

Full Length Research paper

Immunoactive property of acute and subacute administration of high dilution of *Gelsemium sempervirens* L., *Histaminum* and *Poumon histamine*

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Anxiety is associated with subjective distress and many harmful physiological effects including oxidative stress, immunodepressive effect, and an increase in blood pressure. Anxiety is also implicated in a number of psychiatric disorders. The objective of this work was to study the protective property of homeopathic drugs on immunodepression caused by high anxiety level by evaluating the immunological effect of both acute and subacute intraperitoneally administration of high dilutions (9 CH, 15 CH and 30 CH) of *Gelsemium sempervirens* L., *Histaminum* and *Poumon histamine* in mice with contrasting level of anxiety evaluated in the light/dark choice test. Our results showed that these homeopathic drugs at different dilutions exert an important immunotrope effect on the cellular (granulocytes, monocytes, total lymphocytes, TCD4⁺, TCD8⁺ and NK cells) and humoral (immunoglobulins A, E and G) immunity in anxious mice.

Key words: Anxiety, *Gelsemium sempervirens* L., *Histaminum*, *Poumon histamine*, light/dark choice test, cellular and humoral immunity.

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INTRODUCTION

Anxiety affects one-eighth of the total population world-wide and has become an important area of research in psychopharmacology during this decade (Eisenberg et al., 1998). Anxiety is defined as a feeling of apprehension and fear characterized by physical symptoms such as palpitations, sweating, and feelings of stress. Anxiety causes considerable subjective distress (Ayers et al., 2007), reduces life satisfaction (Brenes et al., 2005) and increases the risk for the onset of disability, even in high-functioning older adults (Seeman et al., 1995). Yet, anxiety continues to be under-recognised, and therefore, under treated (Forsell and Winblad, 1998; van Hout et al., 2004), and is likely to be subacute and unremitting (Livingston et al., 1997). Anxiety most likely becomes pathological when it presents an extreme and persistent character (Vautrin et al., 2005). Our recent findings have

shown that anxious mice have the oxidative stress in their central and peripheral systems (Bouayed et al., 2007a; Rammal et al., 2008a and b). In a recent research, we have also found that anxiety exerts a significant suppressive effect on the cellular and humoral immunity (Rammal et al., 2010a), which leads us to study the action of natural products on the peripheral immune status in anxious mice. Currently, there is an increasing evidence that natural products have beneficial effect on anxiety (Vignes et al., 2006; Bouayed et al., 2007b), oxidative stress (Bouayed et al., 2007b and c) and immunity (Bousta et al., 2001; Elhabazi et al., 2006).

G. sempervirens L. is a medicinal plant which possesses sedative and analgesic properties (Valnet, 1992), it decreases seizures (Peredery and Persinger, 2004), and has potent effects on the central and peripheral nervous system (Newall et al., 1996; Ellingwood, 1919). In addition to its anti-inflammatory properties, it was also traditionally believed to have a beneficial influence in epilepsy and chorea. The main active Compounds of *G. sempervirens* (Demarque et al., 1995)

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are sempervirine, gelsemicine and also gelsemine that have both stimulant and depressant actions on central nervous system (Newall et al., 1996; Ellingwood, 1919).

P. histamine is prepared from the lungs of guinea pigs sacrificed during anaphylactic shock after ovalbumin to advance awareness. It contains several inflammatory and allergic mediators such as histamine and leukotriene involving in the regulation of allergic reaction mechanisms (Demarque et al., 1995).

Histaminum possesses some pharmacological actions as excitement of the smooth muscles of arteries, veins, the bronchioles and the intestinal, dilated capillaries. Also, hyperglycemia, polycythemia, leucopenia and some allopathic uses as anaphylactic shock, neuralgia, subacuterheumatism, gastric ulcer, urticaria (Voisin, 1949) have been reported for *Histaminum*. In homeopathic medicine, *Histaminum* is used to treat the acute hypotension, stomach ulcers, asthma, migraine, angioedema, erythema (Voisin, 1949).

In the present investigation, we evaluate the effect of acute and subacute intraperitoneally administration of high dilutions (9 CH, 15 CH and 30 CH) of three homeopathic drugs (*G. sempervirens*, *P. histamine* and *Histaminum*) on the cellular (granulocytes, monocytes, total lymphocytes, TCD4⁺, TCD8⁺ and NK cells) and humoral (immunoglobulins A, E and G) immunity in mice with contrasting levels of anxiety evaluated using the light/dark choice test (Crawley and Goodwin, 1980; Misslin et al., 1989; Belzung and Le Pape, 1994; Bouayed et al., 2007a,b; Rammal et al., 2008a,b; Rammal et al., 2010a).

MATERIALS AND METHODS

Homeopathic drugs were obtained from BOIRON Laboratories, France. Halothane was obtained from Belmont Laboratories, France. Heparin was obtained from Sanafi- Synthelabo, France. Anti mouse antiCD4, CD8, NK, lysing solution, and CellWASH solution were obtained from Becton Dickinson (BD, USA). The monoclonal IgA, IgE, and anti-IgE were obtained from Interchim, France. The monoclonal IgG, anti-IgG, anti-IgA, Phosphate Buffered Saline (PBS) 1X, and Carbonate- bicarbonate buffer were obtained from Sigma Co., St. Louis, MO, USA. Tween 20 and sulfuric acid were purchased from Roth. Bovine Serum Albumin (BSA) and 3,3',5,5'-Tetramethylbenzidine (TMB) were obtained from Merck, Calbiochem, Germany. The 96-well plates were obtained from Nunclon, Delta, Denmark.

Animals

We used Swiss albino male mice that were nine weeks old at the time of delivery from the breeder (Charles River, France) and ranged in weight from 35 to 40 g. They were housed with a 12 h light: 12 h dark schedule (lights on at 8:00 p.m.) with free access to water and food (SDS Dietex - France) and maintained at a constant temperature (21 ± 2°C) and a relative humidity of 55 ± 10%. Experiments began after a 1-week period of acclimatization. All the procedures applied in the experiment, on these mice, were in accordance with the European Communities Council Directive of

November 24th, 1986 (86/609/EEC).

Light-dark choice test

We have employed the light/dark choice test described by Crawley and Goodwin (1980). The light/dark box apparatus consisted of two compartments (light/dark, surface ratio 3:2), divided into 15 squares (9 x 9 cm). The dark box (black PVC, 27 x 18 x 29 cm) was illuminated by a dim red light (50 Lx) and was divided into six squares. The lit box (white PVC, 27 x 27 x 29 cm) was illuminated by a white light located 1.50 m above the device (150 Lx at the level of the floor) and was divided into nine squares. The two compartments were accessible by means of a small door (7 x 7 cm).

To distinguish between anxious (A) and non-anxious mice (NA), we adopted the method previously described by Rammal et al. (2008a, b) and Rammal et al. (2010a). Briefly, testing was performed in a dark room. After each test, the light/dark box was cleaned with a 10% ethanol solution. Each test was 180 s long. At the beginning of the test, each animal was placed in the lit box with its head facing the door of the dark box. The amount of time spent in the lit box, the number of entries into the lit box (all four feet) and the latency time (latency of the first crossing from the dark to the lit box) were recorded after the first entry into the dark box.

The mice were considered anxious when the latency time was elevated (more than 120 s), the time spent in the lit box was low (from 0 to 14 s) and the number of transitions was weak (from 0 to 2). The mice were considered non-anxious when the latency time was low (from 5 to 30 s), the time spent in the lit box was elevated (from 70 to 100 s) and the number of transitions was high (from 8 to 12).

According to these criteria, only 10% of mice were considered as anxious and 10% as non-anxious from a general population of 100 mice. Thus, 80% of mice with intermediate behavior were eliminated in this study.

Administration of various drugs

Acute (1 day) and subacute (15 days) intraperitoneally administration (ip) of high dilutions of three homeopathic drugs were done to the anxious and non-anxious mice. Control group was intraperitoneally injected the H₂O dynamized.

Immunological study

To determine the numbers of granulocytes, monocytes, total lymphocytes, TCD4⁺, TCD8⁺, and NK cells by flow cytometry technique and the immunoglobulins A, E and G by ELISA, all samples were prepared according to the method previously described by (Rammal et al., 2010a).

Study design

A general population of 200 mice was used to evaluate the effect of each homeopathic drug in this study. Immediately after the separation of the anxious (A) mice (40) from the non-anxious (NA) mice (40) using the light/dark box test, the drugs (homeopathic drug and vehicle) were injected. Half an hour after the administration of various drugs, mice (20 A and 20 NA) were anaesthetized with the halothane and then sacrificed. The rest of mice (20 A and 20 NA) were injected daily at the same time during 15 days with various drugs until D15 where their level of anxiety was evaluated. At D15, immediately after the light/dark box test, the mice were killed and their blood was collected to realize the different immunological

Table 1. The effect of *G. sempervirens* on the leucocytes numbers in mice. (n = 10). *P < 0.05, **P < 0.01 between control A and treated A group.

	Groups	Lymphocytes	Granulocytes	Monocytes	CD4	CD8	NK
9 CH	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
	<i>Gelsemium</i> A	3881 ± 355	2502 ± 322	119 ± 15*	2029 ± 210	545 ± 58	331 ± 67
15 CH	Control NA	4420 ± 355	3232 ± 153	151 ± 18	2174 ± 199	564 ± 70	315 ± 93
	Control A	3548 ± 238	3670 ± 313	194 ± 14	1754 ± 153	513 ± 71	318 ± 49
	<i>Gelsemium</i> A	4183 ± 425*	2714 ± 312*	118 ± 12**	2363 ± 250*	509 ± 54	175 ± 29*
30 CH	Control NA	5696 ± 479	2032 ± 298	129 ± 15	2772 ± 353	774 ± 99	377 ± 49
	Control A	3924 ± 221	3145 ± 298	155 ± 14	2015 ± 186	606 ± 30	327 ± 42
	<i>Gelsemium</i> A	4187 ± 234	2561 ± 235	133 ± 12	1991 ± 93	604 ± 29	360 ± 70
9 CH	Control NA	4303 ± 429	1628 ± 345	146 ± 22	2354 ± 239	596 ± 73	236 ± 27
	Control A	3188 ± 345	2393 ± 249	155 ± 37	1754 ± 169	366 ± 31	228 ± 48
	<i>Gelsemium</i> A	4224 ± 207*	1693 ± 264*	88±7*	2096 ± 119*	574 ± 35**	339 ± 87
15 CH	Control NA	4094 ± 472	3787 ± 556	165 ± 53	2489 ± 311	633 ± 76	235 ± 84
	Control A	3548 ± 238	2966 ± 639	145 ± 22	2142 ± 308	578 ± 70	210 ± 30
	<i>Gelsemium</i> A	4240 ± 423	2744 ± 487	114 ± 23	2306 ± 270	628 ± 92	345 ± 96*
30 CH	Control NA	2710 ± 421	2884 ± 697	154 ± 17	1809 ± 272	442 ± 65	241 ± 38
	Control A	3553 ± 275	2945 ± 500	162 ± 40	1673 ± 277	417 ± 61	258 ± 39
	<i>Gelsemium</i> A	2758 ± 352*	1512 ± 262*	107 ± 23**	2029 ± 206	453 ± 66	266 ± 96

studies (cellular and humoral immunity).

Statistical analysis

Results are presented as mean ± S.E.M. Data that did not conform to a Gaussian distribution were analyzed using a Mann–Whitney *U*-test to compare one group to another. Data that conformed to a Gaussian distribution were analyzed using a Student's *t*-test. Mean differences with *P* < 0.05 were considered statistically significant.

RESULTS

Effect of *G. sempervirens* on the cellular immunity in mice

Acute administration of *G. sempervirens* at the dilution 15 CH has induced a significant effect on the total lymphocytes, TCD4⁺. In the same time, it has significantly prevented the action of anxiety on the granulocytes and monocytes and diminished the level of NK cells (Table 1). Subacute administration of *G. sempervirens* at the dilution 9 CH has induced a significant effect on the granulocytes, total lymphocytes, TCD4⁺ and TCD8⁺. In the same time, it has significantly prevented the action of anxiety on the monocytes (Table 1). The dilution 30 CH

has induced a significant immunoactive effect only on the total lymphocytes. It has significantly prevented the action of anxiety on the monocytes (Table 1).

Effect of *G. sempervirens* on the humoral immunity in mice

Results obtained showed that acute administration of *G. sempervirens* at the dilution 9 CH has significantly increased the immunoglobulin (IgA and IgG) concentrations (*P* < 0.05). The dilution 15 CH has significantly increased the IgE only. In the same time, the 30 CH dilutions have significantly increased the IgE and the IgG (*P* < 0.05) (Table 2).

Subacute administration of the different dilutions of *G. sempervirens* has significantly increased the concentration of the IgE (*P* < 0.05) (Table 2). By the same way, only the dilution 9 CH has significantly increased the IgG concentration (Table 2).

Effect of *Histaminum* on the cellular immunity in mice

Acute administration of *Histaminum* at the dilution 15 CH has induced a significant effect on the TCD4⁺ (Table 3).

Table 2. The effect of *Gelsemium sempervirens* on the humoral immunity in mice. (n=10). *P<0.05, **P<0.01 between control and treated group.

	D1						D15					
	IgA		IgE		IgG		IgA		IgE		IgG	
	NA	A	NA	A	NA	A	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	17.2 ± 3	23 ± 7	6 ± 1	4.3 ± 0.8	2.3 ± 0.1	9.2±4
Control 15CH	25.4 ± 5.6	26.3 ± 8	4±0.9	5 ± 1	2.1 ± 0.3	3±0.4	17.4 ± 2	12 ± 0.9	5 ± 0.8	3.3 ± 0.5	2.5 ± 0.3	2.3 ± 0.3
Control 30CH	29 ± 9	21±5	4.3 ± 1.5	4 ± 1	2±0.1	2.4 ± 0.9	19.2 ± 2	13.4 ± 1.6	5 ± 0.7	4±0.6	2.2 ± 0.3	4±0.9
Gelsemium 9CH	45.3 ± 15	37.1 ± 11*	66 ± 34**	37 ± 18**	6±3*	7 ± 3.7*	47±13	23 ± 6	34.2 ± 14**	13 ± 4**	10±4*	3.2 ± 0.3*
Gelsemium 15CH	40.3 ± 12.6	31±9	28 ± 9.8**	23.1 ± 9**	8±4.5	6.4 ± 3.4	42 ± 13.7	22 ± 5	41 ± 21**	14.1 ± 5**	4±0.6	3.4 ± 0.5
Gelsemium 30CH	24 ± 5	18 ± 2.7	18.2 ± 4.5**	18 ± 6**	5.4 ± 2**	6 ± 1.8**	38 ± 8.6	21.4 ± 8	32.5 ± 15*	16 ± 5**	6±1**	4±0.6

Table 3. The effect of *Histaminum* on the leucocytes numbers in mice. (n = 10). *P < 0.05, **P < 0.01 between control A and treated A group.

	Groups	Lymphocytes	Granulocytes	Monocytes	CD4	CD8	NK	
D1	9 CH	Control NA	4222 ± 307	2666 ± 318	176 ± 18	2143 ± 126	531 ± 39	368 ± 68
		Control A	3684 ± 304	2992 ± 188	174 ± 18	1902 ± 183	564 ± 57	416 ± 33
		<i>Histaminum</i> A	4498 ± 446	1785 ± 169***	127 ± 11*	2563 ± 246*	531 ± 56	280 ± 75*
	15 CH	Control NA	4604 ± 330	2451 ± 226	148 ± 19	2356 ± 165	696 ± 92	475 ± 78
		Control A	4394 ± 132	2737 ± 271	170 ± 19	1895 ± 91	626 ± 58	328 ± 68
		<i>Histaminum</i> A	4682 ± 310	1788 ± 252*	125 ± 11*	2279 ± 155*	595 ± 56	309 ± 44
	30 CH	Control NA	3983 ± 363	3251 ± 227	168 ± 20	2013 ± 194	531 ± 68	301 ± 55
		Control A	4265 ± 235	2616 ± 290	181 ± 34	2148 ± 146	700 ± 65	318 ± 57
		<i>Histaminum</i> A	4672 ± 298	1902 ± 169*	119 ± 10*	2293 ± 126	601 ± 59	333 ± 58
D15	9 CH	Control NA	3825 ± 317	2517 ± 167	163 ± 24	2111 ± 212	568 ± 62	132 ± 18
		Control A	2734 ± 464	3655 ± 675	228 ± 54	1375 ± 197	389 ± 62	176 ± 37
		<i>Histaminum</i> A	4266 ± 434*	1770 ± 298*	131 ± 21*	2339 ± 222**	560 ± 67*	237 ± 48
	15 CH	Control NA	3627 ± 326	2767 ± 606	217 ± 45	1980 ± 234	501 ± 49	220 ± 27
		Control A	3101 ± 382	2949 ± 585	242 ± 92	1706 ± 260	499 ± 74	250 ± 95
		<i>Histaminum</i> A	3840 ± 353	1738 ± 275*	107 ± 13*	2027 ± 149	487 ± 49	235 ± 39
	30 CH	Control NA	4109 ± 447	2805 ± 604	149 ± 18	2121 ± 204	585 ± 66	307 ± 65
		Control A	4024 ± 342	2380 ± 296	204 ± 40	1989 ± 197	595 ± 69	296 ± 53
		<i>Histaminum</i> A	4182 ± 396	1818 ± 349	142 ± 38*	2232 ± 198	528 ± 57	263 ± 60

Table 4. The effect of *Histaminum* on the humoral immunity in mice. (n = 10). *P < 0.05, **P < 0.01 between control and treated group.

	D1						D15					
	IgA		IgE		IgG		IgA		IgE		IgG	
	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A
Control 9CH	21 ± 6	15 ± 1.9	4.1 ± 1	4.2 ± 1.6	2 ± 0.1	2.2 ± 0.3	22.2 ± 5	16.4 ± 3.8	6 ± 1	4.2 ± 1	6 ± 2	3.3 ± 0.5
Control 15CH	14.1 ± 1.6	20 ± 4.8	3.5 ± 1	4 ± 0.9	2 ± 0.3	3 ± 0.5	18.3 ± 2.9	10.1 ± 0.6	5.1 ± 0.8	3.1 ± 0.6	4 ± 0.7	3 ± 0.6
Control 30CH	34 ± 9.8	51 ± 17	4.3 ± 1	4.3 ± 1	2.5 ± 0.4	2 ± 0.1	29.2 ± 6.7	12 ± 1.5	6 ± 0.9	3.4 ± 0.5	6 ± 1	3 ± 0.4
Histaminum 9CH	25 ± 7	21.1 ± 4	24 ± 8**	17.1 ± 4.6**	5 ± 2*	2.3 ± 0.2	28 ± 5.7	47.3 ± 22*	18 ± 6.9*	21 ± 8**	3 ± 0.4	3.4 ± 0.5
Histaminum 15CH	22 ± 3.9*	28 ± 10	19 ± 5**	57.1 ± 35**	5 ± 2*	5 ± 1.5*	47 ± 13.9*	34.2 ± 11*	27.5 ± 10.8*	16 ± 6**	3.4 ± 0.5	5 ± 0.9*
Histaminum 30CH	26.4 ± 7	19.1 ± 3*	24 ± 9.3**	22.1 ± 9**	5.2 ± 2	5 ± 1.9**	39 ± 16	34.5 ± 14	24 ± 9*	62 ± 48*	4 ± 0.6	6 ± 1.6*

In the same time, all dilutions of *Histaminum* have significantly decreased the level of the granulocytes and monocytes. The level of NK cells has significantly been decreased by the dilution 9 CH only (Table 3).

Subacute administration of *Histaminum* at the dilution 9 CH has induced a significant effect on the TCD4⁺ and TCD8⁺. In the same time, it has significantly decreased the level of the granulocytes and the monocytes (Table 3). The dilution 15 CH has induced a significant effect only on the TCD4⁺. It has significantly decreased the level of the granulocytes and the monocytes (Table 3). The dilution 30 CH has induced a significant effect only on the monocytes (Table 3).

Effect of *Histaminum* on the humoral immunity in mice

Acute and subacute administration of *Histaminum* at all dilutions has significantly increased the IgE concentration (P < 0.05) (Table 4). In the same time, the dilutions 15 CH and 30 CH have significantly increased the IgG concentration (P < 0.05) (Table 4).

Acute administration of *Histaminum* at the dilution 30 CH has significantly increased the IgA

concentration (P < 0.05) (Table 4). This has significantly been increased by the subacute administration of the dilutions 9 CH and 15 CH (P < 0.05) (Table 4).

Effect of *Poumon histamine* on the cellular immunity in mice

Acute administration of *P. histamine* at the dilution 15 CH has induced a significant immunotrope effect on the TCD4⁺ and NK cells (Table 5). The dilution 30 CH has induced a significant immunotrope effect only on the NK cells (Table 5). Subacute administration of *P. histamine* at the dilution 9 CH has induced a significant decrease of the level of granulocytes and monocytes (Table 5). The dilution 15 CH has induced a significant immunotrope effect on monocytes. In the same time, it has significantly increased the level of the TCD4⁺ and NK cells and it has significantly prevented the action of anxiety on the granulocytes (Table 5). The dilution 30 CH has induced a significant immunotrope effect only on the total lymphocytes. It has significantly decreased the level of the NK cells (Table 5).

Effect of *Poumon histamine* on the humoral immunity in mice

Acute administration of *P. histamine* at the dilution 15 CH has induced a significant increase of the IgA concentration (P < 0.05) (Table 6). Moreover, all the dilutions have significantly increased the IgE and IgG concentrations (Table 6).

Subacute administration of the dilutions 15 CH and 30 CH of *P. histamine* has significantly increased the IgA and IgG concentrations. In the same, all dilutions of this homeopathic drug have significantly increased the IgE concentration (P < 0.05) (Table 6).

DISCUSSION

Application of mice with different extremes of anxiety permit researcher to have a better insight into anxiety (Vautrin et al., 2005; Krömer et al., 2005; Ditzen et al., 2006; Rammal et al., 2008a,b). Mice in this strain with contrasting levels of anxiety have been selected to evaluate their cellular and humoral immunity after the intra-peritoneally administration of high dilutions of the three homeopathic drugs (*G. sempervirens*, *Histaminum* and *P. histamine*). It is well known

Table 5. The effect of *Poumon histamine* on the leucocytes numbers in mice. (n = 10). *P < 0.05, **P < 0.01 between control A and treated A group.

		Groups	Lymphocytes	Granulocytes	Monocytes	CD4	CD8	NK
9 CH		Control NA	4681 ± 397	2483 ± 337	168 ± 22	2463 ± 211	803 ± 175	294 ± 44
		Control A	4414 ± 264	1771 ± 120	160 ± 22	2407 ± 141	676 ± 82	443 ± 93
		<i>P histamine</i> A	4536 ± 210	1907 ± 224	115 ± 8*	2274 ± 140	541 ± 24	374 ± 51
D1 15 CH		Control NA	4611 ± 343	3183 ± 286	192 ± 25	2375 ± 175	636 ± 68	374 ± 63
		Control A	3888 ± 220	2694 ± 325	160 ± 18	1900 ± 97	631 ± 57	243 ± 53
		<i>P histamine</i> A	4093 ± 336	2619 ± 278	137±8	2188 ± 97*	582 ± 99	382 ± 106*
30 CH		Control NA	4701 ± 218	2523 ± 307	161 ± 29	2332 ± 207	642 ± 44	464 ± 53
		Control A	4224 ± 309	2613 ± 432	143 ± 19	1792 ± 147	544 ± 47	277 ± 27
		<i>P histamine</i> A	4412 ± 307	2452 ± 176	196 ± 39	2125 ± 169	568 ± 51	425 ± 69*
9 CH		Control NA	4080 ± 344	2402 ± 389	181 ± 37	1939 ± 200	585 ± 59	365 ± 52
		Control A	3949 ± 514	2180 ± 479	168 ± 38	2059 ± 298	546 ± 64	235 ± 35
		<i>P histamine</i> A	3957 ± 430	1303 ± 138*	83±6*	2050 ± 196	523 ± 73	329 ± 64
D15 15 CH		Control NA	4473 ± 366	1794 ± 325	132 ± 25	1417 ± 262	858 ± 135	227 ± 27
		Control A	3415 ± 540	3263 ± 651	191 ± 37	1361 ± 171	476 ± 93	181 ± 16
		<i>P histamine</i> A	3609 ± 497	2231 ± 440*	126 ± 19*	2138 ± 257*	514 ± 79	299 ± 68*
30 CH		Control NA	3768 ± 527	1837 ± 171	133 ± 18	1828 ± 231	478 ± 72	317 ± 57
		Control A	2885 ± 536	3916 ± 632	147 ± 28	1505 ± 303	383 ± 54	286 ± 63
		<i>P histamine</i> A	3695 ± 445	2189 ± 359*	122 ± 25	2109 ± 270	525 ± 73	161 ± 23*

that the therapeutic effects 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 study, anxious and non-anxious mice have been employed to have a better insight into the effects of homeopathic drugs on immune problems induced by anxiety. Our results demonstrated that there are significant differences in

the immune system of anxious and non-anxious mice with regards to lymphocytes (TCD4⁺ and TCD8⁺) and immunoglobulins (IgE and IgG), suggesting that high level of anxiety exert immunosuppressive effects on peripheral leucocytes of adaptive immune systems, including cellular and humoral immunity.

In the same time, our findings showed that anxiety has increased the numbers of granulocytes, monocytes and NK cells (Rammal et al., 2010a). Moreover, we have found that the high dilutions of the three homeopathic drugs used in our study

have an important immunoactive property on the cellular and humoral immunity of anxious mice. The subacute administration of the three homeopathic drugs at the dilutions 9 CH and 30 CH was more effective than the acute administration of the same dilutions.

However, the acute administration of *G. sempervirens* at the dilution 15 was effective than the subacute administration. Moreover, *Histaminum* has shown a similar effect in acute and subacute administrations at the dilution 15 CH. Acute administrations of *G. sempervirens*,

Table 6. The effect of *Poumon histamine* on the humoral immunity in mice. (n = 10). *P < 0.05, **P < 0.01 between control and treated group.

	D1						D15					
	IgA		IgE		IgG		IgA		IgE		IgG	
	NA	A	NA	A	NA	A	NA	A	NA	A	NA	A
Control 9CH	20±4	28.4 ± 7	4 ± 1	4.3±1	2.2 ± 0.3	2±0.3	21.5 ± 4	13±1	5.3±1	3.4 ± 0.5	3.4 ± 0.7	3±0.5
Control 15CH	22.1 ± 6	13±2	4.4±1	4 ± 1	2±0.1	2±0.3	16.5 ± 2	14±1	4±0.5	4±0.6	3±0.5	4±0.7
Control 30CH	28±9	16±3	6±2.6	5 ± 2	2.1 ± 0.2	2±0.1	25.5 ± 6	13.3 ± 1	5.3±1	3.1 ± 0.3	5 ± 1	3±0.5
PH 9CH	23.4 ± 6	24±9	22.2 ± 6**	16±5*	5±1**	6±1**	29.3 ± 5	14±2	17 ± 6**	8±2*	4±0.8	3±0.2
PH 15CH	29.5 ± 7	38.5 ± 12*	25.2 ± 9**	45 ± 20**	5.4 ± 2*	7.5 ± 2**	38.3 ± 15*	47.5 ± 17*	19±5*	33 ± 16**	3.4 ± 0.4	6 ± 0.8*
PH 30CH	30±10	34.4 ± 13	35 ± 17**	28.5 ± 11***	5.1 ± 2*	7±2**	40.3 ± 12*	48.5 ± 19**	24.3 ± 10**	13 ± 3**	4±0.8	5.4 ± 1*

Histaminum or *P. histamine* at the dilution 15 CH have shown a protective potential on the TCD4⁺. *G. sempervirens* and *Histaminum* at the dilution 9 CH have shown an immunoactive effect on the TCD4⁺ and TCD8⁺. *G. sempervirens* at different dilutions has demonstrated an immunoprotective property and was able to reverse the effect of anxiety on the granulocytes and monocytes. Concerning the antibody production, the three homeopathic drugs at various dilutions have significantly increased the concentration of the immunoglobulins (A, E and G), and more particularly the IgE. So, we can conclude that these homeopathic drugs have an immunostimulant effect on the immunoglobulins.

We could also make some comments on the interest of protecting our functional systems from alterations induced by high level of anxiety which is associated with oxidative stress (Rammal et al., 2008a; Rammal et al., 2010a,b), by products or natural substances coupling the positive effects on the anxiety and also on oxidative stress. Products whose have double profile neuroactive-antioxidant could have beneficial effects cytoprotective and also preventive (Bouayed et al., 2007b). This is how our works on homeopathic drugs or other natural sub-stances such as

polyphenols have resulted in scientific validation of interest we seemed to be very important for health but also nutrition in relation to the environment.

In summary, the data presented in this study showed that high dilutions (9 CH, 15 CH and 30 CH) of *G. sempervirens*, *Histaminum* and *P. histamine* have an important effect on the immune system of mice with contrasting levels of anxiety. The results showed an immunotrope and immunoprotective properties of the high dilution of these homeopathic drugs. In a future study, we will further evaluate the effect of these homeopathic drugs on the oxidative status in central and peripheral systems.

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