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Effect of a Phytochemical formulation on Muscle fatigue, Energy, Recovery in Delayed Onset Muscle Soreness (DOMS), inflammation and Stress in Healthy Subjects: Results from a Randomized Placebo Controlled Study

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Abstract

Objective: To assess the effectiveness and safety of β-caryophyllene (Rephyll®) on muscle fatigue, energy, recovery in delayed onset muscle soreness (DOMS), inflammation and stress in healthy subjects.

Methods: A randomized, double-blind, placebo-controlled, clinical study was conducted by administering Rephyll® capsules or placebo capsules in the treatment of DOMS as daily supplements in 110 subjects, who were untrained in resistance or power exercise. The study subjects were randomized in a 1:1 ratio to receive either Rephyll® or placebo for 30 days. Main outcome measures were changes in muscle fatigue by fatigue index, the rating of perceived exertion (RPE) and Creatine Kinase levels. Respiratory exchange ratio (RER) and VO_{2max}, adenosine-5'-triphosphate (ATP) and lactic acid threshold were measured for endurance of energy supply & recovery. The subjective pain score was measured using the Visual analogue scale (VAS). All evaluations were carried out between baseline and 30 days after the completion of treatment. Safety parameters were assessed by evaluating biochemical parameters for liver function, kidney function & metabolic parameters and monitoring adverse events throughout the study period.

Results: The muscle fatigue was significantly improved with Rephyll® treatment as compared to the placebo after 30 days of treatment. The Rephyll® group had a significantly lower respiratory exchange ratio than the placebo group, indicating better aerobic function. The Rephyll® group had a higher reserve of ATP compared to the placebo group after 30 days of treatment. Additionally, the Rephyll® group consumed less oxygen than the placebo group, demonstrating the product's aerobic efficiency. Increased endurance is a result of Rephyll's capacity to improve the body's ability to expel lactic acid during exercise. The Rephyll® group showed statistically significant pain reduction after 30 days of treatment at each visit as compared to placebo group. No adverse events were recorded during the study period in any of the groups.

Conclusion: Rephyll® was able to reduce muscle fatigue, improve endurance & energy supply, and showed potential for reducing DOMS. It also enhanced neuromuscular activation and reduced inflammation and stress in subjects receiving Rephyll® treatment. It was safe and well tolerated by all subjects.

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Introduction

A cascade phenomenon known as delayed-onset muscle soreness (DOMS) can have detrimental effects on people who are physically active as well as those who have been sedentary for extended periods of time and want to resume professional sports, return to training, or just increase their level of physical activity [1]. DOMS is a combination of muscle pain and stiffness occurring several hours after unaccustomed exercise, particularly when eccentric muscle activity is involved, it can induce muscle damage. An inflammatory response and the reactive oxygen species (ROS) production is generated by this mechanical stress. The reason behind this process is that the mechanical stress stimulates the transcription factors such as nuclear factor-κB (NF-κB) to become activated [2]. NF-κB produces pro-inflammatory cytokines IL-6 and tumor necrosis factor-α (TNF-α) which have been proposed to be involved in DOMS [3,4]. Other studies have shown that DOMS may be linked to other muscular responses to exercise such as injury, high temperatures, spasms, lactic acid levels, and connective tissue damage [1]. DOMS is categorized as a type I muscle strain injury and manifests as movement and/or palpation-induced soreness or stiffness [5].

DOMS is the primary cause of reduced exercise performance, including decreased muscle strength and range of motion for both athletes and non-athletes, and it also results in continual psychological distress [6]. After exercise, muscle soreness commences 12-24 h later, peaks 24-72 h later, and lasts for five-to-seven days [1].

Researchers investigating the mechanisms of DOMS have induced muscle soreness using exercise regimens that are mostly eccentric-such as downhill running, resisted cycling, ballistic stretching, isokinetic dynamometry, stepping and/or eccentric resistance exercise [7].

Recovery from DOMS and muscle damage is becoming increasingly important so that any sports person or athlete or any otherwise healthy individual with interest in exercise may undergo training more frequently to increase long-term performance.

Cryotherapy, stretching, massage, compression, ultrasound, oral nonsteroidal anti-inflammatory medications (NSAIDS), and exercise are some of the therapeutic techniques for the management of DOMS-related symptoms. Several nutritional supplements (e.g., protein powders, vitamin C, fish oil, and chondroitin sulphate) have also been studied, with varying outcomes [8]. Other strategies have been treated to mitigate DOMS such as hyperbaric oxygen, homeopathy, electrotherapeutic modalities, rest, and light exercise [2].

Phytochemicals have accredited consideration in recent years for their diverse biological activities. Phytocannabinoids are one class of phytochemicals that have garnered significant attention due to their anti-inflammatory, cardio protective, and anticancer properties. Among the phytocannabinoids, β- caryophyllene, a bicyclic sesquiterpene plentifully offered in spices, particularly *Piper nigrum* L., *Cinnamomum verum*, *Origanum vulgare*, and *Eugenia caryophyllata* has attracted substantial attention because of its nutritional readiness and extensive care [9].

Recently, β-Caryophyllene (BCP) has the distinction of being the first known "dietary cannabinoid," a common food ingredient that is FDA-approved for use in food and has the designation of "Generally Recognized as Safe" (GRAS) status. BCP is the main sesquiterpene contributing to the spiciness of black pepper; it is also a significant constituent of cloves, hops, rosemary, copaiba, and cannabis [10].

Recent research has shown that BCP has modulatory and pharmacological effects on numerous organs including the liver, kidney and brain. It has been documented that BCP possesses antioxidant, anti-inflammatory and anticancer therapeutic effects [11].

Rephyll® is a novel formulation that is helpful in managing pain and contains liposomal β-caryophyllene. It is formulated using nanofiber weaving (NFW) technology which is a well-organized method of creating nanofiber (NF), expanding the range of applications for BCP, especially in the pharmaceutical and nutraceutical industries [9].

The possible mechanism of action of the Rephyll® (BCP) in DOMS is recommended based on recent studies, which suggest that the pain relieving outcome may be a result of the interaction of the glutamatergic pathway by the way of the opioid system and with L-arginine/nitric oxide. The further probable mechanisms of action for BCP include variation of pain through the endocannabinoid pathways, the hindrance of the NF-κB pathway, and a decrease in cyclooxygenase-2 expression (COX-2) or tumor necrosis factor-α (TNF-α) and prostaglandin E2 (PEG2) release. Concisely, agonist action on CB2 receptors and inhibitor action on the Toll-like receptor complex, cluster of differentiation 14/Toll-like receptor 4/ myeloid differentiation factor 2 (CD14/TLR4/MD2)-triggered pathways, and significant down-regulation of inducible nitric oxide synthase (iNOS) and COX-2 and reduction of proinflammatory cytokines thus attribute to the anti-inflammatory properties of BCP [9].

In order to investigate the effect of Rephyll® on DOMS we conducted a randomized, placebo-controlled clinical trial to evaluate the efficacy and safety of Rephyll® capsules as compared to placebo.

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Methods

Study design

This was a randomised, double-blind, placebo-controlled clinical study with 30 days of treatment. The study was conducted at two different clinical sites in the city of Ahmedabad, India. The study protocol and protocol-related documents were approved by the institutional ethics committee before initiating the trial related activity. The study was registered with Clinical Trials Registry of India (CTRI/2022/06/043370). The study was conducted in accordance with regulatory and ethical guidelines (Declaration of Helsinki, ICH GCP, and New Drug and Clinical Trial Rule, 2019, CDSCO).

Study subjects

Male and female subjects who were untrained or less active otherwise healthy were contacted. All study subjects provided voluntary written informed consent before initiating the screening procedures. All of these subjects were undergone screening. They were screened based on inclusion and exclusion criteria. They were included in the trial if they were aged 19 to 50 years, in good health, never done power or resistance training, if they exercised fewer than four hours a week on a regular basis, agreeable, willing to comply with the study schedule and procedure. The exclusion criteria were a subject who is participating in any additional clinical study, had a history of alcohol consumption or drug abuse in the past year, and had a substantial history of otherwise recent occurrence of treated or untreated bleeding complaints, diabetes mellitus, high blood pressure, thyroid sickness, tachyarrhythmia, heart illness, kidney infection, or liver sickness. The subjects were also excluded who had an identified allergy or sensitivity to any ingredient in the investigational product, subject suffering from a sleep disorder and had a known history of (or is currently being treated for) clinical depression, eating disorder(s) or any other psychiatric condition(s), had any medical condition or uses any medication, nutritional product, dietary supplement or program, which in the opinion of the investigator, might interfere with the conduct of the study or place the subject at risk, had a history of difficulty swallowing large tablets or pills, had used creatine supplements within 9 weeks prior to screening, had a history of orthopedic surgery or injury within the last year. Subjects with any physical condition that reflected a contraindication to the type of exercise performed in the study, and had an abnormal resting ECG were also excluded.

Randomisation, medication dosing and dispensing

Study subjects were randomized in a 1:1 ratio to receive the phytocannabinoid β-caryophyllene (Rephyll® capsules) or indistinguishable placebo capsules. The study medications were dispensed in pre-labelled identical bottles to both the sites according to individual site randomisation schedule generated using a computerised random number generator with mixed block sizes to prevent the identity of treatment assignment. The randomisation codes were kept in an individually sealed, opaque envelope and broken in case of any serious adverse event otherwise, only after the completion of data lock procedures. Each capsule of Rephyll® contained 250 mg β-caryophyllene with inert excipients. In case of placebo, β-caryophyllene was replaced with a food grade starch, which was visually similar to Rephyll®. Subjects were provided with two bottles of study medication. Each bottle contained 30 capsules for a total of 30 days of administration. Subjects were instructed to take two capsules at a time in the morning, after breakfast, with a glass of water. They were instructed to fill up the time of drug administration and the number of capsules taken in the subject diary. Dosage compliance was assessed counting the unused quantity of medication from returned bottles. Medication compliance was considered reached when there was 90 % capsules intake over a period of 30 days capsules administration. Subjects were advised to record the type and amount of food consumed in the subject diary provided.

Study visits

Subjects visited the study center four times for evaluation of study parameters: during the screening and randomization visit 1 (Day -5 to 1), visit 2 follow-up visit (Day 4), visit 3 follow-up visit (Day 15), and the end-of-treatment (EOT) visit 4 (Day 30). There was a telephonic follow-up post-study visit on Day 60.

Eccentric Exercise

Subjects had to perform four eccentric exercises (calf raises, hamstring curls, lying hip lifts, decline board knee bends) at the randomization visit (Day 1) and the end-of-treatment visit (Day 30).

End points and measures of outcomes

The primary efficacy end point was to measure change in muscle fatigue (fatigue index, rating of perceived exertion (RPE- using the Borg Rating Scale), evaluating level of creatine kinase (CK), lactate dehydrogenase (LDH), myoglobin, serum lactic acid, Na^+ and K^+, change in endurance energy supply and recovery (respiratory exchange ratio (RER), maximum oxygen consumption (VO_{2max}), creatine, phosphocreatine, ATP and lactic acid threshold), change in neuromuscular activation (Electromyography (EMG), Vestibular function test), change in stress and anti-inflammatory biomarkers on day 30 as compared to baseline. The change in subjective pain score (visual analogue scale (VAS)) was also evaluated at each visit as a primary outcome. Secondary outcome measures include clinically and physically observing and reporting adverse events (AE) and assessing changes in vital signs and biochemistry parameters.

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Statistical analysis

Mean change in efficacy parameters from baseline to end of treatment visit were analyzed using unpaired "t" test (For between group comparison). For Within group comparison, mean change in efficacy parameters from baseline to end of treatment visit were analyzed using paired "t" test. Normality test (Shapiro-Wilks test) was used to check the distribution of data. All tests were 2 sided. P values of less than 0.05 was considered as statistically significant difference between treatment groups. The data presented as mean (±Standard deviation) with 95% confidence interval of treatment difference.

Results

Disposition of subjects

Of total 110 subjects, one subject from Rephyll® group and four from placebo group withdrew their consent during the course of the study. A total of 105 subjects completed the study. Figure 1 describes the disposition of the study subjects.

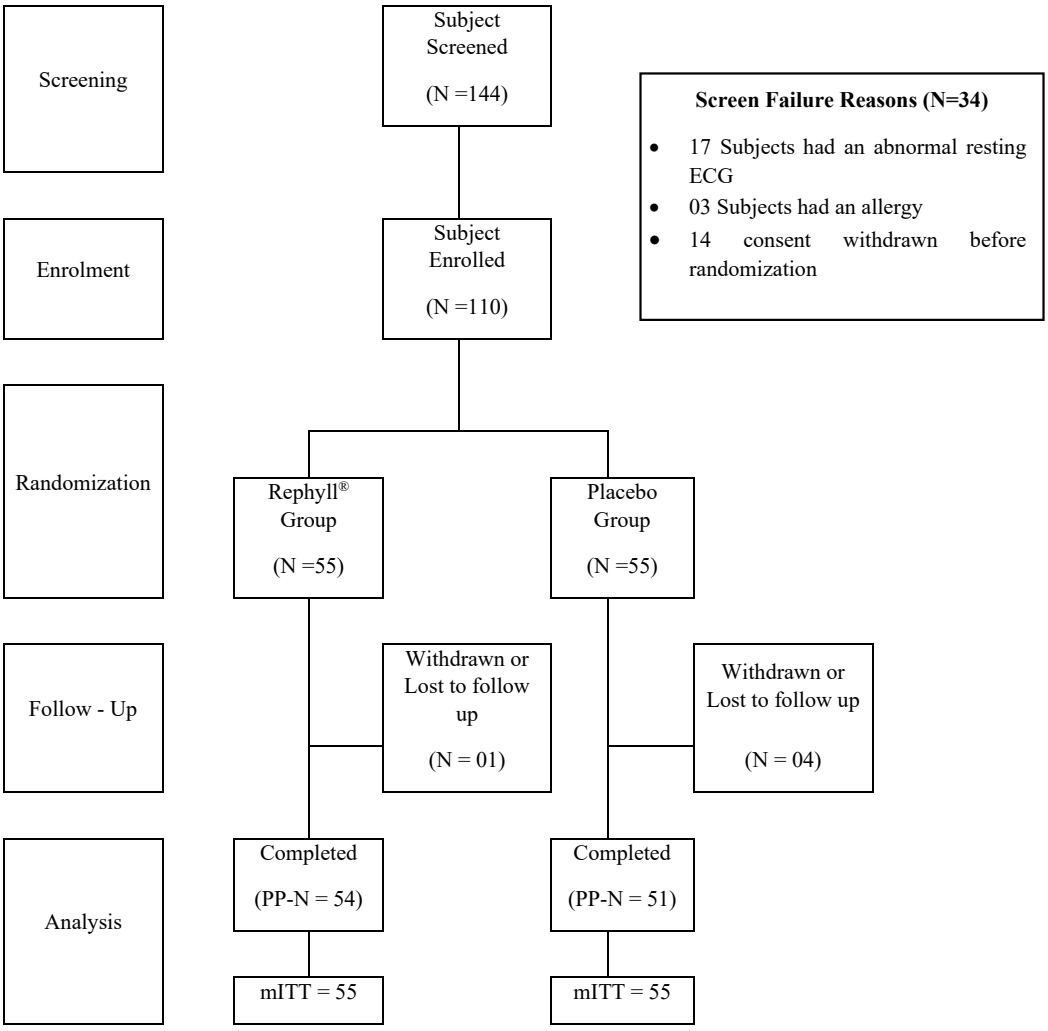
Demography & subject characteristics

Baseline demographic characteristics (mean ± SD) for subjects in the Rephyll® group were, age 37.64 ± 7.57 years; weight and height 64.33 ± 10.85 kg and 163.25 ± 8.67 cm, respectively; and for the placebo group, age 37.31 ± 6.55 years; weight and height 64.65 ± 11.04 kg and 161.69 ± 7.95 cm, respectively. There was no significant difference between the baseline demographics of study subjects in each treatment group (Table 1).

Efficacy analyses

Muscle fatigue

A significant improvement was observed in the fatigue index after 30 days of treatment with Rephyll®. In the Rephyll® group, fatigue index was elevated from



N= number of subjects; PP=per protocol; mITT=modified intended to treat

Figure 1: Disposition of the subject

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166.08 ± 101.02 (mean ± SD) at baseline to 222.13 ± 143.24 (mean ± SD) at the end of 30 days, which was statistically significant ($p<0.05$) as compared with baseline measurements. While in the placebo group, the fatigue index was 152.39 ± 73.11 (mean ± SD) at baseline, which minimally changed to 159.54 ± 74.81 at the end of 30 days of treatment, which was statistically non-significant. The observed change in fatigue index was 56.04 ± 143.47 (mean ± SD) in Rephyll® group, while in the placebo group it was 7.16 ± 79.54 (mean ± SD) (Table 2) (Figure 2).

In Rephyll® group, RPE was 12.26 ± 1.73 (mean ± SD) at baseline which was reduced to 9.24 ± 2.01 (mean ± SD) at the end of the study treatment and this change was statistically significant ($p<0.05$). However, no such difference was observed in placebo group ($p>0.05$) (Table 2). Changes from baseline in RPE is shown in figure 3. Result showed that the exertion during exercise at day 1 was similar between both groups but after 30 days of treatment, subjects in Rephyll® reported less exertion as compared to subjects in placebo group. The level of CK was increased in both Rephyll® and placebo group on day 4 (72hr) of exercise and day 15 from baseline but in placebo group the level increased till day 30. However, in Rephyll® group, there was a reduction in CK level after 30 days of treatment period (Table 3). This shows muscle injury was higher in placebo group as compared to the Rephyll® group, though there was no statistically significant difference. No significant changes in lactate dehydrogenase, myoglobin levels and lactic acid levels were also found during the study period in either group.

There were no statistical significant differences found in electrolyte levels (including glucose, sodium, and potassium) between the groups.

Table 1: Demographics of study subjects

Subject characteristic	Rephyll® (N = 55)	Placebo (N = 55)	Overall (110)
Age (years)	37.64 (7.57)	37.31 (6.55)	37.47 (7.05)
Gender	M=46, F =9	M=39, F =16	M=85, M=25
Height (cm)	163.25 (8.67)	161.69 (7.95)	162.47 (8.32)
Weight (kg)	64.33 (10.85)	64.65 (11.04)	64.49 (10.90)
BMI (kg/m²)	24.18 (3.96)	24.79 (4.18)	24.48 (4.07)

Values are expressed as Mean ± Standard deviation (SD). Gender expressed as absolute number. N = number of subjects. Data are presented as descriptive statistics.

Table 2: Change in muscle fatigue

	Rephyll®	Placebo	Effect Size, 95% CI of treatment difference and P value (Between group comparison)
Fatigue index			
PP population	N=54	N=51	
Baseline (Visit 1)	166.08 (101.02)	152.39 (73.11)	13.6900 (-20.6115 to 47.9915) P=0.4304
End of treatment (EOT) visit	222.13 (143.24)*	159.54 (74.81)	62.5900 (17.9729 to 107.2071), P=0.0064
Change from baseline at EOT visit	56.04 (143.47)	7.16 (79.54)	48.8800 (3.6150 to 94.1450), P=0.0346
RPE score			
Enrollment Visit 1 (Day 1) after exercise	12.26 (1.73)	12.41 (1.81)	-0.1500 (-0.8352 to 0.5352) P= 0.6651
End of treatment (EOT) Visit 4 after exercise (Day 30)	9.24 (2.01)*	11.04 (1.96)*	-1.8000 (-2.5690 to -1.0310), P= 0.0001
Change from Enrollment Visit 1 (Day 1) after exercise at End of treatment (EOT) Visit 4 after exercise (Day 30)	-3.02 (2.47)	-1.37 (2.42)	1.6500 (0.7028 to 2.5972), P= 0.0008

*p<0.05 vs baseline (within group comparison) otherwise not specified. Values are expressed as Mean ± Standard deviation (SD). Abbreviation: N= number of subjects; PP=per protocol

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Table 3: Change in Creatine kinase

	Rephyll®	Placebo	Effect Size, 95% CI of treatment difference and P value (Between group comparison)
Creatine kinase, CK (U/L)			
PP population	N=54	N=51	
Enrollment visit (Day 1) after exercise	97.41 (51.22)	114.92 (53.00)	-17.5100 (-37.2097 to 2.1897) P= 0.0809
Follow up visit # 2 (day 4, 72 hrs.)	106.54 (70.10)	120.04 (48.01)	-13.5000 (-36.8878 to 9.8878), P= 0.2550
Follow up visit # 3 (15 days)	111.70 (66.79)	120.80 (66.56)	-9.1000 (-34.9213 to 16.7213), P= 0.4862
End of treatment (EOT) visit after exercise (Day 30).	107.28 (66.69)	122.69 (58.15)	-15.4100 (-39.6867 to 8.8667), P= 0.2109
Change from Enrollment visit at follow up visit # 2	7.89 (54.65)	7.56 (50.88)	-0.3300 (-20.7976 to 20.1376), P= 0.9746
Change from Enrollment at follow up visit # 3	14.30 (44.82)	8.18 (65.27)	-6.1200 (-27.6872 to 15.4472), P= 0.5748
Change from Enrollment at EOT	10.21 (52.43)	7.42 (45.12)	-2.7900 (-21.7722 to 16.1922), P= 0.7713

*p<0.05 vs baseline (within group comparison) otherwise not specified. Values are expressed as Mean ± Standard deviation (SD). Abbreviation: N= number of subjects; PP=per protocol

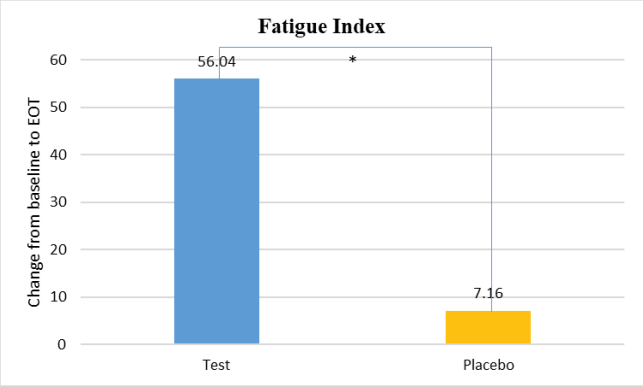

Figure 2: Change in fatigue index from baseline visit to EOT visit. *Significantly greater than placebo.

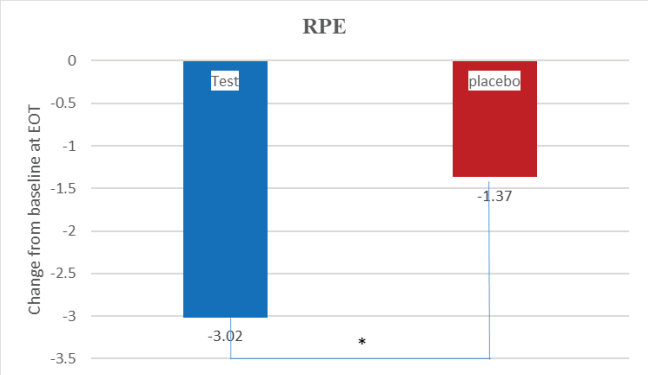

Figure 3: Change in the rating of perceived exertion (RPE) score from baseline visit to EOT visit.

*Significantly greater than placebo.

Endurance energy supply and recovery

There was statistically significant difference found between Rephyll® and placebo subjects for RER after 30 days of treatment. RER increases with exercise intensity. In Rephyll® group, RER was found to be 1.54 ± 0.72 (mean ± SD) at baseline, which improved to 1.36 ± 0.66 (mean ± SD) at end the of the treatment visit, while in the placebo group, a RER was observed similar between the baseline 1.37±0.74 (mean ± SD) and the end of the treatment visit 1.39±0.53 (mean ± SD). Change from baseline in RER is presented in table 4 and figure 4.

Results of VO_{2max}, showed that after 30 days of treatment, less oxygen was consumed during exercise in the Rephyll® group, and the change was statistical significant (Table 4 and figure 5). Additionally, change in ATP was statistically significant from baseline to Day 30 within Rephyll® group and placebo group (Table 4) (Figure 6). At baseline, time for lactic acid threshold was almost similar in both groups (23.23 ± 13.19 (mean ± SD) mins in Rephyll® and 22.45±15.33 mins (mean ± SD) in placebo group). At the end of treatment visit, the time was similar as baseline in placebo group but in Rephyll® group the time was increased (25.52 ± 06.53 mins (mean ± SD) in Rephyll® group and 22.48± 08.13 mins (mean ± SD) in placebo group) (Table 4). Subjects in Rephyll® group took more time to reach threshold than placebo.

There were no significant changes in creatine and phosphocreatine during the study period in either group.

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Table 4: Change in endurance energy supply and recovery

	Rephyll®	Placebo	Effect Size, 95% CI of treatment difference and P value (Between group comparison)
PP population	N=54	N=51	
Respiratory Exchange Ratio, RER			
Enrollment Visit 1 (Day 1) after exercise	1.54 (0.72)	1.37 (0.74)	0.1700 (-0.1126 to 0.4526) P= 0.2356
End of Treatment (EOT) Visit 4 after exercise (Day 30)	1.36 (0.66)	1.39 (0.53)	-0.0300 (-0.2625 to 0.2025), P= 0.7985
Change from Enrollment Visit 1 (Day 1) after exercise at EOT Visit 4 after exercise (Day 30)	-0.18 (0.49)	0.02 (0.54)	0.2000 (0.0006 to 0.3994), P= 0.0493
VO_2max_Max oxygen consumption (mL/kg/min)			
Enrollment Visit (Day 1)	37.98 (3.48)	37.47 (3.32)	0.5100 (-0.8079 to 1.8279) P= 0.4446
End of Treatment (EOS) Visit 4 (Day 30)	36.63 (4.26)	37.91 (2.64)	-1.2800 (-2.6612 to 0.1012), P= 0.0690
Change from Enrollment Visit (Day 1) at EOT Visit 4 (Day 30)	-1.35 (3.19)	0.45 (2.82)	1.8000 (0.6320 to 2.9680), P= 0.0029
ATP (nmol/ml)			
Enrollment Visit 1 (Day 1) before exercise	1.15 (0.45)	1.27 (0.60)	-0.1200 (-0.3245 to 0.0845) P=0.2473
End of Treatment (EOT) Visit 4 before exercise (Day 30)	2.65 (0.91) *	2.43 (1.00)*	0.2200 (-0.1497 to 0.5897), P= 0.2407
Change from Enrollment Visit 1 (Day 1) before exercise at EOT Visit 4 before exercise (Day 30)	1.46 (1.20)	1.00 (1.35)	-0.4600 (-0.9538 to 0.0338), P= 0.0675
Summary of Time to achieve Lactic Acid Threshold (mins)			
Baseline	23.23 (13.19)	22.45 (15.33)	0.7800 (-4.6252 to 6.1852), P=0.7754
EOT	25.52 (06.53)	22.48 (08.13)	3.0400 (0.2529 to 5.8271), P=0.0328

*p<0.05 vs baseline (within group comparison) otherwise not specified. Values are expressed as Mean ± Standard deviation (SD). Abbreviation: N= number of subjects; PP=per protocol

Neuro muscular activation

For neuromuscular activation, mean change from baseline to the end of treatment was statistically significant for muscles such as Gastrocnemius (P = 0.015) and Adductor (P = 0.014) in Rephyll® group. While clinical improvement was observed in Quadriceps, and Hamstring muscles. We also evaluated vestibular function (VFT) parameters and found that subjects' vestibular functions were normal after exercise, with no imbalances observed during vestibular function activities in either group.

Subjective pain score

There was a significant reduction in subjective pain score after 30 days consumption of Rephyll® following eccentric exercise, indicating a beneficial effect on DOMS after eccentric exercise (Table 5). Percentage change in subjective pain score from baseline visit to EOT visit is presented in figure 7.

Stress and anti-inflammatory biomarkers

There were reductions in inflammatory markers such as cortisol, CRP, IL-6, ESR and TNF-α after 30 days in subjects consuming Rephyll® as compared to placebo. Additionally, in the Rephyll® group, there was a reduction of 24% in the level of TNF- α from baseline visit to visit 3 (day 15) and the change was statistically significant within the Rephyll® group (Table 6). There was no significant change observed in other biomarkers.

Safety variable

The blood parameters evaluation showed that the individuals' overall health was not impacted by the treatment in either group.

Looking into the safety profiles of both the groups; it was noted that there was no treatment-induced-emergent adverse event reported in subjects receiving either Rephyll® group or placebo group.

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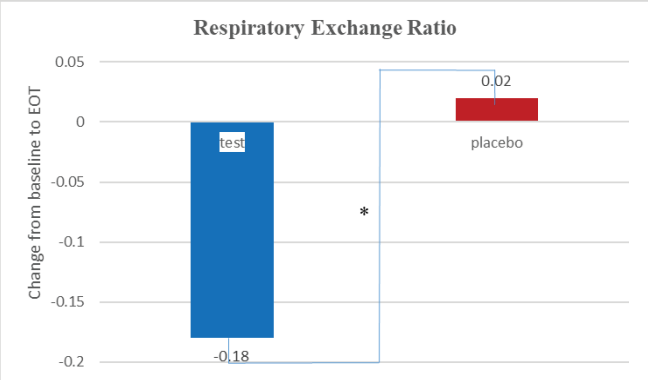

Figure 4: Change in respiratory exchange ratio (RER) from baseline visit to EOT visit

*statically significant difference found between test and placebo.

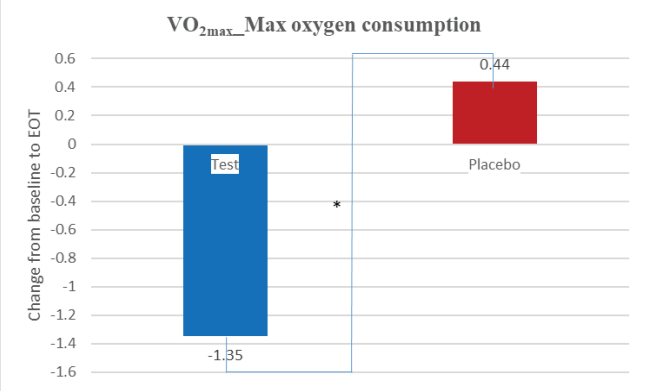

Figure 5: Change in VO_{2max} from baseline visit to EOT visit. *statically significant difference found between test and placebo.

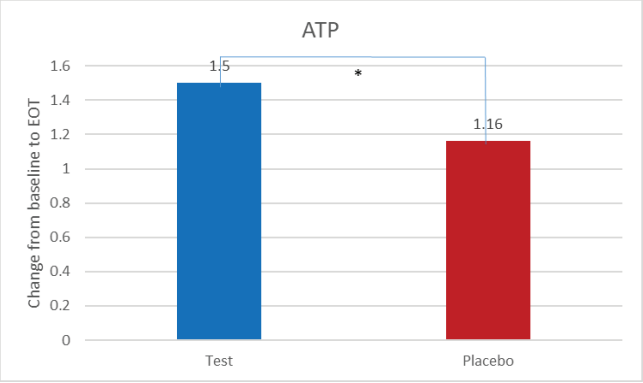

Figure 6: Change in ATP from baseline visit to EOT visit.

*statically significant difference found between test and placebo.

Table 5: Change in Subjective Pain Score

	Rephyll®	Placebo	P value (Between group comparison)
Pain score			
Baseline (Day 1) after exercise	4.09 (1.17)	4.00 (0.89)	0.64
Follow up visit 02 (Day 4)	3.00* (1.27)	3.78 (1.12)	0.001
Follow up visit 03 (Day 15)	1.46* (1.24)	2.14* (1.02)	0.003
End of Treatment (EOT) Day 30 after exercise	2.44* (0.92)	3.80 (0.80)	<0.001
Visit 4 (post exercise)			
Change from Enrolment Visit 1 (Day 1) at follow up visit 2 (Day 4)	-1.08 (1.11)	-0.22 (0.94)	<0.001
Change from Enrolment Visit 1 (Day 1) at follow up visit 3 (Day 15)	-2.65 (1.15)	-1.86 (0.94)	<0.001
Change from Enrolment Visit 1 (Day 1) at EOT Visit 4 (Day 30)	-1.63 (0.92)	-0.20 (0.72)	<0.001

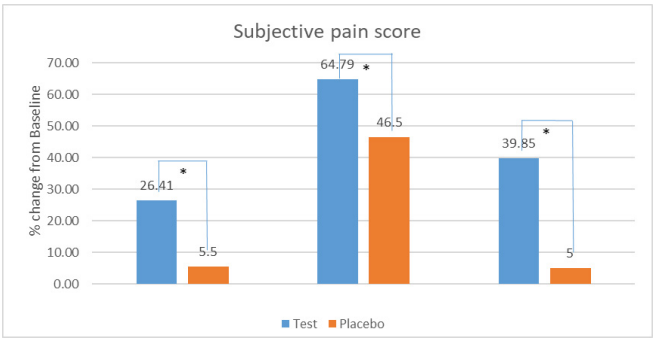

Figure 7: Percentage change in subjective pain score from baseline visit to EOT visit. *Significantly higher in test compared to placebo.

Discussion

This prospective study highlights the beneficial effects of the liposomal β-caryophyllene (Rephyll®, 250 mg, 2 capsules at a time), a sesquiterpene sourced from curcumin and black pepper extract, supplement, on adverse symptoms associated with DOMS, namely eccentric exercise-related parameters of muscle impairment. The eccentric exercise, that was calf raises, Hamstring curls, lying hip lifts, decline board knee bends, successfully induced DOMS, as evidenced by the increase in Creatine kinase after 72 hrs. of exercise (Day 4) in Rephyll® group and placebo group. Increased CK levels after eccentric exercise were associated with muscle injury, with a pronounced increase between 2 and 7 days after exercise.

Citation: Aurea Biolabs, Anupam Paliwal, Blake Kraemer, Dhara Shah, Ishita Basera, Akhil Mukim, Vaishal Sheth, Milan Satia. Effect of a Phytochemical formulation on Muscle fatigue, Energy, Recovery in Delayed Onset Muscle Soreness (DOMS), inflammation and Stress in Healthy Subjects: Results from a Randomized Placebo Controlled Study. Journal of Food Science and Nutrition Research 7 (2024): 131-142.

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Table 6: Change in Stress and Anti-Inflammatory Biomarkers

	Rephyll®	Placebo	Effect Size, 95% CI of treatment difference and P value (Between group comparison)
Cortisol (µg/dL)			
Screening Visit	7.61 (3.02)	7.77 (3.17)	-0.1600 (-1.3580 to 1.0380) P=0.7916
End of Study visit (EOS) Visit 4 (Day 30) before exercise	7.24 (3.36)	7.79 (2.38)	-0.5500 (-1.6829 to 0.5829), P= 0.3379
Change from Screening Visit at EOT Visit 4 (Day 30) before exercise	-0.37 (3.44)	0.02 (3.42)	0.3900 (-0.9384 to 1.7184), P= 0.5617
C-reactive protein (CRP)			
Screening Visit	1.92 (2.99)	3.19 (4.79)	-1.2700 (-2.8063 to 0.2663) P= 0.1042
Follow up visit 02 (Day 4)	2.82 (7.03)	2.49 (3.30)	0.3300 (-1.8162 to 2.4762), P= 0.7610
Follow up visit 03 (15 Days)	2.15 (2.68)	2.22 (2.34)	-0.0700 (-1.0461 to 0.9061), P= 0.8872
End of Treatment (EOT) Visit 4 (Day 30) before exercise	2.13 (3.02)	3.03 (3.90)	-0.9000 (-2.2457 to 0.4457), P= 0.1877
Change from Screening Visit at Follow up visit 02 (Day 4)	2.82 (7.03)	-0.70 (3.64)	2.1200 (-0.0659 to 4.3059), P= 0.0572
Change from Screening Visit at Follow up visit 03 (15 Days)	0.23 (3.15)	-0.97 (4.55)	-1.2000 (-2.7076 to 0.3076), P= 0.1175
Change from Screening Visit at EOT Visit 4 (Day 30) before exercise	0.21 (4.28)	-0.22 (4.72)	-0.4300 (-2.1722 to 1.3122), P= 0.6255
IL-6 (pg/mL)			
Enrollment Visit 1(Day 1)	4.20 (4.60)	4.49 (7.12)	-0.2900 (-2.5972 to 2.0172) P= 0.8036
Follow up visit 03 (15 days)	5.21 (5.57)	4.57 (5.52)	0.6400 (-1.5076 to 2.7876), P= 0.5558
End of Study visit (EOS) Visit 4 (Day 30) before exercise	4.00 (2.21)	6.12 (7.96)	-2.1200 (-4.3537 to 0.1137), P= 0.0626
Change from Enrollment Visit 1(Day 1) at Follow up visit 03 (15 days)	2.49 (6.22)	0.08 (5.09)	2.5700 (0.3629 to 4.7771), P= 0.0229
Change from Enrollment Visit 1(Day 1) at EOT Visit 4 (Day 30) before exercise	-0.28 (4.52)	1.63 (7.36)	1.9100 (-0.4395 to 4.2595), P= 0.1100
ESR (mm/hr)			
Screening Visit	16.07 (11.97)	18.59 (15.83)	-2.5200 (-7.9328 to 2.8928) P=0.3580
Follow up visit 02 (Day 4)	13.87 (12.46)	18.47 (13.72)	-4.6000 (-9.6679 to 0.4679), P= 0.0748
Follow up visit 03 (15 Days)	17.98 (16.54)	19.14 (15.94)	-1.1600 (-7.4534 to 5.1334), P= 0.7154
End of Treatment (EOT) Visit 4 (Day 30) before exercise	20.33 (18.89)	24.32 (16.89)	-3.9900 (-10.9400 to 2.9600), P= 0.2575
Change from Screening Visit at Follow up visit 02 (Day 4)	-2.20 (9.62)	-0.12 (10.78)	2.0800 (-1.8698 to 6.0298), P= 0.2987
Change from Screening Visit at Follow up visit 03 (15 Days)	1.91 (13.96)	0.55 (13.22)	-1.3600 (-6.6289 to 3.9089), P= 0.6098
Change from Screening Visit at EOT Visit 4 (Day 30) before exercise	4.26 (16.28)	5.25 (18.71)	0.9900 (-5.7876 to 7.7676), P= 0.7726
TNF-Alpha (pg/mL)			
Enrollment Visit 1(Day 1) before exercise	23.86 (18.62)	23.30 (18.96)	0.5600 (-6.7148 to 7.8348) P= 0.8790

Citation: Aurea Biolabs, Anupam Paliwal, Blake Kraemer, Dhara Shah, Ishita Basera, Akhil Mukim, Vaishal Sheth, Milan Satia. Effect of a Phytochemical formulation on Muscle fatigue, Energy, Recovery in Delayed Onset Muscle Soreness (DOMS), inflammation and Stress in Healthy Subjects: Results from a Randomized Placebo Controlled Study. Journal of Food Science and Nutrition Research 7 (2024): 131-142.

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Follow up visit 03 (15 days)	17.94 (9.56)*	18.46 (12.03)	-0.5200 (-4.7138 to 3.6738), P= 0.8062
End of Treatment visit (EOT) Visit 4 (Day 30) before exercise	20.40 (13.40)	21.79 (14.70)	-1.3900 (-6.8294 to 4.0494), P= 0.6134
Change from Enrollment Visit 1(Day 1) before exercise at Follow up visit 03 (15 days)	-3.41 (18.80)	-1.52 (13.56)	1.8900 (-4.4865 to 8.2665), P= 0.5579
Change from Enrollment Visit 1(Day 1) before exercise at EOT Visit 4 (Day 30) before exercise	-6.55 (22.40)	-5.27 (25.74)	1.2800 (-8.0447 to 10.6047), P= 0.7860

*p<0.05 vs baseline (within group comparison) otherwise not specified.

Values are expressed as Mean ± Standard deviation (SD). Abbreviation: N= number of subjects; PP=per protocol

The study results showed significant change in terms of fatigue index and rating of perceived exertion (RPE). Rephyll® treatment showed significant improvements by reducing fatigue levels after 30 days of treatment as compared to placebo (observed change in fatigue index was 56.04, while in the placebo group it was 7.16) resulting in a significant rise in EMG score, indicating lesser muscle fatigue.

Crossland BW, et al. [12] reported that the cannabidiol (CBD) supplementation was unable to reduce fatigue, in well-trained female athletes. This may be because CBD has little binding affinity for both CB1 or CB2 receptors [13] while beta-caryophyllene (BCP) is recognized as a full selective functional agonist on CB2 receptors and produces therapeutic effects by activating CB2 and the nuclear receptors, peroxisome proliferator-activated receptors (PPARs).[14] Connolly SE, et al. [15] reported that supplementation with polyphenols from tart cherry juice accelerated strength recovery, but muscle damage, inflammation, or oxidative stress was not measured. Additionally, RPE scores, a subjective measure of exercise difficulty, were also lower in the Rephyll® group after 30 days of study treatment which confirmed that the Rephyll® group had less exertion during exercise as compared to the placebo group. These findings confirm the beneficial effects of Rephyll® in improving fatigue levels, as demonstrated by within-group statistical improvements in the Rephyll® group.

In this study, there was no statistical difference seen in creatine kinase (CK) and myoglobin levels in Rephyll® group. Although, there was an improvement observed in Rephyll® group where CK level dropped from 111. 70 (day 15) to 107.28 (day 30) while in placebo group CK raised from 120.80 (day 15) to 122.69 (day 30) at the end of the treatment visit. This results were similar with literature where Amalraj A, et al. [9] reported no significant changes in CK and myoglobin levels in the Rephyll® group. There was gradual increase in myoglobin levels in the placebo group until the end of the study. Bańkowski S, et al. [16] reported curcumin supplementation did not have a significant effect on subjects' muscle damage markers such as CK, LDH and myoglobin levels in a 6 week trial on middle-aged amateur long-distance runners.

Changes in parameters for endurance energy supply and recovery, showed that RER and increased energy supply were statistically significant which indicated better aerobic function, as CO_2 was cleared and O_2 was consumed, more efficiently. Szymanski MC, et al. [17] reported there were no significant change in RER over the 60 min exercise in Placebo and in Curcumin group. The data from VO_{2max} suggests that the Rephyll® group required less oxygen to perform eccentric exercise as compared to their oxygen consumption levels at the beginning of the study. Bankowski S, et al. [16] reported VO_{2max} did not change significantly in the curcumin supplemented group over 6 weeks of supplementation with curcumin. The rise in plasma ATP level after 30 days of treatment suggested the effect of Rephyll® in terms of availability of ATP in plasma which is source of energy. Bielawiec P, et al. [18] reported CBD enhanced the level of glutathione (GSH), adenosine triphosphate (ATP), and nicotinamide adenine dinucleotide (NAD), which supported the assumption that CBD increases intracellular lipolysis and mitochondrial activity. Moreover, the concentration of lactic acid remained low and less O_2 was consumed after exercise after 30 days of treatment with Rephyll® and no such change was observed in placebo group. This showed that the point of exhaustion was achieved late in Rephyll® group as compared to that for placebo group. This indicated that Rephyll® can help in clearance of lactic acid during exercise, which can aid in endurance-boosting effect.

The main symptom related to DOMS and for which scientific researches are converging is pain, which is known to limit both recovery and subsequent athletic performances. The origin of pain linked with DOMS is hypothesized to be induced by the release of prostaglandin E2 (PGE-2), which sensitizes types III and IV afferent nerve fibers through nociceptors. Furthermore, inflammatory responses to eccentric exercise would contribute to the sensation of pain [15]. Amalraj A, et al. [9] reported significantly decreased VAS score after day 2 in the Rephyll® group leading to conclusion that Rephyll® can effectively alleviate DOMS induced by maximal voluntary contraction exercise. The present study observed statistically significant improvements in pain scores between Rephyll® and placebo on days 4, 15 and

30. Moreover, within-group analysis revealed a statistically significant reduction in pain score among subjects in the Rephyll® group, whereas such reductions were not observed in the placebo group. Specifically, the pain VAS score in the Rephyll®-treated subjects decreased from an initial score of 4.09 to 2.44 by the end of the study. In contrast, the placebo group showed a minimal difference in score, from 4.00 to 3.80, which was not statistically significant. This might be due to the cannabimimetic anti-inflammatory activity of Rephyll® which can be achieved by better reabsorption of interstitial fluid and cells into the bloodstream, leading to a reduction in edema; reducing the development of prostaglandins and involving other eicosanoids in the inflammatory response to damage. These mechanisms of decreased inflammation could further reduce the pain.

The majority of the prior studies confirmed the capacity of nutritional supplements to improve severe symptoms associated with DOMS due to their anti-inflammatory properties [9]. In this study, there was a more pronounced reduction in inflammatory markers, including cortisol, CRP, IL-6, ESR and TNF-α after 30 days in subjects consuming Rephyll® as compared to the placebo group. McFarlin BK, et al. [19] reported curcumin supplementation resulted in significantly smaller increases in TNF-α (−25%) following EIMD compared to placebo. However, no significant differences in IL-6 was observed. Rephyll® treatment provided positive responses in terms of neuromuscular activity following 30 days of treatment.

No difference in safety parameters was observed between the Rephyll® and placebo groups. Also, none of the subjects reported any adverse event throughout and after the completion of study treatment and none of them reported having taken any concomitant medication.

Thus, this study observed that Rephyll® had the potential to reduce DOMS, muscle fatigue, improvement in endurance energy supply and recovery, and neuromuscular activation, without any adverse effects in healthy, untrained subjects. Rephyll® can be recommended for muscle strengthening, energy endurance, and recovery from inflammation due to DOMS.

Conclusion

In summary, we conclude that Rephyll® containing β-Caryophyllene was able to reduce muscle fatigue, improve endurance and energy supply, and demonstrated potential for reducing DOMS in healthy individuals, after 30 days of treatment as compared to placebo. It should also be taken into account that Rephyll® significantly reduced the VAS score for pain and can be an alternative supplement for pain management related to DOMS. Rephyll® was found to be safe and well tolerated by all study subjects.

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We would like to thank Ethics committee for approval of this study.

Conflicts of interest

There are no conflicts of interest to declare.

References

1. Torre MF, Martinez-Ferran M, Vallecillo N, et al. Supplementation with Vitamins C and E and Exercise-Induced Delayed-Onset Muscle Soreness: A Systematic Review. Antioxidants (Basel) (2021): 279
2. Rattanaseth N, Panyarapeepat P, Muljadi JA, et al. Effect of curcumin supplement or placebo in delayed onset muscle soreness: a systematic review and meta-analysis. Bulletin of the National Research Centre (2021): 1-6.
3. Liu T, Zhang L, Joo D, et al. NF-κB signaling in inflammation. Signal Transduction Targeted Therapy (2017): 17023
4. Dos Santos RS, Veras FP, Ferreira DW, et al. Involvement of the Hsp70/TLR4/IL-6 and TNF-α pathways in delayed-onset muscle soreness. Journal of Neurochemistry (2020): 29-44.
5. Burnett D, Smith K, Smeltzer C, et al. Perceived Muscle Soreness in Recreational Female Runners. International Journal Exercise Science (2010): 108-116
6. Kim J, Lee J. A review of nutritional intervention on delayed onset muscle soreness. Part I. Journal Exercise Rehabilitation (2014): 349-356
7. Cheung K, Hume PA, Maxwell L. Delayed onset muscle soreness: Treatment Strategies and Performance Factors. Sports medicine (2003): 145-164.
8. Rynders CA, Weltman JY, Rynders SD, et al. Effect of an herbal/botanical supplement on recovery from delayed onset muscle soreness: a randomized placebo-controlled trial. Journal of the International Society of Sports Nutrition (2014): 1-9
9. Amalraj A, Jacob J, Varma K, et al. Preparation and Characterization of Liposomal β-Caryophyllene (Rephyll)

by Nanofiber Weaving Technology and Its Effects on Delayed Onset Muscle Soreness (DOMS) in Humans: A Randomized, Double-Blinded, Crossover-Designed, and Placebo-Controlled Study. ACS Omega (2020): 24045-24056.

10. Hartsel JA, Eades J, Hickory B, et al. Cannabis sativa and Hemp. InNutraceuticals (2016): 735-754.
11. Scandiffio R, Geddo F, Cottone E, et al. Protective effects of (E)-β-caryophyllene (BCP) in chronic inflammation. Nutrients (2020): 3273.
12. Crossland BW, Rigby BR, Duplanty AA, et al. Acute Supplementation with Cannabidiol Does Not Attenuate Inflammation or Improve Measures of Performance following Strenuous Exercise. Healthcare (Basel). (2022): 1133
13. Vučković S, Srebro D, Vujović KS, et al. Cannabinoids and Pain: New Insights From Old Molecules. Frontiers in Pharmacology Volume 9 (2018): 1259
14. Jha NK, Sharma C, Hashiesh HM, et al. A Natural Dietary CB2 Receptor Selective Cannabinoid can be a Candidate to Target the Trinity of Infection, Immunity, and Inflammation in COVID-19. Frontier in Pharmacology (2021): 590201
15. Romain C, Freitas TT, Martínez-Noguera FJ, et al. Supplementation with a Polyphenol-Rich Extract, TensLess®, Attenuates Delayed Onset Muscle Soreness and Improves Muscle Recovery from Damages After Eccentric Exercise. Phytotherapy Research (2017): 1739-1746.
16. Bańkowski S, Petr M, Rozpara M, et al. Effect of 6-week curcumin supplementation on aerobic capacity, antioxidant status and sirtuin 3 level in middle-aged amateur long-distance runners. Redox Report (2022): 186-192.
17. Szymanski MC, Gillum TL, Gould LM, et al. Short-term dietary curcumin supplementation reduces gastrointestinal barrier damage and physiological strain responses during exertional heat stress. Journal of Applied Physiology (2018): 330-340.
18. Bielawiec P, Harasim-Symbor E, Chabowski A. Phytocannabinoids: Useful Drugs for the Treatment of Obesity? Special Focus on Cannabidiol. Frontier in Endocrinology (2020): 114
19. McFarlin BK, Venable AS, Henning AL, et al. Reduced inflammatory and muscle damage biomarkers following oral supplementation with bioavailable curcumin. BBA Clinical (2016): 72-78.