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Glucagon-like peptide-1 agonist and Quercetin improve skeletal muscle performance and pain threshold in male rats with fibromyalgia via activating the Nrf2/HO1/NQO1 pathway

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Keywords

- Liraglutide
- Quercetin
- Skeletal muscle tension
- Fibromyalgia
- Nrf2

Abstract

Background: Chronic fatigue and musculoskeletal pain, compromising the quality of life, are hallmarks of fibromyalgia (FM). Yet, there is no proven treatment for these health issues associated with FM. Nuclear factor erythroid 2-related factor 2 (Nrf2) agonists reduce pain. As well, they enhance muscle activity. Nevertheless, little information is available on how Liraglutide and Quercetin, Nrf2 agonists, impact FM patients' motor functions and pain. **Objective**: The current research explored how Liraglutide and Quercetin might affect pain threshold and muscular function in FM rats. **Methods:** Four groups including 24 male Wistar rats were equally distributed as Control (CTRL), FM, FM + Liraglutide and FM + Quercetin. We evaluated the body weight, mechanical pain pressure threshold, skeletal muscle contractions, serum substance P and glucose, muscle tissue catalase, malondialdehyde, and gene expression of Nrf2, NAD(P)H: quinone oxidoreductase 1 (NQO1) and hemoxygenase1 (HO1). **Results**: Liraglutide and Quercetin substantially enhanced the muscle performance, and pain threshold, via enhancing the antioxidant activity through upregulation of Nrf2, HO1 and NQO1 genes. **Conclusion**: Liraglutide and Quercetin comparably showed favorable efficacy on the motor dysfunctions and pain associated with FM.

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1. Introduction

Fibromyalgia (FM), an increasingly prevalent disease, manifests as generalized pain and tenderness along with morning stiffness, significant fatigue, insomnia, and psychosocial issues[1]. Although the exact aetiology of FM is unknown, disorders of the immunological, endocrine, neurological, and musculoskeletal systems as well as genetic predisposition have all been linked to the condition. Additionally, decreased serotonin and elevated substance P, which mediate the pain control system, are contributors[2]. There has rarely been a cure for FM patients, even with multidisciplinary care. Therefore, to create novel, efficient treatments, it is imperative to comprehend the potential culprits of FM[3].

Nuclear factor erythroid 2-related factor 2 (Nrf2) is a crucial supervisory of antioxidants elements. After dissociating from Kelch-like ECH-associated protein 1 (Keap1), Nrf2 moves to nucleus where it attaches itself to the antioxidant response element (ARE). This is accomplished by regulating a variety of protective enzymes, namely NAD(P)H: quinone oxidoreductase 1 (NQO1) and hemoxygenase1 (HO1), both of which possess potent antioxidant features[4] and lessen pain-like symptoms[5].

The small intestine's L cells release glucagon-like peptide-1 (GLP-1) consequent to meal consumption. GLP-1 - GLP-1 receptor (GLP-1R) complex improves insulin production, suppresses glucagon release, stomach evacuation, and intake of food, as well as stimulates the division of pancreatic cells[6]. Furthermore, activation of GLP-1R has been shown to reduce pain hypersensitivity[7].

Among the many flavonoid components found in fruits and vegetables is Quercetin. There is increasing evidence that Quercetin has anti-nociceptive properties in chronic pain-stricken animal models[8].

Thus, given their capacity to reduce pain, we theorized that GLP-1R agonists and Quercetin can be able to alleviate muscular dysfunction as well as tiredness in FM patients. This could potentially alleviate the two primary FM manifestations without the need for numerous drugs with different adverse effects. Therefore, this work's objective was to ascertain how GLP-1R agonist (Liraglutide) and the flavonoid (Quercetin) affect the pain and performance of the skeletal muscles in FM male rats, as well as whether these effects resulted from activation of Nrf2/HO1/NQO1 pathway theyby damping the oxidative stress (OS).

2. Materials and Methods

2.1. Animals' acclimation

Twenty-four mature male Wistar rats averaging 220 ± 7 g were procured from Faculty of Medicine, Cairo University, Egypt's animal house. For 7 days, rats were allowed for acclimation in the animal house in well-aerated plastic cages (3 in a cage) at 25±5°C and regular cycles of light and dark. Ad-lib meals with unrestricted access to water were supplied daily. The Cairo University Ethical Animal Research Committee approved this study with approval number (CU III F 65 24).

2.2. Drugs and chemicals

We bought Liraglutide, Quercetin, and reserpine from Sigma Aldrich (St. Louis, MO). If not specified otherwise, all chemical kits were acquired from My BioSource, Inc. in San Diego, USA.

2.3. Establishment of FM model

Reserpine (1 mg/kg) was injected SC once a day into 18 rats at different locations on the flank for 3 days in succession. Reserpine was diluted with glacial acetic acid until its ultimate concentration was 0.5% acetic acid in distilled water [1].

2.4. Animal groups

The animals were categorized into four equal groups: Negative control group **(CTRL)**: animals administered distilled water (1 mg/kg/day/3 days, SC) and subsequently were given intraperitoneal (i.p) saline[9] concomitant with oral orange-flavored Tang [10] for the next 21 days. Fibromyalgia group **(FM)**, FM and Liraglutide group **(FM + Liraglutide)**: FM rats received daily Liraglutide (0.6 mg/kg, single dose, i.p) for 21 days[9], and FM and Quercetin group **(FM + Quercetin)**: FM animals were treated with oral Quercetin (25 mg/kg/day/21 days)[11].

2.5. The subsequent parameters were examined after the study:

2.5.1 Body weight was measured using the digital scale.

2.5.2 Mechanical pressure pain threshold

The animals were kept on a raised steel scaffolding inside a clear plexiglass container. Rats' middle plantar skin was subjected to progressive vertical pressures using a mechanical plantar-induced pain instrument; the maximum stimulation value was set at 50 g. Once the duration exceeds eight seconds, the pressure is raised until the response happens. When the rat's foot is lifted, the device electronically registers the force imparted to the sole. The mechanical stimulation pain threshold is then determined by averaging the three test results over 10 minutes[12].

Afterwards, the rats were sacrificed after they had been sedated by ketamine and xylazine (60 and 6 mg/kg, respectively)[13]. Blood samples were drawn from jugular veins and processed carefully to measure substance P and glucose levels.

Following the research and safety disposal guidelines, all the remains of sacrificed animals were disposed ~~of~~ in the general incinerator of Cairo University's Faculty of Medicine in a sanitary and ethical way.

2.5.3 ELISA measurement of plasma glucose level (Cat#MBS7233226) and substance P (Cat#MBS703659) according to the manufacturer's instructions.

2.5.4 Skeletal muscle activities record by power lab 26 T.

As described before, the proximal tendon of the extensor digitorum longus (EDL) muscle was explored (1). The anterior crural compartment was made apparent. Distal fasciotomy was used to remove connective tissue and ligaments from the foot, allowing the distal tendons of the EDL to be separated undamaged. The whole EDL muscle was mounted utilizing the Harvard Apparatus, Holliston, MA (25 ml organ bath system) incorporating Krebs-Ringer bicarbonate buffer. The system was maintained at 30 °C and bubbled continually with an oxygen and CO2 mixture (95% versus 5%, respectively). A non-absorbable surgical silk thread was utilized to unite the four distal EDL tendons before being secured to a support. The force transducer, which was wired to

the iWorx advanced data collection unit AHK/214 (Harvard apparatus), was placed on the proximal tendon. The electrodes were put parallel to the longitudinal axis of the muscles, then the muscles were stimulated to supramaximal intensities. Utilizing a micromanipulator, the length of the muscles was manipulated to achieve the highest possible isometric-twitch tension by applying 1Hz electric stimuli interrupted by a 1-minute break interval. Then the durations it took to achieve the greatest tension and relaxation to 50% of it were recorded. The muscles were then stimulated at escalating frequencies for one second to create the highest feasible fused-tetanic tension. Responses were then recorded. After a 3-minute rest, for a whole duration of five minutes, muscles were stimulated for one second at the ideal frequency—achieved in the preceding step—followed by five seconds of rest. After allowing the muscles to rest for five minutes, tetanic tension was measured to determine the degree of recovery from fatigue. Wet muscle mass was measured and normalized muscular tensions were given as Newtons per gram (N/g)[14].

2.5.5 Muscle homogenate CAT and MDA.

Considering the manufacturer's manuals the skeletal muscle catalase (CAT) activity was determined utilizing a CAT assay kit (Cat#MBS2548442) and Malondialdehyde (MDA) concentration was evaluated Colorimetrically by MDA assay Kit (Cat#ab233471; Abcam, Cambridge, Massachusetts, USA).

2.5.6 *Real-time quantitative polymerase chain reaction (RT-qPCR) measurement of skeletal muscle homogenate relative Nrf2, HO1 and NQO1gene expression.*

Following the supplier's guidelines "Thermo Fisher Scientific Inc. Waltham, MA USA)", the entire amount of RNA was obtained using Direct-zol RNA Miniprep Plus (Cat#12183555). The subsequent primer sequences were used to create cDNA utilizing reverse transcription re-action and PCR in a single step using the SuperScript™ IV One-Step RT-PCR kit (Cat# 12,594,100). Primers were Nrf2: Forward; GCTATTTTCCATTCCCGAGTTAC, Reverse ATTGCTGTCCATCTCTGTCAG, HO1: Forward; GAGCGCCCACAGCTCGACAG, Reverse; CGGCTCCATGTACTCTCTGC, NQO1: Forward; TCCTGCAGACCAAGAACTATGACATCG, Reverse; TCTTCCAGCCTTCCTTCCTG, and GAPDH: Forward; TTCACCACCATGGAGAAGGC, Reverse; TGATGGCATGGACTGTGGTC as an endogenous reference control. The StepOnePlus™ RT-PCR System was employed to analyze the samples. The 2ΔΔCt was applied to assess the expression of genes.

2.6. Statistical analysis

The statistical software program SPSS 26 was used for processing the data, and for quantitative variables, the results were summarized in mean ± standard deviation then the analysis of variance followed by the post hoc test was carried out. The significance value was set at $p \leq 0.05$.

3. Results

3.1. Body weight and serum glucose level

As displayed in **Table 1**, statistical insignificance was recorded among all study groups regarding either their body weight or blood glucose level.

Table 1: Body weight and serum glucose level.

Variable	CTRL	FM	FM + Liraglutide	FM + Quercetin
Body weight	220.83±7.36	215±5.44	217±5.44	216.66±8.14
Serum glucose	89.7±1.11	91.27±2.68	88.43±2.10	90.60±2.89

Parameters were represented in mean ± standard deviation. CTRL: Control, FM: Fibromyalgia.

3.2. Mechanical pressure pain threshold (PPT)

As displayed in **Figure 1A**, the muscle mechanical PPT significantly diminished in those with FM relative to CTRL (p=0.000). However, this PPT significantly increased in the FM + Liraglutide and FM + Quercetin groups (p=0.005) in contrast to FM. Still, a significant difference exists in contrast with CTRL (p=0.005 and 0.006, correspondingly). Liraglutide's and Quercetin's impacts on the mechanical PPT showed comparable effect.

3.3. Substance P results

As displayed in **Figure 1B**, the serum substance P concentration significantly boosted in FM group relative to CTRL. However, it significantly decreased in the FM + Liraglutide and FM + Quercetin groups in comparison to FM (p=0.000). Although substance P attained the normal levels "displaying insignificant difference from CTRL" in FM + Quercetin rats, however, a significant variance in FM + Liraglutide rats (p= 0.007) in contrast to CTRL was detected. Overall, the effects of either Liraglutide or Quercetin on the substance P were comparable with no significant difference recognized between them.

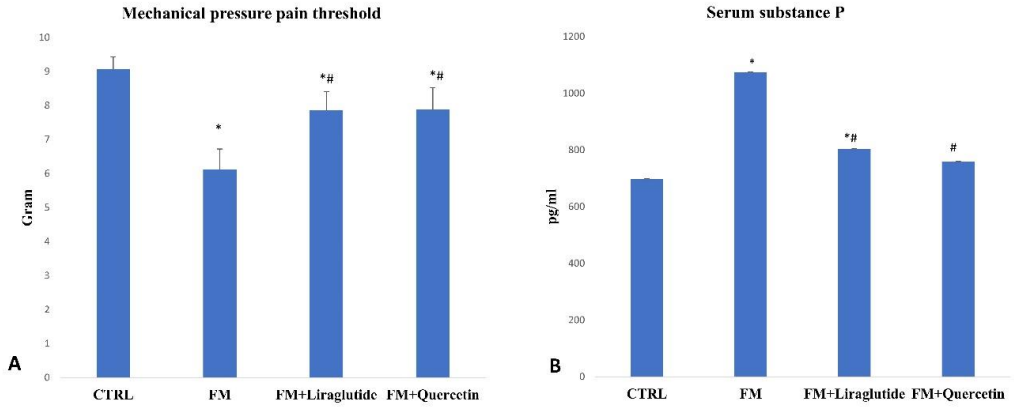

Figure 1: A) Mechanical pressure pain threshold, and **B)** Serum substance P concentration in all study groups. Parameters were represented in mean ± standard deviation. CTRL: Control, FM: Fibromyalgia. *: significant from CTRL, #: significant from FM. Statistical significance means p-value ≤ 0.05.

3.4. Skeletal muscle function results

As displayed in **Figure 2A**, neither time to peak twitch tension nor time taken to relax from max twitch tension to 50% demonstrated any significant change among all groups. As shown in **Figure 2B**, the maximum isometric twitch tension of the FM group was statistically lower than its value in the CTRL (p=0.000). While comparing to FM, this tension significantly enhanced in FM + Liraglutide and FM + Quercetin groups (p=0.005 and 0.002, correspondingly), however, these beneficial effects of both groups did not reach the CTRL value (p=0.003 and 0.009 respectively). Worth noting that Liraglutide and Quercetin influences were comparable with no significant difference documented between them.

Regarding the maximum fused tetanic tension, it was substantially lower in FM group compared to CTRL (p=0.008). However, this tension significantly improved in the groups FM + Liraglutide and FM + Quercetin in contrast to FM (p=0.000 and 0.026, correspondingly).

Although most of the tensions recovered after Liraglutide treatment, however regarding max. isometric twitch tension, a significant difference still exists in contrast to CTRL result (p=0.000). Of note, the effect of Liraglutide was significantly superior to Quercetin (p=0.000). When juxtaposing the FM group to the CTRL, there was a significant drop in the maximal fused tetanic tension following fatigue (p=0.000). However, this tension significantly improved in the FM + Liraglutide and FM + Quercetin groups relative to the FM (p=0.000) attaining the CTRL values reflecting that both Liraglutide and Quercetin had equivalent effects without any significant difference. Following a 5-minute rest period, the FM group's tetanic tension was considerably lower than the CTRL group's (p=0.000). However, this tension significantly improved in the FM + Liraglutide and FM + Quercetin groups relative to FM (p=0.003 versus 0.004, respectively) attaining CTRL values reflecting that both Liraglutide and Quercetin had similar effects without any significant difference between their effects.

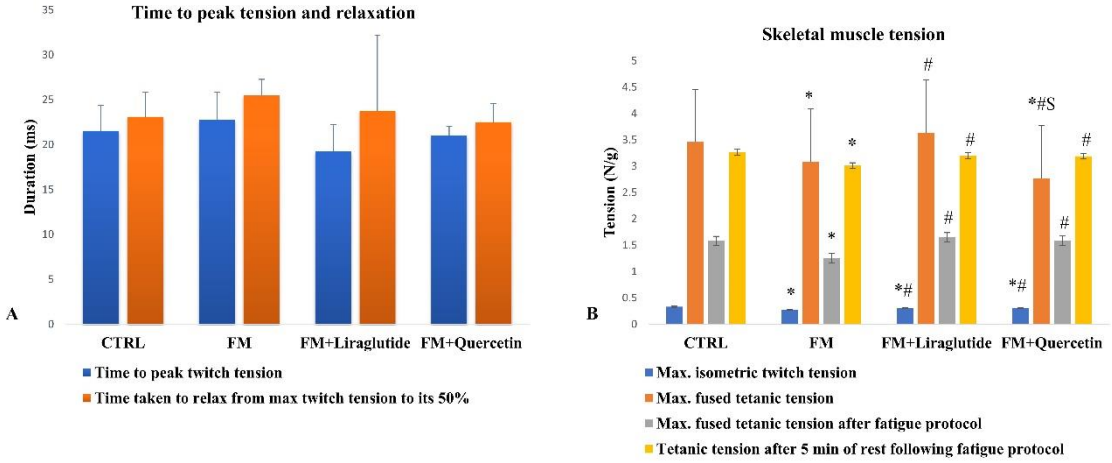

Figure 2: A) Time to peak tension and relaxation, and **B)** Skeletal muscle tension in all study groups. Parameters were represented in mean ± standard deviation. CTRL: Control, FM: Fibromyalgia. *: significant from CTRL, #: significant from FM, $: significant from FM + Liraglutide. Statistical significance means p-value ≤ 0.05.

3.5. Oxidative stress biomarkers results

As displayed in **Figure 3A**, in comparison to the CTRL group, the muscular tissue MDA level significantly increased in the FM group, concomitantly with a reduction in muscle tissue CAT (p=0.000). However, compared to the FM group, the OS homeostasis significantly improved (decreased MDA and increased CAT, p=0.000) in the FM + Liraglutide attaining the normal values of the MDA when compared to the CTRL, but regarding CAT a significant difference is still existing (p=0.026).

The MDA and CAT exhibited a notable improvement in FM + Quercetin group in comparison to FM **(**p=0.002 and 0.007, respectively) although MDA significantly improved attaining the standard normal values however the CAT was not completely recovered when compared to CTRL rats (p=0.001). Nevertheless, the valuable effects of Liraglutide and Quercetin on OS were similar (p=0.000).

3.6. Relative expression of Nrf2, HO1and NQO1 results

As revealed in **Figure3B**, the gene expression of *Nrf2*, *HO1*and *NQO1* significantly declined in FM group in contrast to CTRL (p= 0.000). However, their gene expression significantly improved in the FM + Liraglutide (p= 0.000, 0.000 and 0.002, respectively) and FM + Quercetin (p= 0.000, 0.000 and 0.014, respectively) groups. In contrast to CTRL, in the FM + Liraglutide group, *NQO1* gene expression was normalized while Nrf2 and *HO1* gene expression did not realize the normal values (p= 0.001 and 0.000, correspondingly). Nrf2, HO1and NQO1 gene expression of the Quercetin group although being improved, were not normalized relative to the CTRL values (p= 0.000, 0.000 and 0.021, respectively). It is noteworthy that there was a lack of statistical significance between the implications of Liraglutide and Quercetin.

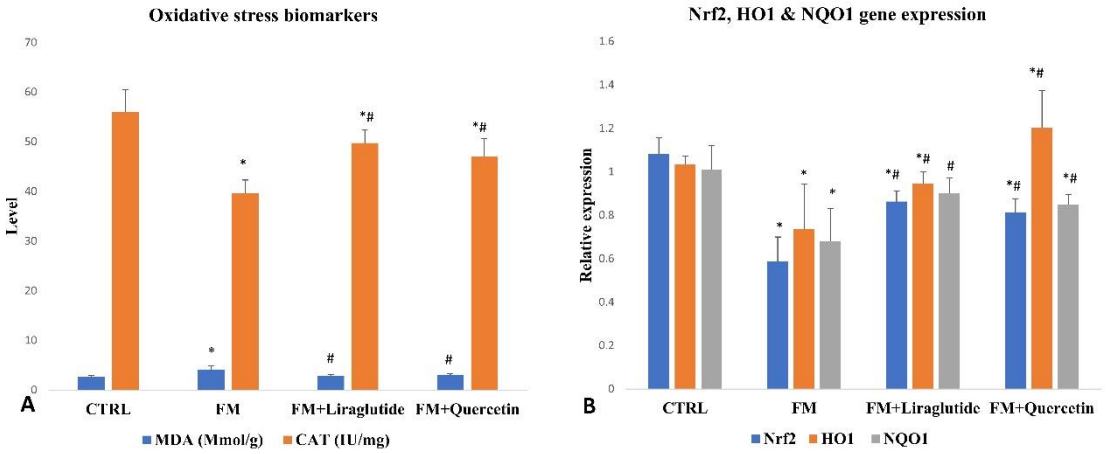

Figure 3: A) Oxidative stress biomarkers, and **B)** Nrf2, HO1 and NQO1 in all study groups. Parameters were represented in mean ± standard deviation. CTRL: Control, FM: Fibromyalgia, MDA: Malondialdehyde, CAT: Catalase, Nrf2: Nuclear factor erythroid 2-related factor 2, HO1: Hemoxygenase1, NQO1: NAD(P)H: quinone oxidoreductase 1. *: significant from CTRL, #: significant from FM. Statistical significance means p-value ≤ 0.05.

3.7. Correlations

Figure 4A revealed a significant strong inverse relationship between Nrf2 relative expression and serum substance P concentration (r=-0.834). However, strong significant positive relationships between the Nrf2 relative expression and the mechanical PPT (r=0.798), max. isometric twitch tension (r=0.755), max. fused tetanic tension after fatigue (r=0.686), and tetanic tension after 5 min of rest after the fatigue procedure (r=0.713) were illustrated in **Figures 4B-E.** All p values were equal to 0.000.

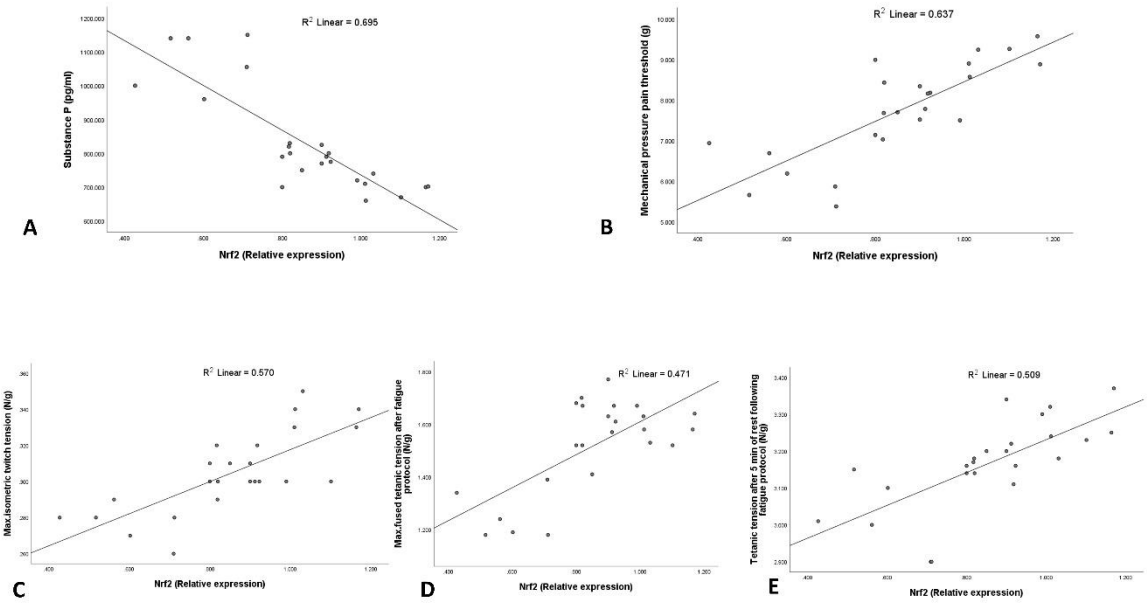

Figure 4: Correlation between Nrf2 relative expression and each of the following: **A)** Serum substance P concentration (r= -0.834), **B)** Mechanical pressure pain threshold (r=0.798), **C)** Max. isometric twitch tension (r=0.755), **D)** Max. fused tetanic tension after fatigue protocol (r=0.686) and **E)** Tetanic tension after 5 min of rest following fatigue protocol (r=0.713). All p- values were equal to 0.000.

4. Discussion

Widespread musculoskeletal pains and exhaustion are hallmarks of FM along with other physical and cognitive symptoms. The present research found that reserpine reduced mechanical PPT concurrent with an increase in serum substance P level and caused musculoskeletal deficits that resembled FM, as evidenced by compromised hindlimb muscle motor performance in rats and OS imbalance (decreased CAT and increased MDA) attributed to diminished expression of muscle Nrf2, HO1, and NQO1.

When monoaminergic neurotransmission malfunctions in FM, excitatory neurotransmitters like substance P, which stimulate N-methyl-D-aspartate receptors that transmit pain, are trebly elevated[15]. Our findings largely corroborated those of *Hassan et al.*, who found that reserpine reduced the contractile capabilities of skeletal muscle in correlation with elevated OS indicators, which were caused by a reduced expression in peroxisome proliferator-activated receptor-gamma (PPAR-γ) [1]. While *Favero et al.* linked the musculoskeletal disorders to constitutive markers implicated in OS, myogenesis, and inflammation including cyclooxygenase (COX)-1 and sirtuin 3[16]. Furthermore, the lipid peroxidation in FM was made worse by the down-

regulation of PPAR-γ coactivator 1-α (PGC1α) and its associated transcription factors Nrf2 and NQO1[17].

Even with a multitude of treatment plans available, managing FM effectively is still difficult. This work aimed to investigate the potential of new therapies such as Liraglutide and Quercetin to manage the musculoskeletal symptoms and pain in rats with FM.

In the current research, the motor activity of the hindlimb muscles of rats was enhanced by both Liraglutide and Quercetin. Additionally, there was a significant improvement in the muscle OS homeostasis (increased CAT and decreased MDA) due to raised expression of muscle Nrf2, HO1, and NQO1. Both drugs improved mechanical PPT concurrent with decreased serum substance P level.

GLP-1R is extensively expressed in various tissues, among them muscles as well as neurons[18]. Research has shown that GLP-1R agonists can be effectively used to treat conditions other than usual metabolic objectives. These include neuroprotection, synaptic plasticity, and neuroinflammation regulation. They have also been shown to reduce pain and other motor and non-motor manifestations[7]. In pain models, GLP-1R agonists reduced sensitization[19], thermal and mechanical hyperalgesia by upregulating β-endorphins, interleukin (IL)-4 and -10 expression in spinal microglia and downregulating prostaglandin E2 (PGE2), nitric oxide and IL-6. Additionally, it caused a switch in macrophages from pro- (M1) to anti- (M2) inflammatory subtypes, along with an increase in superoxide dismutase[20–24].

To the best of our knowledge, no prior research has examined how Liraglutide might affect the skeletal muscles' contractile powers in FM. However, in skeletal muscle atrophy, Liraglutide demonstrated a myogenic aptitude by promoting MyoD, myogenin, and myosin-heavy chain expression in either myoblasts or myotubes or both of them which are rich in GLP-1R [25].

Moreover, GLP-1 analogues cause myogenesis along with a sharp increase in cyclic adenosine monophosphate (cAMP), which is essential for triggering p38, extracellular signal-regulated kinase (ERK), and protein kinase B and directing all the downstream effectors through protein kinase A (PKA) and exchange proteins directly activated by cAMP (EPAC)-dependent pathways. Furthermore, Liraglutide promotes faster healing in many atrophy models by downregulating atrogin-1 and MuRF1 and upregulating cAMP-response element binding protein (CREB), AKT, FoxO1/3, and glycogen synthase kinase-3 beta (GSK3β) phosphorylation expression [26]. The cumulative effect of these strong restorative and protective properties with established skeletal anabolic properties may account for the Liraglutide’s potent restorative attributes on skeletal muscle's mechanical performance as seen in this study.

Nrf2 couples with AREs to influence several detoxification genes expression and antioxidants. Thus, treatments that target the Nrf2 signaling pathway offer a prospective treatment option for many oxidative damage-related disorders[4]. GLP-1 exhibits antioxidative properties and influences several markers of OS (lipid peroxidation, glutathione reductase, CAT, superoxide dismutase, and nonenzymatic

glycosylated proteins). The stimulation of Nrf-2 and receptor-mediated cAMP, phosphoinositide 3-kinase, protein kinase C (PKC) and cAMP-mediated PKA/ERK pathways was determined to be the mechanism by which GLP-1 reduces OS[27,28].

Herbal medications are frequently used to treat pain, particularly when traditional treatments have failed or when side effects are a consideration. Some plant-based chemical molecules, such as the phytochemicals in food, provide a variety of beneficial biological attributes for human health[29].

Quercetin, a phytochemical, is commonly present in fruits and vegetables. Quercetin inhibits the enzyme that produces PGE2 and the COX-2-cascade, as well as regulates neuronal excitability, involving nociceptive sensory transmission via voltage-gated potassium, sodium, and calcium channels. Consequently, Quercetin may have potential as a treatment for nociceptive and/or pathological pain[30,31]. Additionally, it has been observed that Quercetin inhibits the synthesis of neuropeptides including substance P and nerve growth factor[31].

As an adenosine A1 receptor antagonist, Quercetin has been shown to improve exercise tolerance and lessen fatigue[32]. In addition to recovering the neuromuscular activity by limiting reactive oxygen species generation, lipid oxidative degradation, C-reactive protein and IL-6, Quercetin can additionally defend against muscle injury by decreasing the incidence of decline in strength attributed to deterioration in action potential dissemination propagation as well as myofibrils in eccentric exercise-induced muscle damage[33,34]. In addition, Quercetin's obvious impacts on the brain play a part in clarifying the neuromuscular function resurgence accompanying eccentric exercise-induced muscle damage[33].

Because Nrf2 affects mitochondrial biogenesis and dysfunction, its depletion exacerbates muscle atrophy and frailty. It has been observed that phytonutrient interventions activate the Nrf2/HO1 pathway[35]. Quercetin is one of the phytonutrients that reduce the degree of muscle wasting caused by metabolic disorders by blocking the atrophic factors (MuRF1 and MAFbx/atrogin-1) that are generated by tumor necrosis factor-α through the Nrf2/HO1 pathway and therefore deactivating nuclear factor- kappa B [36,37]. Likewise, by triggering the Nrf2/HO1 pathway, Quercetin protected against glutamate-induced oxidative HT22 rat hippocampus neuronal cell death[38] and on central neurons to protect them from long-term hyperglycemia by enhancing the Nrf2/ARE/glyoxalase-1 pathway, which is mediated by GSK-3 suppression or PKC stimulation[39].

5. Conclusion

In conclusion, our research demonstrated the intricate relationship between FM-associated muscle dysfunction and OS. Notably, by modifying the Nrf2/HO1 and NQO1 signaling, the introduction of Liraglutide and Quercetin as therapeutics produced substantial changes in motor activity, pain threshold, and OS indicators.

6. Limitations

Among the limitations of this study is the use of a rat model, which may not adequately capture the complexities of FM in humans. Additionally, the sample size was relatively small, which may impact the generalizability of the results. Additionally, quantifying the proteins of the

examined genes would highlight our findings even more. In later research, we will investigate at more fundamental molecular mechanisms to overcome these limitations.

Declarations

Authors' contributions: All the authors fairly contributed to the Conceptualization, Methodology, Investigation, Formal analysis, Writing – Review and Editing of the manuscript.

Consent to participate: Not relevant.

Consent for publication: All the authors reviewed and agreed to the publication of the manuscript in the current format.

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Data availability:

All information supporting the results of the current investigation is available upon request.

Competing interests: None.

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