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*Full Length Research paper*

# The 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.

## 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 $24^{th}$, 1986 (86/609/EEC).

### Light-dark choice test

We have employed the light/dark choice test described by Crawely 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 $H_2O$ dynamized.

### Immunological study

To determine the numbers of granulocytes, monocytes, total lumphocytes, $TCD4^+$, $TCD8^+$, 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 |
|---|---|---|---|---|---|---|---|---|
| D1 | 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 |
| | | *Gelsemium* 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 |
| | | *Gelsemium* 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 |
| | | *Gelsemium* A | 4187 ± 234 | 2561 ± 235 | 133 ± 12 | 1991 ± 93 | 604 ± 29 | 360 ± 70 |
| D15 | 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 |
| | | *Gelsemium* 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 |
| | | *Gelsemium* 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 |
| | | *Gelsemium* 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 |
| | | *Histaminum* 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 |
| | | *Histaminum* 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 |
| | | *Histaminum* 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 |
| | | *Histaminum* 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 |
| | | *Histaminum* 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 |
| | | *Histaminum* 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 intraperitoneally 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 |
|---|---|---|---|---|---|---|---|---|
| D1 | 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 |
| | | *P histamine* A | 4536 ± 210 | 1907 ± 224 | 115 ± 8* | 2274 ± 140 | 541 ± 24 | 374 ± 51 |
| | 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 |
| | | *P histamine* 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 |
| | | *P histamine* A | 4412 ± 307 | 2452 ± 176 | 196 ± 39 | 2125 ± 169 | 568 ± 51 | 425 ± 69* |
| D15 | 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 |
| | | *P histamine* A | 3957 ± 430 | 1303 ± 138* | 83±6* | 2050 ± 196 | 523 ± 73 | 329 ± 64 |
| | 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 |
| | | *P histamine* 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 |
| | | *P histamine* 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. simpervirens* at the dilution 15 was effective than the subacute admini -stration. 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 immune-stimulant 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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