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The effects of *Eleutherococcus senticosus* and *Panax ginseng* on steroidal hormone indices of stress and lymphocyte subset numbers in endurance athletes

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Abstract

A clinical trial was undertaken to investigate the effects of *Eleutherococcus senticosus* (ES) and *Panax ginseng* (PG) on competitive club-level endurance athletes engaged in their normal in-season training. Participants were matched for training stress and received a 33% ethanolic extract (8 mL/day) containing either ES, PG (equivalent to 4 g and 2 g/day of dried root, respectively), or a placebo. A pre-test and post-test were used to evaluate the effects of six weeks of supplementation on cortisol, testosterone, and testosterone to cortisol ratio (TCR) as well as circulating numbers of total T-cells, T-helper cells (CD4), T-suppressor cells (CD8), CD4 to CD8 ratio, natural killer cells, and B lymphocytes. None of the immune system variables changed significantly nor showed any clear trend from pre to post test in any of the treatment groups. No significant change in testosterone, cortisol or TCR was observed in the PG group. In the ES group, however, TCR decreased by 28.7% from 0.0464 to 0.0331 ($P=0.03$). The main contribution to this decrease appeared to be a non-significant ($P= 0.07$) 31% trend towards increased cortisol rather than a very small non-significant ($P= 0.36$) 7% decrease in the calculated mean for testosterone. This result suggested that contrary to initial expectation, ES increased rather than decreased hormonal indices of stress, which may be consistent with animal research suggesting a threshold of stress below which ES increases the stress response and above which ES decreases the stress response.

Keywords: Stress; *Panax ginseng*; *Eleutherococcus senticosus*; Cortisol; Testosterone; Testosterone to cortisol ratio; Lymphocyte subsets

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Introduction

Stress is a convenient, but imprecise term which refers either to an external event that is interpreted as threatening, or to the internal response to that event. When wishing to make a distinction between the two meanings, the former is referred to as a stressor and the latter as a stress response (1). Selye's (2,3) observation that stressors produced a similar pattern of organ and tissue damage (adrenal hypertrophy, thymus involution, and gastrointestinal ulceration) irrespective of the specific type of stressor, established the idea of stress *per se* as a cause of disease in its own right. Since Selye's seminal work many different lines of research have identified stress as a causative agent in a plethora of disease processes including, hyperlipidemia, arterial disease, diabetes, amenorrhoea, infertility, suppression of anabolic processes, immunosuppression, hippocampal deterioration, depression, dementia, and psychosis (4,1,5,2,3,6,7,8). The identification of compounds that can effectively and safely treat the damaging effects of stress remains a major unmet challenge in life-science research.

A class of herbal medicines which may be useful in decreasing the damaging effects of stress are the so-called "adaptogens", of which *Panax ginseng* (PG) and *Eleutherococcus senticosus* (ES) are the best known and most researched examples. *Panax ginseng*, commonly known as Korean Ginseng, has been highly esteemed in Chinese medicine for 4000 years, where it is said to increase "Chi" energy, the deficiency of which is believed to allow disease processes to predominate (9,10). Animal studies conducted in the latter half of the 20th century demonstrated that PG and a plant more recently discovered by Russian scientists, *Eleutherococcus senticosus* (ES), attenuated organ and tissue damage induced by various stressors and, in addition, increased the length of time until exhaustion in forced exercise trials (11,12,13,14). This suggested that ES and PG altered an organism's response to stress in a manner which improved adaptation to stress (15,16). Hence the term "adaptogen" was adopted to classify ES and PG and their respective active constituents, eleutherosides and ginsenosides. Recently, Nörr (17) subjected rats to the stress of saline injection and observed that pre-treatment with ES (3.0mg/Kg/day for seven weeks) decreased corticosterone levels by 45% compared to unsupplemented controls. The dose of ES administered to these rats was, however, 50 times greater than the upper end of the dose range recommended for human consumption (0.06 mg/Kg/day) (18). Indeed it appears that all of the animal studies which have scientifically verified the effectiveness of adaptogens used dosages which were at least an order of magnitude higher than the upper limits of the recommended normal human dose (11,12,16,13,14,17,18). The present study examined the effects of ES and PG at normal human dosage levels on endurance athletes who, as part of their training, voluntarily place themselves under stress.

There is evidence to suggest that training stress in endurance athletes can produce temporary endocrine and immune system perturbation (19,20,21). One measure of endocrine perturbation, which has been used experimentally as a marker of training stress, is a decrease in the resting plasma total testosterone to cortisol ratio (TCR), while possible indicators of immune system perturbation include a decrease in resting numbers of lymphocyte subsets and a decrease in T-helper to T-suppressor cell ratio (22,23,24,25,26). A significant number athletes take ES or PG in the belief that these agents will attenuate immune and endocrine perturbation and thereby decrease the incidence of colds and infection and improve recovery (27).

Furthermore, some competitive athletes and coaches have expressed the need for more scientific investigation into ES and PG which may represent a legal and safe way to augment recovery from the heavy physical exertion of training and competition (27,28). The present study used a six week double blind placebo controlled pre-test post-test trial to investigate the ability of ES or PG to influence endocrine measures of the stress response (cortisol, total testosterone, and TCR) and selected markers of immune system status (lymphocyte subset numbers) in competitive club-level endurance athletes engaged in their normal in-season training. Ethics committee approval for the trial was received from the Royal Melbourne Institute of Technology (RMIT) before commencement of the trial.

Methods

Trial participants

Groups of three male training partners (30 in all) were recruited from a number of endurance sporting clubs and from respondents to advertisements placed in a number of metropolitan University fitness centers. The criteria for inclusion in the study were that the participants: had at least two years of endurance training experience, had trained on average at least five hours/week for the previous three months, were between the ages of 18 and 40 years, did not use medications likely to affect the dependent variables, were training in preparation for competition within the season that the study was undertaken, and were within a group of three training partners matched for training volume and intensity. Training partners were deemed to be matched on the basis that they trained once or more times per week with the other two participants in their group. The level of matching was assessed via a recall questionnaire filled out by participants at the post-test which recorded average weekly training volume and intensity as well as competitions over the previous six weeks. Each participant was telephoned twice, once at two weeks and again at four, to check that their training was progressing according to their plan and had not been affected by illness, injury or any other reason. Of the 18 participants who successfully completed the study, nine were cyclists, six were tri-athletes, and three were endurance kayakers. All were competitive club-level athletes, i.e., they belonged to a club and were training for competition within the season they were tested. Participants were required to read and sign an informed consent form before participation.

Supplementation

Each member of a matched group was randomly assigned a 500 mL bottle containing a 35% ethanolic extract of either ES, PG, or a placebo solution. ES and PG were supplied courtesy of Mediherb Pty. Ltd. (Warwick Queensland, Australia) (henceforth referred to simply as “Mediherb”). The ES supplement consisted of 8 mL per day of Mediherb’s 35% ethanolic ES extract (equivalent to 4g/day of dried root), while the PG supplement consisted of 4 mL per day of Mediherb’s 60% ethanolic PG extract (equivalent to 2g/day of dried root) diluted with distilled water and drinking ethanol (Polish Pure Spirit, United Liquors Pty. Ltd., Poland) to make it equivalent to the ES supplement in terms of volume and ethanol concentration. The doses for both herbs were commensurate with the upper limit of the normal human dose range as recommended by Baranov (9), Baldwin et al. (18), and Mediherb. The placebo

preparation was adapted from that used by Asano et al., (29) and contained 0.5 mL of gobo flavour (International Flavours and Fragrances Pty. Ltd., Sydney, Australia) 0.15 mL French vermouth essence (International Flavours and Fragrances Pty. Ltd. Sydney, Australia), 0.5 mL Parisian colour (Queen Fine Foods Pty. Ltd. Brisbane, Australia), 35 mg of brown HT colour (Ajanta Chemical Industries Pty. Ltd. New Delhi, India) 25 mL of glycerol (Faulding Healthcare Pty. Ltd., Rydalmere, NSW, Australia), 178 mL of 80% drinking ethanol, and 292 mL of distilled water. Participants were given a marked measuring cylinder and were instructed to take eight mL of their treatment before breakfast every morning for the next six weeks and to record the event on a calendar provided on the bottle. Each participant was telephoned twice, once at two weeks and again at four, to check compliance. At the post-test, participants were asked to bring in the bottle of supplement so that supplementation compliance could be assessed via the calendar provided and the volume remaining.

Blood collection and analysis

Prior to random assignment of supplement, all participants reported to the Austin Hospital (Heidelberg Victoria, Australia) at a prearranged time (between 8 and 11 a.m.) for the pre-test when 15 mL of blood was drawn from an antecubital vein. The blood collection procedure was repeated at the same time of day six weeks later at the post-test. Participants were instructed not to compete on the day before testing, nor to exercise at all on the morning of testing, and to eat their normal breakfast on the morning of testing. Ten mL of the blood was collected into EDTA lined plastic tubes and kept at room temperature prior to whole blood count and lymphocyte analysis which were conducted on the same day. The remaining five mL was collected into plain plastic tubes and allowed to clot prior to being centrifuged at 3000 rpm for ten minutes. The serum layer was then pipetted off and separated into five aliquots before storage at minus 80° C until testosterone and cortisol determinations were performed. In all nine dependant variables were measured: cortisol concentration, total testosterone concentration, total testosterone to cortisol ratio (TCR), total T-cell count, T-helper cell count, T-suppressor cell count, T-helper to T-suppressor cell ratio, Natural killer cell (NKC) count, and B-cell count.

All endocrine determinations, lymphocyte subset counts and whole blood counts were conducted in the Haematology Department, Austin Hospital, Heidelberg Victoria, Australia. All of the serum cortisol and testosterone determinations were undertaken together (one run each) at the end of the trial. Determination of serum cortisol was via a competitive chemiluminescent immunoassay (Ciba Corning ACS™). All samples were measured once only (according to the operating procedures for cortisol and testosterone determination followed by the Austin Hospital). Intra-assay coefficients of variation determined for high (858.7 nmol/L,) and low (85.2 nmol/L) cortisol concentrations were 6.4 and 8.9 %, respectively. The assay had a minimum detectable concentration of 5.52 nmol/L and was accurate up to 2069 nmol/L. Determination of serum total testosterone was also via chemiluminescent immunoassay (Ciba Corning ACS™). The assay used a steroid releasing agent to release bound testosterone from its binding proteins, yielding total testosterone concentration. Intra-assay coefficients of variation determined for high (38.00 nmol/L,) and low (2.73 nmol/L) testosterone concentrations were 13.15 and 6.14 %, respectively. The assay had a minimum detectable concentration of 0.347 nmol/L and was accurate up to 52.05 nmol/L.

Flow cytometric evaluation of the proportion of each lymphocyte subset as a percent of the total lymphocyte population was conducted using a Coulter Epics Elite ESP flow cytometer (Coulter Electronics Pty. Ltd., Brookvale NSW, Australia) with an argon laser set at an excitation wavelength of 488 nm. Whole blood was prepared for cytometric analysis by lysing red blood cells with formaldehyde followed by stabilization and fixation of white blood cells with polyethylene glycol, and one percent paraffin aldehyde, respectively. Next, monoclonal antibodies conjugated with fluoresceinisothiocyanate (FITC) or phycoerythrin (PE) were mixed with 100 mL of the prepared white blood cell suspension. The monoclonal antibodies and the fluorescent marker used to specify each class of lymphocyte subset along with the coefficient of variation (CV) for each procedure were as follows: CD3 FITC/CD4 PE for discrimination of T helper/inducer cells (CD4) from pan T cells (CD3) (CV = 1.45 %), CD3 FITC/CD8 PE for discrimination of T suppressor/cytotoxic (CD8) from pan T cells (CV = 9.70 %), CD3 FITC/CD16+ 56 PE for discrimination of natural killer cells from pan T cells (CV = 22.99%), CD19 PE to specify B lymphocytes (CV = 17.37 %), IgG1 FITC/IgG1 PE (mouse Ig) was used as a control for possible immunoglobulin interference (no quality control data provided), CD45+ FITC/CD14+ PE for discrimination of monocytes (CD14) from all white blood cells (leucocytes CD45) (CV = 0.00 %). The lymphocyte subset tests were conducted throughout the study period from 1994 to 1997, however, the coefficients of variation reported above were determined from quality control data taken from a two week period at the end of the study and are, therefore, representative only of the variation expected in these procedures. Software on the cytometer corrected for potential false positive lymphocyte count errors produced by monocyte presence, while the absolute number of circulating lymphocyte subsets was calculated by multiplying the fraction of each lymphocyte subset (as a proportion of the total lymphocyte population as determined by flow cytometry) by the total number of lymphocytes determined from the whole blood counts (Coulter Electronics Pty. Ltd., Brookvale NSW, Australia).

Statistical analysis

Statistical analysis was carried out using a two by three factorial design with matched samples on one vector and the second factor fully repeated (30). The number of matched groups (six) allowed for five (number of matched groups minus one) pre verses post test planned comparisons (30). The five dependent variables chosen for comparison before the commencement of the study were testosterone concentration, cortisol concentration, TCR, T helper/T suppressor cell ratio and NKC count. The remaining four non planned comparisons, total T-cell count, T-helper cell count, T-suppressor cell count, and B-cells count, were made using Newman-Keuls post-hoc tests if found to be significant on T-test. An alpha level of 0.05 (single tailed comparison) was chosen for assessing significance of comparisons.

Results

Compliance

The nature of the two by three matched sample factorial design employed for statistical analysis required that (a) participants be in matched groups of three and (b) data collection be

fully repeated at the post-test. Hence groups of three that contained one or more members who did not complete either the pre-test or post-test, or who failed to comply with supplementation, were eliminated from the study. The most common reason for elimination from the study was failure to complete the post-test, usually due to time constraints from a participant's work or sporting commitments. One participant, however, was not able to complete the endocrine component of the pre-test due to insufficient blood collection (collapsed vein), while another participant, taking the placebo preparation was unable to comply with supplementation because it appeared to give him stomach cramp. None of the other participants reported any difficulty taking their supplement daily. Of the ten groups (of three) original trial participants, six groups successfully completed all requirements for the lymphocyte subset component of the trial. The mean weekly training time for these six groups was 18.9 ± 7.4 (SD) hrs/wk. There was no significant difference between the hours trained in the ES (18.1 ± 7.9 hrs), PG (21.4 ± 6.4 hrs), or placebo (17.2 ± 8.6 hrs) groups (all $P>0.05$). One of these six groups was eliminated for the endocrine analysis component of the trial due to insufficient blood collection at the pre-test.

Endocrine data

ES treatment decreased the testosterone to cortisol ratio (TCR) by 28.7% from 0.0464 to 0.0331 ($P = 0.03$). The main contribution to this decrease appeared to be a non-significant 31.4% trend towards increased cortisol ($P= 0.07$) rather than a very small non-significant 7.1% decrease in the calculated mean for testosterone ($P= 0.36$). PG treatment did not significantly change TCR, cortisol or testosterone. In the placebo group their was no significant change in TCR, testosterone, or cortisol (see Table 1). There were no significant differences between ES, PG, or placebo means for any of the endocrine parameters at pre-test (all $P> 0.05$).

Lymphocyte data

T-tests carried prior to Newman-Keuls analysis revealed that neither ES nor PG had any statistically significant effect on any of the measured lymphocyte subsets. Few of the lymphocyte variables displayed trends from pre-test to post-test with the exception of T suppressor

Table 1
Effect of ES or PG supplementation on testosterone, cortisol, and TCR

	ES		PG		Placebo	
Parameter	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Serum cortisol ($nmol \cdot L^{-1}$)	357 ± 42	469 ± 72	352 ± 35	433 ± 58	419 ± 31	436 ± 41
Serum testosterone ($nmol \cdot L^{-1}$)	15.6 ± 1.0	14.5 ± 1.7	15.1 ± 1.1	17.7 ± 1.8	16.9 ± 1.2	17.7 ± 2.3
Serum TCR ($\times 10^{-3}$)	46.4 ± 6.5	33.1 ± 5.2*	45.5 ± 7.2	42.8 ± 4.9	41.0 ± 3.6	40.6 ± 3.5

(Mean ± Standard Error) serum testosterone, cortisol, and TCR before and after six weeks of ES, PG or placebo supplementation in competitive club-level endurance athletes ($N= 15$) engaged in their normal in-season training. "*" denotes a significant difference ($P<0.05$) between the change in the treatment group compared to the placebo group. TCR, testosterone to cortisol ratio.

Table 2
Effect of ES or PG supplementation on lymphocyte subsets

	ES		PG		Placebo	
Parameter	Pre-test	Post-test	Pre-test	Post-test	Pre-test	Post-test
Lymphocyte count ($\times 10^9 L^{-1}$)	1.81 ± 0.26	1.81 ± 0.24	1.96 ± 0.12	2.00 ± 0.15	2.13 ± 0.13	2.01 ± 0.22
T-cell CD3+ count ($\times 10^9 L^{-1}$)	1.35 ± 0.21	1.30 ± 0.13	1.47 ± 0.16	1.43 ± 0.13	1.73 ± 0.12	1.43 ± 0.13
T-helper cell CD3+\CD4+ count ($\times 10^9 L^{-1}$)	0.76 ± 0.16	0.67 ± 0.09	0.82 ± 0.06	0.74 ± 0.10	0.94 ± 0.12	0.81 ± 0.07
T-suppressor cell CD3+\CD8+ count ($\times 10^9 L^{-1}$)	0.49 ± 0.06	0.50 ± 0.07	0.47 ± 0.06	0.76 ± 0.18	0.63 ± 0.04	0.65 ± 0.10
T helper : T suppressor ratio	1.53 ± 0.20	1.46 ± 0.26	1.84 ± 0.23	1.29 ± 0.27	1.55 ± 0.26	1.35 ± 0.18
Natural killer cell count CD3−\16+56+ ($\times 10^9 L^{-1}$)	0.23 ± 0.08	0.28± 0.09	0.32 ± 0.08	0.26 ± 0.04	0.25 ± 0.06	0.30 ± 0.09
B-cell CD20+ count ($\times 10^9 L^{-1}$)	0.26 ± 0.06	0.29 ± 0.12	0.26 ± 0.03	0.28 ± 0.09	0.22 ± 0.05	0.23 ± 0.04

(Mean ± Standard Error) plasma lymphocyte subset counts and T helper : T suppressor cell ratio before and after six weeks of ES, PG, or placebo supplementation in competitive club-level endurance athletes (N= 18) engaged in their normal in-season training.

cells in the PG group which displayed a non-significant 61.7% trend towards increase ($P>0.12$). No changes were detected in any lymphocyte subset in the placebo group (see Table 2). There were no significant differences between ES, PG, or placebo means for any of the lymphocyte parameters at pre-test (all $P>0.05$).

Discussion

Endocrine data

The significant ($P=0.030$) 28.7% decrease in TCR in the ES supplemented group would appear to represent a substantial perturbation in steroid hormone levels, considering that a decrease of 30% in TCR was considered by Adlercreutz et al. (22) as diagnostic of overtraining syndrome in athletes. It appeared unusual that ES, supposedly beneficial in attenuating the ill-effects of stress, should elicit a change in a biological marker indicative of increased stress. A search of the relevant literature, however, revealed another example of ES increasing the stress response. Nörr (17) observed a decrease in corticosterone levels with ES administration in rats exposed to the stress of saline injection, however, when administered to unstressed rats, i.e., those not exposed to the stress of saline injection, ES increased corticosterone concentration by 102% over the level observed in unsupplemented controls. This suggests that there may be a threshold of stress severity below which ES increases the stress response and above which ES decreases it. In turn, this suggests the possibility that in the

present study, the increase in the stress response produced by ES may have been because the severity of stress endured by the participants, was beneath this hypothetical threshold. It is worth noting that there is also evidence supporting the concept of such a threshold for the other principal adaptogen, PG. Kim et al. (31) measured the effect of PG on the stress response (as indicated by adrenal ascorbate loss) over a nine hour period in mice subjected to continuous heat stress. During the initial stages of the experiment (one hour after stressor onset) the stress response in PG treated mice was approximately 104% higher than in unsupplemented controls ($P<0.05$), however, when the stress response reached a certain threshold (approximately 84% of peak adrenal ascorbate loss, occurring one hour and 40 minutes after stressor onset) the stress response in PG supplemented animals dropped below unsupplemented controls (by up to 43% [$P < 0.05$]), a process which continued for the next three hours.

With regard to how ES and PG could produce a biphasic response Gaffney (32) suggested that ES and PG may exaggerate an already existing biphasic response by increasing the occupancy of stress hormone receptors at positive and negative feedback regulatory sites. It is hypothesized that this increased occupancy could occur in one of three ways; (a) ES and PG may increase receptor affinity by binding to a secondary site on the noradrenergic or glucocorticoid receptor, respectively, (b) ES and PG may inhibit specific enzymes which serve to decrease the occupancy of stress hormone receptors, or (c) ES and PG may induce specific enzymes which serve to increase the occupancy of certain stress hormone receptors. With regard to the enzyme inhibition hypothesis, it is suggested that ES may inhibit catechol-O-methyl tranferase (COMT), an enzyme which catalyses the methylation of noradrenaline into its inactive metabolite, normetanephrine. By inhibiting COMT, ES may allow more noradrenaline to bind to noradrenaline receptors including those responsible for positive and negative feedback of the stress response, i.e., alpha-one and alpha-two receptors, respectively, thereby exaggerating an already existing biphasic response (33). With regard to the enzyme induction hypothesis, it is proposed that PG may induce 11-beta hydroxysteroid dehydrogenase isozyme one (11-HSD1), an enzyme that resides in close proximity to type II glucocorticoid receptors and catalyses the resynthesis of glucocorticoids from their inactive oxidized, to their active reduced form (34). If PG does induce 11-HSD1, it would be acting in an analogous way to glucocorticoid, which has recently been shown to induce the enzyme in *vivo* (35). By inducing 11-HSD1, PG may allow more cortisol to bind to glucocorticoid receptors including those responsible for positive (36) and negative feedback (37) of the stress response.

It is not clear why only ES caused a statistically significant reduction in TCR. The dose of each adaptogen was chosen in accordance with the upper end of the normal human dose, as recommended by Baranov et al. (9), Baldwin et al. (18), and Mediherb. It has been suggested that the upper end of the recommended normal human dose for PG is set at a more conservative, and less physiologically active, level than ES due to a perception that long term PG use at high doses is associated with a greater incidence of side effects than ES (personal communication with Kerry Bone, Director of Research and Development, Mediherb). Hence, the difference in response might be due simply to a lower effective dose of PG. The possibility should also be considered that ES and PG may have different effects at different time points after administration. Thus, for example, PG may have had an effect at an earlier point in the trial, which was not evident after six weeks.

Since the doses used in this study were, in part, set at a level to avoid side effects, it is

worthwhile briefly documenting what some of these side effects are and what type of studies reported them. Siegel (38) used questionnaire based studies to investigate side effects associated with long term (2 year) PG (or ES – the study did not distinguish between the two) use on participants who took self prescribed unspecified doses (there was no control group). The side effects included hypertension (17%), nervousness (19%), sleeplessness (20%), skin eruptions (25%), and diarrhoea (35%). In a later study, Siegel (39) used a similar approach to investigate the side effects experienced by participants during and after 12 weeks of oral administration PG. Again, dose was not reported, although it was noted that side effects, which were similar to Siegel's (38) earlier study, occurred predominantly in participants who also used psychomotor stimulants, e.g., caffeine. Side effects similar to those reported by Siegel (38, 39) were reported by Cartwright (40), who conducted a clinical trial using 22 students (age 18–25 years) to whom PG (0.5 g/day) was administered over a 30 day period to examine its influence on fatigue and concentration. The side effects reported were insomnia (17%), skin eruptions (17%) and diarrhoea (8%).

Lymphocyte data

Neither ES nor PG had any statistically significant effect on any of the measured lymphocyte subsets which remained essentially unchanged. These results are not consistent with the results of Bohn et al. (41) who found that ES (2 g/day) increased lymphocyte numbers, especially T-helper cells (60%, $P<0.0001$). This discrepancy is difficult to reconcile since the dose of adaptogen was similar in both trials. The only obvious difference was that Bohn et al. (41) used middle aged sedentary participants while the present study used younger endurance trained athletes, suggesting the possibility that age or training status may be important factors influencing the effects of adaptogens on the immune system. On the other hand, these results are consistent with Srisurapanon et al. (42) who observed no change in lymphocyte subset numbers after eight weeks of PG supplementation in young healthy adult males. It is worth noting that the dose used by Srisuraponanon et al. (41), 300 mg/day, was approximately 6.7 times lower than that used in the current trial.

With regard to comparisons of the null results of the present study with the results of animal trials, where adaptogens have been shown to profoundly modify immunity, the differing dose of adaptogen may provide an explanation, e.g., the dose of PG used by Singh et al. (43) which was observed to increase natural killer cell activity in mice and to protect them from semliki forest virus was 660 mg/Kg/day given orally for four days. While the dose of PG root saponins used by Luo et al. (44), observed to stem the decline in phagocytic function of macrophages after cold stress, was 100 mg/Kg via intraperitoneal injection. These doses are, respectively 22,000 and 3,000 times greater than the upper end of the dose range recommended for human consumption (0.03 mg/Kg/day, the approximate dose used in the present study) (9,18). With regard to where and how the recommended doses for human consumption of PG and ES were derived, in the case of PG it appears to have come more from time tested trial and error on the part of herbal practitioners (9,18), while in the case of ES it appears to be based on the amount producing equivalent effects to PG in animal studies (11, 12).

In addition to dose, other important methodological differences exist between the present human study and animal studies which observed an effect of adaptogens on the immune system. These differences relate to the greater relative intensity of the experimental stressor in

the animal studies. Importantly, the participants in the current study had control over their training and presumably felt that their regular exercise training was for their own benefit. Luo's rats, for example, obviously had no such control nor any perception of benefit. Glavin (45) suggested that inescapability from a stressor rather than the stressor itself is a critical determinant of stressor induced damage. Furthermore, the novelty of a stressor has an effect on the stress response which usually decreases if the stressor is repeated and not too severe (46). Hence, the habituation to endurance exercise training in the participants in the present study may have decreased the effectiveness of endurance training as a stressor to the point where adaptogens had no effect on lymphocyte subset numbers.

Conclusions

Based on the results of the present study, the stress of normal in-season training in endurance athletes may not be severe enough to elicit a stress reducing response from ES or PG. Furthermore, if a 29% reduction in TCR is interpreted as being deleterious to the recovery process, then the possibility should be considered that ES may not be an appropriate supplement for club-level endurance athletes engaged in their normal in-season training. Fulder (10) suggested that ES and PG are not recommended for healthy people under forty years of age, but are instead more useful as treatments for disease where a lack of vitality in one or more organ systems is considered to be a predisposing factor. The results of the current trial, in the case of ES only, may, in part, support this general recommendation. On the other hand, Fulder (10) suggested that the other principal adaptogen, PG, was traditionally kept by soldiers in some countries to be taken in case of serious injury, which suggests that when placed under unusually stressful situations adaptogens may provide a benefit to otherwise healthy young people. A possible way to assess this is by measuring the effects of ES or PG on endocrine and immune system perturbation in athletes or soldiers undertaking highly stressful competition or military training exercises.

The world-wide popularity of ES and PG appears to be linked less to the results of controlled human studies, of which there are relatively few, and more to the value placed upon PG in traditional Chinese medicine and the results of animal studies documenting both decreased damage from severe stressors and an anti-fatigue effect with large doses. The potential value of ES and PG to decrease damage from severe stressors in humans warrants ongoing scientific investigation with controlled human studies.

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