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Full Length Research paper

Effects of high doses of *Gelsemium sempervirens* L. on GABA receptor and on the cellular and humoral immunity in mice

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In this study, our objective was first to evaluate the anxiolytic-like effect of the acute intraperitoneally administration of high doses (9 CH, 15 CH and 30 CH) of *Gelsemium sempervirens* L. in mouse model of anxiety, the light/dark choice test. Secondly, to evaluate the effect of the acute intraperitoneally administration of these same doses on the cellular and humoral immunity in mice with contrasting level of anxiety evaluated also in the light/dark choice test. Our results showed that all doses of *G. sempervirens*, more particularly the 30 CH, presented a significant anxiolytic-like effect comparable to that obtained by the well known anxiolytic diazepam. On the other hand, flumazenil (a benzodiazepine receptor antagonist) significantly blocked the effects induced by all doses of the homeopathic drug *G. Sempervirens* which also exerted an important effect on the cellular (granulocytes, monocytes, total lymphocytes, $TCD4^+$, $TCD8^+$ and NK cells) and humoral (Ig A, E and G) immunity.

Key words: *Gelsemium sempervirens*, anxiolytic-like, cellular and humoral immunity, light/dark choice test, diazepam, flumazenil.

INTRODUCTION

Anxiety is an aversive emotional state, in which the feeling of fear is disproportionate to the nature of the threat (Weinberger, 2001). In response to threatening situations, the feeling of the emotion that constitutes the subjective feature of anxiety is accompanied by emotional stress, which involves behavioral, expressive and physiological features, such as an avoidance of the source of the danger, assuming defensive postures and an increase in blood pressure, respectively (Weinberger, 2001; Leman et al., 2004).

Anxiety is a normal emotional response to a threat or potential threat. When this emotion is inappropriate, extreme and persistent, it is classified as pathological (Weinberger, 2001; Gross and Hen, 2004). Anxiety is implicated in a number of psychiatric disorders, such as depression, panic attacks, phobias, generalized anxiety disorder, obsessive-compulsive disorder and post-traumatic stress disorder (Gross and Hen, 2004). Anxiety disorders affect approximately 28.8% of the U.S population (Kessler et al., 2005), imposing both an individual and a social burden that amounts to a total cost of $42.3 billion in the U.S. in 1990 (Lépine, 2002). It is estimated that one-eighth of the total population worldwide suffers from inappropriate anxiety (Eisenberg et al., 1998).

Recent scientific work highlighted the bond between the anxiety and the immune alteration (Rammal et al., 2010). Predominantly, the research that has been performed on anxiety has focused on the regulatory systems, including gamma-aminobutyric acidergic (GABAergic) and serotoninergic systems among others. Thus, it appears interesting to evaluate the immune effects in parallel to the neuroactive effects of *G. sempervirens* which is a medicinal plant that possesses many important properties (Valnet, 1992; Peredery and Persinger,2004; Newall et al., 1996) . Currently, very few studies could highlight the biological mechanisms of the neurotropic effects of *G. sempervirens* in animals. In this study, we reported the effect of high doses (9 CH, 15 CH and 30 CH) of *G.*

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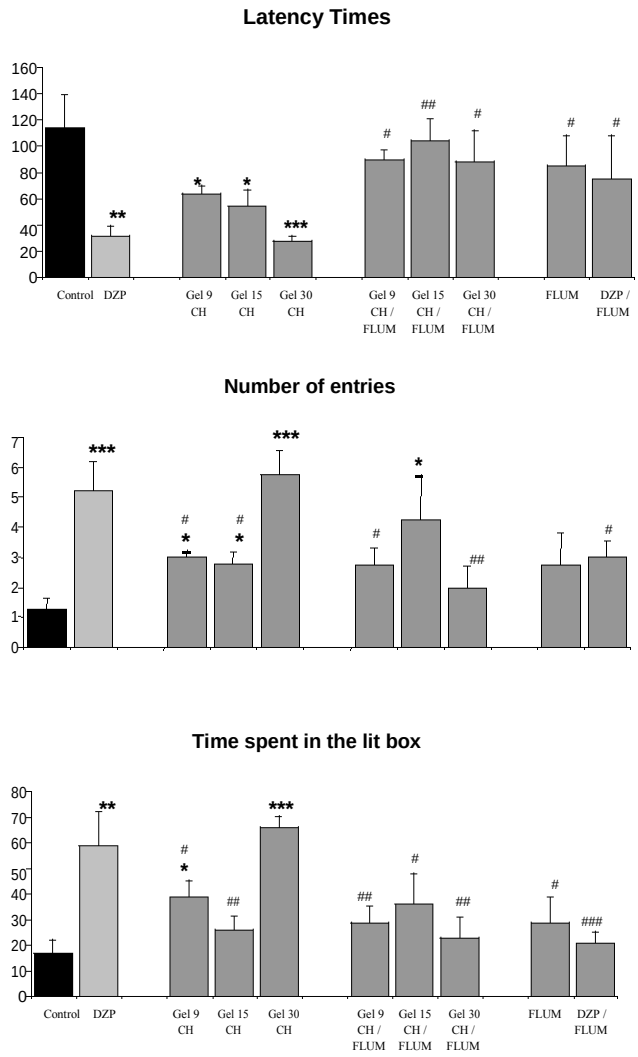

Figure.1. Effects of intraperitoneal administration of high doses of *G. sempervirens* (9 CH, 15 CH and 30 CH), diazepam at 1 mg/kg (Dzp) and administration of the combination of both *G. sempervirens* and flumazenil (5 mg/kg) (Gel/Flum), and diazepam (1 mg/kg) and flumazenil (5 mg/kg) (Dzp/Flum) compared to control on the behavior of Swiss mice in light/dark box test. Data represent mean ± SEM. * in comparison to control group. # in comparison to DZP group. *p<0.05, **p<0.01, ***p<0.001. #p<0.05, ##p<0.01, ###p<0.001.

sempervirens on behavior and on the cellular and humoral immunity in mice and its interaction with GABA receptors. The light/dark choice test was used to estimate the level of anxiety in mice (Crawley and Goodwin 1980; Misslin et al., 1989; Belzung and Le Pape, 1994; Bouayed et al., 2007; Rammal et al., 2008a,b, 2010).

MATERIALS AND METHODS

All animal experiments were in accordance with the European Communities Council Directive of November 24th, 1986 (86/609/EEC). Swiss albino mice male (n=200) weighing 35-40 g were delivered by Charles River in France have been used in this study. We have employed the light/dark choice test described by Crawely and Goodwin (1980). All behavioural tests were carried out as previously described by Bouayed et al. (2007), Rammal et al. (2008a,b, 2010).

Gelsemium sempervirens L. has been provided by BOIRON Laboratories, France. It is prepared from climbing shrub of the family of Loganiaceae. The dying mother is essentially prepared from the root of the plant. Acute (1 day) intraperitoneally injection of different doses of *G. sempervirens* (9 CH, 15 CH and 30 CH 'Centesimal Hahnemaniennes') was done to mice. These doses were prepared by centesimal dose 1:100 (1 ml of product / 99 ml of alcohol) and have been provided by the BOIRON Laboratories, France without any contribution of our laboratory. Control group of mice was intraperitoneally injected with the H_2O dynamized (9 CH, 15 CH and 30 CH) provided also by BOIRON Laboratories, France. The numbers of granulocytes, monocytes and total lymphocytes were evaluated by flow cytometry technique. The characterization of lymphocytes $TCD4^+$, $TCD8^+$, and NK cells was performed using the monoclonal antibodies antiCD4 (PerCP), antiCD8 (PE), and antiNK (FITC) (Elhabazi et al., 2006; Rammal et al., 2010) obtained from Becton Dickinson (BD, USA).

The total immunoglobulin concentrations were determined and quantified in mouse serum by ELISA as previously described by Rammal et al. (2010).

Study design

In this study, a general population of 100 mice was used to evaluate the effect of high doses of *Gelsemium sempervirens* (9 CH, 15 CH and 30 CH) on behavior. Half an hour after the injection of various drugs (*G. sempervirens*, diazepam, and flumazenil), the behavioral study using the light/dark choice test was performed as previously described by Bouayed et al. (2007), Rammal et al. (2008a,b, 2010).

Another population of 100 mice was used to evaluate the effect of *G. sempervirens* on the cellular and humoral immunity. Their level of anxiety was measured by the light/dark choice test. Immediately, anxious and non anxious mice have been treated with different doses of *G. sempervirens* and control injections. Half an hour after the injection, mice were decapitated and their blood were collected and used to measure the various immune parameters.

Statistical analysis

Behavioral data with a Gaussian distribution were analyzed by ANOVA followed by PLSD of Fisher. Data are reported as mean ± S.E.M. Level of significance was set at $p<0.05$.

RESULTS

Results showed significant differences between groups of mice with respect to latency time ($F(9,71)=2.69$; $p=0.0094$), number of transitions ($F(9,71)=3.1$; $p=0.0034$) and the time spent in the lit box ($F(9,71)=4.17$; $p=0.0002$) (Figure 1).

Mice injected with *G. sempervirens* at different doses took significantly less time to leave the dark box, so the latency time was decreased in comparison to control group ($p<0.05$) (Figure 1). Among these doses, the 30 CH was the most effective. Its effect was comparable to

Table 1. Effect of acute administration of high dilutions (9 CH, 15 CH and 30 CH) of *Gelsemium sempervirens* on the cellular immunity (granulocytes, monocytes, total lymphocytes, $TCD4^+$, $TCD8^+$ and NK cells). (n=10). *P<0.05, **P<0.01 between control A and treated A group.

	Groups	Lymphocytes	Granulocytes	Monocytes	CD4	CD8	NK
	Control NA	4048 ± 352	3092 ± 328	171 ± 17	2172 ± 221	500 ± 55	269 ± 24
	Control A	3769 ± 314	3040 ± 294	171 ± 26	2056 ± 180	557 ± 70	320 ± 60
	Gelsemium A	3881 ± 355	2502 ± 322	119 ± 15 *	2029 ± 210	545 ± 58	331 ± 67
9 CH	**Control NA**	4420 ± 355	3232 ± 153	151 ± 18	2174 ± 199	564 ± 70	315 ± 93
9 CH	**Control A**	3548 ± 238	3670 ± 313	194 ± 14	1754 ± 153	513 ± 71	318 ± 49
9 CH	**Gelsemium A**	4183 ± 425 *	2714 ± 312 *	118 ± 12 **	2363 ± 250 *	509 ± 54	175 ± 29 *
15 CH	**Control NA**	5696 ± 479	2032 ± 298	129 ± 15	2772 ± 353	774 ± 99	377 ± 49
15 CH	**Control A**	3924 ± 221	3145 ± 298	155 ± 14	2015 ± 186	606 ± 30	327 ± 42
30 CH	**Gelsemium A**	4187 ± 234	2561 ± 235	133 ± 12	1991 ± 93	604 ± 29	360 ± 70

A: anxious;NA: non anxious

Table 2. Effect of acute administration of high dilutions (9CH, 15CH and 30CH) of *Gelsemium sempervirens* on the humoral immunity (IgA, E and G) in mice. (n=10). *P<0.05, **P<0.01 between control A and treated A group.

	D1					
	IgA		IgE		IgG	
	NA	A	NA	A	NA	A
Control 9CH	30±9	11±0.8	4±0.8	4±1	2±0.1	2.1±0.3
Control 15CH	25.4±5.6	26.3±8	4±0.9	5±1	2.1±0.3	3±0.4
Control 30CH	29±9	21±5	4.3±1.5	4±1	2±0.1	2.4±0.9
Gelsemium 9CH	45.3±15	37.1±11 *	66±34 **	37±18 **	6±3 *	7±3.7 *
Gelsemium 15CH	40.3±12.6	31±9	28±9.8 **	23.1±9 **	8±4.5	6.4±3.4
Gelsemium 30CH	24±5	18±2.7	18.2±4.5 **	18±6 **	5.4±2 **	6±1.8 **

A: anxious:NA: non anxious

that of diazepam. This decrease was significantly blocked by flumazenil (p<0.05), an antagonist of GABA receptor (Figure 1). On the other hand, these mice had done significantly more transitions and have spent significantly more time in the lit box in comparison to control group (Figure 1). In the same time, this increase was significantly blocked by flumazenil.

The dose 15 CH of *G. sempervirens* has induced a significant increase of the number of total lymphocytes, $TCD4^+$, and the IgE concentration. However, it has significantly diminished the numbers of the granulocytes, monocytes and NK cells in comparison to the control anxious group (Table 1). The dose 9 CH has significantly increased the immunoglobulin (IgA and IgG) concentrations. In the same time, the dose 30 CH has significantly increased the IgE and the IgG concentrations (Table 2).

DISCUSSION

Since homeopathic preparations are much diluted mixtures of natural substances, they are completely safe and without undesirable side-effects. In the United States, homeopathic preparations are currently regulated by the FDA as over-the-counter medications. It is well known that the therapeutic effects and the lack of side effects of natural products and substances depend upon their unique biochemical matrix, and the synergistic actions of the multiple constituents within each natural extract (Hoffman, 1991; Serrentino, 1991). These effects are often difficult to mimic with a single, isolated and pure pharmaceutical substance (Heinermann, 1984; Mills, 1993). In this context, we have chosen to evaluate the neuroactive and immunoactive properties of the *G. sempervirens* and not its pure molecules. Firstly, we have found that the well-known anxiolytic diazepam at 1 mg/kg and *G. sempervirens* caused a similar spectrum of behavioral change compared to the vehicle. The both increased the number of transitions from the dark to the lit box; the cumulative time spent in the lit box and decreased the latency time. According to effects obtained on anxiety-related behavior in mice at different doses more particularly at the dose 30 CH, our results suggest that *G. sempervirens* displays an anxiolytic-like effect. These results agree with others that showed that a decrease in transitions between the light and dark box is considered to be indicative of increased anxiety-like behavior (Jones et al., 1988; Costall et al., 1989; Dailly et al., 2002), and an increase in the time spent in the lit box and an increase in the number of transitions are all viewed as indices of reduced anxiety (Clement and Chapouthier, 1998). The results showed that *G. sempervirens* acts on the benzodiazepine receptors since its effect has been significantly blocked by the flumazenil. Secondly, *G. sempervirens* showed an important immunoactive effect. The dose 15 CH has induced an important effect on the cellular and humoral (IgE) immunity. Our results showed that this dose has inversed the effect of anxiety on the granulocytes and monocytes. The dose 9 CH has acted on the monocytes only. However, it has significantly stimulated the immunoglobulin (A, E and G) concentrations. The dose 30 CH has significantly stimulated the immunoglobulins (E and G). All these results are in agree with those obtained by Bousta (2001) who demonstrated the anxiolytic effect of *G. sempervirens* in stressed mice. These results enable us to conclude that the anti- stress effect of *Gelsemium* would be mainly central. In the same time, our results enable us to suppose that the observed immunoactive effect of *G. sempervirens* may be due to its anxiolytic-like effect. This conclusion has been proved by a recent scientific work highlighted the bond between the anxiety and the immune alteration (Rammal et al., 2010). In a future study, we will evaluate the effect of *Gelsemium sempervirens* on the oxidative status of the central (neurons and glial cells) and peripheral (leukocytes) systems of mice with high level of anxiety to show if this homeopathic medicine, by decreasing the anxiety level, acts on the oxidative status.

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