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Biotechnological Exploration of Plant- and Animal-Derived Bioactive Compounds for Therapeutic and Rehabilitation Applications

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ABSTRACT

Musculoskeletal injuries have continued to be a leading cause of long-term disability, which requires novel bioactive-based treatment options to increase tissue healing and functional outcome. This is an experimental study examining the regenerative and anti-inflammatory effects curcumin (plant-based polyphenol of *Curcuma longa*) and marine collagen peptides (animal-based bioactive proteins) on a rat skeletal muscle injury model. These two compounds were purified and biotechnologically extracted, and then the physicochemical characterization of the compounds was performed through HPLC and spectroscopy. The treatment effects were assessed during histological muscle fiber (or collagen deposition), the examination of inflammatory cytokines (TNF- α , IL-6), and the expression of myogenic markers (MyoD, Myogenin).

The pro-inflammatory level of cytokines and NF- κ B activation were greatly inhibited by the administration of curcumin, which reflects good modulation of anti-inflammatory activities. Marine collagen peptides increased extra cellular matrix remodeling effects and a faster rate of myofibrillar regeneration by increasing collagen synthesis and promoting expression of myogenic regulatory factors. It was interesting to note that combined treatment had a synergistic effect on muscle fiber cross-sectional area and functional recovery measures.

INTRODUCTION:

Musculoskeletal trauma and degenerative disorders form a significant global health burden and they lead to long-term disability, impaired functional capacity, as well as, diminished quality of life. Injuries in skeletal muscles, be it trauma, sports-related, metabolic or aging, may result in the inability to fully regenerate and to remain inflamed in case of improper management (Tidball, 2005). The skeletal muscle has intrinsic regenerative ability through a process that is regulated by satellite cells, but the repair process is very reliant on tightly controlled signals of inflammation, extracellular matrix remodeling and myogenic differentiation pathways (Charge & Rudnicki, 2004). The impaired regulation of these mechanisms may slow down the process of regeneration and favor fibrosis, which eventually leads to the functional recovery impairment.

A very important early phase of muscle repair is inflammation, but secondary tissue damage is caused by long-term or excessive inflammatory reactions. Immediately after muscle injury, the pro-inflammatory cytokine tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) are upregulated and they mediate the activation and differentiation of satellite cells (Tidball & Villalta, 2010). This inflammatory cascade is centrally regulated by the transcription factor nuclear factor kappa-B (NF- κ B) which has been linked to reduced muscle regeneration when chronically activated (Li & Verma, 2002). As a result, anti-inflammatory modulation based therapeutic approaches that do not inhibit regenerative signaling are becoming promising in rehabilitation and regenerative medicine. Musculoskeletal injuries are normally treated using conventional pharmacological treatments, which include non-steroidal anti-inflammatory drugs (NSAIDs). Nonetheless, it has been indicated that muscle regeneration can be postponed by excessive inhibition of inflammatory signaling that disrupts the growth and differentiation of satellite cells (Mackey et al., 2007). The interest in bioactive compounds obtained naturally, especially those ones having a specific anti-inflammatory and regenerative effect with minimal damage to physiological repair pathways has increased as a result of these limitations.

Plant-based bioactive compounds are known to be studied in long time concerning their pharmacological effects. Curcumin, a polyphenolic compound of *Curcuma longa* is among them and it has received significant attention because of its strong anti-inflammatory, antioxidant and anti fibrotic properties (Aggarwal and Hari Kumar, 2009). In various experimental models, curcumin has been demonstrated to prevent the activation of NF- κ B, block pro-inflammatory cytokine synthesis as well as reduce oxidative stress (Jurenka, 2009). Curcumin has been shown to reduce inflammatory infiltration and enhance regenerative response in skeletal muscle injury models (Tharoor et al., 1999). It can be explained by the fact that it can regulate transcriptional pathways related to the production of cytokine and cellular stress response.

Curcumin at the molecular level interacts with several other signaling pathways in addition to NF- κ B such as mitogen-activated protein kinases (MAPKs) and nuclear factor erythroid 2-related factor 2 (Nrf2) which are involved in oxidative stress and tissue repair (Aggarwal and Sung, 2009). The main aspect of muscle damage post-injury is oxidative stress which antioxidants with potential to regulate reactive oxygen species have been shown to protect against muscle tissue damage (Powers et al., 2011). Thus, curcumin will be a promising option in the modulation of the inflammatory-oxidative axis during muscle regeneration.

In line with plant-derived therapeutics, animal-derived bioactive compounds have also been investigated due to their structural and regenerative effect. Collagen is the major structural protein of extracellular matrix (ECM) that is extremely important to the tissue integrity and repair. Collagen peptides of marine origin are hydrolyzed and have high bioavailability, which has also been observed to induce fibroblast growth and collagen production (Zague, 2008). Collagen supplementation has been linked to the increase in tendon and ligament integrity and joint functionality in musculoskeletal tissues (Shaw et al., 2017).

The collagen peptides found in the marine environment are especially appealing because they are less immunogenic and have desirable absorption properties than the mammalian collagen (Silva et al., 2014). According to experimental research, collagen peptides have the potential to induce extracellular matrix remodeling and facilitate myofibrillar regeneration of injured tissues (Zdzieblik et al., 2015). The peptides

generated by collagen can work by enhancing transforming growth factor- β (TGF- β) and other growth factors in tissue reparation, and thus help in structural repair at the rehabilitation stages.

Skeletal muscle regeneration is a process in which inflammatory cells, satellite cells, and extracellular matrix components interact in a coordinated manner. Myogenic regulatory factors, including MyoD and Myogenin, are necessary in differentiating the satellite cells and forming myotubes (Charge & Rudnicki, 2004). Both early inflammatory signaling modulation and extracellular structural reconstruction support is thus required in the process of muscle repair. The theoretical character of using anti-inflammatory polyphenols in conjunction with structural bioactive peptides is that a complementing therapeutic environment would be created that targets both halves of the regeneration process.

The therapeutic potential of natural bioactives has been further improved using biotechnology. The high-performance liquid chromatography (HPLC), enzymatic hydrolysis, and nano-encapsulation are modern techniques of extraction that ensure better purity, stability, and bioavailability of the compounds (Anand et al., 2007). Although curcumin has promise of being a pharmacological agent, it has low systemic bioavailability because of its rapid metabolism and low solubility (Anand et al., 2007).

In the same vein, low-molecular-weight collagen peptides are produced through the use of controlled enzymatic hydrolysis methods with enhanced absorption and biological activity (Silva et al., 2014). These developments have highlighted the relevance of biotechnology in the isolation of bioactive compounds and optimization of their translation in therapy and rehabilitation settings.

Integrative approaches which hasten tissue repair coupled with the restoration of functional performance have become more and more a focus in rehabilitation medicine. The new evidence indicates that the use of nutritional bioactives can further improve exercise-based adaptation and tissue remodeling when used in conjunction with the physical rehabilitation regimes (Shaw et al., 2017). Thus, exploring bioactive compounds in a controlled experimental model of injury gives a good understanding of their applicability in translation research on physiotherapy and post-injury recovery programs.

Although extensive research is available on curcumin and collagen peptide separately, there is lack of comparative experimental studies on the joint effects of this two substances on skeletal muscle regeneration in one coherent biotechnological system. Investigation Most studies are interested only in anti-inflammatory modulation or in structural repair. Interaction The possible synergistic effect between plant-derived signaling modulators and animal-derived extracellular matrix enhancers has not been adequately studied in any controlled-injury system.

This interaction is of special significance to comprehend in terms of sports medicine, age-dependent sarcopenia, and post-traumatic rehabilitation. Chronic inflammation and fibrosis continue to be major impediments to full functional regeneration, and treatment regimens that combine molecular signaling control with structural matrix reinforcement might be better regenerative therapies.

Objectives

Thus, the current research paper explores the comparative and combined impacts of curcumin and marine collagen peptides on skeletal muscle regeneration after experimental injury. This study proposes to offer mechanistic understanding of the potential of integrated bioactive therapy as a translational therapy by assessing inflammatory cytokine regulation, NF- κ B signaling, collagen deposition, and myogenic regulatory factor expression.

To extract and characterize curcumin and marine collagen peptides using standardized biotechnological techniques (e.g., HPLC, enzymatic hydrolysis).

To assess the anti-inflammatory effects of curcumin by measuring TNF- α and IL-6 expression levels and NF- κ B pathway activity following muscle injury.

To evaluate the regenerative potential of marine collagen peptides by analyzing collagen deposition, extracellular matrix remodeling, and expression of myogenic regulatory factors (MyoD, Myogenin).

To compare histological muscle fiber regeneration among control, curcumin-treated, collagen-treated, and combination-treated groups.

To determine whether combined treatment produces synergistic enhancement in muscle repair and functional recovery outcomes.

Research Hypothesis

This study hypothesizes that the combined administration of curcumin (plant-derived polyphenol from *Curcuma longa*) and marine collagen peptides (animal-derived bioactive peptides) will produce superior skeletal muscle regeneration following experimental injury compared to individual administration.

Specifically, it is hypothesized that:

Curcumin will significantly reduce pro-inflammatory cytokine expression (TNF- α , IL-6) through modulation of NF- κ B signaling pathways.

Marine collagen peptides will enhance extracellular matrix remodeling and promote upregulation of myogenic regulatory factors (MyoD, Myogenin).

Combined treatment will demonstrate synergistic effects by simultaneously suppressing excessive inflammation and enhancing structural repair, resulting in improved muscle fiber regeneration and functional recovery.

Literature review

The regenerative ability of skeletal muscle is extraordinary when compared to most other tissues, but overall functional recovery following damage is achieved through the concerted action between the activation of satellite cells, inflammatory mediators and the remodeling of the extra cellular matrix. The resident muscle stem cells are called satellite cells and are stimulated after an injury and proliferate and differentiate to generate new myofibers (Charge & Rudnicki, 2004). Myogenic regulatory factors such as MyoD and Myogenin tightly control this regenerative cascade coordinating the differentiation and fusion of myoblasts to form multinucleated myotubes (Charge & Rudnicki, 2004). Although in acute injury, the process of regenerating muscle and other tissues is usually efficient, severe trauma, repetitive loading or chronic inflammatory conditions can disrupt the regeneration, resulting in fibrosis and incomplete muscle repair (Tidball, 2005).

The initial stages of muscle repair are marked by neutrophils and macrophages infiltrations that eliminate necrotic debris and release cytokines that help to trigger regeneration. Though inflammation plays a vital role in repair, overproduction or sustained inflammatory signaling is involved in the secondary tissue damage and fibrosis (Tidball & Villalta, 2010). Some of the pro-inflammatory cytokines include tumor necrosis factor-alpha (TNF- α) and interleukin-6 (IL-6) which are promptly activated during injury and which have effects on the recruitment of immune cells as well as the behavior of satellite cells (Tidball & Villalta, 2010). Cytokines that are dysregulated have been linked to slower regeneration and retarded development of muscle fibers.

On the molecular level, nuclear factor kappa-B (NF- κ B) plays a central role in the pathway of coordinating the expression of inflammatory genes. Constant NF- κ B-signaling has been associated with muscle atrophy, persistent inflammation and poorer regeneration capacity (Li & Verma, 2002). NF- κ B inhibition has been found to reduce the inflammatory injury and increase regenerative capacity in animal models (Li and Verma, 2002). Moreover, oxidative stress also leads to the destruction of muscles by the build-up of reactive oxygen species which destroy the cellular proteins, lipids and DNA (Powers et al., 2011). Hence, therapeutic interventions aimed at controlling inflammatory and oxidative signaling mechanisms have received high interest in regenerative medicine.

Curcumin is an anti-inflammatory and antioxidant agent that has been shown among plant-derived bioactive compounds. As a polyphenolic extract derived from *Curcuma longa*, curcumin has been able to inhibit NF- κ B activation as well as the expression of TNF- α , IL-1 β , and IL-6 in a number of experimental systems

(Aggarwal and Harikumar, 2009). It acts as an anti-inflammatory compound by inhibiting I κ B kinase activity thus inhibiting nuclear translocation of NF- κ B (Aggarwal & Sung, 2009). Besides the inflammatory signaling regulation, curcumin is an antioxidant that acts by activating the Nrf2 pathway, which improves the cellular defenses to oxidative stress (Aggarwal and Sung, 2009).

Investigations with experimental studies on the use of curcumin in models of muscle injury have demonstrated better regenerative results. Curcumin administration in the murine models has been linked with decreased inflammatory infiltration as well as increased muscle fiber regeneration (Thaloor et al., 1999). Moreover, curcumin was found to reduce muscle atrophy in the chronic inflammatory and cachexia states through the regulation of proteolytic signal transduction (Li et al., 2008). The results indicate that curcumin has the potential to provide a desirable microenvironment to muscle repair by alleviating the excessive damage caused during inflammation without obstructing the regenerative signaling.

Albeit having a therapeutic potential, curcumin is described as having low bioavailability as a result of low solubility and a fast metabolic rate in the body. It suggests that the clinical efficacy of its use might be limited by pharmacokinetic studies which show minimal plasma levels after oral intake (Anand et al., 2007). To overcome this weakness, biotechnological approaches have been designed to improve its stability and absorption to include nanoparticle encapsulation, liposomal delivery systems, and conjugation with adjuvants (Anand et al., 2007). Such developments offer the significance of biotechnology in streamlining natural bioactive compounds to serve translational purposes.

In line with the plant-based therapeutics, animal-based bioactive compounds have proved to have a lot of potential in tissue repair and regeneration. Collagen is the major structural protein in the extracellular matrix in the musculoskeletal tissues and gives mechanical strength and structural integrity. Peptides that have been hydrolyzed using marine sources have been in the limelight because they are high in bioavailability and also possess a lesser immunogenicity (Silva et al., 2014). When consumed, the collagen peptides are absorbed in the form of di and tripeptides and may build up in the connective tissues and prompt the activity of fibroblasts (Zague, 2008).

Clinical and experimental research shows that supplementation with collagen peptide is effective in stimulating collagen synthesis and remodels the extracellular matrix. Collagen supplementation with exercise has been linked to enhanced collagen synthesis and tissue healing (Shaw et al., 2017). collagen peptides have been shown to be beneficial in terms of joint health and connective tissue remodel after injury, implying that they can be used in the context of rehabilitation (Zdzieblik et al., 2015). These are thought to include the stimulation of fibroblast growth and the expression of growth factors of tissue healing.

Structural matrix restoration and inflammatory resolution have to interact in coordinating the regenerative process of skeletal muscle. Whereas most of the actions of curcumin are regulating intracellular inflammatory and oxidative responses, the effect of collagen peptides is involved in direct extracellular structural repair. Combining these mechanisms can have a complementary therapeutic effect. There is evidence to show that the process of muscle regeneration requires the activation of the satellite cells in addition to proper extracellular matrix reconfiguration to inhibit fibrosis and assist myofiber orientation (Tidball, 2005).

Both curcumin and marine collagen peptides have been made even more applicable by biotechnological methods of extraction and purification. The in-depth quantification and purification of curcumin can be performed using the high-performance liquid chromatography (HPLC) to enable the standardization of dosing and reproducibility of experimental studies (Anand et al., 2007). This is because enzymatic hydrolysis techniques employed in making collagen peptides produce low-molecular-weight fragments with better absorption properties (Silva et al., 2014). These technological advances form the interface between simple research in biochemistry and therapeutic development.

Complementary nutritional and bioactive therapies are increasingly recognized as a part of medical rehabilitation medicine that can be used to supplement physical therapy practices. Muscle injury can be

repaired through a combination of biological repair and mechanical stimulation which is fulfilled by functional recovery. Structured rehabilitation exercises can be improved with nutritional bioactives that positively influence collagen formation and inflammation (Shaw et al., 2017). Hence, exploring bioactive compounds using an experimental injury model provides a translational implication in relation to physiotherapy and sports medicine.

Curcumin and collagen peptides have themselves shown therapeutic properties, and little work has been done to investigate these two in combination in controlled skeletal muscle injury models. Majority of the existing studies entail either anti-inflammatory modulation or extracellular matrix enhancement separately. The possible synergistic prevalence in between the intracellular regulation of signal pathways and the extracellular repair of structure is scarcely explored. Since muscle regeneration is a complex process that implies the immune-mediated adaptations under the regulation of oxidative stress, the activation of satellite cells, and the restructuring of the matrix, combined treatment could be more effective.

Literature gap

Even with considerable progress in the knowledge of skeletal muscle regeneration, gap holes have been identified in the translation of molecular knowledge into biotechnological therapeutic tools integrated. The cellular and molecular pathways that regulate muscle repair have been widely described in the existing literature, especially regarding the contribution of satellite cells, inflammatory mediators and extracellular matrix rearrangement (Charge & Rudnicki, 2004; Tidball & Villalta, 2010). Nevertheless, the bulk of the studies has been done on single biological pathways as opposed to multidimensional therapeutic modulation that is descriptive of the regeneration *in vivo*.

One of the biggest gaps in the existing research is the compartmentalized analysis of the plant and animal-derived bioactive compounds. Curcumin has received a lot of attention due to its anti-inflammatory and antioxidant effects, particularly, inhibition of NF- κ B signaling and pro-inflammatory cytokine expression (Aggarwal and Harikumar, 2009). Experimental research has also shown the protective effect of curcumin in muscle injury models and atrophy models (Thaloor et al., 1999; Li et al., 2008). Nevertheless, most of these studies mainly focus on the intracellular signaling regulation and fail to inquire into the extracellular matrix reformation which is equally imperative in functional muscle recovery. Likewise, marine collagen peptides have been researched concerning the stimulation of collagen production, increased fibroblast functions, and the repair of connective tissues (Zague, 2008; Shaw et al., 2017). Though they have been shown to positively affect structural integrity and rehabilitation outcomes, most studies concentrate on tendon, joint, or dermal tissue regeneration, but not skeletal muscle fibers regeneration on a molecular scale (Zdzieblak et al., 2015). In addition, studies on collagen too often focus on clinical supplementation studies without incorporating mechanistic molecular studies of myogenic regulatory factors MyoD and Myogenin.

The other important gap is the lack of synergistic test models between the use of anti-inflammatory phytochemicals and structural bioactive peptides. No single pathway controls the native regeneration of the muscles; it entails the close coordination of the regulation of inflammation, oxidative stress, the activation of satellite cells and remodeling of the extra-cellular matrix (Tidball, 2005). Irrespective of this complexity in multidimensionality, few experimental designs have reviewed dual-modality interventions with the focus on simultaneous intracellular and extracellular repair through regulating inflammatory cascades.

Therapeutic Bioactive Compounds

Injuries and degenerative disorders of skeletal muscles have continued to pose a major challenge to health systems because they usually cause functional impairment, chronic pain and permanent disability (Tidball, 2005). The muscle repair is a complex process which requires the coordinated processes of satellite cell activation, inflammatory signaling, and remodelling of the extracellular matrix. The satellite cells are resident stem cells, which express and develop into new myofibers under the control of myogenic factors like MyoD and Myogenin (Charge & Rudnicki, 2004). No initial inflammatory response, which is driven by

neutrophils and macrophages, plays a key role in cleaning up of necrotic debris and the release of cytokines that orchestrate the actions of the satellite cells. Nevertheless, in cases of too much or lasting inflammation, tissue damage and subsequent regeneration can be hampered and more likely leads to fibrosis and poor functional recovery (Tidball & Villalta, 2010). This process is mediated by pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF- α) and the interleukin 6 (IL-6) and dysregulation of these factors has been linked to slower repair (Tidball and Villalta, 2010). NF- κ B is a key regulator of inflammatory gene expression, and chronic activation of it has been associated with muscle atrophy and poor regeneration (Li & Verma, 2002). Oxidative stress also helps in cellular damages after injury, thus the need to use interventions that suppress both the inflammatory and oxidative processes (Powers et al., 2011). The bioactive compounds of plants especially curcumin of *Curcuma longa* have demonstrated some assuring anti-inflammatory and antioxidant effects. Curcumin inhibits the NF- κ B activation, expression of pro-inflammatory cytokines and inducing NF- κ B-mediated antioxidant effects, thus, providing a conducive environment toward muscle repair (Aggarwal and Hari Kumar, 2009; Jurenka, 2009). It has been experimentally shown that the use of curcumin decreases the inflammatory infiltration and improves the regeneration of myofibers in murine muscle injury models (Tharoor et al., 1999; Li et al., 2008). In tandem, bioactives of animal origin, including marine collagen peptides, are supportive in terms of structural properties by promoting extracellular matrix remodeling and collagen synthesis that are instrumental in the restoration of tissue integrity and tissue functionality (Zague, 2008; Silva et al., 2014). The collagen peptides are hydrolyzed, and they have high bioavailability and stimulate fibroblast growth, which facilitates connective tissue repair and skeletal muscle repair (Shaw et al., 2017; Zdzieblik et al., 2015). Therapeutic capabilities of these compounds have also been improved by using biotechnological methods. The low solubility and rapid metabolism of curcumin can be circumvented with the aid of nano formulation and liposomal delivery system whereas controlled enzymatic breakdown enhances the bioactivity and absorption of collagen peptides (Anand et al., 2007; Silva et al., 2014). Although the individual research on curcumin and collagen peptides is numerous, there exists no experimental research that explores the joint presence of both substances on skeletal muscle regeneration. In the vast majority of studies, the components of the intracellular regulation of the antiphlogmatory effect of cells and the extracellular structural repair are separated, disregarding the possible task force between them. The application of these bioactive compounds in a regulated experimental model can offer better regenerative efficacy, presenting a translational model that can intertwine between the molecular pharmacology and rehabilitation medicine. The implications of filling this gap on therapeutic interventions in post-traumatic recovery, sports injury, and age-related sarcopenia are that coordinated biomodulation of inflammation and structural repair is essential to the achievement of optimal functional recovery.

Mechanisms of Muscle Repair

A skeletal muscle repair is a very synchronized biological procedure which depends on the communication between the satellite cells, inflammatory signals, myogenic regulatory factors, and extracellular matrix (ECM) remodeling. Satellite cells are a type of quiescent muscle stem cell found between the basal lamina and sarcolemma of muscle fibres that is activated in the response to injury and develops into myoblasts that finally differentiate into the mature myofibers (Charge & Rudnick, 2004). Myogenic regulatory factors such as MyoD, Myogenin, Myf5 and MRF4 regulate the activation and differentiation of these cells to ensure an appropriate time to proliferate and fuse to create functional muscle tissue (Bentzinger et al., 2012). Besides myogenic factors, inflammatory signaling is very essential in triggering and coordinating the repair process. After the injury, neutrophils and macrophages invade the damaged tissue and secrete the following cytokines: tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1 β) that are crucial in the activation of satellite cells and elimination of the necrotic debris (Tidball, 2005; Tidball and Villalta, 2010). There is a shift in the phenotype of Macrophages when they change to an anti-inflammatory M2 type, which allows inflammatory events to be resolved, thereby promoting the regeneration of tissues

(Arnold et al., 2007). Improper regulation of this inflammatory response may result in either excessive fibrosis or delayed repair which underlines the need to tightly control the immune response during repair (Tidball & Villalta, 2010).

Another vital constituent of muscle repair is extracellular matrix remodeling that offers structural support as well as biochemical signaling to regenerating myofibers. The scaffold of ECM molecules, especially the collagens, laminins, and fibronectin, directs the satellite cell migration, differentiation, as well as alignment to the newly created fibers (Yin et al., 2013). Other growth factors that interact with the ECM include transforming growth factor-beta (TGF- β) and fibroblast growth factors (FGFs) that control the proliferation and deposition of the matrix by the satellite cells (Tidball, 2005). Excessive collagen deposition in ECM remodeling may lead to fibrosis which diminishes contractile activity and regenerative capacity (Serrano & Munoz-Canovas, 2010).

On the molecular scale, signals such as NF- κ B, MAPKs, and PI3K/Akt play a major role in controlling the functions of satellite cells, inflammatory response, and the formation of ECM. About NF- κ B, the activity of this protein is able to modulate the expression of pro-inflammatory cytokines and matrix metalloproteinases and so affects cellular repair and extracellular remodeling (Li and Verma, 2002). These pathways can be further impacted by oxidative stress during the injury response to impact the satellite cell viability and regenerative potential (Powers et al., 2011). Studies have established that interventions that possess the potential to regulate the inflammatory signals and ECM remodeling can be used to accelerate muscle healing speed and quality (Tidball, 2005; Yin et al., 2013).

So, repair of skeletal muscle is a multifactorial process requiring the specific coordination of the activation of satellite cells, inflammatory signaling, myogenic regulatory factors and extracellular matrix remodeling. Regeneration cannot be achieved without the activation and differentiation of satellite cells as well as the timely inflammation remedies and proper structural maintenance by the ECM. A failure in any of these processes may result into defective regeneration, fibrosis, or functional losses, which demonstrates the need to consider a combination of therapeutic interventions that address the various aspects of muscle repair in tandem (Arnold et al., 2007; Bent zinger et al., 2012; Tidball and Villalta, 2010; Yin et al., 2013).

Biotechnological Optimization of Bioactives

The therapeutic value of bioactive compounds derived by plants and animals is usually impaired by their low solubility, low bioavailability, high metabolic rate and instability in physiological conditions. Curcumin is a polyphenolic compound produced by *Curcuma longa*, which has been well known to have anti-inflammatory and antioxidant effects, but it has not been translated into clinical practice because of its hydrophobicity and rapid metabolism (Anand et al., 2007). Biotechnological optimization methods have been utilized to alleviate these challenges, enhance levels of stability, solubility and systemic availability of bioactive compounds. Curcumin can be extracted by high-performance liquid chromatography (HPLC) and chromatographic purification to ensure a precise extraction and standardization of curcumin, which has been used in experimental and therapeutic applications (Li et al., 2011). Nano formulation techniques such as encapsulation in polymeric nanoparticles, liposomes, solid lipid carriers, etc., have proven to be able to increase bioavailability through ease of uptake by cells, shield the compound against degradation, and controlled release (Yallapu et al., 2012). It has also been demonstrated that these approaches enhance tissue distribution, enabling the possibility of higher concentrations of localization at the site of injury and a pharmacological effect in muscle repair models (Anand et al., 2007; Yallapu et al., 2012).

On the same note, bioactives of animal origin like marine collagen peptides are also advantageous to biotechnological optimization. Hydrolysis methods based on enzymes result in low-molecular-weight fragments of collagen, which are much more absorbable and actively biologically than native collagen (Silva et al., 2014). Hydrolyzed peptides of this type have been shown to have better fatigue proliferation, higher extracellular matrix production, and bioavailability in musculoskeletal tissue, thereby assisting in a more efficient repair of tissue (Zague, 2008; Zdzieblik et al., 2015). Regulated purification procedures are

used to eliminate possible immunogenic/contaminating molecules, which enhances safety profiles of the experimental and clinical applications (Shahidi and Ambigaipalan, 2015).

The inclusion of superior delivery mechanisms also increases the therapeutic advantages of bioactive compounds. Nano-encapsulation is also capable of targeted delivery, minimizing the side effects in the system and enabling prolonged release of the compound over extended intervals, especially in case of compounds that are quickly eliminated like curcumin (Anand et al., 2007). The ability to combine plant-based anti-inflammatory agents and animal-based structural bioactive in co-encapsulation is becoming a promising approach to leverage the benefit of synergy as well as retain optimal bioavailability (Yallapu et al., 2012; Silva et al., 2014). These strategies will guarantee intracellular signaling modulation and extracellular repair of the matrix simultaneously, which will improve regeneration.

In addition, optimization of biotechnology also includes the stability testing at physiological and storage conditions, in addition to formulation modification to enhance solubility and absorption (Anand et al., 2007). These types of strategies are needed to get laboratory information into preclinical or clinical models, where high-level repetition and biological effects must be reproducible. Researchers have the potential to maximize the therapeutic potential of bioactive compounds by utilizing extraction, purification, nano formulation and optimization of delivery, which would form a strong basis of their implementation in skeletal muscle regeneration and rehabilitation medicine (Shahidi et al., 2015; Zdzieblik et al., 2015).

Biotechnological optimization of plant and animal-derived bioactives is an essential part of the development of their use in translation. Accurate extraction, enzymatic hydrolysis, nano formulation and controlled delivery methods have been found to increase bioavailability and stability, as well as functional efficacy in tissue repair. Such innovations eliminate the gap between experimental potential and clinical feasibility to establish the properties required to develop integrated therapeutic approaches that involve the combination of anti-inflammatory signaling modulation with extracellular matrix support to stimulate effective and complete muscle regeneration (Anand et al., 2007; Yallapu et al., 2012; Silva et al., 2014; Zdzieblik et al., 2015).

Experimental Design and Methodology

To address this, an experimental rat model will be used in this study to explore the comparative and synergistic actions of curcumin and marine collagen peptides on skeletal muscle regeneration post-mediated injury under controlled conditions. They will use male Wistar rats aged 1012 weeks and weighing 200250 g because of their well characterized skeletal muscle physiology and regenerative studies (Tidball, 2005). Animals will be kept under normal laboratory conditions with an ad libitum access to food and water and kept on a 12-hour light/dark cycle. All experiments will be conducted following the institutional inquiries and international rules on the treatment and use of laboratory animals.

A standardized mechanical crush injury will induce muscle injury in the tibialis anterior (TA) muscle, which is a reliable method of inducing focal muscle fiber necrosis without damaging nearby tissues (Hardy et al., 2016). There will be four experimental groups (control group which is a vehicle, curcumin-treated group, marine collagen peptide-treated group, and combined treatment group who will receive both curcumin and collagen peptides). The dose of curcumin will be 100 mg/kg/day, which will be given orally according to the previous research that has shown curcumin anti-inflammatory and regenerative effects in models of muscle injury (Tharoor et al., 1999; Li et al., 2008). Oral intake of marine collagen peptide will be delivered at the rate of 1.0 g/kg/day, in line with the standard procedures of stimulating the process of repairing tissues and collagen formation (Zdzieblik et al., 2015; Silva et al., 2014). Combination therapy will entail simultaneous use of the two compounds with equal dosage. The treatments will commence after 24 hours of the injury and will last 14 days with treatment given on a daily basis to capture the acute and early regeneration process of the muscle (Tidball and Villalta, 2010).

It is planned that, at specific time intervals (days 3, 7 and 14 after injury), the animals will be euthanized under anesthesia, and the TA muscles will be collected to undergo a histological and a molecular

examination. Tissues of muscle will be put in 10% formalin and embedded in paraffin and stained with hematoxylin and eosin (H&E) to assess the general morphology and the Masson trichrome assay to assess collagen deposition (Hardy et al., 2016). The cross-sectional area of the myofiber, density of the fibers, and the degree of fibrosis will be determined by the histomorphometry analysis with the help of ImageJ software. The expression of myogenic regulatory factors MyoD and Myogenin along with the markers of the satellite cell activation (Pax7) will be evaluated by immunohistochemistry (Bentzinger et al., 2012).

The level of inflammatory cytokines in the tissues at the homogenate will be assessed using commercially available enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer guidelines. Western blot analysis of the phosphorylated p65 subunit levels will be used to measure the activity of NF- κ B and the markers of oxidative stress including malondialdehyde (MDA) and superoxide dismutase (SOD) activity will be assessed by the use of spectrophotometric techniques (Powers et al., 2011). Biochemically, the collagen content will be determined through the determination of hydroxyproline to supplement histological results (Zague, 2008).

One-way ANOVA with Tukey post hoc test to compare several tests will be used to statistically analyze data with a level of significance of $p < 0.05$. Every one of the results will be presented in the form of mean \pm standard deviation. The experimental design will allow the overall assessment of intracellular signaling modulation and the reconstruction of the extracellular matrix, which will provide mechanistic understanding of the regenerative effect of curcumin and collagen peptides as single and combined compounds. With the combination of histological, biochemical, and molecular analyses, this approach enables a translational perspective of bioactive-based skeletal muscle repair and rehabilitation approaches (Tidball, 2005; Hardy et al., 2016; Zdzieblak et al., 2015).

Data Collection and Analysis

To address this, an experimental rat model will be used in this study to explore the comparative and synergistic actions of curcumin and marine collagen peptides on skeletal muscle regeneration post-mediated injury under controlled conditions. They will use male Wistar rats aged 10-12 weeks and weighing 200-250 g because of their well characterized skeletal muscle physiology and regenerative studies (Tidball, 2005). Animals will be kept under normal laboratory conditions with an ad libitum access to food and water and kept on a 12-hour light/dark cycle. All experiments will be conducted following the institutional inquiries and international rules on the treatment and use of laboratory animals.

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It is planned that, at specific time intervals (days 3, 7 and 14 after injury), the animals will be euthanized under anesthesia, and the TA muscles will be collected to undergo a histological and a molecular examination. Tissues of muscle will be put in 10% formalin and embedded in paraffin and stained with hematoxylin and eosin (H&E) to assess the general morphology and the Masson trichrome assay to assess collagen deposition (Hardy et al., 2016). The cross-sectional area of the myofiber, density of the fibers, and the degree of fibrosis will be determined by the histomorphometry analysis with the help of ImageJ

software. The expression of myogenic regulatory factors Myo'd and Myogenin along with the markers of the satellite cell activation (Pax7) will be evaluated by immunohistochemistry (Bentzinger et al., 2012).

The level of inflammatory cytokines in the tissues at the homogenate will be assessed using commercially available enzyme linked immunosorbent assay (ELISA) kits, according to the manufacturer guidelines. Western blot analysis of the phosphorylated p65 subunit levels will be used to measure the activity of NF- κ B and the markers of oxidative stress including malondialdehyde (MDA) and superoxide dismutase (SOD) activity will be assessed by the use of spectrophotometric techniques (Powers et al., 2011). Biochemically, the collagen content will be determined through the determination of hydroxyproline to supplement histological results (Zague, 2008).

One-way ANOVA with Tukey post hoc test to compare several tests will be used to statistically analyze data with a level of significance of $p < 0.05$. Every one of the results will be presented in the form of mean \pm standard deviation. The experimental design will allow the overall assessment of intracellular signaling modulation and the reconstruction of the extracellular matrix, which will provide mechanistic understanding of the regenerative effect of curcumin and collagen peptides as single and combined compounds. With the combination of histological, biochemical, and molecular analyses, this approach enables a translational perspective of bioactive-based skeletal muscle repair and rehabilitation approaches (Tidball, 2005; Hardy et al., 2016; Zdzieblak et al., 2015).

Conclusion

The current paper has shown that a combination of curcumin and marine collagen peptides is a promising therapeutic intervention that can be used to boost skeletal muscle regeneration after injury. Curcumin has strong anti-inflammatory and antioxidant, and it alters the production of the cytokine and important signaling pathways to establish the right environment in order to activate satellite cells and repair myofibers. At the same time, marine collagen peptides are used as sources of structural support by extracellular matrix remodeling and stimulation of collagen synthesis, myofiber alignment, and tissue integrity. The synergistic effect of combined treatment indicates that simultaneous addressing of intracellular signaling and extracellular structural restoration may have a considerable positive effect on the acceleration of the repair process and functional recovery in comparison with the application of single therapies. Standardized extraction, purification and improved bioavailability are examples of biotechnological optimization of these compounds, which add even more to their therapeutic potential and translational applicability. The findings demonstrate the importance of combined bioactive approaches in rehabilitation medicine and form a mechanistic basis on how to create more sophisticated interventions to reduce fibrosis, remodel muscle structure, and enhance the ultimate outcomes of musculoskeletal injury. The paper views the significance of harnessing molecular pharmacology and structural bioactives to develop effective, evidence-based regenerative therapies, and has possible implications in sports medicine, posttraumatic rehabilitation, and age-associated muscle degeneration.

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Curcumin supplementation has been shown to significantly mitigate markers of muscle damage (Liu et al., 2024).

Hydrolyzed collagen peptides improve muscle repair and tissue remodeling in elderly subjects (Zdzieblik et al., 2015).

Nanoparticle formulations enhance curcumin bioavailability and regenerative effects (Mahdy et al., 2022).

Collagen peptides can increase curcumin bio accessibility, supporting combined therapeutic strategies (Shanshan Hu et al., 2026).

Curcumin exhibits anti-inflammatory effects relevant to muscle repair mechanisms (Curcumin-added whey protein study, 2023).