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Effect of ThymoQuin Black Cumin Seed Oil as a Natural Immune Modulator of Upper-Respiratory Tract Complaints and Psychological Mood State

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ABSTRACT

We conducted a randomized, double blind, placebo-controlled study to evaluate the effects of dietary supplementation with black cumin seed oil (Nigella sativa) on upper-respiratory tract complaints (URTCs) and psychological mood state. Thirty-seven runners (13.1-mile half-marathon and 26.2-mile marathon distance) consumed 500mg of black cumin seed oil extract (commercial name ThymoQuin®3%) or placebo daily during the 4 week supplementation period (3 weeks before and 1 week following a marathon or half-marathon competition). We collected subjective and objective measures before and after supplementation. Before and after supplementation, each subject completed two subjective surveys; (1) the profile of mood state (POMS) psychological assessment and (2) a standardized health log assessing health status and URTCs, as well as provided saliva samples and fecal samples for objective measurement of cortisol and microbiome balance, respectively. Compared to placebo, subjects in the ThymoQuin supplementation group reported significantly fewer upper-respiratory tract complaints (URTCs such as cough, sore throat, sinus congestion, etc) and better overall well-being (e.g. lower stress, increased energy), as well as lower stress hormones (cortisol) and superior microbiome diversity (S. thermophilus). These results suggest that ThymoQuin black cumin seed oil extract may improve immune system vigilance and overall well-being following the stress of endurance training and competition, and that these immune-mood benefits may be mediated through the Gut-Immune-Axis.

Keywords

Immune system, Black cumin seed, Respiratory Tract Complaints.

Introduction

The immune system is traditionally considered to be "merely" our primary defense against external pathogens such as viruses, bacteria, cancer, and other pathogens and toxins. Increasingly, research is demonstrating an expanded role of the immune system as both a "shield" against viruses and as a "communication organ" via its contribution to psychological mood state and overall well-being.

Numerous studies have shown the close links between psychological factors such as stress, sleep deprivation, and immune suppression leading to poor vaccine responses and increased upper-respiratory tract infections (URTIs) and non-infection upper-respiratory tract complaints (URTCs). For example, athletes undergoing heavy exercise or intense training are often at risk for increased susceptibility to URTI [1-4]. As a unique physical stressor, intense exercise causes significant reductions in key immune system components such as neutrophils, natural killer cells, T cells and B cells [1,5,6]. Interestingly, during the one to two-week recovery period after competitive endurance events, athletes are at a particularly elevated risk for suppressed immune system function, illness and injury – partially due to elevations in hormones such as cortisol that coordinate the stress response [1,2,4]. The net effect of an ongoing immune challenge is a weakened immune system, which often results in URTIs/URTCs as well as detrimental effects on psychological mood state.

As a "physical" stress, exercise is similar in certain ways to other stressors, such as psychological or mental stress, which can lead to a weakened immune system and increased susceptibility to URTI/URTC and other disease states [5]. Although moderate-intensity regular exercise is generally regarded as a buffer against many

detrimental effects of stress, the combined effects of very intense physical training with the psychological stress of competition, often leads to noticeable deteriorations in mood state during peak training periods [7]. Lifestyle factors, including sleep patterns, nutritional intake, recovery periods, and dietary supplements may all influence the immune/mood response to exercise [8,9].

A variety of intervention techniques can be used to ameliorate psychological and physical stress, such as administering selective dietary supplements containing immune modulating compounds [1,2,4,10]. In ultra-marathon runners, 600mg of vitamin C, taken 21 days before and 14 days after a 90 km race, reduced URTI symptoms [2]. Biological response modifiers such as beta-glucan, enhance the innate immune response, helping to prime immune system function during and following various forms of chronic stress.

Black cumin seed (*Nigella sativa*) oil is widely used in various traditional Eastern and Ayurvedic systems of medicine as a therapeutic tool for many different ailments and conditions. Nigella sativa seeds have been used medicinally in the Middle East and Southeast Asia for over 2,000 years. Black cumin seeds are known to have been buried with Egyptian pharaohs and are mentioned in the Bible and the Koran where they are referred to as "the blessing seed." In traditional medicine, black seeds are used to manage fatigue and chronic headache [11]. Black seed oil is used as an antiseptic and analgesic remedy and for treatment of joint's pain and stiffness and can be mixed with sesame oil to treat dermatosis, abdominal disorders, cough, headache, fever, liver ailments, jaundice, sore eyes, and hemorrhoids [12]. Thymoquinone, the main constituent in black seed volatile oil, has been shown to suppress carcinogenesis [13].

Extensive preclinical, animal, and clinical research has been conducted on N. sativa's properties and it is among the top ranked evidence-based herbal medicines. Most of the therapeutic properties of this plant are attributed to its essential oil constituent, ThymoQuinone (TQ), and modern black seed extracts can be standardized for TQ content (e.g. ThymoQuin, 3% TQ; TriNutra, Israel). A number of laboratory and animal studies have demonstrated immune modulation of black cumin seed via effects on hematopoietic stem cells, lymphocytes, macrophages, T cells, dendritic cells (DCs), natural killer (NK) cells, and a variety of cytokines (IFN, IL-2, TNF-alpha, IL-6, and others). Stress-related immune alterations can be consequential for health; they can enhance susceptibility to infectious agents and influence the severity of infectious disease, diminish the strength of immune responses to vaccines, reactivate latent viruses, and slow wound healing.

Materials and Methods

This study protocol and subject informed consent documents were reviewed and approved by an external scientific and ethics advisory board (WCG-IRB, Puyallup, WA; Protocol #20202070) and registered as a clinical trial with ClinicalTrials.gov (NCT05508529).

Our was to recruit 40 healthy volunteers to participate in a research study investigating the effects of dietary supplementation for one-month with black cumin seed oil extract (500mg ThymoQuin; standardized to 3% ThymoQuinone and 1.8% Free Fatty Acid, TriNutra, Israel; N=20) that may be immunomodulatory for improving immune system vigilance and psychological stress versus Placebo (500mg Maltodextrin; N=20).

Subjects

We recruited healthy, fit, recreational runners who were experienced in training and competing for half-marathon to marathon-distance events. Our subject pool completing all phases of baseline pre-supplementation measurements, training, competition, and final post-supplementation measurements included 37 subjects (Table 1). One subject in the ThymoQuin group and two subjects in the Placebo group were lost to follow up. There were no adverse events reported for either group.

Health log

Subjects completed a physical health questionnaire at baseline (pre-supplementation) and 4-weeks (post-supplementation). The health log was a daily health perception log containing questions related to overall health status and specific upper-respiratory tract complaints (URTCs). The URTC-related symptoms measured included nasal congestion, runny nose, sore throat, sneezing, cough, fatigue, headache, general malaise and body aches. Reported symptoms were totaled for each assessment period.

Mood Assessment

Changes in psychological mood state were assessed using the Profile of Mood States (POMS) survey. POMS assesses 5 primary "negative" psychological factors (tension, depression, anger, fatigue, and confusion) plus the "positive" mental states of vigor and global mood state as an indication of overall subjective well-being. The POMS methodology has been used in ~3,000 studies, and its validity is well established. The POMS profile uses 65 adjective-based intensity scales scored on a 0–4 hedonic scale (e.g. "not at all" to "extremely").

Salivary Cortisol

Cortisol is the major glucocorticosteroid stress hormone produced in the adrenal cortex and is actively involved in regulating many aspects of metabolism related to sports performance, immune vigilance, and psychological mood state. Cortisol levels peak in the early morning and drop to the lowest concentration at night. Levels rise independently of circadian rhythm in response to stress. In the blood, only 1 to 15% of cortisol is in its unbound or biologically active form, with the remaining cortisol bound to serum proteins. Unbound serum cortisol enters the saliva via intracellular mechanisms, and in saliva the majority of cortisol remains unbound to protein where it can be easily collected and assayed as an index of overall stress exposure and recovery. Each subject provided "first morning" saliva samples (upon awakening) for analysis of free cortisol at baseline (pre-supplementation) and 4-weeks (post-supplementation). The first morning cortisol is routinely used as an indication of cortisol sensitivity and stress reactivity.

Microbiome Assessment

Volunteers were provided with a take-home kit to obtain fecal samples in the privacy of their home for analysis. The kit included detailed instructions and postage paid packaging for return directly to the lab. Each kit was numerically coded so that samples were blinded to the lab. Microbiome analysis of fecal samples was carried out using the BiomeTracker system (Wasatch Scientific, Murray, UT). Briefly, fecal samples were obtained by nylon swab and placed into preservative binding buffer to lock the composition of bacteria in place. DNA was then purified using DNA columns and ~20ng of DNA from each sample was added to the reaction mixtures. Samples were processed on an ABI 7500 Fast (Applied Biosystems) instrument in duplicate. A "microbiome composite score" was generated as an overall average of many different aspects of microbiome balance, including Bifidobacterium, Lactobacillus, Akkermansia, S. Thermophilus, Firmicutes/Bacteroidetes (F/B) ratio, and others.

Data Management and Analysis

All questionnaires were hand-delivered or mailed to a central location and transcribed to a central database. Subjects who did not complete the questionnaires or who submitted incomplete questionnaires were dropped from the study and not included in the study analysis (3 subjects; 1 from the Supplement group and 2 from the Placebo group). Data were identified by subject number and examined for accuracy and completeness. Tabulated data were analyzed with JMP 14.0 (JMP Statistical Discovery, Cary, NC) using standard parametric paired t tests, and significance was assessed with a 2-tailed alpha level set at 0.05. Data are presented as average values for each group (Placebo and ThymoQuin) before and after supplementation.

Table 1: Subject Demographics.

Group	Average Age	Men	Women
ThymoQuin	35 ± 6	10	9
Placebo	36 ± 5	10	8

Results

Following 4 weeks of supplementation with ThymoQuin, (3 weeks before and 1 week following an intense endurance run), we observed the following differences between the Supplement and Placebo groups:

Subjective Measures

As expected, both groups reported dramatically more subjective upper-respiratory tract complaints (URTCs) following the endurance run compared with before (Figure 1). However, URTCs, including the total number of symptoms reported such as cough, sore throat, sniffles, stuffiness, etc., were 62% lower in the ThymoQuin group compared to placebo (Figure 1).

There was no significant change in Global Mood State (e.g. "overall well-being"), following the endurance run in the Placebo group (Figure 2), while the ThymoQuin group demonstrated a 11% improvement (a lower number indicates a less negative psychological mood state).

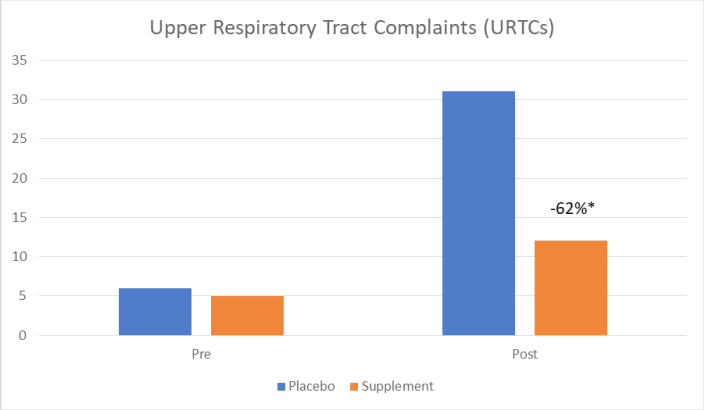

Figure 1: Subjects in the ThymoQuin group had significantly fewer self-reported upper respiratory tract complaints (URTCs) compared to Placebo. (*significantly different from post-supplementation placebo value, $p<0.05$).

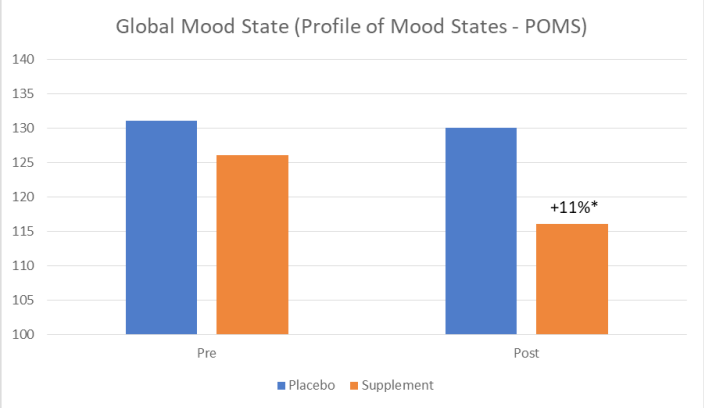

Figure 2: Subjects in the ThymoQuin group had significantly better Global Mood State compared to Placebo (lower score indicates higher well-being index). (*significantly different from post-supplementation placebo value, $p<0.05$).

Objective Measures

There was no significant difference in microbiome indices following the endurance run. However, following supplementation, *Streptococcus thermophilus* values were 66% higher in the ThymoQuin group, suggesting improved immune system regulation (Figure 3) and overall microbiome composite score was 8% higher, indicating improved microbiome diversity (Figure 4).

Cortisol, the primary stress hormone related to both mood and immune function, was slightly but not significantly elevated in the Placebo group following the endurance run, but was 44% lower in the ThymoQuin group post-supplementation compared to Placebo (Figure 5).

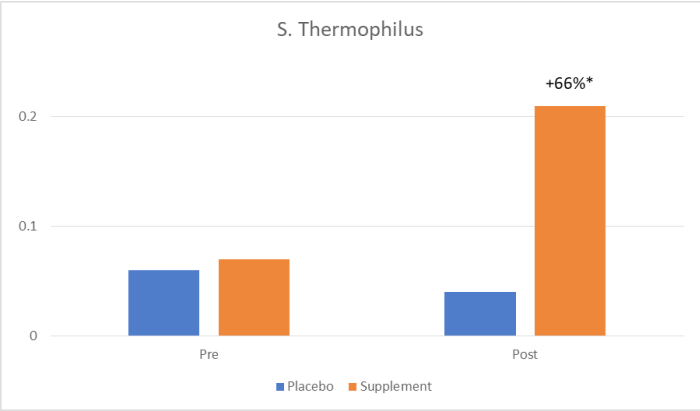

Figure 3: Subjects in the ThymoQuin group had significantly higher relative abundance of *Streptococcus thermophilus* bacteria compared to Placebo. (*significantly different from post-supplementation placebo value, $p<0.05$).

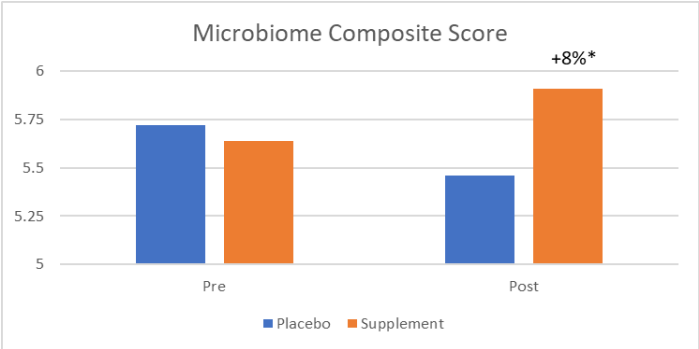

Figure 4: Subjects in the ThymoQuin group had significantly higher Microbiome Composite Score compared to Placebo. (*significantly different from post-supplementation placebo value, $p<0.05$).

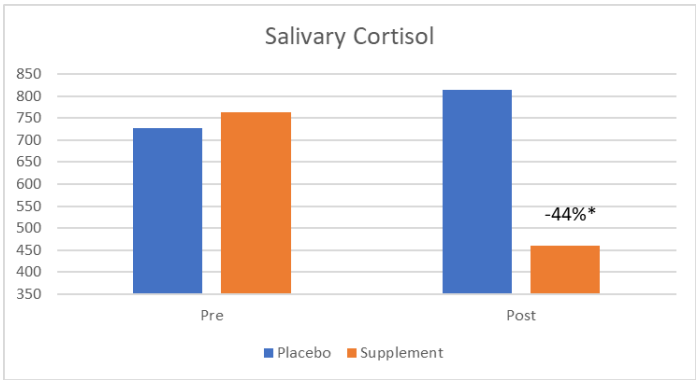

Figure 5: Subjects in the ThymoQuin group had significantly lower Salivary Cortisol (ng//ml) compared to Placebo. (*significantly different from post-supplementation placebo value, $p<0.05$).

Discussion

These results demonstrate a significant and meaningful benefit of ThymoQuin supplementation for the immune system as both a "shield" (protection from upper-respiratory complaints) and as a "communication organ" (signaling well-being between body and mind and resulting in superior psychological mood state). This linkage between body and mind across the Gut-Brain-Axis involves many aspects of a coordinated and interconnected communication system linking the gut microbiome (*S. thermophilus*) to the brain (psychological mood state) across the axis (immune and stress response pathways). When the entire system is balanced, as evidenced here in the ThymoQuin group, there is a noticeable benefit for physical health and mental wellness.

Volunteers in our study were experienced endurance runners who trained for and competed in a strenuous half-marathon (13.1 miles) or marathon (26.2 miles). Endurance running is an accepted model for ethically inducing both physical and mental stress as a way to temporarily "stress and suppress" the immune system. Such immune system suppression creates a "susceptibility window" whereby a higher risk for upper-respiratory tract complaints (URTCs) is more likely to be observed in the control/placebo group. Our hypothesis was that the group supplementing with ThymoQuin as a natural immune modulator would demonstrate fewer URTC symptoms and lower indices of mental/physical stress.

Rather than being an "immune-booster" to stimulate immune system activity, ThymoQuin may be considered as a natural "immune-modulator" that can help to balance overall immune system activity. Such natural substances represent an emerging approach to immunotherapy that either elevates a suppressed immune system "up" to optimal – or calms an over-activated immune system "down" to optimal – in a paradigm that we refer to as "priming" the immune system. This priming effect is analogous to the concept of "adaptogens" in herbal medicine, whereby adaptogenic herbs such as cordyceps, ashwagandha, and rhodiola help to restore various body systems toward "balance" after stressful events. In this way, a properly primed immune system is better at focusing on pathogens (e.g. viruses, bacteria, cancer cells, etc), and less likely to attack non-harmful entities (e.g. pollen, mucus membranes, joint cartilage, etc).

The majority of both the microbiome and the immune system reside in the gut – forming a symbiotic relationship and ensuring that the human body is protected from harmful pathogens. Over time, our immune system shapes the diversity of our microbiome, and our gut influences the development and vigilance of our immune system. For example, the gut microbiome acts as a gatekeeper, and a trainer, and increasingly as a communication organ. In addition, the gut microbiome interacts with the brain in multi-directional ways that involve the immune system using neural, inflammatory, and hormonal signaling pathways [14]. These immune-mediated signals from gut to brain have been implicated in many aspects of mental health and well-being, including depression, anxiety, and overall psychological mood states [14].

In this study, we report the effect of supplementing with ThymoQuin for 4 weeks on the physical and psychological well-being of long-distance runners. The current study employed a series of subjective self-assessment questionnaires that addressed overall health status and URTCs. In addition to evaluation of subjects for physical health, a psychological assessment known

as the Profile of Mood States (POMS) was conducted to assess mood state. We also collected objective markers of microbiome balance (*Streptococcus thermophilus*) and stress hormones (salivary cortisol), both of which are associated with immune system vigilance and psychological stress response, and which may represent a possible mechanism by which immune function and psychological mood state are related.

During the course of the 4-week treatment period (3 weeks before and 1 week after an intense endurance run), subjects in the ThymoQuin group reported fewer URTCs, better overall health and a more positive mood state compared to Placebo. In addition, supplemented runners also showed higher levels of *Streptococcus thermophilus* (S. thermophilus) and lower stress hormone exposure (cortisol) – both of which being associated with immune vigilance and psychological mood state.

Runners and other athletes, whose athletic activities cause significant physical stress, are more susceptible to URTI (infections) and URTC (complaints). Previous research has reported that athletes training for a marathon experience a deterioration in global mood state [7], and a number of studies have reported that nutritional supplementation can modulate their health status [1-4].

Physical and psychological factors of subjects undergoing stressful situations are reported to increase URTI and URTC [8,9,15]. In all cases, the subjects supplemented with ThymoQuin experienced better physical health and a significantly improved psychological status (Global Mood State), than those in the placebo group. ThymoQuin participants reported both fewer URTC symptoms and a better overall health status. The URTC symptoms reported by subjects are typical of cold and flu symptoms, and analogous to symptoms reported in other studies [8,9].

Previous work has shown benefits of beta-glucan for improving overall immune function, including following intense endurance exercise [4,16], and a range of other dietary supplements may help reduce URTI symptoms in athletes [2,17,18], i.e., zinc treatment reduced duration and severity of cold symptoms [19]; probiotics (*Lactobacillus fermentum*) reduced the severity and duration of URTI in athletes [17]; and vitamin C supplementation in ultramarathoners reduced the duration and severity of URTI [2].

Conclusion

In this study, ThymoQuin significantly decreased upper-respiratory tract complaints (URTCs) and improved psychological mood state (Global Well-Being) following intense endurance training and competition. Additionally, ThymoQuin subjects had lower stress hormones (cortisol) and superior microbiome parameters (*S. thermophilus*), suggesting that immune vigilance and mental well-being are linked through the microbiome and stress response pathways. These results add to the growing scientific literature and natural armamentarium for immune-modulation to both reduce respiratory symptoms and improve psychological mood state in "stressed" individuals (endurance athletes in this study).

Conflicts of Interest and Funding Statement

This study was funded by TriNutra, which manufactures and sells ThymoQuin black cumin seed oil, and conducted by 3Waves Wellness, which was compensated to carry out the trial. ST is an employee of Amare Global, which sells a multi-ingredient dietary supplement that includes black cumin seed oil. ST and JT are owners of 3Waves Wellness, an independent research organization.

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