*Original Article*

# *Morus alba* Leaves Extract Improves Memory Impairment, Oxidative Stress and Neurodegeneration in Hippocampus of the VPA-Rat Model of Autism

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***Background:*** Due to limitation of the current therapy against autism, the novel regimen to improve autism is required. Based on crucial roles of oxidative stress on the pathophysiology of autism and the benefit against autism of the medicinal plants possessing neuroprotective effect, the health benefit of *Morus alba* leaves for autism was focused. However, no supported evidence for this issue is available.

***Objective:*** To determine the effect of *Morus alba* (*M. alba*) leaves extract on autism-like behaviors and to explore the possible underlying mechanisms in animal model of autism induced by valproic acid (VPA).

***Materials and Methods:*** VPA rat model of autism were orally given *M. alba* leaves extract at doses of 25, 50 and 100 mg.kg$^{-1}$ between PND14-PND40. Behaviors were assessed using hot plate, open-field, elevated plus-maze, Morris water maze and social behavior tests at the end of study, MDA level and the activities of SOD, CAT and GSH-Px together with the alteration of neuron density in the involved brain area were also evaluated.

***Results:*** All doses of the extract improved memory impairment and the elevated oxidative stress in hippocampus. The increased neuron density in hippocampus was also observed in VPA rats treated with low and medium doses of the extract.

***Conclusion*:** *Morus alba* leaves extract can improve memory impairment in autism model. The memory enhancing effect of the extract may occur partly via the improvement of oxidative stress status and the increased neuron density in hippocampus. However, further research is still necessary to understand the precise underlying mechanism.

***Keywords:*** Autism, *Morus alba*, Oxidative stress, Neurodegeneration

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Autism, an important neurodevelopment disorder, is characterized by deficit in language development, social interactions, motor skills, and abnormal sensitivity to sensory stimuli[1]. In addition, memory is also broadly decline[2]. It has been reported that cognitive and sensory/motor development may progress symptom free for several months to years and followed by a period of retardation or a period of regression or a period of intrusion[3]. To date, autism has gained much attention due to the high prevalence and the great impact on both social cost and family. Although autism is an important neurodevelopmental disorder, no effective treatment for autism patient is available until now. Most treatments are symptomatic treatments and the adverse effects are still observed. Therefore, the novel regimen to improve autism is required.

Autism is the multi-etiology disease and its pathogenesis is still unclearly known. Accumulative lines of evidence have demonstrated that the toxicant-induced oxidative stress may play a role[4]. Many substances possessing antioxidant and neuroprotective activities such as *Bacopa monnieri*[5,6], resveratrol[7], green tea[8], piperine[9] can also mitigate autism-like behavior in animal model of autism induced by valproic acid (VPA) exposure[10-13]. Therefore, we hypothesized that leaves extract of *Morus alba*, a polyphenol-rich plant, with potent antioxidant and neuroprotection[14] which is widely consumed in Northeast of Thailand should provide the beneficial effect for autism. To test this hypothesis, we aimed to determine the effect of *M. alba* leaves extract on autistic liked behavior and on oxidative stress status in hippocampus of an animal model of autism induced by VPA.

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## Materials and Methods

### *Plant material and preparation*

*Morus alba* leaves of Burirum 60 cultivar were collected during October to November, 2013 from Khon Kaen province. They were dried, cut into small pieces and ground as powder. Then, the powder was extracted with 50x (w/v) of hot water (85°C) for 3 h and filtered with Whatman No. 1 filter paper. The filtrate was concentrated under vacuum at 25°C. The percentage yield of extract was 17.49. The extract contained total phenolic compounds via Folin-Ciocalteu method was 303.97±5.51 mgGAE/g extract.

### *Animals*

Female pregnant Wistar rats were purchased from National Laboratory Animal Center, Salaya, Nakhon Pathom. Rat pups both male and female were housed together in cage, maintained in a 12: 12 h light: dark cycle, and given ad libitum access to food and water. The experimental protocols had been approved by the Institutional Animal Care and Use Committee Khon Kaen University, Thailand (AEKKU 56/2556).

### *Autism-like condition induction*

Sodium valproate (VPA) (Sigma Aldrich, USA) at dose of 400 $mg.kg^{-1}$ BW was injected to both male and female rat pups at 14 days old (18 to 30 g) via subcutaneous route. The following assessments were performed in order to confirm the development of autism-like symptoms; the reduced weight gain measured weekly interval, impaired olfactory discrimination on PND 9, delayed eye opening on PND 13 and 14 and impaired motor development (swim performance) on PND 8 and 12 as initial activities reflecting the abnormal neuron development in pups[15].

### *Experimental protocol*

Rats were assessed and the day of birth was recorded as PND 0. They were divided into various groups (8 rats each):

Group 1: Naive control group; the offspring rats received no treatment.

Group 2: VPA: All rats in this group were administered VPA alone.

Group 3: VPA+ vehicle: The animals in this group were administered VPA and treated with vehicle.

Group 4 to Group 6: VPA+ *M. alba* leaves extract : VPA-treated rats in these groups were rats which showed autism-like symptoms and treated with *M. alba* leaves extract at doses of 25, 50 and 100 mg.kg-1 BW respectively.

The assigned substances were administered once daily during PND 14 to PND 40. Based on the previous findings that rat pups which showed autism-like behavior usually showed developmental disorders as retardations, regressions and intrusions, the battery tests that assess autism-like behavior including hot plate, open-field, elevated plus-maze, Morris water maze and social behavior tests were performed. On PND 41, hippocampus was isolated and the oxidative stress markers, including malondialdehyde (MDA) level and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes together with the neuron density in hippocampus were carried out.

### *Determination of behavior disorders*

#### *Hot plate test*

Since the decreased pain sensitivity was a common feature of children with autism[16], the pain response to thermal stimuli during PND 37 to PND 39 was evaluated by using a plantar test apparatus (UgoBasile, Comerio, Italy) according to Hargreaves' method[17]. The withdrawal response latency was recorded.

#### *Open field test*

On the basis of the decreased environmental exploration of children with autism, the general locomotor activity and willingness to explore in rodents was investigated via open field test[18]. On PND 40, each animal was placed in a center of large Plexiglas open field chamber (72x72x30 cm) and permitted to explore the apparatus for 5 minutes. Grooming, rearing, time spent in the periphery or in the center of the open field were assessed using a video tracking system[19].

#### *Social interaction*

Since the impairment of social interaction is an important clinical manifestation in autism[20], the effect of *M. alba* leaves extract on social behavior was also determined. An acrylic plastic circular cage for rat pups consisting of an acrylic plastic circular cage was used for an evaluation tool. The animals were separated and housed individually the night before the experiment to enhance social interactions. All rats were matched for their gender and weight. On PND 40, two animals from the same group but different litters and cages were placed into the test cage under red light for 20 min. Pairs were tested in a randomized order for groups and the animals did not differ by more than 15 g in body weight. Pining frequency (one rat lies on its back, the other stands with two paws on top of it), following, grooming each other, sniffing of any body part besides of anogenital parts were used as indicators of social engagement[21].

#### *Elevated plus maze test*

Based on the information that anxiety is a real difficulty for autism[22], anxiety was evaluated using elevated plus maze test. The animals were placed directly on the elevated plus maze consisting of two opened and two closed arms (50 cm length x 12 cm wide x 30 cm height) which was elevated approximately 50 cm from the floor. Each rat was placed in the center of the maze facing an open arm and allowed to freely explore for 5 min. Percent of time spent in the opened arm and percent of time in opened arm entries were recorded.

#### *Morris water maze test*

The effect of *M. alba* leaves extract on memory

was evaluated based on the previous finding that autism patients often display memory deficit[23]. In brief, a metal pool (170 cm in diameter x 58 cm tall) filled with tap water (25°C, 40 cm deep) which covered with non-toxic milk was divided into 4 quadrants (Northeast, Southeast, Southwest, and Northwest).The removable platform was immersed below the water level at the center of one quadrant. Each animal was trained to memorize to location of the immersed platform by using the specific visual cues placed around the outside of the tank. The position of platform was maintained unchanged throughout the training session. Each animal must be exposed to four consecutive trials each day with a gap of 5 min for four consecutive days. During the training session, the rat was gently placed in the water between quadrants, facing the wall of pool with drop location changing for each trial, and allowed 120 sec to locate submerged platform. If the rat failed to find the platform within 120 s, it was guided gently on to the platform and allowed to remain there for 20 s. The time spent to climb on the immersed platform was recorded as escape latency. 24 hours later, the animal was re-exposed to the same condition except that the immersed platform was removed. The mean time spent in the target quadrant in order to search for the missing platform was noted as retention time and used as index of retrieval memory. Both escape latency and retention time in a 5 minute-exposure time were used as indices of learning and memory. The blind observer always stood at the same position. Care was taken not to disturb the relative location of water maze with respect to other objects in the laboratory. The determination of spatial memory capacity via this test was performed on PND 40[24].

### *Oxidative stress markers assays*

On PND 41, all rats were sacrificed, hippocampai were isolated and prepared as homogenate for the determination of oxidative stress markers including malondialdehyde (MDA) levels and the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) enzymes. Thiobarbituric acid reacting substances (TBARS) assay was used to determine MDA levels[25]. The determination of SOD was carried out via xanthine/xanthine oxidase reaction[26] whereas CAT and GSH-Px were performed based on the decomposition rate of $H_2O_2$[26] and based on the amount of reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidized per minute respectively.

### *Morphological evaluation*

Neuronal density in hippocampus was performed at a 40x magnification of light microscope with a final field of 255 $\mu m^2$ from five coronal sections of each rat in each group by a blind observer. Viable stained neurons were identified on the basis of a stained soma with at least two visible processes. All data were expressed as number of neurons per 255 $\mu m^2$.

### *Statistical analysis*

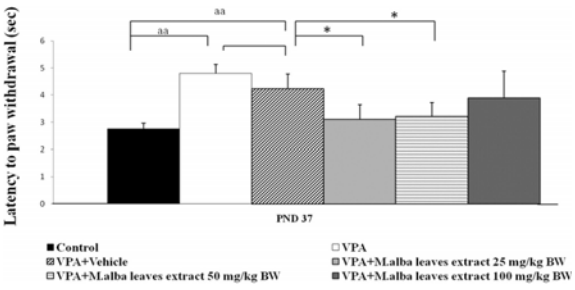
Data were presented as means ± SEM and the statistical analysis was performed by one-way ANOVA, followed by Post-hoc (LSD) test. The *p*-value <0.05 were considered statistically significant.

## Results

### *Effect of M. alba extract on autistic-like behavior*

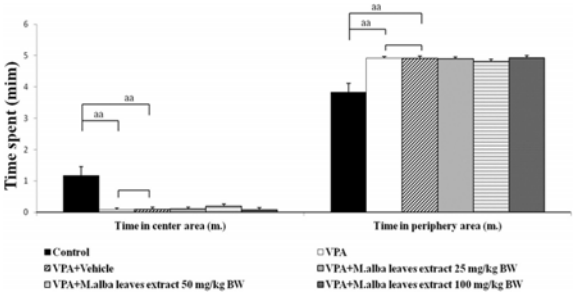
VPA administration significantly increased paw withdrawal latency (*p*-value <0.01; compared to control rats). VPA treated rats which received vehicle showed no significant change of paw withdrawal latency when compared to VPA treated rats. *M. alba* leaves extract at doses of 25, 50 $mg.kg^{-1}$ BW failed to improve the elevation of paw withdrawal latency induced by VPA on PND 37 as shown in Figure 1.

Since motor behavior is regarded as the important clinical manifestation of autism, we also investigated the effect of *M.alba* leaves extract on motor behaviors in VPA-rat model of autism. The results were shown in Figure 2. VPA treated rats significantly decreased time spent in the central area but increased time spent in the peripheral area (*p*-value <0.01 all; compared to control rats). VPA treated rats didn't produce the significant changes of this parameter when compared to




[aa] *p*<0.01 compared with control group. * *p*<0.05 compared with VPA plus vehicle group.
PND = postnatal day, VPA=valproic acid

**Figure 1.** Effect of *M. alba* leaves extract on pain response to thermal stimuli in rats exposed to VPA. Values are expressed as mean ± SEM (n = 5).




[aa] *p*<0.01 compared with control group
PND = postnatal day, VPA = valproic acid

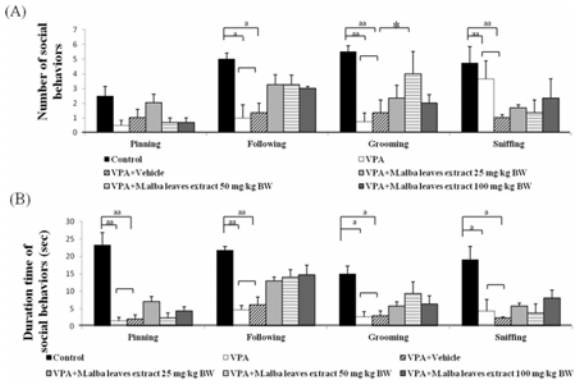
**Figure 2.** Effect of *M. alba* leaves extract on motor behaviors in VPA-rat model of autism. Values are expressed as mean ± SEM (n = 5).

VPA treated rats. *M. alba* leaves extract failed to modify the effect of VPA on exploration activity in VPA treated rats.

The effect of *M. alba* leaves extract on social behaviors was also observed. In Figure 3, it was found that VPA significantly decreased number of following, grooming and sniffing ($p$-value <0.5, 0.01, and 0.01 respectively; compared to control rats). In addition, the reduction of pinning, following, grooming and sniffing were also observed in VPA treated rats ($p$-value <0.01, 0.01, 0.05, and 0.01 respectively; compared to control rats). *M. alba* extract at dose of 50 mg.kg$^{-1}$ BW significantly increased grooming behavior in VPA treated rats ($p$-value <0.05; compared to VPA plus vehicle treated rats) and no other changes were observed as shown in Figure 3.

On PND40, the VPA treated rats significantly decreased both % of time spent in the opened arm and % of time sent in the opened arm entries ($p$-value <0.001 and 0.01 respectively; compared to control rats). Vehicle failed to produce the significant change of this parameter in VPA treated rats. *M. alba* also showed no significant change on the VPA treated treats as shown in Figure 4.

The effect of *M. alba* on spatial memory was shown in Figure 5. VPA treated rats significantly increased escape latency but decreased retention time ($p$-value <0.01 and 0.001 respectively; compared to control rats). Vehicle produced no change of this parameter in VPA treated rats. However, *M. alba* extract at doses of 25, 50 and 100 mg.kg$^{-1}$ BW used in this study significantly decreased escape latency ($p$-value <0.01 all; compared to vehicle treated rats) but enhanced retention time ($p$-value <0.01, 0.01 and 0.05 respectively; compared to VPA plus vehicle treated rats) of VPA treated rats.

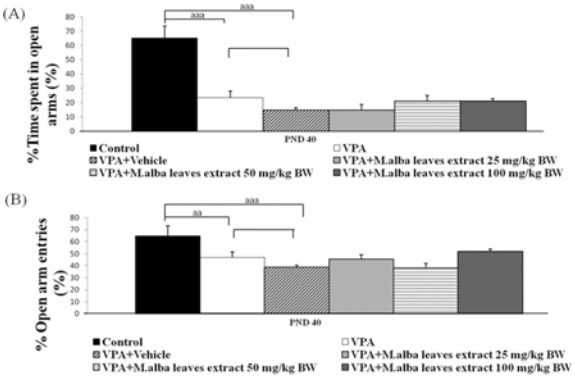



a,aa $p$<0.05, 0.01 respectively; compared with control group, * $p$<0.05 compared with VPA plus vehicle group
PND = postnatal day, VPA = valproic acid

**Figure 3.** Effect of *M. alba* leaves extract on social behaviors in VPA-rat model of autism (A) number of social behaviors, (B) duration time of social behaviors. Values are expressed as mean ± SEM (n = 5).

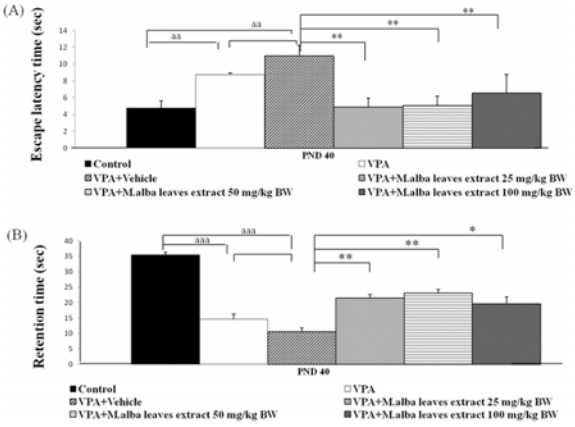
### *Effect of M. alba extract on oxidative stress*

Our data clearly demonstrated that *M. alba* significantly improve spatial memory, a hippocampus dependent memory, in VPA treated rats. Therefore, the alteration of oxidative stress in hippocampus was investigated and results were shown in Table 1.Our results showed that VPA significantly enhanced MDA level but decreased the activities of SOD, CAT and GSH-Px enzymes in




aa,aaa $p$<0.01 and 0.001 respectively, compared with control group
PND = postnatal day, VPA = valproic acid

**Figure 4.** Effect of *M.alba* leaves extract on anxiety using elevated plus maze test in VPA-rat model of autism (A) % time spent in open arm (B) % open arm entries. Values are expressed as mean ± SEM (n = 5).




aa,aaa $p$<0.01, 0.001 respectively, compared with control group, *,** $p$<0.05, 0.01 respectively; compared with VPA plus vehicle group
PND = postnatal day, VPA = valproic acid

**Figure 5.** Effect of *M. alba* leaves extract on spatial memory using Morris water maze test in VPA-rat model of autism (A) escape latency time and (B) retention time. Values are expressed as mean ± SEM (n = 5).

**Table 1.** Effect of *M. alba* leaves extract on the oxidative stress marker including MDA level and the activities of SOD, CAT, and GSH-Px enzymes in hippocampus. Values are expressed as mean ± SEM (n = 5)

| Treatment groups | MDA (nmol/mg protein) | SOD (U/mg. Protein) | CAT (U/mg. protein) | GPx (U/mg protein) |
|---|---|---|---|---|
| Control | 0.182±0.011 | 7.122±0.342 | 6.581±0.118 | 8.519±0.271 |
| VPA | 0.422±0.051$^{aa}$ | 3.180±0.433$^{a}$ | 2.148±0.51$^{aa}$ | 4.256±0.286$^{a}$ |
| VPA + Vehicle | 0.462±0.016$^{aa}$ | 2.961±0.131$^{a}$ | 1.762±0.304$^{aa}$ | 3.761±0.314$^{a}$ |
| VPA + *M. alba* leaves extract 25 mg/kg BW | 0.209±0.081 | 3.633±0.297 | 2.239±0.047 | 6.087±0.448$^{*}$ |
| VPA + *M. alba* leaves extract 50 mg/kg BW | 0.255±0.091$^{**}$ | 3.826±0.254 | 3.623±0.431$^{*}$ | 6.943±0.356$^{*}$ |
| VPA + *M. alba* leaves extract 100 mg/kg BW | 0.219±0.062$^{**}$ | 3.088±0.430 | 1.056±0.245 | 5.077±0.352 |

$^{a, aa}$ $p$<0.05, 0.01 respectively; compared with control group, *,** $p$<0.05, 0.01 respectively, compared with VPA plus vehicle group.
PND = postnatal day, VPA = valproic acid

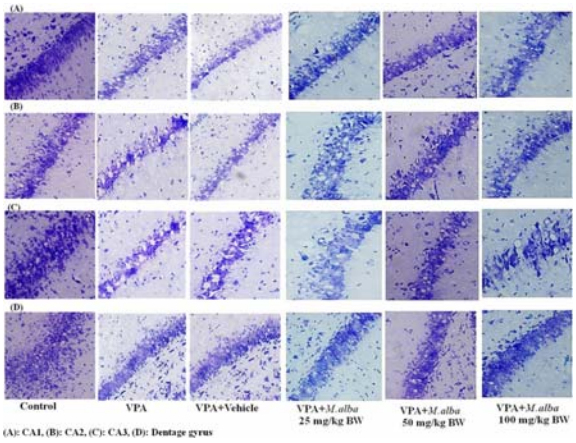
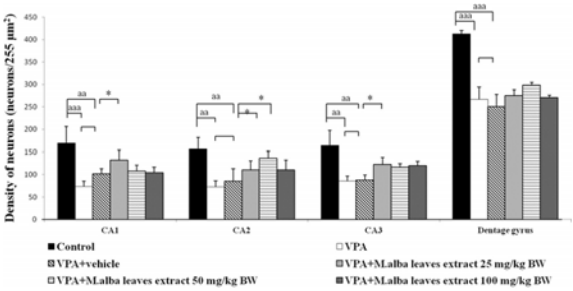



**Figure 6.** Photographic image of neurons with cresyl violet stained in various subregions of hippocampus. (a) CA1, (b) CA2, (c) CA3, and (d) dentate gyrus.




$^{aa,aaa}$ $p$<0.01, 0.001 respectively; compared with control group, * $p$<0.01 compared with VPA plus vehicle group
PND = postnatal day, VPA = valproic acid.

**Figure 7.** Effect of *M. alba* leaves extract on the neurons density in various subregions of hippocampus after treatments. Values are expressed as mean ± SEM (n = 5).

hippocampus ($p$-value <0.01, 0.05, 0.05, 0.01 respectively; compared to control rats). No changes of the parameters mentioned earlier in VPA rats which received vehicle. However, *M. alba* extract at doses of 25, 50 and 100 mg.kg-1 BW significantly mitigated the elevation of MDA induced by VPA ($p$-value <0.01 all; compared to vehicle treated rats). VPA treated rats which received low dose of extract significantly enhanced CAT activity in hippocampus while those which received the medium dose of extract enhanced both CAT and GSH-Px activities in the area just mentioned ($p$-value <0.05 all; compared to VPA plus vehicle treated rats).

### *Effect of M. alba extract on neuron density in hippocampus*

The current data have clearly demonstrated that VPA significantly decreased neuron density in CA1, CA2, CA3 and dentate gyrus ($p$-value <0.001, 0.01, 0.01 and 0.001 respectively; compared to control rats). Vehicle failed to produce significant changes of neuron density in all areas mentioned earlier. *M.alba* leaves extract at dose of 25 mg.kg$^{-1}$ BW significantly increased neuron density in CA1, CA2 and CA3 ($p$-value <0.05 all; compared to VPA plus vehicle treated rats) while medium dose of extract produced the significant increase in neuron density only in CA2 of hippocampus ($p$-value <0.05; compared to VPA plus vehicle treated rats) as shown in Figure 6, 7.

## Discussion

Our data showed that *M. alba* leaves extract at doses of 25, 50 and 100 mg.kg$^{-1}$ BW could improve memory impairment in VPA rat model of Alzheimer's disease and increased neurons density in hippocampus, the area contributing the important role on spatial memory, together with the decreased oxidative stress in the area just mentioned.

Brain is an area which is vulnerable to oxidative stress due to the high lipid content, high energy consumption and a limited capacity to detoxify oxidative stress[27,28]. The increased oxidative stress can decrease the neuronal survival especially during critical period so the antioxidant is required[29]. It was been clearly demonstrated that *M. alba* leaves extract at all doses used in this study decreased oxidative stress and improved spatial memory but only the low and medium doses could enhance neuron density in hippocampus.

Therefore, we suggested that the oxidative stress might play a pivotal role on the improved spatial memory. In addition, the decreased oxidative stress might partly play the role on the survival of neuronal cell during postnatal period. However, other factors such as the distribution of growth factor especially neurotrophin[30-32] and apoptosis[31,33] might also involve. In addition to the neuron survival, the structural alterations at spiny synapses or dendritic spines also contribute the crucial role on the pathogenesis of autism[34] and memory[35]. The survival of dendritic spines is also under the influence of growth factors[36]. Based on these pieces of evidence, the possible explanation for the lack of a tight association between the density of neuron survival cell and the improved memory observed in VPA rats which received *M. alba* leaves extract might partly due to the influence of growth factor which in turn exerted the influence on the development of dendritic spine and memory. However, further researches are required to clarify the precise underlying mechanism.

Accumulative lines of evidence have demonstrated that polyphenolic compounds can decrease oxidative stress and neurodegeneration[37,38]. In addition, it can also enhance growth factor in the brain[39]. Taken all data together, we suggested that the polyphenolic compounds in *M. alba* leaves extract might decrease oxidative stress but enhanced growth factor leading to the increased neuron density in hippocampus resulting in the improved memory in VPA rat model of autism. In addition, the polyphenolic compounds in *M. alba* leaves extract might also enhance dendritic spine in hippocampus giving rise to the improved memory in VPA treated rats.

The improved oxidative stress reflecting by the decreased MDA level also failed to show a tight association with the improved antioxidant enzymes. The VPA rats which received low dose of extract showed the decreased MDA level with no changes in antioxidant enzymes activities. This might occur because the polyphenolic compounds in the extract decreased oxidative stress formation in the cells[40] which in turn improved oxidative stress status in the cells including neuronal cells reflecting by the decreased MDA level.

In the present study, it was found that different regions of hippocampus showed different sensitivity to *M. alba* extract. This phenomenon might be due to the different distribution of growth factor which exerts the influence on plasticity[40], the different distribution of antioxidant enzymes, different brain metabolism and the different in blood supply. Moreover, no dose dependent effect of *M. alba* leaves extract was observed because the relationships between the concentration of *M. alba* leaves extract and the observed parameters such as spatial memory, oxidative stress and neuron density are not the simple linear relationship. All observed parameters are involved multi-factors. In addition, the extract used in the present study was the crude extract which contained numerous substances and can produce masking effect and interaction effect which in turn influence on the observed parameters.

## Conclusion

The current study has demonstrated that *M. alba* leaves extract is the potential food to improve memory impairment and neurodegeneration in VPA rat model of autism. The possible underlying mechanism might occur partly via the decreased oxidative stress. Other factors such as the enhanced growth factor and the decreased apoptotic factor might also be involved. However, further research is necessary to elucidate this issue.

## What is already known on this topic?

Autism is the multi-etiology disease and its pathogenesis is still obscurely known. The toxicant-induced oxidative stress may play a role in its pathogenesis. Many substances possessing antioxidant and neuroprotective activities such as Bacopa monnieri, resveratrol, green tea can also mitigate autism-like behavior in animal model of autism induced by valproic acid (VPA) exposure.

## What this study adds?

Morus alba leaves, a polyphenol-rich plant, with potent antioxidant and neuroprotection provide the beneficial effect for autism. M.alba leaves extract improved memory impairment and neurodegeneration in VPA rat model of autism partly via the decreased oxidative stress.

## Acknowledgements

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## Potential conflicts of interest

The authors declare no conflicts of interest.

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## สารสกัดใบหม่อนทำให้ความจำบกพร่อง ความเครียดออกซิเดชันและการตายของเซลล์ประสาทในฮิปโปแคมปัสในแบบจำลองภาวะออติซึมที่เหนี่ยวนำโดยกรดวาลโพรอิก

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***ภูมิหลัง:*** สืบเนื่องจากความจำกัดของการรักษาออติซึมในปัจจุบัน จึงทำให้สารใหม่ ๆที่สามารถช่วยทำให้ภาวะออติซึมดีขึ้นเป็นที่ต้องการ เนื่องจากภาวะความเครียดออกซิเดชันมีบทบาทบาทสำคัญต่อการเกิดพยาธิสรีรวิทยาของภาวะออติซึมและประโยชน์ในการต้านต่อภาวะออติซึมของสมุนไพรที่มีฤทธิ์ปกป้องสมองทำให้มีมีการให้ความสนใจต่อประโยชน์ของใบหม่อนต่อภาวะออติซึม อย่างไรก็ตามยังไม่มีหลักฐานสนับสนุนในประเด็นนี้

***วัตถุประสงค์:*** เพื่อประเมินผลของสารสกัดใบหม่อนต่อพฤติกรรมที่คล้ายกับที่พบในภาวะออติซึมและสำรวจกลไกการออกฤทธิ์ที่น่าจะเป็นไปได้ในแบบจำลองภาวะออติซึมในสัตว์ทดลองที่เหนี่ยวนำด้วยกรดวาลโพรอิก (วีพีเอ)

***วัสดุและวิธีการ:*** สัตว์ทดลองที่เป็นแบบจำลองภาวะออติซึมที่เหนี่ยวนำด้วยกรดวาลโพรอิกจะได้รับการป้อนสารสกัดใบหม่อนในขนาด 25, 50 และ 100 มิลลิกรัมต่อกิโลกรัมน้ำหนักตัวระหว่างช่วงวันที่ 14 หลังคลอด ถึง วันที่ 40 หลังคลอด และทำการประเมินพฤติกรรมด้วย hot plate, open-field, elevated plus-maze, Morris water maze และ social behavior test เมื่อสิ้นสุดการทดลองจะประเมินระดับ MDA และการทำงานของ SOD, CAT และ GSH-Px ร่วมกับการเปลี่ยนแปลงความหนาแน่นของเซลล์ประสาทในสมองส่วนที่เกี่ยวข้อง

***ผลการศึกษา:*** สารสกัดทุกขนาดช่วยทำให้การเรียนรู้และความจำและการเพิ่มความเครียดออกซิเดชันในสมองส่วนฮิปโปแคมปัสดีขึ้น สารสกัดขนาดต่ำและกลาง ยังเพิ่มความหนาแน่นของเซลล์ประสาทในฮิปโปแคมปัสของหนูที่ได้รับกรดวาลโพรอิกด้วย

***สรุป:*** สารสกัดใบหม่อนช่วยทำให้ความบกพร่องความจำในแบบจำลองออติซึมดีขึ้น กลไกการเพิ่มความจำของสารสกัดส่วนหนึ่งเกิดผ่านการทำให้ความเครียดออกซิเดชันและการเพิ่มความหนาแน่นเซลล์ประสาทในฮิปโปแคมปัส อย่างไรก็ตามยังต้องการงานวิจัยต่อไปเพื่อให้เข้าใจกลไกออกฤทธิ์ที่แท้จริง