International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2021.180.189

Research Article
Asiatic Acid Improves Diabetes-Induced Muscle Atrophy in Mice

[1]Liu Xianchu, [2]Peng Huan, [1]Deng Beiwang, [1]Cheng Changhao and [3]Liu Ming

[1]Department of Human Movement Science, Institute of Physical Culture, Hunan University of Arts and Science, 415000 Changde, China
[2]Department of Hematology, Xiangya Changde Hospital, 415000 Changde, Hunan, China
[3]Department of Biochemistry, College of Life and Environmental Sciences, Hunan University of Arts and Science, 415000 Changde, China

Abstract

Background and Objective: Diabetic amyotrophy is one of serious muscular complications in diabetes. Asiatic Acid (AA) possessed multiple pharmacological actions in diabetic complications. However, the potential role of AA on diabetic myopathy has been not reported to date. Current study aimed to estimate improvement effect of AA on diabetic-associated muscle atrophy in mice. **Materials and Methods:** Mice were intraperitoneal injection with streptozocin (STZ, 50 mg kg^{-1}) for 5 days to establish diabetic model. When blood glucose concentration was above 16.7 mmol/L, mice were treated with AA (30 mg/kg/day) for 8 weeks. Then, mice were euthanized to collect blood and muscle samples for further research. **Results:** Our data demonstrated that AA significantly reduced blood glucose concentration and led to a significant decrease in serum glucose (Glu), triglyceride (TG), cholesterol (TC), Creatine Kinase (CK), lactate dehydrogenase (LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea (BUN) and creatinine (Cre) in diabetic mice. Moreover, AA significantly increased myofiber size and weight of gastrocnemius and enhanced grip strength to improve muscle atrophy. In skeletal muscle, AA significantly relieved Atrogin-1, MuRF-1, TNF-a, IL-6 and Bax expressions and elevated Nrf-1, Pgc-1α and Bcl-2 expressions in diabetic mice. **Conclusion:** AA produced beneficial effects against diabetic amyotrophy through its accommodation of inflammatory response, mitochondrial dysfunction and apoptosis in mice. These biological functions suggested AA could be used as a novel medicine for improvement of diabetes-induced skeletal muscle atrophy.

Key words: Asiatic acid, diabetes, muscle atrophy, apoptosis, inflammation, mitochondria

Citation: Xianchu, L., P. Huan, D. Beiwang, C. Changhao and L. Ming, 2021. Asiatic acid improves diabetes-induced muscle atrophy in mice. Int. J. Pharmacol., 17: 180-189.

Corresponding Author: Liu Ming, Department of Biochemistry, College of Life and Environmental Sciences, Hunan University of Arts and Science, 415000 Changde, China Tel: +8615073178916

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

Type 1 diabetes mellitus (T1DM) is a prevalent autoimmune disorder, which is mediated by many factors, such as heredity and environment[1]. The key etiology of T1DM is beta-cell destruction[2]. Hyperglycemia is considered an important characteristic of T1DM, which causes multiple organ damages and complications[3]. Skeletal muscle, as a largest tissue of body, is not only involved in movement but also maintaining glucose homeostasis[4]. Hence, skeletal muscle is a target tissue of hyperglycemia. The uncontrolled hyperglycemia that occurs in skeletal muscle reduced the mass of muscle and size of myofiber, resulting in muscle atrophy[5]. In turn, skeletal muscle damage induces a higher concentration of blood sugar, brining about vicious cycle[6].

Muscle atrophy is a common myopathy in T1DM and primary cause of muscle dysfunction[7]. Although the etiology of muscle atrophy has not been completely clarified to date, abnormal protein metabolism is now considered a key factor of impairments of myofiber size[8]. Clinically, diabetic patient exhibits a dramatic loss of skeletal muscle because of protein degradation[9]. The ubiquitin-proteasome system is closely related to protein degradation. In a general way, Atrogin-1 and MuRF-1as indicator of protein degradation are elevated in muscle tissues of diabetes[10]. Patient with T1DM require insulin therapy to maintain vital movement. However, administration of insulin easily leads to allergic or adverse reactions in muscle. Hence, to understand molecular mechanisms and search effective therapy method in diabetes-associated muscle atrophy has become a trend of current research.

In molecular mechanisms, diabetes-associated muscle atrophy is complicated[11]. Inflammatory response is responsible for deterioration of muscle atrophy[12]. Mitochondrial biology is closely associated with growth and development of skeletal muscle[13]. Subsequently, inflammatory response and mitochondria dysfunction contribute to myopathy which induced muscle damage and cell apoptosis[14]. Besides, asiatic acid, a as a pentacyclic triterpene, has multiple pharmacological both in diabetes and muscle biologic efficacies. It was reported that AA increased GLUT4 expression in skeletal muscle to ameliorate glucose response in diabetic rats[15]. However, there was no research elucidated that AA exhibited the protective effect on STZ-induced muscle atrophy. Therefore, we hypothesized that AA exerted improvement on diabetic myopathy via ameliorating inflammation, mitochondria and apoptosis.

MATERIALS AND METHODS

The study was carried out at biochemistry Laboratory, college of life science, Hunan University of Arts and Science (January, 2021 to April, 2021).

Animals: 8-week-old C57BL/6 mice (male, 20±2 g) were obtained from Hunan SJA Laboratory animal (Changsha, China). 60 mice were kept under SPF condition with humidity (50±10%), temperature (23±2), light-dark (12:12 hrs) cycle. Experimental mice were fed with standard chow diet and water. In this study, animal experiments were inspected by the Ethics Committee of Hunan University of Arts and Science (No. HUAS-2021-TY-133).

Chemicals and reagents: Asiatic acid (purity: ≥98%) were acquired from Chengdu Biopurify Phytochemicals (Chengdu, China). Streptozotocin and qRT-PCR primer sequences were acquired from Sangon Biotech (Shanghai, China). The biochemical detection kits of Glu, TG, TC, CK, LDH, AST, ALT, BUN and Cre were acquired from Nanjing Jiancheng Institute of Biological Engineering (Nanjing, China).The q-PCR kits were acquired from TaKaRa Bioengineering (Dalian, China). The ELISA kits of TNF-α and IL-6 were acquired from BOSTER Biological Technology (Wuhan, China). The antibodies of Bax, Bcl-2 and β-actin were acquired from proteintech (Wuhan, China). The antibodies of Atrogin-1, MuRF-1, Nrf-1 and Pgc-1α were acquired from Sangon Biotech (Shanghai, China).

Experimental design: All mice were assigned into 3 different groups at random. Each group consisted of 20 mice. The Diabetic Mice (DM) group was administrated with STZ (50 mg kg^{-1}) for 5 days by intraperitoneal injection. Blood was obtained from tail to measure glycemia. Blood glucose concentration was ≥16.7 mmol L^{-1}, which was permitted as applicable diabetic model. After administrated with STZ, mice were treated with AA (30 mg/kg/day) by gavage for a period of 8 weeks to establish DM+AA group. The control (CON) group was induced by administration of physiological saline.

Grip strength: Grip strength was analyzed by a dynamometer (YLS13, Anhui Zhenghua Bioinstrumentation). The measuring method of grip strength was described previously[16]. Briefly, for muscle force test, mouse was allowed to engage the grip with all limbs. Then, mouse pulled backward to observe peak grip strength. The observed value was repeated in triplicate to calculate average grip strength.

Table 1: Primers were used in this study

Gene	Accession number		Primer sequences
TNF-α	NM_013693	Forward primer	GCCACCACGCTCTTCTGCCT
		Reverse primer	GGCTGATGGTGTGGGTGAGG
IL-6	NM_031168	Forward primer	AGTTGCCTTCTTGGGACTGA
		Reverse primer	CCACGATTTCCCAGAGAAC
GAPDH	NM_001289726	Forward primer	AGGTCGGTGTGAACGGATTTG
		Reverse primer	TGTAGACCATGTAGTTGAGGTCA

Sample preparation: After administration of AA, mice were euthanized with pentobarbital and sacrificed by cervical decapitation. Blood was collected from eyeball and centrifuged to obtain serum. Biochemical markers were detected using commercial kits. The skeletal muscle was isolated and weighed. The skeletal muscle was freezed in liquid nitrogen immediately and preserved at -80°C for detecting mRNA and protein expressions. Meanwhile, part of gastrocnemius was stored in 4% paraformaldehyde to measure myocyte cross-sectional areas.

Histological analysis: The histomorphology of gastrocnemius was analyzed through Haematoxylin and Eosin staining. After 24 hrs of fixation with paraformaldehyde, gastrocnemius was thoroughly dehydrated with and xylene was regard as transparent additives. Then gastrocnemius was embedded in paraffin for pathological section. The gastrocnemius was cut into 5 μm by rotary microtome. After stained with haematoxylin and eosin, slices were sealed with neutral balsam. The staining was diagnosed under light microscope (200×magnification) to evaluate the pathological feature. The images were captured to analyze myocyte cross-sectional area by Image J.

qRT-PCR: Gastrocnemius was homogenized by motor-driven tissue grinder. After centrifugation, RNA was extracted with Trizol method to synthesis in synthesis in cDNA by reverse transcription. The target genes were detected by Bio-Rad real-time PCR. The specific primers of TNF-α and IL-6 were showed in Table 1. The mRNA was amplified with SYBR PCR mix. GAPDH was regard as a house keeping gene. The mRNA expression was evaluated and exhibited by CT method.

Western blot: Gastrocnemius was ground gradually with lysis buffer containing RIPA and proteinase inhibitors. The protein concentration was measured by BCA method. Equal amount of protein was separated by SDS-PAGE. Wet electroblotting method was used to transfer protein onto PVDF membrane. 5% milk sealant was applied for blocking nonspecific binding site for 1 hr at 25°C. The primary antibodies combined specific binding overnight at 4°C. The membrane was washed thrice with PBST and incubated with corresponding HRP-conjugated antibodies for 2 hrs at 25°C. The image capturing of protein band was performed with ECL method. β-actin was regard as an internal control. The density of band was normalized to β-actin. The signal was exhibited as relative density ratio to β-actin.

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

RESULTS

Properties of AA on biochemical alterations in diabetes: Biochemical alterations of STZ-induced diabetic mice with AA treatment were showed in Table 2. The levels of Glu, TG, TC, CK, LDH, AST, ALT, BUN and Cre were increased in DM group. However, AA significantly restrained these parameters, suggesting that AA might be effective in general features of diabetic mice.

Properties of AA on skeletal muscle atrophy in diabetes: As shown in the Fig. 1, Haematoxylin and Eosin staining assay demonstrated that the fiber size of gastrocnemius was significantly decreased in DM group, while AA significantly increased the representative myocyte cross-sections (Fig. 1a-b). In addition, gastrocnemius weight and grip strength were markedly reduced in DM group, while AA significantly reversed these changes (Fig. 1c-d).

Properties of AA on ubiquitin E3 ligase in diabetic-associated muscle atrophy: To estimate regulation of AA on diabetic-associated protein degradation, Atrogin-1 and MuRF-1 expressions were detected in skeletal muscle. As shown in the Fig. 2, Atrogin-1 and MuRF-1 expressions were markedly enhanced in DM group. By contrast, AA significantly inhibited the expressions of these specific ubiquitin ligases in skeletal muscle.

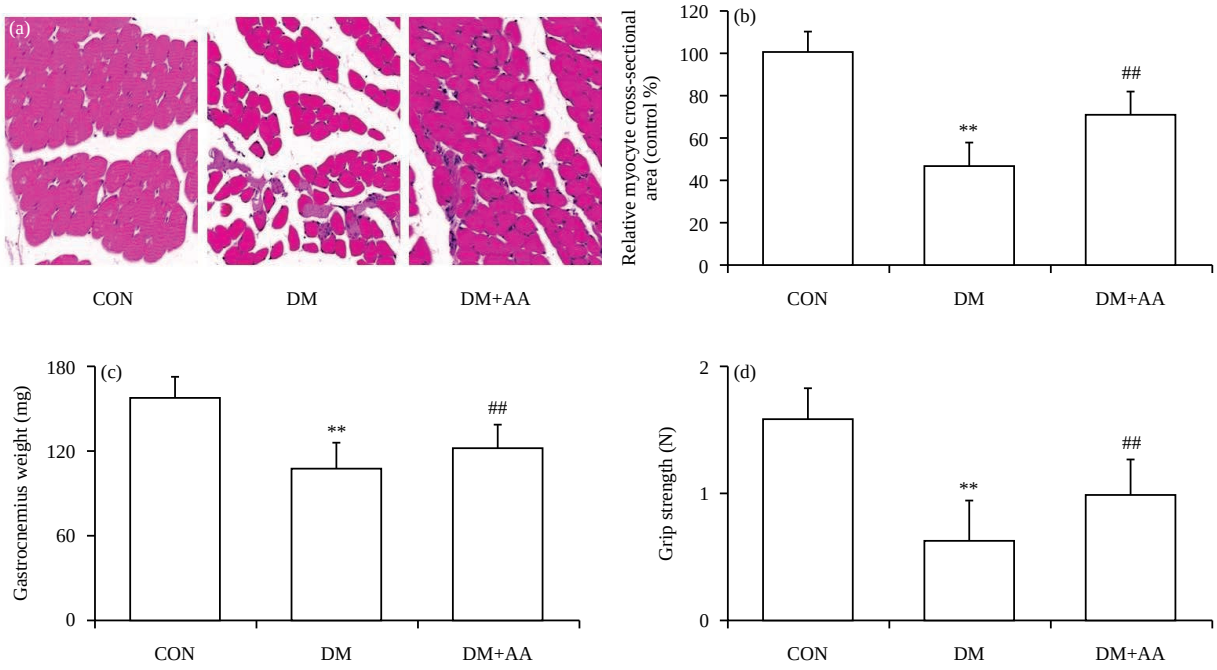

Fig. 1(a-d): Properties of AA on (a) Histopathological features (Hematoxylin and Eosin, 200X), (b) Myofiber size, (c) Weight of gastrocnemius and (d) Grip strength in diabetes

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

Table 2: Biochemical markers of diabetic mice

	CON	DM	DM+AA
Glu (mmol L^{-1})	8.18±0.73	19.77±1.69**	16.88±1.77##
TG (mmol L^{-1})	0.24±0.06	0.78±0.07**	0.57±0.11##
TC (mmol L^{-1})	1.45±0.18	4.10±0.63**	2.65±0.46##
CK (U mL^{-1})	0.47±0.13	2.50±0.27**	1.71±0.34##
LDH (U mL^{-1})	0.82±0.16	1.89±0.29**	1.41±0.31##
AST (U L^{-1})	105.35±17.65	205.46±19.74**	155.58±18.93##
ALT (U L^{-1})	31.68±7.58	116.31±15.93**	77.47±16.27##
BUN (mmol L^{-1})	9.26±1.11	16.44±2.30**	12.03±1.77##
Cre (μmol L^{-1})	34.49±10.13	95.80±15.22**	76.53±13.02##

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

Properties of AA on anti-inflammatory effect in diabetic-associated muscle atrophy: Inflammatory injury induced by diabetes was further confirmed by assessing TNF-α and IL-6 levels in skeletal muscle and serum. As shown in the Fig. 3, TNF-α and IL-6 levels were significantly elevated in DM group. By contrast, AA significantly relieved diabetic-induced activation of inflammatory response in skeletal muscle and serum.

Properties of AA on mitochondrial biogenesis in diabetic-associated muscle atrophy: As shown in the Fig. 4, Nrf-1 and Pgc-1α expressions were markedly inhibited in DM group. By contrast, AA significantly promoted diabetic-induced inactivation of Nrf-1 and Pgc-1α in skeletal muscle of diabetic mice, suggesting that AA might have protective efficacy on STZ-induced mitochondrial dysfunction.

Properties of AA on anti-apoptosis effect in diabetic-associated muscle atrophy: As shown in the Fig. 5, Bax expression was dramatically enhanced in DM group, while AA dramatically relieved diabetic-induced activation of Bax in skeletal muscle (Fig. 5a-b). However, Bcl-2 expression was markedly reduced in DM group, while AA dramatically increased diabetic-induced inactivation of Bcl-2 in skeletal muscle (Fig. 5c-d).

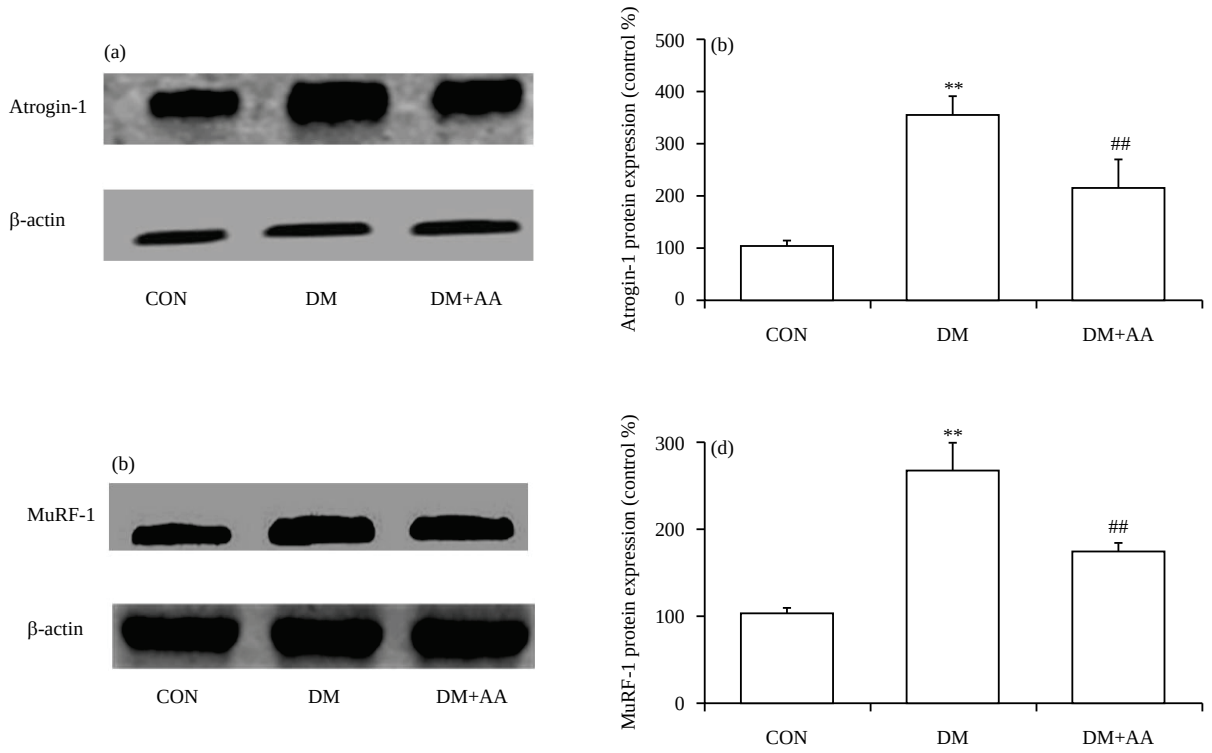

Fig. 2(a-d): Properties of AA on the activities of (a-b) Atrogin-1 and (c-d) MuRF-1

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

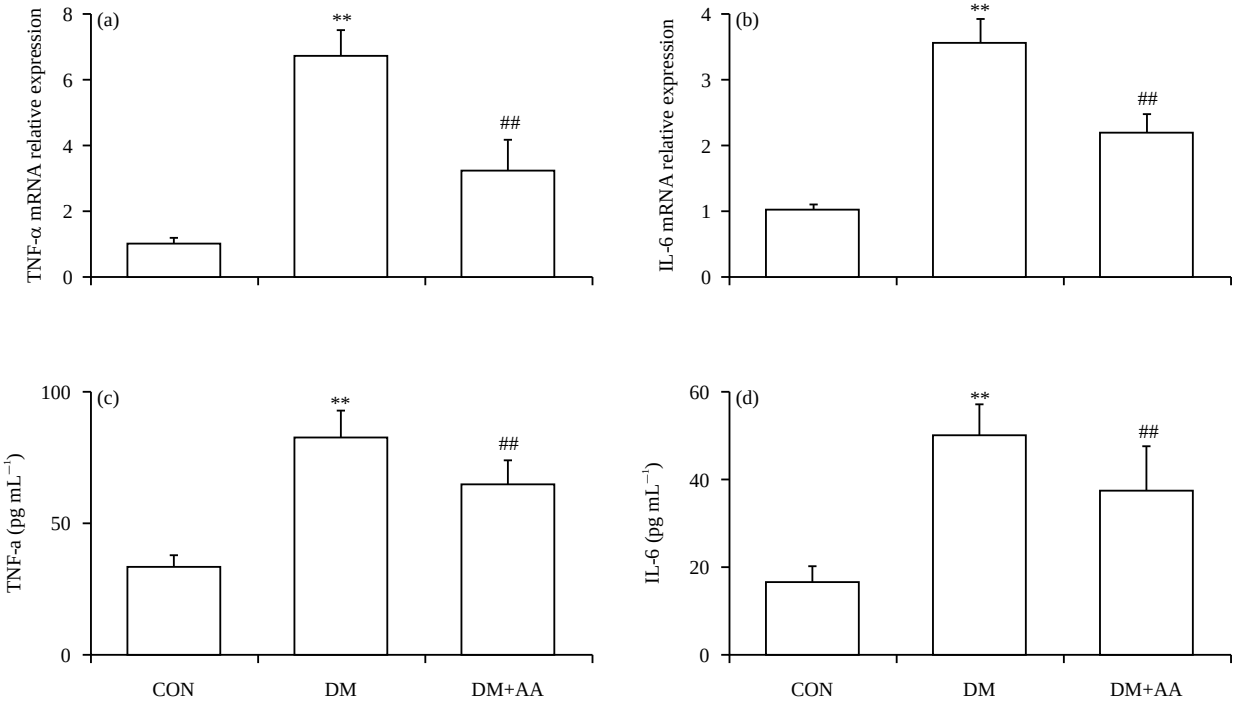

Fig. 3(a-d): Properties of AA on the levels of (a, c) TNF-α and (b, d) IL-6 in skeletal muscle and serum

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

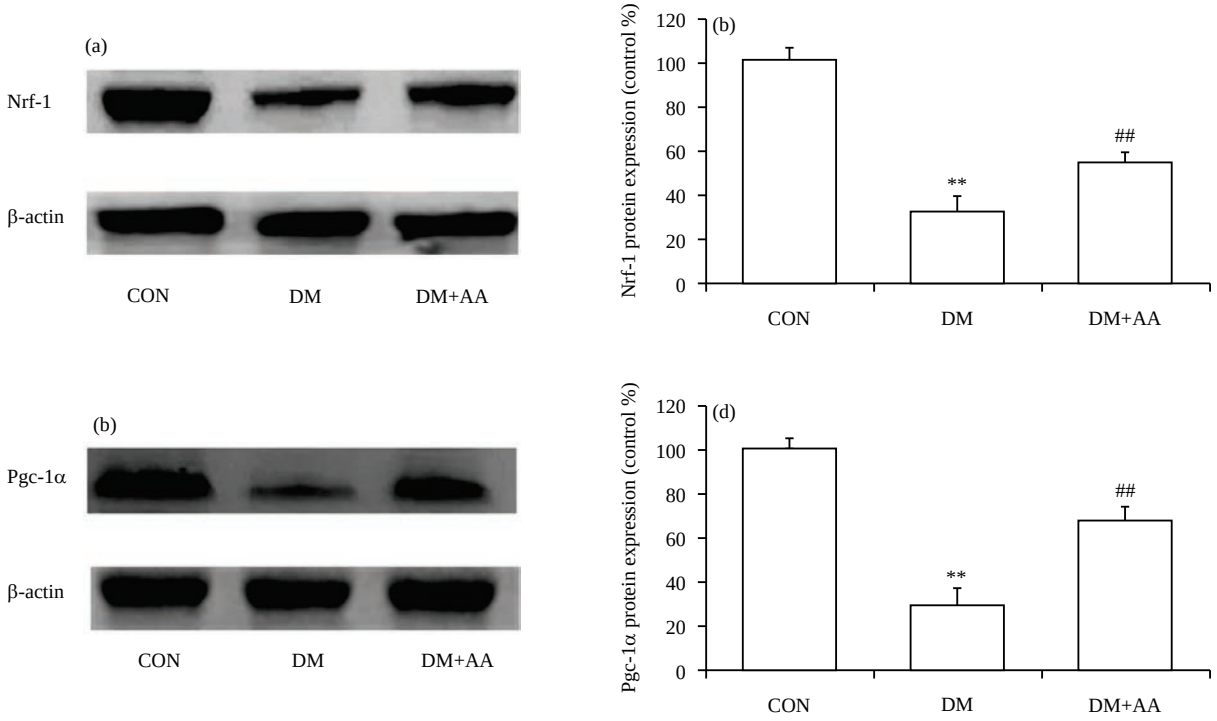

Fig. 4(a-d): Properties of AA on the expressions of (a-b) Nrf-1 and (c-d) Pgc-1α

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

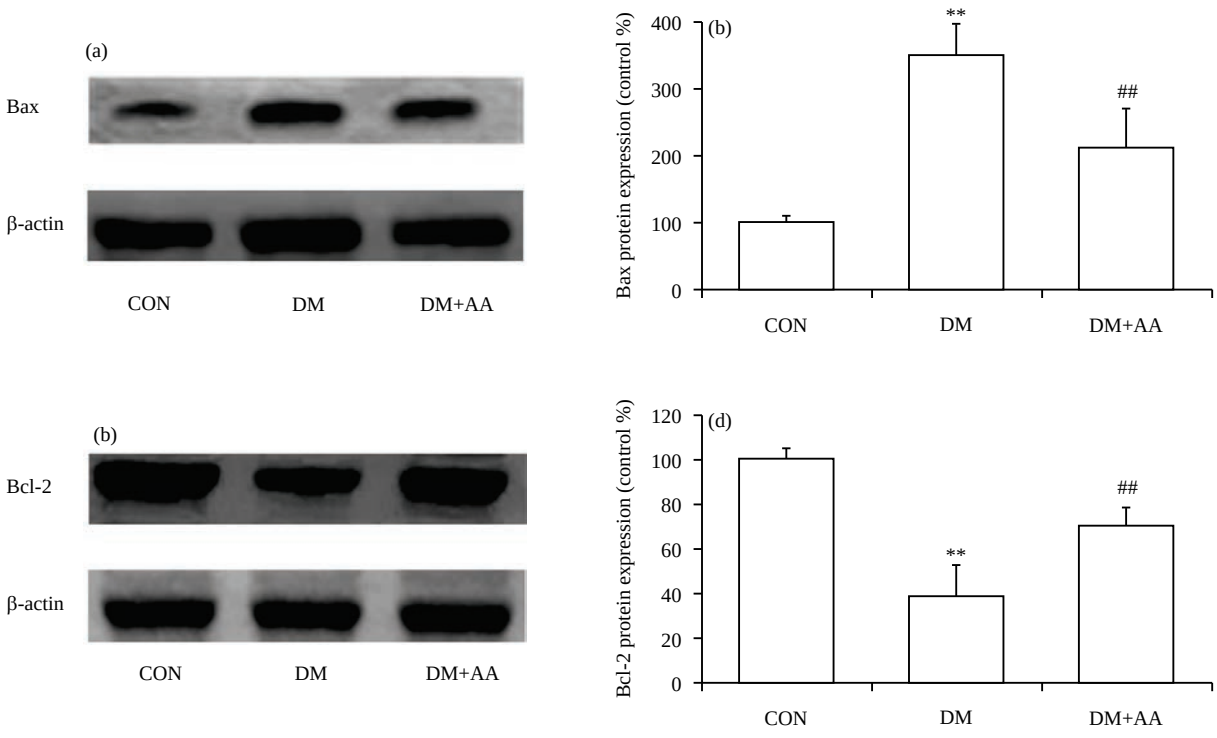

Fig. 5(a-d): Properties of AA on the expression of (a-b) Bax and (c-d) Bcl-2

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

DISCUSSION

The present results demonstrated that AA not only reduced blood glucose concentration but also recovered biochemical alterations in serum to improve general features of diabetic mice. Previous researches showed that AA was involved in improvement of cardiac and hepato-renal toxicities by ameliorating biochemical alterations in serum[17,18]. In diabetes, AA attenuated glucose-6-phosphatase and fructose-1,6-bisphosphatase activates in carbohydrate metabolism, which was involved in regulation of blood glucose level[19,20]. In addition, diabetic myopathy is one of metabolic diseases induced by high blood sugar, which causes muscle atrophy, as represented by impairments of fiber size of skeletal muscle[21,22]. AA was further demonstrated to maintain glucose homeostasis via enhancing the protein expression of IR, PI3K and GLUT-4 in skeletal muscle of STZ-induced diabetic rats[15]. In this study, biochemical alterations were proved to be consistent with muscle histopathology, as represented by myofiber size of skeletal muscle. Besides, treatment with AA enhanced gastrocnemius weight and grip strength in STZ-induced mice. These results suggested that AA possessed important biological functions and pharmacological effects in diabetic muscular dystrophy.

Treatment with AA ameliorated diabetes-induced increase of Atrogin-1 and MuRF-1 expressions in skeletal muscle. Muscle atrophy is a severe type of myopathy which impairs proper functioning of muscle fibers[23]. Previous research showed that ubiquitination system is involved in developing and pathological process of muscle atrophy[24,25]. Atrogin-1 and MuRF-1, as ubiquitin E3 ligases, are indicators for the appraisal of muscle atrophy[26]. Hence, Atrogin-1 and MuRF-1 are used as valuable markers of myocyte degradation and physiological function of skeletal muscle. Our results agree with O'Neill *et al.,* who reported that the increase of Atrogin-1 and MuRF-1 expressions can interfere with protein metabolism to trigger myocyte degradation in the course of muscle atrophy[27]. Moreover, Patient with DM aggravates muscular disorder as a result of muscle atrophy[28,29]. Interestingly, treatment with AA relieved these ubiquitin ligases in gastrocnemius. These results suggested that AA has muscular protective effects in diabetic mice, in line with previous researches, which showed that suppression of Atrogin-1 and MuRF-1expressions is beneficial to recover muscle function and improve muscle atrophy[30].

AA was demonstrated to decrease TNF-α and IL-6 levels in skeletal muscle and serum. Inflammatory response is risk factor in organ damage. AA was proved to alleviate organ damage, such as hepatic failure and pulmonary fibrosis, by preventing inflammatory signaling pathways[31,32]. It seems that the occurrence and progression of diabetes accompanies with excessive inflammation. Diabetic myopathy, as one of diabetic complications, stimulates elevation of inflammatory cytokines[33]. In reverse, overproduction of inflammatory cytokines disturbs metabolism to cause protein degradation[34,35]. Moreover, the levels of TNF-α and IL-6 were increased in hyperglycemia-induced muscle atrophy[36]. However, our results matched with Han *et al.*, who reported that AA mitigated the levels of inflammatory cytokines, such as TNF-α and IL-6, to improve its complication[37]. These data implied that AA could protect muscular system through its anti-inflammatory mechanisms.

Furthermore, current study results revealed diabetes decreased Nrf-1 and Pgc-1α expressions in skeletal muscle of diabetic mice, indicating mitochondrial dysfunction promoted muscular lesions and atrophy. Interestingly, treatment with AA elevated Nrf-1 and Pgc-1α expressions in skeletal muscle. Previous research showed that hyperglycemia perturbed mitochondria function in various tissues, such as skeletal muscle and cardiac muscle[38,39]. It is known that mitochondria dysfunction is harm for energy metabolism, which interferes with vital activities[40, 41]. In skeletal muscle, mitochondrial dysfunction is strongly linked to myopathy[42]. Moreover, Nrf-1 and Pgc-1α are valuable indicators of mitochondrial function[43]. Likewise, AA was proved to elevate glutamate-induced the inactivation of Pgc-1α and Sirt-1 in SH-SY5Y cells[44]. Current Our results matched with Wang *et al.*[30] who reported that mitochondrial dysfunction was improved in diabetic mice, as evidenced by increasing Nrf-1 and Pgc-1α expressions[45]. These data implied that AA was involved in regulation of mitochondrial bioactivities to ameliorate diabetic-associated muscle atrophy.

In this study, AA inhibited Bax expression and elevated Bcl-2 expression. Previous researches showed that apoptotic pathway was involved in regulation of atrophy in skeletal muscle[45,46]. In addition, inflammatory response and mitochondrial dysfunction leads to excessive apoptosis[47,48]. During hyperglycemia, the mechanism of muscle atrophy is probably associated with aberrant expressions of apoptosis-related factors[29]. Bax and Bcl-2 are used as common detection markers to appraise apoptosis[49]. In high glucose-induced toxicity, AA increased Bcl-2 mRNA expression and cell viability in HUVE cells[50]. This study results suggested that AA alleviated muscle atrophy in diabetes, in line with previous researches, which showed that inhibition of apoptosis was conducive to improvement of physiological function in skeletal muscle[51].

CONCLUSION

The results of current study demonstrated and confirmed that treatment with AA effectively ameliorated diabetic myopathy which was linked to its accommodation of inflammation, mitochondrial bioactivity and apoptosis. Therefore, these possible mechanisms revealed that AA is an available agent for improving muscle atrophy in diabetes.

SIGNIFICANCE STATEMENT

This study identified the ameliorative efficacy of AA against muscle atrophy in diabetic mice. Treatment with AA increased myofiber size, weight and grip strength to alleviate diabetic myopathy. In skeletal muscle, AA also restrained amyotrophy by regulating ubiquitin-proteasome system. Additionally, AA attenuated STZ-induced inflammatory response, mitochondrial disorder and apoptosis to mitigate muscle atrophy. These beneficial effects suggested AA could be developed as a therapeutic drug for diabetes-induced muscle atrophy.

ACKNOWLEDGMENT

We greatly appreciate the librarians of Hunan University of Arts and Science for their help in collecting literatures. This study was supported in part by a project supported by Scientific Research Fund of Hunan Provincial Education Department (Grant No. 18B402 & No.19C1274) and Hunan Provincial Natural Science Foundation of China (Grant No. 2020JJ5391).

REFERENCES

1. Bluestone, J.A., K. Herold and G. Eisenbarth, 2010. Genetics, pathogenesis and clinical interventions in type 1 diabetes. Nature, 464: 1293-1300.
2. Burghardt, K., U.A. Müller, N. Müller, C. Kloos, G. Kramer, V. Jörgens and N. Kuniss, 2020. Adequate structured inpatient diabetes intervention in people with type 1 diabetes improves metabolic control and frequency of hypoglycaemia. Exp. Clin. Endocrinol. Diabetes, 128: 796-803.
3. Mallick, C., R. Maiti and D. Ghosh, 2006. Antidiabetogenic effects of separate and composite extract of seed of jamun (*Eugenia jambolana*) and root of kadali (*Musa paradisiacal*) in streptozotocin-induced diabetic male albino rat: A comparative study. Int. J. Pharmacol., 2: 492-503.
4. Evans, P.L., S.L. Mcmillin, L.A. Weyrauch and C.A. Witczak, 2019. Regulation of skeletal muscle glucose transport and glucose metabolism by exercise training. Nutrients, Vol. 11. 10.3390/nu11102432.
5. Muramatsu, K., M. Niwa, T. Tamaki, M. Ikutomo and Y. Masu *et al*., 2017. Effect of streptozotocin-induced diabetes on motoneurons and muscle spindles in rats. Neurosci. Res., 115: 21-28.
6. Liu, S.H., Y.H. Chang and M.T. Chiang, 2010. Chitosan reduces gluconeogenesis and increases glucose uptake in skeletal muscle in streptozotocin-induced diabetic rats. J. Agric. Food Chem., 58: 5795-5800.
7. Jurisic-Erzen, D., G. Starcevic-Klasan, D. Ivanac, S. Peharec, D. Girotto and R. Jerkovic, 2018. The effects of alpha-lipoic acid on diabetic myopathy. J. Endocrinol. Invest., 41: 203-209.
8. Tang, L., N. Li, W. Jian, Y. Kang and B. Yin *et al*., 2017. Low-intensity pulsed ultrasound prevents muscle atrophy induced by type 1 diabetes in rats. Skeletal Muscle, Vol. 7. 10.1186/s13395-017-0145-7.
9. Krause, M.P., M.C. Riddell and T.J. Hawke, 2011. Effects of type 1 diabetes mellitus on skeletal muscle: clinical observations and physiological mechanisms. Pediatr. Diabetes, 12: 345-364.
10. Rusbana, T.B., A.Z. Agista, W.D. Saputra, Y. Ohsaki and K. Watanabe *et al*., 2020. Supplementation with fermented rice bran attenuates muscle atrophy in a diabetic rat model. Nutrients, Vol. 12. 10.3390/nu12082409.
11. Hernández-Ochoa, E.O., P. Llanos and J.T. Lanner, 2017. The underlying mechanisms of diabetic myopathy. J. Diabetes Res., Vol. 2017. 10.1155/2017/7485738.
12. Zebrowska, A., B. Hall, A. Maszczyk, R. Banas and J. Urban, 2018. Brain-derived neurotrophic factor, insulin like growth factor-1 and inflammatory cytokine responses to continuous and intermittent exercise in patients with type 1 diabetes. Diabetes Res. Clin. Pract., 144: 126-136.
13. Alway, S.E., 2019. Mitochondrial dysfunction: Linking type 1 diabetes and sarcopenia. Exercise Sport Sci. Rev., 47: 63-63.
14. Nguyen, T.T.N., H. Choi and H.S. Jun, 2020. Preventive effects of dulaglutide on disuse muscle atrophy through inhibition of inflammation and apoptosis by induction of Hsp72 expression. Front. Pharmacol., Vol. 11. 10.3389/fphar.2020.00090.
15. Ramachandran, V. and R. Saravanan, 2015. Glucose uptake through translocation and activation of GLUT4 in PI3K/Akt signaling pathway by asiatic acid in diabetic rats. Hum. Exp. Toxicol., 34: 884-893.
16. 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.
17. Kamble, S.M. and C.R. Patil, 2018. Asiatic acid ameliorates doxorubicin-induced cardiac and hepato-renal toxicities with Nrf2 transcriptional factor activation in rats. Cardiovasc. Toxicol., 18: 131-141.
18. Liu, J., L. Chen and H. Lu, 2018. Asiatic acid enhances antioxidant and anti-inflammatory activity to suppress isoproterenol induced cardiotoxicity. Int. J. Pharmacol., 14: 1038-1045.

19. Ramachandran, V. and R. Saravanan, 2013. Efficacy of asiatic acid, a pentacyclic triterpene on attenuating the key enzymes activities of carbohydrate metabolism in streptozotocin-induced diabetic rats. Phytomedicine, 20: 230-236.
20. Hung, Y.C., H.T. Yang and M.C. Yin, 2015. Asiatic acid and maslinic acid protected heart via anti-glycative and anti-coagulatory activities in diabetic mice. Food Funct., 6: 2967-2974.
21. Ishida, T., M. Iizuka, Y. Ou, S. Morisawa and A. Hirata *et al*., 2019. Juzentaihoto suppresses muscle atrophy in streptozotocin-induced diabetic mice. Biol. Pharm. Bull., 42: 1128-1133.
22. Yin, L., X. Chen, N. Li, W. Jia and N. Wang *et al*., 2021. Puerarin ameliorates skeletal muscle wasting and fiber type transformation in STZ-induced type 1 diabetic rats. Biomed. Pharmacother., Vol. 133. 10.1016/j.biopha.2020.110977.
23. 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.
24. Khalil, R., 2018. Ubiquitin-proteasome pathway and muscle atrophy. Adv. Exp. Med. Biol., 1088: 235-248.
25. Yadav, A., A. Singh, J. Phogat, A. Dahuja and R. Dabur, 2021. Magnoflorine prevent the skeletal muscle atrophy via Akt/mTOR/FoxO signal pathway and increase slow-MyHC production in streptozotocin-induced diabetic rats. J. Ethnopharmacol., Vol. 267. 10.1016/j.jep.2020.113510.
26. Bodine, S.C. and L.M. Baehr, 2014. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx//atrogin-1. Am. J. Physiol.-Endocrinol. Metab., 307: E469-E484.
27. O`neill, B.T., G. Bhardwaj, C.M. Penniman, M.T. Krumpoch and P.A.S. Beltran *et al*., 2019. FoxO transcription factors are critical regulators of diabetes-related muscle atrophy. Diabetes, 68: 556-570.
28. Glenn, M.D. and D. Jabari, 2020. Diabetic lumbosacral radiculoplexus neuropathy (diabetic amyotrophy). Neurol. Clin., 38: 553-564.
29. 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.
30. 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.
31. Lv, H., Z. Qi, S. Wang, H. Feng, X. Deng and X. Ci, 2017. Asiatic acid exhibits anti-inflammatory and antioxidant activities against lipopolysaccharide and D-galactosamine-induced fulminant hepatic failure. Front. Immunol., Vol. 8. 10.3389/fimmu.2017.00785.
32. Dong, S.H., Y.W. Liu, F. Wei, H.Z. Tan and Z.D. Han, 2017. Asiatic acid ameliorates pulmonary fibrosis induced by bleomycin (BLM) via suppressing pro-fibrotic and inflammatory signaling pathways. Biomed. Pharmacother., 89: 1297-1309.
33. Narasimhulu, C.A. and D.K. Singla, 2021. Amelioration of diabetes-induced inflammation mediated pyroptosis, sarcopenia and adverse muscle remodelling by bone morphogenetic protein-7. J. Cachexia Sarcopenia Muscle, 12: 403-420.
34. Mastrocola, R., P. Reffo, F. Penna, C.E. Tomasinelli and G. Boccuzzi *et al*., 2008. Muscle wasting in diabetic and in tumor-bearing rats: Role of oxidative stress. Free Radical Biol. Med., 44: 584-593.
35. Webster, J.M., L.J.A.P. Kempen, R.S. Hardy and R.C.J. Langen, 2020. Inflammation and skeletal muscle wasting during cachexia. Front. Physiol., Vol. 11. 10.3389/fphys.2020.597675.
36. Ono, T., S. Takada, S. Kinugawa and H. Tsutsui, 2015. Curcumin ameliorates skeletal muscle atrophy in type 1 diabetic mice by inhibiting protein ubiquitination. Exp. Physiol., 100: 1052-1063.
37. Han, Y., Y. Jiang, Y. Li, M. Wang and T. Fan *et al*., 2019. An aligned porous electrospun fibrous scaffold with embedded asiatic acid for accelerating diabetic wound healing. J. Mater. Chem. B, 7: 6125-6138.
38. Wood, N., S. Straw, M. Scalabrin, L.D. Roberts, K.K. Witte and T.S. Bowen, 2021. Skeletal muscle atrophy in heart failure with diabetes: From molecular mechanisms to clinical evidence. ESC Heart Fail., 8: 3-15.
39. Fujimaki, S. and T. Kuwabara, 2017. Diabetes-induced dysfunction of mitochondria and stem cells in skeletal muscle and the nervous system. Int. J. Mol. Sci., Vol. 18. 10.3390/ijms18102147.
40. Ji, L.L. and D. Yeo, 2019. Mitochondrial dysregulation and muscle disuse atrophy. F1000Research, Vol. 8. 10.12688/f1000research.19139.1.
41. Rendon, D.A., 2006. Mitochondrial cardiac energy metabolism after one treatment with benzonidazole-rochagan®. Int. J. Pharmacol., 2: 623-627.
42. Gan, Z., T. Fu, D.P. Kelly and R.B. Vega, 2018. Skeletal muscle mitochondrial remodeling in exercise and diseases. Cell Res., 28: 969-980.
43. Ye, L., M. Li, Z. Wang, Z. Yang and J. Zhang *et al*., 2020. Depression of mitochondrial function in the rat skeletal muscle model of myofascial pain syndrome is through down-regulation of the AMPK-PGC-1α-SIRT3 axis. J. Pain Res., 13: 1747-1756.
44. Xu, M.F., Y.Y. Xiong, J.K. Liu, J.J. Qian, L. Zhu and J. Gao, 2012. Asiatic acid, a pentacyclic triterpene in *Centella asiatica*, attenuates glutamate-induced cognitive deficits in mice and apoptosis in SH-SY5Y cells. Acta Pharmacol. Sinica, 33: 578-587.

45. Dupont-Versteegden, E.E., 2006. Apoptosis in skeletal muscle and its relevance to atrophy. World J. Gastroenterol., 12: 7463-7466.
46. Reddy, V.S., S. Jakhotia, P.Y. Reddy and G.B. Reddy, 2015. Hyperglycemia induced expression, phosphorylation and translocation of αB-crystallin in rat skeletal muscle. IUBMB Life, 67: 291-299.
47. Cauwels, A., E. Rogge, B. Vandendriessche, S. Shiva and P. Brouckaert, 2014. Extracellular ATP drives systemic inflammation, tissue damage and mortality. Cell Death Dis., Vol. 5. 10.1038/cddis.2014.70.
48. Rizwan, H., S. Pal, S. Sabnam and A. Pal, 2020. High glucose augments ROS generation regulates mitochondrial dysfunction and apoptosis via stress signalling cascades in keratinocytes. Life Sci., Vol. 241. 10.1016/j.lfs.2019.117148.
49. Li, M., H. Qingxiang, X. Lingli, Z. Mei, L. Dan and L. Yongwang, 2019. Treatment with tinosporaside attenuates the uterine fibroid by stimulating the apoptosis. Int. J. Pharm., 15: 50-55.
50. Yan, S.L., Z.H. Wang, M.C. Mong, Y.C. Yang and M.C. Yin, 2019. Combination of carnosine and asiatic acid provided greater anti-inflammatory protection for HUVE cells and diabetic mice than individual treatments of carnosine or asiatic acid alone. Food Chem. Toxicol., 126: 192-198.
51. Reddy, S.S., K. Shruthi, D. Joy and G.B. Reddy, 2019. 4-PBA prevents diabetic muscle atrophy in rats by modulating ER stress response and ubiquitin-proteasome system. Chem. Bio. Interact., 306: 70-77.