Original Research Article

Crude and Partition Extracts of *Newbouldia laevis* Leaves Attenuate Excitotoxin-induced Stereotypy in Mice

ABSTRACT

Aims: This study was designed to investigate the effects of crude ethanol and partition extracts of *Newbouldia laevis* leaves on excitotoxin-induced stereotypy in mice.
Place and Duration of Study: The study was carried out in the Laboratory of the Department of Pharmacology and Therapeutics, College of Health Sciences, Ladoke Akintola University of Technology, Ogbomoso, Nigeria, between July and October, 2020.
Methodology: Following pretreatment of mice with graded doses (150 - 600 mg/kg b.w) of crude extract (NLE) and partition fractions [n-butanol partition fraction (BPE), n-hexane partition fraction (HPE) and ethylacetate partition fraction (EAPE)] of *Newbouldia laevis* leaves, stereotypy was induced by the administration of methamphetamine (35 mg/kg b.w) and apomorphine (5mg/kg b.w.), and stereotypy scores were recorded thereafter. The effects of BPE and NLE on catalepsy were also determined. Statistical significance was taken at $P<0.05$
Results: In both apomorphine and methamphetamine models, stereotyped behavior was significantly decreased ($P<0.05$) in the treated mice compared to the control. The degree of protection offered by *Newbouldia laevis* extracts against excitotoxin-induced stereotypy was in the order: BPE>NLE>EAPE>HPE. In the catalepsy test, BPE (600 mg/kg b.w) significantly potentiated haloperidol-induced catalepsy compared to control ($P<0.05$). Likewise, NLE significantly increased catalepsy compared to control ($P<0.05$).
Conclusion: Findings from this study indicate that crude and partition extracts of *Newbouldia laevis* leaves attenuated stereotypy in methamphetamine and apomorphine models, and thus could be effective remedy for schizophrenia-like psychosis.

Keywords: stereotypy, psychosis, excitotoxins, *Newbouldia laevis*, catalepsy

1. INTRODUCTION

The term “psychosis” denotes a variety of mental disorders. One of the mental disorders included in psychosis is schizophrenia. Symptoms of schizophrenia include delusions, incoherence, hallucinations, affective disturbances, catatonia, and disorganized behavior. It is not necessary and is unlikely that one person exhibits all these symptoms. The hallucinations in schizophrenia are characteristically auditory, usually experienced as hearing one or more voices. Incoherence is a marked loosening of associations in which thinking becomes highly illogical. Common affective disturbances include flat affect, extreme ambivalence, or affective incongruity. Catatonia is a state of elevated muscle tone and rigid posture [1]. Schizophrenia is an episodic condition. Some individuals experience recurrent

acute episodes (commonly referred to as nervous breakdown) separated by periods of more or less normal activity.

In spite of many drugs available for this disorder, a sizable number of schizophrenic patients have committed suicide [2]. Risk factors that were identified for suicide include drug abuse, depression, and previous suicide attempt. Negative symptoms experienced by schizophrenic patients include anhedonia and anergia. These symptoms undoubtedly result in low quality of life [3]. It has been widely reported that antipsychotic drugs cause weight gain and thus put schizophrenic patients at greater risk of associated health problems, including osteoarthritis, stroke, and certain cancers. Olanzapine and clozapine are among the neuroleptic drugs with this side effect [4]. Antipsychotic drugs are known to be associated with dyslipidemia and diabetes. Left unattended to, these disorders may ultimately lead to cardiovascular and renal problems [5]. Antipsychotic drugs are also known for their extrapyramidal side effects. This may contribute to noncompliance and have negative impact on the patient [6]. Stigmatization, lack of motivation, and social withdrawal are other factors that reduce quality of life and self esteem in schizophrenia [7].

Since antipsychotic drugs presently used in schizophrenia have these side effects, there is need to intensify efforts towards discovering new agents that are free of these drawbacks. In addition to their side effects, conventional neuroleptics are not easily accessible to many patients in rural areas. The high cost of these drugs also makes them unaffordable by many patients [8]. As a result, many patients in developing countries use alternative medicines, particularly herbal preparations to treat neurological and neuropsychiatric disorders, including psychosis.

Newbouldia laevis is one of the medicinal plants employed for the treatment of psychosis, convulsions, diabetes and other diseases in many West African countries, including Nigeria, Republic of Benin, and Ghana [9]. *Newbouldia laevis* is an angiosperm of medium size, and belongs to the family *Bignoniaceae*. Its common names are ‘African Border Tree’ and ‘Fertility Tree’. In spite of the widespread use of the leaves of *Newbouldia laevis* as herbal remedy in psychosis, its efficacy as antipsychotic agent has not been scientifically validated. To fill this gap, the effects of crude and partition extracts of *Newbouldia laevis* leaves on excitotoxin-induced stereotypy were investigated in this study.

2. METHODOLOGY

2.1 Preparation of crude extract and fractions

Leaves of *Newbouldia laevis* was pulverized and extracted in 80% ethanol using a Soxhlet apparatus and concentrated by a rotary evaporator (Heidolph-Rotacool, Germany) to give the crude extract (NLE). Then, solvent-solvent partition was carried out with *n*-hexane, *n*-butanol, and ethyl acetate to give *n*-hexane (HPE), *n*-butanol (BPE) and ethyl acetate (EAPE) fractions respectively.

2.2 Experimental animals

Male Swiss albino mice (20-25 g) were obtained from the Animal Holding Unit of the Department of Pharmacology and Therapeutics, Ladoke Akintola University of Technology (LAUTECH). The animals were housed in polypropylene cages inside a well-ventilated room. A maximum of six animals were kept in one cage. The animals were maintained under standard laboratory conditions of temperature (22 ± 2°C), relative humidity (55-65%) and 12 hour light/dark cycle. During the study period, animals were fed with standard commercial pellet diet and potable tap water *ad libitum*. All experimental procedures and protocols were as outlined in the "Guide for the Care and Use of Laboratory Animals" published by the National Research Council [10]. All procedures were carried out as approved by the Ladoke Akintola University of Technology, Ogbomoso, Nigeria in line with the provisions for animal care and use.

2.3 Excitotoxin-induced stereotypy

Mice that had fasted for 12 hours were divided into 5 groups of six animals each. The groups were treated as follows: Group I: distilled water {10 mL/kg b.w (p.o)}; Group II: chlorpromazine {2 mg/kg b.w (i.p)}; Group III: NLE {150 mg/kg b.w (p.o)}; Group IV: NLE {300 mg/kg b.w (p.o)}; Group V: NLE {600 mg/kg b.w (p.o)}. The extract, chlorpromazine and distilled water were administered 30 minutes before intraperitoneal administration of methamphetamine (35 mg/kg b.w) or apomorphine (5 mg/kg b.w). Thereafter, each mouse was put in an observation chamber and observed individually for 2 minutes at 15, 30, 60, and 90 minutes after methamphetamine administration [11, 12]. Stereotypy was scored as: Absence of stereotype behavior = 0; Presence of stereotyped movement of the head and occasional sniffing = 1; Occasional sniffing and gnawing = 2; Frequent gnawing = 3; Intense continuous gnawing = 4; Intense gnawing and staying on the same spot = 5. A global score was obtained for each mouse by calculating the mean stereotype scores. In three separate experiments, the effects of the partition extracts (HPE, BPE, and EAPE) were evaluated using the same method and the same doses.

2.4 Screening for *Newbouldia laevis*-induced catalepsy in mice

Mice were assigned into groups of 6 animals each and treated as follows:
Group I: Distilled water {10 mL/kg b.w (p.o)}; Group II: Haloperidol {1 mg/kg b.w (i.p)}; Group III: BPE {150 mg/kg b.w (p.o)}; Group IV: BPE {300 mg/kg b.w (p.o)}; Group V: BPE {600 mg/kg b.w (p.o)}. The mice were then subjected to catalepsy test at 60th, 120th, 180th, and 240th min after the treatment. The set-up for the test consists of a Perspex rod elevated to a height of 3.5 cm. The time a mouse spent when placed on the rod with its fore limb was recorded. The test was terminated when the mouse removed the fore limbs from the rod and placed them on the floor or when it climbed the rod [13].

2.5 Effect of *Newbouldia laevis* extracts on haloperidol-induced catalepsy

Mice were assigned into groups of 6 animals each and pretreated with the extract as follows:
Group I: Distilled water {10 mL/kg b.w (p.o)}; Group II: NLE {150 mg/kg b.w (p.o)}; Group III: NLE {300 mg/kg b.w (p.o)}; Group IV: NLE {600 mg/kg b.w (p.o)}
Thirty minutes later, the mice were injected intraperitoneally with haloperidol (1 mg/kg b.w), and then subjected to catalepsy test as described above [14].

2.6 Statistical analysis

Data obtained from the experiments are expressed as mean ± standard error of mean (SEM). The data were subjected to one-way analysis of variance (ANOVA) and Student's - Newman-Keul test to determine the statistical significance of differences between groups. Differences were considered to be significant when $p<0.05$. GraphPad Prism version 5.0 for windows was used for these statistical analyses (GraphPad software, San Diego California USA).

3. RESULTS

3.1 Effect of *Newbouldia laevis* on excitotoxin-induced stereotypy

The crude and partition extracts attenuated the stereotype behavior induced by methamphetamine. Compared with the control, stereotype behavior was significantly reduced following administration of NLE ($p<0.05$), HPE ($p<0.05$), EAPE ($p<0.05$), BPE and chlorpromazine ($P<0.01$). Similarly, the extracts significantly decreased ($p<0.01$) apomorphine-induced stereotype behavior in mice compared with the control. The results are depicted in Figures 1 and 2

3.2 Effects of *Newbouldia laevis* extracts on catalepsy in mice

Administration of BPE (600 mg/kg b.w) induced catalepsy in mice. Lower doses of the extract (150 and 300 mg/kg b.w) did not produce catalepsy as shown in Table 1. With 600 mg/kg, there was significant difference ($p<0.05$) between the duration of catalepsy in the extract-treated mice and the control at 60, 120, 180, and 240 minutes. Haloperidol also significantly increased duration of catalepsy ($p<0.01$) compared with the control at all the time of the experiment. Administration of NLE (600 mg/kg b.w) caused significant potentiation of haloperidol-induced catalepsy in mice at 120, 180, and 240 min of the experiment. This is depicted in Figure 3

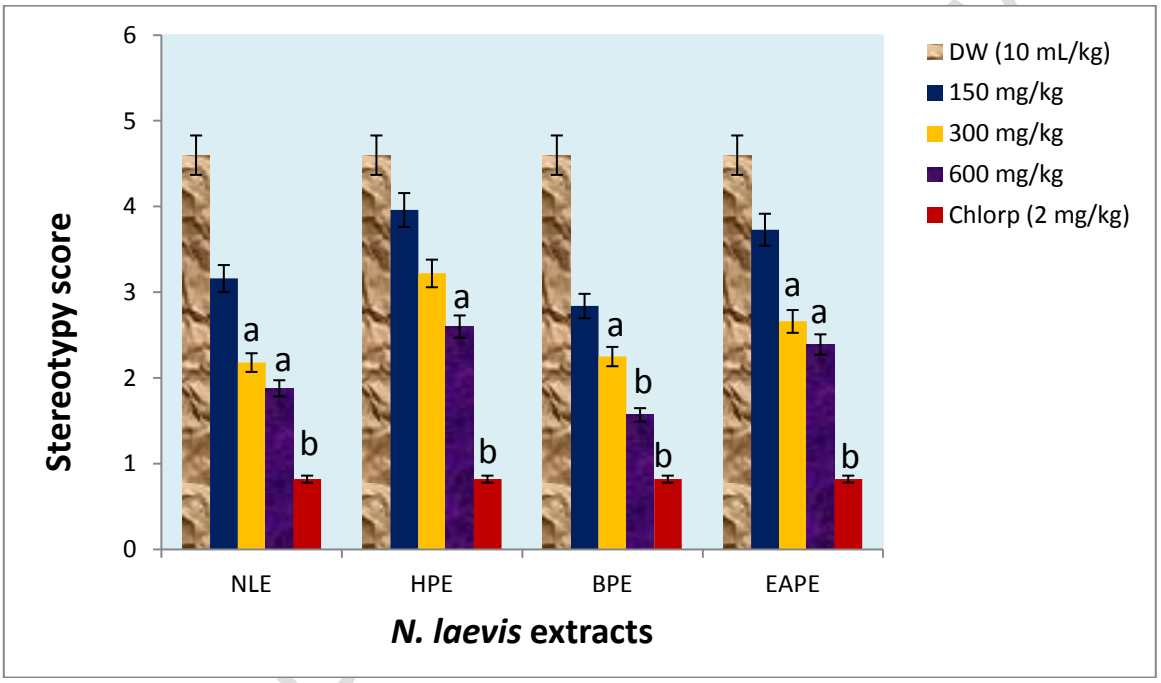

Figure 1: Effect of *N. laevis* extracts on methamphetamine-induced stereotypy
Values represent mean ± SEM (n = 6), [a]$p<0.05$ compared with distilled water-treated mice, [b]$p<0.01$ compared with distilled water-treated mice. DW = distilled water, Chlorp = chlorpromazine (standard drug).

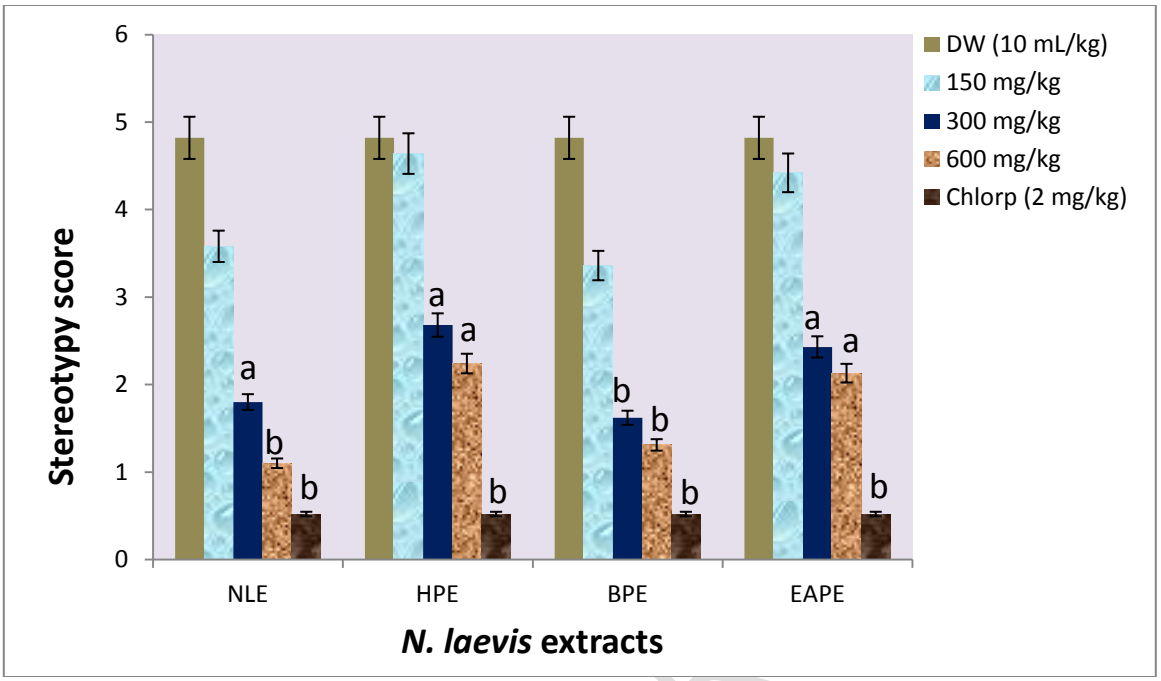

Figure 2: Effect of *N. laevis* extracts on apomorphine-induced stereotypy
Values represent mean ± SEM (n = 6), [a]p<0.05 compared with the control, [b]p<0.01 compared with the control. DW = distilled water (control), Chlorp = chlorpromazine (standard drug)

Table 1: Effect of butanol fraction of *N. laevis* (BPE) on catalepsy in mice

Treatment	Dose	Duration of catalepsy (s)			
	(mg/kg b.w)	60th min	120th min	180th min	240th min
Control	-	4.04±0.31	3.22±0.20	5.70±0.52	4.88±0.93
HPD	1	38.42±28.30[a]	54.33±24.24[a]	66.46±27.55[a]	62.60±21.40[a]
BPE	150	5.62±0.41	6.34±0.17	8.51±0.20	6.24±0.63
BPE	300	6.66±0.40	8.24±0.32	7.08±0.85	8.58±0.68
BPE	600	28.84±0.73[a]	32.62±0.60[a]	34.41±0.79[a]	42.62±0.66[a]

Values represent mean ± SEM (n = 6), [a]p<0.05 compared with the control. Distilled water (10 mL/kg b.w) served as the control. HPD = haloperidol

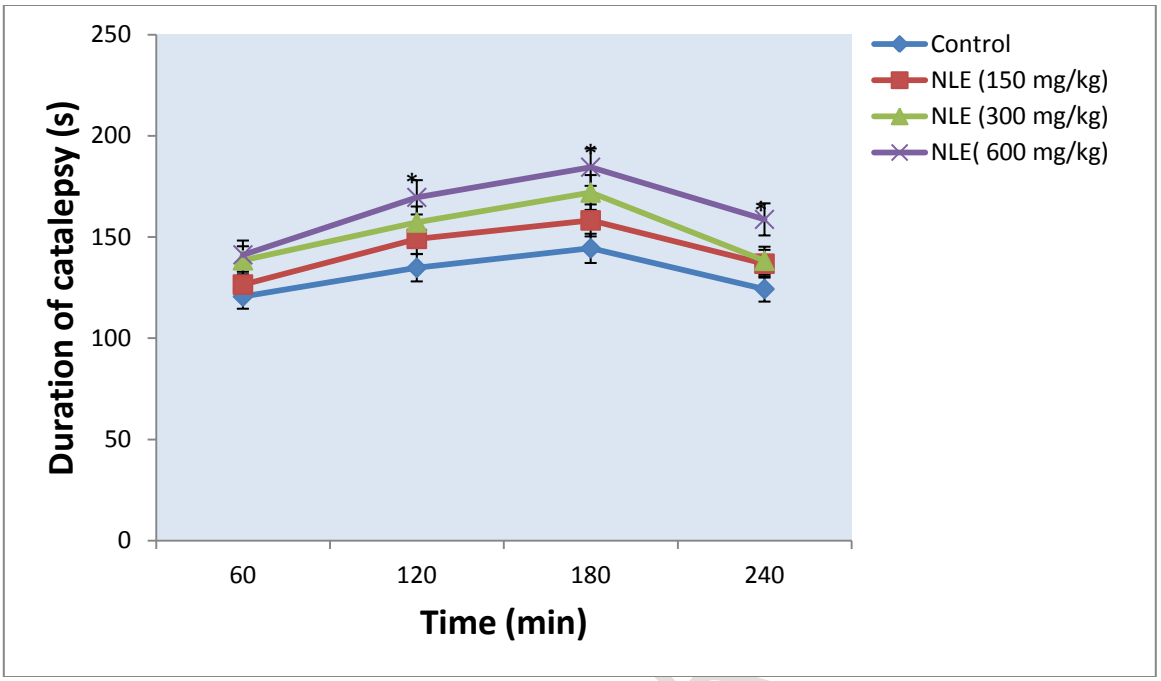

Figure 3: Effect of *N. laevis* extract (NLE) on haloperidol-induced catalepsy in mice
Values represent mean + SEM (n = 6). *p<0.05 compared with the control. Distilled water (10 mL/kg b.w) served as the control.

4. DISCUSSION

The results obtained from this study indicate that *Newbouldia laevis* extracts have modulatory effects on methamphetamine- and apomorphine-induced stereotypy in mice. The extracts significantly reduced the stereotyped behavior induced by the two drugs. Methamphetamine- and apomorphine-induced stereotyped behaviors are models of schizophrenia-like disorder generally used to evaluate antipsychotic drugs [15, 16]. The effects of both apomorphine and methamphetamine are mediated through dopaminergic activity in the brain. Apomorphine is a direct dopamine agonist, while methamphetamine enhances dopamine release and inhibits its reuptake [17]. Activation of D_1 and D_2 receptors in the brain elicits behavioral responses such as stereotyped behavior in animals [18]. Activation of the postsynaptic D_1 receptors through the direct pathway increases neuronal excitability, resulting in the amplification of excitatory corticostriatal input which manifest as increased stereotyped behaviors in the animals. On the other hand, suppression of the direct pathway through the blockade of D_1 receptors results in reduced stereotypic behavior [19]. In the indirect pathway, when postsynaptic D_2 receptors in the striatum are activated, the major inhibitory output nuclei are inhibited, and the thalamus becomes uninhibited, leading to increased activity of the cortex which manifests as increased stereotypy [20, 21]. Dopamine agonists such as methamphetamine and apomorphine activate D_2 receptors to increase stereotypy, whereas dopamine antagonists such as chlorpromazine block D_2 receptors to prevent or attenuate stereotypy [22]. In this study, *Newboulsia laevis* extracts attenuated stereotypy induced by the administration of apomorphine and methamphetamine in mice. There are five identified dopamine pathways in the brain, and each accounts for an effect of antipsychotic drugs. The mesolimbic dopamine pathway is probably involved in the antipsychotic efficacy of neuroleptics. The nigrostriatal dopamine pathway is implicated in the extrapyramidal side effects. The mesocortical pathway is involved in the sedative action of neuroleptics. The hypothalamic-hypophyseal and incertohypothalamic pathways are

responsible for the neuroendocrine side effects caused by neuroleptics [23]. D_2 receptors appear to be the more important of the two important classes of dopamine receptors, for both schizophrenia and the extrapyramidal side effects of antipsychotic drugs, since the drugs with the greatest affinity at the D_2 receptors also have the greatest clinical potency in both respects [24]. The results of this study suggest that *Newbouldia laevis* leaves contain some active compounds which act as dopamine D_2 or D_1 receptor blockers. Many antipsychotic drugs are known to have extrapyramidal side-effects. Therefore it is important to determine whether a drug being screened for antipsychotic property has this side effect using catalepsy test [25]. In this study, the results of catalepsy tests showed that BPE and NLE at 600 mg/kg b.w caused catalepsy and potentiated haloperidol-induced catalepsy in mice, suggesting that *Newbouldia laevis* extracts might have extrapyramidal effects at high doses.

5. CONCLUSION

In conclusion, crude and partition extracts of *Newbouldia laevis* leaves protect mice against stereotyped behavior induced by methamphetamine and apomorphine. This indicates that this medicinal plant possesses antipsychotic property as claimed by traditional healers. Further scientific investigations are required to identify, characterize, and isolate the active compounds responsible for these effects.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee

REFERENCES

1. Tron T, Peled A, Grinsphoon A, Weinshall D. Differentiating facial incongruity and flatness in schizophrenia using structured light camera data. Proceedings: IEEE Annual International Conference of the Engineering in Medicine and Biology Society *(EMBC)* 2016

2. Hor K, Taylor M. Suicide and schizophrenia: a systematic review of rates and risk factors. J. Psychopharmacol 2010; 24(4): 81–90

3. Marder SR, Galderisi S. The current conceptualization of negative symptoms in schizophrenia. World psychiatry 2017; 16(1): 14–24

4. Dayabandara M, Hanwella R, Ratnatunga S, Seneviratne S, Suraweera C, de Silva VA. Antipsychotic-associated weight gain: management strategies and impact on treatment adherence. Neuropsychiatr Dis Treat. 2017; 13: 2231-2241

5. Holt R. Association between antipsychotic medication use and diabetes. Curr Diab Rep. 2019; 19(10): 96

6. Peluso MJ, Lewis SW, Barnes TR, Jones PB. Extrapyramidal motor side-effects of first- and second-generation antipsychotic drugs. Br J Psychiatry. 2012; 200(5): 387-92.

7. Tesfaw G, Kibru B, Ayano G. (2020). Prevalence and factors associated with higher levels of perceived stigma among people with schizophrenia Addis Ababa, Ethiopia. International Journal of Mental Health Systems 2020; 14: 19.

8. Hou CL, Wang SB, Wang F, Xu MZ, Chen MY, Cai MY, et al. Psychotropic medication treatment patterns in community-dwelling schizophrenia in China: comparisons between rural and urban areas. BMC Psychiatry. 2019; 19(1): 242.

9. Kolawole OT, Akanji MA, Akiibinu MO. Toxicological assessment of ethanolic extract of the leaves of *Newbouldia laevis* (P. Beauv). Am. J. M. Sc. 2013; 3(4): 74-80

10. National Research Council (NCR). Guide for the Care and Use of Laboratory Animals (National Academies Press), 2011; 1-220

11. Bourin M, Poison L, Larousse C. Piracetam interaction with neuroleptics in psychopharmacological tests. Neuropsychobiol. 1986; 19: 93-96

12. Umukoro S. Ashorobi RB. Anti-stress potential of aqueous seed extract of *Aframomum melegueta*. Afr J Biomed Res. 2005; 8: 119-121

13. Taiwe GS, Bum EN, Talla E, Dawe A, Clarisse F, Moto O. et al. Antipsychotic and sedative effects of the leaf extract of *Crassocephalum bauchiense* (Hutch.) Milne-Redh (Asteraceae) in rodents. J. Ethnopharmacol. 2012; 143(1): 213-220

14. Pemminati SV, Dorababu NP, Gopalakrishna HN, Pai MRS. Effect of ethanolic leaf extract of *Ocimum sanctum* on haloperidol-induced catalepsy in albino mice. Indian J Pharmacol 2007; 39(2): 87-89

15. Amoateng P, Adjei S, Osei-Safo D, Kukuia K, Bekoe EO, Karikari TK. et al. Extract of *Synedrella nodiflora* (L) Gaertn exhibits antipsychotic properties in murine models of psychosis. BMC Complement Altern Med. 2017; 17(1): 389

16. Pandy V, Vijeepallam K. Antipsychotic-like activity of scopoletin and rutin against the positive symptoms of schizophrenia in mouse models. Experimental Animals 2017; 66(4): 417-423

17. Pandy V, Narasingam M, Mohamed Z. Antipsychotic-like activity of Noni (*Morinda citrifolia* Linn.) in mice. BMC Complement Altern Med. 2012; 12: 186

18. Claude MJ, Silvia GM. Dopamine receptor subtypes, physiology and pharmacology: New ligands and concepts in schizophrenia. Front Pharmacol. 2020; 11: 1003

19. Callum H, Peng H, Linnet R, Sunil UN, Yohanka C, Allen BR, et al. Dopamine D1-like receptor agonist and D2-like receptor antagonist (−)-stepholidine reduces reinstatement of drug-seeking behavior for 3,4-methylenedioxypyrovalerone (MDPV) in rats. ACS Chem Neurosci. 2018; 9(6):1327-1337.

20. Aliane V, Perez S, Bohren Y, Deniau JM, Kemel ML. Key role of striatal cholinergic interneurons in processes leading to arrest of motor stereotypies. Brain 2011; 134(Pt 1): 110–118.

21. Langen M, Kas MJ, Staal WG, van Engeland H, Durston S. The neurobiology of repetitive behavior: Of mice … Neurosci Biobehav Rev. 2011; 35(3): 345-55

22. Yael D, Zeef D, Sand D, Moran A, Katz D, Cohen D, et al. Haloperidol-induced changes in neuronal activity in the striatum of the freely moving rat. Front Syst Neurosci. 2013; 7: 110.

23. Prashant T, Rajnikant P, Arin B, Dheeraj A, Anish C. Evidence of possible side effects of neuroleptic drugs: A systematic review. Asian Pac J Reprod. 2012; 35: 345-355

24. Sykes DA, Moore H, Stott, L, Holliday N, Javitch JA, Lane JR, et al. Extrapyramidal side effects of antipsychotics are linked to their association kinetics at dopamine D_2 receptors. Nat Commun. 2017; 8(1): 763

25. Phattanarudee S, Maher TJ, Towiwat P. Catalepsy and Comparing Gamma-Hydroxybutyrate,1,4-Butanediol, and Gamma-Butyrolactone. In: Preedy VR editor. Neuropathology of Drug Addictions and Substance Misuse, Academic Press; 2016