Effects of the *Spirulina platensis* and *Panax ginseng* oral supplementation on peripheral blood cells in rats

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**SUMMARY**

The effects of *Spirulina platensis* and *Panax ginseng* dietary supplementations were analysed in 30 female adult Wistar albino rats divided in 3 equal groups. In the first and second groups, animals were treated with *Spirulina platensis* (300 mg/kg/day) and with *Panax ginseng* (400 mg/kg/day) respectively in drinking water for 30 days, whereas the third group served as control. Red Blood Cell (RBC) and White Blood Cell (WBC) counts, enumeration of leukocyte types, Packed Cell Volume (PCV) and haemoglobin concentrations were determined by haemocytometric methods on blood samples collected on days 0, 15 and 30, and Mean Globular Volumes (MGV) and Mean Corpuscular Haemoglobin Concentrations (MCHC) were calculated. Populations of B and T lymphocytes were counted by the *α* naphthyl acetate esterase (ANAE) staining method. *Spirulina platensis* and *Panax ginseng* treatments markedly stimulated the erythrocyte formation and the haemoglobin synthesis on day 30 and small erythrocytes (microcytosis) greatly loaded with haemoglobin (increases of MCHC) were obtained especially with *Panax ginseng*. Dramatic increases of WBC counts since the 15th day were also observed in both treated groups. In *Spirulina*-treated rats, the neutrophil count was enhanced precociously (since the 15th day). The overall lymphocyte population as well the T cell number has gradually augmented according to the treatment duration in all treated rats. Furthermore, *Panax ginseng* treatment has exhibited significant greater effects on lymphocyte and T cell counts than *Spirulina platensis* treatment. These results suggest that these 2 bio-medicines positively interfere with bone marrow cellular production and with immune cellular response and may be useful as adjuvant treatment of anaemia or of immune deficiency.

**Keywords:** *Spirulina platensis*, *Panax ginseng*, blood cells, ANAE staining.

**Introduction**

*Spirulina platensis* and *Panax ginseng* are alternative bio-medicines that have recently become widely available on the market and pharmacy in tablet and powder form. *Spirulina* species (blue-green algae) are rich in proteins, lipids, carbohydrates, and in oligoelements (zinc, magnesium, manganese and selenium) [27]. They also contain anti-oxidants, such β-carotene, riboflavin, α-tocopherol and α-lipoic acid, and also SOD enzymes that has a significant effect on scavenging free radicals, thereby protecting body organs from damage caused by exposure to lead [10, 23]. In addition, *Spirulina* spp. decreases the number of mast cells increased by lead in the cortex and medulla of rat ovary [9] and reduces the incidence of mast-cell mediated immediate-type allergic reactions by preventing the release of histamine [11]. The blue-green algae accelerates production of antibodies and stimulates immune cells (the bone marrow stem cells, macrophages, T-cells and natural killer cells) [7]. Furthermore, blue-green algae can restore the white cell and nucleated cell counts and also haemoglobin concentrations in patients treated with chemotherapy and/or radiotherapy [8, 30].

In the Far East, the plant *Panax ginseng* (ginseng) is regarded as an adaptogenic agent, which enhances physical performance, promotes vitality, increases resistance to stress and ageing and possesses immunomodulatory activity [29]. Ginseng is a plant with low haemolytic effect, which increases the cellular immune functions of peripheral blood mono-
nuclear cells in spleen and thymus and may alleviate the symptoms of anaemia, poisoning and immunodeficiency [25]. SUN et al. [25] and QINA et al. [17] have reported that Panax notoginseng enhanced significantly not only the mitogene-stimulated splenocyte proliferation, but also IgG, IgG1 and IgG2b productions specific for T cells. The prolonged administration of red ginseng extract significantly inhibits the incidence of carcinogenesis induced by various chemical carcinogens such as ethane, aflatoxin B1 and tobacco smoke condensates [29]. Long-term intake of Korean red ginseng delays disease progression in human immunodeficiency virus type 1 (HIV-1)-infected patients and significantly increases the number of circulating neutrophils, natural killer cells, CD4+ T-cells, and interleukin-2 concentrations [26].

The peripheral circulating T- and B-lymphocytes are classified on the basis of both their immunological and their enzyme histochemical properties [6, 20]. Separation of T- and B- lymphocytes are possible by Antibody Complement rosette method, Erythrocyte rosette method and immunofluorescence. Because these methods are expensive and tricky and cannot be applied to tissue sections [4, 12, 28], researchers have preferred to study differential enzyme profiles between B and T cells. In this way, the presence of ANAE (x-naphthyl acetate esterase) was reported in T lymphocytes and not in B lymphocytes. Consequently, ANAE staining is a simple, sensitive, inexpensive, easily and rapid method for identifying and quantifying the population of the T lymphocytes [1, 2, 4, 5, 16, 18, 20].

Small amounts of Spirulina platensis and Panax ginseng increase both the humoral and cellular defense of the immune system due to improvement activity of bone marrow stem cells. However, there are few studies on the effects of these biomedicines on the peripheral blood cells. The aim of this study is to determine the direct effects of Spirulina platensis and Panax ginseng on circulating peripheral blood cells and ANAE-positive cells in rats.

Materials and Methods

ANIMALS AND EXPERIMENTAL DESIGN

Thirty adult female Wistar albino rats weighing about 200-250 g were used. The animals were given standard rat pellets and tap water ad libitum and were housed in stainless steel cages (360 x 200 x 190 mm³), each containing 2 or 3 animals, from 15 days before the start of the experiment. All animals were kept under standard laboratory conditions (light period 07.00h a.m. to 8.00h p.m., 21 ± 2°C, relative humidity 55%), and received human care according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Academy of Sciences and published by the National Institute of Health.

Rats were divided into three equal groups (n = 10) (Control group, Spirulina platensis - treated group and Panax ginseng - treated group). The control group received normal food and water during the experiment whereas the Spirulina platensis and Panax ginseng - treated groups received normal food plus Spirulina platensis (300 mg/kg per day) or Panax ginseng (400 mg/kg per day) dissolved in water during the experiment (30 days). Spirulina platensis and Panax ginseng were purchased from the Sigma Chemical Co.

HAEMATOLOGICAL ANALYSES

In both control and experimental groups, blood samples from tail veins of the animals were taken into heparinized (100 IU heparin/ml blood) tubes on 0, 15, 30 days of the experimental period. From each sample, ten blood films were prepared and air-dried, and five of these were stained with May Grunwald-Giemsa’s stain [22], whereas the other five were fixed in glutaraldehyde acetone fixative (3 minute at -20°C) for ANAE demonstration [14]. Erythrocytes (RBC) and leukocytes (WBC) were counted by the haemocytometric method, the haemoglobin concentration was determined spectrophotometrically, packed cell volume (PCV) by the microhematocrit tube method, and percentage rates of leukocytes were determined with the May Grunwald-Giemsa’s staining method [22]. For erythrocytes, the mean corpuscular haemoglobin concentration (MCHC) and the mean globular volume (MGV) were calculated with the following formulas:

\[ \text{MCHC} \text{ (in g/L)} = \frac{\text{haemoglobinemia (g/L)}}{\text{PCV (L/L)}} \]
\[ \text{MGV} \text{ (in fL)} = \frac{\text{PCV (L/L)}}{\text{RBC (10^{12}/L)}} \]

To determine the ANAE activity, an incubation solution constituted by phosphate buffer hexazotized paraarsonaline (pH 5.0, 67 mM) containing the enzyme substrate (0.25% alpha naphthyl acetate dissolved in acetone) was prepared according to the method of MUELLER et al. [14]. The incubation solution was adjusted to three different pH values (pH 5.8, 6.8 and 7.2) and sample films were stained for 3 hours for each pH value. The films were then counterstained with 1% methylene green for 10 minutes. Lymphocytes with red-brown granules, giving dot-like positivity, were regarded as ANAE-positive in blood films. The positive lymphocyte rates were determined by counting 200 lymphocytes in every smear (x 40 magnification).

STATISTICAL ANALYSIS

Mean ± Standard Error values were calculated for each group to determine the significance of inter-group differences. Each parameter was analysed separately by using one-way analysis of variance (ANOVA). For determining differences between groups, the Duncan test was used. A p value of <0.05 was considered to be significant.

Results

Haematological parameters observed in the control and experimental groups are shown in figures 1 and 2.

In controls, Red Blood Cell (RBC) counts (figure 1a) and haemoglobin concentrations (figure 1b) remained relatively constant during the whole period of the experiment whereas Packed Cell Volume (PCV) (figure 1c) and Mean Globular
HAEMATOLOGICAL EFFECTS OF SPIRULINA PLATENSIS OR PANAX GINSENG ORAL TREATMENTS IN RATS

Figure 1: Effects of Spirulina platensis (300 mg/kg/day) and Panax ginseng (400 mg/kg/day) treatment for 30 days on erythrocyte haematological parameters [Red Blood cells (RBC, figure 1a), Haemoglobin concentration (Hb, figure 1b), Packed Cell volume (PCV, figure 1c), Mean Globular Volume (MGV, figure 1d) and Mean Corpuscular Haemoglobin concentration (MCHC, figure 1e)] in female adult Wistar rats (n = 10 in each group). Results are expressed as Mean ± Standard errors.

RBC: Red Blood Cells; Hb: Haemoglobin concentration; PCV: Packed Cell volume; MGV: Mean Globular Volume; MCHC: Mean Corpuscular Haemoglobin Concentration. Different superscripts for a given parameter indicate significant differences according to time and to group (p < 0.05).

Figure 2: Effects of Spirulina platensis (300 mg/kg/day) and Panax ginseng (400 mg/kg/day) treatment for 30 days on leukocyte haematological parameters [White Blood Cells (WBC, figure 2a), Neutrophils (figure 2b), Lymphocytes (figure 2c) and monocytes (figure 2d)] in female adult Wistar rats (n = 10 in each group). Results are expressed as Mean ± Standard errors.

WBC: White Blood Cells. Different superscripts for a given parameter indicate significant differences according to time and to group (p < 0.05).
**TABLE 1. Effects of Spirulina platensis (300 mg/kg/day) and Panax ginseng (400 mg/kg/day) treatment for 30 days on lymphocyte counts and ANAE positive lymphocytes (T cells) in female adult Wistar rats (n = 10 in each group). Results are expressed as Mean ± Standard errors.**

<table>
<thead>
<tr>
<th>Parameters and dates</th>
<th>Control</th>
<th>Spirulina platensis</th>
<th>Panax ginseng</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Lymphocytes (10^6/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>6260 ± 16.75 a</td>
<td>6549 ± 55.25 a</td>
<td>6213 ± 4.80 a</td>
</tr>
<tr>
<td>Day 15</td>
<td>6399 ± 17.75 a</td>
<td>7607 ± 27.90 b</td>
<td>8712 ± 8.10 c</td>
</tr>
<tr>
<td>Day 30</td>
<td>6772 ± 21.90 a</td>
<td>8647 ± 53.35 c</td>
<td>9870 ± 57.35 d</td>
</tr>
<tr>
<td><strong>T cells (10^6/L)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0</td>
<td>4031 ± 12.56 a</td>
<td>4273 ± 22.10 a</td>
<td>3961 ± 7.20 a</td>
</tr>
<tr>
<td>Day 15</td>
<td>4212 ± 21.30 a</td>
<td>5024 ± 23.71 b</td>
<td>5954 ± 9.85 c</td>
</tr>
<tr>
<td>Day 30</td>
<td>4495 ± 19.71 a</td>
<td>6260 ± 21.34 c</td>
<td>7898 ± 20.03 d</td>
</tr>
</tbody>
</table>

Different superscripts for a given parameter indicate significant differences according to time and to group (p <0.05).

On day 0, all haematological parameters were similar between the both treated groups and the control group. But, on Day 15 and 30, significant increases of haemoglobin concentrations were observed in the 2 treated groups compared to the control group (p <0.05). This parameter gradually increased according to the treatment duration and maximal values were obtained on Day 30 (Day 15 vs. Day 0 and Day 30 vs. Day 15 and Day 0: p <0.05). At this date, haemoglobin concentrations were similar in the both treated groups (figure lb). RBC counts and PCV were also markedly enhanced by the applied treatments (Spirulina platensis and Panax ginseng) compared to controls on Day 30 (p<0.05) for each of the treated groups: Day 30 vs. Day 0: p < 0.05). Whereas
erythrocyte numeration was precociously significantly modified in the Panax ginseng-treated group (p < 0.05 on Day 15), PCV was not still altered. By contrast, in the Spirulina platensis-treated group, PCV significantly increased while RBC count was not significantly modified at the 15th day (figures 1a and 1c). Moreover, the PCV changes in this group were time dependent, the highest values being obtained on Day 30 (Day 15 vs. Day 0 and Day 30 vs. Day 0: p < 0.05). Consequently, the alterations of the erythrocyte cytology (i.e. MGV and MCHC) have significantly differed according to the nature of treatment (Spirulina platensis vs. Panax ginseng) (figures 1d and 1e). With Spirulina platensis supplementation, the erythrocyte volume declined more slowly (Day 30 vs. Day 15 and Day 0: not significant) and the corpuscular haemoglobin concentration increased more rapidly (Day 30 vs. Day 15 and Day 0: not significant) and the cor-puscular haemoglobin concentration increased more rapidly (Day 15 and Day 30 vs. Day 0: p < 0.05) than in control rats. On the other hand, with Panax ginseng supplementation, the erythrocytes were smaller than those of controls and of Spirulina platensis-treated rats (Days 15 and 30; p < 0.05) but they were dramatically more loaded with haemoglobin (Day 30, Panax ginseng-treated group vs. Spirulina platensis-treated group and vs. control group: p < 0.05).

The White Blood Cell (WBC) counts and lymphocyte numbers significantly increased in both treated groups at the 15th and the 30th days compared to controls (p < 0.05) and reached maximal values on Day 30 (Day 30 vs. Day 15 and vs. Day 0: p < 0.05) (figures 2a and 2c). Furthermore, the increases of these 2 parameters were significantly greater in the Panax ginseng-treated group than in the Spirulina platensis-treated group for the 2 dates (p < 0.05). Marked increases of neutrophil counts were observed in the Spirulina platensis-treated group since the 15th day (p < 0.05) whereas in the Panax ginseng-treated group, significant modifications of the neutrophil population compared to controls were only evidenced on the 30th day (p < 0.05) (figure 2b). Furthermore, no significant fluctuation of the monocyte numbers was noted at the 15th days (figure 2d) while they were significantly enhanced in both treated groups at the 30th day compared to controls (p < 0.05).

Neutrophil and eosinophil granulocytes and monocytes exhibited diffus ANAE staining in the cytoplasm (figures 3b and 3d) while T lymphocytes generally contained 1 - 2 specific granules (figure 3a and 3c) or more rarely 3-5 red brown ANAE positive granules (figure 3a) and B lymphocytes remained ANAE negative (figure 3b). A diffuse ANAE staining was also observed in platelets (figure 3c). Consequently, the specific ANAE staining pattern in T cells has allowed their quantification in peripheral blood (Table I). Total lymphocyte counts increased gradually during the treatment (for the 2 treated groups, Day 15 vs. Day 0 and Day 30 vs. Day 15: p < 0.05), and maximal values were obtained in the Panax ginseng-treated group compared to the Spirulina platensis-treated group and to the control group on Day 30 (p < 0.05). Like for the total lymphocyte population, supplementation with Spirulina platensis or with Panax ginseng induced significant increases of T cell numbers on Days 15 and 30 compared to control values (p < 0.05). The variations of this parameter were time (for the 2 treated groups, Day 15 vs. Day 0 and Day 30 vs. Day 15: p < 0.05) and treatment dependent, the greatest T cell population being obtained in the Panax ginseng-treated group on the Day 30 (Panax ginseng-treated group vs. Spirulina platensis-treated group: p < 0.05).

Discussion

In the present study, Spirulina platensis or Panax ginseng supplementation in drinking water for 30 days has induced significant positive effects on erythropoiesis in adult rats evidencing by increases of RBC counts and of haemoglobin concentrations. Moreover, with Panax ginseng treatment, microcytosis was observed but erythrocytes were dramatically loaded with haemoglobin. Previous studies have already reported that Spirulina platensis may reduce the severity of anaemia and the UV-induced damage of bone marrow [18, 30], and increase blood haemoglobin concentrations [8, 30]. On the other hand, the effects of Panax ginseng on erythrocytes are more scarcely explored and only low-haemolytic actions of Panax ginseng were reported [17, 25]. By contrast, the effects of these 2 biomedicines on leukocytes are more documented. Spirulina platensis is reported to enhance the phagocytosis activity of bronchoalveolar and abdominal macrophages [19] and to increase the numbers of white cells and nucleated cells [8, 30]. In agreement with that, total leukocyte, neutrophil and lymphocyte counts were significantly increased in Spirulina platensis-treated rats since the 15th day of treatment and remained markedly elevated until the 30th day, whereas the monocye population appeared unaltered on the 15th day by the algae supplementation. In the same way, increases of the numbers of total leukocytes, lymphocytes and alveolar macrophages have been reported in Panax ginseng-treated animals [3, 21], although these findings were debated by SRISURAPANON et al. [24]. However, other authors have reported that ginseng significantly increased the numbers of circulating neutrophils, macrophages, natural killers and CD4+ and/or CD8+ T cells as well as the IL2 and IL10 concentrations [25, 26]. Nevertheless, in the present study, the Panax treatment of the rats has also induced significant modifications of leukocyte haematological parameters: the numbers of total WBC were markedly increased on the days 15 and 30, neutrophil and monocyte populations were significantly greater on day 30 than those observed in controls and more interestingly, increases of the overall lymphocyte population and more particularly of the numbers of ANAE positive cells (i.e. T cells) were time dependent and were more severe in rats treated with Panax ginseng than in those receiving Spirulina platensis.

Peripheral blood cells diversely contain the ANAE enzyme. The ANAE positive reaction of T lymphocytes generally appears as 1 - 2 localised granules (more scarcely 3-5 stained granules) while B lymphocytes in humans and animals give a negative reaction [2, 14]. Neutrophils also give a negative reaction in some animal species (camels [20], cattle [28]) or show a diffuse staining in other species (rats, dogs, cats, horses, sheep, goats [1] and rabbits [16]). Eosinophils, monocytes and platelets also present a diffuse localisation of the enzyme in the cytoplasm [1, 16]. Consequently, the ANAE staining pattern coupled to cytological parameters allows the identifi-
culation and the quantification of T lymphocytes among the ove-
ral peripheral blood lymphocytes. However, The ANAE stai-
ing properties of the T lymphocytes have been shown to vary
according to the stain pH in humans and in various animal spe-
cies [15]. The optimal staining pH values ranged from 5.0 [14]
to 6.4 in rats [1], were 5.8 in chickens, sheep, goats and dogs,
6.2 in horses and cows and 6.4 in cats [1]. These differences
depend on both distinction of organism and duration of
staining. In the present study, ANAE positive reaction fairly
appeared more long time in the pH 6.8 to 7.2 than in the pH
5.8 (3 hours). The rates of T lymphocytes have been found to
be 81.33% in camels [20], 63% in cattle [28] and 56% in chik-
ens [13]. In this study, T cell proportions ranged from 64.4 ±
0.75 % to 66.38 ± 0.90 % (4031 ± 12.56. 106/L to 4495 ±
19.71. 106/L) in control rats. The total lymphocyte counts gra-
dually increased according to the treatment duration (Day 15
to Day 30 vs Day 30 vs Day 15: p <0.05) and maximal values
were obtained in the Panax ginseng - treated group com-
pared to the Spirulina platensis - treated group and the con-
trol group on the Day 30 (9870 ± 57.35. 106/L, 8647 ±
53.35. 106/L and 6772 ± 21.90 106/L respectively).
These results suggest that Spirulina platensis and Panax
ginseng treatments may stimulate the activity of the bone
marrow stem cells [7] and consequently strengthen systemic
and particularly immune cellular defences of the organism.
Such nutritional supplementations with Spirulina platensis
or Panax ginseng may be beneficial in humans and in ani-
mals suffering from anaemia or from immune deficiency but
further investigations are required for identifying active
drugs supplied by these 2 biomedicines and for investigating
their molecular actions on the regulation of the immune sys-
tem and of the activity of bone marrow stem cells.

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