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Jobelyn® pretreatment ameliorates symptoms of psychosis in experimental models

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Abstract

Background: Psychosis is a chronic neurological disorder and it remains a major medical and social problem in most African countries. Individuals with psychotic illness in this region tend to seek help from traditional medical practitioners, who prescribe herbal remedies as alternative forms of treatment for the disease. Jobelyn® (JB) is a commercial polyherbal formulation that has been acclaimed to show beneficial effects in neurological disorders. However, its usefulness in psychosis has not been scientifically validated. Thus, this study was undertaken to evaluate its effects on animal models predictive of human psychosis.
Methods: Antipsychotic activity of JB was assessed based on the inhibition of stereotyped behavior induced by amphetamine or apomorphine in mice. Amphetamine-induced hyperactivity and lethality in aggregated mice were additional tests employed to further evaluate the antipsychotic property of JB. The effect of JB on catalepsy was also assessed, using the inclined plane paradigm.
Results: JB (5–50 mg/kg, p.o.) significantly ($p < 0.05$) inhibited stereotypy induced by amphetamine (10.0 mg/kg, i.p.) or apomorphine (1 mg/kg, i.p.), which suggests antipsychotic activity. Furthermore, JB (5–50 mg/kg, p.o.) reduced lethality in aggregated mice and inhibited hyperactivity induced by amphetamine, respectively. However, JB (5–50 mg/kg, p.o.) did not cause cataleptic behavior, as it failed to alter the duration of stay of the animals on the inclined plane.
Conclusions: Taken together, these findings suggest that JB exhibits antipsychotic-like activity, devoid of the adverse effect of cataleptic behavior, and may offer some beneficial effects in the symptomatic relief of psychotic ailments.

Keywords: antipsychotic; hyperactivity; Jobelyn®; lethality; stereotypy.

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Introduction

Psychosis is a mental disorder characterized by multiple symptoms affecting thought, perceptions, emotion, and volition and generally impairs the quality of life of the patients [1]. Psychotic manifestations are on the increase as a neurological disease in most African countries including Nigeria and remain a major medical and social problem globally. Although hyperdopaminergic activity is generally believed to play a crucial role in the symptomatology of the disease, most African people believe that the disease is due to supernatural forces at work against the mental well-being of individuals and as such cure cannot be found through modern medicine or orthodox medical practice [1–3]. Thus, patients with the disease or their relatives tend to have more confidence in traditional healers than their orthodox medical counterparts [2, 3]. The scarcity of accessible and high quality mental health-care services in most African countries also contributes to the increased patronage of traditional healers by patients with psychotic disorders in most African settings [2–4].

Plant has offered mankind the first medicine, reserpine, clinically used for the treatment of psychotic disorders before the advent of chlorpromazine [5]. Currently, especially in developing countries such as Nigeria, most patients with psychosis still depend on medicinal plants for the treatment of the disease [6]. The rich and diverse plant sources of Nigeria offer traditional healers a wide range of effective herbal remedies for the therapy of psychosis and a number of these plants or their preparations have been evaluated and confirmed to have antipsychotic effects in animal models [6, 7]. One of the best approaches in the search for new medicine from plant sources is the selection of plants based on ethnomedicinal leads.

Jobelyn® (JB), a unique polyherbal preparation that has won international recognition as being antianemic, an immune booster, and an energizer, is widely used by

natives of Western Nigeria for the treatment of neurological disorders, especially epilepsy in children [8, 9]. JB is one of the fastest selling herbal medicines in Nigeria and is available as capsules and suspensions for the treatment of anemia and rheumatoid arthritis [10]. It is also used to combat stress and to promote general well-being [9, 10]. Each capsule of JB consists of 250 mg and the recommended dose by the manufacturer is one or two capsules (1–3 times daily) depending on the ailment.

JB is rich in several phytochemicals including proanthocyanidins, anthocyanidins, apigenidins, proapigenidins, apigenins, luteolins, naringenins, flavonoids, and polyphenols [8, 10]. Most of these compounds are present in *Sorghum bicolor* plant and these phytochemicals have been found to exhibit a wide range of biological activities [11, 12]. JB also contained other phytochemicals obtained from *Parquetina nigrescens* (Periplocaceae) and *Harungana madagascariensis* (Clusiaceae). In particular, apigenin, luteolin, and naringenin have been found to exhibit neuroprotection and to reduce neuroinflammation, which indicate their therapeutic efficacy in central nervous system (CNS) disorders [11, 12]. Although previous studies have confirmed the antianemic effect of JB [8, 10], no studies have shown its usefulness in CNS disorders. In this study, we present the results of the psychopharmacological evaluation of antipsychotic activity of JB in animal models predictive of human psychosis.

Materials and methods

Experimental animals

Male albino Swiss mice (19–21 g) were obtained from the Central Animal House, University of Ibadan. The animals were housed in plastic cages at room temperature with a 12:12 h light/dark cycle. They had free access to commercial food pellets and water ad libitum. They were acclimatized for at least 1 week before use. All procedures in this study were performed in compliance with the World Medical Association Declaration of Helsinki regarding ethical conduct of research involving the Care and Use of Laboratory Animals [13].

Drugs and chemicals

JB (Health Forever Ltd., Lagos, Nigeria), amphetamine (AMP; Sigma-Aldrich, St. Louis, MO, USA), apomorphine (APO; Sigma-Aldrich), and haloperidol (HP; Sigma-Aldrich) were employed in this study. JB and other drugs were dissolved in distilled water just prior to the start of the experiments. Doses of 5, 10, and 50 mg/kg JB used in the study were selected based on results obtained from preliminary investigations.

Experimental paradigms

Effect of JB on APO-induced stereotypy

APO-induced stereotyped behavior was employed in this study as the animal paradigm predictive of human psychosis as previously described by Bourin et al. [14]. The animals were divided into five treatment groups (n=6/group). The first three groups were pretreated with JB (5, 10, or 50 mg/kg, p.o.), whereas the fourth and fifth groups received HP (1 mg/kg, p.o.) and distilled water (10 mL/kg, p.o.), respectively. Sixty minutes later, each animal received intraperitoneal (i.p.) injection of APO (1 mg/kg) and was placed immediately in a transparent observation chamber (20 cm×20 cm×23 cm). Thereafter, stereotype behaviors were observed for 2 min at 10, 15, 30, 45, and 60 min after APO injection. Stereotype behaviors were scored as: 0=absence of stereotype behavior; 1=presence of stereotype movements of the head; 2=intermittent sniffing; 3=chewing; 4=intense licking.

Effect of JB on AMP-induced stereotypy

AMP-induced stereotyped behavior, a widely used animal model for psychosis, was further employed to screen for the antipsychotic effect of JB in this study. Mice (n=6) were given JB (5, 10, or 50 mg/kg, p.o.), HP (1 mg/kg, p.o.), or distilled water (10 mL/kg, p.o.) for 60 min before induction of stereotyped behaviors with i.p. injection of AMP (10 mg/kg). Each mouse was observed for stereotype behaviors in a transparent chamber (20 cm×20 cm×23 cm) for 2 min at time 10, 15, 30, 45, and 60 min, respectively. Stereotype behaviors were scored as described above [14].

Antagonism of AMP-induced hyperactivity

The performance of JB in the open field test was employed to screen for its effect on AMP-induced hyperactivity in mice. Mice (n=6/group) were given JB (5, 10, or 50 mg/kg), HP (1 mg/kg), or distilled water (10 mL/kg) orally. Sixty minutes later, the animals received i.p. injection of AMP (1 mg/kg) and were placed individually at the center of an open field chamber (35 cm×30 cm×23 cm). The duration of ambulation (s) and number of line crosses were recorded for 5 min using a digital camera [15].

Effect of JB on AMP-induced lethality test

Protection against AMP-induced lethality in grouped mice is a valid animal model predictive of antipsychotic property of drugs [14]. In this test, AMP is known to cause death in aggregated animals within 24 h or 48 h. The animals (10 mice per group) were treated with JB (5, 10, or 50 mg/kg, p.o.), HP (1 mg/kg, p.o.), or distilled water (10 mL/kg, p.o.) 60 min before administration of APM (10 mg/kg, i.p.). Mice in each group were placed immediately in a transparent cage (20 cm×20 cm×23 cm) and number of death in each group was recorded 24 h later.

Assessment of cataleptic behavior

The cataleptic effect of JB was investigated according to the modified version previously described by Costall and Naylor [16]. Mice (n=6/group) were pretreated orally with JB (5, 10, or 50 mg/kg), HP (1 mg/kg), or distilled water (10 mL/kg) 60 min before testing for catalepsy. The test was done by gently placing the fore limbs of each animal on a horizontal plane wood surface (H=6 cm; W=4 cm; L=16 cm) and the duration of akinesia (period of time the animal remained in one position, before initiating any active movement) in seconds was recorded.
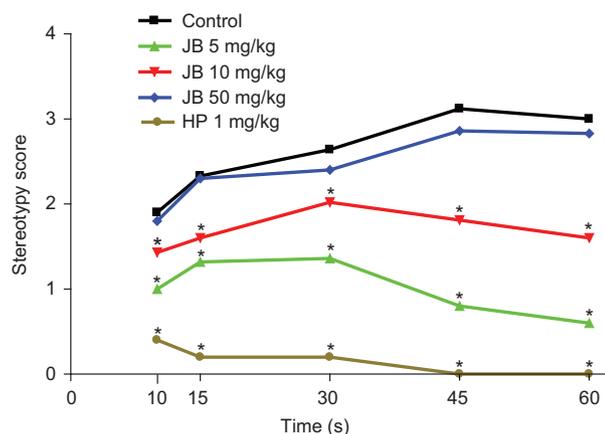
Statistical analysis

The data in this study were analyzed using Primer of Biostatistics version 3.01 (Glantz, New York, NY, USA) and Graph Pad Prism software version 4 (JR Miller, San Diego, CA, USA). Statistical analysis of data was done by the Kruskal-Wallis test and one-way analysis of variance (ANOVA), followed by the Dunnett post-hoc test where appropriate. A level of $p<0.05$ was considered as statistically significant.

Figure 2 Effects of Jobelyn® on amphetamine-induced stereotyped behaviors in mice.
Values represent the mean of six animals/group. *$p<0.05$ compared with the control group (Kruskal-Wallis test).

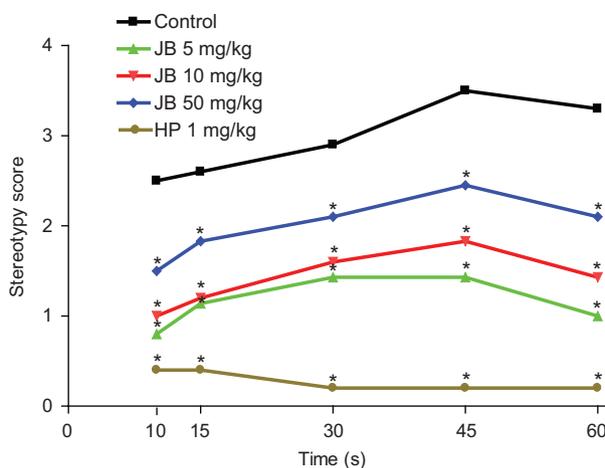inhibitory activity against stereotypy when compared with JB (Figures 1 and 2).

Results

Anti-stereotypic effect of JB

Intraperitoneal injection of APO (1 mg/kg) or AMP (10 mg/kg) produced stereotyped behaviors characterized by head movements, persistent sniffing, chewing, and intense licking in mice. As shown in Figures 1 and 2, JB (5 or 10 mg/kg, p.o.) significantly ($p<0.05$) inhibited these behavioral deficits, suggesting antipsychotic activity. However, HP (1 mg/kg, p.o.) demonstrated greater

Effects of Jobelyn® on amphetamine-induced hyperactivity

Table 1 shows the effect of JB on AMP-induced hyperactivity as assessed by the open field test in mice. One-way ANOVA revealed that there were significant differences between treatment groups: total number of lines crossed [$F_{(4,20)}=296.10$, $p=0.702$] and duration of ambulation [$F_{(4,20)}=204.4$, $p=0.0114$]. Post-hoc analysis by the Dunnett test revealed that JB (5, 10, and 50 mg/kg, p.o.) produced a significant ($p<0.05$) reduction in hyperactivity as shown by a decrease in the number of line crosses and duration of ambulation(s) induced by AMP (1 mg/kg, i.p.). However, HP (1 mg/kg, p.o.) demonstrated a greater inhibitory effect than JB against hyperlocomotion produced by AMP (Table 1).

Figure 1 Effects of Jobelyn® on apomorphine-induced stereotypy paradigm in mice.
Values represent the mean of six animals/group. *$p<0.05$ compared with the control group (distilled water) (Kruskal-Wallis test).

Table 1 Effects of Jobelyn® on amphetamine-induced hyperactivity in mice.

Treatment	Dose, mg/kg	Number of lines crossed	Duration of ambulation, s
Control	–	240.20±5.95	277.80±3.51
JB	5	143.60±4.74[a]	168.60±2.09[a]
JB	10	172.40±4.06[a]	215.20±11.84[a]
JB	50	205.00±5.20[a]	242.60±6.71[a]
HP	1	29.80±2.76[a]	37.00±3.45[a]

Each value represents the mean±SEM of six animals/group. [a]$p<0.05$ compared with the control group (ANOVA followed by the Dunnett post-hoc test).

Effect of JB on AMP-induced lethality in grouped mice

As shown in Figure 3, AMP (20 mg/kg, i.p.) produced 100% death in aggregated mice 24 h post-administration. JB (5 and 10 mg/kg, p.o.) was found to exhibit significant (p<0.05) protection against the lethal effect of AMP (20 mg/kg, i.p.) in the grouped mice. The protective effect shown by JB (5 mg/kg, p.o.) was similar to that produced by HP (1 mg/kg, p.o.), a standard antipsychotic drug (Figure 3).

Discussion

The behavioral studies using antagonism of stereotypy, hyperlocomotion, and lethality in aggregated animals induced by dopaminergic agents have traditionally been used to detect antipsychotic activity of novel agents [17, 18]. Stereotyped behavior as a prominent symptom observed in patients with psychosis is characterized by performance of strange gestures in a purposeless fashion and it is susceptible to the blocking effect of antipsychotic drugs [18]. In this study, JB was found to demonstrate significant inhibitory activity against stereotypic behavior induced by APO or AMP, suggesting antipsychotic activity. Hyperactivity produced by AMP is closely related to psychotic agitation seen in patients with this mental abnormality [19]. The findings that JB inhibited AMP-induced hyperlocomotion activity further indicate its antipsychotic activity.

The antipsychotic effect of JB was also revealed by the antagonism of AMP-induced lethality in grouped mice, a model that had served in the routine screening for compounds with neuroleptic activity [20, 21]. Antipsychotics

Figure 3 Effect of Jobelyn® on amphetamine-induced aggregate toxicity test in mice.
Values represent the mean±SEM of six animals/group. **p<0.001, *p<0.05 compared with the control group (ANOVA followed by the Dunnett post-hoc test).

have been found to specifically antagonize grouped AMP toxicity at dose levels which had little effect on the toxicity of AMP to isolated mice [21, 22]. Protection against the lethal effect of AMP to grouped mice has therefore been considered as a suitable test for assessing substances with antipsychotic effects [14, 21, 22]. Although it has been shown that the LD_{50} of AMP to grouped mice is dependent both on environmental temperature and degree of aggregation of mice, the underlying cause for greater toxicity of AMP to grouped mice is not yet fully understood [23]. However, it has been suggested that the major events leading to death in grouped mice, given AMP, is closely connected with marked hyperactivity, followed by a phase of exhaustion in those mice which died [24]. Previous studies have shown that a significant association exists in grouped mice between increased lethality and increased motor activity [25]. However, Burn and Hobbs [21] reported that increased excitement may be the critical factor in enhanced lethality of AMP to grouped animals. Hyperactivity or agitation and displays of aggressive behaviors are integral components of psychomotor excitement commonly seen in most patients with psychotic disorders [1, 26]. Patients in the state of psychomotor excitement constitute sources of danger to themselves and others, thus requiring the use of drugs with a tranquillizing effect such as antipsychotics [1, 26]. The finding that increased dopamine brain levels is the major neurochemical pathway involved in the mediation of the lethal effect of AMP in grouped mice further support its relevance as an animal model predictive of antipsychotic activity [20].

Studies have shown that stereotypies induced by high doses of AMP or APO are mediated through the stimulation of dopamine D_2 receptors in the striatum [18, 27]. However, whereas APO mediates its action through direct activation of dopamine receptors in the striatal region of the brain, AMP, by contrast, acts indirectly through the liberation of dopamine from dopaminergic neurons [18]. The neurochemical mechanism underlying AMP-induced hyperlocomotion or hyperactivity at moderate doses is closely connected with the activation of dopamine D_2 receptors located in the limbic region of the brain [18]. Thus, preferential blockade of D_2 receptors in the limbic system will confer antipsychotic effects with little or no propensity to cause extrapyramidal symptoms (EPSs) [18, 28]. EPSs are thought to result from decreased dopamine activity in the striatum, a preferential action of a novel agent against AMP-induced hyperactivity might serve as an earlier indicator of a lower propensity to induce EPSs in patients [18]. Although first-generation or typical antipsychotics such as HP inhibit AMP-induced hyperactivity and stereotypies, the atypical counterparts
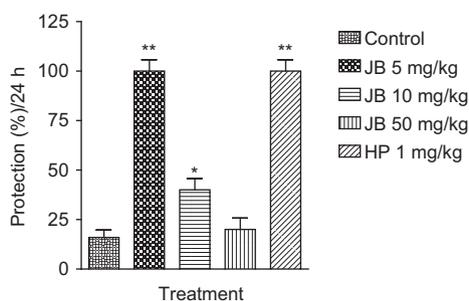
such as clozapine are known to be less effective against stereotypies [28–30]. These observations further confirm the notion that the atypical antipsychotics show preferential action on D_2 receptors in the limbic system [28]. Thus, the findings that JB inhibits both AMP-induced hyperlocomotion and stereotypy suggest an action that may resemble those of HP-like antipsychotic drugs. However, the test for catalepsy revealed that JB did not impair sensorimotor performance of the animals, suggesting an action that differs from HP-like antipsychotic drugs. The catalepsy test is a paradigm established in rodents that reveals the tendency of antipsychotics such as HP and chlorpromazine to induce EPSs based on prolongation of the duration of akinesia [31–33].

Although the mechanism responsible for the antipsychotic effect of JB needs further investigation, the present data suggest that JB contains phytochemical constituents with antidopaminergic activity. The major finding of interest in this study was that the most effective antipsychotic dose of JB is within the same range recommended by the manufacturer [8]. Previous studies have shown that JB contained several phytochemicals that readily cross the blood–brain barrier (BBB) to exert various CNS activities [11, 12]. These phytochemicals, in particular apigenidins, apigenins, luteolins, and naringenins, readily penetrate the BBB due to their high lipophilicity to exert various pharmacological activities including anti-amnesia, anti-depressant, anti-neuroinflammation, and neuroprotection [33]. However, it is yet to be determined which are the primary components responsible for the antipsychotic activity of JB.

Conclusion

This study shows that JB demonstrated antipsychotic activity devoid of the cataleptic effect of traditional neuroleptic agents and this may be suggestive of a preferential antagonism of D_2-mediated dopaminergic activity.

Conflict of interest statement

References

1. Davis KL, Kahn RS, Grant KO, Davidson M. Dopamine in schizophrenia: a new review and conceptualization. Am J Psychiatry 1991;148:1474–86.
2. Ewhrudjakpor C. Conceptualizing Africans' perception of disease as distinct from Euro-America practice. J Soc Policy Issues 2007;4:8–11.
3. Adebowale TO, Ogunlesi AO. Beliefs and knowledge about aetiology of mental illness among Nigerian psychiatric patients and their relatives. Afr J Med Med Sci 1999;28:35–41.
4. Kabir M, Iliyasu Z, Abubakar IS, Aliyu MH. Perception and beliefs about mental illness among adults in Karfi village, northern Nigeria. BMC Int Health Hum Rights 2004;4:3–7.
5. Bleuler M, Stoll WA. Clinical use of reserpine in psychiatry: comparison with chlorpromazine. Ann NY Acad Sci 1955; 61:167–73.
6. Sonibare MA, Soladoye MO, Subuloye TA. Ethnobotanical survey of anti-psychotic plants in Lagos and Ogun states of Nigeria. Eur J Sci Res 2008;19:634–44.
7. Sonibare MA, Umukoro S, Shonibare ET. Antipsychotic property of aqueous and ethanolic extracts of Lonchocarpus cyanescens (Schumach and Thonn.) Benth. (Fabaceae) in rodents. J Nat Med 2012;66:127–32.
8. Okochi VI, Okpuzor J, Okubena MO, Awoyemi AK. The influence of African herbal formula on the haematological parameters of trypanosome infected rats. Afr J Biotechnol 2003;2:312–6.
9. Oshikoya KA, Senbanjo IO, Njokanma OF, Soipe A. Use of complementary and alternative medicines for children with chronic health conditions in Lagos, Nigeria. BMC Complement Altern Med 2008;8:66.
10. Erah PO, Asonye CC, Okhamafe AO. Response of Trypanosoma brucei brucei-induced anaemia to a commercial herbal preparation. Afr J Biotechnol 2003;2:307–11.
11. Awika JM, Rooney LW. Sorghum phytochemicals and their potential impact on human health. Phytochemistry 2004;65:1199–221.
12. Heo HJ, Kim M, Lee J, Choi S, Cho H, Hong B, et al. Naringenin from Citrus junos has an inhibitory effect on acetylcholinesterase and a mitigating effect on amnesia. Dement Geratr Cogn Disord 2004;17:151–7.
13. American Physiological Society. Guiding principles for research involving animals and human beings. Comp Physiol 2002;283:R281–3.
14. Bourin M, Poisson L, Larousse C. Piracetam interaction with neuroleptics in psychopharmacological tests. Neuropsychobiology 1986;19:93–6.
15. Brown RE, Corey SC, Moore AK. Differences in measures of exploration and fear in MHC-congenic C578L/61 and B6-H-2K mice. Behav Genet 1999;26:263–71.
16. Costall B, Naylor RJ. On catalepsy and catatonia and the predictability of the cataleptic test for neuroleptics activity. Psychopharmacology (Berlin) 1974;34:233–41.

17. Janssen PA, Niemegeers CJ, Smellekens KH. Is it possible to predict the clinical effects of neuroleptic drugs (major tranquilizers) from animal data? Drug Res 1965;15:56–69.

18. Porsolt RD, Moser PG, Castagné V. Behavioral indices in antipsychotic drug discovery. J Pharmacol Exp Therap 2010;333:632–8.

19. Swerdlow NR, Braff DL, Taaid N, Geyer MA. Assessing the validity of an animal model of deficient sensorimotor gating in schizophrenic patients. Arch Gen Psychiatry 1994;51:139–54.

20. Duterte-Boucher D, Duhamel F, Costentin J. Dopaminergic transmission and (+)amphetamine-induced lethality in aggregated mice. Fundam Clin Pharmacol 1992;6:21–7.

21. Burn JH, Hobbs R. A test for tranquillizing drugs. Arch Int Pharmacodyn 1958;113:290–5.

22. Lasagna L, McCann WP. Effect of tranquilizing drugs on amphetamine toxicity in aggregated mice. Science 1957;125:1241–2.

23. Hogn R, Lasagna L. Effects of aggregation and temperature on amphetamine toxicity in mice. Psychopharmacologia 1960;1:210–20.

24. Askew BM. Hyperpyrexia as a contributory factor in the toxicity of amphetamine to aggregated mice. Br J Pharmacol 1962;19:245–57.

25. Greenblat TE, Osterberg AC. Correlations of activating and lethal effects of excitatory drugs in grouped and isolated mice. J Pharmacol Exp Ther 1961;131:115–9.

26. Mullen PE. Schizophrenia and violence: from correlations to preventive strategies. Adv Psychiatric Treat 2006;12:239–48.

27. Sams-Dodd F. A test of the predictive validity of animal models of schizophrenia based on phencyclidine and D-amphetamine. Neuropsychopharmacology 1998;18:293–304.

28. Leite JV, Guimarães FS, Moreira FA. Aripiprazole, an atypical antipsychotic, prevents the motor hyperactivity induced by psychotomimetics and psychostimulants in mice. Eur J Pharmacol 2008;578:222–7.

29. O'Neill MF, Shaw G. Comparison of dopamine receptor antagonists on hyperlocomotion induced by cocaine, amphetamine, MK-801 and the dopamine D1 agonist C-APB in mice. Psychopharmacology (Berlin) 1999;145:237–50.

30. Castagné V, Moser PC, Porsolt RD. Preclinical behavioral models for predicting antipsychotic activity. Adv Pharmacol 2009;57:381–418.

31. Geyer MA, Ellenbroek B. Animal behavior models of the mechanisms underlying antipsychotic atypicality. Prog Neuropsychopharmacol Biol Psychiatry 2003;27:1071–9.

32. Hoffman DC, Donovan H. Catalepsy as a rodent model for detecting antipsychotic drugs with extrapyramidal side effect liability. Psychopharmacology (Berlin) 1995;120:128–33.

33. Liu R, Gao M, Qiang GF, Zhang TT, Lan X, Ying J, et al. The anti-amnesic effects of luteolin against amyloid β_{25-35} peptide-induced toxicity in mice involve the protection of neurovascular unit. Neuroscience 2009;162:1232–43.