

# **Effect of an oral supplementation of a proprietary melon juice concentrate rich in superoxide dismutase on stress and tiredness in healthy people : a pilot, double-blind, placebo-controlled clinical trial**

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## **Abstract**

### **Background**

Recent studies have demonstrated a correlation between perceived stress and oxidative stress. As SOD is the main enzyme of the enzymatic antioxidant defence system of the body, we evaluated the effect of an oral daily intake of a proprietary melon juice concentrate rich in SOD (EXTRAMEL®) on the signs and symptoms of stress and tiredness in healthy volunteers.

### **Methods**

This randomized, double blind, placebo controlled clinical study was conducted with seventy healthy volunteers between the ages of 30 and 55 years, who have an everyday life stress and tiredness. They took the dietary supplement based on the melon juice concentrate (140 IU SOD per capsule) or identical placebo one time daily for 4 weeks. Stress and tiredness were measured using four observational psychometric scales: FARD, PSS-14; SF 12 and Epworth scale. The study was conducted by Isoclin, a clinical research organization, located in Poitiers, France.

### **Results**

No adverse effect was noted. The supplementation of 140 IU SOD/day with the melon juice concentrate significantly improved signs and symptoms linked to performance even physical (pain, sleep troubles), cognitive (concentration, weariness) or behavioural (attitude, irritability, difficulty of contact) compared to the placebo. In the same way, quality of life and perceived stress were significantly improved with SOD supplementation.

### **Conclusion**

This pilot study showed that an oral supplementation with a melon juice concentrate rich in SOD may have a positive effect on several signs and symptoms of perceived stress and tiredness.

## **Background**

In developed countries, people from all social classes are concerned about tiredness, stress and anxiety. It is estimated that 30% of the population will suffer from these symptoms at one point in their life. It is known also that professional activity generates stress and tiredness, which are closely linked. According to medical sources, the percentage of working people treated for depression is around 10% and people who perceive stress and tiredness is constantly increasing.

Several studies show that there is an emerging link between individual stress and intracellular oxidative stress. In a clinical trial on 42 women in the work force, a correlation between perceived stress and intracellular oxidative stress was demonstrated. Inflammatory markers like TNF- $\alpha$  or HbA1C were enhanced in women with a high burnout, indicating a role of inflammatory responses and oxidative stress in the pathophysiology of burnout [1]. In the same way, a clinical study on workers of a prehospital emergency service, showed a positive correlation between malondialdehyde (MDA), a biomarker of lipid peroxidation and burnout levels [2]. Finally, a recent study on mice showed a positive correlation between the glutathione reductase activity and anxiety, suggesting that cellular oxidative stress seen mice might be clinically translated as anxious and stressed behaviour in patients [3].

SOD is one of the main antioxidant enzymes found in living cells and organisms. Known since 1969 [4], SOD from erythrocytes was largely studied and used as a treatment to certain diseases like arthritis [5] or in chronic radiotherapy damage [6]. Since 2000, a melon juice concentrate containing high level of SOD has been developed, its use as a dietary supplement has been a new subject of interest and its antioxidant and anti-inflammatory properties have been demonstrated [7]. In healthy volunteers, this melon juice concentrate combined with wheat gliadin polymers, showed a protective effect against DNA damage resulting from oxidative stress induced by hyperbaric oxygen [8]. Recently same authors showed that this melon juice concentrate combined with gliadin polymer reduced cell injury affiliated with aortic cross-clamping in pigs [9].

It seemed then very interesting to study the effect on perceived stress of EXTRAMEL® (Bionov, Avignon, France), this melon juice concentrate containing high concentration of SOD which usually acts on oxidative stress as the cellular level,

The present pilot study determined if a daily intake of 140 IU of melon SOD is able to decrease the signs and symptoms of perceived stress and tiredness in healthy volunteers.

## **Materials and methods**

### **Test Material**

EXTRAMEL® is a proprietary freeze-dried melon juice concentrate obtained by physical treatment (crushing the melon, recovery of the pulp, centrifugation, filtration, freeze-drying) of a specific variety of melon (not GMO) which contains high levels of SOD and other antioxidants (US Patent No 5 616 323). As SOD is very sensitive to oxygen, high temperature and acidity, the melon juice concentrate has to be coated. The product used in the study (EXTRAMEL® microgranules) was coated with palm oil. The dietary supplements given to the volunteers were small hard capsules containing 140 IU of SOD through the coated melon juice concentrate and starch for the *verum* (EXT) and starch only for the placebo (P).

The volunteers assigned by randomisation in two groups of 35 were given a food supplement or a placebo for 28 days: one capsule per day taken in the morning. The capsules were indistinguishable and were administered in a double blind approach.

### **Analyses**

In order to evaluate the signs and symptoms of perceived stress and tiredness, the neurobehaviour sphere of the volunteers was explored. Moreover, their own perception of stress and its impact on their quality of life and sleep were evaluated. Four validated and well-known evaluation scales were then chosen:

1. **Ferreri Anxiety Rating Diagram** (FARD) [11,12] explores four poles divided in three items:

- Somatic pole (attitude, neurovegetative troubles, pain),
- Relational pole (apprehension, internal tension, difficulty of contact),
- Vigilance pole (irritability, sensorial troubles, sleep troubles),
- Cognitive pole (concentration, weariness, doubt-indecision),

with a total of 12 questions and a score from 0 to 72;

2. **Cohen Perceived Stress scale** (PSS-14) [14] evaluates the perceived stress during the last two weeks with 14 questions and a score from 14 to 70;
3. **SF-12® Health Survey** [15] allows to measure eight aspects of the quality of life : general physical and mental health state, physical and social functioning, physical and emotional health, pain and vitality. The SF-12 questionnaire is composed of 12 questions with a score from 0 to 12;
4. **Epworth Sleepiness Scale** [16] is a questionnaire intended to measure daytime sleepiness composed by eight questions with a score from 0 to 18.

The **Beck Depression Inventory** (BDI) [17], a 13 question multiple choice self-report inventory that is one of the most widely used instruments for measuring the severity of depression, was used to avoid the inclusion of depressive volunteers.

### **Study Design**

The clinical trial was an observational study based on individual evaluation scales. The protocol followed was randomized, double blind and placebo controlled. It was approved by the Comité de Protection des Personnes Ouest III, the ethical committee of Poitiers, France under the n° 2006-A00677-44 to be conducted by Dr Thierry Cantin.

A call for volunteers was made in the region of the investigation centre (Poitiers area, Poitou, 86, France) and the volunteers for the study were pre-screened by the investigator.

The inclusion criteria were to be between 30 to 55 years old, to have a BMI (Body Mass Index)  $\leq 30$ , to have a stable professional activity for more than one year, to perceive stress & tiredness, to be in full health, not taking any drugs or dietary supplements, not taking tisane or drinks for anti-stress or anti-tiredness.

The exclusion criteria were to be pregnant or breast-feeding, to have a previous case of psychiatric disease, to have pathologies on going or active during the last month, to have received medical treatment (allopathic or homeopathic) during the previous month, to have taken a dietary supplement during the last month, to be in a stressful situation during the next month (wedding, birth, scheduled hospitalisation, important exam...).

Acceptable volunteers were called in for a screening and baseline evaluation using the fifth evaluation scales previously described: FARD, Epworth, PSS-14, SF-12 and BDI's inventory scale. Then, 70 volunteers were definitively included and participated to the clinical trial. These volunteers provided a written informed consent.

The volunteers were tested three times during a visit to the doctor. The first time was before the supplementation (D0), corresponding to the screening evaluation. A test was planned 7 days (D7) after taking the supplement and another one at the end of the trial (D28). The evaluation scales were filled out by the volunteers themselves. During every consultation, the doctor made a general clinical exam (arterial tone and cardiac frequency measure) and filled up an event journal with remarks on the volunteers, eventual undercurrent effects, the eventual pathologies and their associated treatment.

### **Method of Screening the Volunteers**

Axis were determined by combining the different scales :

- Physical Axis composed by FARD somatic pole (0 – 18), FARD vigilance pole (0 – 18) and SF-12 scale (0 – 12) with a total score from 0 to 48;
- Cognitive Axis composed by FARD cognitive pole (0 – 18) with a total score from 0 to 18;
- Relational Axis composed by FARD relational pole (0 – 18) with a total score from 0 to 18;
- Perceived Stress Axis composed by Epworth sleepiness scale (0 – 18) and Cohen's PSS-14 perceived stress scale (14 – 70) with a total score from 14 to 88.

To be included, the volunteers had to get scores within the following parameters: BDI < 5; Physical Axis > 15/48; Cognitive Axis > 5/18; Relational Axis > 5/18; Perceived Stress Axis > 33/88.

The means of each score for the volunteers were: Physical Axis = 24.95; Cognitive Axis = 6.27; Relational Axis = 5.89; Perceived Stress Axis = 40.7.

### **Statistical Analysis**

The data were expressed as means  $\pm$  SD of the evolution of the scores on each scale between D0 and D7 and between D0 and D28 in absolute and relative values. The paired Student's t-Test were used to analyse each group relative to the amplitude of

variation between D0 and D7 and D0 and D28 and to compare results between groups at D0, D7 and D28.

## **Results**

### **Study Population**

Seventy volunteers aged between 30 and 55 years (mean 40.26) with a BMI from 17 to 42 (7 volunteers were included even if they have a BMI over 30) were enrolled and randomized into two test groups and no subject was dropout. There were no statistical difference between the two groups at baseline in scores for age, ethnicity, ...

During the 4 weeks of the study, the doctor filled up a file of events and no adverse effects were noted.

As preliminary results, scores used for the statistical analysis are reported in Table 1. The two groups are not equivalent in their base level score but this difference is not statically different except for vigilance pole, irritability and apprehension items of the FARD scale for which the scores are worst for the EXT group.

Table 1. Scores of all scales in P and EXT groups at D0, D7 and D28

### **FARD Scale**

The statistical analysis on somatic, vigilance and cognitive poles showed that scores of these poles were significantly improved at D28 in both groups (Figure 1). This clearly indicates that an important placebo effect is present in the study. Nevertheless, when the scores of the groups are compared at D28, a significant difference between them appears: the three poles are much more efficiently improved in the EXT group than in the P group (Figure 2) with respectively 18% more for the somatic pole, 25% more for the vigilance pole, and 20% more for the cognitive pole.

Concerning the relational pole, its improvement is not significant in either group.

**Figure 1.** Scores of FARD scale poles : comparison between EXT and P groups before and after supplementation

**Figure 2.** Comparison of the Scores of FARD scale poles : comparison between EXT and P groups after supplementation

The further analysis of each pole also gave interesting results. In the P group, all items were significantly improved from D7 excluding sleep troubles and doubt-indecision (Figure 3). In the EXT group, all items were also significantly improved from D7, excluding only doubt-indecision, but contrary to the P group, the improvement of certain items continued to increase in D28 (Figure 4).

**Figure 3.** Scores of FARD scale items for P group before, during and after supplementation

**Figure 4.** Scores of FARD scale items for EXT group before, during and after supplementation

At D28, the results (Figure 5) showed that the pain, irritability, sleep troubles and the weariness scores were significantly improved in the EXT group as compared to the P group, being respectively, 30, 22, 38 and 26% higher. The attitude, difficulty of contact and concentration items also tended to be significantly more improved in the EXT group than in the P group, being respectively, 26, 26 and 22% higher. All other items (neurovegetative troubles, sensorial troubles, apprehension, internal tension and doubt-indecision) scores were not significantly different at D28 between the EXT and P groups, and seemed then to be only improved by a placebo effect.

**Figure 5.** Scores of FARD scale items between EXT and P groups after supplementation

### **Perceived Stress Scale**

The analysis of the results of the PSS-14 scale (Figure 6) showed that a significant decrease in perceived stress occurred between D0 and D7 in both groups. However, after D7, in the P group, the placebo effect seemed to have reached its maximum level and perceived stress stopped to decrease, whereas in the EXT group, perceived stress continued to decrease with a statistically significant improvement of 12% ( $p=0.01$ ) compared to the P group at D28 and a global decrease of 30%.

**Figure 6.** Scores of PSS-14 scale (Mean  $\pm$  SD) before, during and after supplementation

### **Quality of Life Scale**

The analysis of the results of the SF-12 scale (Figure 7) showed that a high and significant improvement in the quality of life occurred between D0 and D7 in both groups. However, after D7, in P group the placebo effect seemed to have reached its maximum level and the score stopped to decrease whereas in the EXT group, the quality of life continued to improve with a statistically significant difference of 9% ( $p=0.049$ ) compared to the P group at D28 and a global improvement of 42%.

**Figure 7.** Scores of SF-12 scale (Mean  $\pm$  SD) before, during and after supplementation

### **Sleepiness Scale**

The analysis of the results of the Epworth's scale (Figure 8) gave non significant difference between the P and EXT groups.

**Figure 8.** Scores of Epworth's scale (Mean  $\pm$  SD) before, during and after supplementation

### **Discussion**

The FARD scale showed that the symptoms linked to performance, even physical (pain, sleep troubles), cognitive (concentration, weariness, sleep troubles) or behaviour (attitude, irritability, difficulty of contact), were visibly improved with the use of the melon SOD supplementation compared to placebo whereas psychological behaviour (neurovegetative and sensorial troubles, internal tension and doubt-indecision) were not improved over the placebo effect. Perceived stress and quality of life were also improved by the melon SOD supplementation compared to the placebo whereas no improvement was shown on sleepiness.

Tiredness and stress were not very high and corresponded to a normal everyday level of tiredness and stress of working people in developed countries.

Because of this low base level of stress and tiredness, the placebo effect seemed to be very high whereas with a higher base level the difference between melon SOD supplemented and placebo groups might be more significant. Nevertheless the study highlighted that the placebo effect was only present during the first 7 days of supplementation and not beyond.

It was interesting to note that, despite the high placebo effect and the low base level of stress and tiredness of the volunteers, the study highlighted that melon SOD supplementation had a significantly higher effect compared to placebo on most of the variables of the study and tended to have a significantly higher effect on some others. This may indicate that melon SOD supplementation has an effect on signs and symptoms of perceived stress and tiredness such as pain, irritability, sleep troubles, weariness, perceived stress and quality of life, covering physical and mental health; moreover it has a probable effect on others such as attitude, concentration and difficulty of contact; and it has no effect on signs and symptoms linked to relational behavior and sleepiness. Finally, it was interesting to discover that the melon SOD supplementation has a significant effect on sleep troubles but not on sleepiness, which could be a side effect of many sedatives. This result added to the positive results on perceived stress, quality of life (physical and mental health) and overall physical and cognitive performances could suggest that melon SOD supplementation acts as a cognitive and physical tonic.

## **Conclusion**

In conclusion, this pilot trial highlights that an oral supplementation with 140 IU of SOD through EXTRAMEL®, which is known to have an antioxidant activity on cellular level and fighting against oxidative stress, could have a positive effect on several signs and symptoms of perceived stress and tiredness, and particularly perceived stress, quality of life and some aspects of neurobehaviour. Further studies with a larger number of volunteers and a longer duration would be interesting to confirm these effects and better understand the action of an oral intake of SOD on stress and tiredness.

## **Competing interests**

This research was supported by Seppic (Air Liquide Group), Paris, France and Bionov, Avignon France. The consultation and manuscript preparation was also funded by Seppic and Bionov.

## **Authors' contributions**

MAM and DL conceived the study and participated to the design of the study. HB designed and coordinates the study. DD did the statistical analysis. MAM and DL drafted the manuscript.

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## References

1. Grossi G, Perski A, Evengard B, Blomkvist V, Orth-Gomer K: **Physiological correlates of burnout among women.** *J Psycho Res* 2003, **55**:309-316.
2. Casado A, De Lucas N, Lopez-Fernandez E, Sanchez A, Jimenez JA: **Lipid peroxidation, occupational stress and aging in workers of a prehospital emergency service.** *Eur J Emerg Med* 2006, **13**(3):165-17.
3. Hovatta I, Tennant RS, Helton R, Marr RA, Singer O, Redwine JM, Ellison JA, Schadt EE, Verma IM, Lockhart DJ, Barlow C: **Glyoxalase 1 and glutathione reductase 1 regulate anxiety in mice.** *Nature* 2005, **438**(7068):662-666.
4. McCord JM, Fridovich J: **Superoxide dismutase.** *J Biol Chem* 1969, **244**:6049-6055.
5. Corvo LM, Jorge JC, Van't Hof R, Cruz ME, Crommelin DJ, Storm G: **Superoxide dismutase entrapped in long-circulating liposomes: formulation design and therapeutic activity in rat adjuvant arthritis.** *Biochim. Biophys Acta* 2002, **1564**(1):227-236.
6. Delanian S, Martin M, Bravard A, Luccioni C, Lefaix JL: **Cu/Zn superoxide dismutase modulates phenotypic changes in cultured fibroblasts from human skin with chronic radiotherapy damage.** *Radiother Oncol* 2001, **58**(3):325-331.
7. Vouldoukis I, Lacan D, Kamate C, Coste P, Calenda A, Mazier D, Conti M, Dugas B: **Antioxidant and anti-inflammatory properties of a *Cucumis melo* LC. Extract rich in superoxide dismutase activity.** *J Ethnopharma* 2004, **94**:67-75.
8. Muth CM, Glenz Y, Klaus M, Radermacher P, Speit G, Lerverve X: **Influence of an orally effective SOD on hyperbaric oxygen-related cell damage.** *Free Rad Res* 2004, **38**:927-932.
9. Kick J, Hauser B, Bracht H, Albicini M, Öter S, Simon F, Ehrmann U, Garrel C, Sträter J, Brückner UB, Lerverve X, Schelzig H, Speit G, Radermacher P, Muth CM: **Effects of a**

- cantaloupe melon extract/wheat gliadin biopolymer during aortic cross-clamping. *Intensive Care Med* 200, **33**:694-702.**
10. Oberley LW, Spitz DR: **Nitroblue tetrazolium.** In *Handbook of methods for oxygen radical research.* Edited by Greenwald RA: CRC Press, Inc., Boca Raton, Fla; 1985:217-220.
  11. von Frenckell R, Ferreri M, Tawil S, Bonnet D, Girre JP, Alby JM: **Construction and preliminary validation of an anxiety scale, the FARD (Ferreri anxiety rating diagram).** *Acta Psychiatr Belg* 1987, **87**:704-713.
  12. Ferreri M, Von Frenckell R, Mirabel V, Tawil S, Alby JM: **A new scale of quantitative and qualitative evaluation of anxiety: the Ferrerri Anxiety Rating Diagram (FARD).** *Encephale* 1988, **14**(5):385-393.
  13. Cohen S, Kamarck T, Mermelstein R: **A global measure of perceived stress.** *J Health Soc Behav* 1983, **24**(4):385-396.
  14. Ware JE, Kosinski M, Keller SD: **A 12-Item Short-Form Health Survey: Construction of scales and preliminary tests of reliability and validity.** *Med Care* 1996, **34**(3):220-233.
  15. Johns MW: **A new method for measuring daytime sleepiness: the Epworth Sleepiness Scale.** *Sleep* 1991, **14**:540-545.
  16. Beck AT, Ward C, Mendelson M: **Beck Depression Inventory (BDI).** *Arch Gen Psych* 1961, **4**:561-571.

## Figure legends

### Figure 1

Scores of FARD scale poles : comparison between EXT and P groups before and after supplementation.

Somatic pole (attitude, neurovegetative troubles, pain), Relational pole (apprehension, internal tension, difficulty of contact), Vigilance pole (irritability, sensorial troubles, sleep troubles) and Cognitive pole (concentration, weariness, doubt-indecision).

EXT group : melon SOD supplemented

P group : placebo supplemented

\*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$

### Figure 2

Comparison of the Scores of FARD scale poles : comparison between EXT and P groups after supplementation.

Somatic pole (attitude, neurovegetative troubles, pain), Relational pole (apprehension, internal tension, difficulty of contact), Vigilance pole (irritability, sensorial troubles, sleep troubles) and Cognitive pole (concentration, weariness, doubt-indecision).

EXT group : melon SOD supplemented

P group : placebo supplemented

\* =  $p < 0.05$

### **Figure 3**

Scores of FARD scale items for P group before, during and after supplementation.

P group : placebo supplemented

\* =  $p < 0.05$ , \*\* =  $p < 0.01$ , \*\*\* =  $p < 0.001$

### **Figure 4**

Scores of FARD scale items for EXT group before, during and after supplementation.

EXT group : melon SOD supplemented

\*  $p < 0.05$ , \*\*\*  $p < 0.001$

### **Figure 5**

Scores of FARD scale items between EXT and P groups after supplementation.

EXT group : melon SOD supplemented

P group : placebo supplemented

\*  $p < 0.05$ , \*\*  $p < 0.01$

### **Figure 6**

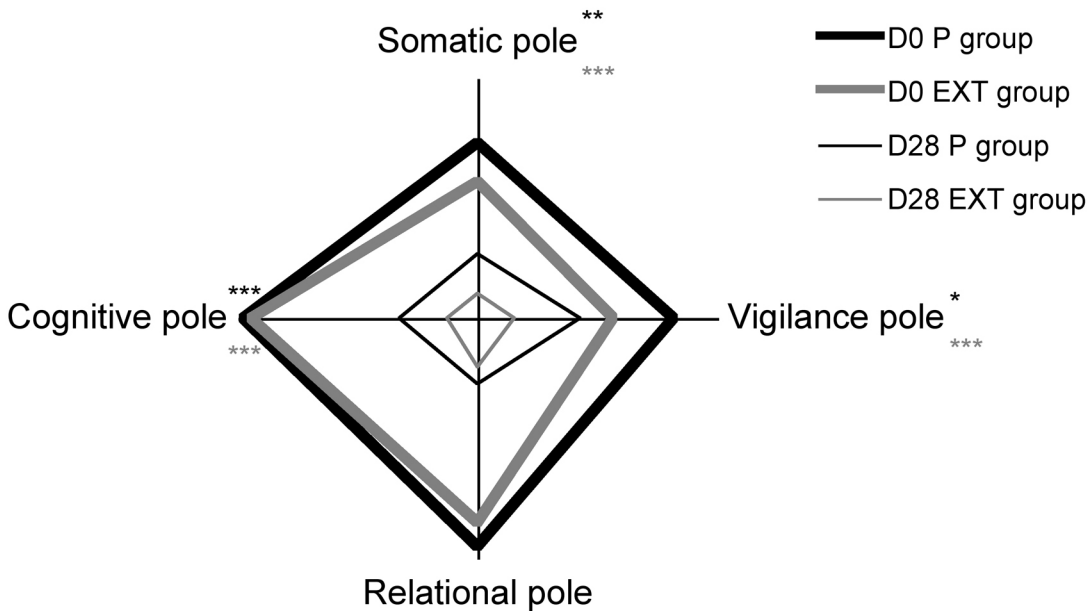
Scores of PSS-14 scale (Mean  $\pm$  SD) before, during and after supplementation

### **Figure 7**

Scores of SF-12 scale (Mean  $\pm$  SD) before, during and after supplementation

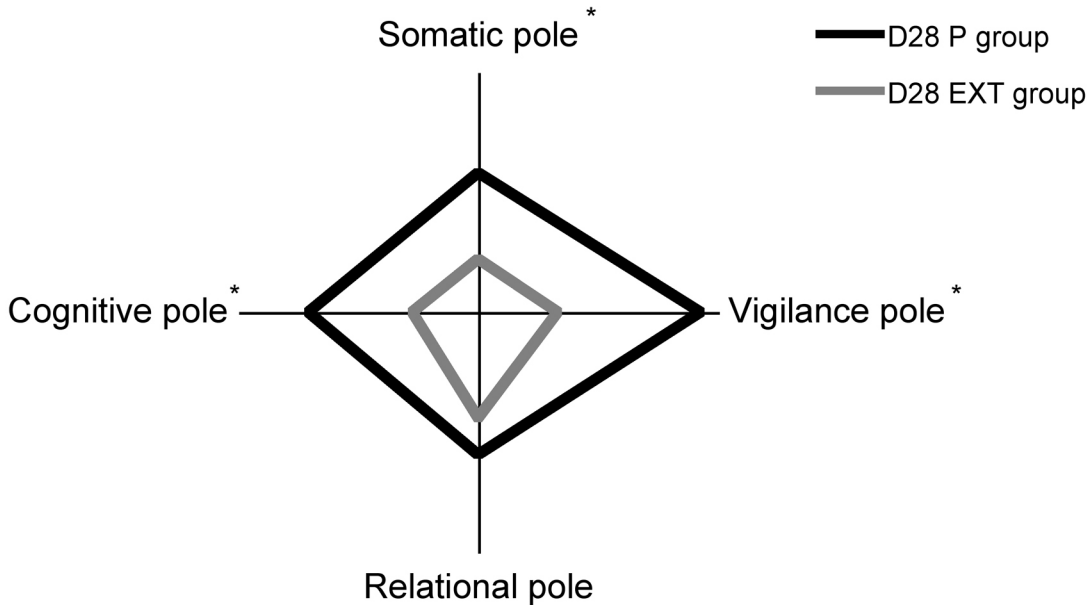
### **Figure 8**

Scores of Epworth's scale (Mean  $\pm$  SD) before, during and after supplementation

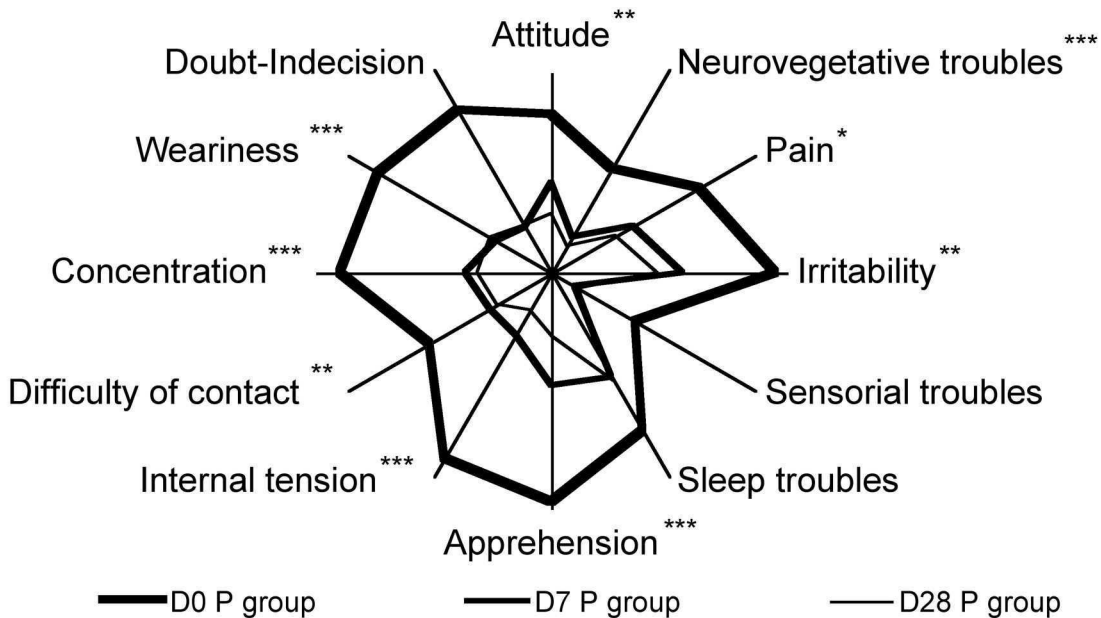


\* Differences between D28 and D0 scores are statistically significant

Figure 1

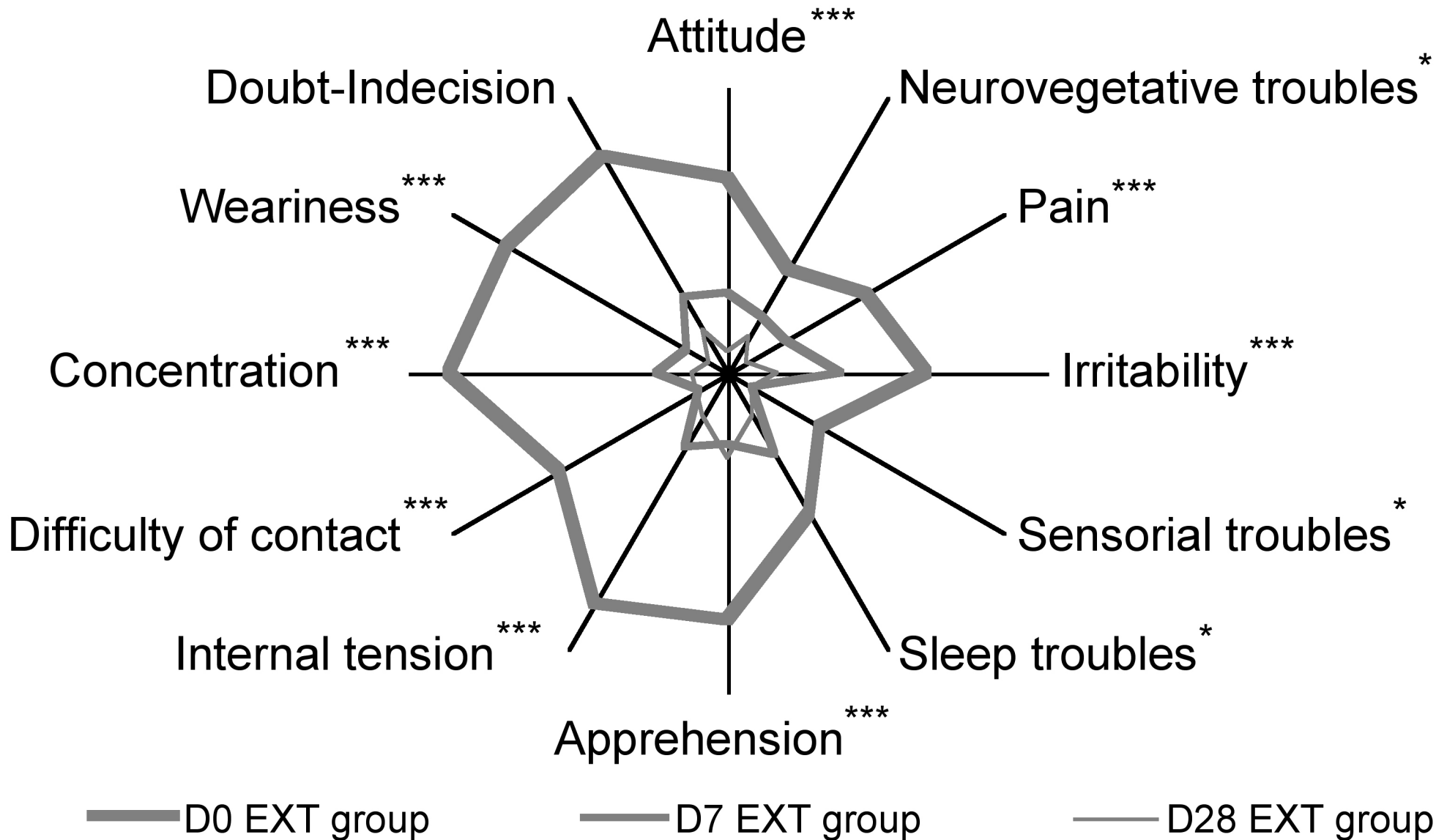


\* Differences between EXT and P group scores are statistically significant



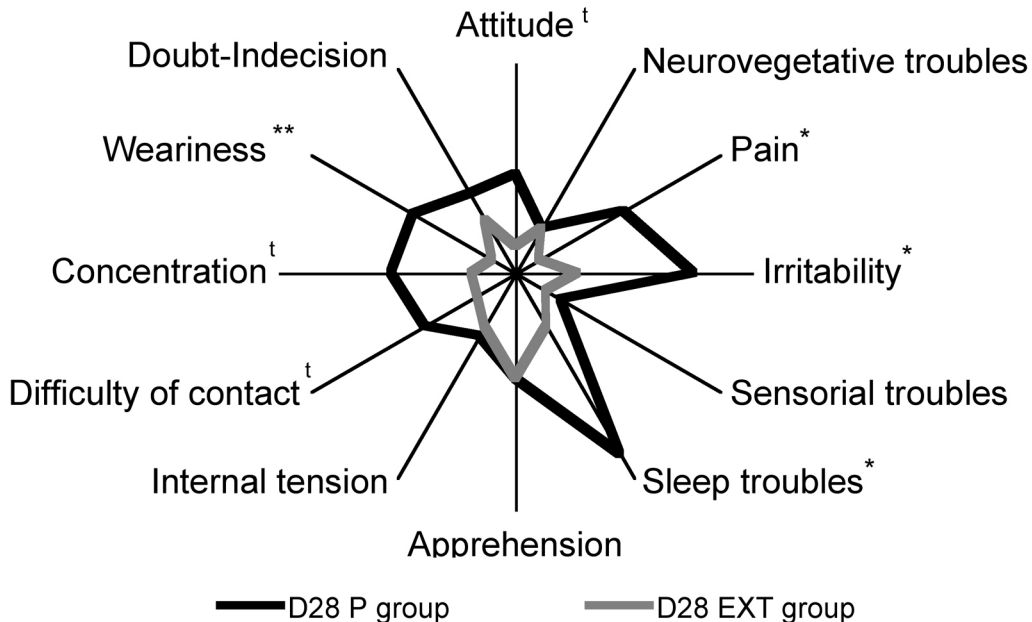
\* Differences between D28 and D0 scores are statistically significant

Figure 3



\* Differences between D28 and D0 scores are statistically significant

Figure 4



\* Differences between EXT and P group scores at D28 are statistically significant

t Differences between EXT and P group scores at D28 have a trend to be statistically significant

Figure 5

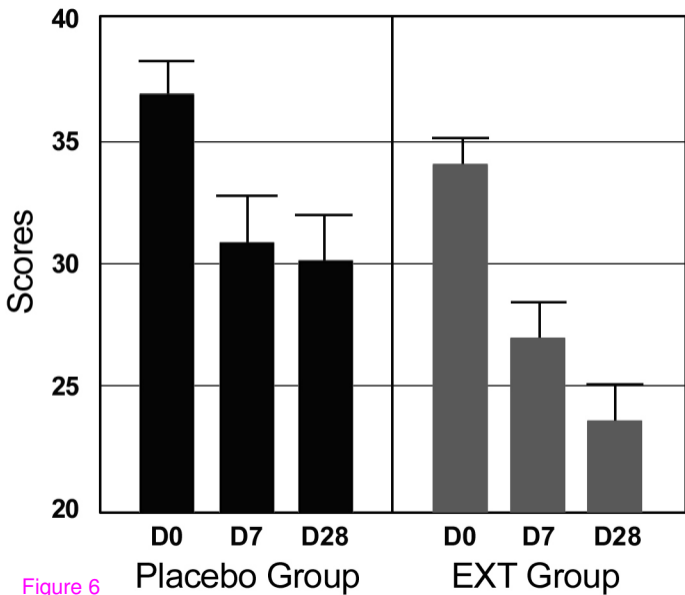


Figure 6

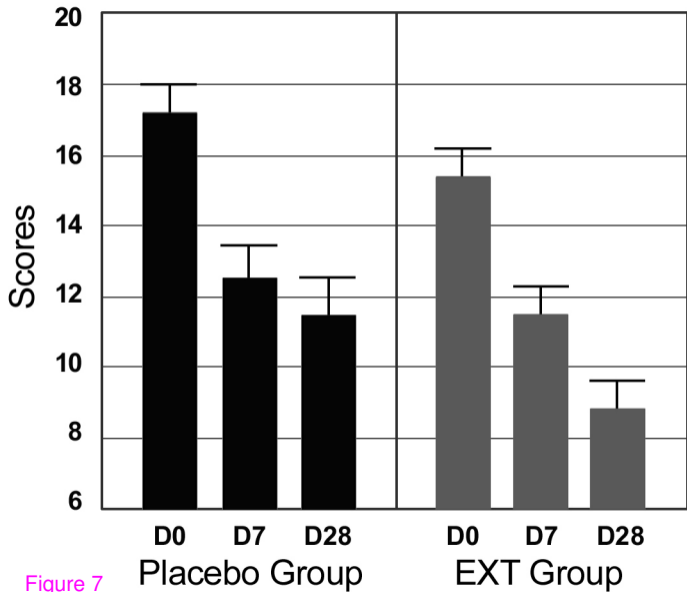


Figure 7

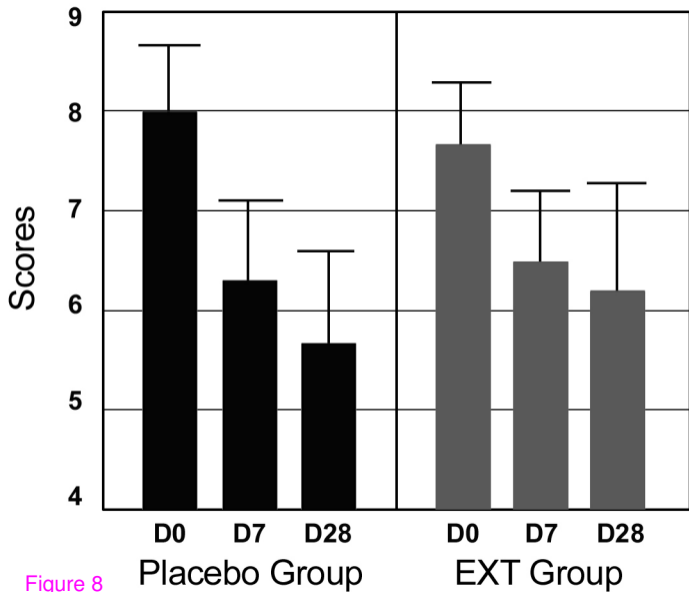


Figure 8

**Additional files provided with this submission:**

Additional file 1: table 1.jpg, 438K

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