International Journal of Pharmacology

ISSN 1811-7775

science alert

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OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2022.1420.1428

Research Article
Protective Effects of Apocynin on Streptozotocin-Induced Diabetic Muscular Atrophy

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Abstract

Background and Objective: Diabetes plays a vital role in the pathological process of muscular atrophy. Apocynin (APO) was proved to possess multiple biological effects in diabetes. However, its improvement in diabetic muscular atrophy has not been reported. Hence, the current article investigates the improvement of APO on diabetic muscular atrophy and its underlying mechanism. **Materials and Methods:** Haematoxylin and Eosin (HE) staining was utilized to observe gastrocnemius histopathology. Muscle force was tested according to the grip strength meter. The levels of ALT, AST, CK, LDH, T-AOC and MDA were measured by spectrophotometer. TNF-α and IL-6 levels were analyzed by ELISA kits. The expressions of Atrogin-1, MuRF-1, CHOP, GRP-78, BAX and BCL-2 were detected by Western blot. **Results:** Data demonstrated that APO markedly improved diabetic muscular atrophy, as represented by enhancing myofiber size and weight of gastrocnemius and promoting grip strength. In serum, APO markedly promoted T-AOC levels, while restrained ALT, AST, CK, LDH, MDA, TNF-α and IL-6 levels. In gastrocnemius, diabetes markedly increased the expressions of Atrogin-1, MuRF-1, CHOP, GRP-78 and BAX and reduced BCL-2 expression. However, treatment with APO reversed these changes. **Conclusion:** Results revealed that APO could attenuate Endoplasmic Reticulum (ER) and apoptosis in the gastrocnemius of diabetic mice and could be used as a novel therapeutic in the prevention and treatment of muscular atrophy.

Key words: Apocynin, diabetes, muscle atrophy, endoplasmic reticulum, apoptosis, hyperglycemia, athletic ability

Citation: Xianchu, L., C. Changhao, D. Beiwang and L. Ming, 2022. Protective effects of apocynin on streptozotocin-induced diabetic muscular atrophy. Int. J. Pharmacol., 18: 1420-1428.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Hyperglycemia has become a serious public health problem that is closely connected with the increased incidence of diabetes. Chronic hyperglycemia brings many complications, which are harmful to tissues and organs in patients. In skeletal muscle, poorly controlled hyperglycemia can predispose to diabetic myopathy. It contributes to a reduction of muscle mass, myofiber area and strength[1,2]. These features are independent indexes for muscle atrophy during diabetes. Although, many therapeutic agents are effective against hyperglycemia, muscle atrophy is still a pathogenic factor for dyskinesia and quality of life in diabetic patients.

Skeletal muscle is a locomotive organ that plays a significant role in bodily movement[3]. Any interruption to skeletal muscle influences physical capacity and athletic ability. In diabetes, skeletal muscle was also proved to regulate glucose homeostasis[4]. Recent results showed that diabetes was a pathogenic factor for muscle atrophy[5,6]. Up to now, the molecular mechanisms that link diabetes to muscle atrophy remain elusive. Hyperglycemia can disturb muscle homeostasis by activation of the ubiquitin-proteasome system (UPS)[7]. ER stress and apoptosis play a crucial role in aggravating muscle injury[8]. The indicators of ER stress were increased during the process of diabetes[9]. Furthermore, these adverse stresses can trigger myocyte apoptosis which contributes to skeletal muscle disorder[10,11]. Therefore, modulation of ER stress and suppression of apoptosis is considered potential prevention and treatment of muscle atrophy in diabetes.

Apocynin is an acetophenone compound isolated from the root of *Picrorhiza kurroa*. APO is used as an NADPH oxidase inhibitor which can prevent superoxide production in many disease models[12]. With the deepening of research, various biological and pharmacological activities of APO were exhibited in diabetes[13]. It was demonstrated that APO alleviated inflammation via suppressing TLR4/NF-κB pathway in gestational diabetes mellitus[14]. APO was also described to inhibit ER stress by disruption of Rac1 signalling in diabetic hearts[15]. In diabetes-induced testes injury, APO ameliorated apoptosis-related genes and attenuated apoptosis by TUNEL assay[16]. What is more, APO was shown to improve ANG II-induced skeletal muscle injury, cigarette smoking-induced loss of tibialis anterior muscle weight and Duchenne muscular dystrophy[17-19]. However, there was no report on the protective effects and mechanism of APO on skeletal muscles atrophy in diabetes. Taking these functions into account, APO was hypothesized to possess improvement effects on diabetic atrophy.

MATERIALS AND METHODS

Study area: The study was carried out at the Human Movement Science Laboratory, Hunan University of Arts and Science (January to October, 2021).

Animals: Eight week-old C57BL/6 male mice with an average body weight of 20 g were purchased from Hunan SJA Laboratory Animal Co., Ltd (Changsha, China). Mice were kept under SPF conditions. The temperature is 23±2 and the humidity is 50±10%. The number of experimental animals is 60. All mice lived in cages with the freedom to eat diet and drink water. All animal experiments were inspected according to the Ethics Committee of Hunan University of Arts and Science (No. HUAS-2021-TY-133).

Chemicals and reagents: Apocynin (purity: ≥98%) and streptozotocin were obtained from Sangon Biotech (Shanghai, China). The assay kits of ALT, AST, CK, LDH, T-AOC and MDA were obtained from Nanjing Jiancheng Biotechnology Institute (Nanjing, China). The ELISA kits of TNF-α and IL-1β were obtained from MultiSciences (Hangzhou, China). The antibodies of CHOP, BAX and BCL-2 were obtained from Proteintech (Wuhan, China). The antibodies of Atrogin-1, MuRF-1 and GRP-78 were obtained from Sangon Biotech (Shanghai, China).

Experimental design: To study the improvement of APO on diabetic muscular atrophy, mice were intervened by STZ (ip, 200 mg kg^{-1}) to establish a diabetic model. Then, blood samples were acquired through the tail to test glucose levels. Mice with blood glucose concentrations were ≥16.7 mmol L^{-1}, which was retained in the diabetes mellitus group (DM). Diabetic mice were administered APO (16 mg/kg/day) for 8 weeks to serve as APO+DM group. Mice in the control group (CON) were intraperitoneally administrated with citrate buffer (pH 4.5).

Grip strength: Muscle force was tested according to the grip strength meter, which was acquired through Anhui Zhenghua Bioinstrumentation (Huaibei, China). During the muscle force test, mice were allowed to grasp by pulling a backward plate. Muscle force was immediately recorded when the mouse spontaneously released as previously reported[20]. After three repeated tests, the average value was defined as grip strength.

Skeletal muscle tissue harvest: At the end of the experimental period, mice were intraperitoneally injected with pentobarbital sodium (50 mg kg^{-1}). Then, mice were decapitated. Gastrocnemius was quickly isolated and

weighed. A part of gastrocnemius was stored in 4% paraformaldehyde to observe its histopathology. Another gastrocnemius was placed at -80 to detect gene expressions.

Biochemical assay: The blood samples were centrifuged and utilized for various biochemical determinations. The levels of ALT, AST, CK, LDH, T-AOC and MDA were measured with commercial kits, while TNF-α and IL-6 levels were analyzed by ELISA kits.

Histological analysis: Haematoxylin and Eosin (HE) staining were utilized to observe gastrocnemius histopathology. After being fixed in paraformaldehyde, the gastrocnemius was dehydrated by alcohol. And more, xylems served as a transparent reagent. Then, the gastrocnemius was embedded in paraffin. Gastrocnemius was cut into 5 um by a rotary microtome. The section was stained with haematoxylin and eosin to distinguish the nucleus and cytoplasm. The image was captured under the light microscope (200X) for histological analysis.

Western blotting: Gastrocnemius samples were washed with pre-cooled PBS and ground with RIPA. The homogenate was vibrated at 4 every 5 min. The supernatant was collected by centrifuge method and its concentration was measured according to the BCA method. SDS-PAGE was performed to separate proteins, which were transferred to the PVDF membrane by electroblotting. Then, the membranes were incubated with 5% milk (25, 1 hr), primary antibodies (4°C, overnight) and corresponding HRP-coupled secondary antibodies (25, 2 hrs), respectively. The visualisation was done with the ECL system. The β-actin was an internal control that was used to standardize band intensity quantification. The protein expression was analyzed and quantified by Image J.

Statistical analysis: All data were shown as Mean±SD. The results were analyzed with SPSS 16.0 software. Statistical difference was demonstrated by the ANOVA test with Tukey's post hoc test. The $p<0.05$ was deemed statistically significant.

RESULTS

Properties of APO on diabetic muscular atrophy: To evaluate whether APO ameliorated diabetic myopathy, the myocyte cross-sectional area was assayed according to HE staining. In Fig. 1a-b, APO markedly ($p<0.01$) enhanced the representative myocyte cross-sections of gastrocnemius in diabetic mice. To corroborate the results of HE staining, the weight and grip strength of skeletal muscle was detected. In Fig. 1c-d, APO markedly ($p<0.01$) prevented diabetes-induced decreases of gastrocnemius mass and grip strength.

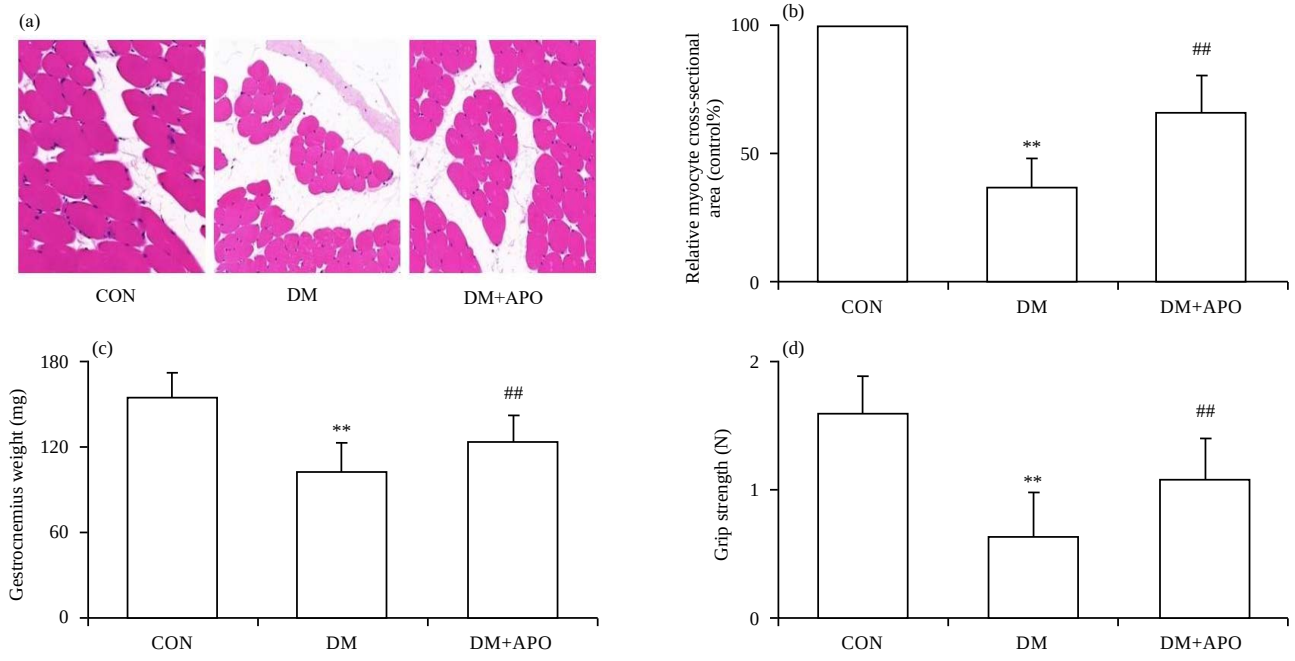

Fig. 1a-d: Properties of APO on diabetic muscular atrophy, (a) HE staining (200X), (b) Relative myocyte cross-sectional area, (c) Gastrocnemius weight and (d) Grip strength

**p<0.01 compared with CON group and ##p<0.01 compared with DM group

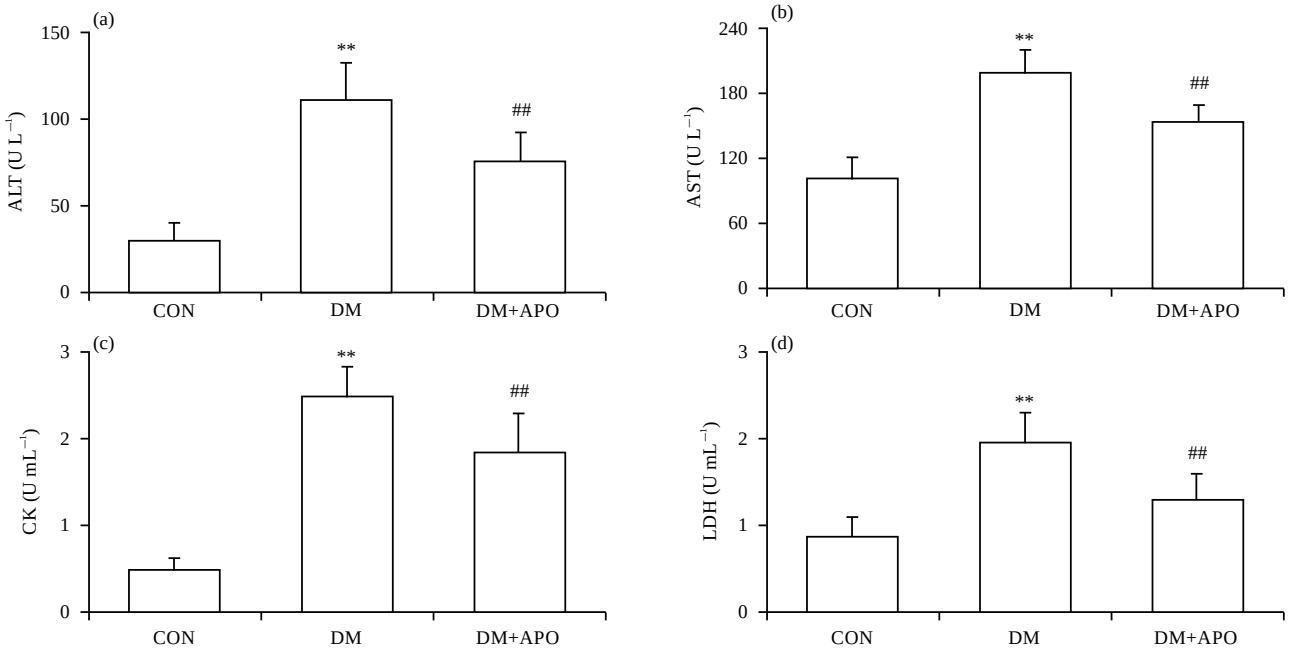

Fig. 2(a-d): Properties of APO on different levels, (a) ALT, (b) AST, (c) CK and (d) LDH in serum of diabetic mice

**p<0.01 compared with CON group, #p<0.05 and ##p<0.01 compared with DM group

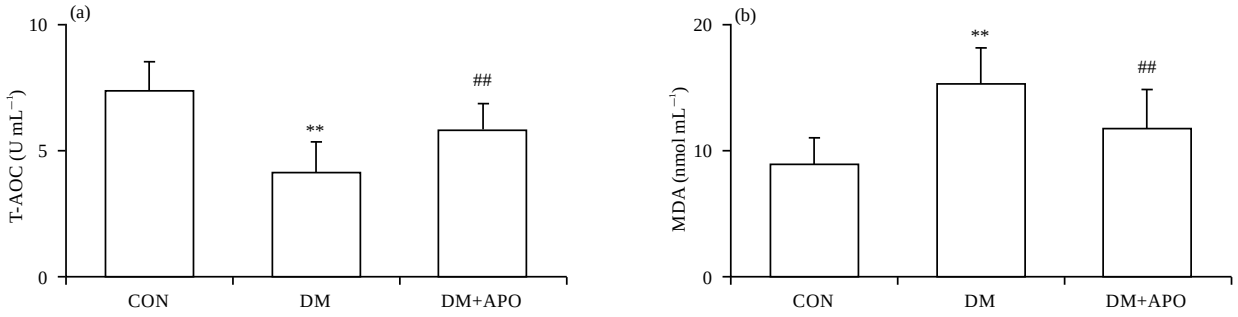

Fig. 3(a-b): Properties of APO on oxidative stress, (a) T-AOC and (b) MDA levels were examined by spectrophotometer in serum of diabetic mice

**p<0.01 compared with CON group, #p<0.05 and ##p<0.01 compared with DM group

Properties of APO on ALT, AST, LDH and CK: To investigate the regulation of APO in diabetes, the levels of ALT, AST, CK and LDH were examined in serum. In Fig. 2a-d, ALT, AST, LDH and CK levels were markedly ($p<0.01$) increased in the DM group. In contrast, treatment with APO obviously ($p<0.01$) restrained these parameters to improve general features in STZ-induced diabetes.

Properties of AA on oxidative stress: To investigate the anti-oxidation effect of APO, T-AOC and MDA levels were examined in serum. In Fig. 3a-b, the T-AOC level was markedly ($p<0.01$) decreased, while the MDA level was markedly ($p<0.01$) increased in the DM group. However, treatment with APO obviously ($p<0.01$) improved the above indicators to reduce oxidative stress.

Properties of APO on inflammatory response: To investigate the anti-inflammatory effect of APO, TNF-α and IL-6 levels were tested in serum. In Fig. 4a-b, TNF-α and IL-6 levels were markedly ($p<0.01$) increased in the DM group, while treatment with APO obviously ($p<0.01$) reduced these inflammatory factors levels.

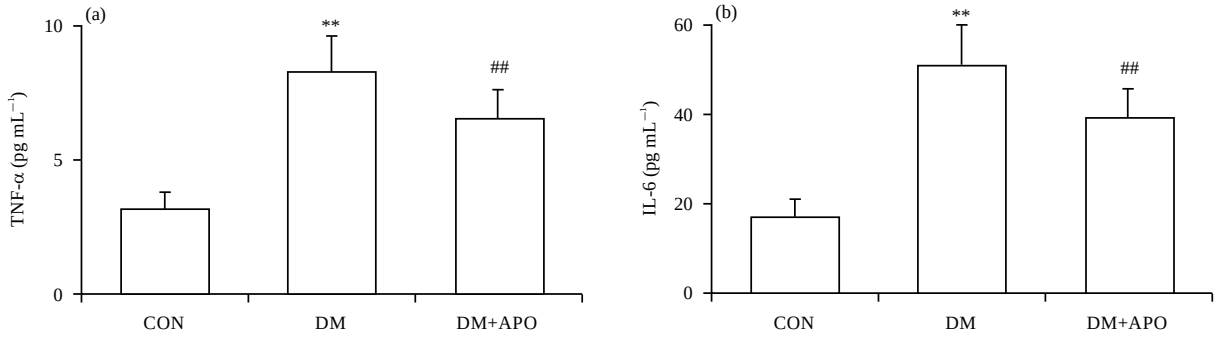

Fig. 4(a-b): Properties of APO on the inflammatory response, (a) TNF-α and (b) IL-6 levels were tested by ELISA in the serum of diabetic mice

**p<0.01 compared with CON group, #p<0.05 and ## p<0.01 compared with DM group

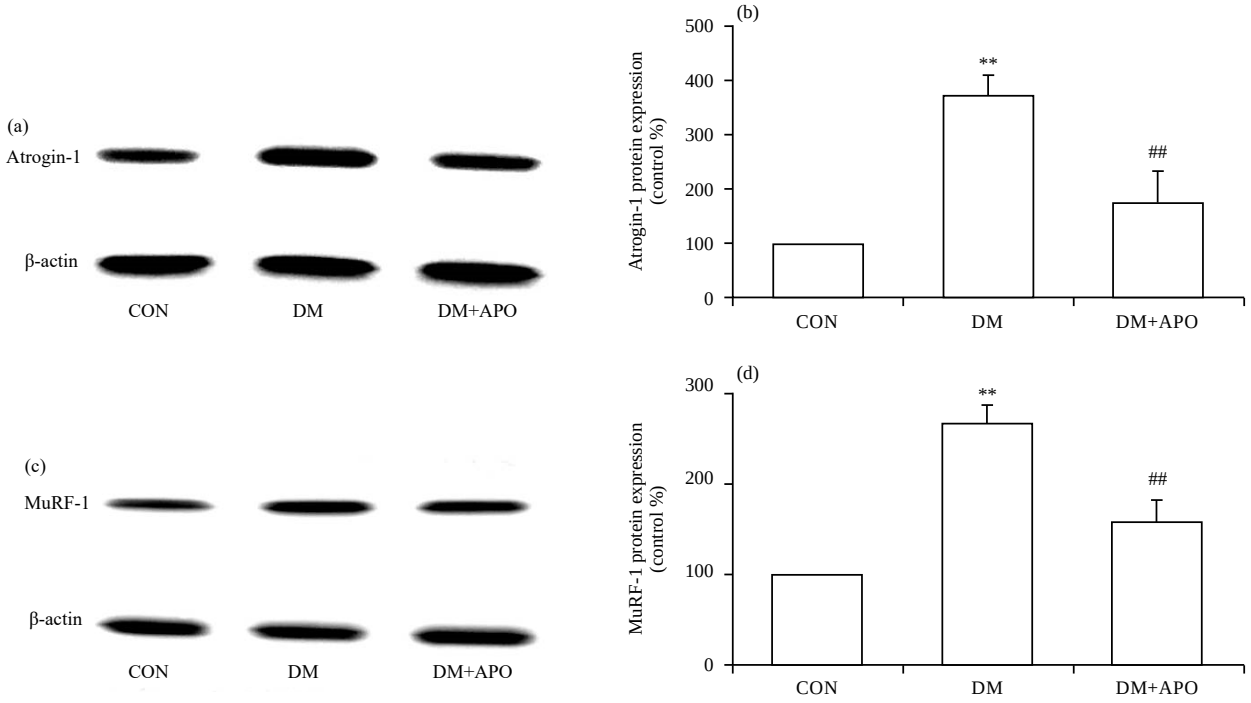

Fig. 5(a-d): Properties of APO on proteostasis, (a and c) Western blot was utilized to detect Atrogin-1 and MuRF-1 expressions in gastrocnemius of diabetic mice, (b and d) Quantifications of Atrogin-1 and MuRF-1 levels

**p<0.01 compared with CON group and ##p<0.01 compared with DM group

Properties of APO on proteostasis: To analyze the findings diabetic-evoked skeletal muscle atrophy, the UPS system was examined in gastrocnemius. As shown in Fig. 5a-d, APO markedly (p<0.01) abrogated diabetic-induced Atrogin-1 and MuRF expressions in gastrocnemius. In addition, these results were consistent with changes of myocyte cross-sectional area, gastrocnemius mass and grip strength, suggesting that APO prevented proteostasis induced by diabetes in skeletal muscle to improve muscle atrophy.

Properties of APO on ER stress: To analyze modulation of diabetic-evoked ER stress, CHOP and GRP-78 levels were tested in gastrocnemius. In Fig. 6a-d, diabetes-induced muscle atrophy involved ER stress, as represented by markedly (p<0.01) increasing CHOP and GRP-78 levels. In contrast, APO obviously (p<0.01) reduced weakened diabetic-evoked activation of CHOP and GRP-78 in gastrocnemius, suggesting that APO protected gastrocnemius against muscle atrophy by inhibition of ER stress.

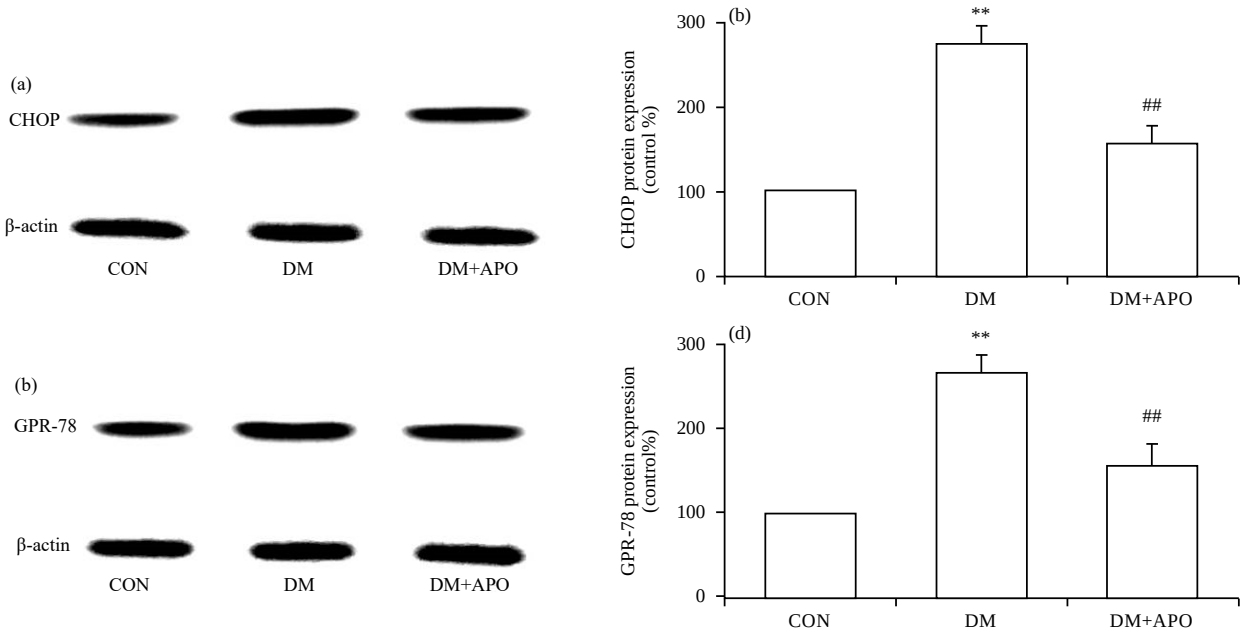

Fig. 6(a-d): Properties of APO on ER stress, (a and c) Western blot was utilized to detect CHOP and GRP-78 expressions in gastrocnemius of diabetic mice, (b and d) Quantifications of CHOP and GRP-78 levels

**p<0.01 compared with CON group and ##p<0.01 compared with DM group

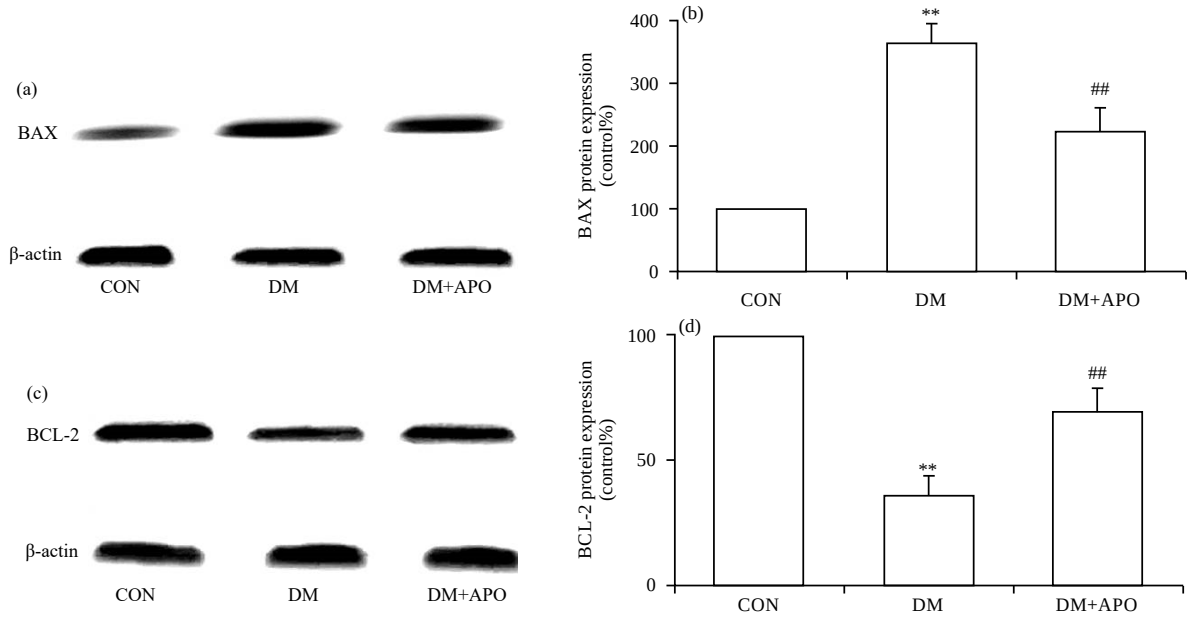

Fig. 7(a-d): Properties of APO on the apoptotic pathway (a and c) Western blot was utilized to detect Bax and BCL-2 expressions in gastrocnemius of diabetic mice, (b and d) Quantifications of Bax and BCL-2 levels

**p<0.01 compared with CON group and ## p<0.01 compared with DM group

Properties of APO on apoptotic pathway: To analyze the anti-apoptosis effect of APO in gastrocnemius, Bax and BCL-2 expressions were examined. In Fig. 7a-d, Bax expression was markedly ($p<0.01$) increased, while expression was markedly ($p<0.01$) decreased in the DM group. However, treatment with APO obviously ($p<0.01$) reduced reversed the changes of

these apoptosis-related gen in gastrocnemius, suggesting that APO alleviated diabetes-associated muscle atrophy via suppression of the apoptotic pathway.

DISCUSSION

This study revealed that APO preserved proteostasis, represented by inhibition of Atrogin-1 and MuRF-1 expressions, expressions, to attenuate muscle loss and dysfunction. Further studies suggested that APO inhibit CHOP and GRP-78 expressions to prevent ER stress. Moreover, the resistance to muscular dystrophy of APO might be concerned to its anti-apoptosis effects by regulating Bax and BCL-2 levels. In turn, the induction of ER stress and apoptotic pathway perturbs proteostasis via strengthening UPS activation to cause muscle loss and dysfunction. Current results demonstrated that APO ameliorated hyperglycemia-associated muscle atrophy which was linked with its modulation of ER stress and apoptosis.

Muscle atrophy is one of the common manifestations of myopathy. Diabetes was proved as a risk factor to induce muscles atrophy, which leads to morphological lesions and dysfunction of skeletal muscles. The main feature of muscle atrophy is muscle mass loss, which causes muscle weakness [21]. In diabetes, the weight of skeletal muscles was decreased[20]. Furthermore, hyperglycemia causes deterioration of muscle function, which was characterized by myocyte cross-sections change and grip strength attenuation[22]. Previous studies showed that APO prevented loss of tibialis anterior muscle mass and attenuated muscle weakness by increasing contractile force in cigarette smoking exposure[18]. However, its regulating effect on diabetes-induced muscles atrophy was unclear. In this study, APO was shown to improve diabetic muscle atrophy by reducing loss of gastrocnemius mass, increasing myocyte cross-sections and reinforcing grip strength.

The mechanism of muscle atrophy in diabetes is complicated and many precipitating factors intensify its pathogenesis. UPS is known as an important proteolytic system, which is a negative regulator of muscle mass[23]. An increase in UPS production caused by hyperglycemia-induced diabetes is deemed an important factor in triggering skeletal muscle dysfunction. Atrogin-1 and MuRF-1, as markers of muscle-specific E3 ubiquitin ligases, are involved in several cell signalling pathways to regulate the synthesis and degradation of protein in tissue and organ[24]. Several experimental types of research showed that the expressions of Atrogin-1 and MuRF- were increased in skeletal muscle of diabetes model[25]. In this study, APO relieved Atrogin-1 and MuRF-1 expressions in gastrocnemius, indicating APO could suppress UPS against diabetes-induced muscle dysfunction.

ER stress, which is a risk factor for muscle atrophy, is known to rise in correlation with declining skeletal muscle function[26]. CHOP and GRP-78 are the markers of ER stress[27]. Therefore, suppression of CHOP and GRP-78 expressions which have been implicated in diabetic myopathy could be applied as an available treatment method in muscular atrophy[8]. In type 1 diabetes, APO could inhibit ER stress to reduce fibrosis and hypertrophy in hearts[15]. In this study, hyperglycemia produced excessive ER stress in gastrocnemius. Treatment with APO alleviated CHOP and GRP-78 expressions, indicating that APO could prevent ER stress against diabetic muscular dystrophy. Hence, targeted suppression of ER stress may be used to prevent muscle atrophy associated with diabetes.

The apoptotic pathway is involved in the regulation of muscle health as a programmed cell death system[28]. Bax and BCL-2 are widely used to denote cellular senescence and death in biology. In addition, many factors such as inflammatory response and ER stress are closely related to the progress of apoptosis[25,29]. Therefore, targeting these factors contributes to the pathophysiological mechanisms of diabetic myopathy. Previous studies indicated that APO could modulate IRE1α engagement to prevent endoplasmic reticulum stress-induced apoptosis in endothelial cells[30]. In diabetes, APO could also regulate apoptosis-related genes, such as Bax and BCL-2, to ameliorate diabetes-induced testis injury[16]. In this study, APO was proved to mediate apoptotic pathways against diabetic atrophy by alleviating Bax and BCL-2 expressions in gastrocnemius.

CONCLUSION

Research demonstrated that APO improved proteostasis caused by hyperglycemia exposure in skeletal muscle. The underlying mechanism for muscle protection was related to its suppression of ER stress and inhibition of apoptosis. These results showed APO may be utilized and developed to attenuate muscular dystrophy associated with diabetes.

SIGNIFICANCE STATEMENT

This study revealed the improvement of APO against muscle atrophy in STZ-induced diabetic mice. The APO enhanced fibre size and weight of gastrocnemius and promoted grip strength to alleviate muscle atrophy. In serum,

APO restrained ALT, AST, CK, LDH, MDA, TNF-α and IL-6 levels and promoted T-AOC levels. In skeletal muscle, APO inhibited protein degradation by mitigating the UPS system. Moreover, APO increased the expressions of Atrogin-1, MuRF-1, CHOP, GRP-78 and BAX and reduced BCL-2 expression to attenuate ER stress and apoptosis. These results suggested APO could be used in the prevention and treatment of muscular atrophy in diabetes.

ACKNOWLEDGMENTS

We would like to thank all the people who participated in the study and the researchers. This study was supported in part by a project supported by the Scientific Research Fund of Hunan Provincial Education Department (Grant No. 19C1274) and Hunan Provincial Natural Science Foundation of China (Grant No.2020JJ5391 and No. 2021JJ40376).

REFERENCES

1. Andersen, H., P.L. Poulsen, C.E. Mogensen and J. Jakobsen, 1996. Isokinetic muscle strength in long-term IDDM patients in relation to diabetic complications. Diabetes, 45: 440-445.
2. Monaco, C.M.F., C.G.R. Perry and T.J. Hawke, 2017. Diabetic myopathy: Current molecular understanding of this novel neuromuscular disorder. Curr. Opin. Neurol., 30: 545-552.
3. Sartori, R., V. Romanello and M. Sandri, 2021. Mechanisms of muscle atrophy and hypertrophy: Implications in health and disease. Nat. Commun., Vol. 12. 10.1038/s41467-020-20123-1.
4. Deshmukh, A.S., 2016. Insulin-stimulated glucose uptake in healthy and insulin-resistant skeletal muscle. Hormone Mol. Biol. Clin. Invest., Vol. 26. 10.1515/hmbci-2015-0041.
5. Hirata Y., K. Nomura, Y. Senga, Y. Okada and K. Kobayashi *et al*., 2019. Hyperglycemia induces skeletal muscle atrophy via a WWP1/KLF15 axis. JCI Insight, Vol. 4. 10.1172/jci.insight.124952.
6. Nomura, T., T. Kawae, H. Kataoka and Y. Ikeda, 2018. Assessment of lower extremity muscle mass, muscle strength, and exercise therapy in elderly patients with diabetes mellitus. Environ. Health Preventive Med., Vol. 23. 10.1186/s12199-018-0710-7.
7. Marfella, R., M. D'. Amico, C. di Filippo, M. Siniscalchi and F. Sasso *et al*., 2007. The possible role of the ubiquitin proteasome system in the development of atherosclerosis in diabetes. Cardiovasc. Diabetology, Vol. 6. 10.1186/1475-2840-6-35.
8. Reddy, S.S., K. Shruthi, D. Joy and G.B. Reddy, 2019. 4-PBA atrophy in rats by modulating ER stress response and ubiquitin-proteasome system. Chem. Bio. Interact., 306: 70-77.
9. Hotamisligil, G.S., 2010. Endoplasmic reticulum stress and the inflammatory basis of metabolic disease. Cell, 140: 900-917.
10. Frank, D. and J.E. Vince, 2019. Pyroptosis versus necroptosis: Similarities, differences, and crosstalk. Cell Death Differ., 26: 99-114.
11. Sano, R. and J.C. Reed, 2013. ER stress-induced cell death mechanisms. Biochim. Biophys. Acta (BBA)-Mol. Cell Res., 1833: 3460-3470.
12. Tan, Y.C. M.A. Sattar, A.F. Ahmeda, N.A.K. Khan and V. *et al*., 2020. Apocynin and catalase prevent hypertension and kidney injury in cyclosporine nephrotoxicity in rats. PLOS ONE, Vol. 15. 10.1371/journal.pone.0231472.
13. Peng, J.J., S.Q. Xiong, L.X. Ding, J. Peng and X.B. Xia, 2019. Diabetic retinopathy: Focus on NADPH oxidase and its as therapeutic target. Eur. J. Pharmacol., 853: 381-387.
14. Liu, T., W. Zheng, L. Wang, L. Wang and Y. Zhang, 2020. TLR_4/NF-κB signaling pathway participates in the protective effects of apocynin on gestational diabetes mellitus induced placental oxidative stress and Inflammation. Reprod. Sci., 27: 722-730.
15. Li, J., H. Zhu, E. Shen, L. Wan, J.M.O. Arnold and T. Peng, 2010. Deficiency of Rac1 blocks NADPH oxidase activation, inhibits *Endoplasmic reticulum* stress, and reduces myocardial remodeling in a mouse model of type 1 diabetes. Diabetes, 59: 2033-2042.
16. Li, M., Z. Liu, L. Zhuan, T. Wang and S. Guo *et al*., 2012. Effects of apocynin on oxidative stress and expression of apoptosis-related genes in testes of diabetic rats. Mol. Med. Rep., 7: 47-52.
17. Capogrosso, R.F., A. Cozzoli, P. Mantuano, G.M. Camerino and A.M. Massari *et al*., 2016. Assessment of resveratrol, apocynin and taurine on mechanical-metabolic uncoupling and oxidative stress in a mouse model of duchenne muscular dystrophy: A comparison with the gold standard, α-methyl Prednisolone. Pharmacol. Res., 106: 101-113.
18. Chan, S.M.H. Bernardo, C. Mastronardo, K. Mou and S.N.D. Luca *et al*., 2021. Apocynin prevents cigarette smoking induced loss of skeletal muscle mass and function in mice by preserving proteostatic signalling. J. Pharmacol., 178: 3049-3066.
19. Inoue, N., S. Kinugawa, T. Suga, T. Yokota, K. Hirabayashi *et al*., 2012. Angiotensin II-induced reduction in exercise capacity is associated with increased oxidative stress in skeletal muscle. Am. J. Physiol. Heart. Circ. Physiol., 302: H1202-H1210.
20. Guo, S., Q. Chen, Y. Sun and J. Chen, 2019. Nicotinamide protects against skeletal muscle atrophy in Streptozotocin-induced diabetic mice. Arch. Physiol. Biochem., 125: 470-477.
21. Guadagnin, E., D. Mázala and Y.W. Chen, 2018. STAT3 in skeletal muscle function and disorders. Int. J. Mol. Sci., Vol. 19. 10.3390/ijms19082265.

22. Yang, J., L. Sun, X. Fan, B. Yin, Y. Kang, S. An and L. Tang, 2018. Pulsed electromagnetic fields alleviate Streptozotocin-induced diabetic muscle atrophy. Mol. Med. Rep., 18: 1127-1133.
23. Tanaka Morifuji, M. Yoshikawa, R. Nakanishi Fujino 2018. Effects of combined treatment with blood flow restriction and low-intensity electrical stimulation on diabetes mellitus-associated muscle atrophy in rats. J. Diabetes, 11: 326-334.
24. Ono, T., S. Takada, S. Kinugawa and H. Tsutsui, 2015. Curcumin ameli atrophy in type 1 diabetic mice by inhibiting protein ubiquitination. Exp. Physiol., 100: 1052-1063.
25. Wang, D., H. Sun, G. Song, Y. Yang, X. Zou, P. Han and S. Li, 2018. Resveratrol improves muscle atrophy by modulating mitochondrial quality control in STZ-induced diabetic mice. Mol. Nutr. Food Res., Vol. 62. 10.1002/mnfr.201700941.
26. Afroze, D. and A. Kumar, 2017. ER stress in skeletal muscle remodeling and myopathies. FEBS J., 286: 379-398.
27. Rana, S.V.S., 2019. Endoplasmic reticulum stress induced by toxic elements-a review of recent developments.. Biol. Trace Elem. Res., 196: 10-19.
28. Elmore, S., 2007. Apoptosis: A review of programmed cell death. Toxicol. Pathol., 35: 495-516.
29. Ciciliot, S., A.C. Rossi, K.A. Dyar, B. Blaauw and S. Schiaffino, 2013. Muscle type and fiber type specificity in muscle wasting. Int. J. Biochem. Cell Biol., 45: 2191-2199.
30. Wu, J., W. Zhang, X. Liu, L. Wu and G. He *et al*., 2018. Apocynin protects endothelial cells from endoplasmic reticulum stress-induced apoptosis via IRE1α engagement. Mol. Cell. Biochem., 449: 257-265.