Title: COMPOSITIONS FOR ALLEVIATING: STRESS, ANXIETY, DEPRESSION AND AIDING WITH WEIGHT LOSS

Figure 1: Mean Differences in CAR Levels over the study period compared to Baseline

Abstract: The present invention provides specific formula for producing compositions for nutritional support to enhance alertness and reduce the symptoms of stress, anxiety and depression. The compositions of this invention consist primarily of the following ingredients olive oil supplemented with extra hydroxytyrosol, phosphatidyserine (PS), lecithin (from soya), as well as the herbal extract from Magnolia bark. The present invention also relates to the administration of these specific formulated compounds to persons for weight loss.
Compositions for alleviating: Stress, Anxiety, Depression and aiding with Weight Loss

Cross-reference to Related Applications

This application claims the benefit of Irish Provisional Patent Application No. 2011/0008, filed in Ireland on January 12, 2011, the entirety of which is incorporated herein by reference as if set forth in its entirety.

This application claims the benefit of U.S. Provisional Patent Application No. 61/435,524, filed January 24, 2011, the entirety of which is incorporated herein by reference as if set forth in its entirety.

Field of the Inventive Subject Matter

This invention pertains, in general, to the field of nutritional-dietary/herbal-botanical, neuro-endocrine support factors designed for the reduction of stress, anxiety and depression. In particular, the present invention provides formulas for producing compositions for the structural/functional nutritional support of those who want to limit the neurological and immunological destructive effects of excessive Cortisol secretions. In addition, the present invention provides compositions comprising nutritional/botanical factors formulated for those who want to reduce their weight gained as a result of elevated Cortisol plasma levels.

Brief Summary

This invention provides unique formula of nutrients and herbal extracts designed to provide specific dietary-nutritional and herbal-botanical support factors for reducing circulating Cortisol levels and subsequently alleviating feelings of stress, anxiety and depression. Administration of the compositions based on this formula in the double blinded study of 40 patients documented herein resulted in statistically significant reduction in Cortisol levels in the treatment arm. The formula according to this patent has increased bioavailability of
constituent components and therefore has an enhanced effect on the reduction of stress, anxiety and depression as evidenced from the results and graphs attached.

As a convenient addition to the daily diet, the formula of the present invention provides a unique combination of neurological support molecules from the herbal extract of magnolia, neurological effective antioxidant in the form of hydroxytyrosol in olive oil and nutrients for cell wall and other cell membrane support in the phosphatidylserine and lecithin, these ingredients are formulated in their most bio available/absorbable form to provide enhanced efficacy.

This invention presents the specific anxiolytic effects of the formulation of magnolia bark extract combined with olive oil enriched with additional hydroxytyrosol and phosphatidylserine, lecithin and beta carotene:

**Brief Description of the Drawing Figures**

Figure 1 is a plot of mean differences in CAR Levels over a study period compared to baseline.

Figure 2 is a plot of pattern of waking Cortisol levels over the study period for a treatment group and a placebo group.

Figure 3 is a plot of pattern of daily diary psychological measures over the study period for the treatment group.

Figure 4 is a plot of pattern of daily diary stress over the study period for both groups.

Figure 5 is a plot of pattern of daily diary anxiety over the study period for both groups.

Figure 6 is a plot of pattern of daily diary depression over the study period for both groups.
Figure 7 is a plot of pattern of daily diary alertness and focus over the study period for both groups.

Figure 8 is a plot of pattern of weekly diary stress over the study period for both groups.

Figure 9 is a plot of pattern of weekly diary anxiety over the study period for both groups.

Figure 10 is a plot of pattern of weekly diary depression over the study period for both groups.

**Detailed Description**

These are the results of a randomized, double-blind, placebo-controlled clinical trial of Formula 1 (see page 15, below) of this patent in human subjects.

Stress is a familiar aspect of our lives and we all encounter stress in one form or another. Nearly a century of stress research has suggested that stress is associated with reduced well-being and has been linked to increased disease states, for example, hypertension, diabetes and gastrointestinal disorders (Drossman, D.A. (1998), Gastrointestinal illness and the biopsychosocial model. *Psychosomatic Medicine*, 60, 258-267; Gabry, K.E., Chrousos, G.P., Rice, K.C., Mostafa, R.M., Sternberg, E., Negrao, A.B., Webster, EX., McCann, S.M., & Gold, P.W. (2002), Marked suppression of gastric ulcerogenesis and intestinal responses to stress by a novel class of drugs. *Molecular Psychiatry*, 1, 474-483; Milde, A.M., Sunberg, H., Røsseth, A.G., & Murison, R. (2003), Proactive sensitizing effects of acute stress on acoustic startle responses and experimentally induced colitis in rats: Relationship to corticosterone. Stress, 6, 49-57), and increased susceptibility to common viral and bacterial infections (Segerstrom and Miller, 2004). There is also evidence that stress adversely affects various aspects of psychological well-being, including memory and learning impairment (Talbott, S., (2002), *The Cortisol Connection: Why Stress Makes You Fat and Ruins Your Health- and What You Can Do About It*. *Hunter House Publishers*, USA), general fatigue (Michielsen, H. I., Vries, J., & Van Heck, G. L. (2002), Psychometric qualities of a brief self-rated fatigue...

In today's fast paced society, people are often overbooked and overworked, making it increasingly difficult to find the time for psychological therapies for stress. Although numerous pharmacological therapies exist for the treatment of stress that are effective in reducing symptoms, they are often associated with several undesirable side-effects including lethargy, sedation, dizziness, gastrointestinal discomfort, and weight gain (Everly, G.S., & Lating, J.M. (2002) A Clinical Guide to the Treatment of the Human Stress Response. Kluwer Academic, New York).

Magnolia extract is a standardised herb extract made from the bark of the Magnolia officinalis tree. The bark contains two active phytochemicals: honokiol and magnolol. These constituents have several health benefits. The activities of these compounds often complement each other, with honokiol exerting an anti-stress effect, and magnolol exerting an anti-depressant effect. Using well-validated models of depression in rodents, oral administration of a mixture of honokiol and magnolol in rats has been reported to have strong antidepressant-like effects (Xu, Q., et al. (2008), Antidepressant-like effects of the mixture of honokiol and magnolol from the barks of Magnolia officinalis in stressed rodents. Progress in Neuro Psychopharmacology & Biological Psychiatry, 32, 715-725). The activity of honokiol has been compared to diazepam (commonly known as Valium), with honokiol demonstrating anxiolytic effects, but without the common side effects (Kalman, D.S., Feldman, S., Feldman, R., Schwartz, H.I., Krieger, D. R., Garrison, R. (2008), Effect of a proprietary Magnolia and Phellodendron extract on stress levels in healthy women: a pilot, double-blind, placebo-controlled clinical trial. Nutrition Journal, 7, 1-6). Xu and colleagues (2008) found that elevated levels of the stress hormone corticosterone induced by a chronic mild stress model
where ameliorated following administration of this mixture of chemicals. These findings provide evidence that the mixture of honokiol and magnolol are effective at normalizing hypothalamic-pituitary-adrenal (HPA) dysfunction in rats (the neuroendocrine regulator of the stress response).

There has been limited research investigating the anxiolytic activity of magnolia bark extract amongst humans. Kalman and colleagues (2008) measured the effects of a proprietary blend of magnolia and phellodendron extracts on anxiety, stress and sleep in healthy premenopausal women. The 16 patients in treatment group received 750mg of the mixture, daily for six weeks. They found the blend was not effective in reducing long standing feelings of anxiety or depression. Other assessments conducted in this study, including salivary Cortisol and amylase levels, were not significantly changed in comparison to placebo.

Several studies have demonstrated magnolia bark extract to exhibit a range of health benefits including anti-inflammatory, anti-bacterial, and anti-allergic activities (Lee, Y.K., et al. (2009), Protective effect of the ethanol extract of Magnolia officinalis and 4-0-methylhonokiol on scopolamine-induced memory impairment and the inhibition of acetylcholinesterase activity. *Natural Medicines*, 63, 274-282). Other research has demonstrated a role of magnolia bark extract in the treatment of cancer (Crane, C., Panner, A., Pieper, R.O., Arbiser, J., & Parsa A.T. (2009), Honokiol-mediated inhibition of PBK/mTOR pathway: a potential strategy to overcome immunoresistance in glioma, breast, and prostate carcinoma without impacting T cell function. *Journal of Immunotherapy*, 32, 585-92) and memory impairment (Lee et al., 2009). The active chemicals from magnolia bark Honokiol and magnolol have both been reported to show powerful anti-oxidative activities, and may therefore have a role in the protection against cardiovascular disease (Shen, C.C., et al. (2009), Phenolic Constituents from the Stem Bark of *Magnolia officinalis*. *Journal of Natural Products*, 72, 168-171).

Several studies have demonstrated a relationship between perceived stress and Cortisol levels, with findings providing evidence that higher Cortisol secretion is related to higher levels of stress (Ockenfels, M. C., Porter, L., Smyth, J., Kirschbaum, C., Hellhammer, D. H., & Stone,

Several studies have also demonstrated that alpha-amylase is a sensitive biomarker of the stress response (Nater, U.M., & Rohleder, N. (2009), Salivary alpha-amylase as a non-invasive biomarker for the sympathetic nervous system: Current state of research. *Psychoneuroendocrinology*, 34, 486-496). Salivary alpha-amylase reflects the activity of the sympathetic nervous system, with studies showing increases in alpha-amylase during times of stress (Nater et al., 2009).

As mentioned above, one of the adverse effects of this increasingly fast paced, highly stressed lifestyle is burnout and mental exhaustion (Talbott, 2002). This can lead to lack of concentration and focus, and loss of motivation and energy. There are many commercial products readily available to consumers targeting the relief of these symptoms. These often include large amounts of caffeine and other herbal stimulants such as guarana and ginseng.
However, these strong stimulants can often make one feel jittery and tense for a short time, followed by a let-down with a crash, often leaving one feeling worse than before.

Phosphatidylserine is a therapeutic agent used to improve concentration and alertness, without the negative side-effects of harsh stimulants. Phosphatidylserine is a phospholipid (including amino and fatty acids) that is vital to brain cells. Phosphatidylserine works by enabling brain cells to metabolize glucose and to release and bind with neurotransmitters, which are important to learning, memory and other cognitive functions (Kennedy, D.O., Haskell, C.F., Mauri, P.L., & Scholey, A.B. (2007), Acute cognitive effects of standardised Ginkgo biloba extract complexed with phosphatidylserine. Human psychopharmacology, 22, 199-210). Furthermore, phosphatidylserine has been shown to have a direct effect on improvements in stress and depression. Intravenous administration of phosphatidylserine has been shown to inhibit physical stress-induced increases in Cortisol (Hellhammer, J., Fries, E., Buss, C., Engert, V., Tuch, A., Rutenberg, D., & Hellhammer, D. (2004), Effects of soy lecithin phosphatidic acid and phosphatidylserine complex (PAS) on the endocrine and psychological responses to mental stress. Stress, 7, 119-26) and produce significant improvements in depressive symptoms (Castilho, J.C., Perry, J.C., Andreatini, R., & Vital, M. (2004), Phosphatidylserine: an antidepressive or a cognitive enhancer? Progress in Neuro-Psychopharmacology & Biological Psychiatry, 28, 731-738).

Hydroxytyrosol is a phytochemical with very powerful antioxidant properties, eg. ten times higher than green tea and two times higher than CoQ10. In nature, hydroxytyrosol is found in olive oil. We discovered that the level of hydroxytyrosol in the olive oil sourced for the formulation had an effect on efficacy of the total formulation of this patent in relation to Cortisol reducing effect we decided that this aspect of the formulation could not be left to vary dependent on the olive oil sourced but needed to be standardized. The concentration of hydroxytyrosol in olive oil varies depending on the variety of olive oil used, geographical area, ripeness of the fruit and the method of olive oil pressing, among other factors. For example hydroxytyrosol (HT) concentration in Manzanilla variety of olive oil contains 2.5 to 3 times as much (HT cone 479.6 umol/kg oil) as the Hojiblanca (HT cone 159.2 umol/kg oil), Picual (HT cone 176.8 umol/kg oil) Cornicabra (HT cone 164.1 umol/kg oil) or
Arbequina (HT cone. 179.6 umol/kg oil). For to produce the effects required from this patented formulation we determined that the level of Hydroxytyrosol should be brought to a level of 450-500 umol/kg olive oil regardless of the origin of the olive oil so as to standardize the effect on Cortisol reduction.

The clinical trial documented herein was carried out to validate the unique formulation of this patent regarding the anxiolytic (anti-stress, anti-anxiety, and anti-depression) effects of the specifically formulated magnolia bark extract in phosphatidylsene (from soybean)/olive oil supplemented with hydroxytyrosol/lecinthin (from soybean) and beta carotene to enhance brain delivery, in humans. Specifically this study documents the mechanisms underlying the beneficial effects of magnolia bark extract formulated in the manner outlined within this patent by evaluating the changes in biological (salivary Cortisol and alpha-amylase) and psychological (e.g., concentration, mood, perceived stress, anxiety and depression) processes amongst a human population.

This study was a double-blind, placebo-controlled trial, conducted over a consecutive 4-week period. Participants were randomly allocated to either the treatment group or the placebo-control group. Participants in the treatment group were administered the proprietary supplement as formulated according to this patent. Participants in the placebo control group were administered an inactive, matched capsule containing olive oil, identical in size, shape and color.

The protocol was approved by a Human Research Ethics Committee and an informed consent form was signed by all the subjects who agreed to participate in the protocol.

To be eligible to participate in the study, participants had to be aged 18 years or over; have no history of significant psychological or physiological conditions; not be allergic to magnolia bark extract or other ingredients in the supplement; not taking any stimulant medication including MAO-I, anxiolytics, psychotropics, or steroid hormones; not taking any dietary supplement purported to alter stress levels; not pregnant, breast feeding, or planning to become pregnant during the study.
Participation in the study was over a period of four consecutive weeks, including:

Baseline period: week 1; during this week participants were asked to complete several questionnaires, including an initial Demographic Questionnaire, Daily Diary Questionnaires, and a Weekly Questionnaire. They were also asked to provide several saliva samples. The purpose of this period was to obtain a 'normal' or baseline measure of well-being before treatment.

Experimental period: week 2 and week 3; during these two weeks participants were asked to complete Daily Diary Questionnaires, and a Weekly Questionnaire. Participants were asked to provide several saliva samples. Participants were also asked to take two capsules of the specific formulation of this patent immediately upon awakening each day throughout this period. The purpose of this period was to assess the effects of treatment on well-being measures.

Follow-up period: week 4; during this week participants were asked to complete Daily Diary Questionnaires and a Weekly Questionnaire. Participants were also asked to provide several saliva samples. The purpose of this period was to assess possible continuing effect on well-being measures after the treatment had ceased.

**Questionnaire Measures**

Demographic Questionnaire: assessed demographic information (e.g., age, sex). To be completed at the onset of the study (approx 2 min).

Daily Diary Questionnaire: a series of scales assessing daily incidences of health-related symptoms, and daily mood, stress, depression, anxiety, and alertness, measured on a 10cm visual analog scale. To be completed in the evening of any 3 selected days during baseline week 1, experimental week 2, experimental week 3, and follow-up week 4 (approx 2 min).

Weekly Questionnaire: assessed weekly stress, depression, and anxiety, scoring on a 4-point
response scale (The Depression, Anxiety, and Stress Scales, DASS21; Lovibond & Lovibond, 1995) according to how much the statement applied over the past week (from 0 = "Did not apply to me at all" to 3 = "Applied to me very much") giving a theoretical range between 0-21. To be completed at the end of each week of testing (approx 2 min).

5 Saliva Sampling

Saliva samples were used to measure the biological markers of the stress response, Cortisol (provides a measure of the hypothalamus-pituitary-adrenal axis/ the hormonal regulator of the stress response), and alpha-amylase (provides a measure of the sympathetic nervous system). Participants were asked to provide 3 timed saliva samples on any 3 selected days during each week of testing; baseline week 1; experimental week 2 and week 3; and follow-up week 4. For each day of sampling, a saliva sample was to be provided immediately upon awakening, 30 minutes post-awakening, and at 9 PM.

A written protocol instructing participants how to collect saliva samples was provided. Participants were asked to provide as uncontaminated a sample as possible, and refrain from eating or drinking 1-hour prior to the saliva collection. Timed 3-min samples of whole unstimulated saliva were collected by placing the saliva sampling device, Salivette (Sarstedt, Niimbrecht, Germany) cotton dental swab under the tongue for an accurately timed 3-min period. Participants were instructed not to swallow during this sampling period. After sampling, the swab was returned to the Salivette tube and immediately place in the freezer. Upon receipt at the laboratory, saliva samples were stored at -20°C, until assayed.

Before analysis, saliva samples were thawed and centrifuged (15 min at 3,000 g). Salivary alpha-amylase was assayed with a Salivary Alpha-Amylase Assay kit (Salimetrics, Biocore Pty. Ltd., Cat. No. 1-1902). A 1:20 dilution was made with Assay Buffer. A competitive enzyme immunoassay kit (Salimetrics, Biocore Pty. Ltd, Cat. No. 1-3002) was used for the determination of Cortisol. Analyses were carried out according to the manufacturer’s specifications. All samples from a participant were analysed in one assay. Absorbance measurements were carried out with an Emax micro-plate reader (Molecular Devices, Victoria, Australia) at 405 nm. The
concentration of samples was calculated using an immunoassay software package (SoftMAX Pro), utilising a four parameter logistic curve fitting program.

**Statistical Methods**

Descriptive data are presented as mean ± SD when applicable. Due to the small sample size, all tests were performed using non-parametric techniques. Differences were tested for significance using a non-parametric Friedman one-way within subjects ANOVA. A non-parametric within subjects ANOVA was also used to test the reproducibility of the phenol/chloroform extraction method. Statistical significance was defined at a p value of less than 0.05. Data are presented as means. All statistical analyses were performed using Statistical Package for Social Sciences (SPSS) for Windows Statistical Package Version 18.0.

**Study Population**

The intent-to-treat population consisted of 40 participants; however 34 participants aged between 19-45 years (M=26.09, SD=6.09) completed the study. The sample had an equal number of females and males. Based on random assignment, two groups were established, the treatment group and the control group. Eighteen in the treatment group and 16 in the control group completed the study. Six subjects (two in the treatment group and four in the control group) terminated the study early for various reasons. Baseline characteristics were similar in both groups. The only notable difference between groups was that the control group showed lower waking Cortisol levels at baseline, but this was not considered of clinical importance as all values were within the normal range of human salivary Cortisol levels. Participants with missing observations were removed from relevant analyses.

**Laboratory measures**

**Salivary Cortisol**

Paired sample t-tests were conducted to compare difference in scores for the Cortisol awakening response (CAR) over the study period, with comparisons made with the baseline condition for the treatment group and control group (Table 1 and Figure 1).
Table 1: Mean Differences in CAR Levels over the study period compared to Baseline Condition.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Treatment Group (N = 17) M (SD), p value</th>
<th>Control Group (N = 16) M( SD), p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Change from Baseline to Week 2</td>
<td>0.064 (0.16), p = 0.100</td>
<td>-0.034 (0.11), p = 0.241</td>
</tr>
<tr>
<td>Change from Baseline to Week 3</td>
<td>0.075 (0.15), p = 0.060</td>
<td>-0.028 (0.10), p = 0.263</td>
</tr>
<tr>
<td>Change from Baseline to Week 4</td>
<td>0.082 (0.17), p = 0.080</td>
<td>-0.050 (0.13), p = 0.159</td>
</tr>
</tbody>
</table>

A non-parametric Friedman within subjects ANOVA was conducted to compare the change in the CAR across the study period for the treatment group and control group. Statistical analysis indicated there was no significant change in the CAR over the study period for the control group, χ² (3, N = 14) = 1.63, p = 0.653. However, in the treatment group, statistical analyses indicated a trend towards significance, χ² (3, N = 16) = 4.21, p = 0.240. Further examination of the CAR showed that it tended to decrease following Baseline week for the treatment group, with this drop in CAR maintained throughout the study. However, the control group showed the opposite pattern, with the CAR increasing following the Baseline week and throughout the study period.

Closer examination of Cortisol levels from saliva samples provided immediately upon waking was performed, as the process of awakening results in HPA-axis activity, often characterised by a marked increase in Cortisol levels, therefore providing an accurate measure of the stress response. Mean waking Cortisol levels over the study period for the treatment group and control group are given in Table 2 and presented graphically in Figure 2.
Table 2: Mean and Standard Deviation for Waking Cortisol Levels over the study period for both treatment and control groups.

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Treatment Group (N = 18) M (SD)</th>
<th>Control Group (N = 16) M (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline week 1</td>
<td>0.32 (0.15)</td>
<td>0.25 (0.11)</td>
</tr>
<tr>
<td>Experimental week 2</td>
<td>0.24 (0.11)</td>
<td>0.25 (0.10)</td>
</tr>
<tr>
<td>Experimental week 3</td>
<td>0.28 (0.19)</td>
<td>0.28 (0.10)</td>
</tr>
<tr>
<td>Follow-up week 4</td>
<td>0.25 (0.18)</td>
<td>0.29 (0.12)</td>
</tr>
</tbody>
</table>

A non-parametric Friedman one-way within subjects ANOVA found that there was no significant difference in waking Cortisol levels across the study period for the control group $\chi^2 (3, N = 14) = 0.86, p = 0.836$. However, there was a significant effect for the study sampling period for waking Cortisol levels for the treatment group $\chi^2 (3, N = 17) = 8.08, p = 0.044$. Pairwise comparisons with non-parametric t-tests (Wilcoxon Signed Rank Test) indicated that the amount of waking Cortisol detected in the baseline condition was significantly greater than the amount detected in experimental week 1 ($Z = -2.33, p = 0.02$) and follow-up week 4 ($Z = -2.20, p = 0.028$) of the treatment group.

**Salivary Alpha-amylase**

A non-parametric Friedman one-way within subjects ANOVA was conducted to compare the levels of alpha-amylase detected from evening saliva samples over the study period for the treatment and control groups. For both groups, there were no significant differences in salivary alpha-amylase levels over the study period, $\chi^2 (3, N = 18) = 6.02, p = 0.111$ and $\chi^2 (3, N = 15) = 4.36, p = 0.225$, respectively.

**Questionnaire Measures of Psychological Well-Being**

**Daily Diary Questionnaire**

A non-parametric Friedman within subjects ANOVA was conducted to compare the changes in the daily diary questionnaire measures of psychological well-being over the study. For the control
group, statistical analysis found there was no significant change in the daily measures of stress, depression, anxiety, and alert and focus over the study period, (χ² (3, N = 16) = 7.53, p = 0.057, χ² (3, N = 16) = 6.21, p = 0.102, χ² (3, N = 16) = 0.95, p = 0.814, χ² (3, N = 16) = 3.38, p = 0.337, respectively. However, in the treatment group, statistical analyses found a significant difference in the daily measure of stress (χ² (3, N = 18) = 11.01, p = 0.012). There was no significant difference in the daily measures of depression, anxiety and alert-and-focus in the treatment group (χ² (3, N = 18) = 5.20, p = 0.157, χ² (3, N = 18) = 4.24, p = 0.237, χ² (3, N = 18) = 0.83, p = 0.843, respectively). However, further examination of these daily measures (Figures 3-7) showed an interesting pattern for the ill-being measures of stress, depression, and anxiety, with levels continuing to decrease up until experimental week 3, followed by a rise in levels in the follow-up week of the treatment group. However, the well-being measure of alert-and-focus remained relatively stable over the study period.

**Weekly Questionnaire**

A non-parametric Friedman within subjects ANOVA was conducted to compare the changes in the weekly questionnaire measures of psychological well-being over the study. For the control group, statistical analysis found there was no significant change in the daily measures of stress, depression, and anxiety, (χ² (3, N = 16) = 0.58, p = 0.901, χ² (3, N = 16) = 0.31, p = 0.959, χ² (3, N = 16) = 3.88, p = 0.275, respectively. However, in the treatment group, statistical analyses found a significant difference in the weekly measure of depression (χ² (3, N = 18) = 8.79, p = 0.032). There was no significant difference in the weekly measures of stress and anxiety in the treatment group (χ² (3, N = 18) = 4.83, p = 0.185, χ² (3, N = 18) = 0.773, p = 0.856, respectively). However, further examination of these daily measures (Figures 8-10) showed that all weekly measures of ill-being showed a similar pattern, with levels continuing to decrease up until experimental week 3 in the treatment group.

This study is the first to demonstrate the beneficial effects of the anxiolytic dietary supplement of magnolia bark extract formulated as per this patent on both psychological and biological processes amongst a human population. Initial findings showed that this formulation was effective in reducing the measures of daily stress and weekly depression. In the treatment group,
statistical analyses of salivary CAR indicated a trend towards significance. The CAR response showed a decreasing trend following baseline week for the treatment group, with the drop in CAR maintained throughout the study period. The control group showed the opposite pattern. This observation that formulation was effective in attenuating the CAR provides evidence for its anxiolytic effects through targeting the HPA-axis stress marker, and is in agreement with previous research showing chronic stress is related to increase CAR. There was a significant beneficial effect during the study sample period for waking Cortisol levels in the treatment group; there was no such improvement in the control group.

The beneficial effects of the patented formulation on the stress response through targeting the HPA-axis is further supported by the findings from the questionnaire measures. From the data collected in the Daily Diary Questionnaire, statistical analyses found a significant beneficial difference in the daily measure of stress in the Treatment group but not in the control group. From the Weekly Questionnaire statistical analyses found a significant beneficial difference in the weekly measure of depression in the treatment group. The formulation of this patent (Formula 1 - see below) was also effective in reducing HPA-axis activation of the stress response as indicated by a significant reduction in salivary Cortisol. However, the patented formulation did not appear to have any effect on the sympathetic nervous system, indicated by no significant changes in salivary alpha-amylase.

Formula 1:

<table>
<thead>
<tr>
<th>Material</th>
<th>Capsule soft gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg Magnolia Bark 40%</td>
<td>25 mg</td>
</tr>
<tr>
<td>50 mg Phosphatidylserine 70%</td>
<td>50 mg</td>
</tr>
<tr>
<td>50 mg Olive Oil (Hydroxytyrosol supplemented to between 450-500 umol/kg oil)</td>
<td>50 mg</td>
</tr>
<tr>
<td>500 IU Vit A Betacarotene (as Betacarotene 30%) ARTG 1046</td>
<td>1.1 IU Vit A</td>
</tr>
<tr>
<td>350 mg Lecithin</td>
<td>350 mg</td>
</tr>
</tbody>
</table>
Formula 2:

<table>
<thead>
<tr>
<th>Material</th>
<th>Capsule soft gel</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg Magnolia Bark 40%</td>
<td>25 mg</td>
</tr>
<tr>
<td>50 mg Phosphatidylserine 70%</td>
<td>50 mg</td>
</tr>
<tr>
<td>50 mg Olive Oil (hydroxytyrosol supplemented to between 450-500 umol/kg oil)</td>
<td>50 mg</td>
</tr>
<tr>
<td>500 IU Vit A Betacarotene (as Betacarotene 30%) ARTG 1046</td>
<td>1.1 IU Vit A</td>
</tr>
<tr>
<td>350 mg Lecithin</td>
<td>350 mg</td>
</tr>
</tbody>
</table>

Additional ingredients for Formula 2:

Include the following:

- Phosphatidylcholine : 25mgs
- DHA : 25mgs s to be included in Formula 2:
- DMAE : 25 mgs
- Phosphatidylcholine : 25mgs
- DHA : 25mgs.
Claims

1. A composition designed to reduce stress, depression and anxiety and increase alertness wherein the composition comprises: A: Olive oil supplemented by the addition of extra hydroxytyrosol. B: Phosphatidylserine (PS); C: Lecithin from soyabean; D: Beta carotene, E: Herbal extracts from Magnolia Bark.

2. The composition of claim 1 further comprising at least one antioxidant complex selected from the group consisting of: Vitamin E, Vitamin C and proanthocyanidin.

3. The composition of claim 2 wherein the antioxidant proanthocyanidin is derived from the grape or the seed of Vitis vinifera.

4. The composition of claim 1 further comprising at least one mineral complex selected from the group consisting of calcium, copper, iron, iodine, lithium, magnesium, manganese, potassium, vanadium and zinc.

5. The composition of claim 4 wherein the mineral complex comprises magnesium, calcium, zinc and iron.

6. The composition of claim 5 wherein the magnesium, calcium, zinc and iron are present as Krebs Cycle Intermediates.

7. The composition of claim 1 wherein the composition further comprises at least one B-complex Vitamin selected from the group consisting of Vitamin Bl, Vitamin B2, Vitamin B3, Vitamin B5 and Vitamin B6.

8. The composition of claim 1 further comprising an herbal extract wherein the herb is Luo Han Guo (Siraitia grosvenorii).

10. A method of reducing stress, anxiety and depression comprising administering a supplement consisting of the composition of claim 1 twice daily.

11. A composition comprising the ingredients of Formula I.

12. A composition comprising the ingredients of Formula II.

13. A method of reducing stress, anxiety and depression comprising administering a daily supplement consisting of the composition of claim 11 or 12.


15. A method of reducing body weight comprising administering a daily supplement consisting of the composition of claim 11 or 12.

16. A method of reducing body weight comprising administering a daily supplement consisting of the composition of claim 11 or 12.

17. A method of enhancing alertness and focus in a human, comprising administering a daily supplement consisting of the composition of claim 11 or 12.

18. A method of enhancing alertness and focus in a human, comprising administering a daily supplement consisting of the composition of claim 11 or 12.

19. A method of attenuating the Cortisol Awakening Response (CAR) comprising administering a daily supplement consisting of the composition of claim 11 or 12.
20. A method of attenuating the Cortisol Awakening Response (CAR) comprising administering a daily supplement consisting of the composition of claim 11 or 12.

21. A method of reducing waking Cortisol levels comprising administering a daily supplement consisting of the composition of claim 11 or 12.

22. A method of reducing waking Cortisol levels comprising administering a daily supplement consisting of the composition of claim 11 or 12.
Figure 1: Mean Differences in CAR Levels over the study period compared to Baseline

Figure 2: Pattern of waking cortisol levels over the study period for treatment Group and Placebo Group.
Figure 3: Pattern of Daily Diary Psychological Measures over the study period for the Treatment Group.

Figure 4: Pattern of daily diary stress over the study period for both groups

Daily stress experienced in the Experimental and placebo groups
Treatment period

Figure 5: Pattern of daily diary anxiety over the study period for both groups
Daily ANXIETY experienced in the TC&F™ and placebo groups

Figure 6: Pattern of daily diary depression over the study period for both groups
Daily DEPRESSION experienced in the TC&F™ and placebo groups
Figure 7: Pattern of daily diary alertness and focus over the study period for both groups

Daily ALERT AND FOCUS experienced in the TC&F™ and placebo groups

Figure 8: Pattern of weekly diary stress over the study period for both groups

Weekly STRESS experienced in the TC&F™ and placebo groups
Figure 9: Pattern of weekly diary anxiety over the study period for both groups
Weekly ANXIETY experienced in the experimental and placebo groups

Figure 10: Pattern of weekly diary depression over the study period for both groups
Weekly DEPRESSION experienced in the TC&F™ and placebo groups
INTERNATIONAL SEARCH REPORT

International application No
PCT/IB2012/00003 1

A. CLASSIFICATION OF SUBJECT MATTER

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category* Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No.


Further documents are listed in the continuation of Box C. See patent family annex.

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Date of the actual completion of the international search
24 April 2012

Date of mailing of the international search report
08/05/2012

Name and mailing address of the ISA/European Patent Office, P.B. 5818 Patentdienst 2 NL-2280 HV Rijswijk
Tel (010-70) 340-2040, Fax (010-70) 340-3016

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Schnack, Anne
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