Research Article

iMedPub Journals
www.imedpub.com

Journal Nutraceuticals and Food Science

2021
Vol.6 No.8:34

Beneficial Effect of Gremin, a Proprietary Dual Antioxidant Formulation on Skeletal Muscle Injury - *In vivo*

Shankaranarayanan Jeyakodi[1*], Arunkanth Krishnakumar[1], Dinesh Kumar Chellappan[2], Rajesh Subbanna[3], Sachin Bansal[3]

1 ZeusHygia Life Sciences Pvt. Ltd., Hyderabad, India
2 Department of Life Sciences, School of Pharmacy, International Medical University, Kuala Lumpur, Malaysia
3 Palamur Biosciences Pvt. Ltd., Telangana, India

***Corresponding author:**
Shankaranarayanan Jeyakodi

shankar@zeushygia.com

ZeusHygia Life Sciences Pvt. Ltd., Hyderabad, India

Citation: Jeyakodi S, Krishnakumar A, Chellappan DK, Subbanna R, Bansal S (2021) Beneficial Effect of Gremin, a Proprietary Dual Antioxidant Formulation on Skeletal Muscle Injury - *In vivo.* J Nutraceuticals Food Sci Vol.6 No.8:34

Abstract

Background: Tissue repair capabilities in mammals are relatively limited, and there is a growing interest to discover newer therapeutic interventions for the muscle regeneration process. While Curcumin and Green Coffee extract has previously shown to have beneficial effect individually, there is no study to examine the synergistic effects of these compounds. We, therefore, attempted to study the effect of Gremin, a proprietary encapsulated ingredient formulation in a mouse model with $BaCl_2$ induced muscle injury.

Methods and Findings: Adult male Swiss Albino mice were divided into four groups (n=6 per group) as vehicle control, $BaCl_2$ control, Gremin and reference compound group. Animals were administered with $BaCl_2$ injection to induce muscle injury, except for vehicle control. Gremin and the reference compound was administered at a dose level of 200 mg/kg/b.w daily, for up to 17 days via the oral route. Biochemical markers viz., TNF-α, Creatine Kinase (CK), Lactate Dehydrogenase (LDH), and histopathology for necrosis, were assessed.

Results: Histopathology analysis of the mouse muscle tissues demonstrated significant protective effects of Gremin on muscle injury. Gremin group exhibited reduced levels of LDH and CK enzyme biomarkers indicating muscle recovery.

Conclusion: Gremin Supplementation has beneficial effects on muscle injury and could be of immense value during post-injury rehabilitation.

Keywords: Gremin; Muscle injury; Biochemical markers; Histopathology

Received: September 04, 2021; **Accepted:** September 27, 2021; **Published:** September 30, 2021

Introduction

Muscle repair and regeneration is closely linked processes that relies on the precise and timely sequencing of the pro-inflammatory and anti-inflammatory signals that occur post-muscle injury [1,2]. Following injury, resident stem (satellite) cells (SCs), re-enter the cell cycle and generate myoblasts that will participate in myofiber reconstitution or repair [3]. There are two types of skeletal muscle injury, one caused by direct destruction of muscle tissue and the other one by a contractile overload [4]. The healing phases for an injured muscle include inflammation, degeneration, regeneration and remodelling, which are considered to be common among the injury types, even though the initiating mechanism of damage most probably differs depending on the type of injury [5-7]. There is also considerable crosstalk between fibro-adipogenic, endothelial, and myogenic cells to coordinate connective tissue formation, angiogenesis, remodelling and myogenesis for the efficient reconstitution of functional muscle [8,9]. Studies have reported that the tissue repair capabilities in mammals are relatively limited, and hence there is a growing interest to discover newer therapeutic interventions for the skeletal muscle regeneration process [10]. Presently, the available treatment options for muscle injuries are limited and lead to significant muscle wasting as well as delay in muscle recovery.

Recent evidence suggests that various herbal extracts including green coffee bean extract from Coffee Robusta and curcumin from *Curcuma longa* have potent health benefits [11-13]. Green coffee Robusta beans contain a high amount of Chlorogenic Acids (CGA) which have been reported for their potent antioxidant,

anti-diabetic, anti-obesity, anti-inflammatory and antimicrobial properties [13-17]. However, most of the CGA content is lost when coffee beans are heated at very high temperatures during the process of making coffee. Another interesting herbal molecule, curcumin, a natural dietary polyphenol, has been shown to have beneficial effects on skeletal muscle injury and also possesses numerous pharmacological properties, including anti-inflammatory, immunomodulatory, antioxidant, and antiarthritic effects [18-21]. Oral supplementation of curcumin suppresses oxidative stress by decreasing the hydrogen peroxide levels in mouse skeletal muscles after downhill running [22]. Additionally, curcumin supplementation reduces serum CK activity, a marker for skeletal muscle damage [23]. Thus, curcumin has been established to have beneficial effects on skeletal muscle injury. As there is a growing interest in exploring the potential benefits of natural products as therapeutics for muscle injury, a combination of curcumin and green Coffee Robusta may therefore be a good treatment option.

We developed a proprietary ingredient formulation called Gremin, a combination of green coffee extract from Coffee Robusta and curcumin from *Curcuma longa* to treat muscle injury. We used a barium chloride ($BaCl_2$) induced muscle injury model, which has been largely considered as a locally specific method for the study of muscle regeneration [24]. The main advantages of chemical injury with $BaCl_2$ include both ease of use and its ability to reproducibly damage myofibers while preserving their associated satellite cells [25-26]. While curcumin and Green Coffee have been shown to have beneficial effects individually, there are no studies yet to examine the synergistic effects of these compounds at an optimized ratio. We, therefore, attempted to study the effect of Gremin, in a mouse model of $BaCl_2$-induced muscle injury.

Methods

Animal experiments

Adult male Swiss Albino mice (aged 6 weeks-8 weeks) were conveniently housed in polypropylene cages in an animal room maintained with a 12-hrs light/dark cycle at 22°C ± 2°C and 55% ± 5% humidity. Animals were allowed free access to water and pelleted rodent feed. The feed and water were routinely analyzed and were considered not to contain any contaminants that we expect could reasonably affect the purpose or integrity of the study. The animals used in the present study were maintained according to the principles and guidelines of the Committee for Control and Supervision of Experiments on Animals (CPCSEA). All the protocols and procedures were approved by the Institutional Animal Ethics Committee (IAEC) (Project approval number: PAL/IAEC/2020/5/01/05).

Animals were grouped based on stratified randomization by using body weights (G1-G4). Randomization was performed one day before the initiation of treatment. Additional animals were taken for randomization procedure. Animals at randomization were ensured to be within ± 20% of the mean body weight for each group. The study groups, detailed study plan and treatment schedule are presented in **Table 1.**

Induction of muscle injury

To induce the muscle injury, 50 µL of a 1.2% (w/v) $BaCl_2$ solution dissolved in Phosphate Buffered Saline (PBS) was injected to the right Tibialis Anterior (TA) muscles of the anaesthetized mice. Left TA muscle served as a contralateral control. The $BaCl_2$ injection was administered to all animals on Day 7 of the treatment.

Test and reference compound treatment phase

The test agent, Gremin, was formulated using a platform technology where in Chlorogenic acid (Green coffee bean extract) was infused with Curcuminoids (*Curcuma longa* extract) under optimal processing conditions. The reference compound was a physical blend of green coffee bean extract and curcumin extract. Both test and reference agents were given once daily for 17 days by oral administration. Dose formulations of test compound Gremin was administered to the G3 group and the reference compound was administered to G4 at a dose level of 200 mg/kg/b.w daily, up to 17 days by oral route and the dose-volume was maintained at 10 mL/kg bodyweight for 17 days. Group G2 was only administered with barium chloride ($BaCl_2$). The vehicle, distilled water was administered to the vehicle control (G1) group at an equivolume of 10 mL/kg body weight, for up to 17 days.

The animals were observed for clinical signs (daily cage side observation); body weights and feed consumption; during the entire treatment period. All animals were observed for general clinical signs, morbidity/viability, twice daily throughout the observation period. Body weights of the animals were recorded on the day of receipt, on the day of randomization, on the day of dosing (day 1) and thereafter on days 4, 7, 10, 13, 16 for all groups. Blood samples for clinical biochemistry were collected from all animals from groups G1, G2 G3 and G4 on day 18. Blood

Table 1: Experimental study plan and treatment schedule

	Group	Dose (mg/kg b.w)	Dose frequency	Barium Chloride ($BaCl_2$)-induction	No of animals/ group
Grouping & Dose	G1 (Vehicle control)	0.0	Daily for 17 Days (Distilled Water)	-	6
	G2 (Bacl2 control)	0.0	Daily for 17 Days (Distilled Water)	Induction of muscle injury on 7th day	6
	G3 (Gremin treatment)	200	Daily for 17 Days (Gremin)	Induction of muscle injury on 7th day	6
	G4 (Reference compound treatment)	200	Daily for 17 Days (Reference)	Induction of muscle injury on 7th day	6

samples were drawn from the retro-orbital plexus using a non-heparinized glass capillary tube. After centrifugation, the serum was separated in the pre-labelled vials and stored at -80°C for further analysis.

Histopathological studies

Animals were sacrificed humanely by cervical dislocation on day 18 of the study after blood collection. Right, and Left TA muscle samples were collected and fixed with 10% buffered neutral formalin solution. Post-fixation, tissue samples were paraffin-embedded, 5-micron sections prepared and stained with Hematoxylin and Eosin (H&E).

Quantitative analysis of TNF-alpha, CK and LDH

TNF Alpha was measured by quantitative sandwich Enzyme-Linked Immunosorbent Assay (ELISA) (Krishgen Bio, USA) according to the manufacturer's instructions. The values are expressed in pg/mL. CK and LDH were measured by a Beckman Coulter AU analyzer and the values were expressed as U/L. The intra- and inter-assay coefficients of variation were <5% and <10% respectively.

Statistical analysis

Differences were evaluated by one-way Analysis of Variance (ANOVA) using GraphPad Prism software, Version 5 (GraphPad Software Inc., CA, USA) and a p-value<0.05 was considered as statistically significant.

Results

The test and reference compounds were orally administered for 7 days after which $BaCl_2$ injection was injected into the right tibialis anterior muscle of the animals to induce muscle injury. Treatment with the test compound, Gremin and the reference compound were continued for another 10 days after which the animals were euthanized, and tissue samples were collected and processed for further analysis. We assessed whether $BaCl_2$ and Gremin treatment affected the body weight and found that these treatments did not induce any difference in body weights between the groups (Supplementary Data). Further 50 µl injection of 1.2% $BaCl_2$ onto the right tibialis anterior muscle did not result in any mortality (Data not shown).

Further, to assess the effects of the test compound on $BaCl_2$ induced injury, we carried out a histopathological analysis on H&E-stained tissue sections. We found typical necrotic lesions in the $BaCl_2$ treated groups and found no such necrotic changes either in Gremin or reference compound treated animals **(Figure 1).**

Localized muscle injury could result in inflammation which has been shown to increase TNF-alpha levels [27]. To assess whether the test compound has an effect in reducing inflammation, we measured the TNF-alpha levels in the serum after 10 days of $BaCl_2$ induced muscle injury. $BaCl_2$ treatment did not increase TNF-alpha levels in all study groups **(Figure 2)**. TNF-alpha levels were comparable to that of the vehicle in the Gremin and reference treated mice.

Creatine kinase, a well-established marker of muscle injury was assessed in the blood samples of mice from the control and treatment groups [28]. It was found that $BaCl_2$ injection resulted in creatine kinase level-increase by 2.9-folds ($p<0.01$) in comparison to vehicle. Treatment of mice with the test compound, Gremin showed a significant 3-fold reduction when compared with the $BaCl_2$-Control group ($p<0.01$). However, there was only a moderate 1.6-fold reduction in creatine kinase levels in the reference group ($p<0.01$) **(Figure 3).**

Similarly, $BaCl_2$ injection resulted in increased levels of LDH by 1.8-fold (p value <0.01) when compared to vehicle control animals. Treatment of Gremin reduced the LDH levels by 1.5-fold ($P<0.01$) when compared to $BaCl_2$-control treatment while the

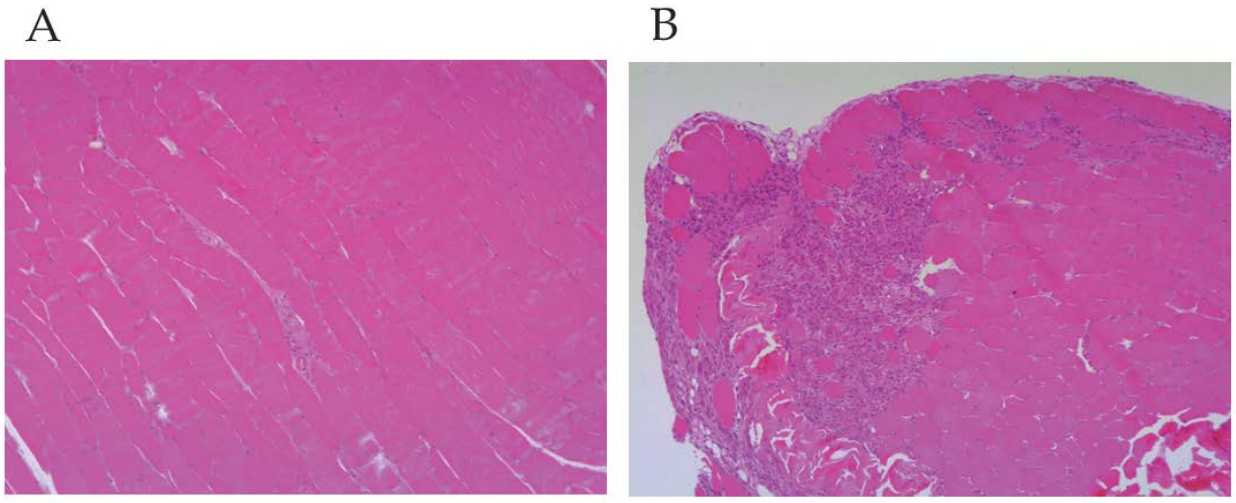

Figure 1 Representative H & E staining images of the tibialis anterior tissue sections of the leg; A): $BaCl_2$ induced necrosis; B): Protection of $BaCl_2$ induced muscle injury by Gremin.

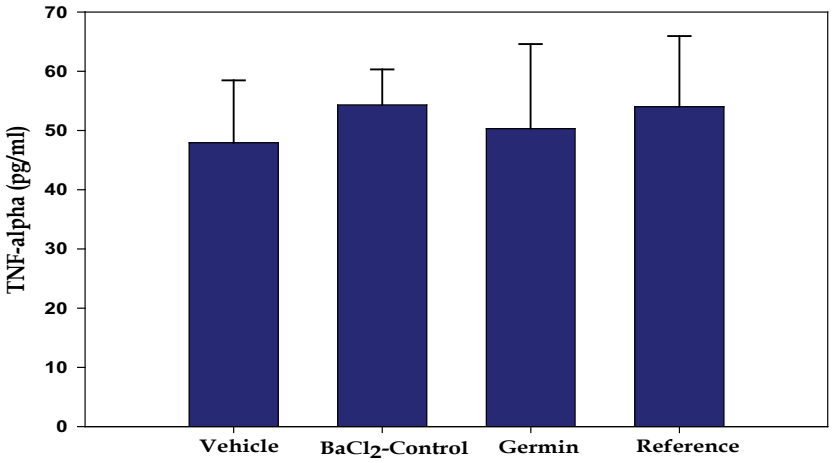

Figure 2 Measurement of TNF-alpha levels at the study endpoint, Data represents Mean ± SEM, n=6 in each group.

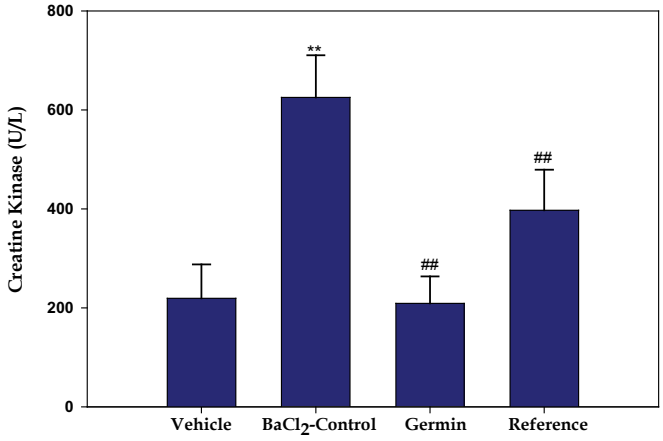

Figure 3 Measurement of creatin kinase enzyme activity at the study end point. Data are expressed as Mean ± SEM. ** p value<0.01 when compared with vehicle group. ## p value<0.01 when compared with $BaCl_2$ control group.

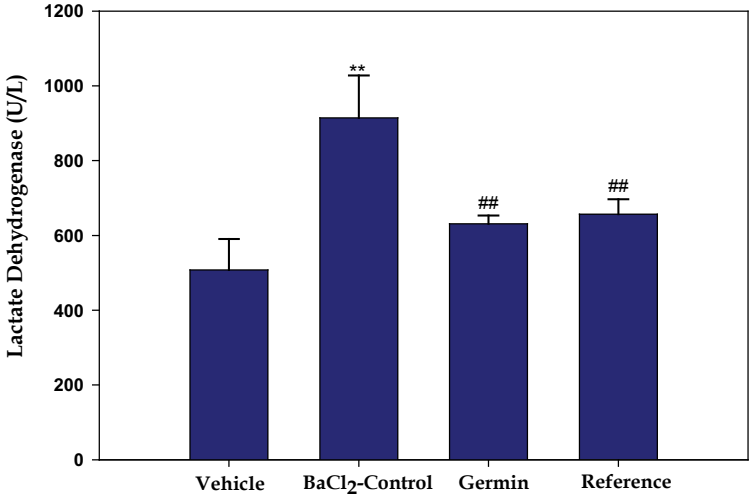

Figure 4 Measurement of lactate dehydrogenase enzyme activity at the study endpoint. Data are expressed as Mean ± SEM. **p value<0.01 when compared with the vehicle group. ## p value<0.01 when compared with $BaCl_2$ control group.

effect of the reference compound was similar to that of the test compound resulting in a reduction of LDH levels by a factor of 1.4-fold (p value<0.01) **(Figure 4).**

Discussion

Skeletal muscle injury and repair are ongoing processes. This homeostatic equilibrium is altered due to defective tissue regeneration capacity in many pathological states involving various organs such as the heart, lung, and tendons [29-31]. Tissue regeneration involves multiple pathways including antioxidant generation, inflammation, and recruitment of stem cells [32]. Ageing is associated with skeletal muscle loss or muscle wasting that could potentially involve one or multiple pathways. Skeletal muscle alterations are known to occur in several disease states such as COPD [30], and peripheral arterial disease [33]. Mostly, tissue injury occurs because of contractile overload occurring during physical workouts and sports. Drugs that can aid in tissue regeneration will be of immense addition to the existing treatment arsenal of anti-inflammatory drugs.

Curcuma longa and Coffee extract have been shown earlier to have enormous impact as natural antioxidants. In this report, we have demonstrated that a novel therapeutic "Gremin", a proprietary infusion of Curcumin and Chlorogenic acid (Green Coffee Robusta) is better in the resolution of skeletal muscle injury as compared to the simple physical mixture of curcumin extract and green coffee bean extract. We induced skeletal muscle injury by standard $BaCl_2$ model. The necrosis of the skeletal muscle induced by $BaCl_2$ was completely resolved when the mice were treated with Gremin. Further, enzymes that were released during muscle injury such as, LDH and creatine kinase were significantly lowered in Gremin treated animals compared to the reference compound animals, which is indicative of the greater performance of this formulation in resolving skeletal muscle injury.

Curcumin has been shown to increase antioxidant activity by elevating Superoxide Dismutase (SOD), glutathione peroxidase and catalase in a parallel decrease of pro-oxidant molecules such as malondialdehyde, inflammatory molecules-IL-6 and TNF-alpha [34]. In our study, $BaCl_2$ injury did not induce inflammation as observed TNF-alpha levels were similar to the vehicle group. Consequently, there was no effect on TNF-alpha levels by Gremin as well as the reference compound. Previous studies have shown that curcumin ameliorates myocardial infarction in a model induced by β-adrenoreceptor antagonists isoproterenol hydrochloride (ISO) [35]. Similar to our study, curcumin treatment decreased the levels of skeletal muscle markers namely LDH and creatine kinase induced by ISO insult on myocardial tissue. The green coffee extract has several beneficial effects and has been shown to reduce inflammation evidenced by a decrease in CRP levels [36]. Chlorogenic acids (CGA), a key ingredient of coffee extract has been earlier demonstrated for bioavailability and CGA has been shown to down regulate several inflammatory genes [37]. The cytoprotective effects observed in this study is perhaps due to the synergistic effects of both curcumin and green tea extract.

One of the limitations of curcumin treatment is the limited bioavailability of oral administration. Pharmacokinetic studies on curcumin have revealed the oral bioavailability of curcumin was 0.06 µg/ml [38]. This level of curcumin is inadequate to achieve desirable effects *in vivo*. Hence, numerous attempts are being made to obtain a formulation that increases the oral bioavailability of curcumin [39,40]. Our formulation has shown significant biological activity on oral administration in a mouse model of muscle injury. Further, the platform technology-driven infusion process of curcumin with green coffee bean possibly plays a synergistic role in the efficient resolution of muscle injury. These effects could be replicated for various other diseases in which Gremin has shown positive biological effects including cancer, heart disease and inflammation. Another possible limitation of this study is the lack of data on pharmacokinetics and pharmacodynamics. However, the demonstration of biological activity of this novel Gremin formulation assures a positive development in this area of natural medicine.

Conclusion

Currently, there are limited treatment options for muscle injury aside from symptomatic treatment. In this study, we have demonstrated that a supplement of Gremin is highly beneficial for muscle injury treatment and could be of immense value for post-injury rehabilitation.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Credit Authorship Contribution Statement

Shankaranarayanan Jeyakodi: Conceptualization, Methodology, Data curation, Writing (original draft, review & editing). Arunkanth Krishnakumar: Data Curation, Validation. Dinesh Kumar Chellappan: Data Curation, Validation, Writing (Original Draft). Rajesh Subbanna: Data Curation, Validation, Writing (Original Draft). Sachin Bansal: Conceptualization, Methodology, Data curation, Writing (original draft, review & editing).

Declaration of Competing Interest

The authors report no declarations of interest.

References

1. Aurora AB, Olson EN (2014) Immune modulation of stem cells and regeneration. Cell Stem Cell 15, 14-25.
2. Perdiguero E, Sousa Victor P, Ruiz Bonilla V, Jardí M, Caelles C, et al. (2011) p38/MKP-1-regulated AKT coordinates macrophage transitions and resolution of inflammation during tissue repair. Journal of Cell Biology 195: 307-322.
3. Snijders T, Nederveen JP, McKay BR, Joanisse S, Verdijk LB, et al. (2015) Satellite cells in human skeletal muscle plasticity. Front Physiol 6:283.

4. Warren GL, Summan M, Gao X, Chapman R, Hulderman T, et al. (2007) Mechanisms of skeletal muscle injury and repair revealed by gene expression studies in mouse models. J Physiol 582: 825-841.

5. Huard J, Li Y, Fu FH (2002) Muscle injuries and repair: current trends in research. J Bone Joint Surg Am 84: 822-883.

6. Jarvinen TAH, Jarvinen TLN, Kaariainen M, Kalimo H, Jarvinen M (2005) Muscle injuries: biology and treatment. Am J Sports Med 33: 745-764.

7. Warren GL, Ingalls CP, Lowe DA, Armstrong RB (2001) Excitation contraction uncoupling: major role in contraction-induced muscle injury. Exerc Sport Sci Rev 29: 82-87.

8. Murphy MM, Lawson JA, Mathew SJ, Hutcheson DA, Kardon G (2011) Satellite cells, connective tissue fibroblasts and their interactions are crucial for muscle regeneration. Development 138: 3625-3637.

9. Arnold L, Henry A, Poron F, Baba-Amer Y, van Rooijen N, et al. (2004) Inflammatory monocytes recruited after skeletal muscle injury switch into antiinflammatory macrophages to support myogenesis. J Exp Med 204: 1057-1069.

10. Krafts KP (2010) Tissue repair: The hidden drama Organogenesis 6: 225-233.

11. Ms SA, Waldman HS, Krings BM, Lamberth J, Smith JW, et al. (2020) Effect of curcumin supplementation on exercise-induced oxidative stress, inflammation, muscle damage, and muscle soreness. Journal of dietary supplements 17: 401-414.

12. Avci G, Kadioglu H, Sehirli AO, Bozkurt S, Guclu O et al. (2012) Curcumin protects against ischemia/reperfusion injury in rat skeletal muscle. J Surg Res 172: e39-e46.

13. Tharwat AGE (2021) Health Benefits of Green Coffee Beans. Int J Dent Ora Hea 7: 1.

14. Ríos Hoyo, Gutiérrez Salmeán A G (2016) New dietary supplements for obesity: What we currently know. Curr Obes Rep 5: 262-270.

15. Chaves Ulate E, Esquivel Rodríguez P (2019) Chlorogenic acids present in coffee: antioxidant and antimicrobial capacity. Agron Mesoam 30: 299-311.

16. Shin HS, Satsu H, Bae MJ, et al. (2015) Anti-inflammatory effect of chlorogenic acid on the IL-8 production in Caco-2 cells and the dextran sulphate sodium-induced colitis symptoms in C57BL/6 mice. Food Chem 168: 167-175.

17. Sarriá B, Martínez López S, Mateos R, Bravo Clemente L (2016) Long-term consumption of a green/roasted coffee blend positively affects glucose metabolism and insulin resistance in humans. Food Res Int 89: 1023-1028.

18. Sahebkar A (2014) Are curcuminoids effective C-reactive protein-lowering agents in clinical practice? Evidence from a meta-analysis. Phytother Res 28: 633-642.

19. Srivastava RM, Singh S, Dubey SK, Misra K, Khar A (2011) Immunomodulatory and therapeutic activity of curcumin. Int Immunopharmacol11: 331-341.

20. Panahi Y, Ghanei M, Hajhashemi A, Sahebkar A (2016) Effects of Curcuminoids-Piperine combination on systemic oxidative stress, clinical symptoms and quality of life in subjects with chronic pulmonary complications due to sulfur mustard: a randomized controlled trial. J Diet Suppl 13: 93-105.

21. Panahi Y, Rahimnia AR, Sharafi M, Alishiri G, Saburi A et al. Curcuminoid treatment for knee osteoarthritis: A randomized double-blind placebo-controlled trial. Phytother Res 28: 1625-1631.

22. Kawanishi N, Kato K, Takahashi M, Mizokami T, Otsuka Y et al. (2013) Curcumin attenuates oxidative stress following downhill running-induced muscle damage. Biochem Biophys Res Commun. 44: 573-578.

23. McFarlin BK, Venable AS, Henning AL, Sampson JN, Pennel K, et al. (2016) Reduced inflammatory and muscle damage biomarkers following oral supplementation with bioavailable curcumin. BBA Clin 5: 72-78.

24. Jung HW, Choi JH, Jo T, Shin H, Suh JM (2019) Systemic and Local Phenotypes of Barium Chloride Induced Skeletal Muscle Injury in Mice. Ann Geriatr Med Res 23: 83-89.

25. Tierney MT, Sacco A (2016) Inducing and evaluating skeletal muscle injury by notexin and barium chloride. Methods Mol Biol 1460: 53-60.

26. Hardy D, Besnard A, Latil M, Jouvion G, Briand D et al. (2016) Comparative study of injury models for studying muscle regeneration in mice. PLoS One 11: e0147198.

27. Collins RA, Grounds MD (2001) The role of tumor necrosis factor-alpha (TNF-alpha) in skeletal muscle regeneration. Studies in TNF-alpha(-/-) and TNF-alpha(-/-)/LT-alpha(-/-) mice. J Histochem Cytochem 49: 989-1001.

28. Brancaccio P, Lippi G, Maffulli N (2010) Biochemical markers of muscular damage. Clin Chem Lab Med 48: 757-767.

29. Anker SD, Ponikowski P, Varney S, Chua TP, Clark AL et al. (1997) Wasting as independent risk factor for mortality in chronic heart failure. Lancet 349: 1050-1053.

30. Gea J, Agustí A, Roca J (2013) Pathophysiology of muscle dysfunction in COPD. J Appl Physiol. 11: 1222-1234.

31. Liu W, Zhai Y, Heng X, Che FY, Chen W et al. (2016) Oral bioavailability of curcumin: problems and advancements. J Drug Target 24: 694-702.

32. Blau HM, Cosgrove BD, Ho AT (2015) The central role of muscle stem cells in regenerative failure with aging. Nat Med 21: 854-862.

33. McDermott MM, Ferrucci L, Gonzalez Freire M, Kosmac K, Leeuwenburgh C et al. (2020) Skeletal Muscle Pathology in Peripheral Artery Disease: A Brief Review. Arterioscler Thromb Vasc Biol 40: 2577-2585.

34. Zia A, Farkhondeh T, Pourbagher Shahri AM, Samarghandian S (2021) The role of curcumin in aging and senescence: Molecular mechanisms. Biomed Pharmacother 134: 111119.

35. Rahnavard M, Hassanpour M, Ahmadi M, Heidarzadeh M, Amini H et al. (2019) Curcumin ameliorated myocardial infarction by inhibition of cardiotoxicity in the rat model. J Cell Biochem 120: 11965-11972.

36. Chen H, Huang W, Huang X, Liang S, Gecceh E et al. Effects of green coffee bean extract on C-reactive protein levels: A systematic review and meta-analysis of randomized controlled trials. Complement Ther Med 52: 102498.

37. Morton K, Knight K, Kalman D, Hewlings S (2018) A Prospective Randomized, Double-Blind, Two-Period Crossover Pharmacokinetic Trial Comparing Green Coffee Bean Extract-A Botanically Sourced Caffeine-With a Synthetic USP Control. Clin Pharmacol Drug Dev 7: 871-879.

38. Liu W, Zhai Y, Heng X, Che FY, Chen W et al. (2016) Oral bioavailability of curcumin: problems and advancements. J Drug Target 24: 694-702.

39. Maiti K, Mukherjee K, Gantait A, Saha BP, Mukherjee PK (2007) Curcumin–phospholipid complex: Preparation, therapeutic evaluation and pharmacokinetic study in rats. Int J Pharm 330: 155-163.

40. Gupta NK, Dixit VK (2011) Bioavailability enhancement of curcumin by complexation with phosphatidyl choline. J Pharm Sci 100: 1987-1995.