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Aloe vera, a phytochemical ameliorates Huntington's disease pathology in *Drosophila* model

Biomedical

KEYWORDS : Huntington's disease, Aloe vera, Drosophila

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ABSTRACT

Huntington's disease (HD) is a neurodegenerative disorder caused by an abnormal expansion of polyglutamine (polyQ) stretch in huntingtin (Htt) protein. Progressive degeneration of neurons and impaired motor function has been implicated in the pathogenesis of HD. HD pathology is complex and may affect multiple cellular processes. At present, no cure or effective treatment for HD exists. Additionally, the currently available treatments cause undesirable and severe side effects.Therefore, we investigated the effects of the 'miracle plant' or 'natural healer' plant 'Aloe vera' on HD pathogenesis. Aloe vera is an ancient plant which has an excellent safety profile and a broad range of medicinal properties, including anti-oxidant, anti-inflammatory and immunomodulatory effects. Here we show that Aloe vera leaf gel extract significantly suppresses disease symptoms in a Drosophila model of HD which mimics the key features of the agonizing, fatal human disease. Therefore, we propose that Aloe vera can be an excellent and effective phytochemical for the treatment of HD symptoms with the possibility of least or no side effects.

Introduction

Huntington's disease (HD) is an autosomal dominant, progressive neurodegenerative disorder caused by expansion of polyQ stretch and characterized primarily by progressive cognitive, behavioural and motor dysfunctions. PolyQ repeat expansions above 35Qs lead to disease condition and the degree of toxicity conferred on the mutant protein is directly proportional to polyQ length (Marsh et al., 2003).

The clinical features and pattern of neurodegeneration differ among the polyQ diseases. However, these diseases share important pathogenic features such as abnormal protein folding and aggregation (Paulson et al., 2000). PolyQ pathology is complex and may affect multitude cellular events such as transcription, protein modification, oxidative stress, mitochondrial dysfunction and impaired protein homeostasis (Bates et al., 2002; Marsh and Thompson, 2004). Unfortunately, there is no cure or effective treatment for polyQ diseases including HD. Moreover, administration of synthetic drugs against these devastating diseases elicits undesirable and severe side effects (Monroy et al., 2013). Thus, therapeutic agents that can target multiple cellular mechanisms are expected to provide greater relief from disease symptoms. The use of 'natural products' which elicit less or no side effects as an alternative to conventional therapeutic strategy has gained tremendous momentum in the last few decades. With this approach, we investigated the effects of an ancient medicinal plant *Aloe vera* (*Aloe barbadensis*) on HD pathogenesis using transgenic *Drosophila*. *Aloe vera* has been used for centuries to treat various ailments due to its curative and therapeutic properties. Till date, over 75 active components of *Aloe vera* with medicinal properties have been identified and these bioactive components have been suggested to possess strong synergistic action in alleviating various disease symptoms (Scalbert and Williamson, 2000; Hamman, 2008; Nejatzadeh-Barandozi, 2013). *Aloe vera* possesses a multitude of biological properties such as immunomodulation, anti-depressant, anti-inflammatory, anti-oxidant and memory enhancing effects (Nejatzadeh-Barandozi, 2013; Parihar, et al., 2004; Halder, et al., 2012; Rathor, et al., 2012). A recent study has demonstrated the extension of adult longevity in *Drosophila* on *Aloe vera* supplementation possibly due to its anti-oxidant activity (Chandrashekara and Shakarad, 2011). In another study, *Aloe vera* was shown to have protective effects on mitochondria of neuronal cells and increase the weight of rat brain (Wang et al., 2010). It has also been reported to improve lipid profile status in rats with streptozotocin-induced diabetes (Rajasekaran et al., 2006). The plethora of biological activities of *Aloe vera* has been attributed to its various chemical components or phytonutrients that include vitamins, enzymes, triterpenes, glyconutrients, polysaccharides, acemannan, phenolic glycosides and dihydrocoumarias (Choi and Chung, 2003; Eshun and He, 2004).

Transgenic *Drosophila* models of neurodegenerative diseases including HD exhibit most of the key pathogenic features of the diseases in humans, for instance, late onset, progressive neurodegeneration, reduced longevity and motor dysfunction (Marsh and Thompson, 2004; Marsh et al., 2003; Bonini and Fortini 2003). Therefore, these are excellent in vivo models for testing potential therapeutic compounds. Here, we show that *Aloe vera* leaf gel extract significantly suppressed degeneration of photoreceptor neurons and motor neuron dysfunction in a transgenic *Drosophila* model expressing Htt exon1 with 93 glutamine residues (Httex1p Q93) suggesting that *Aloe vera* can be a promising agent for the treatment of HD and other neurodegenerative diseases.

MATERIALS AND METHODS

Drosophila stocks and crosses. The polyglutamine expressing transgenic stock used in this study is *w*;P{UAS-Httex1p Q93}4F1. These flies were mated with the pan-neuronal driver *w*; P {$w^{+mW.hs}$ =GawB} elavC155. Cultures were raised at 25°C.

***Aloe vera* extracts preparation.** *Aloe vera* leaf was taken from a healthy plant (well watered and exposed to adequate sunlight). After thorough washing of leaf,, the *Aloe* gel was extracted from the middle of the leaf by ripping off the skin. The desired amount of extracted *Aloe* gel was supplemented to *Drosophila* food media as per the concentrations to be prepared.

Crawling assay. Eggs were transferred to vials containing standard *Drosophila* food supplemented with different concentrations of *Aloe vera* crude extract (0%, 2.5%, 5%, and 7.5%). The crawling ability of a total of ten wandering third-instar larvae were monitored for each concentration in a track (2 mm wide, 30 mm long

and 5 mm deep) created in a petridish (dimensions: 100 mm x 10 mm) containing 3.3% agar. The distance travelled in a fixed time of 30 seconds by each larva was recorded.

Climbing Assay. Climbing ability of 1, 3 and 7 day-old female flies were monitored using a vertical tube (diameter, 2.2 cm). For each condition, two groups with 10 flies in each group were monitored in the vertical tube. The fraction of flies crossing the maximum marked height in 10 sec was recorded.

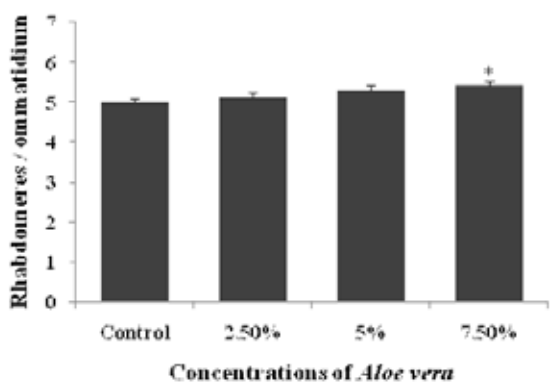
Pseudopupil analysis. Seven-day-old female flies were decapitated and mounted in a drop of nail polish on a microscopic slide. The head was then covered with immersion oil and examined under Nikon Eclipse (Ni-E) microscope with 50X oil objective. At least 200 ommatidia in six flies were examined, and the number of visible rhabdomeres was counted for each.

Statistical anlaysis. In the result, error bars in the graph indicate standard error of the mean (SEM). Significance was established by using Student's t test (*=P<0.05, **=P<0.01, ***=P<0.001).

Results

***Aloe vera* suppresses degeneration of photoreceptor neurons.** The ommatidia of the fly compound eye are organized in a repeating pattern of 9 neuronal cells (8 photoreceptors and 1 mechanosensory) and 11 support cells (pigment cells and cone cells). A popular measure of neurodegeneration is the integrity of the photoreceptor cells that produce a repeating trapezoidal arrangement of 7 visible rhabdomeres which carry light-gathering rhodopsins (Truman et al., 1993; Franceshini, 1972). Flies expressing Httex1p Q93 in neurons exhibit progressive loss of photoreceptor cells which is indicative of more widespread neurodegeneration (Agrawal et al., 2005). Thus, flies were scored for neurodegeneration at 7 day post eclosion by counting the rhabdomeres in each ommatidium. Adult flies fed with a dose of 7.5% significantly suppressed degeneration of photoreceptor neurons (Figure 1A and B).

A



B

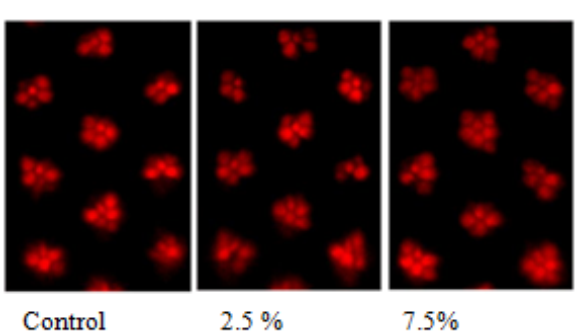
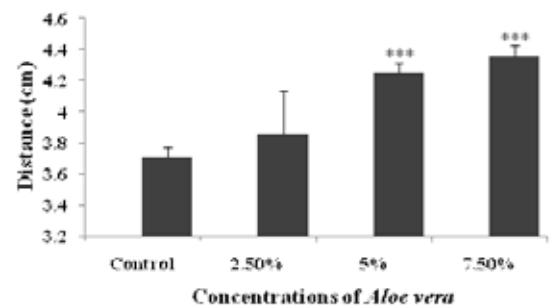


Fig.1. *Aloe vera* suppresses photoreceptor degeneration in HD flies. (A) Seven day old flies when fed standard *Drosophila* food supplemented with different concentrations of *Aloe vera* leaf gel extract post eclosion, showed suppression of neurodegeneration at 7.50 % dose. (B) Photographic images from ommatidia of seven day old flies fed different concentrations of the extract show the effect observed. *, p< 0.05, compared with control.

***Aloe Vera* improves locomotor activity in a *Drosophila* HD model.** To determine the effect of *Aloe vera* on motor neuron function of *Drosophila* expressing Httex1p Q93 peptide under the control of *elav*-Gal4 driver, larval crawling and adult climbing ability was monitored. We found that feeding 5 and 7.5 % of *Aloe vera* leaf gel extract significantly improved larval motor function (Figure 2A). We also found no significant difference between the effects of these two doses.

The adult climbing ability was monitored at days 1, 3 and 7 post-eclosion. Our results showed that all doses of *Aloe vera* administered since larval stage markedly improved adult climbing ability and thus decreased abnormal movements (Figure 2B). There was no significant difference between the effects of all doses administered.

A



B

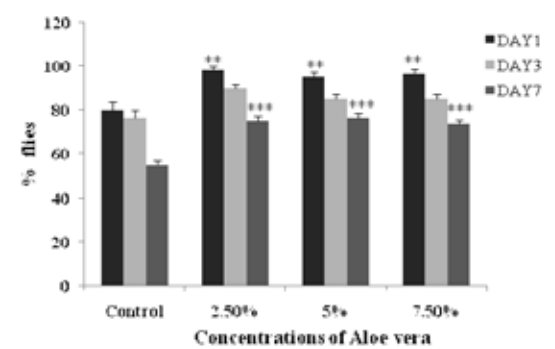


Fig.2. *Aloe vera* improves motor function of *Drosophila* HD model. (A) Crawling ability of larvae treated with different concentrations of *Aloe vera* leaf gel extract was determined. Larvae fed 5% and 7.5% doses exhibited a significant improvement of crawling ability. ***, p << 0.001. (B) Climbing ability of adult flies cultured on standard food supplemented with different concentrations of *Aloe vera* was evaluated at days 1, 3 and 7. Marked improvement in locomotor function was observed when compared to control. **, p << 0.01; ***, p<<0.001.

DISCUSSION

Neurodegenerative diseases are devastating conditions affecting millions of people worldwide. Although scientific and technolog-

ical advances in the past 20 years have moved us toward a better understanding of the molecular and cellular mechanisms of disease process, yet there is no cure or treatment that can reverse or slow disease progression. As synthetic drugs exert undesirable side effects, the potential of using natural products to treat neurodegenerative diseases is warranted.

The multiple biological effects of the medicinal herb *Aloe vera* have been attributed to its various chemical components such as anthraquinones, glycoproteins, polysachharides, vitamins and enzymes (Choi and Chung, 2003; Eshun and He, 2004). These constituents are postulated to act synergistically in maintaining the integrity of the plant's antioxidant status and anti-tumor activity (Saada et al., 2003; Kametani et al., 2007). The gel portion of *Aloe vera* leaves has been employed in most research studies. Additionally, various studies have shown that the polysaccharides in *Aloe vera* leaf gel have a plethora of therapeutic properties such as immunomodulation, anti-inflammatory and anti-oxidant effects, promotion of radiation damage repair, anti- viral, anti-fungal, anti-diabetic and anti-neoplastic actions (Talmadge et al., 2004; Ni and Tizard, 2004; Reynolds and Dweck, 1999). It has been reported that *Aloe vera* enhances anti-oxidant activity within the hippocampus and cerebral cortex thereby leading to improvement of motor function and memory in diabetic mice model (Parihar et al., 2004). A recent study demonstrated that *Aloe vera* has antioxidant and neuroprotective effects in mice models of Parkinson's disease (Bagewadi and Rathor, 2004).

Some of the most common measures of neuronal dysfunction include crawling and climbing ability, and integrity of photoreceptor cells of the fly compound eye. In our present study, we found that *Aloe vera* leaf gel at the dose of 5 % and 7.5 % fed since larval period improved motor function of a transgenic *Drosophila* model of HD as demonstrated by larval crawling behaviour and adult climbing ability. We also found that feeding a higher dose of 7.5 % during adult period suppresses degeneration of photoreceptor neurons in the *Drosophila* HD model. Our results clearly indicate that *Aloe vera* has the potential to alleviate disease symptoms when administered at the time of disease onset or even after progression.

CONCLUSION

Our results strongly employ Aloe vera leaf gel extract as one of the most suitable phytochemicals that can affect disease pathogenesis if administered at the time of disease-onset and even after progression of disease. As neurodegenerative diseases share most of the pathogenic features such as late-onset, abnormal protein folding and accumulation of abnormal structural forms of disease-specific proteins, Aloe vera can be an excellent candidate for the treatment of multiple neurodegenerative diseases including HD.

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