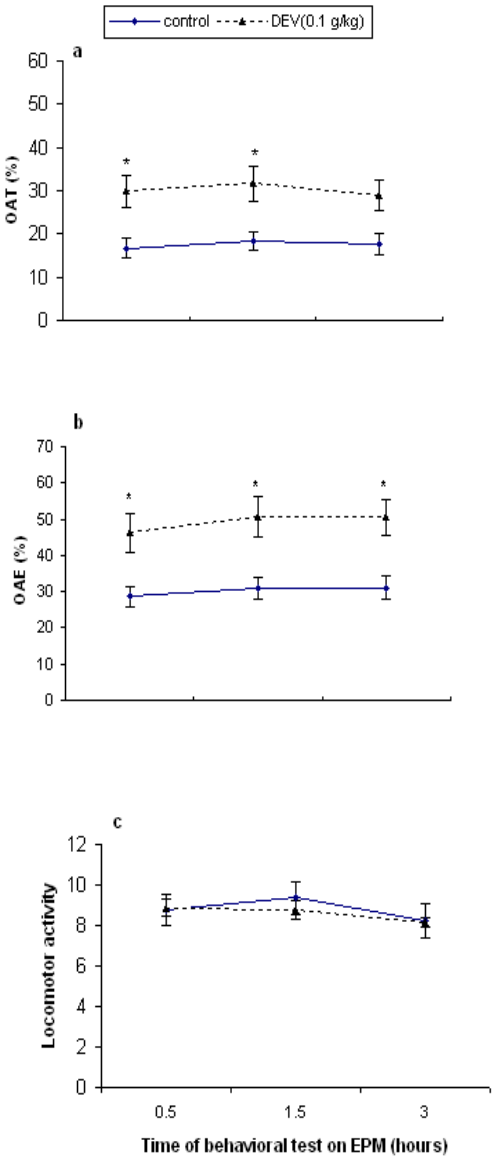

Fig. 1 Effects of DEV, 0.5, 1.5 and 3 hours after oral administration in the elevated plus-maze. Rats were treated with either DMSO (1 ml/rat) or with DEV (0.1 g /kg). Each bar is mean ± S.E.M. %Open Arm Time (a), %Open Arm Entries (b) or locomotor activity (c). $N = 8$. *$P < 0.05$, when compared to the DMSO treated control rats.

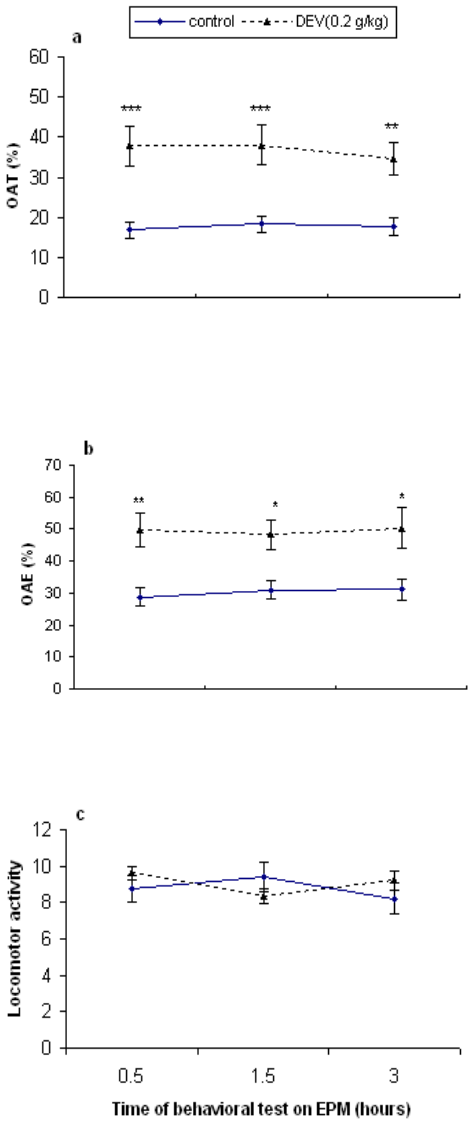

Fig. 2 Effects of DEV at dose of 0.2 g/kg (0.5, 1.5 and 3 hours after oral administration) in the elevated plus-maze. Rats were treated with either DMSO (1 ml/rat) or with DEV (0.2 g /kg). Each bar is mean ± S.E.M. %Open Arm Time (a), %Open Arm Entries (b) or locomotor activity (c). $N = 8$. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ when compared to the DMSO treated control rats.

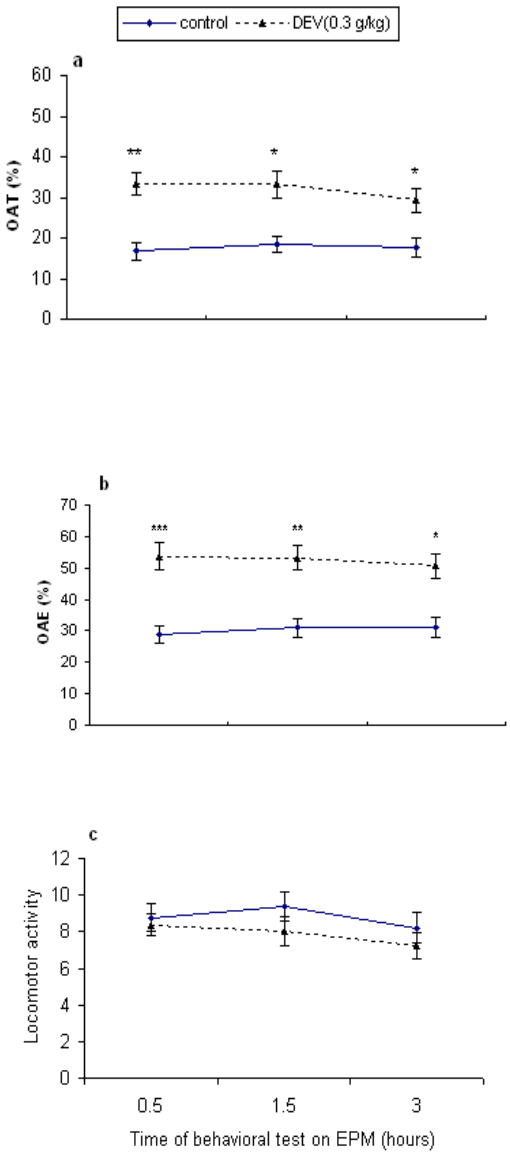

Fig. 3 Effects of DEV at dose of 0.3 g/kg (0.5, 1.5 and 3 hours after oral administration) in the elevated plus-maze. Rats were treated with either DMSO (1 ml/rat) or with DEV (0.3 g /kg). Each bar is mean ± S.E.M. %Open Arm Time (a), %Open Arm Entries (b) or locomotor activity (c). $N = 8$. *$P < 0.05$, **$P < 0.01$, when compared to the DMSO treated control rats.

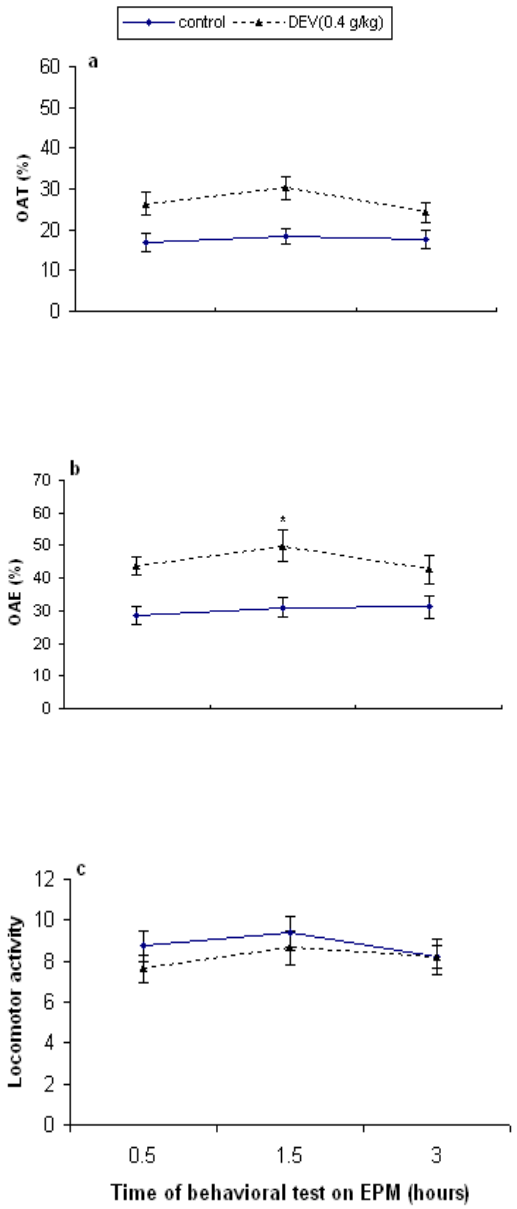

Fig. 4 Effects of DEV at dose of 0.4 g/kg (0.5, 1.5 and 3 hours after oral administration) in the elevated plus-maze. Rats were treated with either DMSO (1 ml/rat) or with DEV (0.4 g /kg). Each bar is mean ± S.E.M. %Open Arm Time (a), %Open Arm Entries (b) or locomotor activity (c). $N = 8$. $^*P < 0.05$ when compared to the DMSO treated control rats.

IV. DISCUSSION

Valerian is one of the most commonly used herbal medicines in Iran that used for the treatment of tachycardia, anxiety and insomnia.

In the present study, the effects of valepotriates extracted from valerian on anxiety behavior in the elevated plus-maze have been investigated.

The elevated plus-maze is one of the many tests for the identification of anxiolytic or anxiogenic effect of a drug in rodents [14]. However, there may be other methods such as the Vogel conflict test [15].

The result of this research shows that DEV (0.1, 0.2 and 0.3 g/kg) increased %OAT (% Open Arm Times) and %OAE (% Open Arm Entries), without locomotor impairment in the elevated plus maze, indicating the induction of anxiolytic-like response by Valerian. Previous studies show that valerian concludes tranquilizing effects and can treat sleep disorders in human [16], [17]. There is not more published information about physiological mechanism of DEV anxiolytic effects.

However, pervious studies show that Valerian or its constituents can induce their effects by interacting with central GABAergic system [18], [19]. Chun-Su Yuan and colleagues suggest that the pharmacological effects of valerian extract and valerenic acid are mediated through modulation of GABAA receptor function [19]. It has been shown that valerian extract, aqueous or hydroalcoholic, ontained GABA and other amino acids that can displace labeled muscimol [18], [20], suggesting that specific constituents of valerian extract can directly bind to GABAA receptors [21], [22]. Valerian extracts can also be responsible for the stimulated release and reuptake of GABA[21]. Aqueous extract of valerian inhibited the uptake and stimulated the release of [3H]GABA, either in the absence or in the presence of K+ depolarization. It is concluded that valerian extract releases [3H]GABA by reversal of the GABA carrier [22]. Derivatives of valerenic acid can also inhibit the local catabolism of GABA by inhibition of the enzyme GABAse, which can also increase GABA concentration [23]. The γ-aminobutyric acid (GABA) is the main inhibitory neurotransmitter, which acts through different receptor sites, termed $GABAA_A$, $GABAB_B$ and $GABAC_C$ [24], [25]. The anxiolytic role of GABAergic system and $GABA_A$ receptors in the limbic system has been well established [26]-[28].

V. CONCLUSION

The results show that DEV have anxiolytic-like effects. Therefore after complementary experiments, it can be used as treatment to anxiety.

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