Multi–Enzyme Complex for the Management of Delayed Onset Muscle Soreness after Eccentric Exercise: A Randomized, Double Blind, Placebo Controlled Study

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Abstract

Background: Delayed onset muscle soreness (DOMS) results from muscle overload or strenuous exercise that goes beyond the intensity or duration for which the muscle is accustomed to perform. It is accompanied with the sensation of pain, tenderness, deep ache, and stiffness in muscles that usually begins several hours after the unaccustomed exercise. The aim of this study was to compare the efficacy of multi enzyme complex with a matching placebo in reducing pain associated with DOMS induced by standardized eccentric exercise.

Methods: Twenty healthy males (10 pairs) were randomized in this double blind, placebo controlled trial to receive a placebo or multi enzyme complex capsule (50 mg) thrice a day for a period of 3 days. Mean differences within the group and between groups were assessed at each data collection time-point using Analysis of Covariance (ANCOVA) and Wilcoxon signed rank sum test for all outcome measures.

Results: In this controlled clinical study, intake of multi enzyme complex for 3 days resulted in no statistically significant changes in the descriptive statistics and efficacy analysis in muscle power and grip strength measured by hand held dynamometer. Algometer readings of thigh muscle showed statistical significance (p<0.043). Decrements were observed in McGill Pain Questionnaire showing high statistical significance. Reducing trend was observed in bio markers of muscle damage (creatinine kinase and lactate dehydrogenase) as well.

Conclusion: The study results suggest that compared to placebo, Multi enzyme complex supplementation improves the outcome measures related to DOMS induced by standardized eccentric exercise.

Keywords: Delayed onset muscle soreness (DOMS); Muscle soreness questionnaire (MSQ); Pressure pain threshold (PPT); Hand held dynamometer; Illinois agility run test; Multi-enzyme complex

Introduction

Delayed onset muscle soreness is related to muscle damage occurring several hours after unaccustomed exercise, particularly when eccentric muscle activity is involved [1,2]. Contracting muscles are forcibly lengthened with eccentric exercise like downhill running which limits physical function for several days [3,4]. This triggers an inflammatory response and the production of reactive oxygen species (ROS) that sustain inflammation and oxidative stress by promoting the activation of transcription factors like the nuclear factor-κB (NF—κB), a pro-inflammatory master switch that controls the production of inflammatory markers and mediators [5]. The inflammatory response ensures musculoskeletal injury; uncontrolled inflammation may prolong skeletal muscle recovery [4].

Delayed onset muscle soreness (DOMS) is a well-documented phenomenon, often occurring as the result of the unaccustomed or high intensity eccentric exercise. Associated symptoms include muscle shortening, increased passive stiffness, swelling, decreases in strength and power, localized soreness and disturbed proprioception. Symptoms will often occur within 24 h post-exercise and typically subside after 3–4 days. The severity of damage and soreness varies as a function of several factors [6].

Considerable amount of research on the treatment of DOMS has been carried out till date but no single treatment has been proven successful in consistently preventing or treating DOMS. Treatment strategies have often integrated multiple therapeutic approaches such as cryo therapy, ultrasound, compression therapy, stretching and deep tissue massage [7-11]. There is some evidence that ibuprofen, naproxen, and massage may accelerate the resolution of DOMS [12]. In addition, several dietary supplements have been tested in the treatment of DOMS including protein, vitamin C, proteases (enzymes), phosphatidylserine, chondroitin sulphate, and fish oil, all with variable success [4,12-18].

Non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen are used widely as anti-DOMS recourse. NSAIDs are known to interfere with chemo taxis of monocytes as well as inhibit neutrophil aggregation [19]. Monocytes produce cytokines, which are responsible for most of the physiological responses accompanying injury, and neutrophils produce elastase and collagenase, which increase vascular permeability via degradation of the vasculature and healthy tissue near the injury site [20]. It is possible that the use of NSAIDs may impair and lengthen the healing process.

In spite of inconsistencies, dose and timing of various NSAIDs also differ in different studies there are side effects such as gastrointestinal distress and hypertension. Hence NSAIDs are not an optimal choice for treating DOMS [12]. Using enzymes to combat DOMS is also well established.

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A study by inner field in 1957 examined the anti-inflammatory effects of protease enzyme therapy and showed that protease enzyme supplementation may have anti-inflammatory effects [21].

Commercially, digestive enzymes are isolated from various sources such as the pancreas of higher animals (swine and cattle), higher vegetables (barley), and microorganisms including bacteria and fungi. The multi enzyme complex (DigeZyme®) consists of MO free broad acting enzymes obtained from the fermentation process with Aspergillusoryzae, including amylase, protease and lipase; this group of enzymes breaks carbohydrates, proteins and fats and all three groups of enzymes are resistant to the action of gastric juices, while retaining their digestive activity. DigeZyme® was also clinically evaluated for enhanced absorption of minerals and vitamins.

Absorption issues and the destruction of enzymes in the gut have severely limited the effectiveness of traditional anti-DOMS enzyme therapy. Sitosterols -- plant sterols and protease enzymes can help reduce the inflammation associated with DOMS. Earlier research has suggested protease oral supplementation to shorten recovery time post injury through the inhibition of arachidonic cascade. Protease is also believed to inhibit the biosynthesis of pro-inflammatory agents [22], increase tissue permeability, facilitate resorption of oedema and accelerate the restructuring of damaged tissue [23]. Protease utilization is generally considered safe.

The current clinical study aims at investigating the efficacy of multi-enzyme complex, a multi-enzyme complex which is a combination of five digestive enzymes (alpha-amylase, neutral protease, cellulase, lactase and lipase), as a dietary supplement in the management of DOMS.

Therefore, the current randomized, double blind placebo controlled trial was conducted to evaluate the effect of multi-enzyme complex (amylase, lipase, lactase, cellulose and neutral protease) for the management of delayed onset muscle soreness after eccentric exercise.

### Materials and Methods

#### Product description

DigeZyme® is an "off - white to creamy white powder of multi-enzyme complex". This multi-enzyme complex consists of amylase, protease, lipase, cellulase and lactase. Placebo capsules containing equivalent weight of maltodextrin. No differences in colour, taste, texture or packaging were detectable between the two products. Capsules were sealed in identically-appearing, high-density polyethylene bottles with desiccant. The enzyme activity of multi-enzyme complex was determined by following standard methods as per Food Chemicals Codex guidelines (Table 1).

Enzyme units were defined as per Food Chemical Codex (FCC), 5th ed. 2004. The National Academy Press Washington DC.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Enzyme</th>
<th>Enzyme activity (Units/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alpha-amylase</td>
<td>24000 DU/g (Dextrinizing Unit/gram)</td>
</tr>
<tr>
<td>2</td>
<td>Cellulase</td>
<td>1100 CU/g (Cellulase Unit/gram)</td>
</tr>
<tr>
<td>3</td>
<td>Lipase</td>
<td>200 FIP/g (FédérationInternationalePharmaceutiqueUnit/gram)</td>
</tr>
<tr>
<td>4</td>
<td>Lactase</td>
<td>4000 ALU/g (Acid Lactase Unit/gram)</td>
</tr>
<tr>
<td>5</td>
<td>Neutral Protease</td>
<td>6000 PC/g (Protease Unit on L-tyrosine basis/gram)</td>
</tr>
</tbody>
</table>

Table 1: Composition detail of multi-enzyme complex (DigeZyme®) formulation.

#### Ethics approval

Ethics approval was obtained through the Sparsh Hospital for Advanced Surgeries, #146, Infantry Road, Bangalore prior to the initiation of the study. The study documents reviewed were (protocol, informed consent form, investigator brochure, and case record form). The aforesaid Ethics Committee was registered under central drugs standard control organization as per the gazette notification number F.28-10/45-H(1), dated 21 Dec 1945 and last amended vide notification number G.S.R. 76(E) dated 08 FEB 2012.

### Study design

For the DOMS model under study, we selected the following design features: prospective, double blind, randomized, and placebo controlled. The analgesic efficacy of DigeZyme® was compared with a matching placebo. The pre specified efficacy end points of the study were assessed on Day 0 (pre exercise) and 72 hours post exercise.

#### Participants

Twenty healthy males with no known musculoskeletal pathology participated in the study. Subjects were excluded if they met one or more of the following exclusion criteria: treatment with anti-inflammatory/analgesic/antioxidant drugs in the previous month, abnormal liver or renal function tests, laboratory findings suggestive of an active inflammatory or infectious process and presence of any known disease.

The participants also completed a health history questionnaire designed to identify the degree of risk for cardiovascular or orthopaedic complications during exercise.

### Supplementation with multi enzyme complex

All participants in the experimental group received multi enzyme complex supplementation over a 3 day period. During this period, they consumed one capsule of multi enzyme complex thrice a day. The participants in the placebo group received capsules of similar size and colour. They were given identical instructions on dosage of study supplement to be followed. Study supplements were prepared and provided by Sami Labs Limited, Bangalore. The administration of treatment and placebo was blinded for both the participants and the investigator. There were no side effects reported by the participants as a result of the supplementation.

#### Data collection protocol

Subjects visited the clinic on day 0 (baseline visit) and subsequent visits on day 1, day 2 and day 3. After recording vital signs (blood pressure, pulse rate, heart rate and respiratory rate), medical and medication history, physical examination was conducted.

### Randomisation and allocation concealment

The randomisation sequence was prepared by an independent statistician, independent of the sponsoring organization and not involved in conduct or reporting of the study. An alpha numeric code was generated for both the active and placebo to improve the
isometric contraction (MVC) of the muscle. Each participant was asked to perform three repetitions of maximal knee extension and flexion contractions (MVC) at three different positions of knee range of motion (0 degrees, 90 degrees, 120 degrees). The test angles were chosen to account for changes in muscle strength that could occur due to alteration in the length of the muscle at different positions of the knee [25].

Illinois agility run test

Agility was measured using Illinois agility run test. The tests were conducted on a course 10 meters in length and 5 meters in width. Agility time was recorded using a stopwatch. The start, finish and the two turning points were marked with four cones and another four cones were placed down the center equal distance spaced 3.3 meters apart. Subjects were made to lie on their front (head to the start line) and hands by their shoulders. The run started from a standing start on the command ‘Go’ the stopwatch was started, and the subjects were instructed to get up as quickly as possible and run around the course in the direction indicated, without knocking the cones over, to the finish line, at which the timer was stopped [26].

Outcome measures

McGill pain questionnaire comprising of 10 subscales from 0 (no pain) to 10 (worst possible pain) was used to assess pain [27,28]. An algorimeter was used to experimentally induce pain on a predefined point on the patellar tendon five centimetres above the center of the patella, tenderness was assessed.

Subjects ranked their pain perception on a scale from 0 to 10. On day 3, assessments were taken at baseline (pre-exercise), post-exercise and further at 24, 48 and 72 hours post-exercise for each arm of the study. Secondary outcomes included assessments of inflammation, muscle damage, flexibility, and the amount of energy expended prior to exercise. Biomarkers creatine kinase and lactate dehydrogenase were monitored pre exercise and 72 hours post exercise.

Safety profile of the supplement was assessed by routine haematology, kidney and liver function tests. Adverse events were monitored throughout the study.

Statistical analysis

All testing was done using Statistical Analysis Software (SAS) having version 9.2. All analyses were conducted using the intent-to-treat population. Patients with no data recorded for a particular parameter were automatically excluded from the analyses of that parameter. For all the data set variables analysis of covariance (ANCOVA) and Wilcoxon signed rank sum test were used and the level of significance was set as P<0.05.

Results

Subject disposition

A total of 20 subjects were enrolled into the study. There were no subject withdrawals or dropouts in this study.

Physical characteristics

The population was essentially healthy without significant concomitant disease or medication intake. Screening characteristics of participants are presented in (Table 2). There were no statistically significant differences between subjects in the placebo (n=10) and the DigeZyme® (n=10) group. On the day of screening, the mean weight of

For muscular strength and power using hand held dynamometer

A Hand-Held Dynamometer (HHD) was used to quantify changes in muscle strength by measuring strength during maximal voluntary isometric contraction (MVC) of the muscle. Each participant was asked to perform three repetitions of maximal knee extension and flexion contractions (MVC) at three different positions of knee range of motion (0 degrees, 90 degrees, 120 degrees). The test angles were chosen to account for changes in muscle strength that could occur due to alteration in the length of the muscle at different positions of the knee [25].

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all the enrolled subjects was 59.3 ± 4.64 kgs; mean height was 163.8 ± 4.99 cm and the mean BMI was 22.2 ± 1.50 kg/m².

**Efficacy evaluation**

Delayed Onset Muscle Soreness-quality of life was analyzed throughout the study period as primary efficacy measure. The 'p' value suggests that there was a statistically significant change in these symptoms from baseline to final visits, between the placebo and active arms. Statistical analysis using Analysis of Co-Variance (ANCOVA) showed the primary efficacy parameters were statistically significant (p<0.05) between the multienzyme complex and placebo groups (Table 3). Furthermore, comparative mean values of efficacy assessments between Multi enzyme complex and placebo groups across various visits (baseline, day 1, day 2 and day 3) are presented for efficacy parameters (Figures 1-8). Patients who were on Multi enzyme complex had statistically significant difference for the efficacy parameters on day 3 when compared with placebo.

**Pain assessment**

McGill pain questionnaire is a multidimensional pain instrument; the final score is a sum of all the ten individual pain questions. The first nine McGill pain questionnaires were based on assessment of pain (current pain, least pain, and worst pain) while the tenth on the degree of numbness and its interference with function.

When differences between multi enzyme complex and placebo were compared, the pain score showed high statistical significance (p=0.0061).

### Demographics Values

<table>
<thead>
<tr>
<th>Demographics</th>
<th>Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Height (cm)</strong></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Mean(SD)</td>
<td>163.8 (4.99)</td>
</tr>
<tr>
<td>Median</td>
<td>164.5</td>
</tr>
<tr>
<td>Min, Max</td>
<td>154, 172</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Mean(SD)</td>
<td>59.3 (4.64)</td>
</tr>
<tr>
<td>Median</td>
<td>59.2</td>
</tr>
<tr>
<td>Min, Max</td>
<td>51, 67</td>
</tr>
<tr>
<td><strong>Body Mass Index (kg/m²)</strong></td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>Mean(SD)</td>
<td>22.2 (1.50)</td>
</tr>
<tr>
<td>Median</td>
<td>22.3</td>
</tr>
<tr>
<td>Min, Max</td>
<td>20, 25</td>
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<tr>
<td><strong>Race</strong></td>
<td></td>
</tr>
<tr>
<td>Central American</td>
<td>0</td>
</tr>
<tr>
<td>East Asian</td>
<td>0</td>
</tr>
<tr>
<td>South Asian</td>
<td>20</td>
</tr>
<tr>
<td>South American</td>
<td>0</td>
</tr>
<tr>
<td>South East Asian</td>
<td>0</td>
</tr>
<tr>
<td>Western European</td>
<td>0</td>
</tr>
<tr>
<td>White</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2: Subject demographics characteristics.

### Subjective Parameters

<table>
<thead>
<tr>
<th>Measure</th>
<th>Investigational Products</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hand held Dynamometer readings result [grip strength] kg</td>
<td>DigeZyme® Baseline 36.2 (7.86) Post Exercise 37.6 (8.32)</td>
<td>0.4956</td>
</tr>
<tr>
<td>Thigh muscle (point of pain) Algometer Reading's kg/cm²</td>
<td>9.5 (0.58) 5.3 (1.18)</td>
<td>0.0436*</td>
</tr>
<tr>
<td>Calf muscle (point of pain) Algometer Reading's kg/cm²</td>
<td>9.1 (1.09) 3.9 (1.20)</td>
<td>0.1397</td>
</tr>
<tr>
<td>Total Time Taken Illinois Agility Run test (Seconds)</td>
<td>25.6 (3.43) 24.6 (4.11)</td>
<td>0.7246</td>
</tr>
<tr>
<td>The McGill pain questionnaire (total pain score)</td>
<td>29.5 (2.55) 48.7 (8.86)</td>
<td>0.0061*</td>
</tr>
</tbody>
</table>

Table 3: Efficacy analysis.
Tenderness assessment

On the tenderness quotient, subjects taking multi enzyme complex demonstrated significantly less tenderness, 72 hours after exercise (p=0.042).

Muscle damage assessments

Liberation of biochemical substances such as creatine kinase, lactate dehydrogenase, protein metabolites and myoglobin occurs from muscle cells approximately 24 hours post exercise and have been found in plasma up to 48 hours [29]. Creatine kinase being a surrogate index of muscle damages more indicative of damage or gaps in the sarcolemma [30]. The CK response was less in the multi enzyme complex group suggesting the membrane integrity was maintained to greater extent than the placebo group.

Flexion and extension measurements

24 hours post exercise, subjects on the placebo group showed trend towards a higher level of CK than the multi enzyme complex group. This trend continued through the 72 hour assessment. Difference between the multi enzyme complex group and placebo in CK values was not statistically significant. Lactate dehydrogenase also showed a similar phenomenon, which trended higher at 24 and 72 hours post-exercise in the placebo group (Figures 7-8).

Safety evaluations

Vital signs such as Blood Pressure, Respiratory Rate, Pulse Rate and any abnormal laboratory parameters were considered for safety evaluations. No clinically significant changes were recorded for descriptive physical examination in both the groups (Multi enzyme complex and placebo). The safety of multi enzyme complex was assessed using adverse event data (occurrence, intensity, and relationship to study drug). No adverse events were noticed in the study.

Discussion

Multi enzyme complex capsules contain alpha-amylase, neutral protease, lipase, lactase and cellulase. The capsule contains free broad acting enzymes obtained from the fermentation process.
In the present study multi-enzyme complex capsules demonstrated significant improvement in subjective pain and tenderness, with no significant improvement in levels of markers of inflammation, muscle damage or muscle flexion. Multi-enzyme complex contains a multiple enzymes that are indicated for relieving the symptoms of DOMS.

The findings of this study suggest that multi enzyme complex can have several potential clinical applications. Protease supplementation when coupled with a well-managed training programme can result in more rapid recovery of the damage caused to contractile mechanism by DOMS.

**Registration**

The trial was registered on the Clinical Trial Registry of India with the registration number CTRI/2015/09/006148.

**References**


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