Article

The effects of a pine bark extract on exercise performance and post exercise inflammation, oxidative stress, muscle soreness and damage

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Abstract: The purpose of the present study was to examine if 14 days of supplementation with a pine bark extract leading up to and following an exercise test would increase performance and reduce biomarkers associated with muscle damage, inflammation and oxidative stress. The study used a double-blind, placebo controlled, cross-over design. Participants ingested either 800mg pine bark extract or placebo for 14 days prior to the first exercise trial and for 2 days post-exercise. On the exercise day, participants submitted a pre-exercise blood sample, then completed a VO_2 peak test until volitional failure. A post-blood sample was collected 1 hour after completion of exercise. Participants returned at 24 & 48 hours after the exercise testing for measures of muscle pain in the lower body using an algometer. Participants then had a 7-day washout period before beginning to crossing over to the alternate treatment. Analysis via ordinal regression demonstrated a significant difference in oxidative stress in the pine bark extract group compared to placebo (ChiSq = 2.63; p=0.05). The pine bark extract was effective at affording protection from oxidative stress post exercise. Further work should be undertaken to evaluate the findings with other exercise modes or in participants with known metabolic syndrome.

Keywords: Pine bark extract, Oxidative stress, Muscle damage

1. Introduction

While exercise has multiple known health benefits, there are consequences to prolonged bouts of exercise. High muscle forces damage the sarcolemma initiating the release of cytosolic enzymes and myoglobin, further damaging muscle contractile fibrils and noncontractile structures. Metabolites such as calcium accumulate to abnormal levels in the muscle cell to produce more cell damage and reduced force capacity[1,2]. At this point, the inflammatory process begins, allowing muscle tissue to heal and adapt to protect from subsequent exercise.

The contraction of skeletal muscle also generates free radicals, and resulting in oxidative damage to the cell [3]. The primary free radicals generated in cells are superoxide ($O_2^{\bullet-}$) and nitric oxide (NO). Dismutation of superoxide provides a major source of hydrogen peroxide (H_2O_2) in cells. Hydrogen peroxide is cytotoxic, and readily generates hydroxyl radicals in specific circumstances. Hydroxyl

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radicals damage molecules close to the site of their generation and are considered the most damaging reactive oxygen species (ROS) present in biological materials[3].

1.1 Description of French Maritime pine bark

French Maritime pine bark (*Pinus P.)* has many purported pharmacological benefits on multiple physiological functions. Most commercial formulations contain 65-75% procyanidins, with phenolic acids making up the remainder [4]. Procyanidins are biopolymers of catechin and epicatechin subunits which are recognized as important constituents in human nutrition. The phenolic acids are derivatives of benzoic and cinnamic acids, specifically, feurlic acid and taxifolin [5].

1.2 Pinus P. potential benefits during exercise

The primary mechanism of *Pinus P.* is to increase serum levels of Nitric Oxide (NO) via conversion of NO to superoxide and prolonging the half-life of NO [6], and stimulating Nitric Oxide Synthase (NOS) enzyme [7]. Standard dosing for *Pinus P.* is 100-200 mg daily, but doses as low as 40-60 mg daily have been shown as beneficial if taken over long periods of time. Nishioka et al (2007) found "180 mg daily for 2 weeks is associated with an augmentation of an acetylcholine-induced blood vessel relaxation via NO *in vivo"*[8]. During inflammation, *Pinus P.* metabolites inhibit nitrite production with near absolute suppression of NO at 50mcg/mL, which is a 20-fold increase in potency over hydrocortisone [9].

Similarly, *Pinus P.* is able to sequester superoxide, hydroxyl, and free oxygen radicals [10-12]. Protective effects against hydrogen peroxide and lipid peroxidation in red blood cells [13], and a reduction in the accumulation of oxidatively modified proteins as also been purported [14]. 200mg of *Pinus P.* in coronary artery disease (CAD) patients is able to decease levels of 15-F(2t)-Isoprostane by 7% after 8 weeks, suggesting a lowering of oxidation [15].

1.3 Other Purported Health Benefits

1.3.1 Cardiovascular and Neurological

In patients with CAD, daily dosing of *Pinus P.* was associated with an improvement in blood flow of 32% [15]. *Pinus P.* also demonstrated a 25% inhibition of platelet aggregation [16], and higher doses (>350mg) noted a decrease in LDL-C and total cholesterol [17}.

In a study of 61 children with confirmed ADHD, a dose of 1mg/kg daily for 4 weeks was associated with positive changes in hyperactivity and attention versus baseline and placebo [18], and the results were replicate again in an 8-week study of otherwise healthy students [19].

1.3.2 Obesity and Oxidation

Pinus P. was previously found to possess lipolytic capabilities [20] but the mechanism is concentration-dependent [21]. The proposed mechanism of this property in suppression of the mRNA levels of fat accumulation genes, specifically CEBP-A, PPAR-y, aP2, along with G6PDH mRNA suppression during adipogenesis with dosages of 100-200 mcg/mL [22]. Increases in superoxide dismutase and glutathione peroxide at these same dose level were also noted in this study.

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1.3.3 Diabetes

While a 12-week study failed to demonstrate any alteration in basal insulin or secretion rates in Type II diabetics, a reduction was shown in HbA1c (0.8%) and overall blood glucose [23}. A follow-up 12-week study noted a similar decrease in HbA1c, with a reduction in blood glucose beginning as early as week 8 of the study [24]. In an animal model study of Type I diabetes, Diabetes was induced in rats via injection of streptozotocin followed by free access to 5% glucose. *Pinus P.* was then given to the rats for 4 weeks, then sacrificed for pancreas and liver assays. The group of rats injected daily with *Pinus P.* demonstrated attenuation changes in blood glucose, HbA1c, hepatic glycogen and insulin of the rats [25].

1.4 Purpose of the Study

The purpose of the study was to determine if 14 days if supplementation with *Pinus P.* leading up to an exercise test and two days of post-exercise supplementation would increase performance and reduce biomarkers associated with muscle damage inflammation and oxidative stress.

2. Materials and Methods

2.1 Participants

For the investigation, 20 apparently healthy college males were recruited out of various kinesiology classes at a university in the south United States. Participants were 22.7 years (± 3.9 yrs), with body height of 178.1 cm (± 7.9 cm), and body mass of 82.9 kg (± 13.5 kg). The study group was physically active and engaged in regular exercise 4.3 days/week (± .8 days/week), a relative body fat of 16.7% (± 7.4%), and average cardiovascular capacity of 30.0 ml/kg/min (±7.0 ml.kg/min).

The participants gave written informed consent and reported to the Human Performance Lab for an initial visit where anthropometric measures were collected. All participants gave written informed consent to take part in the study and the methods were reviewed and approved by the Institutional Review Board at the University of Louisiana at Lafayette.

2.2 Procedures

The investigation was conducted to determine if 14 days of supplementation with *Pinus P.* leading up to an exercise test with and additional two days of post exercise supplementation would increase performance and reduce biomarkers associated with muscle damage, inflammation and oxidative stress. During the initial visit, participants gave written informed consent after having had the experimental procedures explained to them. Afterwards, they will filled out a health questionnaire, the PAR-Q+, and the Leisure and Physical Activity Survey [26}. Based upon these surveys, the participants were deemed fit to continue with the experimental procedures. The participants also completed the ASA 24 (Automated Self-Administered 24-hour Recall) diet questionnaire, from which no dietary deficiencies were noted. The participants were then asked to maintain a diet consistent with the 24-hour recall from this point in the study forward.

Following completion of the questionnaires, participant's height and weight was determined via stadiometer, and body fat percentage were measured using air displacement plethysmography (Bod Pod Gold Standard System, Rome, Italy). Finally, to finish data collection on the initial visit the participants underwent a VO_2 peak test on a cycle ergometer. The test consisted of a 25 watt ramp

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protocol on a COSMED E100 P. During this test expired gases was collected continuously as well as heart rate and SpO_2 using a COSMED QUARK CPET. The participant continued to exercise until their VO_2 failed to increase by 100ml/min for an increase in wattage or volitional failure occurred.

2.2.1 Assignment of Order

Participants were randomly assigned to an order of treatment (*Pinus P.* and placebo). The study will be conducted double-blind, 16 days (14 pre – exercise, 2 days post exercise) were pre-packaged into coded bottles (unique numeric codes) and provided to participants. The participants were asked to take 4 pills per day during the 14 days leading up to the exercise trial and the 2 days after. This delivered a 200mg per day dose of *Pinus P*. Participants received their first doses after initial visit and were reminded that exercise was restricted 48 hours prior to the exercise trial until the end of the 48 hour follow up visit.

2.3 Pre-Exercise Trial Visit

The participants were asked to report to the Human Performance Lab in the evening prior to the exercise trials. The participants were feed a standard meal for the evening (pre-packaged to maintain consistency) and sent home with a 240 calorie evening snack consisting of 10g of protein, 41g of carbohydrates, and 4g of fat. The participants were provided with an actigraph sleep monitor to wear and asked to note the time they went to bed. The data from this monitor was collected the next morning prior to the start of the exercise trial. Finally, from this point until the end of the 48-hour follow up NSAIDS (non-steroidal anti-inflammatory drugs) were restricted due to the measurement of inflammatory markers.

2.4 Exercise Trials

Participants reported in the morning hours (0600-0800) fasted. They had blood drawn from the antecubital space (pre-exercise sample) and then repeated the 25-watt ramp protocol similarly to the VO_2 peak test. During this trial expired gases were monitored in a similar fashion, and additionally near-infrared spectroscopy (NIRS) sensors (Moxy Oxygen Monitor 3, Hutchinson, MN) were secured to the quadriceps muscles, one to the vastus lateralis and another to the vastus medialis, to monitor muscle oxygenation. Participants exercised until volitional failure. One-hour post exercise participants again donated a blood sample from the antecubital space. Participants were given water to drink during the recovery phase and were reminded to continue to take their assigned doses through the 24 and 48 hour recovery visits. Participants were also reminded that the restrictions on NSAIDS and exercise are also active through the conclusion of the 48 hour follow up. The participants had a one week wash-out period prior to beginning the next supplementation phase with the opposite treatment.

2.4.1 24 and 48 hour Follow-Up Visits

Participants reported to the lab in the morning hours fasted (0600-0800) and again donated a blood sample from the antecubital space. Following this muscle pain was assessed using an algometer. This is a device that provides a constant low force pressure (50N) to a small (1cm diameter) surface. The use of these devices enhances the collection of post-exercise muscle soreness and is an improvement over the traditional method of using visual analogue scales alone. Three specific

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locations were used, the vastus lateralis 25% and 50% of the distance between the superior border of the patella and greater trochanter of the femur, the vastus medialis 25% of the distance between these same landmarks. The exact locations of the tests were marked with a permanent marker and the participants was given a marker to maintain the marks with for the remainder of the study.

2.5 Blood Collection

Blood donated by the participants during the study was collected in 7.5 ml serum separator tubes. This was allowed to stand at room temperature for 15 minutes then centrifuged at 4° C at 3500 rpm for 10 minutes. Supernatant was removed and stored in micro-centrifuge tubes for later analysis.

Serum was analyzed for oxidative stress via MDA/TBARS colorimetric assays. Additionally serum was tested for Lactate Dehydrogenase and Creatine Kinase activity via colorimetric assays to examine muscle damage. The absorption endpoints of these assays was read with a BioTek ELX 808 microplate reader with Gen5 software for data analysis.

Finally, a multiplex chemilumenescent assay was run to examine inflammation (IL-1α, IL-1β, IL 2, IL 4, IL 6, IL 8, IL 10, INF-Υ, TNF-α). At the conclusion of the assay procedures the plate was imaged with a CCD imager (18 megapixel) and the data analyzed with Qview Pro Software.

2.6 Statistical Analysis

The principal investigator entered all data into JMP 11.0 pro software at the conclusion of the study. Data was grouped according to research question and analyzed via repeated measures Anova with post-hoc analysis where necessary or via non-parametric means provided data deviated from a normal distribution. Statistical significance was set a prior at alpha <0.05 and trend in the data at alpha <0.10.

3. Results

3.1 Human Performance Data

3.1.1 Oxygen Consumption, Muscle Oxygenation and Power from exercise trials

Anova did not reveal a significant difference between maximum VO_2 achieved during the 25 watt ramp protocols by treatment type (F=0.482, p=0.492 *Pinus P.*: 25.8 +/- 4.8 ml O_2/kg*min Placebo: 27.2 +/- 6.9 ml O_2/kg*min). Similarly, statistical Analysis of muscle oxygenation data from NIRS sensors (F=0.833, p=0.367) and Total Hemoglobin (blood flow) (F= 0.610, p=0.439) did not reveal and significant difference by treatment during the exercise trial. Finally, Anova did not reveal a significant difference between watts (power) achieved during the 25 watt ramp protocols by treatment type (F=0.571, p=0.454 *Pinus P.*: 189 +/- 34.8ml O_2/kg*min Placebo: 181.3 +/- 29.8 ml O_2/kg*min).

3.1.2 Muscle Pain at 24 and 48 hours

Vastus Lateralis 25% of the distance between the superior border of the patella and greater trochanter of the femur on the right leg did not reveal any main effects for treatment (F=0.111, p=0.742) nor interaction effects for treatment*time (F=1.34, p=0.253). Vastus Lateralis 25% of the distance between the superior border of the patella and greater trochanter of the femur on the left leg did not reveal any main effects for treatment (F=0.756, p=0.390) nor interaction effects for treatment*time (F=0.352, p=0.555).

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Vastus Lateralis 50% of the distance between the superior border of the patella and greater trochanter of the femur on the right leg did not reveal any main effects for treatment (F=0.237, p=0.628) nor interaction effects for treatment*time (F=0.002, p=0.960). Vastus Lateralis 50% of the distance between the superior border of the patella and greater trochanter of the femur on the left leg did not reveal any main effects for treatment (F=0.125, p=0.724) nor interaction effects for treatment*time (F=0.593, p=0.446).

Vastus Medialis 25% of the distance between the superior border of the patella and greater trochanter of the femur on the right leg did not reveal any main effects for treatment (F=0.052, p=0.821) nor interaction effects for treatment*time (F=0.640, p=0.428). Vastus Medialis 25% of the distance between the superior border of the patella and greater trochanter of the femur on the left leg did not reveal any main effects for treatment (F=0.002, p=0.9617) nor interaction effects for treatment*time (F=0.001, p=0.966).

3.2 Biomarkers of Muscle Damage

3.2.1 Creatine Kinase (CK) and Lactate Dehydrogenase

Analysis of Creatine Kinase data from collected serum did not reveal a main effect for treatment (F=0.172, p=0.681) nor an interaction effect for treatment*time (F=0.223, p=0.878). Analysis of Lactate Dehydrogenase data from collected serum did not reveal a main effect for treatment (F=0.114, p=0.737) nor an interaction effect for treatment*time (F=1.57, p=0.213).

3.2.2 Multiplex Inflammatory Panel

Analysis of IL-1a, IL-1b, IL-2,4,6,8,10, INFg and TNFa data from collected serum did not reveal a main effect for treatment (F<1.0, p>0.4) nor an interaction effect for treatment*time (F<1.2, p>0.3).

3.2.4 Oxidative Stress Results

Analysis via Repeated Measures Anova was undertaken, however the assumption of Anova that residuals will be normally distributed was violated. Therefore, analysis was undertaken with non-parametric means. Chi-square analysis demonstrated that the 48 hour time point was significantly reduced with *Pinus P.* as compared to placebo. (ChiSq = 2.63, p=0.05). The mean for the placebo group ($\bar{x}$ = 0.99nmol/ml, SD = 0.44) , and the mean for the Pinus P. group ($\bar{x}$ = 0.76nmol/ml, SD = 0.38).

4. Discussion

Exercise produces in imbalance between reactive oxygen species (ROS), free radicals and antioxidants {27,28}; this phenomenon is referred to as oxidative stress. Both acute aerobic [29] and anaerobic [30] exercise has the potential to increase free radical production. During low-intensity and duration protocols, antioxidant defenses appear sufficient, but as intensity and duration increase, this is no longer the case [31]. In muscle tissue, eccentric exercises both simple, like arm curls [32] or more complex, as with downhill hill running [33}, demonstrate increases in oxidative stress within the blood plasma. It appears that antioxidant capacity may be temporarily reduced during and immediately post exercise [34], after which time, levels typically increase above basal conditions during the recovery period [35]. In the present study, this response took 24 to 48 hours, as with other studies of post-sprint type exercises [36}, or immediately, in post-endurance marathon runners [36].

A few studies have missed these changes by taking only one sample immediately post-exercise [37], or 20 minutes post-exercise [38]

Conversely, chronic exercise is the same stimulus that is necessary for an up-regulation in endogenous antioxidant defenses [28]. In two studies of exercise trained mice, 8-weeks of exercise, at a rate 3 or more times a week, demonstrated changes in the mitochondria associated with reduced effects of oxidative stress at the 48-hour mark post-exercise [39]. However, prolonged physical activity also produces an excess amount of reactive oxidative species[40], beyond the ability of the body's ability to cope under normal physiological circumstances[41]. Chronic, heavy physical exercise characterized by remarkable increase in oxygen consumption presents a challenge to the antioxidant systems because of the increase production of ROS due to the increased consumption of Vitamin E [42]. The subsequent free radical damage can hamper other body systems if not countered by exogeneous supplementation such as *Pinus P*. Oxidative stress facilitates peroxidation of low-density lipoproteins (LDL) cholesterol, which leads to cytotoxicity and enhanced coronary artery plaque formation [43,44]. It is also involved in many other pathophysiological states including aging, neurodegenerative diseases, and cancer [28]. Certain metabolic conditions and infections, such *H. pylori,* increase the production of reactive oxygen species, which, in turn, can lead to the breakdown of the gastric lining, a precursor to gastric cancer [45].

Additional oral antioxidant supplementation, especially Vitamins C and E, may be a suitable, non-invasive means of reducing oxidative stress, but excess exogenous antioxidants may have detrimental effects on health and performance [28]. Alternatives to supplementation include whole foods that contain antioxidants in natural ratios and proportions [28]. An adequate intake of vitamins and mineral through a varied diet remains an optimal approach[28]. However, food availability, intolerance to certain types of foods, and extreme training regimens where athletes are exposed to high oxidative stress, make exogenous supplementation with *Pinus P.* and other antioxidants necessary.

5. Conclusions

The primary finding of the present investigation was that *Pinus P.* as compared to placebo was effective at affording protection from oxidative stress post exercise. It is suggested that further work be undertaken to evaluate these findings with other exercise modes known to greatly increase lipid peroxidation (marathon, triathlon, road races >10k) and also it could be suggested that clinical evidence be garnered from a study of individuals with metabolic syndrome, as that is known to greatly enhance oxidative stress.

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