

The Beneficial Effect of Asiaticoside on Experimental Neuropathy in Diabetic Rats

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Received 2012-03-28, Revised 2012-08-20; Accepted 2012-08-30

ABSTRACT

Though diabetic neuropathy produces high impact on quality of life, annual cost and morbidities, the therapeutic efficacy is still not in a satisfaction level. Based on the crucial role of oxidative stress on the pathophysiology of diabetic neuropathy and the improvement of this condition induced by antioxidant, we hypothesized that asiaticoside, a substance possessing antioxidant activity, could provide beneficial effect. Therefore, we aimed to determine the effect of asiaticoside on the recovery of sciatic nerve in experimental neuropathy in diabetic rats. Young adult male Wistar rats at 8 weeks old, weighing approximate 180-220 g, were orally given asiaticoside at doses of 0.1 and 1 mg kg⁻¹ BW at a period of 5 days before and 3 weeks after sciatic nerve crush injury. Motor and sensory functions were observed every 3 day until the end of the experiment by using Deminacelli method, walking pattern, muscle power and foot reflex withdrawal test, respectively. Our results showed that both doses of asiaticoside could significantly reverse the enhanced withdrawal threshold intensity elicited by electrical stimuli. However, the rats received asiaticoside at dose of 1 mg kg⁻¹ BW provided optimum benefit. However, no other significant effects were observed. Asiaticoside administration in an experimental model of neuropathy in diabetic rats mitigates some functional impairment of sciatic nerve. Though our data show only the beneficial effect of asiaticoside on the foot withdrawal reflex, it is very much important because it involve the protective mechanism against painful stimuli. Therefore, it is worth for further investigation in order to confirm the improvement of sensori-motor functions and determined the both therapeutic window and possible underlying mechanism.

Keywords: Mechanisms Involving, Sensori-Motor Functions, Underlying Mechanism, Sciatic Nerve Function Index (SFI), Diabetic Polyneuropathy, Scientific Document

1. INTRODUCTION

Diabetic neuropathy, one of the common complications of diabetes mellitus, has been regarded as one of the therapeutic challenges nowadays. It is characterized by a progressive loss of nerve fiber function which can manifest with a wide variety of sensory, motor and autonomic symptoms. The earliest signs of diabetic neuropathy probably reflect the gradual loss of integrity of both large myelinated nerve fiber which give rise to the loss of vibratory sensation and proprioception and the loss of small myelinated and unmyelinated nerve fibers which result in the impairment of pain, light touch and temperature. Therefore, diabetic neuropathy disturbs the quality of life of the sufferers

very much. In addition, the annual cost and morbidities of this condition are very high.

To date, the underlying cause of diabetic polyneuropathy remains controversial. A number of mechanisms involving polyol flux, microangiopathy with ischemia, neurotrophin deficiency, excessive protein glycosylation and heightened oxidative stress are reported to contribute the important role. Though its high prevalence and impact are very high, the therapeutic efficacy is still limited. Therefore, novel strategy is still in required. During the last decade, several preclinical studies have shown that substance possessing antioxidant activity including α -lipoic acid and mulberry leaves can prevent nerve dysfunction (Coppey *et al.*, 2001; Muchimapura *et al.*, 2010). In addition, it has been

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reported that clinical trial study also confirms that substance possessing antioxidant activity can improve sensory symptoms of diabetic polyneuropathy (Ametov *et al.*, 2003). Based on the pivotal role of oxidative stress on the pathophysiology of diabetic neuropathy and the therapeutic effect of substance possessing antioxidant mentioned earlier, the beneficial effect of asiaticoside, a substance possessing antioxidant activity, has been considered.

Asiaticoside, a major triterpenoid glycoside of *Centella asiatica*. (Schaneberg *et al.*, 2003), has been reported to possess wound healing, antiulcer, antioxidant and anti-inflammatory activities (Guo *et al.*, 2004; Shukla *et al.*, 1999). It also decreased oxidative stress and improved cognitive impairment in diabetic condition (Kumar and Gupta, 2003). Moreover, it could accelerate nerve regeneration and neurite elongation (Soumyanath *et al.*, 2005). Based on its role to improve diabetic complication, nerve regeneration and its antioxidant effect, we hypothesized that asiaticoside could enhance the functional recovery of nerve injury in diabetic condition. To date, no scientific document about the mentioned effect is available. Therefore, the present study was set up to elucidate this issue.

2. MATERIALS AND METHODS

2.1. Experimental Animal

Young adult male Wistar rats, 8 weeks old were used as experimental animals. They were obtained from National Animal Center, Salaya. The weights of the animals on the first day of experiment are 180-220 g. They were housed 5 per cage and maintained in 10: 14 light: Dark cycle and given access to food and water ad libitum. The rats were maintained according to the Guidelines of Animal Care described by Animal Center, Faculty of Medicine, Khon Kaen University. All treatments in this study will be performed once daily between 7.00-9.00 a.m.

2.2. Chemicals

Streptozotocin was purchased from Sigma-Aldrich Co., USA. Glucose was assayed using Kits from Sigma-Aldrich Co., USA. One touch gluco-meter (Accu-chek sensor) of Roche Diagnostics, Germany was purchased from Bayer Diagnostics Thailand Ltd. Glycoside was purchased from Sigma-Aldrich Co., USA. All other chemicals were of analytical grade.

2.3. Induction of Diabetes in Rats

Streptozotocin was freshly prepared in 0.1 M citrate buffer, pH 4.5. Then the animals were fasted over night and the single injection of the streptozotocin at dose of 60 mg kg⁻¹ B.W was performed via intraperitoneal route (Akinnuga *et al.*, 2010). Age-matched control rats were

injected with citrate buffer. Forty-eight hours later, blood samples were collected and glucose levels were determined to confirm the development of diabetes. Only the animals which showed hyperglycemia (blood glucose levels >240 mg dL⁻¹) were used in the experiment.

2.4. Surgical Procedures

The animals were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg kg⁻¹). An incision was made at the back of the thigh and the sciatic nerve was carefully exposed at a point immediately distal from the gluteus maximus muscle. The nerve was crushed for 30 sec using hemostatic forceps. A crush lesion was induced on the same site (mid-thigh) in both vehicle and Asiaticoside treated groups.

2.5. Experimental Protocol

The rats were divided into 5 separated groups including (1) control or naïve intact (2) sham operated group or vehicle plus sham operated group (3) vehicle plus nerve injury (4) vitamin C plus nerve injury (5)-6) asiaticoside treated groups at doses of 0.1 and 1 mg kg⁻¹ BW respectively. The animals were treated with the assigned substance 5 days before and 21 days after sciatic crush injury. Then, they were assessed the sciatic functions using Sciatic Function Index (SFI) via De Medinacelli method, walking pattern score, muscle power grade and foot reflex withdrawal threshold via foot reflex withdrawal test, as indices.

2.6. Walking Pattern

All animals in each group were transferred to smooth ground and determined walking, standing and balance. The walking pattern of each animal was graded into 4 grades as following:

- Normal (+4): normal gait (normal standing and walking, no ataxia during walking)
- Mild abnormal (+3): ataxia with wide base gait (can stand but has ataxia and abduction of both legs)
- Moderate abnormal (+2): ataxia with unstable gait (during standing sometime collapse, ataxia and abduction of both legs)
- Severe abnormal (+1): Truncal ataxia (ataxia until cannot walking)

2.7. Muscle Power

The muscle power of the animal was determined using the inclined plane test. In this test, the plane was gradually inclined at different angles ranging from 0, 90, 120 and 180 degrees.

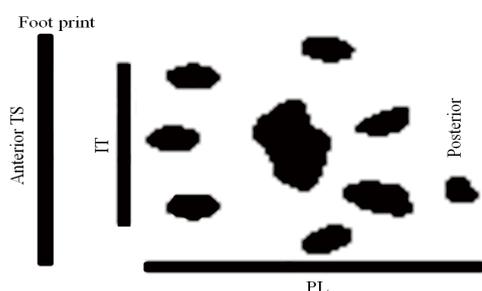


Fig. 1. Walking Track Test (De Medinacelli method)

The ability of animal to maintain its grip on the floor plane was regarded as muscle power. All rats were trained to walk on the inclined floor for 1 week before testing. The evaluation of muscle power was performed after the administration of the assigned substance within 45 m. The time which animals loosed their grips on the textured flooring of the starting area and slipped down the plane since started was recorded and scored at various grades as following:

- 0 = Ability to climb at the angle of 90% less than 15 sec
- 1 = Ability to climb at the angle of 120% less than 10 sec but can climb at the angle of 90 more than 15 sec
- 2 = Ability to climb at the angle of 180% less than 5 sec but can climb at the angle of 120% more than 10 sec
- 3 = Ability to climb at the angle of 180% more than 5 sec

2.8. Walking Track Test (De Medinacelli Method)

In order to measure the ability to use the muscles in the lower paw and foot, the walk track analysis was performed using De Medinacelli method every 3 days throughout the 21 days-after crush injury (**Fig. 1**). The rats are first allowed conditioning trials. According to this method, both hind paws of the animals were dipped in ink and let them walk along the recording paper at 8.2×42 cm which used as walking track. The Sciatic nerve Function Index (SFI) was calculated using the following equation:

$$\begin{aligned} \text{SFI} &= (-38.3 \times \text{PLF}) + (109.5 \times \text{TSF}) + (13.3 \times \text{ITF}) - 8.8 \\ \text{PLF} &= (\text{EPL} - \text{NPL}) / \text{NPL} \\ \text{TSF} &= (\text{ETS} - \text{NTS}) / \text{NTS} \\ \text{ITF} &= (\text{EIT} - \text{NIT}) / \text{NIT} \end{aligned}$$

The SFI were regarded as normal when the values were in the range of -10 to 10%. PL = Print length; TS = Toe spread; IT = Intermediary toe spread; E = Experimental foot; N = Non-operated foot.

2.9. Foot Reflex Withdrawal Test

The recovery of sensory function was determined by the foot reflex withdrawal test every 3 days throughout

21 days after sciatic nerve crush injury. A weak electrical stimulation (0.1mA) was applied to the central portion of the operated foot sole using two stimulation poles (spaced 3 mm apart). The rats subjected to sciatic nerve crush did not retract their paws upon skin contact with the poles while the healthy rats immediately withdrew its foot and spread their toes after stimulation. The threshold reaction to a 0.1 mA electric current was accepted as index for the sensitivity to foot reflex withdrawal test.

2.10. Statistic Analysis

All data were expressed as mean ± SEM value. The differences among various groups were compared by ANOVA and post hoc test. The statistical difference is regarded at p-value <0.05.

3. RESULTS

The effect of asiaticoside on the recovery of motor function of sciatic nerve was assessed using walking pattern analysis. The results were shown in **Fig. 2**.

It was found that vehicle plus sham operation produced no significant change on the walking pattern score. Within 9 days after the sciatic nerve crush injury, the walking pattern score was markedly decreased ($p < 0.05$ all; compared with the vehicle plus sham treated group). Vitamin C and both doses of asiaticoside failed to show significant improvement in walking pattern throughout 21-day experimental period.

In addition to walking pattern score, the muscle power was also assessed and used as motor function index. **Figure 3** showed that vehicle treated group which received crush injury significantly decreased motor power at the third day after crush injury ($p < 0.05$; compared with vehicle+sham operation). Both vitamin C and all doses of asiaticoside also failed to show significant changes on muscle power throughout the experimental period.

The Sciatic nerve Function Index (SFI) or toe spreading score was also determined. The results showed that vehicle plus sham operated group did not show significant change in toe spreading score throughout 21-day experimental period. Crush injury at sciatic nerve decreased toe spreading score within 12 days after crush injury ($p < 0.001$ all; compared between vehicle plus sham operated group and all groups which receive crush injury) then the SFI was gradually improved until no significant changes between vehicle treated group which received crush injury and vehicle plus sham treated group were observed throughout the experimental period. Though the asiaticoside showed more improvement of SFI at 15 days after treatment, it failed to show significant effect. No other significant effects were observed as shown in **Fig. 4**.

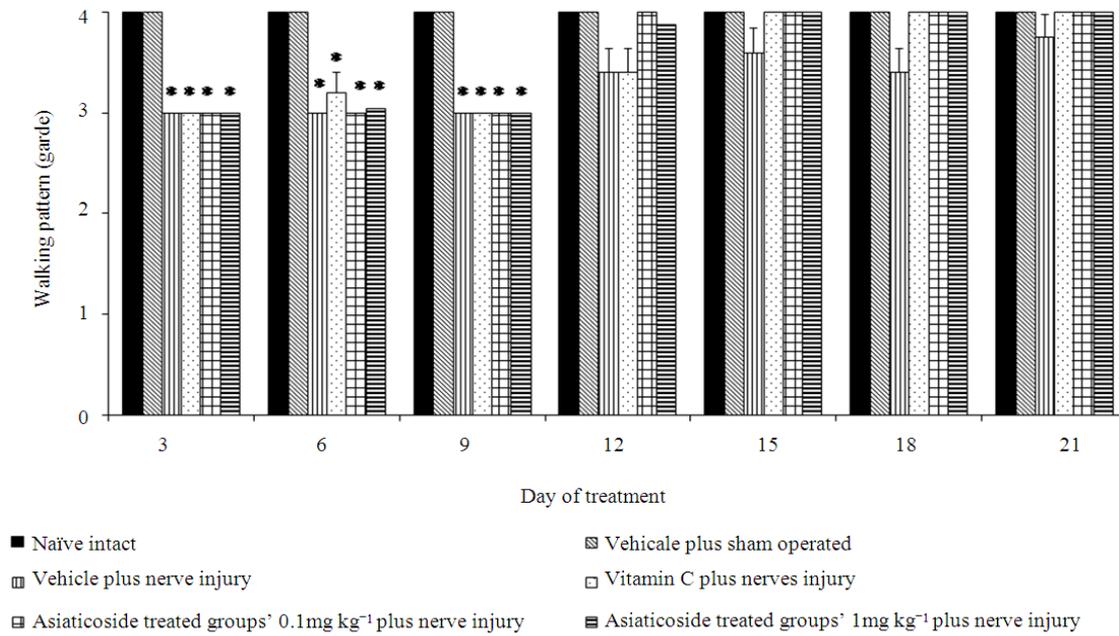


Fig. 2. The effect of asiaticoside on the recovery of motor function of sciatic nerve was assessed using walking pattern analysis. Data are present as mean \pm SEM (n = 6 group⁻¹) * p-value < .05 all; compared with the vehicle plus sham treated group

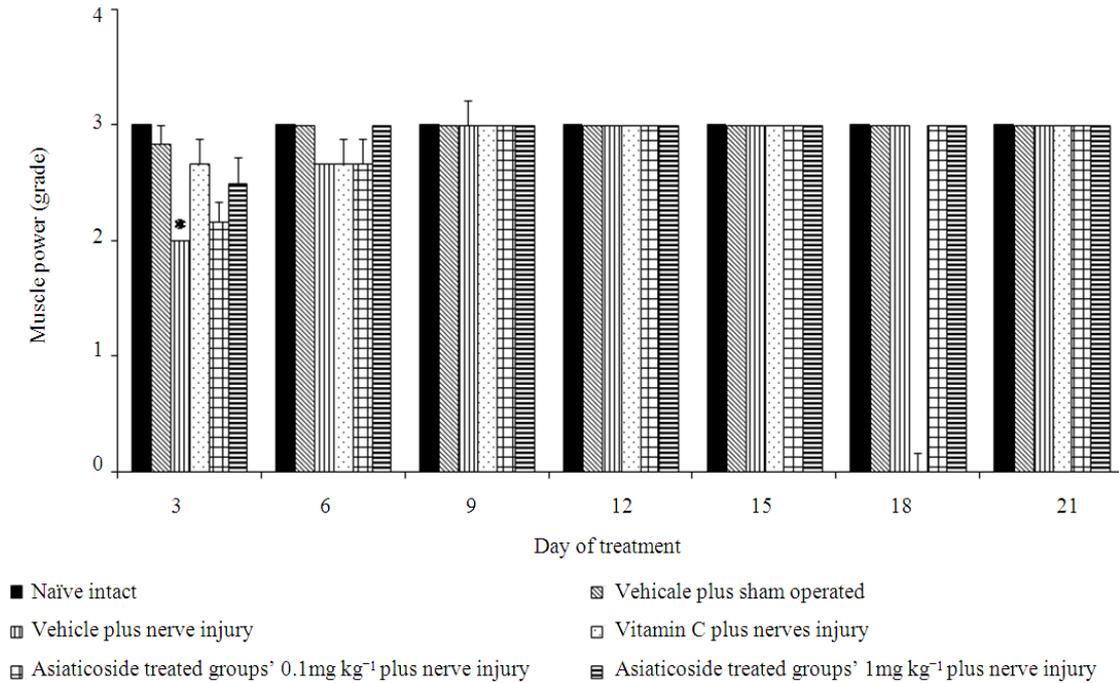


Fig. 3. The effect of asiaticoside on the recovery of motor function of sciatic nerve was assessed using muscle power. Data are present as mean \pm SEM (n = 6 group⁻¹) * p-value < .05 all; compared with the vehicle plus sham treated group

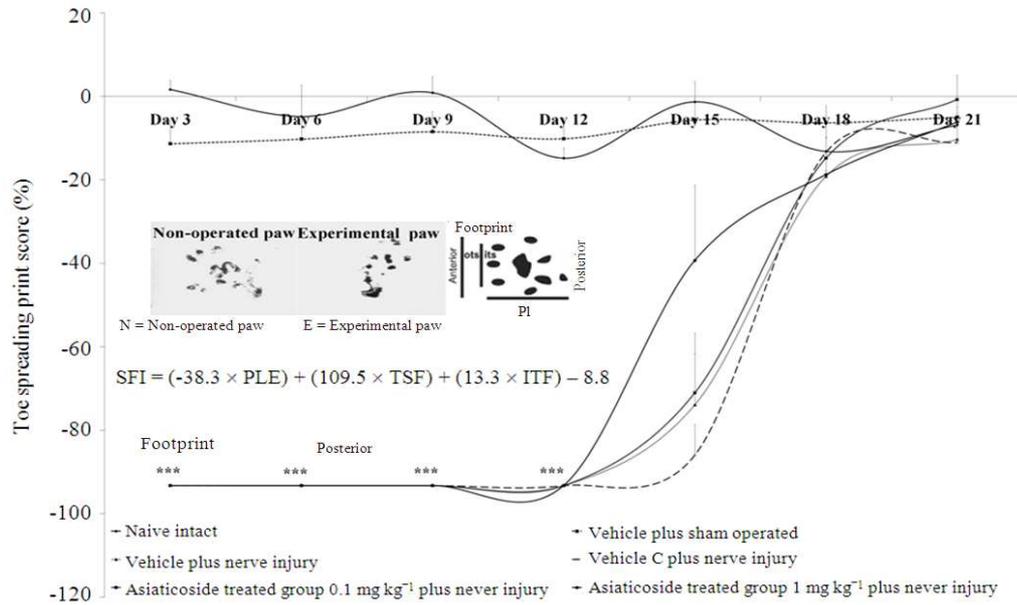


Fig. 4. The effect of asiaticoside on the recovery of motor function of sciatic nerve was assessed using the Sciatic nerve Function Index (SFI) or toe spreading score. Data are present as mean \pm SEM ($n = 6$ group⁻¹) $p < 0.001$ all; compared between vehicle plus sham treated group and all groups which receive crush injury

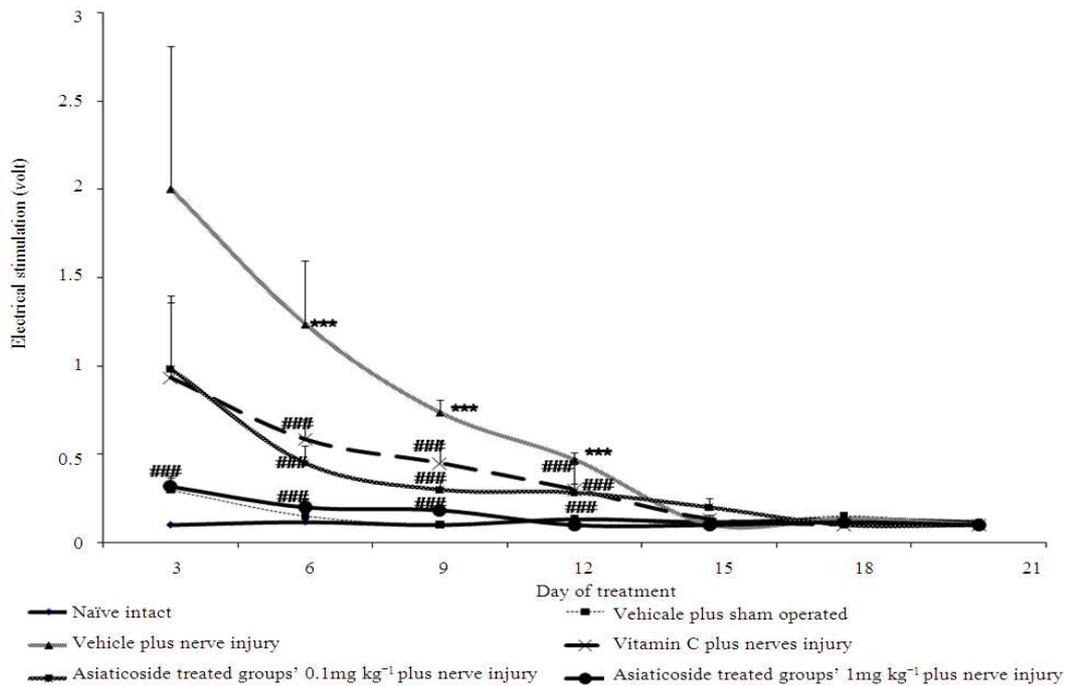


Fig. 5. The effect of asiaticoside on the recovery of motor function of sciatic nerve was assessed using foot withdrawal reflex in response to pain induced by electrical stimuli. Data are present as mean \pm SEM ($n = 6$ group⁻¹) *** p -value $< .001$ all; compared between vehicle plus sham treated group and all groups which receive crush injury, ### $p < 0.001$ all; compared between vehicle plus crush injury

We also determined the foot withdrawal reflex in response to pain induced by electrical stimuli. The results were shown in **Fig. 5**. It was found that rats subjected to vehicle treatment and crush injury showed the difficulty to respond to stimuli. Therefore, the response required higher stimuli intensity in order to induce withdrawal reflex response. However, the significant effect was observed at 6, 9 and 12 days after crush injury ($p < 0.001$ all; compared with vehicle plus sham operation). Rats which received asiaticoside treatment at dose of 1 mg.kg-1BW could reverse the elevation of threshold intensity of stimuli induced by the impairment of sciatic nerve throughout the 12 days after the induction of sciatic nerve damage ($p < 0.001$ all; compared with vehicle plus crush injury). Both vitamin C and asiaticoside at dose of 0.1 mg kg⁻¹BW could reverse the elevation of threshold intensity of stimuli significantly at 6-12 days after the induction of sciatic nerve damage ($p < 0.001$ all; compared with vehicle plus crush injury). However, vitamin C and both doses of asiaticoside had failed to show the significant difference since 15 days after the induction of sciatic nerve damage until the end of experimental period.

4. DISCUSSION

The present study has demonstrated that asiaticoside can restore the impairment of foot withdrawal reflex induced by pain.

Our data had clearly demonstrated that asiaticoside could improve the sensory function of sciatic nerve while no significant effect on motor function was observed. These changes reflected the different susceptibility of sensory and motor nerve fibers to asiaticoside. Our results were also corresponding to previous study which also demonstrated the different survival rate after injury between sensory and motor nerve (Midha *et al.*, 2001).

It has been well established that pain sensation has been conducted by small myelinated nerve fiber (A-delta) and unmyelinated fiber (C-fiber) while the motor function is served by the large myelinated fiber. Therefore, our results have suggested that asiaticoside may selectively improve the function of unmyelinated fiber. Since the function of myelinated fiber is under the influence of myelin sheath, therefore, the lack of improvement of motor function assessed in this study may occur because asiaticoside exerts no effects on myelin sheath. Moreover, it has been reported that sensory and motor axons differ considerably in their expression of genes, proteins and growth factor

receptors, endowing them with differential sensitivity to cellular and molecular influences, such as Schwann cell adhesion molecules, cytokines and neurotrophic factors. Previous study had shown that the depletion of neurotrophic factor such as nerve growth factor which contributed the crucial role on the function of sensory nerve also played the role on diabetic neuropathy (Lindsay and Harmar, 1989; Anand *et al.*, 1996; Dyck *et al.*, 1991). Since the extract of *Centella asiatica* which contained asiaticoside could enhance NGF, it was also possible that the improved function of sensory nerve observed in this study were associated with the elevation of NGF induced by asiaticoside (Soumyanath *et al.*, 2005). In addition, it was also found that antioxidant could decrease both oxidative stress and protein glycation resulted in the decreased risk of diabetic complications (Young *et al.*, 1995). Thus, the antioxidant effect of asiaticoside might also contribute the role.

5. CONCLUSION

In conclusion, asiaticoside could improve the functional impairment with very low dose. Moreover, it did not show toxicity up to dose of 1g kg⁻¹ BW. Therefore, it may provide beneficial effect as the supplement for diabetic neuropathy. However, further researches to confirm the improvement of sensori-motor functions and determined the both therapeutic window and possible underlying mechanism are still required.

6. ACKNOWLEDGEMENT

This study was supported by the National Research Council of Thailand, Research Division of Faculty of Medicine and Integrative Complimentary Alternative Medicine Research and Development Group, Khon Kaen University, Khon Kaen 4002, Thailand.

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