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Asiatic acid ameliorates life and health span in fruit fly

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Abstract: Aging is becoming a prominent health problem in present world. Asiatic acid has multiple health-protecting effects because of its variety biological function. However, relation between asiatic acid and aging is still unknown. In this study, fruit fly was used as model animal to illuminate anti-aging effect of asiatic acid. Our results advised asiatic acid possessed efficacy of promoting health span, as represented by extending lifetime and enhancing locomotor activity both in intrinsic and pathologically aging. In external environment, asiatic acid elevated survival rate against oxidative response and starvation resistance. In aspect of anti-aging mechanism, asiatic acid possessed antioxidant capacity by improvement of super oxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-px) activities and suppression of malondialdehyde (MDA) level to promote health span. In addition, asiatic acid amended energy material storage to meet needs of life process. Moreover, asiatic acid inhibited acetylcholine (AChE) activity to alter cholinergic system in aging process. Lastly, asiatic acid upregulated peroxisome proliferator-activated receptor gamma coactivator 1 (PGC1) and silence information regulator 2 (Sir2) expression in intrinsic aging. In conclusion, asiatic acid exerted health-protecting potential via modulating directly or indirectly antioxidant activity, cholinergic system and longevity genes and could be developed into anti-aging agent.

Keywords: Asiatic acid, aging, oxidative stress, AChE, PGC1, Sir2

INTRODUCTION

Aging is complicated phenomenon which is represented as senescence. In aging process, physiological function is subjected to slow deterioration (Foo *et al.,* 2020). The occurrence of chronic diseases, such as neurodegeneration and metabolic dysfunction, is noteworthy consequences of aging (Butterfield *et al.,* 2020).These physiological changes pose serious threats to health span, which is becoming an internationalization problem. In addition, the number of senior citizens is rising in the world. Hence, aging, as a tremendous public health issue, becomes a hot research spot and front-burner question (Cai *et al.,* 2022).

The account of aging is multiple, a highlighted cause of which is oxidative damage in organisms (Barja. 2019). Excess oxidative stress disturbs internal environment homeostasis to affect health and longevity in human. Previous research reported lutein significantly promoted longevity by reducing MDA level and increasing antioxidant enzyme activities in fruit fly (Zhang *et al.,* 2014). In D-galactose-induced aging, berberine maintained redox balance against kidney senility by alleviating MDA level and enhancing heme oxygenase-1 (HO-1) activity (El-Horany *et al.,* 2020). Trehalose attenuated oxidative stress by activating SOD, GSH and CAT to prevent D-galactose-induced brain tissue damage and behavioral impairment (Sun *et al.,* 2020). In addition, numberous genes have been verified in association with longevity in diverse species (Khan *et al.,* 2019). In fruit fly, thioredoxin-interacting protein (TXNIP) over expression shortened lifespan by aggravating oxidative DNA damage (Oberacker *et al.,* 2018). Modest forkhead box, sub-group O (FOXO) over expression improved nonpathological functional degeneration during aging, while rutin ameliorated fruit fly health span via up regulating FOXO expression (Blice-Baum *et al.,* 2017; Chattopadhyay *et al.,* 2017). Moreover, oxidative stress abnormally regulates biological molecules in anabolism and catabolism to result in organ decline in structure and function (Bandres-Ciga et al., 2020). Hence, to alleviate oxidative stress and regulate longevity genes is effective way to improve physical function changes in aging.

Asiatic acid is one of triterpenes with multiple biological functions. Nowadays, asiatic acid is extensively treated as a food supplement and herb because of its dietary antioxidants. Previous research reported asiatic acid possessed therapeutic outcome in 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-related Parkinson's disease by increasing glutathione level and decreasing ROS generation (Chao *et al.,* 2016). Besides this, asiatic acid is involved in regulating various gene expression in diversified experimental models. In rat, asiatic acid defended against doxorubicin-induced toxicity via enhancing nuclear factor, erythroid derived 2, like 2 (Nrf2) expression (Kamble & Patil. 2018). In neuroblastoma cell, asiatic acid altered PGC1α and Sirt1 expressions to attenuate glutamate-induced toxicity (Xu *et al.,* 2012). Although asiatic acid has been reported to alleviate D-galactose-induced brain aging in mice, little attention regarding mechanism of asiatic acid against aging is paid (Chao *et al.,* 2015). Hence, we assumed asiatic acid was

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involved in modulating lifespan by regulating antioxidant capacity and longevity-associated genes in fruit fly.

MATERIALS AND METHODS

Fruit flies culture

Fruit flies (W1118) were obtained from Hunan Normal University. According to previous method, all fruit flies were cultured in vials with basal diets (Zheng *et al.,* 2015). The diets were renewed once every other day during whole study. Experimental fruit flies were kept in stable environment at 25^{o}C and 12h/12h light/dark period. All animal experiments were inspected according to the Ethics Committee of Hunan University of Arts and Science (No. HUAS-2021-TY-116).

Experimental group

Asiatic acid (purity: ≥95%) was purchased from sigma. After 1-day of hatch, fruit flies were randomly divided into 4 groups: Control group, asiatic acid low-dose group (AA-L), asiatic acid medium-dose group (AA-M) and asiatic acid high-dose group (AA-H). There were 200 flies in each group. The density of asiatic acid in AA-L, AA-M and AA-H group was 0.01, 0.5 and 1mg asiatic acid/100g diet, respectively. Asiatic acid was fed every day throughout whole study.

Lifespan assay

Fruit flies were observed every day to record growing state and survival time. At the end of experiments, survivorship curve was drawn. Lifetime indicators, including median and maximum lifespan, were calculated to appraise anti-aging role of asiatic acid in fruit flies (Zou *et al.,* 2017).

Climbing assessment

Climbing assessment was performed in accordance with previous method (Wen *et al.,* 2016). At 6-week old, fruit flies were placed in tube and crawled with freedom. Then, corresponding distances were recorded to calculate climbing index.

D-Galactose challenge

D-Galactose was used to evoke pathologically aging in numerous researches. In this study, fruit flies were challenged with 6.5% D-galactose in basal diets to accelerate aging (Cui *et al.,* 2004). Survival time was recorded to calculate median and maximum lifespan. At 6-week old, climbing index was measured to appraise motor ability (Wen *et al.,* 2019).

External environment exposure

External environment was performed to estimate health-protection effect of asiatic acid. Fruit flies were starved for 2 hours at 6-week old. Then, fruit flies were exposed at 30% H2O2 in 6% glucose medium for 6 hours to evaluate oxidative injury (Wang *et al.,* 2015). In starvation experiment, fruit flies were left in bottles with 1% agar for 2 days. Similarly, survival situation were recorded to measure healthspan in external environment (Rana *et al.,* 2017).

Biochemical measurement

Fruit flies were collected at 6-week. Then, experimental fruit flies were ground on ice and centrifuged at 12,000g for 15 min. Supernatant was taken to assay intracellular biochemical parameters. The assay kits of AChE, ATP, CAT, Glycogen, GSH-px, MDA and SOD were purchased from Nanjing Jiancheng Biotechnology Institute (Nanjing, China).

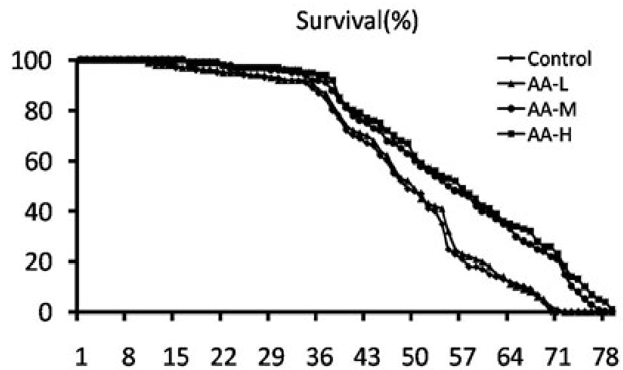

Fig. 1: Efficacy of asiatic acid on lifespan curve in fruit flies. Asiatic acid-L, asiatic acid low-dose group (0.01 mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

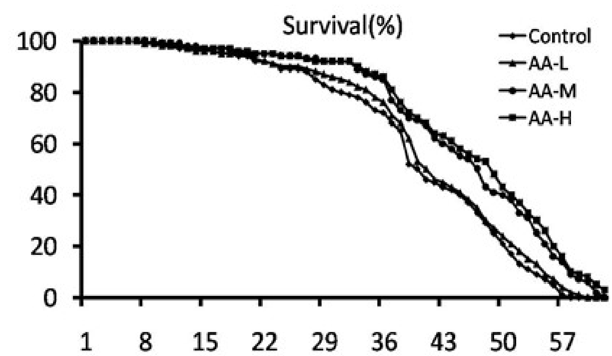

Fig. 2: Efficacy of asiatic acid on lifespan curve in D-galactose-treated fruit flies. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

Gene detection

RNA from fruit flies was extracted by TRIzol. RNA was turned into cDNA to detect gene expression by real-time PCR. Primers for PGC1, Sir2 and Rp49 were synthesized from Sangon Biotech (table 1). Rp49 is an internal gene. mRNA expressions were measured by $2^{-\Delta\Delta Ct}$ method (Wen *et al.* 2019).

Table 1: Primers were used in this study (Wen *et al.* 2019).

Gene		Primer sequences
PGC1	Forward primer	5′-TGTTGCTG CTACTGCTGCTT-3′
	Reverse primer	5′-GCCTCTGCATCACCTACA CA-3′
Sir2	Forward primer	5′-GCAGTGCCAGCCCAATAA-3″
	Reverse primer	5′-AGCCGATCACGATCAGTAGA-3′
Rp49	Forward primer	5′-CTAAGCTGTC GCACAAATGG-3′
	Reverse primer	5-AACTTCTTGAATCCGGTGGG-3′

Table 2: Efficacy of asiatic acid on MLS and MaxLS in fruit flies (n = 800).

	MLS (days)	Percent of increase	Max LS (days)	Percent of increase
Control	47.5±1.4		67.7±1.4	
AA-L	48.2±1.9	1%	68.1±1.9	0.6%
AA-M	54.3±2.1**	14%	74.5±1.6**	10%
AA-H	55.6 ±1.9**	17%	76.7 ±1.5**	13%

Note: Lifelong administration of asiatic acid obviously enhanced MLS and MaxLS in intrinsic aging. **P<0.01 versus control group. MLS, Mean lifespan. MaxLS, Maximum lifespan. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

Table 3: Efficacy of asiatic acid on MLS and MaxLS in fruit flies (n = 800).

	MLS (days)	Percent of increase	MaxLS (days)	Percent of increase
Control	39.5±1.5		55.2±1.2	
AA-L	40.6±1.3	3%	56.3±1.5	2%
AA-M	44.6±1.4**	13%	59.5±1.2**	8%
AA-H	45.5±1.9**	15%	60.5±1.4**	10%

Note: Lifelong administration of asiatic acid obviously enhances MLS and MaxLS in D-galactose-accelerated aging. **P<0.01 versus control group. MLS, Mean lifespan. MaxLS, Maximum lifespan. Asiatic acid-L, asiatic acid low-dose group (0.01 mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

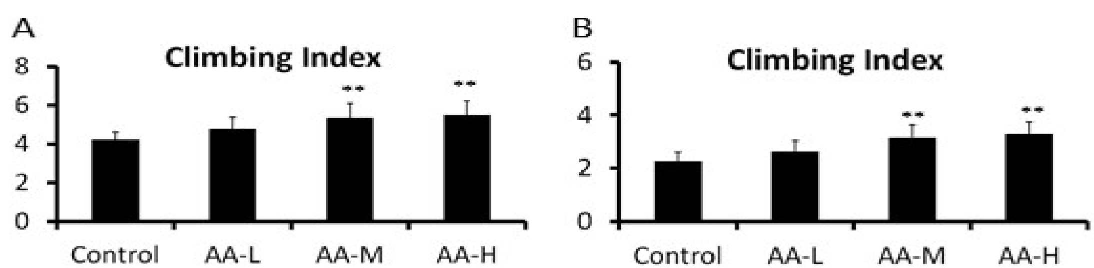

Fig. 3: Efficacy of asiatic acid on locomotor activity in fruit flies. Lifelong administration of asiatic acid obviously visibly promotes locomotor function in (A) intrinsic aging and (B) pathologically aging.**p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5 mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

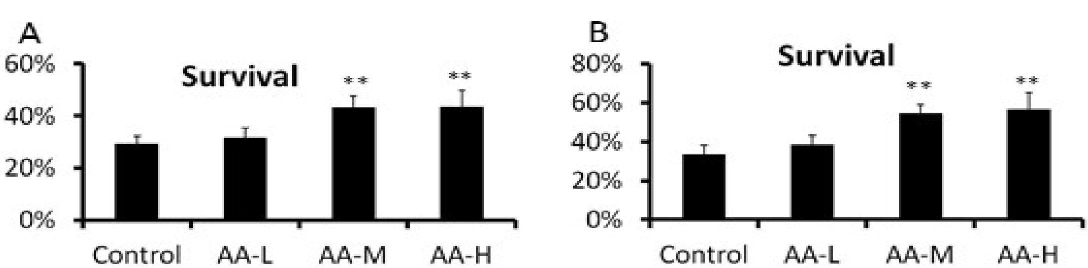

Fig. 4: Efficacy of asiatic acid on external environment stress in fruit flies. Lifelong administration of asiatic acid visibly enhances survival rate in (A) H2O2- and (B) starvation-treated fruit flies. **p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5 mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

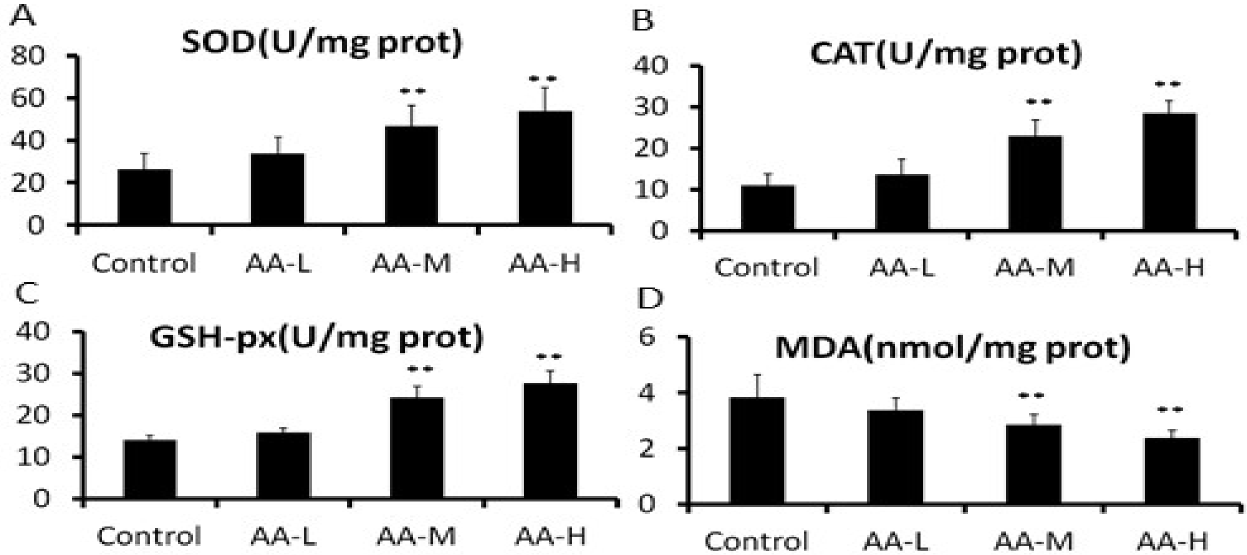

Fig. 5: Efficacy of asiatic acid on oxidation resistance in fruit flies. Lifelong administration of asiatic acid visibly elevates (A) SOD, (B) CAT and (C) GSH-px activities, while alleviates (D) MDA level in fruit flies. **p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

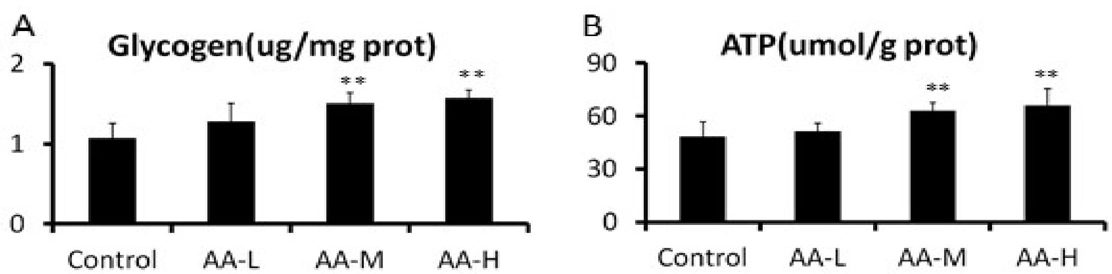

Fig. 6: Efficacy of asiatic acid on energy material storage in fruit flies. Lifelong administration of asiatic acid visibly elevates (A) glycogen and (B) ATP contents in fruit flies. **p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1 mg/100g).

STATISTICAL ANALYSIS

Data was represented as mean ± SD. The data was analyzed with SPSS 16.0 software. Statistical significance was demonstrated by one-way ANOVA test. P<0.05 was deemed significant.

RESULTS

Efficacy of asiatic acid on lifespan

The lifespan is demonstrated in fig. 1. Lifelong administration of asiatic acid at concentration of 0.5 mg/100g and 1mg/100g markedly extends lifespan. Mean lifespan of control, asiatic acid-L, asiatic acid-M and asiatic acid-H groups are 49.1 days, 50.4 days, 55.7 days, and 56.3 days, respectively (table 2). Maximum lifespan was of control, asiatic acid-L, asiatic acid-M and asiatic acid-H groups were 69.1 days, 71.2 days, 76.0 days and 78.9 days, respectively (table 2). These results advise asiatic acid promotes lifespan function in intrinsic aging.

Efficacy of asiatic acid on lifespan in D-galactose-induced pathologically aging

The efficacy of asiatic acid on pathologically aging, which is induced by D-galactose, is demonstrated in fig. 2. Lifelong administration of asiatic acid at concentration of 0.5mg/100g and 1mg/100g markedly extends lifespan in D-galactose-aggravated aging. Mean lifespan of control, asiatic acid-L, asiatic acid-M and asiatic acid-H groups are 39.2 days, 40.2days, 45.4 days and 47.4 days, respectively (table 3). Maximum lifespan is of control, asiatic acid-L, asiatic acid-M and asiatic acid-H groups are 55.2 days, 57.4 days, 60.8 days and 62.4 days, respectively (table 3). These results advise asiatic acid mitigates D-galactose-evoked toxicity to promoted lifespan.

Efficacy of asiatic acid on locomotor activity
The efficacy of asiatic acid on age-related locomotor impairment is demonstrated in fig. 3. The climbing indexes are obviously extended in asiatic acid-M and asiatic acid-H groups both in intrinsic aging and D-galactose-exacerbated aging. These results advise asiatic acid facilitates movement capacity in aging process.

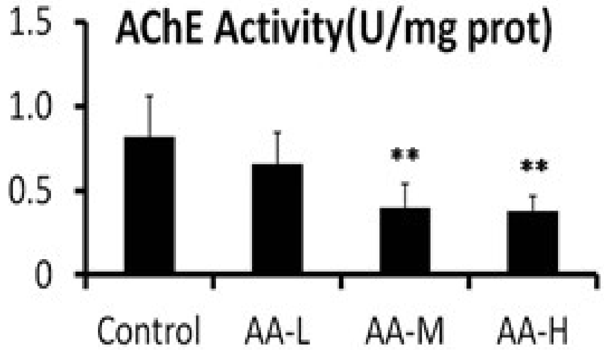

Fig. 7: Efficacy of asiatic acid on AChE level in fruit flies. Lifelong administration of asiatic acid visibly relieves protein level of AChE in fruit flies. **p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01 mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

Efficacy of asiatic acid on external environment stress
The efficacy of asiatic acid on external environment stress, including oxidative response and starvation resistance are demonstrated in fig. 4. In H2O2-induced acute toxicity experiment, survival rate is obviously augmented in asiatic acid-M and asiatic acid-H groups. Moreover, lifelong administration of asiatic acid at concentration of 0.5mg/100g and 1mg/100g obviously increases survival rate in starvation resistance. These results advise asiatic acid ameliorates healthspan party through suppression of oxidative stress and improvement of energy homeostasis.

Efficacy of asiatic acid on oxidation resistance
The efficacy of asiatic acid on antioxidant ability is demonstrated in fig. 5. The activities of SOD, CAT and GSH-px are obviously elevated in asiatic acid-M and asiatic acid-H groups. While lifelong administration of asiatic acid at concentration of 0.5mg/100g and 1 mg/100g obviously decreases MDA level. These results advise asiatic acid ameliorates antioxidant power to promote lifetime in intrinsic aging.

Efficacy of asiatic acid on energy material storage
The efficacy of asiatic acid on energy material storage, which is related to survival in starvation resistance, is demonstrated in fig. 6. The contents of glycogen and ATP were obviously increases in asiatic acid-M and asiatic acid-H groups. These results advise asiatic acid regulates energy supplier to attenuate exercise-induced fatigue.

Efficacy of asiatic acid on AChE level
The efficacy of asiatic acid on AChE level, which is relevant to degenerative disease as represented by locomotor impairment in aging, is demonstrated in fig. 7. The AChE level is obviously inhibited in asiatic acid-M and asiatic acid-H groups. These results advise asiatic acid modulates AChE level to attenuate aging-relatived locomotor disorder.

Efficacy of asiatic acid on aging-associated gene expressions
The efficacy of asiatic acid on PGC1and Sir2, which are related to longevity, is demonstrated in fig. 8. The mRNA expressions of PGC1 and Sir2 are obviously aggrandized in asiatic acid-M and asiatic acid-H groups. These results advise asiatic acid prolongs lifespan via accommodating PGC1/Sir2 pathway.

DISCUSSION

Aging is a spontaneous biological efficacy decline course in living body. Here we framed a round of researches to prove the properties of dietary asiatic acid on health span in fruit fly. Fruit fly is a preeminent model organism to study longevity as for its low lifetime, abundant plentiful offspring, and easy cultivation (Ajagun-Ogunleye & Ebuehi. 2020). In particular, the mechanism connected with aging, such as oxidative reaction, is highly conservative in fruit fly and mammalian because most of their genes are homology. In addition, D-galactose is universally acknowledged to be applied to aging model in many organisms (Liu *et al.,* 2022). In this study, we demonstrated that asiatic acid could prolong lifespan during physiological process, represent as mean lifespan and maximum lifespan, both in natural and d-gal-induced aging.

Negative geotaxis is a very important behavior characteristic in fly. Locomotor capacity is highly associated with normal physical fitness. The forceful inverse relevance between aging and locomotor capacity was proved in many model organism. Moreover, the vital indicator of degenerative diseases, as one of senile diseases, is locomotor impairment (Grice & Liu. 2022). Previous result reported asiatic acid served as a neuroprotective agent to improve cerebral ischemia-induced behavioral deficits in mice (Krishnamurthy *et al.,* 2009). Hence, degradation in locomotor function was detected to assess anti-aging efficacy of asiatic acid. In this study, asiatic acid alleviated locomotor impairment both to regulate pathological features of neurodegenerative disease in intrinsic aging and D-galactose-exacerbated aging.

Oxidative stress is well familiar to be a crucial factor of aging. Alleviation of antioxidant activity and accumulation of oxidative metabolite, which lead to oxidative injury, become serious with aging (Foo *et al.,* 2020).

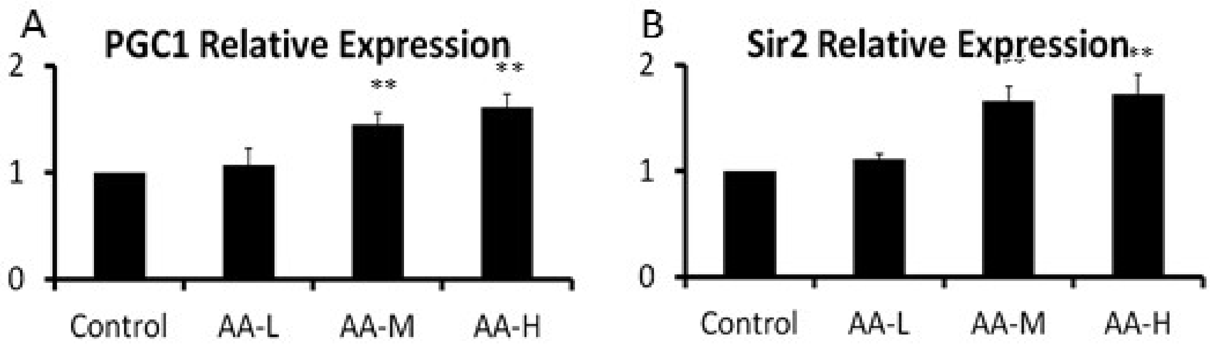

Fig. 8: Efficacy of asiatic acid on aging-relative gene expressions in fruit flies. Lifelong administration of asiatic acid visibly regulates (A) PGC1 and (B) Sir2 mRNA expression in fruit flies. **p<0.01 versus control group. Asiatic acid-L, asiatic acid low-dose group (0.01mg/100g). Asiatic acid-M, asiatic acid middle-dose group (0.5mg/100g). Asiatic acid-H, asiatic acid high-dose group (1mg/100g).

In organism, antioxidant substance is mainly represented as endogenous enzymes, such as SOD, CAT and GSH-Px, involved in antioxidant defense system, while MDA, as an oxidation indictor, is lipid metabolism in oxidation reaction. In particular, oxidative stress aggravates aging process because of its damage on lipid, protein, and DNA. Hence, to restrain Oxidative damage is effective way to delay aging. Nowadays, direct food additives with antioxidant have become an effective way to fight aging. A stable positive connection was validated between antioxidant ability and asiatic acid. Previous results showed consumption of dietary asiatic acid played antioxidant action in early embryonic development by enhancing GSH content and attenuating H2O2-induced ROS production (Qi *et al.,* 2020). In age-related disease, asiatic acid ameliorated SOD, GSH and CAT activities to restrain oxidative stress in aluminum chloride (AlCl3)-related Alzheimer's disease like model (Ahmad *et al.,* 2019). In this study, asiatic acid elevated SOD, CAT and GSH-px activities and decreased MDA level to enhance antioxidant ability in ageing process.

To verify the anti-oxidative performance of asiatic acid during aging process, H2O2-induced acute experiments were used to evaluate oxidative injury. In this study, asiatic acid increased survival rate in H2O2-relevant oxidative stress. In addition, previous result showed that exposure to external environment stress, such as starvation resistance, is in connection with longevity, and chosen as a parameter to evaluate anti-aging function (Wongchum & Dechakhamphu. 2021). In this study, asiatic acid increased survival rate in starvation environment. Moreover, asiatic acid aggrandized glycogen and ATP contents in aged flies, which was closely linked to starvation responses. These results showed that asiatic acid possess protective action against external adverse stimulus closely related to life span in the life cycle.

Cholinergic system is a crucial indicator of aging and its related degenerative diseases. Neurodegenerative diseases, such as Parkinson's disease and Alzheimer's disease, are features of aging. AChE, as a serine protease, is involved in cholinergic system and its abnormal expression is noxious for cholinergic neurotransmission by hydrolyzing acetylcholine into choline (Craig *et al.,* 2011; Hai *et al.,* 2013). In scopolamine-associated Alzheimer's disease like model, deferiprone relieved AChE activity to protect against cognitive disorders (Fawzi *et al.,* 2020). In fruit fly, curcumin alleviated AChE expression to extend lifespan (Akinyemi *et al.,* 2018). Moreover, asiatic acid ameliorated AChE activities to mitigate AlCl3-induced brain injury in rat (Ahmad *et al.,* 2018). In this study, asiatic acid was proved to delaye senescence by inhibiting AChE activity.

Aging-associated gene is well known regulator for mediating longevity performance. Sirtuin family proteins are critically important factors involved in life activities. In current aging research, Sir 2, as mammalian ortholog of Sirt1, was proved to regulate aging and longevity in fruit fly (Hoffmann *et al.,* 2013). In mice, sirt 1 promoted longevity and delayed senescence via mediating Nkx2-1/Ox2r pathway (Satoh *et al.,* 2013) PGC1 is also a crucial determinant of life span. Over expressing PGC1 could effectively enhance mitochondrial activity and slow aging in fruit fly (Michael *et al.,* 2011). In this study, asiatic acid exerted anti-aging potential via accommodating PGC1/Sir2 pathway.

CONCLUSION

Our study demonstrated asiatic acid possessed potential benefit on facilitating health span and slowing senescence in fruit fly via modulating directly or indirectly antioxidant activity, cholinergic system and longevity genes. Therefore, asiatic acid might be a novel anti-aging agent.

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