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Annatto-Derived Geranylgeraniol- and δ-Tocotrienol-Rich Chronic® Formulations Attenuate Dexamethasone-Induced Dynapenia-Like Muscle

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Abstract

Dynapenia, the age-related decline in muscle strength and physical performance, is driven by mechanisms including oxidative stress, low-grade inflammation, mitochondrial dysfunction, and activation of catabolic pathways. Bioactive compounds from *Bixa orellana* L. (annatto), particularly geranylgeraniol and δ-tocotrienol, may counteract these processes through antioxidant, anti-inflammatory, and metabolic actions. This study evaluated whether two annatto-derived formulations, Chronic® and Chronic In®, attenuate dexamethasone-induced dynapenia-like dysfunction in male Wistar rats. Animals were assigned to testosterone, Chronic In®, oral Chronic®, distilled water, injectable vehicle, or healthy normal groups. Dynapenia-like impairment was induced by oral dexamethasone (8 mg/kg/day for 7 days), while treatments were administered for 25 days. Motor coordination, strength, and endurance were assessed using weight-holding, inverted screen, rotarod, and forced-swim tests. Gastrocnemius histology and serum biochemical markers were also evaluated. The oil presented a δ-tocotrienol content of 0.725 ± 0.062 mg/mL, and the nanoformulation remained physically stable for 30 days, with particle sizes of 53.15 ± 0.64 nm at day 0 and 59.90 ± 3.63 nm at day 30. Dexamethasone markedly reduced performance in untreated controls, whereas Chronic® and Chronic In® attenuated functional decline, preserved gastrocnemius microarchitecture, and improved hepatic and lipid biochemical profiles relative to the water-treated group. These findings suggest that annatto-derived geranylgeraniol- and δ-tocotrienol-rich formulations exert protective effects in a glucocorticoid-induced model of dynapenia-like muscle dysfunction. Further mechanistic and clinical studies are warranted.

Introduction

Dynapenia is characterized by a clinically relevant decline in muscle strength and neuromuscular performance that is not necessarily proportional to the loss of skeletal muscle mass. It is a major determinant of frailty, disability, falls, and loss of independence in older adults, and it overlaps mechanistically with sarcopenia while emphasizing functional impairment as the central outcome (Clark and Manini 2008; Manini and Clark 2012; Tieland et al. 2018; Alexandre et al. 2019). Current evidence indicates that oxidative stress, chronic low-grade inflammation, impaired mitochondrial quality control, anabolic resistance, and activation of proteolytic signaling pathways contribute to muscle weakness during aging and disuse-like conditions (Musci et al. 2018; Leduc-Gaudet et al. 2021; Saud Gany et al. 2023; Ohinata et al. 2024).

Glucocorticoid exposure is widely used to model skeletal muscle atrophy because it promotes protein degradation, suppresses anabolic signaling, and increases oxidative and inflammatory stress. Dexamethasone-induced muscle dysfunction has therefore become a useful preclinical platform for testing interventions targeting sarcopenia- and dynapenia-like phenotypes (Kim et al. 2015; Xie et al. 2021; Kim et al. 2021; Seo et al. 2022; Wang et al. 2022). In this context, plant-derived nutraceuticals have attracted increasing interest because they may modulate multiple convergent pathways involved in muscle wasting rather than acting through a single hormonal mechanism (Wang et al. 2021; Bagherniya et al. 2022).

Bixa orellana L. is a source of bioactive apocarotenoids, tocotrienols, and isoprenoids with reported anti-inflammatory, antioxidant, and metabolic activities (Pacheco et al. 2019; Batista et al. 2022). Two constituents are particularly relevant to muscle health: δ-tocotrienol, a vitamin E isoform associated with antioxidant and anti-inflammatory effects, and geranylgeraniol, a C20 isoprenoid related to the mevalonate pathway and protein prenylation. Experimental evidence suggests that geranylgeraniol may attenuate muscle atrophy by suppressing Atrogin-1 and improving mitochondrial quality, whereas tocotrienols may reduce oxidative and inflammatory burden in aging-related musculoskeletal disorders (Miyawaki et al. 2020; Jiwan et al. 2022; Saud Gany et al. 2023; Verdaguer et al. 2022). The present study investigated whether Chronic® and Chronic In®, two annatto-derived formulations enriched in these compounds, attenuate dexamethasone-induced dynapenia-like dysfunction in Wistar rats.

Materials and methods

Formulations and chemical characterization

The standardized *B. orellana* oil formulation Chronic® was supplied by Ages Bioactive Compounds (batch 0012/2022). Chemical characterization focused on δ-tocotrienol quantification by gas chromatography coupled to mass spectrometry (GC-MS), using a laboratory-validated analytical procedure adapted for standardized annatto-derived oil formulations. A δ-tocotrienol stock solution was prepared in hexane (Sigma-Aldrich, MKCF5755, GC grade, Germany) at 10 mg/mL. Working solutions at 2.5, 1.0, 0.5, 0.25, and 0.1 mg/mL were used to generate the analytical curve.

Chronic In® was prepared as an injectable nanodispersion derived from the standardized *B. orellana* oil. The nanoparticulated formulation was obtained via nanoprecipitation, as adapted from Carvalho et al. (2023). Physical stability was monitored by visual inspection, pH measurement, particle-size distribution, polydispersity index (PDI), and zeta potential during the evaluation period.

Animals and ethical approval

The experimental protocol was approved by the Ethics Committee on Animal Use of the Federal University of Amapá (CEUA-UNIFAP; protocol no. 021/2022). Male Wistar rats (*Rattus norvegicus*), aged 4–8 months and weighing approximately 250–500 g, were obtained from the Multidisciplinary Center for Biological Investigation in Laboratory Animal Science, University of Campinas (CEMIB-UNICAMP, Brazil). Animals were acclimatized before testing and housed in polyethylene cages under controlled temperature (21 ± 2°C), a 12-h light/dark cycle, and free access to standard rodent chow and water.

Dexamethasone-induced dynapenia-like model

Dynapenia-like muscle dysfunction was induced by dexamethasone (DEX), a well-established glucocorticoid model of skeletal-muscle atrophy and functional decline (Kim et al. 2015; Seo et al. 2022).

DEX was administered orally by gavage at 8 mg/kg/day for 7 consecutive days. Murinometric and functional evaluations were performed before and after induction.

Experimental design and treatments

Animals were treated for 25 days, comprising 15 days before DEX induction, 7 days concomitant with DEX administration, and 3 days after induction. The experimental groups were organized as follows: testosterone-treated dynapenic rats (TES; 10 mg/kg, subcutaneous, every 4 days), Chronic In®-treated dynapenic rats (CHR IN; 5.0 mg/kg, intramuscular, every 3 days), Chronic®-treated dynapenic rats (CHR; 500 mg/kg, oral), distilled-water-treated dynapenic rats (WAT; 1 mL, oral), injectable-vehicle-treated dynapenic rats (VEH; Tween 5%, 0.1 mL, intramuscular, every 3 days), and untreated healthy normal rats (NOR). Doses of Chronic® and Chronic In® were selected based on previous preclinical studies and compatibility assessments of standardized *B. orellana* oil formulations (Batista et al. 2022; Carvalho et al. 2023).

Functional assessment

All behavioral and functional tests were performed after a brief acclimatization period in the experimental room. Animals were allowed to rest between tests to minimize fatigue-related carryover effects.

The inverted-screen test was adapted from established protocols for assessing four-limb strength and resistance (Kondziela 1964; Deacon 2013; Aartsma-Rus and van Putten 2014). Each rat was placed on a 50 × 50 cm wire grid, which was inverted 50 cm above a padded cage. The latency to fall was recorded, with a maximum cutoff of 120 s.

The weight-holding test was performed according to Deacon (2013) and Iannotti et al. (2019). Rats were allowed to grasp weights composed of steel-wire bundles attached to one to five metal nuts, corresponding to total loads of approximately 53, 103, 153, 203, and 253 g. Success was defined as holding the weight for at least 3 s. The final score was calculated from the heaviest weight successfully held and the holding time.

Motor coordination and balance were assessed using the rotarod test (Dunham 1957; Aartsma-Rus and van Putten 2014). The initial rotation speed was 5 rpm, which increased to 30 rpm during the first 15 s and remained constant thereafter. Each animal was given up to three attempts, and the longest latency to fall was used for analysis, with a maximum cutoff of 500 s.

The forced-swim test was used as an endurance-related functional challenge. Animals were placed in a glass tank containing water at 25°C. A 5-min pre-test was followed 24 h later by a 3-min test session in which active swimming and immobility times were recorded. Immobility was defined as the absence of

active escape movements, except those necessary to keep the head above water (Porsolt et al. 1977; Borsini and Meli 1988; Carvalho et al. 2009).

Biochemical analysis

Animals were anesthetized with sodium thiopental (45 mg/kg; Cristália, São Paulo, Brazil), and 1.5 mL of blood was collected from the retro-orbital plexus. Samples were centrifuged for 10 min at 5000 rpm. Serum biochemical markers were measured using Doles® kits (Goiânia, GO, Brazil) and a UV-VIS spectrophotometer (UVmini 1240, Shimadzu Corporation, Kyoto, Japan). The following markers were evaluated: aspartate aminotransferase (AST/TGO), alanine aminotransferase (ALT/TGP), myoglobin, creatine kinase (CK/CPK), lactate dehydrogenase (LDH), triglycerides, total cholesterol, urea, creatinine, glucose, and testosterone.

Histological analysis

After blood collection, animals were euthanized with a lethal dose of sodium thiopental. Gastrocnemius muscles were excised for macroscopic and microscopic analysis. Tissue samples were fixed in 10% buffered formaldehyde for 24 h, dehydrated in increasing ethanol concentrations (70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin at 60°C. Sections of 5 µm were obtained using a rotary microtome (CUT 5062, SLEE) and stained with hematoxylin and eosin.

Statistical analysis

Data were expressed as mean ± standard error of the mean. Statistical analysis was performed using GraphPad Prism version 7.0. Groups were compared using one-way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test. Differences were considered statistically significant at $p < 0.05$.

Results

Chemical characterization and physical stability of the annatto-derived formulations

GC-MS analysis identified the δ-tocotrienol ($C_{27}H_{40}O_2$), peak at a retention time of 28.6 min. The mean δ-tocotrienol concentration was 0.725 ± 0.062 mg/mL, corresponding to 72.0 ± 1.0%, and the analytical curve showed adequate linearity ($R^2 = 0.9973$). These findings confirm that the tested formulation was enriched in δ-tocotrienol, a bioactive component relevant to antioxidant and anti-inflammatory mechanisms.

The Chronic In® nanodispersion remained visually stable throughout the evaluation period, with no evidence of phase separation or precipitation. The formulation showed a slightly yellow appearance and

a pH of 5.8. Mean particle size was 53.15 ± 0.64 nm at day 0 and 59.90 ± 3.63 nm at day 30, with a PDI of 0.574 ± 0.032. Zeta potential remained similar over time, with values of 19.86 ± 0.60 mV at day 0 and 19.66 ± 1.45 mV at day 30. These values indicate short-term physical stability of the injectable formulation. This results were published by Gomes et al. (2025).

Body-weight evolution

Body-weight monitoring during the 25-day protocol showed that DEX-treated groups lost more weight than the healthy NOR group. The WAT and VEH groups exhibited the greatest weight losses, indicating that the vehicle conditions did not prevent the deleterious systemic impact of DEX. TES, CHR IN, and CHR groups also showed reductions in body weight; however, these changes were less pronounced than those observed in the untreated or vehicle-treated dynapenic controls. Area-under-the-curve analysis supported these trends, with the NOR group showing the most stable body-weight profile across the experiment (Fig. 1).

Figure 1.

Weight-holding performance

The weight-holding test showed a marked decline in the WAT group, indicating impaired forelimb strength and resistance after DEX exposure. The VEH group also showed reduced performance, although the decline was less severe than in WAT. In contrast, TES and CHR maintained relatively stable scores throughout the experimental period, whereas CHR IN showed only a mild decrease. These results suggest that both annatto-derived formulations attenuated DEX-related deterioration in weight-supporting capacity, with performance remaining closer to the pharmacological comparator and clearly superior to the water-treated dynapenic group (Fig. 2).

Figure 2.

Inverted-screen test

Before treatment, NOR animals exhibited the longest suspension time, indicating superior baseline strength and resistance. After DEX induction, NOR animals maintained the best performance. TES and VEH showed significantly lower suspension times than NOR, whereas WAT remained among the lowest-performing groups. CHR IN and CHR performed better than WAT after treatment, suggesting partial preservation of four-limb strength. CHR showed a particularly relevant functional pattern, remaining above WAT and closer to the treated groups than to untreated dynapenic controls (Fig. 3).

Figure 3.

Rotarod performance

Rotarod testing demonstrated that DEX impaired motor coordination and balance, particularly in the untreated control groups. WAT showed a 54.08% reduction in latency to fall, decreasing from 39.20 s

before induction to 18.00 s after induction. VEH exhibited a 70.38% reduction, decreasing from 99.00 to 29.33 s. TES showed a smaller decline of 23.82% (42.67 to 32.50 s), consistent with partial protection.

Both annatto-derived formulations attenuated the DEX-induced decline in rotarod performance. CHR IN decreased from 66.20 to 52.00 s, corresponding to a 21.49% reduction. CHR showed the smallest decline among the dynapenic groups, decreasing from 86.60 to 74.75 s, corresponding to a 13.67% reduction. NOR animals increased their latency from 97.00 to 119.33 s, probably reflecting task learning or adaptation (Fig. 4).

Figure 4.

Forced-swim performance

Before DEX induction, active-swimming times were relatively homogeneous among groups, ranging from 84.75 s in VEH to 116.25 s in TES. After induction, WAT showed a clear impairment, with active swimming decreasing from 106.00 to 68.33 s (a 35.54% reduction) and immobility increasing from 74.00 to 111.67 s (a 51.13% increase). TES showed a moderate reduction in active swimming (116.25 to 88.75 s; 23.65% reduction) and an increase in immobility (63.75 to 91.25 s).

CHR IN showed the most stable endurance-related profile among DEX-treated animals. Active swimming decreased only from 99.80 to 97.33 s (2.47% reduction), and immobility increased modestly from 80.20 to 82.67 s. CHR showed a less pronounced protective effect in this test, with active swimming decreasing from 89.40 to 71.75 s and immobility increasing from 90.60 to 108.25 s. NOR animals maintained a stable profile, with active swimming decreasing from 105.00 to 100.33 s and immobility increasing slightly from 75.00 to 79.67 s (Fig. 5).

Figure 5.

Gastrocnemius microarchitecture

Histological examination of the gastrocnemius muscle revealed treatment-dependent differences in skeletal-muscle microarchitecture. TES animals showed muscle-fiber hypertrophy, but this was accompanied by mild structural disorganization and interstitial separation, suggesting that increased fiber size did not necessarily translate into fully preserved tissue organization (Fig. 6).

CHR IN and CHR groups exhibited better preservation of muscle architecture than the WAT and VEH controls. CHR IN showed more uniform fibers than WAT, whereas CHR displayed structural organization closer to NOR, with preserved alignment and no evident edema or inflammatory disorganization. WAT and VEH groups showed fiber disorganization and interstitial separation, particularly in WAT. NOR animals exhibited the expected healthy pattern, characterized by aligned, homogeneous fibers without significant structural disruption (Fig. 6).

Figure 6.

Biochemical markers

Serum biochemical analysis showed a favorable global profile for CHR IN and CHR compared with WAT, especially for markers of hepatic function and lipid metabolism. AST/TGO and ALT/TGP were markedly elevated in WAT (305 ± 105.24 and 268.0 ± 59.8 U/L, respectively), whereas TES, CHR IN, CHR, and NOR showed significantly lower values. CPK was highest in WAT (495 ± 119.5 U/L) and TES (486 ± 114.5 U/L), while CHR IN showed the lowest CPK value among DEX-exposed groups (93.5 ± 56.78 U/L). Total cholesterol, LDL, and triglycerides were also higher in WAT than in most treated groups. C-reactive protein and myoglobin showed limited variation among groups (Table 1).

Table 1

Serum biochemical profile of rats subjected to dexamethasone-induced dynapenia-like dysfunction and treated with annatto-derived Chronic® formulations. Serum concentrations of biochemical markers were measured to assess systemic metabolic, hepatic, renal, and tissue-injury responses after dexamethasone exposure and treatment.

Parameter	TES	CHR IN	CHR	WAT	VEH	NOR
Creatinine (mg/dL)	0.51 ± 0.02	0.41 ± 0.04	0.60 ± 0.02	0.44 ± 0.02	0.46 ± 0.04	0.60 ± 0.07
Urea (mg/dL)	33.3 ± 11.23	25.2 ± 4.03	51.5 ± 57.21	39.20 ± 5.10	30.3 ± 3.04	48.0 ± 8.48
AST/TGO (U/L)	39.0 ± 17.2*	44.8 ± 28.12*	109.2 ± 26.17*	305 ± 105.24	174.3 ± 42.7	130 ± 22.62*
ALT/TGP (U/L)	70.6 ± 25.6*	62.6 ± 9.63*	64.20 ± 16.05*	268.0 ± 59.8	203.3 ± 50.70	95.5 ± 12.26*
CPK (U/L)	486 ± 114.5*	93.5 ± 56.78*	244.5 ± 107*	495 ± 119.5	249 ± 119.5	125 ± 53.74*
C-reactive protein (U/L)	0.23 ± 0.15	0.40 ± 0.10	0.42 ± 0.09	0.50 ± 0.03	0.46 ± 0.10	0.50 ± 0.14
Myoglobin (U/L)	5.20 ± 0.50	5.00 ± 0.10	5.50 ± 0.10	5.25 ± 0.05	5.25 ± 0.05	5.00 ± 0.14
Glucose (mg/dL)	155.3 ± 105	261.2 ± 135.2	259.0 ± 169.4	135.5 ± 7.07	191 ± 63.6	139.5 ± 16.26
Total cholesterol (mg/dL)	107.0 ± 42.5*	103.7 ± 8.95*	124.0 ± 72.8*	193.5 ± 14.84	145.3 ± 16.19	81.5 ± 14.84*
HDL (mg/dL)	17.66 ± 4.16	24.2 ± 6.95	24.5 ± 6.95	27.5 ± 0.70	29.6 ± 2.08	22.5 ± 2.12
LDL (mg/dL)	64.73 ± 19.3*	45.2 ± 136.2*	98.5 ± 62.95*	173.4 ± 10.25	104.3 ± 14.45	27.4 ± 11.59*
Triglycerides (mg/dL)	70.2 ± 13.42*	79.5 ± 11.61*	47 ± 78.26*	196.0 ± 15.0	115.66 ± 14.2	59 ± 12.72*
Creatinine (mg/dL)	0.51 ± 0.02	0.41 ± 0.04	0.60 ± 0.02	0.44 ± 0.02	0.46 ± 0.04	0.60 ± 0.07
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AST/TGO (U/L)	39.0 ± 17.2*	44.8 ± 28.12*	109.2 ± 26.17*	305 ± 105.24	174.3 ± 42.7	130 ± 22.62*

TES, testosterone-treated dynapenic rats; CHR IN, injectable Chronic In®-treated dynapenic rats; CHR, oral Chronic®-treated dynapenic rats; WAT, distilled-water-treated dynapenic rats; VEH, injectable-vehicle-treated dynapenic rats; NOR, healthy normal rats. Values are expressed as mean ± standard error of the mean (SEM). *p < 0.05 versus WAT = Normal rats. Values are expressed as mean ± standard error of the mean. *p < 0.05 versus WAT.

Parameter	TES	CHR IN	CHR	WAT	VEH	NOR
ALT/TGP (U/L)	70.6 ± 25.6*	62.6 ± 9.63*	64.20 ± 16.05*	268.0 ± 59.8	203.3 ± 50.70	95.5 ± 12.26*
CPK (U/L)	486 ± 114.5*	93.5 ± 56.78*	244.5 ± 107*	495 ± 119.5	249 ± 119.5	125 ± 53.74*
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TES, testosterone-treated dynapenic rats; CHR IN, injectable Chronic In®-treated dynapenic rats; CHR, oral Chronic®-treated dynapenic rats; WAT, distilled-water-treated dynapenic rats; VEH, injectable-vehicle-treated dynapenic rats; NOR, healthy normal rats. Values are expressed as mean ± standard error of the mean (SEM). *p < 0.05 versus WAT = Normal rats. Values are expressed as mean ± standard error of the mean. *p < 0.05 versus WAT.

Table 1.

Discussion

The present study shows that two annatto-derived formulations, Chronic® and Chronic In®, attenuated functional, histological, and biochemical alterations induced by dexamethasone in Wistar rats. The main finding is that both formulations preserved clinically relevant dimensions of skeletal-muscle performance, including weight-holding capacity, four-limb suspension, motor coordination, and endurance-related behavior, while also maintaining a more organized gastrocnemius microarchitecture than untreated dynapenic controls. These results support the hypothesis that annatto-derived geranylgeraniol- and δ-tocotrienol-rich formulations may counteract dynapenia-like dysfunction through mechanisms that extend beyond simple anabolic stimulation.

The DEX model used here reproduces key features of muscle-wasting conditions by increasing proteolysis, reducing anabolic signaling, and promoting oxidative and inflammatory stress. Recent preclinical studies using DEX-induced atrophy have identified AKT/mTOR/FOXO3a signaling, modulation

of oxidative stress, and suppression of inflammatory mediators as relevant therapeutic targets (Kim et al. 2021; Seo et al. 2022; Wang et al. 2022). The poor performance of WAT and VEH animals in the rotarod, forced-swim, inverted-screen, and weight-holding tests confirms that the model produced robust functional impairment. In contrast, CHR and CHR IN improved or preserved functional performance across complementary endpoints, suggesting that these formulations mitigated DEX-related decline in strength, coordination, and resistance.

The composition of the formulations provides a biologically plausible explanation for the observed effects. The tested oil was rich in δ-tocotrienol, and annatto-derived products are also recognized sources of geranylgeraniol and tocotrienols. Tocotrienols can reduce lipid peroxidation, inflammatory signaling, and cellular senescence-associated inflammatory pathways, which are increasingly recognized as contributors to skeletal-muscle aging (Malavolta et al. 2018; Khor et al. 2021; Saud Gany et al. 2023). Geranylgeraniol is mechanistically relevant because it participates in the mevalonate pathway and may influence protein prenylation, mitochondrial quality, and proteolysis. Miyawaki et al. (2020) showed that oral geranylgeraniol rescued denervation-induced muscle atrophy through suppression of Atrogin-1, while Jiwan et al. (2022) reported that geranylgeraniol mitigated soleus atrophy in diabetic rats through changes in mitochondrial quality. These mechanisms align with the preservation of muscle performance and tissue organization observed in CHR and CHR IN groups.

The histological findings strengthen this interpretation. Testosterone promoted fiber hypertrophy, as expected from its anabolic action, but mild disorganization and interstitial separation were still observed. By contrast, CHR and CHR IN preserved fiber uniformity and tissue alignment more consistently, especially when compared with WAT and VEH. This distinction is important in dynapenia research because greater muscle size does not always imply better contractile quality or neuromuscular function. The functional benefits observed with the annatto-derived formulations may therefore reflect improved muscle quality, reduced catabolic stress, or better preservation of tissue architecture rather than hypertrophy alone.

Biochemical data also support a broader protective profile. WAT animals showed higher AST/TGO, ALT/TGP, total cholesterol, LDL, triglycerides, and CPK values, indicating systemic metabolic and tissue-stress alterations after DEX exposure. CHR and CHR IN reduced hepatic enzyme and lipid-marker elevations relative to WAT, consistent with previous reports that tocotrienol-rich annatto fractions and geranylgeraniol may improve inflammatory and metabolic parameters (Shen et al. 2021; Chung et al. 2021; Batista et al. 2022). The particularly low CPK value in CHR IN suggests reduced muscle damage or leakage of muscle-associated enzymes, which is compatible with the better endurance performance and preserved histology observed in this group.

The differences in responses between the oral and injectable formulations may reflect formulation-dependent pharmacokinetics, bioavailability, and tissue exposure. CHR showed the smallest decline in rotarod performance, whereas CHR IN provided the most stable forced-swim profile and the lowest CPK level among DEX-exposed groups. Nanometric particle size and physical stability may have contributed

to the injectable formulation's performance by improving dispersion and tissue compatibility, consistent with previous work on standardized *B. orellana* oil nanodispersions (Carvalho et al. 2023). Nevertheless, the current study was not designed to compare pharmacokinetic profiles, and this interpretation requires direct confirmation.

This study has limitations. Molecular markers of oxidative stress, inflammation, mitochondrial function, and muscle protein turnover were not measured, thereby preventing direct confirmation of the proposed mechanisms. In addition, although DEX is a useful model of muscle atrophy, it does not fully reproduce the multifactorial nature of aging-related dynapenia. Future studies should quantify muscle cross-sectional area, grip strength, inflammatory cytokines, oxidative stress markers, mitochondrial dynamics, and catabolic/anabolic signaling proteins such as Atrogin-1, MuRF1, FOXO3a, AKT, and mTOR. Pharmacokinetic studies comparing oral Chronic® and injectable Chronic In® are also warranted.

Conclusions

Chronic® and Chronic In®, annatto-derived formulations enriched in geranylgeraniol and δ-tocotrienol, attenuated dexamethasone-induced dynapenia-like functional decline in Wistar rats. The formulations preserved motor coordination, endurance-related performance, and muscle microarchitecture, and they improved selected hepatic and lipid biochemical markers relative to untreated dynapenic controls. These effects are plausibly associated with the antioxidant, anti-inflammatory, metabolic and anti-catabolic potential of annatto-derived tocotrienols and geranylgeraniol. The findings support further investigation of these nutraceutical formulations as complementary strategies for muscle dysfunction; however, mechanistic studies and clinical trials are required before translation to human dynapenia or sarcopenia.

Declarations

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Data availability

The datasets generated during the current study are available from the corresponding author on reasonable request.

Ethics approval

The animal study was approved by the Ethics Committee on Animal Use of the Federal University of Amapá (CEUA-UNIFAP; protocol no. 021/2022).

Conflict of interest

The authors should declare whether they have any financial or non-financial competing interests related to Chronic®, Chronic In® or Ages Bioactive Compounds before submission.

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Figures

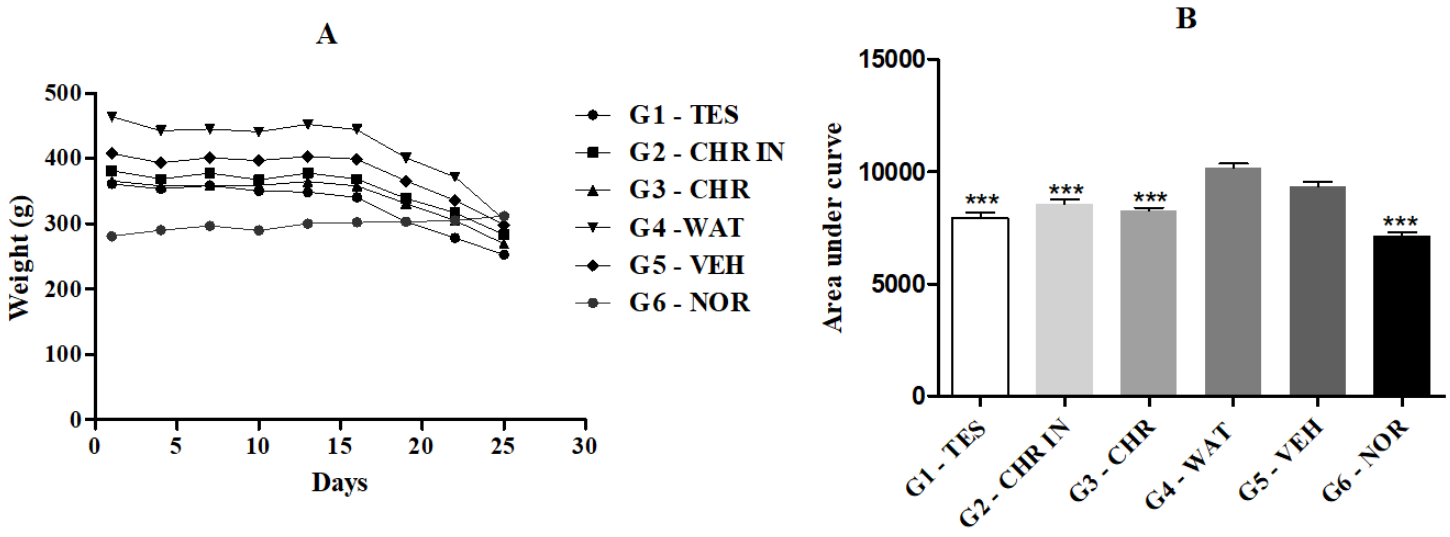


Figure 1

Body-weight trajectory during the dexamethasone-induced dynapenia-like protocol. (A) Longitudinal body-weight variation across the 25-day experimental period, including the pretreatment, DEX-induction, and post-induction phases. (B) Area under the curve summarizing cumulative body-weight change. DEX-exposed control groups showed more pronounced weight loss than healthy normal animals, whereas testosterone, Chronic In®, and oral Chronic® partially attenuated this systemic decline. Data should be presented as mean ± SEM. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group.

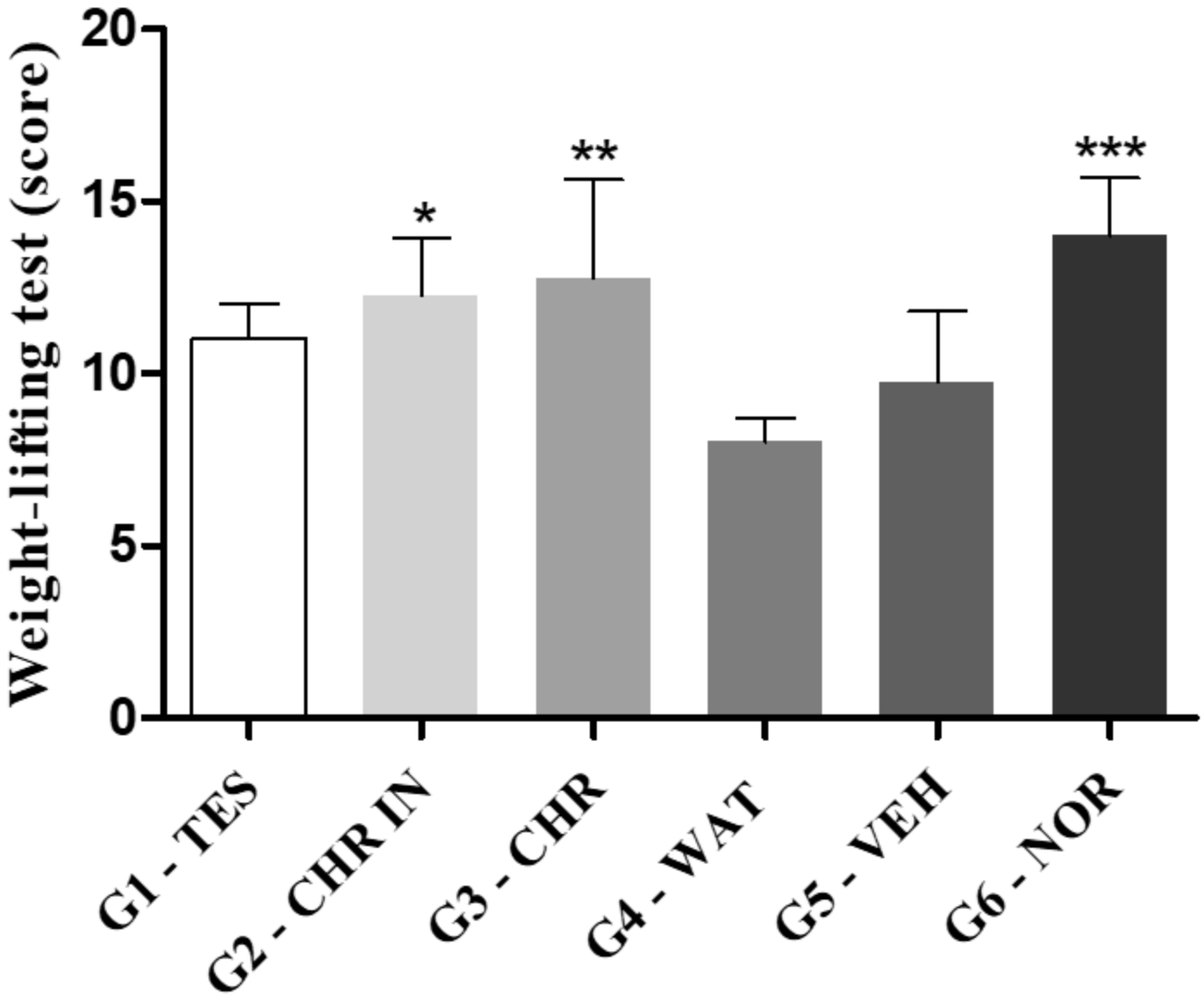


Figure 2

Annatto-derived Chronic® formulations preserve weight-holding capacity after dexamethasone exposure. Weight-holding performance was used as an index of forelimb strength and resistance in rats subjected to DEX-induced dynapenia-like dysfunction. The WAT group showed the greatest functional decline, whereas TES, CHR IN, and CHR maintained higher performance, indicating partial protection against DEX-related loss of weight-supporting capacity. Data are expressed as mean ± SEM. Statistical comparisons were performed by one-way ANOVA followed by Tukey–Kramer multiple-comparison test. *p < 0.05, **p < 0.01 and ***p < 0.001 versus WAT, where indicated. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group.

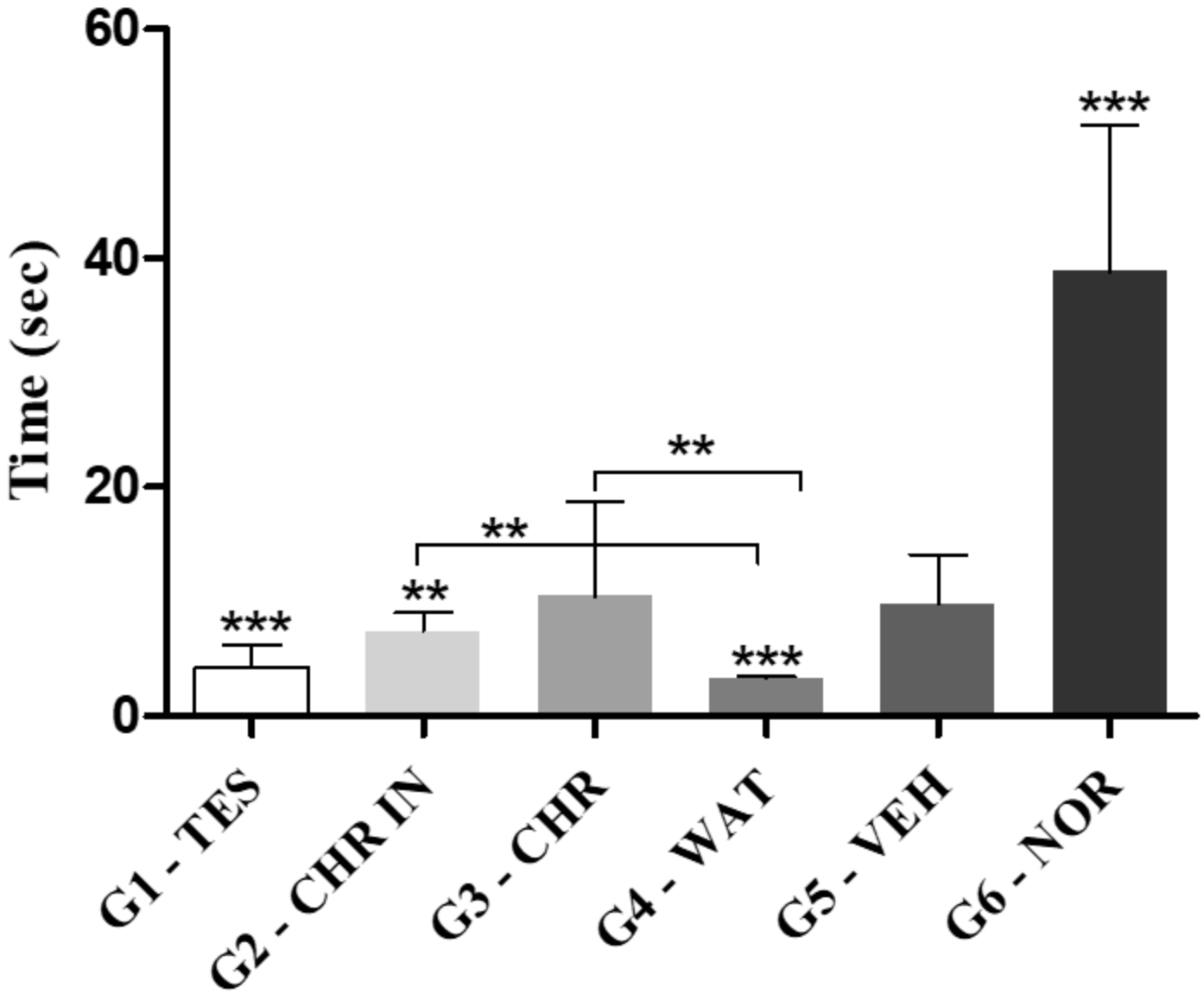


Figure 3

Chronic In® and oral Chronic® improve four-limb suspension performance in dexamethasone-induced dynapenic rats. The inverted-screen test measured the latency to fall as an integrated indicator of four-limb strength, resistance, and neuromuscular coordination. DEX-treated WAT animals exhibited poor suspension performance, whereas CHR IN and CHR showed higher latencies, suggesting partial preservation of muscle function relative to untreated dynapenic controls. Data are expressed as mean ± SEM. When shown, statistical symbols indicate significant differences according to one-way ANOVA followed by Tukey–Kramer multiple-comparison test. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group.

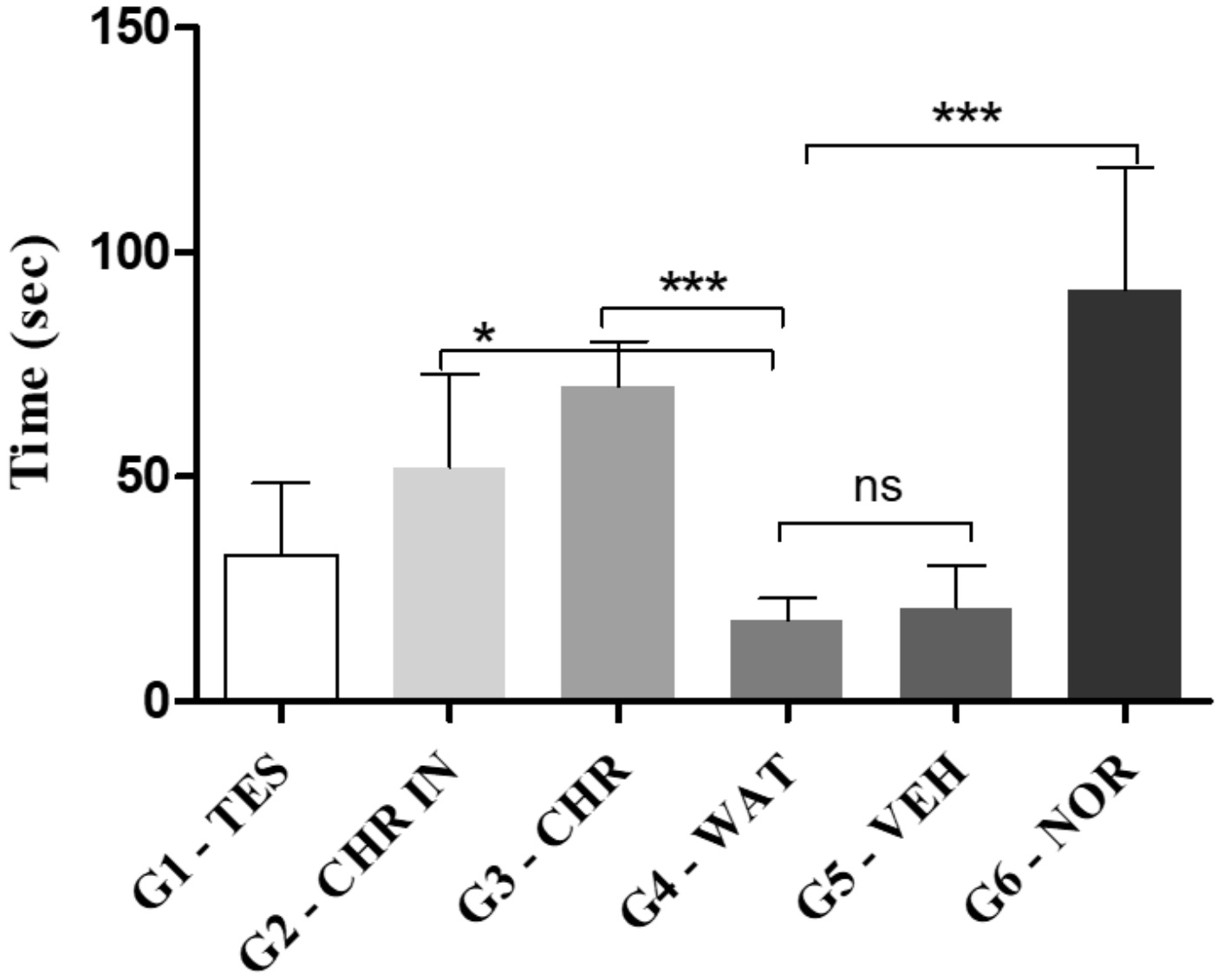


Figure 4

Oral Chronic® shows the strongest preservation of motor coordination in the rotarod test. Rotarod latency to fall was used to assess balance, coordination, and resistance to fatigue before and after DEX induction. Untreated and vehicle-treated dynapenic controls exhibited marked reductions in performance, whereas CHR IN and especially CHR attenuated the DEX-induced decline, with oral Chronic® showing the smallest percentage reduction among dynapenic groups. Data are expressed as mean ± SEM. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group.

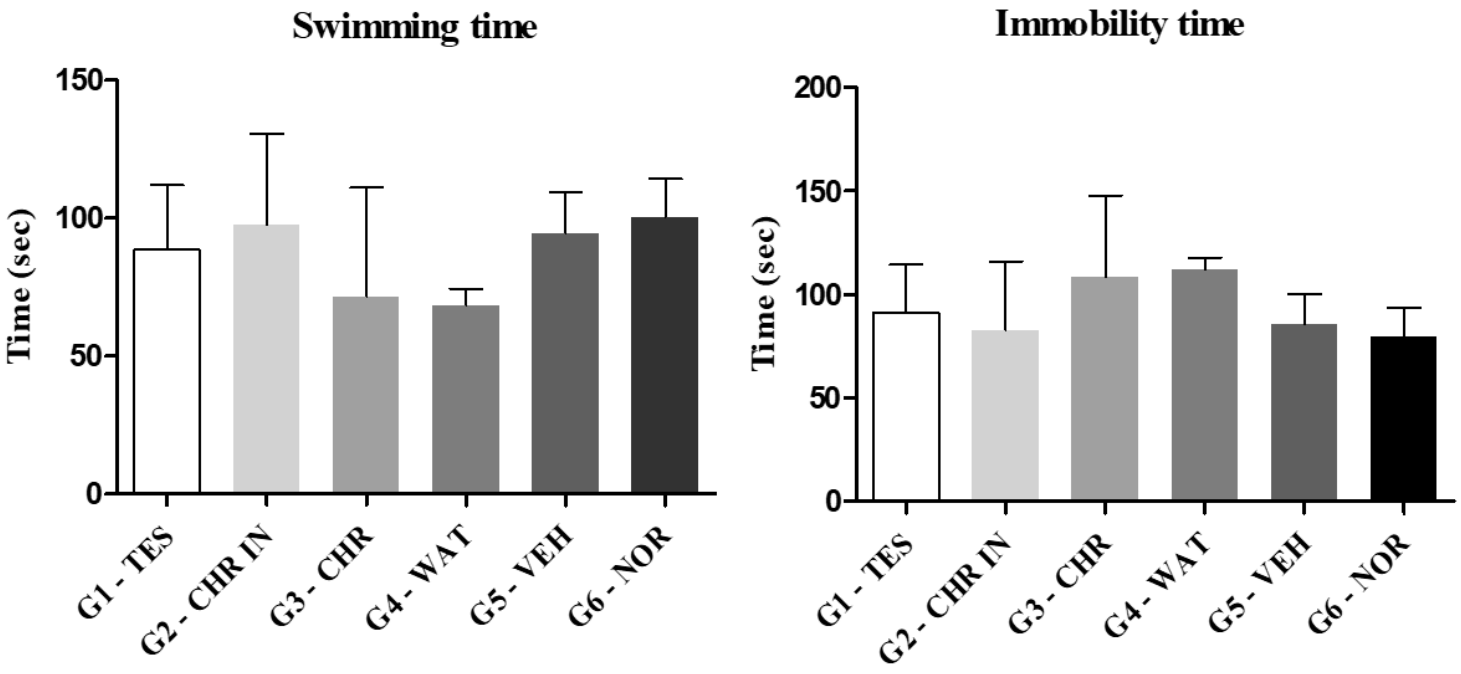


Figure 5

Injectable Chronic In® stabilizes endurance-related behavior in the forced-swim challenge. Active swimming time and immobility were recorded before and after DEX-induced dynapenia-like dysfunction as functional readouts of endurance-related behavior. WAT animals showed reduced active swimming and increased immobility after DEX exposure, whereas CHR IN maintained an almost unchanged active-swimming profile and only a modest increase in immobility. These findings indicate a protective effect of the injectable annatto-derived nanodispersion on fatigue-related functional impairment. Data are expressed as mean ± SEM. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group.

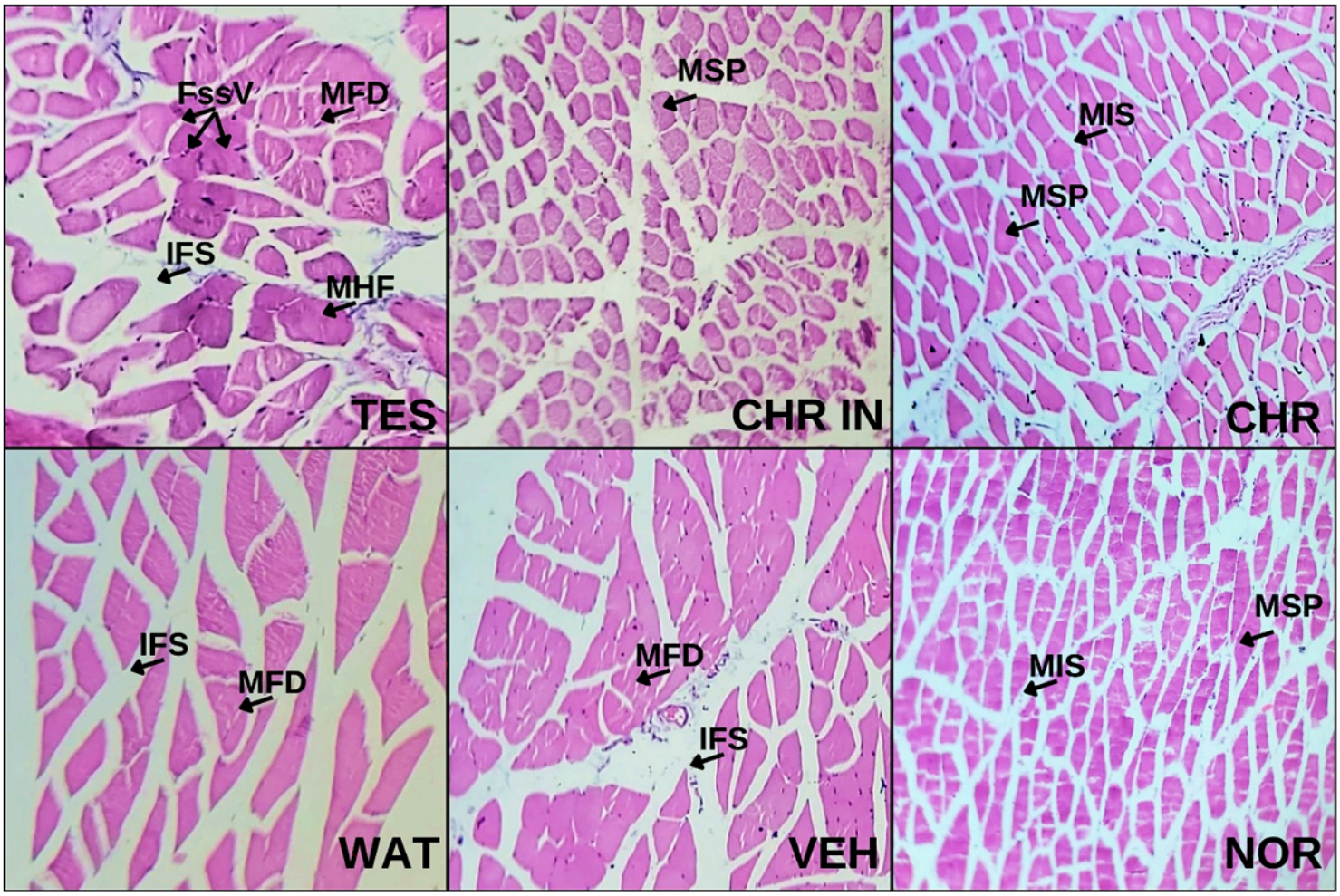


Figure 6

Annatto-derived Chronic® formulations preserve gastrocnemius microarchitecture after dexamethasone-induced muscle dysfunction. Representative hematoxylin-and-eosin-stained photomicrographs of gastrocnemius muscle sections show treatment-dependent differences in skeletal-muscle organization. WAT and VEH animals displayed muscle-fiber disorganization and increased interfascicular spacing, whereas CHR IN and CHR showed better fiber alignment and structural preservation, approaching the healthy pattern observed in NOR animals. TES was associated with fiber hypertrophy but also showed focal structural changes. Original magnification: 10×. TES, testosterone-treated DEX group; CHR IN, injectable Chronic In®-treated DEX group; CHR, oral Chronic®-treated DEX group; WAT, distilled-water-treated DEX group; VEH, injectable-vehicle-treated DEX group; NOR, healthy normal group. MFD, muscle-fiber disorganization; MHF, muscle-fiber hypertrophy; IFS, increased interfascicular space; MIS, minimal interstitial space; MSP, muscle-structure preservation; FSSV, fiber size and shape variation.

Supplementary Files

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