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# The ethyl acetate fraction of a methanolic extract of unripe noni (*Morinda citrifolia* Linn.) fruit exhibits a biphasic effect on the dopaminergic system in mice

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**Abstract:** In earlier *ex vivo* studies, we reported the biphasic effect of a methanolic extract of unripe *Morinda citrifolia* fruit (MMC) on dopamine-induced contractility in isolated rat vas deferens preparations. The present *in vivo* study was designed and undertaken to further explore our earlier *ex vivo* findings. This study examined the effect of the ethyl acetate fraction of a methanolic extract of unripe *Morinda citrifolia* Linn. fruit (EA-MMC; 5–100 mg/kg, p.o.) on the dopaminergic system using mouse models of apomorphine-induced climbing time and climbing behavior, methamphetamine-induced stereotypy (sniffing, biting, gnawing, and licking) and haloperidol-induced catalepsy using the bar test. Acute treatment with EA-MMC at a low dose (25 mg/kg, p.o.) significantly attenuated the apomorphine-induced climbing time and climbing behavior in mice. Similarly, EA-MMC (5 and 10 mg/kg, p.o.) significantly inhibited methamphetamine-induced stereotyped behavior in mice. These results demonstrated that the antidopaminergic effect of EA-MMC was observed at relatively lower doses (<25 mg/kg, p.o.). On the other hand, EA-MMC showed dopaminergic agonistic activity at a high dose (3,000 mg/kg, p.o.), which was evident from alleviation of haloperidol (a dopamine D<sub>2</sub> blocker)-induced catalepsy in mice. Therefore, it is concluded that EA-MMC might possess a biphasic effect on the dopaminergic system, i.e., an antagonistic effect at lower doses (<25 mg/kg, p.o.) and an agonistic effect at higher doses (>1,000 mg/kg, p.o.). However, further receptor-ligand binding assays are necessary to confirm the biphasic effects of *M. citrifolia* fruit on the dopaminergic system.

**Key words:** apomorphine, cage climbing, haloperidol, methamphetamine, stereotypy

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## Introduction

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Noni is the common name for *Morinda citrifolia* Linn, but it is also known as Nono or Nonu, Ba Ji Tian, Indian Mulberry, Nahu, and Cheese Fruit among diverse cultures all over the world. It has been used extensively by the Polynesians in folk medicine for many years. It is normally cultivated in tropical regions for its roots, leaves, and fruits. In recent years, noni fruit juice has

attained a great deal of interest globally for its nutritional and/or medicinal value, and it is commercially marketed as a health drink in many countries, including Malaysia, China, and India.

Extensive preclinical studies using fruit, leaf, and root extracts of noni have been reported regarding their efficacy in many ailments including CNS disorders such as anxiety [7], epilepsy [18], and Alzheimer's disease [17]. Recently, we reported the antipsychotic-like activ-

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ity of a methanolic extract of unripe *M. citrifolia* fruit (MMC) in mouse models of apomorphine/methamphetamine-induced cage climbing/stereotypy behavior which demonstrated the antidopaminergic effect of noni fruit [21]. Similarly, Ekpalakorn *et al.* revealed that a decoction or infusions of roasted mature unripe fruits were effective for relieving the symptoms of nausea and vomiting [8]. According to Traditional Chinese Medicine (TCM), noni is adequate for clearing heat and toxins, invigorating the blood, and tonifying Qi [28]. It has also been reported that a noni fruit extract exhibited prokinetic and antiemetic activity as deduced by a delay in intestinal transit time caused by apomorphine (a potent agonist of the dopamine D<sub>2</sub> receptor) in mice and an effect on the apomorphine-induced emesis in dogs respectively [4], suggesting that noni might contain a weak antidopaminergic component responsible for the observed effects.

Conversely, administration of the ethyl acetate fraction of a crude methanolic extract of *M. citrifolia* (EA-MMC) at a daily dose of 400 mg/kg for 15 days significantly enhanced the levels of monoamines including dopamine in rats [18]. It has been suggested that the opposing effects of *M. citrifolia* fruit extracts on the dopaminergic system could be due to differences in the doses used in previous studies [21]. In an earlier study, we examined the effect of a noni fruit extract (MMC) on dopamine-induced contractile response using isolated rat vas deferens preparations and revealed the biphasic effect of MMC on the dopaminergic system, that is, an antidopaminergic effect at lower concentrations (<40 mg/ml) and dopaminergic agonistic effect at higher concentrations (>60 mg/ml) [22]. The results of that study also revealed the antidopaminergic effect of scopoletin (100 µg/ml), and rutin hydrate (156 µg/ml). Moreover, it has been suggested that scopoletin and/or rutin might also be responsible for the antidopaminergic effect of MMC [21]. In earlier phytochemical investigations of a crude ethanolic extract of *M. citrifolia* fruit and its different fractions, namely, chloroform, ethyl acetate, and butanol fractions, it was revealed that the ethyl acetate fraction possessed a major content of rutin, scopoletin, and quercetin [20]. Therefore, the ethyl acetate fraction of a methanolic extract of unripe *M. citrifolia* fruit (EA-MMC) was chosen to be investigated further in the present study.

In order to strengthen our earlier *ex vivo* study findings, this *in vivo* work was undertaken to elucidate the

neuromodulatory effect of EA-MMC on the dopaminergic system using mouse models, apomorphine/methamphetamine-induced climbing/stereotypy (sniffing, biting, gnawing, and licking) for antidopaminergic activity and haloperidol-induced catalepsy for dopaminergic agonistic activity.

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## Materials and Methods

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### Animals

Male ICR mice weighing 25–30 g were purchased from the Laboratory Animal Center, University of Malaya. Mice were housed and acclimatized in cages (four per cage) in a temperature- and light-controlled vivarium (22 ± 1°C, 12-h light-dark cycle, lights on/off at 7 a.m./7 p.m.). The animals were fed standard food pellets and water *ad libitum*. Behavioral testing was conducted during the light phase. All experiments were conducted in accordance with the Council for International Organization of Medical Sciences (CIOMS) on animal experimentation, guidelines [10] and were approved by the Animal Care and Use Committee (ACUC), University of Malaya, Kuala Lumpur, Malaysia (ACUC Ethics No. FAR/27/01/2012/PV (R)).

### Drugs

R(-)-Apomorphine hydrochloride hemihydrate and sodium metabisulphite (Sigma-Aldrich, St. Louis, MO, USA), methamphetamine hydrochloride (a generous gift from the Department of Chemistry, Ministry of Health, Malaysia), and haloperidol (Manace® Injection, CCM Duopharma (M) SDN BHD, Malaysia) were used. All drugs were freshly prepared using normal sterile saline except for apomorphine (prepared with normal saline containing sodium metabisulphite 0.125% w/v) and EA-MMC, which were suspended in 1% w/v sodium carboxymethyl cellulose (CMC) solution. All drugs were administered intraperitoneally (i.p.) except for EA-MMC. EA-MMC was administered by oral gavage (p.o.). The drug stock solutions were prepared so that the necessary doses were injected at a constant volume of 1 ml/100 g body weight of the animal.

### Plant material

The fresh unripe fruit of *M. citrifolia* used in this study was harvested in Malacca, Malaysia, in January 2012. The species was authenticated by Rimba Ilmu, Institute of Biological Sciences, University of Malaya. A vouch-

er specimen (KLU 47738) was deposited for future reference. The authenticated fruits were cut into thin slices and shade dried at room temperature.

#### *Extraction and fractionation*

The shade-dried plant material (1.8 kg) was ground into powder with the aid of an electric blender. The powdered fruits were extracted with 10 l of methanol (Scharlau, Spain; isocratic HPLC grade) by soaking for 20 h followed by sonication using a water-bath sonicator at 30°C for another 4 h. The resultant solution was evaporated under vacuum in a rotary evaporator to obtain a dry mass of *M. citrifolia* methanolic extract (MMC). In a recent report, we demonstrated the phytochemical characterization of MMC [22]. The obtained MMC was dissolved in distilled water and then further partitioned with ethyl acetate using a separating funnel. The ethyl acetate-soluble fraction (EA-MMC) was later evaporated to obtain a dry mass extract (yield: 9.04% w/w) and stored at 4°C until further use.

#### *Treatments*

Mice were randomly divided into different treatment groups (n=6–8). In a study to evaluate the antidopaminergic activity of EA-MMC, the saline control group received 1% w/v CMC solution orally one hour prior to intraperitoneal saline administration, the vehicle group received 1% w/v CMC solution orally one hour prior to apomorphine (5 mg/kg, i.p.) or methamphetamine injection (5 mg/kg, i.p.), and the test groups received relatively lower doses of EA-MMC (5, 10, 25, 50 or 100 mg/kg, p.o.) one hour prior to apomorphine (5 mg/kg, i.p.) or methamphetamine (5 mg/kg, i.p.) injection.

In another study, which examined the dopaminergic agonistic activity, the saline control group received 1% w/v CMC solution orally one hour prior to intraperitoneal saline administration, the vehicle group received 1% w/v CMC solution orally one hour prior to haloperidol (0.5 mg/kg, i.p.), and the test groups received EA-MMC at relatively higher doses (500, 750, 1,000, or 3,000 mg/kg, p.o.) one hour prior to haloperidol (0.5 mg/kg, i.p.) injection. All the drugs were administered at a constant dose volume of 1 ml/100 g body weight of mice.

#### *Behavioral assessment*

Apomorphine-induced cage climbing in mice: Apomorphine-treated mice showed a peculiar climbing behavior characterized by rearing and then spontaneous

climbing activity [5]. The apparatus, procedure, and scoring pattern used to evaluate climbing behavior were the same as described in our previous study [21]. Immediately after administration of apomorphine, the animals were placed into the corresponding metal cages and examined for climbing behavior. An observer who was blind to drug treatment determined the total climbing time for 30 min after apomorphine treatment. During this period, the climbing behavior on the wall of the cage was scored at 5, 10, 15, 20, 25, and 30 min after apomorphine administration, and the cumulative climbing index was determined. The maximum possible cumulative climbing index is 24.

#### *Methamphetamine-induced stereotypy in mice*

The procedure and apparatus used in the present study were the same as described in our previous report [21] and other reports [23], with slight modifications. The mice were initially acclimatized for 15 min in the test apparatus and then received methamphetamine (METH) intraperitoneally. The METH-treated animals were placed back inside the cage at its base. The intensity of stereotyped behavior of individual mice was scored at 15-min intervals for a period of 60 min as described in our previous report [21].

#### *Haloperidol-induced catalepsy in mice*

Catalepsy is one of the characteristic features of Parkinson's disease. The reduced ability to initiate movement and difficulty in attempting to correct posture are the most common features of catalepsy. In rodents, catalepsy can be easily measured by the bar test [19]. The intensity of catalepsy was measured using the standard bar hanging method by placing naive mice with both forepaws over a horizontal bar (diameter: 3 mm), that was elevated 4.5 cm from the floor [24]. The time at which the forepaws of the mouse touched the floor or climbed over the bar was considered an end point of catalepsy. The catalepsy time (s) was measured 30 and 60 min after haloperidol administration, with a maximum cutoff time of 180 s. The test was repeated three times with an intertrial interval of 1 min. After measurement of catalepsy, animals were returned to their home cages. The experimenter was unaware of the treatments given to the mice in all behavioral studies.

#### *Statistical analysis*

The data are expressed as the mean  $\pm$  SEM. The sta-

tistical significance of differences between groups was evaluated by one-way analysis of variance (ANOVA) followed by *post hoc* comparison using Tukey's multiple comparison test. Stereotyped behavior was analyzed by Kruskal-Wallis test followed by Dunn's multiple comparison test because nonparametric statistics are needed with all or none or rating scale scores. All data analyses were conducted using GraphPad Prism 5 statistical software. A level of  $P < 0.05$  was considered statistically significant.

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## Results

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### *Effect of EA-MMC on apomorphine-induced climbing behavior and methamphetamine-induced stereotypy in mice*

ANOVA results revealed a significant effect of EA-MMC on apomorphine-induced climbing behavior [F (6, 35)=4.807;  $P < 0.01$ ] and climbing time [F (6, 35)=4.601;  $P < 0.01$ ] as shown in Figs. 1a and 1b. The maximum inhibitory effect of EA-MMC on climbing behavior and climbing time was observed at 25 mg/kg. All tested doses of EA-MMC *per se* did not produce either climbing behavior or ataxia in this experiment when compared with the saline control group. Similarly, pretreatment with EA-MMC (5, 10, 25, 50, and 100 mg/kg, *p.o.*) significantly ( $P < 0.0001$ ) reduced methamphetamine-induced stereotypy behavior in a dose-dependent manner, as shown in Figs. 2a and 2b.

### *Effect of EA-MMC on haloperidol-induced catalepsy in mice*

Normal saline-treated control animals did not display catalepsy in the bar test, as they remained on the bar less than 5 s. Intraperitoneal treatment of vehicle control animals with haloperidol at 0.5 mg/kg induced a cataleptic state at 30 min after injection. EA-MMC (500, 750, 1,000, and 3,000 mg/kg, *p.o.*) significantly reduced the duration of haloperidol-induced catalepsy in a dose-dependent manner at 30 min [F (5, 42)=7.606;  $P < 0.0001$ ] and 60 min [F (5, 42)=13.08;  $P < 0.0001$ ], as shown in Fig. 3a and 3b. EA-MMC *per se* at all doses (5–3,000 mg/kg, *p.o.*) did not show any cataleptic behavior or any marked behavioral alterations.

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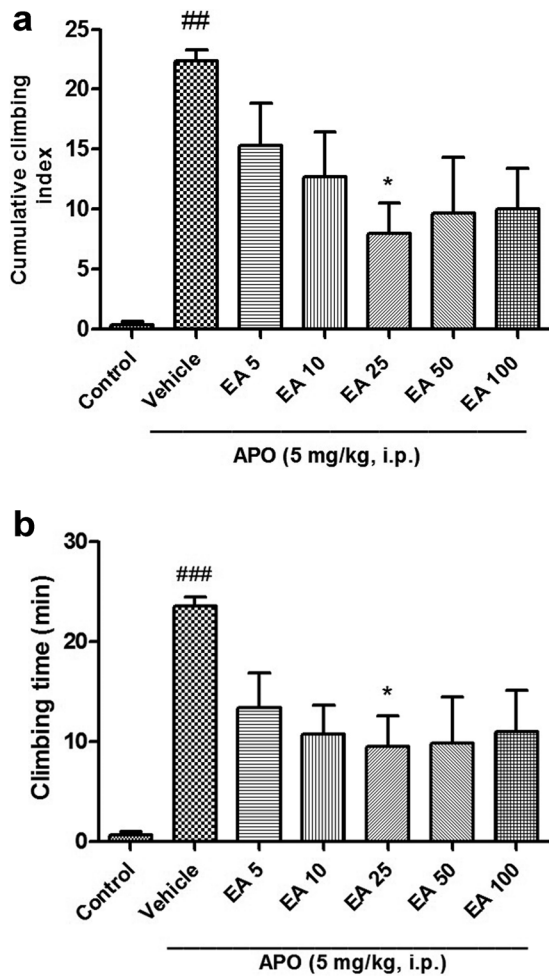
## Discussion

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The present findings revealed the neuromodulatory

effect of EA-MMC on the dopaminergic system in mice. Acute oral pretreatment with EA-MMC at relatively lower doses (<25 mg/kg, *p.o.*) significantly decreased apomorphine-induced climbing behavior and climbing time in mice. Apomorphine is a known nonselective dopamine agonist that causes changes in motor behaviors through the central nervous system. Administration of apomorphine induces locomotor activity, rearing/grooming [15], stereotyped behaviors [1], and cage-climbing behaviors [28] in rodents. Apomorphine-induced climbing behavior is due to stimulation of postsynaptic mesolimbic D<sub>2</sub> and D<sub>1</sub> dopamine (DA) receptors and is an accepted animal model for studying postsynaptic DA activity in the brain [6, 23]. It has been well-established that activation of both D<sub>1</sub> and D<sub>2</sub> receptors is needed to demonstrate climbing behavior in rodents [13]. Neither a pure D<sub>1</sub> agonist, SKF38393, nor pure D<sub>2</sub> agonist, quinpirole, could induce this behavior. On the other hand, either a selective D<sub>1</sub> antagonist or a selective D<sub>2</sub> antagonist successfully alleviated apomorphine-induced climbing behavior [16]. Alleviation of apomorphine-induced climbing in the mouse is indicative of inhibition of D<sub>1</sub> and/or D<sub>2</sub> receptors. In this context, we earlier reported the antidopaminergic effect of a crude methanolic extract of unripe *M. citrifolia* (MMC) fruit at gram doses (3–10 g/kg, *p.o.*) [21]. In the present study, the ethyl acetate fraction of MMC (EA-MMC) showed an antidopaminergic effect at milligram doses (10–25 mg/kg, *p.o.*). The potent antidopaminergic effect of EA-MMC, when compared with MMC, could be due to enrichment of phytochemicals present in the EA-MMC fraction.

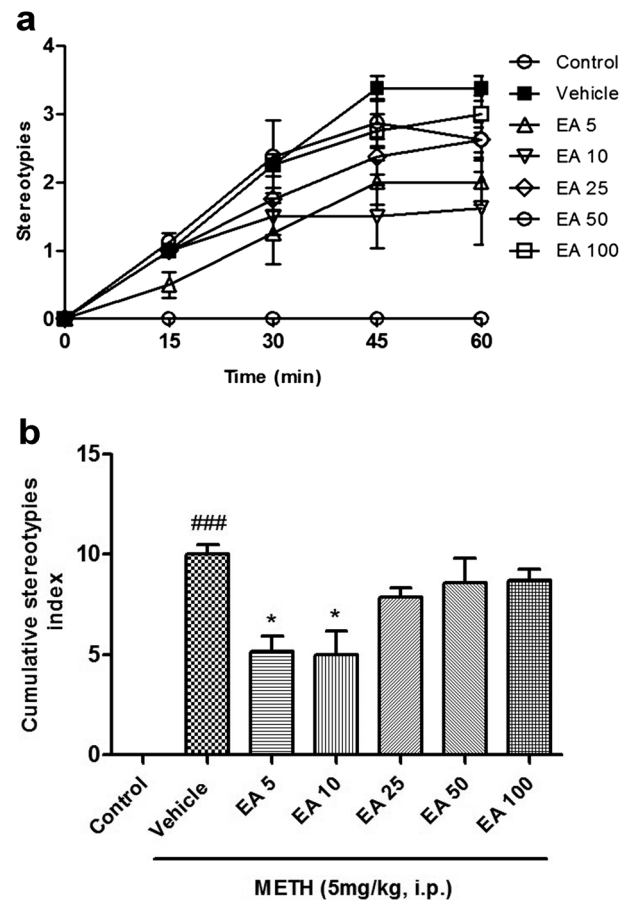
Methamphetamine is a psychostimulant that facilitates the release of newly synthesized DA and inhibits the uptake of DA, causing hyperactivity [14]. Acute administration of methamphetamine activates the nigrostriatal and mesolimbic dopamine systems, leading to hyperlocomotion and stereotyped behavior such as continuous sniffing, licking, biting (or gnawing), circling, and head bobbing in rodents [5, 12, 25]. In the present study, acute pretreatment with EA-MMC (5 and 10 mg/kg, *p.o.*) significantly reduced methamphetamine-induced stereotypy, suggesting an antidopaminergic effect of EA-MMC at lower doses (<10 mg/kg). In our earlier study [21], a crude extract of MMC showed an antidopaminergic effect against methamphetamine-induced stereotypy at doses 1–10 g/kg, *p.o.* Moreover, in our earlier acute oral toxicity study, MMC did not produce any toxic symp-



**Fig. 1.** Effect of EA-MMC (5, 10, 25, 50, and 100 mg/kg, p.o.) on cage climbing behavior and climbing time induced by apomorphine in mice. Each point represents the mean  $\pm$  SEM from the scores obtained from six animals. (a) Cumulative climbing scores were measured for 30 min at 5, 10, 15, 20, 25, and 30 min after apomorphine injection. (b) Total climbing time on the wall of the cage. Levels of statistical significance are depicted as follows: ##,  $P < 0.01$  and ###,  $P < 0.001$  when compared with the saline control group; \*,  $P < 0.05$  when compared with the vehicle control group. When not indicated, the differences were statistically insignificant.

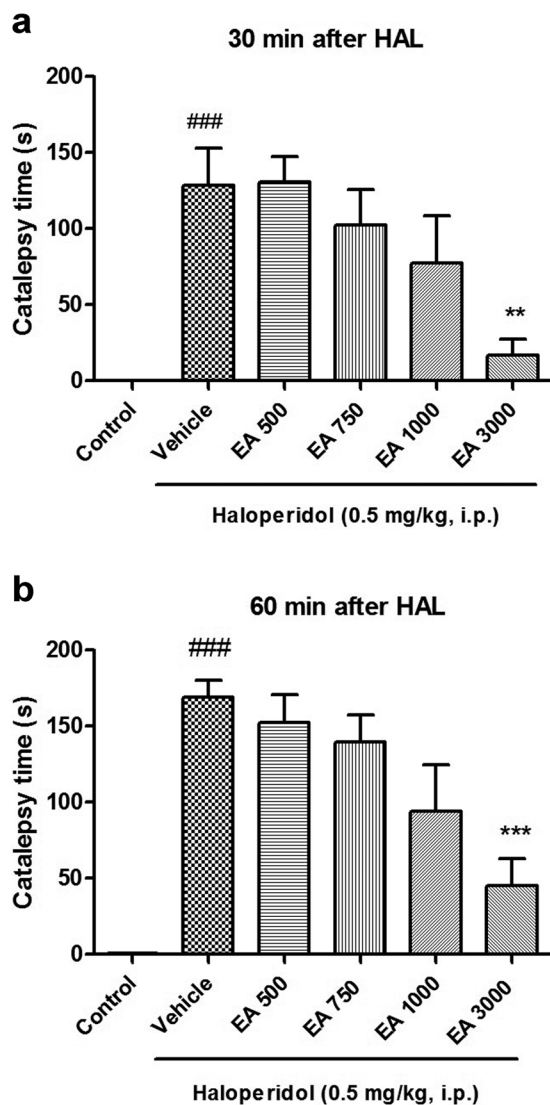
toms or mortality up to a dose level of 20 g/kg body weight in mice [21]. According to OECD Test Guideline 423 for acute oral toxicity, a maximal tolerable dose of 2 g/kg or above is categorized as unclassified and considered safe.

Interestingly, EA-MMC (5–100 mg/kg, p.o.) exhibited a dose-dependent U-shaped trend in the APO-induced cage climbing and METH-induced stereotypy animal models (Figs. 1a and 2b). EA-MMC at 100 mg/



**Fig. 2.** Effect of EA-MMC (5, 10, 25, 50, and 100 mg/kg, p.o.) on stereotypies induced by methamphetamine in mice. (a) Each point represents the mean  $\pm$  SEM from the stereotypy scores. (b) The cumulative stereotypy scores were measured during 30 to 60 min after administration of methamphetamine. Levels of statistical significance are depicted as follows: ###,  $P < 0.001$  when compared with the saline control group; \*,  $P < 0.05$  when compared with the vehicle control group. When not indicated, the differences were statistically insignificant.

kg was found to be ineffective in both animal models. In order to establish the dopaminergic agonistic effect of EA-MMC at higher doses (500–3,000 mg/kg, p.o.), the bar test was used to test the higher doses of EA-MMC against haloperidol-induced catalepsy in mice. Haloperidol is a reputable neuroleptic that specifically acts as a dopamine  $D_2$  receptor antagonist in the mesolimbic-mesocortical pathway. In view of its nonselective action, haloperidol produces a blockade of postsynaptic  $D_2$  receptors in the nigrostriatal pathway, generating extrapyramidal side effects in humans [9] and catalepsy in animals [24]. EA-MMC at higher doses (500–3,000 mg/kg, p.o.) significantly decreased haloperidol-induced cata-



**Fig. 3.** Effect of EA-MMC (500, 750, 1,000, and 3,000 mg/kg, p.o.) on haloperidol-induced catalepsy in mice. (a) 30 min (b) 60 min after haloperidol treatment. Values are expressed as the mean  $\pm$  SEM. Levels of statistical significance are depicted as follows: ###,  $P < 0.001$  when compared with the saline control group; \*\*,  $P < 0.01$  and \*\*\*,  $P < 0.001$  when compared with the vehicle control group. When not indicated, the differences were statistically insignificant.

lepsy in a dose-dependent manner. The maximum reduction of catalepsy was observed at a dose of 3,000 mg/kg (p.o.) of EA-MMC. However, EA-MMC at 500 mg/kg could not alleviate haloperidol-induced catalepsy in mice. These results demonstrate the ineffectiveness of EA-MMC (100–500 mg/kg) on the dopaminergic system; that is, it exhibited neither antidopaminergic nor dopaminergic activities at these doses (100–500 mg/kg).

This could be due to equal activity of EA-MMC at pre- and postsynaptic dopaminergic receptors at the doses tested (100–500 mg/kg).

It has been previously reported that haloperidol-induced catalepsy can be promisingly reversed by  $D_2$  dopamine receptor agonists [19]. Therefore, it can be postulated that the anti-cataleptic effect of EA-MMC at higher doses (500–3,000 mg/kg) could be mediated by its dopaminergic agonistic activity. Thus, these results demonstrated the biphasic effect of EA-MMC on the dopaminergic system, that is, antagonism of dopaminergic transmission at lower doses ( $< 25$  mg/kg), since this extract decreased cage climbing behavior and stereotyped behavior induced by apomorphine and methamphetamine respectively, and a dopaminergic agonistic effect at higher doses ( $> 1,000$  mg/kg). These results are consistent with our earlier *ex vivo* study report that demonstrated the biphasic effect of MMC on the dopaminergic system, that is, the antidopaminergic effect at lower doses ( $< 40$  mg/ml) and dopaminergic agonistic effect at higher doses ( $> 60$  mg/ml) in isolated rat vas deferens preparations [22]. Biphasic effects of drugs on biological systems have been extensively studied and reported earlier in the literature. In our recent report, acute treatment with  $\alpha$ -asarone elicited biphasic actions in the tail suspension test (TST), in which an antidepressant-like effect was seen at relatively lower doses (15 and 20 mg/kg, i.p.) and depressive-like activity was seen at relatively higher doses (50 and 100 mg/kg, i.p.) [3]. Similarly, acute oral administration of a methanolic extract of *Mitragyna speciosa* leaf (MMS; 50–500 mg/kg) showed an inverted bell-shaped dose response in apomorphine-induced cage climbing behavior in mice [27]. MMS at lower doses (75 and 100 mg/kg) was found to be effective in attenuating apomorphine-induced climbing behavior in mice, whereas at higher doses ( $> 125$  mg/kg), it was ineffective; therefore, only lower doses (75 and 100 mg/kg) were suggested for the antidopaminergic effect of MMS [27]. Furthermore, these findings coincide with the conjecture of Wang *et al.* that noni acts as a Yin/Yang regulator from micro-Yin/Yang to macro-Yin/Yang in improving two opposite health conditions such as diarrhea and constipation [28].

Dopamine receptors are broadly classified into  $D_1$  and  $D_2$  families that regulate physiological actions of dopamine [2].  $D_1$ -family dopamine receptors ( $D_1$  and  $D_5$ ) activate the  $G_{\alpha_{s/olf}}$  family of G proteins, thereby stimulating adenylate cyclase and subsequently increasing the

production of cAMP. The D<sub>1</sub> dopamine receptor family is primarily found postsynaptically on dopamine-receptive cells, such as GABAergic medium spiny neurons (MSNs) in the striatum [2]. Activation of postsynaptic D<sub>1</sub> dopamine receptors leads to a moderate stimulatory effect on locomotor activity [2]. Meanwhile, stimulation of D<sub>2</sub>-family dopamine receptors (D<sub>2</sub>, D<sub>3</sub>, and D<sub>4</sub>) coupled with the G $\alpha_{i/o}$  family of G proteins inhibits adenylylate cyclase, thereby decreasing cAMP production. The D<sub>2</sub> and D<sub>3</sub> dopamine receptors are expressed on both pre- and postsynaptic dopaminergic neurons in contrast to the D<sub>1</sub> receptor family. D<sub>2</sub> receptors, located presynaptically on dopaminergic neurons, act as autoreceptors that provide important negative feedback mechanisms such as inhibition of neuronal synthesis, release of the neurotransmitter, and firing rate. Activation of presynaptic D<sub>2</sub> autoreceptors leads to a decrease in locomotor activity due to reduction in dopamine release, whereas activation of postsynaptic D<sub>2</sub> receptors stimulates locomotion [2]. Therefore, dopamine agonists can produce a biphasic effect at different concentrations. At lower concentrations, a dopamine agonist activates the presynaptic D<sub>2</sub> autoreceptors, thereby decreasing dopamine release and consequently impeding behavioral activity. At higher concentrations, it activates the postsynaptic D<sub>2</sub> receptors and stimulates abnormal behavioural activity such as stereotypy [2]. Though the present study could not delineate the actual mechanism of the facilitatory effect of EA-MMC on dopaminergic transmission at higher doses (>1,000 mg/kg), it might be possible that EA-MMC at higher doses blocks presynaptic dopaminergic D<sub>2</sub> autoreceptors, thereby potentiating the release of DA in the synaptic cleft. It has been previously demonstrated that administration of EA-MMC to rats at a daily dose of 200 or 400 mg/kg for 15 days significantly increased the brain levels of monoamines including dopamine [18].

Contrary to the above, a reduction in the levels of neurotransmitters from various part of the brain, including noradrenaline in the amygdala and the hippocampus, serotonin in the amygdala, DOPAC in the hippocampus and substantia nigra, and HVA in the substantia nigra, was found in noni-treated rats (1 ml/day, p.o., for 15 days), and it was hypothesized that this contributes to the anxiolytic effects of noni [26]. The discrepancies in the levels of monoamines (NA, DA, and 5HT) following noni treatment in different studies might be due to the differences in the doses of fruit extract used in the stud-

ies. Increased levels of monoamines in animals treated with chronically high doses of EA-MMC could be due to enrichment of active constituents in the ethyl acetate fraction. Enriched active phytoconstituents of high doses of EA-MMC could block presynaptic D<sub>2</sub> receptors, thereby enhancing the level of monoamines including dopamine. However, there might be other possibilities involved in the biphasic effect of EA-MMC. A schema describing the probable pharmacological mechanisms thought to be underlying in the biphasic effect of EA-MMC at higher and lower doses is illustrated in Fig. 4. In general, the combination of active phytoconstituents present in medicinal herbs is responsible for their therapeutic benefits. An HPLC analysis of the ethyl acetate fractions obtained from a previous study showed the presence of rutin, scopoletin, and quercetin [20]. Based on the information available in the literature, it appears that there is some contribution of these bioactive compounds (rutin, scopoletin, and quercetin) to noni's neurological effects. Importantly, scopoletin has been recommended as a marker constituent for quality control and pharmacokinetic study of noni products [11]. Additionally, our earlier report demonstrated the antidopaminergic effect of scopoletin and rutin *per se* in an *ex vivo* study [22]. Although these phytoconstituents are present in many plants, it is felt that their efficacy in producing an antidopaminergic effect is determined by the quantity of them and their interaction with other phytoconstituents of the plant species. Therefore, these bioactive principles in EA-MMC could mediate the neuromodulatory effect on the dopaminergic system in mice. However, further receptor-ligand binding assays are warranted to ascertain the actual mechanism involved in the biphasic effect of *M. citrifolia* fruit on the dopaminergic system.

In conclusion, acute oral treatment of EA-MMC showed a biphasic effect on the dopaminergic system in *in vivo* animal models. EA-MMC attenuated the cage climbing and stereotyped behavior induced by apomorphine and methamphetamine respectively, thereby demonstrating the antidopaminergic activity of EA-MMC. At higher doses, however, EA-MMC facilitated the dopaminergic system as demonstrated by reversal of haloperidol-induced catalepsy in mice. This atypical biphasic effect of EA-MMC on the dopaminergic system could be utilized for further research to identify the novel therapeutic potential of noni fruit in dopaminergic pathway-mediated neuropsychiatric disorders, such as

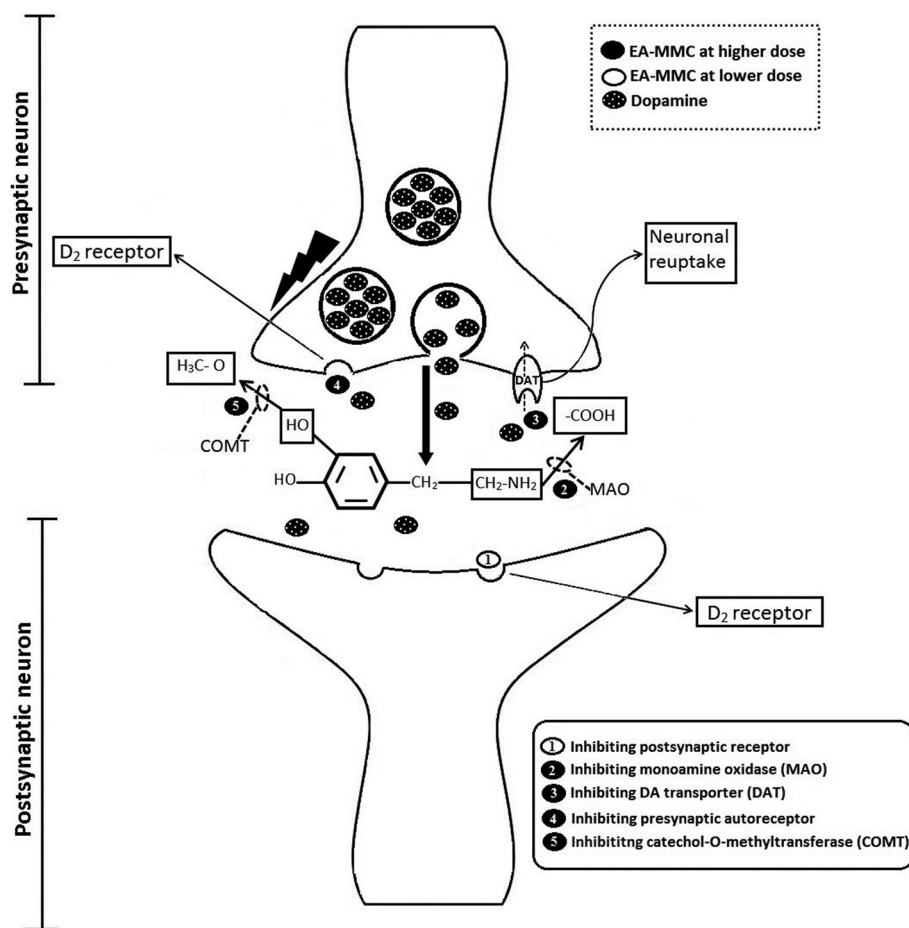


Fig. 4. The proposed mechanisms of the biphasic effect of the ethyl acetate (EA-MMC) fraction of the methanolic extract of noni (*Morinda citrifolia* Linn.) fruit on the dopaminergic system.

schizophrenia, Parkinson's disease, drug addiction, and many others.

### Conflict of Interests

The authors declare that they have no competing interests.

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