


The effects of galangin in prepulse inhibition test and experimental schizophrenia models

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Abstract

Objective: Acetylcholinesterase inhibitors are the focus of interest in the management of schizophrenia. We aimed to investigate the effects of acute galangin administration, a flavonoid compound with acetylcholinesterase inhibiting activity, on schizophrenia-associated cognitive deficits in rats and schizophrenia models in mice. **Methods:** Apomorphine-induced prepulse inhibition (PPI) disruption for cognitive functions, nicotinic, muscarinic, and serotonergic mechanism involvement, and brain acetylcholine levels were investigated in Wistar rats. Apomorphine-induced climbing, MK-801-induced hyperlocomotion, and catalepsy tests were used as schizophrenia models in Swiss albino mice. The effects of galangin were compared with acetylcholinesterase inhibitor donepezil, and typical and atypical antipsychotics haloperidol and olanzapine, respectively. **Results:** Galangin (50,100 mg/kg) enhanced apomorphine-induced PPI disruption similar to donepezil, haloperidol, and olanzapine ($p < 0.05$). This effect was not altered in the combination of galangin with the nicotinic receptor antagonist mecamylamine (1 mg/kg), the muscarinic receptor antagonist scopolamine (0.05 mg/kg), or the serotonin-1_A receptor antagonist WAY-100635 (1 mg/kg) ($p > 0.05$). Galangin (50,100 mg/kg) alone increased brain acetylcholine concentrations ($p < 0.05$), but not in apomorphine-injected rats ($p > 0.05$). Galangin (50 mg/kg) decreased apomorphine-induced climbing and MK-801-induced hyperlocomotion similar to haloperidol and olanzapine ($p < 0.05$), but did not induce catalepsy, unlike them. **Conclusion:** We suggest that galangin may help enhance schizophrenia-associated cognitive deficits, and nicotinic, muscarinic cholinergic, and serotonin-1_A receptors are not involved in this effect. Galangin also exerted an antipsychotic-like effect without inducing catalepsy and may be considered as an advantageous antipsychotic agent.

Significant outcomes

- Galangin, a flavonoid compound, presented antipsychotic-like effects in experimental schizophrenia models in mice.
- Galangin enhanced apomorphine-induced PPI disruption indicating a cognitive enhancement.
- Galangin may be considered as a potential antipsychotic agent in the management of schizophrenia and also schizophrenia-associated cognitive dysfunctions.

Limitations

- This study investigated the acute effects of galangin. It would be better if chronic effects of this agent were also investigated.
- In this study, to investigate the involvement of nicotinic, muscarinic cholinergic, and serotonergic mechanisms in the effects of galangin, we administered the antagonists of these receptors (mecamylamine, scopolamine, serotonin-1_A receptor antagonist WAY-100635, respectively) in combination with galangin. It would be better if the mechanisms of action of galangin were explored on a molecular basis.
- We investigated the effects of galangin on brain acetylcholine levels. It would be better if we also investigated the effects of galangin on brain dopamine levels.

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Introduction

Schizophrenia is a mental disorder characterised by delusions, perceptual disturbances, hallucinations, asociality, anhedonia, and cognitive deficits (Madireddy & Madireddy, 2020). Dopaminergic hyperfunction and glutamatergic hypofunction are suggested hypotheses for

the pathophysiology of schizophrenia. In addition, it has been suggested that subcortical dopamine neurons are modulated by cortical glutamatergic neuronal projections (Rodvelt et al., 2008). Furthermore, the central cholinergic system has been associated with the pathophysiology of schizophrenia (Scarr et al., 2013). This hypothesis originates from the consideration of the interactions between cholinergic, dopaminergic, and glutamatergic systems. In post-mortem studies, it was reported that some subunits of nicotinic and muscarinic receptors were disrupted (Uslu et al., 2014). Nicotinic and muscarinic acetylcholine receptors are suggested to modulate dopamine release and be involved in the pathophysiology of schizophrenia (Zhang et al., 2002; Rodvelt et al., 2008). It has been also suggested that a decrease in muscarinic M₁ receptors led to glutamate hypofunction and a decline in M₁ and/or M₄ receptors enhanced dopamine release (Scarr et al., 2013).

The cholinergic system is crucial for the normal maintenance of cognitive functions. Also, evidence shows that cholinergic dysfunction is associated with cognitive deficits seen in schizophrenia. It was reported that nicotine administration reduced cognitive deficits in patients with schizophrenia (D'Souza & Markou, 2012). Sensorimotor gating is an essential mechanism for healthy cognitive functions, which filters sensory information and integrates with motor responses (Larrauri et al., 2015; Khoja et al., 2019). The cholinergic system is also reported to be involved in the modulation of the sensorimotor gating mechanism (Jin et al., 2019). Prepulse inhibition (PPI) is defined as the attenuation of the startle response to a sensory stimulus (pulse) when it is preceded by a weaker stimulus (prepulse) (Khoja et al., 2019). PPI has been reported to be an operational measure of the sensorimotor gating mechanism reflecting its functioning (Poje & Filion, 2017), and disruption of PPI is considered as one of the typical features of schizophrenia (Uslu et al., 2014).

Inhibition of acetylcholinesterase, which catalyses the degradation of acetylcholine, enhances cholinergic transmission and improves cognitive functions (Ghezzi et al., 2013). The acetylcholine inhibitors donepezil, galantamine, and rivastigmine are commonly used in the symptomatic relief of cognitive dysfunctions in Alzheimer's disease and other dementias (Pohanka, 2014). Recently, these agents have been used for cognitive improvement in schizophrenia (Santos et al., 2018). The clinical data are limited and consist of both positive and negative reports on the use of acetylcholine inhibitors as adjunctive agents to antipsychotics for cognitive enhancement in schizophrenia (Bora et al., 2005; Buchanan et al., 2008; Dyer et al., 2008; Kumar et al., 2017; Santos et al., 2018). Another issue to consider is whether acetylcholinesterase inhibitors affect antipsychotic efficacy. Galantamine has been reported to increase the efficacy of atypical antipsychotics in schizophrenia models (Wadenberg et al., 2017). Similarly, it was reported that galantamine reversed PPI disruptions induced by apomorphine, MK-801, or scopolamine (Hohnadel et al., 2007; Koda et al., 2008). In addition to cholinergic receptors, serotonin-1A (5-HT_{1A}) receptors have also been suggested to be involved in the aetiology of schizophrenia, depression, and anxiety (Ago et al., 2014). Furthermore, it was reported that 5-HT_{1A} agonists alleviated PPI disruption (Matsuda, 2013).

Flavonoids are phytochemicals derived from the secondary metabolisms of plants and are involved in various biologic processes. They are reported to be beneficial molecules for human health (Singh et al., 2014; Zanoaga et al., 2019). Galangin (3,5,7 trihydroxyflavone) is a flavonoid compound and is one of the main active constituents of *Alpinia officinarum* (Chen et al., 2015; Fang

et al., 2019). Besides its antiproliferative, anticancer, and anti-inflammatory features, the acetylcholinesterase inhibiting action of galangin has also been exhibited (Guo et al., 2010; Jung et al., 2014; Chien et al., 2015).

In this study, we aimed to investigate the effects of galangin on apomorphine-induced PPI disruption and the involvement of cholinergic muscarinic, nicotinic, and 5-HT_{1A} receptors in rats and experimental schizophrenia models in mice. The effects of galangin on brain acetylcholine levels in rats were also studied.

Material and methods

Experimental animals

Adult male Wistar rats (250–300 g) and adult male Swiss albino mice (25–35 g) were used in the study (n = 8 in each group for both rats and mice). The animals were sheltered in standard laboratory conditions (12-h light/12-h dark cycle, 22 ± 2°C, with 45–50% relative humidity), and food and water were available *ad libitum*. The study was approved by the Local Ethics Committee for Animal Experimentation at Eskisehir Osmangazi University (approval number: 277/2012). The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

Drugs and chemicals

Galangin (Alfa Aesar), R(-) apomorphine hydrochloride hemihydrate (Sigma), (+)-MK-801 hydrogen maleate (Santa Cruz Biotechnology), donepezil hydrochloride monohydrate (Sigma), mecamlamine hydrochloride (Santa Cruz Biotechnology), (-)-scopolamine hydrobromide trihydrate (Acros Organics), haloperidol (Sigma), olanzapine (Sigma), and WAY-100635 (Sigma) were used in the study. All the drugs and chemicals were dissolved in saline except for galangin, which was dissolved in distilled water: DMSO with 1:1 ratio, and R(-) apomorphine hydrochloride hemihydrate was dissolved in saline containing 0.1% ascorbic acid. Galangin, (+)-MK-801 hydrogen maleate, (-)-scopolamine hydrobromide trihydrate, haloperidol, olanzapine were administered intraperitoneally (i.p.), and R(-) apomorphine hydrochloride hemihydrate, donepezil, mecamlamine hydrochloride, and WAY-100635 were administered subcutaneously (s.c.).

Experimental design

The effects of acute galangin administration on schizophrenia-associated cognitive deficits and the involvement of nicotinic, muscarinic, and 5-HT_{1A} receptors were investigated through apomorphine-induced PPI disruption in rats. The PPI of the acoustic startle reflex test was used for this purpose and is described in detail below. In this test, galangin was administered alone at doses of 50 and 100 mg/kg. Then, for the investigation of the involvement of nicotinic, muscarinic, and 5-HT_{1A} receptors, galangin 50 mg/kg was combined with the nicotinic receptor antagonist mecamlamine (1 mg/kg), the muscarinic receptor antagonist scopolamine (0.05 mg/kg), and the 5-HT_{1A} receptor antagonist WAY-100635 (1 mg/kg). In the combination groups, galangin was administered at a dose of 50 mg/kg because both 50 and 100 mg/kg doses of galangin similarly enhanced apomorphine-induced PPI disruption. Immediately after the PPI test, the rats were sacrificed and their brains were removed and stored at

–80°C until required for the determination of acetylcholine levels. A low dose of scopolamine that does not disrupt PPI was selected because it was reported that scopolamine disrupted PPI (Jones & Shannon, 2000). The effects of galangin on the PPI of the acoustic startle reflex test were compared with the reference drug donepezil (1 mg/kg), a commonly used agent in Alzheimer's disease with acetylcholinesterase inhibiting action, and reference typical antipsychotic haloperidol (1 mg/kg) and atypical antipsychotic olanzapine (3 mg/kg).

The effects of acute galangin administration on animal models of schizophrenia were examined through apomorphine-induced climbing, MK-801-induced hyperlocomotion, and catalepsy tests in mice, as described in detail below. In these tests, galangin was administered at a dose of 50 mg/kg considering the similar effects of 50 and 100 mg/kg galangin in the PPI test. The effects of galangin on animal models of schizophrenia were compared with the reference typical antipsychotic haloperidol (1 mg/kg) and atypical antipsychotic olanzapine (3 mg/kg).

Prepulse inhibition of the acoustic startle reflex test

The test was performed according to the method introduced by Chang et al. (2012) by using four acoustic startle response chambers (SR-LAB Startle Response System, San Diego Instruments, San Diego, CA, USA). Each chamber consisted of a cylindrical plexiglas animal enclosure. Acoustic stimuli were supplied using a speaker mounted 24 cm above the animals in the chambers. Startle responses were transduced by a piezoelectric accelerometer positioned below the cylinder, rectified, digitised, and recorded as data on the computer. Rats were handled daily for a week, and they were placed into chambers for 15 min, 24 h before performing the test sessions for habituation to the test system without any acoustic stimuli. The background noise level was 70 decibels (dB) [A] soundpressure level (SPL) throughout the test sessions. Apomorphine was administered at a dose of 0.5 mg/kg s.c. 30 min after the administration of the drugs (galangin 50 or 100 mg/kg, haloperidol 1 mg/kg, olanzapine 3 mg/kg, donepezil 1 mg/kg), and rats were immediately placed in the chambers and the test sessions were started. Apomorphine dose was determined based on the study of Geyer et al. (1999). In the combination groups, mecamlamine (1 mg/kg, s.c.) was administered 15 min before, scopolamine (0.05 mg/kg, i.p.) was administered 30 min before, and WAY-100635 (1 mg/kg, s.c.) was administered just before the administration of galangin (50 mg/kg, i.p.) or the reference drugs (haloperidol 1 mg/kg, olanzapine 3 mg/kg, donepezil 1 mg/kg). In all the combination groups, apomorphine (0.5 mg/kg, s.c.) was administered 30 min before galangin or the reference drug administrations and immediately the test sessions were started. Mecamlamine, scopolamine, and WAY-100635 doses and administration time schedules were performed according to the related literature (Jones & Shannon, 2000; Krebs-Thomson et al., 2006; Kohnomi et al., 2010; Koda et al., 2011).

At the beginning of the test sessions, a 5-min acclimation period with only 70 dB background noise was started. The prepulse intensities were +4, +8, +16 dB [A] SPL above background noise (74, 78, 86 dB, respectively). After the acclimation period, five consecutive startle pulse-alone trials (120 dB) were applied, which were followed by 10 blocks of trials. Each block consisted of five types of trials in a random order as below:

1. Pulse-alone stimulus (120 dB)
2. Prepulse (74 dB) + pulse stimulus (120 dB)

3. Prepulse (78 dB) + pulse stimulus (120 dB)
4. Prepulse (86 dB) + pulse stimulus (120 dB)
5. No stimulus (70 dB background noise only)

The test session concluded with five consecutive startle pulse-alone trials (120 dB). PPI was described as a reduction in the amplitude of the startle reflex in the presence of the prepulse stimulus, which was calculated for each prepulse intensity with the formula: $PPI (\%) = 100 - (\text{average startle reflex in presence of the prepulse} \times 100 / \text{average startle reflex without a prepulse})$.

Apomorphine-induced climbing test

The test was performed according to the related literature (Akhtar et al., 2006; Mahmood et al., 2012). Apomorphine was administered (1.5 mg/kg, s.c.) 30 min after galangin (50 mg/kg, i.p.) or the reference drug administration (haloperidol 1 mg/kg, olanzapine 3 mg/kg). Then, the mice were immediately placed in a cylindrical wire cage (height 13 cm, diameter 14 cm, and pores 3 mm). The total climbing time on the inside of the wire cage was recorded for 30 min.

MK-801-induced hyperlocomotion test

The test was performed according to the related literature (Bardgett et al., 2003; Sławińska et al., 2013; Canal et al., 2014). MK-801 was administered (0.3 mg/kg, s.c.) 30 min after galangin (50 mg/kg, i.p.) or the reference drug administration (haloperidol 1 mg/kg, olanzapine 3 mg/kg). Then, the mice were immediately placed in activitymeter apparatus (MAY-AMS 02 Animal Activity Monitoring System, Commat Ltd. Ankara/TURKEY), which automatically recorded the total number of movements for 1 h.

Catalepsy test

The test was performed according to the related literature (Kinsey & Cole, 2013; Neves et al., 2013). The mice were positioned with their forepaws on a glass bar 5 cm high from the floor, 30, 60, and 90 min after galangin 50 mg/kg or the reference drug administration (haloperidol 1 mg/kg, olanzapine 3 mg/kg). At each time point, the time that the mice remained in this position, which was defined as the cataleptic posture, was observed for a maximum of 60 s. The test was ended when mice corrupted this position by touching the floor with their forepaws or by climbing on the bar.

Determination of brain acetylcholine levels

Brain acetylcholine concentrations were determined in rats. Immediately after performing the PPI of the acoustic startle reflex test, the rats were sacrificed and their brains were removed. One cerebral hemisphere was weighed and homogenised in phosphate-buffered saline. Acetylcholine concentrations were determined by following the instructions of a commercial kit (Bioassay Systems Enzychrom TM Acetylcholine Assay Kit-EACL-100, CA, USA), and the results were analysed colorimetrically.

Statistical analysis

Data were statistically analysed using the SPSS 21.0 and Sigma Stat 3.5 programmes. The Kruskal–Wallis test was used to analyse the results of the PPI of the acoustic startle reflex, apomorphine-induced climbing, MK-801-induced hyperlocomotion,

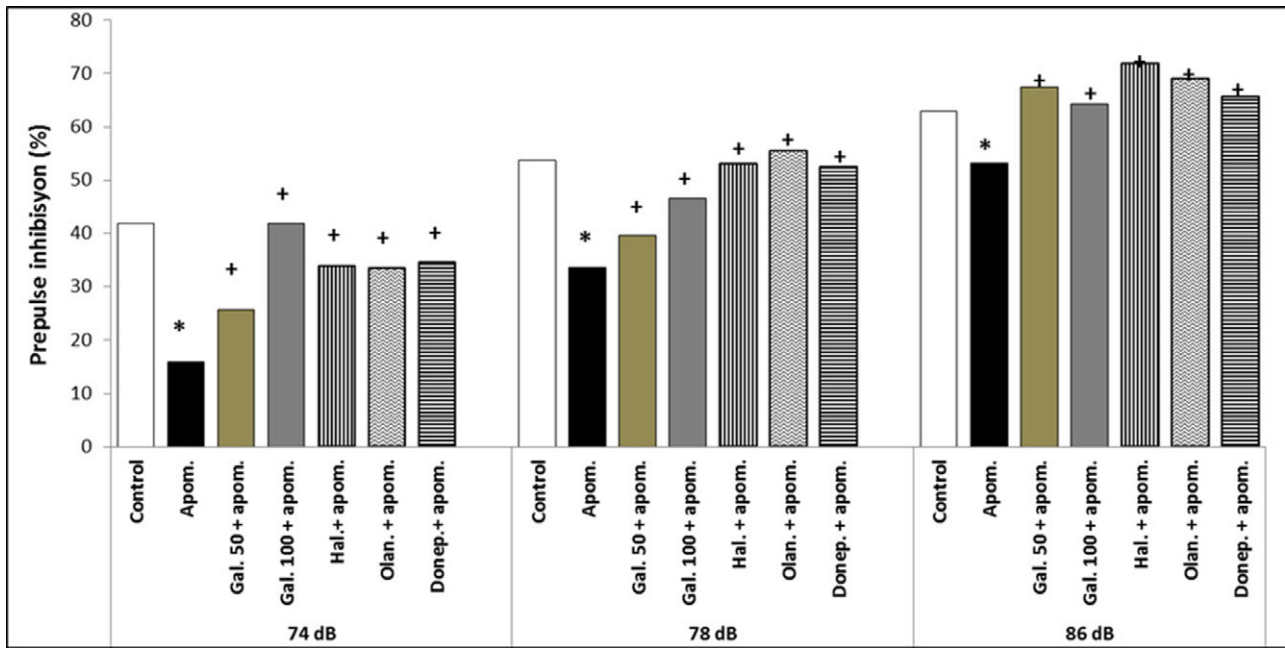


Fig. 1. The effects of a single galangin administration on apomorphine-induced PPI disruption at three prepulse intensity levels (74, 78, and 86 dB) and a comparison with haloperidol, olanzapine, and donepezil in rats. The results are expressed as median. Apom., apomorphine; Gal. 50, galangin 50 mg/kg; Gal. 100, galangin 100 mg/kg; Hal., haloperidol; Olan., olanzapine; Donep., Donepezil. * $p < 0.05$ refers to a statistically significant difference compared with the control group. + $p < 0.05$ refers to a statistically significant difference compared with the apomorphine group.

and catalepsy tests, and multiple comparisons were performed using the Student–Newman–Keuls or Tukey tests. One-way analysis of variance was used to analyse the outcomes of the brain acetylcholine concentrations, and multiple comparisons were made using the Tamhane test. A value of $p < 0.05$ was accepted as statistically significant.

Results

The effects of galangin on apomorphine-induced prepulse inhibition

The results of a single administration of galangin and comparison with haloperidol, olanzapine, and donepezil

Apomorphine significantly decreased PPI% at the three prepulse intensity levels (74, 78, and 86 dB) compared with the control, which meant there was a disruption in PPI ($p < 0.05$) (Fig. 1). Galangin 50 and 100 mg/kg significantly increased PPI% at the three prepulse intensity levels (74, 78, and 86 dB) compared with the apomorphine group, which reflected an enhancement in apomorphine-induced PPI disruption ($p < 0.05$) (Fig. 1). There was no significant difference between galangin 50 and 100 mg/kg doses in PPI% at the three prepulse intensity levels (74, 78, and 86 dB) ($p > 0.05$) (Fig. 1).

Haloperidol, olanzapine, and donepezil also significantly increased PPI% at the three prepulse intensity levels (74, 78, and 86 dB) compared with the apomorphine group ($p < 0.05$) (Fig. 1). There was no significant difference between galangin 50 and 100 mg/kg doses and haloperidol, olanzapine, donepezil in PPI% at the three prepulse intensity levels (74, 78, and 86 dB) ($p > 0.05$) (Fig. 1).

The results of combined administration of galangin with nicotinic, muscarinic, and 5-HT_{1A} receptor antagonists

A galangin 50 mg/kg dose was selected for the combined administrations because there was no significant difference between the 50 and 100 mg/kg doses of galangin in PPI%. There was no significant alteration in the effects of galangin (50 mg/kg) on PPI% at the three prepulse intensity levels (74, 78, and 86 dB) when galangin was administered with the nicotinic receptor antagonist mecamylamine, muscarinic receptor antagonist scopolamine, and 5-HT_{1A} receptor antagonist WAY-100635 ($p > 0.05$) (Fig. 2).

The effects of galangin on apomorphine-induced climbing test

Apomorphine significantly increased the total climbing time compared with the control group ($p < 0.05$) (Fig. 3). Galangin 50 mg/kg significantly reduced the apomorphine-induced total climbing time compared with both the single apomorphine group and the control group ($p < 0.05$) (Fig. 3). Haloperidol (1 mg/kg) and olanzapine (1 mg/kg) also significantly reduced the apomorphine-induced total climbing time compared with both the single apomorphine group and the control group ($p < 0.05$) (Fig. 3). No significant difference was observed in the total climbing time between galangin 50 mg/kg, haloperidol, and olanzapine ($p > 0.05$) (Fig. 3).

The effects of galangin on MK-801-induced hyperlocomotion test

MK-801 significantly increased the total number of movements compared with the control group, indicating hyperlocomotion ($p < 0.05$) (Fig. 4). Galangin 50 mg/kg significantly decreased

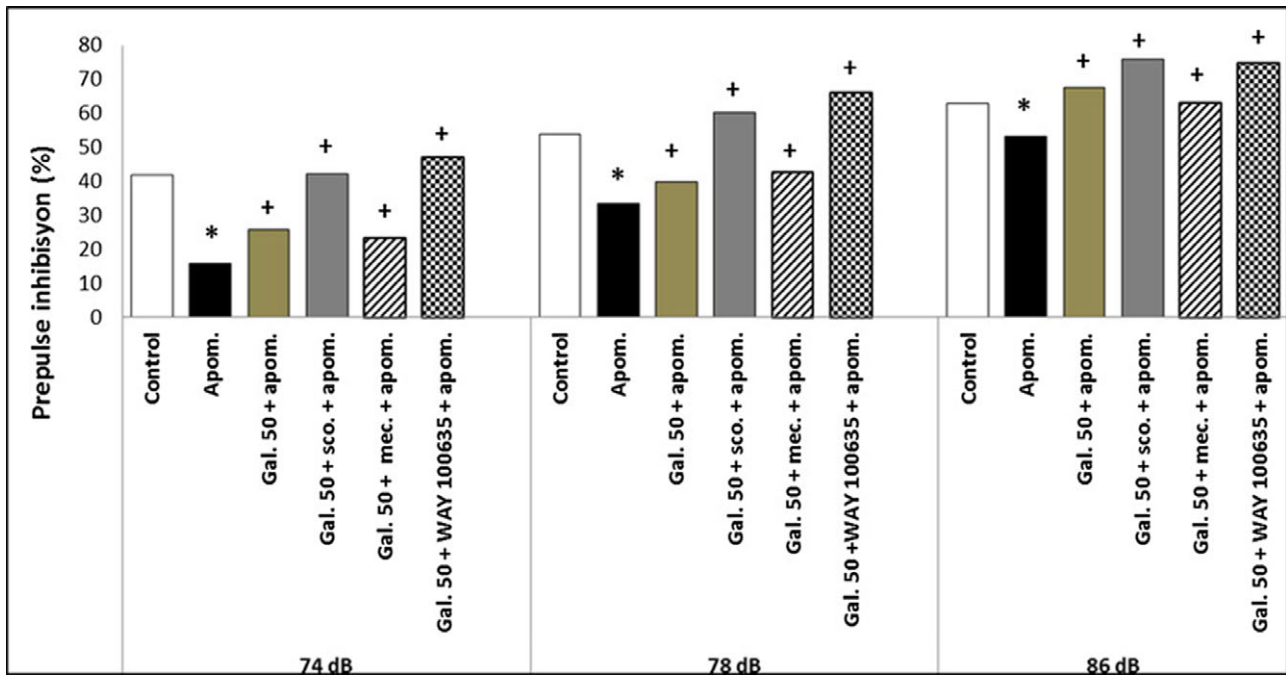


Fig. 2. The effects of galangin with nicotinic, muscarinic, 5-HT_{1A} receptor antagonist combinations on apomorphine-induced PPI disruption at three prepulse intensity levels (74, 78, and 86 dB) in rats. The results are expressed as median. Apom., apomorphine; Gal. 50, galangin 50 mg/kg; sco., scopolamine; mec., mecamylamine. * $p < 0.05$ refers to a statistically significant difference compared with the control group. + $p < 0.05$ refers to a statistically significant difference compared with the apomorphine group.

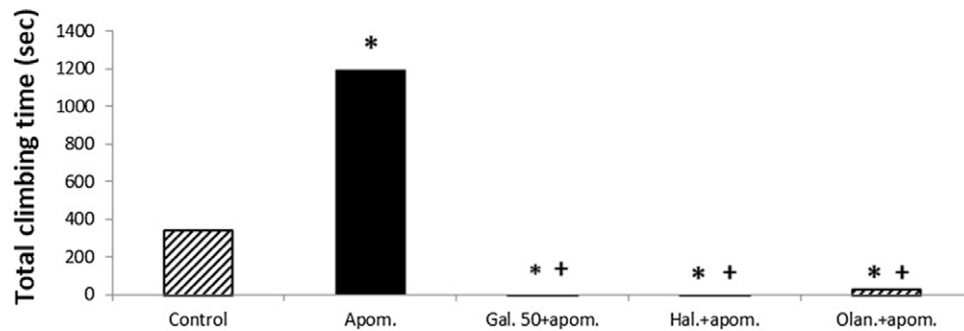


Fig. 3. The effects of galangin on apomorphine-induced climbing test in mice. The results are expressed as median. * $p < 0.05$ refers to a statistically significant difference compared with the control group. + $p < 0.05$ refers to a statistically significant difference compared with the apomorphine group. Haloperidol and olanzapine are used as reference antipsychotic drugs. Apom., apomorphine; Gal. 50, galangin 50 mg/kg; Hal., haloperidol; Olan., olanzapine.

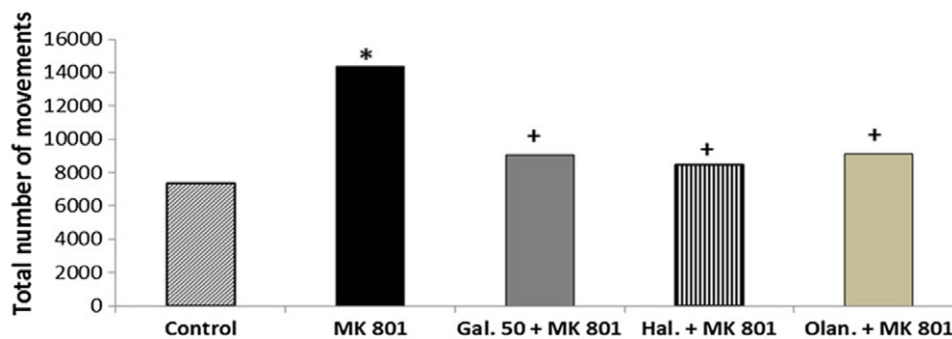


Fig. 4. The effects of galangin on MK-801-induced hyperlocomotion test in mice. The results are expressed as median. * $p < 0.05$ refers to a statistically significant difference compared with the control group. + $p < 0.05$ refers to a statistically significant difference compared with the MK-801 group. Gal. 50, galangin 50 mg/kg; Hal., haloperidol; Olan., olanzapine.

the total number of movements compared with MK-801 ($p < 0.05$) (Fig. 4). Haloperidol (1 mg/kg) and olanzapine (1 mg/kg) also significantly decreased the total number of movements compared

with MK-801 ($p < 0.05$) (Fig. 4). No significant difference was observed in the total number of movements between galangin 50 mg/kg, haloperidol, and olanzapine ($p > 0.05$) (Fig. 4).

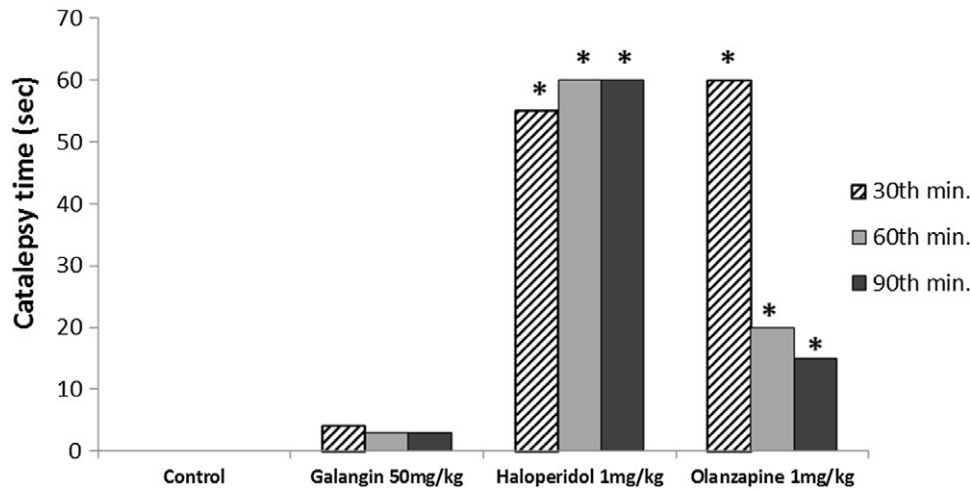


Fig. 5. The effects of galangin in catalepsy test in mice. The results are expressed as median. * $p < 0.05$ refers to a statistically significant difference compared with the galangin 50 mg/kg and control groups.

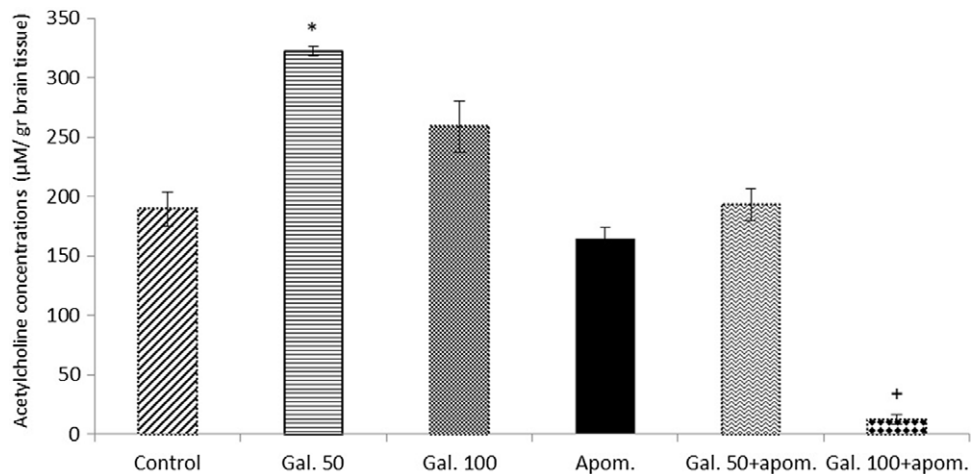


Fig. 6. The effects of galangin alone and in combination with apomorphine on brain acetylcholine concentrations. The results are expressed as mean \pm SEM. Apom., apomorphine; Gal. 50, galangin 50 mg/kg; Gal. 100, galangin 100 mg/kg. * $p < 0.001$ refers to a statistically significant difference compared with the control, Apom., Gal. 50+apom., Gal. 100+apom.; + $p < 0.001$ refers to a statistically significant difference compared with all groups. Acetylcholine concentrations were calculated as $\mu\text{M/g}$ brain tissue.

The effects of galangin on catalepsy test

Galangin 50 mg/kg did not significantly increase the catalepsy time compared with the control group at the 30th, 60th, and 90th min of the test ($p > 0.05$) (Fig. 5). On the other hand, haloperidol and olanzapine significantly increased the catalepsy time compared with both the galangin 50 mg/kg and control groups at the 30th, 60th, and 90th min of the test ($p < 0.05$) (Fig. 5). There was no significant difference between haloperidol and olanzapine in terms of catalepsy time at all time points in the test ($p > 0.05$) (Fig. 5).

The effects of galangin on brain acetylcholine concentrations

Galangin 50 mg/kg significantly increased brain acetylcholine concentrations compared with the control when administered alone ($p < 0.001$) (Fig. 6). An increase in the acetylcholine concentrations was also observed with galangin 100 mg/kg alone administration compared with the control; however, this increase was not statistically significant ($p > 0.05$) (Fig. 6). When galangin 50 and

100 mg/kg were administered in combination with apomorphine, a significant decrease in acetylcholine concentrations was observed compared with galangin 50 and 100 mg/kg alone ($p < 0.001$) (Fig. 6).

Discussion

In this study, we observed that galangin reversed apomorphine-induced PPI disruption in rats and it seemed that muscarinic or nicotinic acetylcholine receptors, and 5-HT_{1A} receptors were not involved in this effect. The reversal of apomorphine-induced PPI disruption by galangin was similar to that of the acetylcholinesterase inhibitor donepezil, typical antipsychotic haloperidol, and atypical antipsychotic olanzapine. Galangin increased brain acetylcholine concentrations when administered alone. On the other hand, the effect of galangin on brain acetylcholine concentrations was inhibited when galangin was administered with apomorphine. In addition, galangin reduced

apomorphine-induced climbing and MK-801-induced hyperlocomotion in mice, similar to haloperidol and olanzapine. Furthermore, galangin did not produce cataleptic behaviour, unlike haloperidol and olanzapine.

It has been suggested that the cholinergic system contributes to the pathophysiology of schizophrenia and some other neurological diseases (Erskine et al., 2019). In addition, the cholinergic system plays an essential role in the modulation of both normal cognitive processes and schizophrenia-associated cognitive deficits (Erskine et al., 2019). This led researchers to focus on the management of cognitive deficits by enhancing cholinergic activity. Hohnadel et al. studied the effects of the acetylcholinesterase inhibitors galantamine and donepezil, indicated in the treatment of Alzheimer's disease, on apomorphine, MK-801, and scopolamine-induced PPI disruption. They reported that galantamine reversed PPI disrupted by all three agents, whereas donepezil only reversed PPI disruption by apomorphine and scopolamine (Hohnadel et al., 2007). Koda et al. (2008) reported that the acetylcholinesterase inhibitors galantamine and donepezil (1 and 3 mg/kg) reversed apomorphine-induced PPI disruption. Ballmaier et al. (2002) reported that the acetylcholinesterase inhibitor rivastigmine reversed PPI disruption in rats with damaged cholinergic neurons. The results of these three studies are in line with our study outcomes. We also observed that galangin (50 and 100 mg/kg), which is reported to have acetylcholinesterase inhibitory activity, reversed apomorphine-induced PPI disruption in rats. We also compared the effects of galangin with donepezil as a reference anticholinesterase drug and observed that donepezil (1 mg/kg) reversed apomorphine-induced PPI disruption in rats and that this effect was comparable to galangin.

The PPI of acoustic startle reflex is a test that evaluates sensorimotor gating, which is disrupted in many neuropsychiatric disorders as well as schizophrenia (Sato, 2020). The cholinergic system, especially M_2 acetylcholine receptors, mediate functional PPI (Sato, 2020). Disruption of PPI refers to abnormal sensorimotor gating functioning, which reflects a cognitive deficit (Koukoulis et al., 2017). Apomorphine, a dopamine receptor agonist, is used to disrupt PPI in experimental schizophrenia models (Wang et al., 2019). In our study, galangin (both 50 and 100 mg/kg) alleviated apomorphine-induced PPI disruption. This suggested that galangin might possess antidopaminergic activity. Besides presenting various biological activities, galangin was also reported to have anticholinesterase activity (Guo et al., 2010). It has also been shown that the cholinergic and the dopaminergic systems interacted in the brain and contributed to the pathophysiology of neuropsychiatric disorders (Zhang et al., 2018). Extracellular acetylcholine levels are associated with dopamine release in the ventral tegmental area, and muscarinic and nicotinic receptors are involved in this process (Zhang et al., 2018).

It was reported that muscarinic receptor antagonists disrupted PPI (Ch'ng et al., 2020). Jones et al. reported that the muscarinic acetylcholine M_1/M_4 receptor agonist xanomeline improved apomorphine-induced PPI disruption in rats and suggested an interaction between cholinergic and dopaminergic systems in the regulation of PPI. They offered xanomeline as a promising agent in the treatment of cognitive dysfunctions in schizophrenia (Jones et al., 2005). In this study, we administered galangin in combination with the muscarinic receptor antagonist scopolamine (0.05 mg/kg, a dose that does not disrupt PPI) to investigate the role of muscarinic receptors in the reversal effect of galangin on apomorphine-induced PPI disruption and observed that there was no alteration in this effect. Accordingly, we suggest

that muscarinic receptors may not be involved in the effect of galangin.

In addition to muscarinic receptors, nicotinic acetylcholine receptors were also reported to mediate PPI and systemic nicotine administration was observed to enhance PPI (Pinnock et al., 2015). It was also reported that activation of nicotinic receptors alleviated the disrupted prepulse (Uslu et al., 2014). In this study, we administered galangin in combination with the nicotinic receptor antagonist mecamylamine to investigate the role of nicotinic receptors in the reversal effect of galangin on apomorphine-induced PPI disruption and observed that there was no alteration in this effect. Accordingly, we suggest that nicotinic receptors may not be involved in the effect of galangin.

5-HT_{1A} receptor agonists were reported to disrupt PPI, and WAY-100635, an antagonist at 5-HT_{1A} receptors, reversed this disruption (Conti, 2012; Tai et al., 2018). However, there are also contradictory results showing that 5-HT_{1A} receptor agonists enhance PPI (Dulawa & Geyer, 2000). In our study, we administered galangin in combination with the 5-HT_{1A} receptor antagonist WAY-100635 to investigate the role of 5-HT_{1A} receptors in the reversal effect of galangin on apomorphine-induced PPI disruption and observed that there was no alteration in this effect. Accordingly, we suggest that 5-HT_{1A} receptors may not be involved in the effect of galangin.

Functional disturbances of glutamate *N*-methyl-D-aspartate (NMDA) receptors are associated with the pathogenesis of schizophrenia (Uno & Coyle, 2019). In addition, it was suggested that cholinergic enhancement in the central nervous system alleviated the reduced NMDA receptor functions and improved cognitive functions (Jones et al., 2012). Furthermore, it was reported that nicotinic acetylcholine receptors and glutamate NMDA receptors were closely located on the nerves and there was a functional interaction between these two receptor types (Zappettini et al., 2014). MK-801 is a glutamate NMDA receptor antagonist that induces hyperactivity in rodents resembling the positive symptoms of schizophrenia (Fan et al., 2018). In this study, we used MK-801-induced hyperlocomotion test in mice as an experimental schizophrenia model and observed that galangin alleviated hyperlocomotion induced by MK-801. It seems that galangin may possess an activity through NMDA receptors.

We used the apomorphine-induced climbing test as one of the experimental schizophrenia models in the mice in our study. Apomorphine is a non-specific dopamine agonist that acts on dopamine D_1 and D_2 receptors and induces climbing behaviour in rodents (Sukhanov et al., 2014). We observed that galangin reduced apomorphine-induced climbing, reflecting an anti-psychotic-like effect. It has been suggested that positive symptoms of schizophrenia result from the overactivity of dopaminergic neurotransmission (Laruelle, 2014). The interaction between cholinergic and dopaminergic systems has been shown (Abudukeyoumu et al., 2019). It was reported that acetylcholine regulated dopaminergic neurotransmission via nicotinic acetylcholine receptors (6). In addition, a functional interconnection between muscarinic acetylcholine receptors and dopaminergic neurotransmission was also reported (Myslivecek, 2021). We may suggest that the effect of galangin on apomorphine-induced climbing may result from the interaction of dopaminergic and cholinergic mechanisms considering the reported anticholinesterase activity of galangin. However, in this study, we also observed that acetylcholine concentrations were not increased in rat brains when galangin and apomorphine were administered in combination. Therefore, it seems that galangin serves the

antipsychotic-like effect by directly inhibiting dopaminergic neurotransmission.

Antipsychotics are the main treatment options for schizophrenia; however, they are associated with extrapyramidal adverse effects (Yoshida & Takeuchi, 2021). These adverse effects are less common with the use of atypical antipsychotics (Chow et al., 2020). The extrapyramidal adverse effects of antipsychotics can be modelled in animals (Gobira et al., 2013). Catalepsy test is one of these models; catalepsy is defined as 'the inability of the animal to change its posture (Hoffman & Donovan, 1995). In this study, we observed that galangin did not induce cataleptic behaviour in the catalepsy test in mice. We may suggest that galangin does not present an extrapyramidal adverse effect. This effect was superior to the typical antipsychotic haloperidol and atypical antipsychotic olanzapine because both agents induced catalepsy in this study.

As a conclusion, we suggest that galangin may possess an antipsychotic-like effect considering its effects on apomorphine-induced PPI disruption in rats and experimental schizophrenia models in mice. These effects were comparable to the reference anticholinesterase donepezil and the antipsychotics haloperidol and olanzapine. Moreover, galangin may be advantageous because it did not induce catalepsy, unlike haloperidol and olanzapine. It seems that direct antidopaminergic actions, as well as the activity on glutamate NMDA receptors, may contribute to the effects of galangin rather than its anticholinesterase activity and effects on cholinergic mechanisms and 5-HT_{1A} receptors. Further studies are needed to elucidate detailed mechanisms of the antipsychotic-like effect of galangin, as discussed in the limitations section of this study.

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Conflict of Interest. None.

References

- Abudukeyoumu N, Hernandez-Flores T, Garcia-Munoz M and Arbuthnott GW (2019) Cholinergic modulation of striatal microcircuits. *European Journal of Neuroscience* **49**(5), 604–622.
- Ago Y, Takuma K and Matsuda T (2014) Potential role of serotonin_{1A} receptors in post-weaning social isolation-induced abnormal behaviors in rodents. *Journal of Pharmacological Sciences* **125**(3), 237–241.
- Akhtar M, Uma Devi P, Ali A, Pillai KK and Vohora D (2006) Antipsychotic-like profile of thioperamide, a selective H₃-receptor antagonist in mice. *Fundamental & Clinical Pharmacology* **20**(4), 373–378.
- Ballmaier M, Casamenti F, Scali C, Mazzoncini R, Zoli M, Pepeu G and Spano PF (2002) Rivastigmine antagonizes deficits in prepulse inhibition induced by selective immunolesioning of cholinergic neurons in nucleus basalis magnocellularis. *Neuroscience* **114**(1), 91–98.
- Bardgett ME, Boeckman R, Krochmal D, Fernando H, Ahrens R and Csernansky JG (2003) NMDA receptor blockade and hippocampal neuronal loss impair fear conditioning and position habit reversal in C57Bl/6 mice. *Brain Research Bulletin* **60**(1–2), 131–142.
- Brimblecombe KR, Threlfell S, Dautan D, Kosillo P, Mena-Segovia J and Cragg SJ (2018) Targeted activation of cholinergic interneurons accounts for the modulation of dopamine by striatal nicotinic receptors. *eNeuro* **5**(5), 0397–17.2018.
- Bora E, Veznedaroglu B and Kayahan B (2005) The effect of galantamine added to clozapine on cognition of five patients with schizophrenia. *Clinical Neuropharmacology* **28**(3), 139–141.
- Buchanan RW, Conley RR, Dickinson D, Ball MP, Feldman S, Gold JM and McMahon RP (2008) Galantamine for the treatment of cognitive impairments in people with schizophrenia. *American Journal of Psychiatry* **165**(1), 82–89.
- Canal CE, Morgan D, Felsing D, Kondabolu K, Rowland NE, Robertson KL, Sakhuja R and Booth RG (2014) A novel aminotetralin-type serotonin (5-HT) 2C receptor-specific agonist and 5-HT_{2A} competitive antagonist/5-HT_{2B} inverse agonist with preclinical efficacy for psychoses. *Journal of Pharmacology and Experimental Therapeutics* **349**(2), 310–318.
- Chang WL, Weber M, Breier MR, Saint Marie RL, Hines SR and Swerdlow NR (2012) Stereochemical and neuroanatomical selectivity of pramipexole effects on sensorimotor gating in rats. *Brain Research* **1437**, 69–76.
- Chen F, Tan YF, Li HL, Qin ZM, Cai HD, Lai WY, Zhang XP, Li YH, Guan WW, Li YB, Zhang JQ (2015) Differential systemic exposure to galangin after oral and intravenous administration to rats. *Chemistry Central Journal* **9**(1), 1152.
- Chien ST, Shi MD, Lee YC, Te CC and Shih YW (2015) Galangin, a novel dietary flavonoid, attenuates metastatic feature via PKC/ERK signaling pathway in TPA-treated liver cancer HepG2 cells. *Cancer Cell International* **15**, 15.
- Ch'ng SS, Walker AJ, McCarthy M, Le TK, Thomas N, Gibbons A, Udawela M, Kusljic S, Dean B, Gogos A (2020) The impact of removal of ovarian hormones on cholinergic muscarinic receptors: examining prepulse inhibition and receptor binding. *Brain Sciences* **10**(2), 106.
- Chow CL, Kadouh NK, Bostwick JR and VandenBerg AM (2020) Akathisia and newer second-generation antipsychotic drugs: a review of current evidence. *Pharmacotherapy* **40**(6), 565–574.
- Conti LH (2012) Interactions between corticotropin-releasing factor and the serotonin 1A receptor system on acoustic startle amplitude and prepulse inhibition of the startle response in two rat strains. *Neuropharmacology* **62**(1), 256–263.
- D'Souza MS and Markou A (2012) Schizophrenia and tobacco smoking comorbidity: nAChR agonists in the treatment of schizophrenia-associated cognitive deficits. *Neuropharmacology* **62**(3), 1564–1573.
- Dulawa SC and Geyer MA (2000) Effects of strain and serotonergic agents on prepulse inhibition and habituation in mice. *Neuropharmacology* **39**(11), 2170–2179.
- Dyer MA, Freudenreich O, Culhane MA, Pachas GN, Deckersbach T, Murphy E, Goff DC and Evins AE (2008) High-dose galantamine augmentation inferior to placebo on attention, inhibitory control and working memory performance in nonsmokers with schizophrenia. *Schizophrenia Research* **102**, 88–95.
- Erskine D, Taylor JP, Bakker G, Brown AJH, Tasker T and Nathan PJ (2019) Cholinergic muscarinic M₁ and M₄ receptors as therapeutic targets for cognitive, behavioural, and psychological symptoms in psychiatric and neurological disorders. *Drug Discovery Today* **24**(12), 2307–2314.
- Fan HR, Du WF, Zhu T, Wu YJ, Liu YM, Wang Q, Wang Q, Gu X, Shan X, Deng S, Zhu T, Xu TL, Ge WH, Li WG, Li F (2018) Quercetin reduces cortical GABAergic transmission and alleviates MK-801-induced hyperactivity. *EBioMedicine* **34**, 201–213.
- Fang D, Xiong Z, Xu J, Yin J and Luo R (2019) Chemopreventive mechanisms of galangin against hepatocellular carcinoma: a review. *Biomedicine & Pharmacotherapy* **109**, 2054–2061.
- Geyer MA, Swerdlow NR, Lehmann-Masten V, Teschendorf HJ, Traut M and Gross G (1999) Effects of LU-111995 in three models of disrupted prepulse inhibition in rats. *Journal of Pharmacology and Experimental Therapeutics* **290**(2), 716–724.
- Ghezzi L, Scarpini E and Galimberti D (2013) Disease-modifying drugs in Alzheimer's disease. *Drug Design, Development and Therapy* **7**, 1471–1478.

- Gobira PH, Ropke J, Aguiar DC, Crippa JA and Moreira FA** (2013) Animal models for predicting the efficacy and side effects of antipsychotic drugs. *Brazilian Journal of Psychiatry* 35(Suppl 2), S132–S139.
- Guo AJ, Xie HQ, Choi RC, Zheng KY, Bi CW, Xu SL, Dong TT and Tsim KW** (2010) Galangin, a flavonol derived from *Rhizoma Alpiniae officinarum*, inhibits acetylcholinesterase activity in vitro. *Chemico-Biological Interactions* 187(1–3), 246–248.
- Hoffman DC and Donovan H** (1995) Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. *Psychopharmacology (Berl)* 120(2), 128–133.
- Hohnadel E, Bouchard K and Terry AV Jr** (2007) Galantamine and donepezil attenuate pharmacologically induced deficits in prepulse inhibition in rats. *Neuropharmacology* 52(2), 542–551.
- Jin J, Cheng J, Lee KW, Amreen B, McCabe KA, Pitcher C, Liebmann T, Greengard P and Flajolet M** (2019) Cholinergic neurons of the medial septum are crucial for sensorimotor gating. *Journal of Neuroscience* 39(26), 5234–5242.
- Jones CK and Shannon HE** (2000) Effects of scopolamine in comparison with apomorphine and phencyclidine on prepulse inhibition in rats. *European Journal of Pharmacology* 391(1–2), 105–112.
- Jones CK, Eberle EL, Shaw DB, McKinzie DL and Shannon HE** (2005) Pharmacologic interactions between the muscarinic cholinergic and dopaminergic systems in the modulation of prepulse inhibition in rats. *Journal of Pharmacology and Experimental Therapeutics* 312(3), 1055–1063.
- Jung YC, Kim ME, Yoon JH, Park PR, Youn HY, Lee HW and Lee JS** (2014) Anti-inflammatory effects of galangin on lipopolysaccharide-activated macrophages via ERK and NF- κ B pathway regulation. *Immunopharmacology and Immunotoxicology* 36(6), 426–432.
- Jones CK, Byun N and Bubser M** (2012) Muscarinic and nicotinic acetylcholine receptor agonists and allosteric modulators for the treatment of schizophrenia. *Neuropsychopharmacology* 37(1), 16–42.
- Koukoulis F, Rooy M, Tziotis D, Sailor KA, O'Neill HC, Levenga J, Witte M, Nilges M, Changeux JP, Hoeffler CA, Stitzel JA, Gutkin BS, DiGregorio DA, Maskos U** (2017) Nicotine reverses hypofrontality in animal models of addiction and schizophrenia. *Nature Medicine* 23(3), 347–354.
- Khoja S, Asatryan L, Jakowec MW and Davies DL** (2019) Dopamine receptor blockade attenuates purinergic P2X4 receptor mediated prepulse inhibition deficits and underlying molecular mechanisms. *Frontiers in Cellular Neuroscience* 13, 331.
- Kinsey SG and Cole EC** (2013) Acute Δ (9)-tetrahydrocannabinol blocks gastric hemorrhages induced by the nonsteroidal anti-inflammatory drug diclofenac sodium in mice. *European Journal of Pharmacology* 715(1–3), 111–116.
- Koda K, Ago Y, Kawasaki T, Hashimoto H, Baba A and Matsuda T** (2008) Galantamine and donepezil differently affect isolation rearing induced deficits of prepulse inhibition in mice. *Psychopharmacology (Berl)* 196(2), 293–301.
- Koda K, Ago Y, Yano K, Nishimura M, Kobayashi H, Fukada A, Takuma K and Matsuda T** (2011) Involvement of decreased muscarinic receptor function in prepulse inhibition deficits in mice reared in social isolation. *British Journal of Pharmacology* 162(3), 763–772.
- Kohnomi S, Suemaru K, Goda M, Choshi T, Hibino S, Kawasaki H and Araki H** (2010) Ameliorating effects of tropisetron on dopaminergic disruption of prepulse inhibition via the α (7) nicotinic acetylcholine receptor in Wistar rats. *Brain Research* 1353(5), 152–158.
- Krebs-Thomson K, Ruiz EM, Masten V, Buell M and Geyer MA** (2006) The roles of 5-HT_{1A} and 5-HT₂ receptors in the effects of 5-MeO DMT on locomotor activity and prepulse inhibition in rats. *Psychopharmacology* 189(3), 319–329.
- Kumar PNS, Mohemmedali SP, Anish PK and Andrade C** (2017) Cognitive effects with rivastigmine augmentation of risperidone: a 12-month, randomized, double-blind, placebo-controlled study in schizophrenia. *Indian Journal of Psychiatry* 59(2), 219–224.
- Larrauri JA, Burke DA, Hall BJ and Levin ED** (2015) Role of nicotinic receptors in the lateral habenula in the attenuation of amphetamine-induced prepulse inhibition deficits of the acoustic startle response in rats. *Psychopharmacology (Berl)* 232(16), 3009–3017.
- Laruelle M** (2014) Schizophrenia: from dopaminergic to glutamatergic interventions. *Current Opinion in Pharmacology* 14, 97–102.
- Madireddy S and Madireddy S** (2020) Regulation of reactive oxygen species-mediated damage in the pathogenesis of schizophrenia. *Brain Sciences* 10(10), 742.
- Mahmood D, Khanam R, Pillai KK and Akhtar M** (2012) Protective effects of histamine H₃ receptor ligands in schizophrenic behaviors in experimental models. *Pharmacological Reports* 64(1), 191–204.
- Matsuda T** (2013) Neuropharmacologic studies on the brain serotonin1A receptor using the selective agonist osetozotan. *Biological and Pharmaceutical Bulletin* 36(12), 1871–1882.
- Myslivecek J** (2021) Two players in the field: hierarchical model of interaction between the dopamine and acetylcholine signaling systems in the striatum. *Biomedicines* 9(1), 25.
- Neves G, Antonio CB, Betti AH, Pranke MA, Fraga CA, Barreiro EJ, Noël F and Rates SM** (2013) New insights into pharmacological profile of LASSBio-579, a multi-target N-phenylpiperazine derivative active on animal models of schizophrenia. *Behavioural Brain Research* 237, 86–95.
- Pinnock F, Bosch D, Brown T, Simons N, Yeomans JR, DeOliveira C and Schmid S** (2015) Nicotine receptors mediating sensorimotor gating and its enhancement by systemic nicotine. *Frontiers in Behavioral Neuroscience* 9, 369.
- Pohanka M** (2014) Inhibitors of acetylcholinesterase and butyrylcholinesterase meet immunity. *International Journal of Molecular Science* 15(6), 9809–9825.
- Poje AB and Filion DL** (2017) The effects of multiphasic prepulses on automatic and attention-modulated prepulse inhibition. *Cognitive Processing* 18(3), 261–270.
- Rodvelt KR, Kracke GR, Schachtman TR and Miller DK** (2008) Ketamine induces hyperactivity in rats and hypersensitivity to nicotine in rat striatal slices. *Pharmacology Biochemistry and Behaviour* 91(1), 71–76.
- Santos B, González-Fraile E, Zabala A, Guillén V, Rueda JR and Ballesteros J** (2018) Cognitive improvement of acetylcholinesterase inhibitors in schizophrenia. *Journal of Psychopharmacology* 32(11), 1155–1166.
- Sato K** (2020) Why is prepulse inhibition disrupted in schizophrenia? *Medical Hypotheses* 143, 109901.
- Scarr E, Gibbons AS, Neo J, Udawela M and Dean B** (2013) Cholinergic connectivity: it's implications for psychiatric disorders. *Frontiers in Cellular Neuroscience* 7, 55.
- Singh M, Kaur M and Silakari O** (2014) Flavones: an important scaffold for medicinal chemistry. *European Journal of Medicinal Chemistry* 84, 206–239.
- Ślawińska A, Wierońska JM, Stachowicz K, Marciniak M, Lason-Tyburkiewicz M, Gruca P, Papp M, Kusek M, Tokarski K, Doller D, Pilc A** (2013) The antipsychotic-like effects of positive allosteric modulators of metabotropic glutamate mGlu4 receptors in rodents. *British Journal of Pharmacology* 169(8), 1824–1839.
- Sukhanov I, Espinoza S, Yakovlev DS, Hoener MC, Sotnikova TD and Gainetdinov RR** (2014) TAAR1-dependent effects of apomorphine in mice. *International Journal of Neuropsychopharmacology* 17(10), 1683–1693.
- Tai YM, Ko CY, Lin CC, Wan YY, Chung JY and Liu YP** (2018) Effects of 5HT_{1A} activation on gating profile following 5HT depletion in rats lacking social attachment since weaning. *Psychiatry Investigation* 15(2), 193–199.
- Uno Y and Coyle JT** (2019) Glutamate hypothesis in schizophrenia. *Psychiatry and Clinical Neurosciences* 73(5), 204–215.
- Uslu G, Savci V, Buyukuysal LR and Goktalay G** (2014) CDP-choline attenuates scopolamine induced disruption of prepulse inhibition in rats: involvement of central nicotinic mechanism. *Neuroscience Letters* 569, 153–157.
- Wadenberg MG, Manetti D, Romanelli MN and Arias HR** (2017) Significance of the nicotinic α 7 receptor in cognition and antipsychotic-like behavior in the rat. *Behavioural Brain Research* 333, 129–134.
- Wang L, Zhang Y, Wang C, Zhang X, Wang Z, Liang X, Alachkar A and Civelli O** (2019) A natural product with high affinity to sigma and 5-HT₇ receptors as novel therapeutic drug for negative and cognitive symptoms of schizophrenia. *Neurochemical Research* 44(11), 2536–2545.

- Yoshida K and Takeuchi H** (2021) Dose-dependent effects of antipsychotics on efficacy and adverse effects in schizophrenia. *Behavioural Brain Research* **402**, 113098.
- Zanoaga O, Braicu C, Jurj A, Rusu A, Buiga R and Berindan-Neagoe I** (2019) Progress in research on the role of flavonoids in lung cancer. *International Journal of Molecular Sciences* **20**(17), 4291.
- Zappettini S, Grilli M, Olivero G, Chen J, Padolecchia C, Pittaluga A, Tomé AR, Cunha RA and Marchi M** (2014) Nicotinic $\alpha 7$ receptor activation selectively potentiates the function of NMDA receptors in glutamatergic terminals of the nucleus accumbens. *Frontiers in Cellular Neuroscience* **8**, 332.
- Zhang C, Liu X, Zhou P, Zhang J, He W and Yuan TF** (2018) Cholinergic tone in ventral tegmental area: functional organization and behavioral implications. *Neurochemistry International* **114**, 127–133.
- Zhang W, Yamada M, Gomeza J, Basile AS and Wess J** (2002) Multiple muscarinic acetylcholine receptor subtypes modulate striatal dopamine release, as studied with M1-M5 muscarinic receptor knock-out mice. *Journal of Neuroscience* **22**(15), 6347–6352.