

Full Length Research Paper

Effect of traditionally designed nutraceutical on stress induced immunoglobulin changes at Antarctica

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This study was conducted to establish the effect of a traditionally designed nutraceutical on stress related changes in selected immunoglobulin levels in the body. The nutraceutical was prepared from different potent herbs described in Ayurveda using standard operative procedures and were tested for heavy metal and microbial load. Initially, 21 subjects were selected in addition to 7 volunteers for control group who did not consume nutraceutical. Sampling was done at zero days and at fortnightly intervals. The levels of selected immunoglobulin IgG, IgA and IgM were estimated with turbidity metric immunoassay at different time intervals. The concentration of immunoglobulin IgA was 146 ± 15.96 at zero day stage. The levels of these immunoglobulins were lower at all stages as compared to the concentration at zero day in trial group subjects whereas the concentration was significantly higher (t stat.>t critic. at $p < 0.05$) in control group subjects. The concentration of IgG was very high to the tune of 3091 ± 705 at zero day stage. The level of IgG was lower in trial subjects as compared to control subjects at all stages except at the 6th week stage where it was higher in trial subjects. Concentration of immunoglobulin IgM was 80.75 ± 30.39 (t stat.>t critic. at $p < 0.05$) at zero day followed by a decrease in both groups at the 2nd week, however the concentration was almost $1/3^{\text{rd}}$ in trial drug subjects as compared to the levels in control subjects followed by an abrupt increase at the 4th week. The levels increased to 106 ± 8.94 at the 4th week stage and 115 ± 9.35 at the 6th week stage in control subjects (even higher than at zero day) whereas the values were 46.15 ± 11.39 and 55.38 ± 15.34 (t stat.>t critic. at $p < 0.05$) at respective stages in trial drug subjects. On the whole the pattern of fall and rise in levels of IgM were similar in the control as well as treatment group subjects at all stages. Studies revealed that the components of the nutraceutical tended to exert significant (t stat.>t critic. at $p < 0.05$) anti-stress effect against stress related changes in immunoglobulin in the body due to the battery of stresses encountered at Antarctica.

Key words: Rasayana, immunoglobulins, *Withania somnifera*, *Tinospora cordifolia*, *Chlorophytum arundinaceum*, *Piper longum*, *Prunus amygdalus*, Antarctica, stress.

INTRODUCTION

An Antarctic expeditioner has to face and cope up with many physical and psychological stresses apart from the stress related to UV radiations, magnetic field, high wind velocity, extreme cold conditions, circadian biorhythms

(Sundaresan et al., 1999) and chemicals. Men living in Antarctica also suffer significant emotional strain as a result of physical isolation and social deprivation (Roy and Deb, 1999) in addition to physiological stress imposed by few factors mentioned earlier. These have been reported to effect immunoglobulin levels also. All these changes might be due to the oxidant stress, which is enhanced by the stressors at the Antarctic region.

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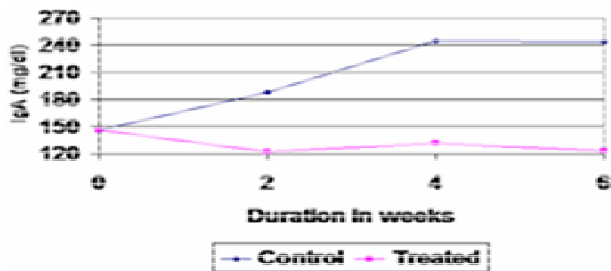


Figure 1. Effect of APY and APP on IgA.

Oxidative stress results from an imbalance in this pro-oxidant antioxidant equilibrium in favour of pro-oxidants. Oxidative stress also leads to some detrimental effects on the immune system. Attempts have been made to study the mechanisms that might be involved in man's physiological responses to polar conditions. Few of them have helped in evaluating the nutritional requirements of men working in Polar Regions.

Ayurveda, the first recorded scientific medicine in the history of the world aims to improve the quality and span of life with its major emphasis on prevention of disease and promotion of health by strengthening tissues so that they can withstand exogenous and endogenous stressors. This is achieved by modulating diet and life style as well as by the appropriate use of drugs that restore the equilibrium of the body (Dahanukar and Thatte, 1989a). Over 600 plants have been described in various Ayurvedic texts like *Charak samhita*, *Sushruta samhita* and various *Nighantu*. There are interesting groups of rasayana herbs that have adaptogenic properties (Dahanukar and Thatte, 1989b). According to Brekhman and Dardymov (1969), an adaptogen must produce a non-specific response that is, increase the power of resistance against multiple stressors. It should have a normalizing influence and be innocuous but should not influence normal body functions more than required. The word "rasayana" means the path that "rasa" takes (*rasa*-plasma; *ayana*-path). It is believed that in Ayurveda, the qualities of "rasa dhatu" influence the health of other "dhatu's" (tissues) of the body (Dahanukar and Thatte, 1989b). Hence any medicine that improves the quality of rasa should strengthen or promote the health of all tissues of the body. A significant part of Ayurvedic therapeutics is preventive in nature. This is the concept of "vyadhirodhak chamataav" that is, the capacity of the body to resist disease. So obviously the immune system as recognized in modern biology, which provides protection against microbes, should be a part of it.

Although there is a provision of very good nutritional diet and pills containing vitamin C and vitamin E pills to the members of the expedition, yet the scientific field lacks the data related to nutritional requirements in the Antarctic climate. At the same time Paul Coates who works in the office of dietary supplements at NIH says that "just because a food with certain compounds in it is

beneficial to health does not mean a pill containing the same compound is". Keeping the above view in mind, a regimen of various rasayana herbs including Ashwagandha (*Withania somnifera*), Guduchi (*Tinospora cordifolia*), Safed musli (*Chlorophytum Arundinaceum*), Pippali (*Piper longum*), Badam (*Prunus Amygdalus*) and some other herbs were prepared and a study was conducted to evaluate the effect of rasayana as a drink and food supplement on prevention of hazards of cold climate on the immune system in Antarctic climatic conditions.

MATERIALS AND METHODS

The study was conducted at Antarctica on Indian station "Maitri" on voluntary accepted subjects out of the 23rd Indian scientific expedition to Antarctica. There were a total of 50 team members out of which twenty one members opted for supporting this study. Initially all the members were briefed about the scope of the project and were given project proformas for their willingness to join the study as per international ethical guidelines. On day zero, blood sampling was conducted and was analysed for biochemical parameters. All estimations were done using standard methods.

Sampling was followed by feeding of subjects with the drug/food supplement "ayush poshak peye (APP)" and "ayush poshak yoga (APY)". Both drugs are coded drugs and were prepared at CCRAS head office, Delhi and supplied to the team visiting Antarctica. The standard operative procedures (SOP) for the preparation of the trial food supplements are under patent process hence can not be revealed at this stage. The trial drug was tested for heavy metals and it was free from cadmium, lead and arsenic. The food supplement was also tested for microbial load and total bacterial count was 7.3×10^4 CFU/g whereas total fungal count, enterobacteria count and *salmonella* spp. was within permissible limits.

All the subjects were provided with the food supplements daily. The "ayush poshak peye" was prepared daily at various timings depending on the convenience of members because they used to be busy with their own scientific projects. The investigators made it pertinent that all the subjects are dispensed with APP (125 ml) and APY (50 g) once daily. The subjects who could not take the drug regularly were put under dropouts. Out of 21 subjects, 8 were dropped out due to different reasons. Three of them went to ship due to some assignment and could not continue taking the drugs. Three of them had some physical problem and discontinued the drug. Two subjects who missed the food supplements continuously for more than two days were dropped out.

In addition to 21 subjects, a control group consisting of 7 subjects was maintained throughout the study period. These subjects were also team members, but were not fed with the supplement. The sampling for all the parameters was done at fortnightly intervals for up to 6 weeks. The effect of the food supplements was assessed from the levels of IgG, IgA and IgM. Immunoglobulins were estimated with turbidity metric immunoassay by using kits from Tulip Diagnostics Pvt. Ltd. A standard curve was prepared for all the immunoglobulins under study using different concentrations of the standard known controls from the same batch of the company for calibration and calculations.

RESULTS

The concentration of immunoglobulin IgA was 146 ± 15.96 at zero day stage (Figure 1). The levels increased continuously at 2nd and 4th week stages in the control group. The level of IgA was similar at the 4th and 6th weeks in the

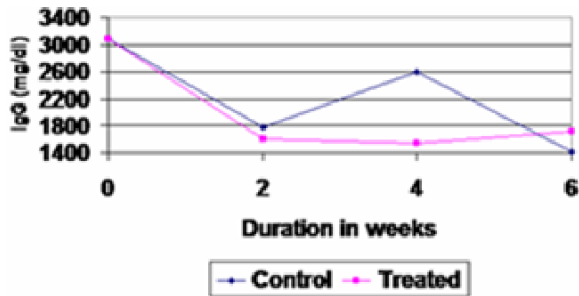


Figure 2. Effect of APY and APP on IgG.

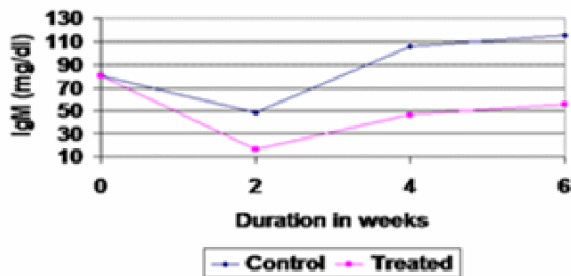


Figure 3. Effect of APY and APP on IgM.

control group. In contrast to it, the levels decreased in the trial drug group subjects at the 2nd week stage but depicted slight increase at the 4th and 6th week as compared to levels at the 2nd week stage. On the whole the levels of these immunoglobulins were lower at all stages as compared to concentrations at zero day in the trial group subjects whereas the concentration was higher in the control group subjects (t stat.>t critic. at p<0.05).

The concentration of IgG was very high to the tune of 3091±705 at the zero day stage (Figure 2). It was lower at all stages in all the subjects irrespective of the trial drug and control as compared to levels at zero day. The level was decreased at the 2nd week, increased at the 4th week and again decreased at the 6th week stage in the control group subjects. The level of IgG was lower in trial subjects as compared to control subjects at all stages except at the 6th week stage where it was higher in trial subjects (t stat.>t critic. at p<0.05). The concentration of IgG decreased till the 4th week stage and slightly increased at the 6th week stage in trial drug subjects.

Concentration of immunoglobulin IgM was 80.75±30.39 at the zero day stage (Figure 3). At the 2nd week stage, the level decreased in both groups, however the concentration was almost 1/3rd in trial drug subjects as compared to the levels in control subjects. At the 4th week stage the level increased abruptly in subjects. The levels increased to 106±8.94 at the 4th week stage and 115±9.35 at the 6th week stage in control subjects (even higher than at zero day stage) whereas the values were 46.15±11.39 and 55.38±15.34 at respective stages in trial drug subjects (t stat.>t critic. at p<0.05). On the whole the

pattern of fall and rise in levels of IgM were similar in control as well as treatment group subjects at all the stages.

DISCUSSION

The concentration of secretory immunoglobulin IgA increased till the 4th week stage and was maintained at same level till the 6th week stage in the control group however the levels decreased or were more or less equal to zero day values in the trial drug subjects. This depicts development of tolerance in treatment group subjects in stress conditions.

Levels of IgG decreased at the 2nd week stage in both groups indicating stress related changes in the 2nd week stage of Antarctica stay. The levels in the trial group subjects were still lower as compared to the control group indicating better resistance developed in the body as an adaptive mechanism to stress. At the 4th week stage, the levels in the control group were raised significantly indicating the effect of stress on the body; however in the trial group the lower level depicted more tolerance of the body. At the 6th week stage, the levels of this immunoglobulin decreased in the control group even lower as compared to the trial drug group probably due to development of resistance of the body due to adaptation and acclimatization to the environment; however the values were almost comparable at the 2nd, 4th and 6th week stages in the trial drug group subjects.

Concentration of IgM was decreased at the 2nd week stage in both groups as compared to zero day values. This shows that the body became less prone to infections or it could be the effect of the bacteria free environment of Antarctica on the immune system. The concentration of immunoglobulin increased gradually till the 6th week stage in both groups; however the values were significantly lower in the treatment group as compared to the control group probably due to better development of resistance in the trial drug subjects against stress induced changes in immunoglobulin levels.

Amongst many medical problems immuno-suppression has been observed during Antarctica sojourn (Muller et al., 1995a; Muller et al., 1995b). There is a wide body of literature demonstrating the detrimental effects of stress on the immune system (Tingate et al., 1997; Boneau, 1990; Khansari et al., 1990). Antarctica expedition team members encounter stress, which is thought to influence immune function through the autonomic nervous system innervating lymphoid cells (Rivolier et al., 1988, Singh et al., 2004). The stress related changes in the level of these immunoglobulins in this study are in accordance with earlier Antarctic studies in which Muchmore et al. (1974) found a decline in serum IgG and IgM, whereas Tashpulatov (1974) observed a rise in serum IgG and IgM levels in Antarctic workers. However the lower level of immunoglobulins in the trial group subjects at almost all the stages in this study demonstrates that a better

adaptive mechanism was adopted by these subjects in relation to stress.

A number of *Rasayana* drugs have been elaborated in *Materia Medica* of Ayurveda for the enhancement of the body's resistance. Some rasayana plants are said to prevent aging, re-establish youth, strengthen life and brain power and prevent disease (Sharma, 1983; Ghanikar, 1981) all of which imply that they increase the resistance of the body against any onslaught. The food supplement consisted of a number of herbs so its anti-stress effect on stress-induced changes in immunoglobulin levels cannot be pinpointed but can be hypothesized. As *Amygdalus prunus* (Badam) is a rich source of natural vitamin E and has been part of the food supplement, it would have supplied vitamin E in sufficient quantity to meet the requirements of the body (Chopra, 2004). Many of the adaptogen rasayana have already been shown to possess immuno-stimulant activity (Wagner, 1994). Whether this immuno-stimulation is responsible for the adaptogenic potential is not clear till date. As mentioned earlier during stress, prostaglandin and antioxidant systems of target organs serve as natural defence mechanisms to counteract the deleterious effect of stress-induced lipid peroxidation in the target organ. In case of its depletion, stress manifestations occur; however prevention of their depletion may be a mechanism for counteracting stress and the beginning of the adaptation process (Meerson, 1994). *Tinospora cordifolia* has also been known to show remarkable immunostimulant and immunomodulatory properties by many workers (Sainis et al., 1998, Deshmukh and Usha, 2002, Dubey et al., 2002). In a clinical study of three months duration guduchi and ashwagandha (*Withania somnifera*) were found as antioxidants. Guduchi was found to be a more effective natural antioxidant over others (De and Tripathi, 1996). Guduchi is known to be a rich source of trace elements (zinc and copper) which act as antioxidants and protects cells from the damaging effects of oxygen radicals generated during immune activation (Shankar and Prasad, 1998). Phenyl propene diasaccharides (Cordifolioside A and B) have been identified as active principles for immunostimulant action of the herb (Salil, 1997). In another study a combination of rasayana herbs including Guduchi and Ashwagandha have been shown to increase cellular and humoral components of immunity (Chatterjee and Das, 1996). Guduchi has been shown to enhance cellular and humoral immunity (Kapil and Sharma, 1997). Pippali has also been hypothesized to improve the immune status of patients (Abbas and Pandey, 1997). Pippali as a part of a polyherbal formulation has also been shown to have antistress and adaptogenic effects (Ramachandran et al., 1990).

Ashwagandha has been shown to increase non-specific general immunity in children (Parbhakar et al., 1994). It contains withanolides that have been reported to possess both immunosuppression and immunostimulatory properties of activating immune responses in various test

studies (Uniyal, 2002). Findings of another study indicate that increase in oxidative free radical scavenging activity of WSG may be responsible for the antistress immunomodulatory and antiaging effects (Salil, 1997). These similar types of trends have been found in the present study. *Withania* as a part of a polyherbal formulation has been shown to have antistress and adaptogenic effects (Ramachandran et al., 1990).

The role of antioxidants in the immune system is multifaceted and well known as they can serve to either suppress or enhance immune response. Depending on the desired response, different antioxidants can play different roles in balancing immune response effectively in an individual. In fact many immune cells such as phagocytes produce free radicals in order to destroy an antigen. If an individual suffers tissue damage and at the same time due to infectious phagocytes are releasing free radicals to destroy an antigen, the Fenton reaction will produce a hydroxyl radical. In order to clear these oxidants there must be a balance for optimal response. Too many antioxidants in our diet would limit the immune cells while not enough would cause phagocytes to produce so many radicals, that they would kill themselves and again be ineffective against any antigen present. In the present study, there was stress on all the subjects and free radicals were probably generated which is also evidently reflected from the elevated serum MDA levels studied as part of the effect of the drug on the antioxidant system of the body (Bansal et al., 2007). However due to food supplements in the treatment group, the level of antioxidants was enough to give resistance to the body. In the treatment group probably due to more resistance, immunoglobulin levels are less; however in the control group, due to more production of free radicals, antioxidant level decreased and could not provide enough resistance to antigens and therefore the level of immunoglobulins were more. In the present study, the level of free radicals as demonstrated by higher MDA levels, vitamin E level and level of Immunoglobulins seem to be part of the same tale. According to a study in humans, whenever you remove antioxidants from the system, cellular integrity is hampered and the immune response is decreased and after repletion, as vitamin E levels increase, the immune response returns to have enhanced killing power. It has also been observed that vitamin E may normalize immune abnormalities in mice with murine retrovirus (Wang et al., 1994). The higher levels of vitamin E in trial group subjects also support the levels of immunoglobulin in the present study (Bansal et al., 2007).

The rasayana herbs seem to exert their effect through immunosuppressant, immunostimulant and immunoadjuvant activities or by affecting the effector arm of the immune response. It has been found that the nervous, endocrine and immune systems are all interrelated. Immune products like various cytokines have been found to stimulate the hypothalamus-pituitary-adrenal axis and

corticotrophin release factor (CRF), which ultimately enhances the production of adrenal corticotropic hormone (ACTH) resulting into increased secretion of glucocorticoids which have an overall suppressive effect on the immune system. Stress also acts on the same axis and brings about changes in the immune status of the body. These rasayana drugs probably reduce stress levels by affecting antioxidant levels. So these rasayana drugs act as potent antioxidants and neuroendocrine immunomodulators (Sehrawat et al., 2007).

Conclusion

Studies carried out on regimen prepared from different rasayana herbs described in Ayurveda revealed that the components of the drug tended to exert significant anti-stress effect against a battery of stresses encountered at Antarctica. The mechanism of action of these rasayana seems to be through its antioxidant effect. From the results obtained it may be concluded that the drug seems to have a potential adaptogenic antistress effect.

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